REVIEW

For reprint orders, please contact: reprints@futuremedicine.com

Next-generation sequencing and personalized genomic medicine in hepatobiliary malignancies

Hepatic Oncology

Arturo Loaiza-Bonilla¹, Emma E Furth² & Jennifer JD Morrisette^{*,2}

Practice points

- Hepatobiliary malignancies are common cancers due to multiple etiologies with a poor prognosis.
- There is significant genomic variation between and within subtypes of these malignancies.
- Actionable genomic changes include: detection of a mutation associated with a targeted therapy, alteration in prognosis, assist in diagnosis or alter therapeutic decisions.
- There are rare mutations within hepatocellular carcinoma subtypes that are targetable.
- Additional targeted therapies are becoming available through clinical trials and US FDA clearance, so therapeutic interventions are continuously changing.

Liver cancer is a heterogeneous group of tumors characterized by significant molecular and genomic heterogeneity. The advent of powerful genomic technologies has allowed detection of recurrent somatic alterations in liver cancer, including mutations, copy number alterations as well as changes in transcriptomes and epigenomes, with the potential to translate these data into clinically relevant predictive and prognostic factors. In this review, we discuss recent advances in the application of high-throughput genomic technologies in liver cancer and the integration of such cancer genome profiling data, highlighting specific relevant subgroups and explain how this knowledge can be used in translational clinical research, 'basket trials', molecular tumor boards, targeted therapy and for personalized genomic medicine applications.

Liver cancer is the third leading cause of cancer-related mortality worldwide, including hepatocellular carcinoma (HCC) and cholangiocarcinoma (CC) [1,2]. CC is the second most common primary hepatic malignancy after HCC [1]. CC is divided into extrahepatic and intrahepatic categories; furthermore, intrahepatic cholangiocarcinomas are further subclassified into bile duct and cholangiolar types each with corresponding etiologic, prognostic and molecular correlates. Since the advent of the 21st century, advances in genomic technologies have led to a comprehensive understanding of pathophysiology and natural history of several tumor models. The molecular and 'omics' analysis of cancer utilizing whole genome and transcriptome approaches has great promise to advance the current classification system for liver cancer, and it has allowed for identification of substantial distinct

KEYWORDS

- cholangiocarcinoma
- genomic medicine
- hepatocellular carcinoma
- liver cancer
- molecular tumor board
- next-generation sequencing
- personalized medicine
- targeted therapy

¹Abramson Cancer Center, Perelman Center for Advanced Medicine, University of Pennsylvania, 3400 Civic Center Boulevard, Philadelphia, PA 19104, USA

²Department of Pathology & Laboratory Medicine, University of Pennsylvania School of Medicine, 3400 Spruce Street, Philadelphia, PA 19104, USA

*Author for correspondence: Tel.: +1 215 898 5578; Fax: +1 215 898 9817; jennifer.morrisette@uphs.upenn.edu

genetic and histologic features that correlate with known risk factors, epidemiology, presentation and outcomes [3–5]. Surgical resection represents the only curative modality for patients suffering from liver cancer; however the majority of cases are diagnosed at advanced stages, with median overall survival of less than 2 years from the time of diagnosis despite palliative chemotherapy and other measures [5–10]. In the particular case of HCC, it is typically resistant to systemic chemotherapy, and only sorafenib-targeted therapy has shown to provide a survival advantage over best supportive care [11].

In an effort to improve the outcomes of these patients, several molecular studies have been performed in HCC and CC, focusing on the correlation of cancer-related genomic changes and consequent alterations in molecular signaling pathways, aiming to detect targetable oncogenic driving mutations, as well as predictive and prognostic genomic markers of response. This active research also includes novel fields of bioinformatics, small RNAs and epigenome mapping. More recently, improvements of sophisticated next-generation sequencing (NGS) and high-throughput microarray-based technologies have provided a high-yield perspective on cancer genomics (e.g., coding mutations, DNA insertions and deletions, copy number variations, chromosomal translocations or inversions, gene amplifications and gene expression) at the single nucleotide resolution level, transforming paradigms of the incipient translational genomic medicine field [12–14].

Herein we describe the most commonly associated molecular changes in hepatobiliary malignancies, and also highlight promising targets for pathway-driven personalized cancer therapy.

Genomic variation, carcinogenesis, allelic imbalance & signaling oncogenic pathways

• HCC

Given the natural history of HCC arising in a background of chronic liver inflammation and cirrhosis in the majority of cases, several somatic alterations and structural variation of genes important for cell cycle and apoptosis are associated with primary liver cancer such as c-MYC, RAS and TP53 [15]. Powerful analytical tools such as array comparative genomic hybridization (CGH) have allowed high-yield DNA copy number data, which provides a background on the molecular changes involved in HCC pathogenesis.

• Mutation interpretation relies on annotated genomic databases

Databases such as OncoDB.HCC [16] are currently available and open access validated catalogs of common aberrations from both human and murine HCC models, including loss of heterozygosity (LOH), gene expression, proteomics and CGH data [17]. This website demonstrates the complexity of HCC at the chromosomal level (Figure 1) and allows the user to search regions of interest for more detailed analysis of the involved genomic regions. The challenge remains in the appropriate interpretation of such rich amount of information. In general, variants are reported according to Human Genome Variation Society (HGVS) nomenclature and classified into three categories: pathogenic, variants of uncertain significance and benign. Categorization of variants is dependent upon literature review, and variant existence in other publicly available databases including the Single Nucleotide Polymorphism Database (dbSNP), the Catalogue Of Somatic Mutations In Cancer (COSMIC) [18] and the 1000 Genomes Project. In addition, academic medical centers host genomic medical knowledge resources online that characterize specific genetic mutations, including ‘My Cancer Genome’ [19], which describes genes, mutations and US FDA and clinical trial information. The Broad Institute maintains a research database of somatically mutated genes linked to a clinical action, updated yearly that includes genes, rationale, mutation type (inclusive of translocations) and examples of potential therapeutic agents (tumor alterations relevant for genomics-driven therapy) (TARGET) [20]. Although this database is not curated for clinical or diagnostic use, the site can be used as a springboard for discovery of mutation-directed therapies.

• Genetic & genomic changes detected in HCC

HCCs are a diverse set of tumors whose pathogenesis has been described as a complex process that requires the accumulation of several epigenetic and genetic changes by sequential alterations, which have not been yet completely established [22]. Specific tumor microarray studies have been pursued to evaluate the transition from a ‘dysplastic nodule (DN)’ (a controversial entity that by some investigators is viewed as a precursor to HCC) to early HCC [22,23]. Gene expression profiling of those lesions identified 460 differentially expressed genes, with MYC

Figure 1. Common genomic aberration regions of hepatocellular carcinoma (per chromosome).

CGH: Comparative genomic hybridization; HCC: Hepatocellular carcinoma; LOH: Loss of heterozygosity; QTL: Quantitative trait locus. Data taken from [16,21].

oncogene activation considered as a potential signaling driver to malignant transformation. Epigenetic analysis of HCC development has also reported progressive CpG island hypermethylation in key genes, as well as distinct methylation patterns based on specific etiology leading to malignant transformation [24–26].

Other efforts have been focused on determining different global gene expression profiles that

correlated with prognosis. One of these studies [27] revealed two separate subclasses of HCC via algorithms and Cox regression models that were predictive of survival. Expression of proliferating cell nuclear antigen, and CDK4, CCNB1, CCNA2 and CKS2 (cell cycle regulators) was greater in the poor survival subclass (cluster A), as well as high expression of antiapoptosis, sumoylation and ubiquitination-related genes.

These findings may provide the rationale for recent efforts to use proteasome inhibitors and inhibition of other mediators of ubiquitin-dependent protein degradation, particularly in poor prognosis subtypes.

Chromosome aberrations (gains or losses) in human HCC have been demonstrated to correlate with tumor grade and specific etiologic factors (e.g., hepatitis B–HBV vs C–HCV viral infection) [28]. The most commonly reported chromosome losses in HCC per a meta-analysis [29] are in 8p (38%), 16q (35.9%), 4q (34.3%), 17p (32.1%) and 13q (26.2%). HBV infection has been correlated with 4q, 16q, 13q and 8p deletions (in the absence of HCV infection). Gain of 1q appeared to be an early event developing in DN, possibly predisposing affected cells to acquire additional chromosomal aberrations, and gain of 3q22–24 is a potential late genomic event associated with tumor recurrence and poor survival. The most frequent chromosomal amplifications involve 1q (57.1%), 8q (46.6%), 6p (22.3%) and 17q (22.2%). These chromosomal aberrations are commonly associated to gene loci implicated in the WNT signaling pathway (e.g., AXIN2, FZD3, SIAH-1, WISP1), as well as oncogene activation sites (e.g., MYC on 8q24) and as inactivating mutations of tumor suppressor genes (e.g., RB1 on 13q14). Poorly differentiated HCC have chromosomes 13q and 4q losses, and gains of 1q correlate with other high-frequency alterations. In DN, amplifications were most frequent in 1q and 8q, whereas deletions occurred in 8p, 17p, 5p, 13q, 14q and 16q [29].

• Mutations in HCC occur in common signaling pathways

Since the advent of the concepts of ‘oncogene-addiction phenomenon’ and compensatory or escape pathways [30–32], the identification of specific signaling driving pathways and key oncogenes and tumor suppressors regulating hepatocarcinogenesis has become a subject of active investigation [15,33–35]. Signaling pathways and mutations described in HCC include (Figure 2) TP53 [34,36–37], Wnt/ β -Catenin (e.g., MYC, Cyclin D1 and E-Cadherin) [38,39], HGF/c-MET [40,41], EGFR (via AKT, STATs, RAS/RAF) [42–44], IGF [45,46], TGF- β [47], NF- κ B [48,49], VEGF [11,50–51] and Hippo [52,53], among other potential candidates.

Transcriptome analysis of HCC by Boyault *et al.* [56] identified six different

subgroups of HCC (G1–G6) that were characterized by specific clinical and genetic features. G1 tumors had low HBV copy number and increased expression of genes controlled by parental imprinting in hepatoblasts. G2 tumors included HBV-related HCC and PIK3CA and TP53 mutations. G1 and G2 were prominent in AKT pathway activation. G3 tumors had cell-cycle control related gene over expression, as well as TP53 mutations. G4 included HCC and hepatocellular adenomas with TCF1 mutations. G5 and G6 groups had Wnt/ β -catenin pathway mutations. This particular study highlighted the fact WNT and AKT pathways were activated in approximately 50% of the analyzed HCC samples, opening up potential therapeutic targets [56].

A meta-analysis of gene expression profiles by Hoshida *et al.* analyzing data sets from eight independent patient cohorts across the world, including 603 patients with HCC, also reported different HCC subclasses (S1, S2, and S3). S1 was prominent on WNT signaling pathway aberrant activation, S2 had MYC and AKT pathways activation, and S3 was associated with hepatocyte differentiation. S1 tumors-related WNT pathway activation was noted as a result of transforming growth factor- β activation (unlike its common association with β -catenin mutation), thus representing a new mechanism of WNT pathway activation in HCC [57].

A recent study that utilized exome sequencing of 243 hepatocellular carcinomas identified relationships between different types of exposures and mutational signatures [14]. This study identified recurrent mutations in 161 genes and grouped tumors into specific mutational signatures based on the patterns of mutations identified. Further analysis identified 14 genes that were significantly mutated (TP53, CTNNB1, AXIN1, ALB, ARID1A, ARID2, ACVR2A, NFE2L2, RPS6KA3, KEAP1, RPL22, CDKN2A, CDKN1A, RB1), commonly deleted (CFH, IRF2, CDKN2A, PTPN3, PTEN, AXIN1, RPS6KA3) and amplified genes (TERT, VEGFA, MET, MYC, FGF-CCND1 JAK3, CCNE1). Analysis of the 161 commonly mutated genes found that they fell into 11 common pathways–telomerase activating mutations (including TERT promoter mutations [60%]), WNT/ β -catenin pathway (54%), PI3K–AKT–mTOR pathway (51%), TP53 and cell cycle alterations (49%), MAPK (43%), hepatic differentiation (34%), epigenetic regulation (32%), chromatin remodeling (28%),

oxidative stress (12%), IL-6 and JAK-STAT (9%) and transforming growth factor (TGF)- β (5%) – with analysis showing that in 28% of cases there was at least one potentially actionable gene mutation identified per tumor [14]. These findings suggest that genomic analysis gene mutations and other alterations can offer the benefit of off-label approved therapies or access to clinical trials to a significant portion of patients.

• CC

As with HCC, CC are an even more diverse set of tumors. In an effort to improve the outcomes of these patients, several molecular studies of CC have been performed, aiming to detect targetable oncogenic driving mutations, as well as predictive and prognostic genomic markers of response [58–65]. The substantial genomic variability noted in CC (Figure 3) is a reflection of its diversity but as well provides the rationale for such approaches based on each patient's CC's particular genetic makeup [66,67].

• Genetic & genomic changes in CC

A study by Miller *et al.* [67] investigated changes in gene expression and copy number

in biliary cancers and correlated those changes with clinical features and outcomes. Gene expression and CGH analysis on 34 biliary tract cancer specimens found 545 genes with altered expression in extrahepatic cholangiocarcinoma (EHC) and 2,354 in intrahepatic cholangiocarcinoma (IHC).

Despite considerable heterogeneity in the extent of chromosomal instability between patients even within specific cancer subtypes, there appeared to be selected chromosomal regions that were commonly altered. CGH analysis revealed that short segments of chromosomes 1p, 3p, 6q, 8p, 9p, and 14q were commonly deleted across all cancer subtypes. Commonly amplified regions included segments of 1q, 3q, 5p, 7p, 7q, 8q, and 20q. Overrepresentation analysis revealed an association between altered expression of functional gene groupings and pathologic features, including mutated expression of a large number of cell cycle regulators including UBD, BCL2L2, CDC2, MCM2, and CDKN1C in all subtypes, as well as genes involved with regulation of cellular metabolism and biosynthesis and high pathologic grade. Vascular invasion was associated

Figure 2. Most common gene mutations in hepatocellular carcinoma.

Mutation data taken from [54,55]

Figure 3. Most common gene mutations in biliary cancers.

Mutation data taken from [54,55].

with mutated expression of genes involved with electron transport and cellular metabolism [67].

Moreover, two main biological classes of CC have recently been described by Sia *et al.* [66]. A group named the ‘proliferation class’ (62% of CCs in this study) had specific copy number alterations. These were high-level amplifications in five regions, including 1p13 (9%) and 11q13.2 (4%), and several focal deletions, such as 9p21.3 (18%) and 14q22.1 (12% in coding regions for the SAV1 tumor suppressor). Many features of the poor-prognosis signatures described for HCC were also noted in this class, as well as a worse outcome ($p < 0.001$) when compared with the other group of CCs. This group was also prominent on DNA amplifications at 11q13.2, deletions at 14q22.1, mutations in KRAS and BRAF, as well as characterized by activation of oncogenic signaling pathways (including RAS, MAP kinase and MET) [65].

The second group described by Sia was the ‘inflammation class’ (38%), which was characterized by activation of inflammatory signaling pathways, overexpression of cytokines, and STAT3 activation [66]. The STAT-1 has been reported as overexpressed nearly 9-fold in cases

of cholangiocarcinoma [18]. These results emphasize the significant genetic diversity of CC and provide specific identifiers for classifying such tumors.

• Common & targetable mutations in CC

In the largest cohort of compiled CC cases to date, Ross *et al.* performed DNA sequencing of hybridization-captured libraries for 3320 exons of 182 cancer-related genes and 36 introns of 14 genes frequently rearranged in cancer. Two-thirds of patients in this study harbored targetable genomic alterations that have the potential to personalize therapy selection for individual patients [60]. The potential clinical applicability and relevance of next generation sequencing tumor profiling is particularly compelling in cases of rare or resistant tumors such as CC, where treatment guidelines are frequently limited and current clinical trial data is often insufficient. Given evidence of significant pleiotropic and biologic heterogeneity, there is a clear need for appropriate classification of subsets of CC patients during treatment choice selection, as these signatures seem not only prognostic but predictive for response to targeted therapy.

Table 1. Clinical utility for targeted mutation testing in hepatocellular carcinoma.

Gene (ordered by frequency)	Type of alteration (estimated frequency)	Examples of therapeutic agents [†]	Rationale
<i>AXIN1</i>	LOF (liver: 7%)	None at this time	Wild-type protein inhibits activation of Wnt/ β -catenin signaling; may be sensitive to Wnt inhibitors
<i>ARID1A</i>	LOF (CC: 15–21%; liver: 14.3%)	None at this time	<i>ARID1A</i> interacts with EZH2 and inhibition of EZH2 acts as a synthetic lethal manner in <i>ARID1A</i> -mutated cells. EZH2 inhibitors [‡]
<i>ARID2</i>	LOF (liver: 3%; CC: 2.5%)	None at this time	<i>ARID2</i> signaling is involved in IFN signaling; may predict insensitivity to IFN if mutated [‡]
<i>ATM</i> [§]	Biallelic inactivation (liver: 5.2–19%; CC: 5%)	PARP inhibitors	Biallelic inactivation predicts sensitivity to PARP inhibitors
<i>BAP1</i>	LOF (liver: 0.4%; CC: 17.5%)	None at this time	HDAC inhibitors [‡] ; EZH2 inhibitors [‡]
<i>BRAF</i>	Mutation, amplification, translocation (liver: 0.4%)	Vemurafenib, dabrafenib, RAF inhibitors MEK inhibitors	Sensitivity to RAF inhibitors; amplification may predict resistance to MEK inhibitors
<i>BRCA1</i> [§]	LOF mutation, deletions (liver: 3.6%)	PARP inhibitors	Biallelic inactivation predicts sensitivity to PARP inhibitors
<i>BRCA2</i> [§]	LOF mutation, deletions (liver: 3.6%)	PARP inhibitors	Biallelic inactivation predicts sensitivity to PARP inhibitors
<i>CDKN2A</i>	LOF mutation, biallelic deletion (liver: 5.6%; CC: 12.5%)	CDK4/6 inhibitors	Biallelic inactivation leads to uncontrolled growth through decreased binding to CDK4/6
<i>CTNNB1</i>	GOF mutation (liver: 14.3–27.4%)	WNT inhibitors	May predict sensitivity to inhibitors of WNT signaling; resistance to EGFR, PI3K and AKT inhibitors
<i>EGFR</i>	Mutations, amplifications, intragenic deletions (liver: 4.2%)	Erlotinib, gefitinib, new-generation EGFR inhibitors	Mutations targetable with TKIs; mutations predict resistance to TKIs. Amplification associated with response to anti-EGFR therapies
<i>FBXW7</i>	Biallelic inactivation (liver: 0.4%; CC: 6.7%)	MTOR pathway inhibitors	May predict sensitivity to MTOR inhibitors; may predict resistance to antitubulin chemotherapies
<i>FGFR2</i>	Amplification, GOF mutations (liver: 1.7%; CC: 10%)	Dovitinib, FGFR inhibitors	May predict response to targeted TKI inhibitors
<i>HNF1A</i>	LOF (liver: 1.6%)	None at this time	Unknown at this time
<i>IDH1</i>	GOF mutations (liver: 1.6%; CC: 20%)	IDH inhibitors, vaccine	May predict sensitivity to IDH inhibitors
<i>IDH2</i>	GOF mutations (liver: 3.7%; CC: 5%)	IDH inhibitors, vaccine	May predict sensitivity to IDH inhibitors
<i>IL6ST</i>	GOF (liver: 3.6%)	None at this time	Unknown at this time
<i>JAK1</i>	GOF mutation (liver: 3.2%)	Ruxolitinib	Activating <i>JAK1</i> mutations activate STAT signaling; ruxolitinib inhibits <i>JAK1/2</i> signaling
<i>KRAS</i>	GOF mutations (liver: 1.3%; CC: 37.5%)	None at this time	Mutations can predict resistance to therapies (EGFR, cetuximab); may show sensitivity to combined therapies with MEK inhibitors
<i>MLL3 (KMT2C)</i>	LOF (liver: 9.4%; CC: 25%)	None at this time	May be sensitive to HDAC inhibitors [‡]
<i>NFE2L2 (NRF2)</i>	Complex: LOF GI (GOF other tumors) (liver: 5.7%)	Tecfidera	Tecfidera mechanism of action is through activation of the NRF2 pathway; mechanism unknown
<i>PBRM1</i>	LOF (liver: 0.9%; CC: 17.5%)	None at this time	Unknown at this time
<i>PIK3CA</i>	GOF mutations (liver: 4.2%; CC: 7.5%)	PI3K/AKT/MTOR inhibitors	Sensitivity to PI3K, AKT, MTOR inhibitors
<i>PTEN</i>	Loss of function mutation, biallelic deletion (liver: 1.7–6.7%; CC: 12.5%)	Inactivation may predict sensitivity to PI3K, AKT, MTOR inhibitors	Biallelic inactivation
<i>RB1</i>	Biallelic inactivation (liver: 5.5–11.3%; CC: 6.1%)	None at this time	Resistance to CDK4/6 inhibitors

Percentages shown here are in whole or part based upon data generated by the TCGA Research Network [82].

[†]Targeted therapy may be in research setting only and may not have current trial open.

[‡]Speculation based on *in vitro* data, untested functionally and in model systems/humans.

[§]May signal the presence of a germline mutation.

GOF: Gain of function; HDAC: Histone deacetylase; IFN: Interferon; LOF: Loss of function; PARP: PolyADP ribose polymerase.

Table 1. Clinical utility for targeted mutation testing in hepatocellular carcinoma (cont.).

Gene (ordered by frequency)	Type of alteration (estimated frequency)	Examples of therapeutic agents [†]	Rationale
<i>RNF43</i>	Biallelic inactivation (CC: 37.5%)	None at this time	Mutations derepress Wnt/ β -catenin signaling; may predict sensitivity to WNT inhibitors [‡]
<i>SMAD4</i>	Biallelic inactivation (liver: 0.9%; CC: 2.5%)	None at this time	Mutation or deletion may lead to sensitivity to TGF- β inhibitors [‡] ; resistance to 5-FU therapy
<i>SMARCA4</i>	LOF (liver: 1.7%; CC: 6.7%)	None at this time	Unknown at this time
<i>TERT</i>	Amplification (liver: 6.2%)	Immunotherapy, telomerase inhibition [‡]	Mutations lead to increased telomerase expression
<i>TP53</i> [§]	LOF mutation, deletion (liver: 61.9%; CC: 20%)	Wee1 inhibitors, Chk1 inhibitors, kevetrin, APR-246, nutlins, gene therapy	Biallelic inactivation

Percentages shown here are in whole or part based upon data generated by the TCGA Research Network [82].
[†]Targeted therapy may be in research setting only and may not have current trial open.
[‡]Speculation based on *in vitro* data, untested functionally and in model systems/humans.
[§]May signal the presence of a germline mutation.
GOF: Gain of function; HDAC: Histone deacetylase; IFN: Interferon; LOF: Loss of function; PARP: PolyADP ribose polymerase.

The most commonly described activating mutations in CC genomic studies are KRAS [61,62], HER2 [63], MET [64], as well as hotspot activating missense mutations in genes downstream to EGFR, such as the BRAF [65].

A recent study using Caris Molecular Intelligence™ (Caris Life Sciences, TX, USA) to analyze tumors from 42,000 patients, covering 150 histological subtypes, reported the benefits of tumor profiling of common and rare types of cancer such as CC in order to identify potential treatment targets. Out of these patients, 14,700 were categorized as having rare tumors, defined as a patient segment that is typically underserved by current guidelines. Results from the study included a 16.3% frequency of KRAS mutations in cholangiocarcinoma [68].

Saha *et al.* [69] further investigated the presence of mutant IDH1 and IDH2 mutations in the liver of genetically engineered mice as a potential biomarker, finding expression of mutant IDH in liver cells to cause impaired hepatocyte differentiation, and markedly elevated levels of cell proliferation. Mutant IDH proteins were associated with activated KRAS, thus promoting malignant transformation and tumor progression to metastatic CC [69]. Interestingly, IDH mutations are present only in intrahepatic CC and enriched in the cholangiolar type.

The MAPK pathway comprises several important targets for targeted therapy. Specific mutations in BRAF can stimulate this pathway, and the presence of a V600E or K mutation predicts responsiveness to BRAF inhibitors or MEK inhibition. BRAF is located on chromosome 7q34. It

is a member of the Raf kinase family that plays a critical role in regulating the cell proliferation through the MAPK pathway. BRAF mutation-driven CCs have been reported at a frequency between 0 and 22% [70–73]. Our group recently reported the successful use of dual BRAF and MEK inhibition for the management of a V600E mutant ICC [73].

Combined hepatocellular-cholangiocarcinoma
Combined hepatocellular-cholangiocarcinoma (CHC) is an intermediate form between HCC and CC, suggesting phenotypic overlap between these tumors, with poorly understood pathogenesis. Woo *et al.* [74] performed gene expression profiling of human HCC, CHC, and CC, identifying a novel HCC subtype, described as cholangiocarcinoma-like HCC (CLHCC), which expressed cholangiocarcinoma-like traits (CC signature). CLHCC co-expressed embryonic stem cell-like expression traits suggesting a bi-potent hepatic progenitor cell origin. The study suggested that the acquisition of cholangiocarcinoma-like expression traits was a crucial step in progression of HCC, associated to worse prognosis [12,74].

• **Promises & pitfalls of next-generation sequencing**
Genomic sequencing through the use of targeted gene panels, exome, whole genome and transcriptome sequencing has changed the landscape of medical oncology. The identification of tumors containing mutations with targeted therapeutics

available continues to grow, as has detection of mutations with a predictive or prognostic effect (e.g. KRAS in colorectal carcinoma). However, the identification of these mutations can be dependent on the type of methodology and bioinformatics analysis tools utilized.

The majority of tumors sent for sequence analysis are heterogeneous with varying mixtures of neoplastic and non-neoplastic cells. In addition, clonal evolution, loss of heterozygosity and aneuploidy can compound the difficulty in detection and interpretation of results. The annotation of clinical knowledge for less common genes and mutations is often lacking with case reports difficult for treating medical oncologists to utilize for patient treatment. These deficiencies have been recognized and large national and international trials are beginning to address some of these concerns.

• Translating the data: molecular tumor boards & promising targets

Several evidence-based commercial tumor profiling services are fast becoming part of current management of oncology patients, and adoption, knowledge and integration of these genomic data for development of personalized regimens is extremely relevant. Many centers across the globe are incorporating multidisciplinary molecular tumor boards to address those needs, making decisions on the most appropriate regimens based on genomic tumor information [73,75].

The identification of promising targets in hepatobiliary cancer that are being discussed include tyrosine kinase growth factor receptors overexpression such as HER2, EGFR, and MET/HGF, (summarized in **Table 1**), which as previously described may play important roles in the development and pathogenesis of HCC and CCs [40–44]. Nakasawa *et al.* [76] reported significant overexpression of ERBB2 (HER2/neu) in extrahepatic bile duct tumors (5.1%). EGFR overexpression (8%) and gene amplification (77%) were also noted. MET overexpression was most frequent in IHC (21.4%) but was not associated with gene amplification. In addition, it has been reported that patients with CC may also harbor gene rearrangements of FGFR2 that could be targetable by agents such as Regorafenib [77–79]. Sia *et al.* reported that 45% of CC patients harbored at least 1 FGFR2 fusion [66]. Gu *et al.* confirmed the presence of ROS kinase fusions in CC patients [80]. The expression of ROS fusions confers a transforming

ability both *in vitro* and *in vivo* and is responsive to its kinase inhibitor, similar to the effect noted in lung adenocarcinoma [81].

STAT-1 protein transcription factors are activated by the JAK, and JAK/STAT pathway inhibitors is another potential therapeutic approach [67,77–78]. Similar to HCC, ubiquitin-related genes have been reported as significantly altered in IHC [27,67,79]. Proteasome inhibitors and inhibition of other mediators of ubiquitin-dependent protein degradation are currently under investigation [80,83]. Those pathways are the major subject of current ‘basket’ trial designs involving targeted therapies incorporating the genotype-to-phenotype concept, enrolling patients harboring specific mutations in pertinent Phase II trials exploring molecular targeted therapies or chemotherapeutical regimens, unlike a disease-specific (or phenotype-to-genotype) approach [81,84–85].

Conclusion & future perspective

Recent advances in NGS, transcriptomics, proteomics and epigenomics in liver cancer have exponentially expanded our knowledge about the molecular events involved in the pathogenesis and natural history of HCC and CC [86]. Despite being at an early development phase, continuous efforts in translational oncology will undoubtedly continue to integrate these data into the clinic and subsequent personalized genomic approaches [87–91].

Particularly regarding HCC, and unlike other malignancies, the notion to have tumor biopsies to undergo NGS studies is a relatively novel concept, as the diagnosis of HCC still relies on imaging studies and serum alpha-fetoprotein levels in most cases, except in the rare event where liver cirrhosis is not documented. This has led to a significant gap between the progress of genomic study and its clinical application for patients with liver cancer, which should be overcome by the rapid implementation of diagnostic algorithms that include procurement of tissue for diagnostic, prognostic and therapeutic purposes.

One major challenge remains in the validity of these tests and platforms to guide treatment decisions, given the heterogeneity of the data and lack of prospective proof-of-concept studies. The development and integration of basket clinical trials with such data is critical and warranted for that purpose. Strong bioinformatics support and homogenization of NGS platforms are also essential in achieving that goal [92].

The ever-growing availability and affordability of DNA microarray technologies and profiling, and the advancement of other global initiatives such as the 1000 Genomes Project and the Cancer Genome Atlas will certainly improve our understanding of the pathophysiology of liver cancer and influence future treatment decisions in clinical oncology, with the ultimate goal of improving our patient's outcomes.

Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

References

Papers of special note have been highlighted as:
• of interest; •• of considerable interest

- Bleachacz BR, Gores GJ. Cholangiocarcinoma. *Clin. Liver Dis.* 12(1), 131–150 (2008).
- Khan SA, Thomas HC, Davidson BR *et al.* Cholangiocarcinoma. *Lancet* 366(9493), 1303–1314 (2005).
- Sempoux C, Jibara G, Ward S *et al.* Intrahepatic cholangiocarcinoma: new insights in pathology. *Semin. Liver Dis.* 31(1), 104–110 (2011).
- Khan SA, Taylor-Robinson SD, Toledano MB *et al.* Changing international trends in mortality rates for liver, biliary and pancreatic tumours. *J. Hepatol.* 37(6), 806–813 (2002).
- Singh P, Patel T. Advances in the diagnosis, evaluation and management of cholangiocarcinoma. *Curr. Opin. Gastroenterol.* 22(3), 294–299 (2006).
- Jarnagin WR, Fong Y, DeMatteo RP *et al.* Staging, resectability, and outcome in 225 patients with hilar cholangiocarcinoma. *Ann. Surg.* 234(4), 507–517 (2001).
- Jarnagin WR, Ruo L, Little SA *et al.* Patterns of initial disease recurrence after resection of gallbladder carcinoma and hilar cholangiocarcinoma: implications for adjuvant therapeutic strategies. *Cancer* 98(8), 1689–1700 (2003).
- Weber SM, Jarnagin WR, Klimstra D, DeMatteo RP, Fong Y, Blumgart LH. Intrahepatic cholangiocarcinoma: resectability, recurrence pattern, and outcomes. *J. Am. Coll. Surg.* 193(4), 384–391 (2001).
- Tan JC, Coburn NG, Baxter NN *et al.* Surgical management of intrahepatic cholangiocarcinoma – a population-based study. *Ann. Surg. Oncol.* 15(2), 600–608 (2008).
- Valle J, Wasan H, Palmer DH *et al.* Cisplatin plus gemcitabine versus gemcitabine for biliary tract cancer. *N. Engl. J. Med.* 362(14), 1273–1281 (2010).
- Llovet JM, Ricci S, Mazzaferro V *et al.* Sorafenib in advanced hepatocellular carcinoma. *N. Engl. J. Med.* 359(4), 378–390 (2008).
- DeVita VT, Lawrence TS, Rosenberg SA (Eds). *Primer of the molecular biology of cancer*. In: *Cancer: Principles & Practice of Oncology*. Lippincott Williams & Wilkins, Philadelphia, PA, USA (2011).
- Pinkel D, Albertson DG. Array comparative genomic hybridization and its applications in cancer. *Nat. Genet.* 37(Suppl.), S11–S17 (2005).
- Schulze K, Imbeaud S, Letouze E. Exome sequencing of hepatocellular carcinomas identifies new mutational signatures and potential therapeutic targets. *Nat. Genet.* 47(5), 505–511 (2015).
- Shows mutational signatures associated with different environmental etiologies of hepatocellular carcinoma, showing the extensive landscape of altered genes and common pathways.
- Villanueva A, Newell P, Chiang DY, Friedman SL, Llovet JM. Genomics and signaling pathways in hepatocellular carcinoma. *Semin. Liver Dis.* 27(1), 55–76 (2007).
- OncoDB.HCC. <http://oncodb.hcc.ibms.sinica.edu.tw>
- Su WH, Chao CC, Yeh SH *et al.* HCC: an integrated oncogenomic database of hepatocellular carcinoma revealed aberrant cancer target genes and loci. *Nucleic Acids Res.* 35, D727–D731 (2007).
- Shows how the use of genomics and bioinformatics may lead to network discoveries.
- Forbes SA, Beare D, Gunasekaran P *et al.* COSMIC: exploring the world's knowledge of somatic mutations in human cancer *Nucleic Acids Res.* 43(D1), D805–D811 (2015)
- Extremely valuable source of genomic information regarding mutations, frequencies in tumors and references for the functional and clinical relevance.
- My Cancer Genome. www.mycancergenome.org
- TARGET. www.broadinstitute.org/cancer/cga/target
- Su WH, Chao CC, Yeh SH, Chen DS, Chen PJ, Jou YS. OncoDB.HCC: an integrated oncogenomic database of hepatocellular carcinoma revealed aberrant cancer target genes and loci. *Nucleic Acids Res.* 35, D727–D731 (2007).
- Kojiro M, Roskams T. Early hepatocellular carcinoma and dysplastic nodules. *Semin. Liver Dis.* 25(2), 133–142 (2005).
- Kaposi-Novak P, Libbrecht L, Woo HG *et al.* Central role of c-Myc during malignant conversion in human hepato-carcinogenesis. *Cancer Res.* 69(7), 2775 (2009).
- Um TH, Kim H, Oh BK *et al.* Aberrant CpG island hypermethylation in dysplastic nodules and early HCC of hepatitis B virus related human multistep hepatocarcinogenesis. *J. Hepatol.* 54(5), 939–947 (2011).
- Lambert MP, Paliwal A, Vaissiere T *et al.* Aberrant DNA methylation distinguishes hepatocellular carcinoma associated with HBV and HCV infection and alcohol intake. *J. Hepatol.* 54(4), 705–715 (2010).
- Hernandez-Vargas H, Lambert MP, Le Calvez-Kelm F *et al.* Hepatocellular carcinoma displays distinct DNA methylation signatures with potential as clinical predictors. *PLoS ONE* 5(3), e9749 (2010).
- Shows that epigenetics is also important and a feature not detected by usual sequencing technologies.
- Lee JS, Chu IS, Heo J *et al.* Classification and prediction of survival in hepatocellular carcinoma by gene expression profiling. *Hepatology* 40(3), 667 (2004).
- Moinzadeh P, Breuhahn K, Stutzer H, Schrmacher P. Chromosome alterations in human hepatocellular carcinomas correlate with aetiology and histological grade – results of an explorative CGH meta-analysis. *Br. J. Cancer* 14(5), 9235–9241 (2005).

- 29 Poon TCW, Wong N, Lai PBS *et al.* A tumor progression model for hepatocellular carcinoma: bioinformatics analysis of genomic data. *Gastroenterology* 131(4), 1262 (2006).
- 30 Weinstein IB, Joe AK. Mechanisms of disease: oncogene addiction – a rationale for molecular targeting in cancer therapy. *Nat. Clin. Pract. Oncol.* 3(8), 448–457 (2006).
- 31 Loaiza-Bonilla A, Glück S. Using modern molecular markers to tailor breast cancer treatment: a new era for personalized medicine. *Breast Cancer Manag.* 1(2), 105–108 (2012).
- 32 Yarden Y. The biological framework: translational research from bench to clinic. *Oncologist* 16(Suppl. 1), 23–29 (2011).
- 33 Aravalli RN, Steer CJ, Cressman EN. Molecular mechanisms of hepatocellular carcinoma. *Hepatology* 48(6), 2047–2063 (2008).
- 34 Farazi PA, DePinho RA. Hepatocellular carcinoma pathogenesis: from genes to environment. *Nat. Rev. Cancer* 6(9), 674–687 (2006).
- 35 Marquardt JU, Galle PR, Teufel A. Molecular diagnosis and therapy of hepatocellular carcinoma (HCC): an emerging field for advanced technologies. *J. Hepatol.* 56(1), 267–75 (2012).
- 36 Woo HG, Wang XW, Budhu A *et al.* Association of TP53 mutations with stem cell-like gene expression and survival of patients with hepatocellular carcinoma. *Gastroenterology* 140(3), 1063–1070 (2011).
- 37 Farazi PA, Glickman J, Horner J, Depinho RA. Cooperative interactions of p53 mutation, telomere dysfunction, and chronic liver damage in hepatocellular carcinoma progression. *Cancer Res.* 66(9), 4766–4773 (2006).
- 38 Calvisi DF, Factor VM, Ladu S, Conner EA, Thorgeirsson SS. Disruption of beta-catenin pathway or genomic instability define two distinct categories of liver cancer in transgenic mice. *Gastroenterology* 126(5), 1374–1386 (2004).
- 39 Ishizaki Y. Immunohistochemical analysis and mutational analyses of [beta]-catenin, Axin family and APC genes in hepatocellular carcinomas. *Int. J. Oncol.* 24(5), 1077–1083 (2004).
- 40 Stella GM, Benvenuti S, Comoglio PM. Targeting the MET oncogene in cancer and metastases. *Expert Opin. Invest. Drugs* 19(11), 1381–1394 (2010).
- 41 Kaposi-Novak P, Lee JS, Gomez-Quiroz L, Coulouarn C, Factor VM, Thorgeirsson SS. Met-regulated expression signature defines a subset of human hepatocellular carcinomas with poor prognosis and aggressive phenotype. *J. Clin. Invest.* 116(6), 1582–1595 (2006).
- **Demonstrates how prognostic features may be determined by gene expression profiling.**
- 42 Ito Y. Expression and clinical significance of erb-B receptor family in hepatocellular carcinoma. *Br. J. Cancer* 84(10), 1377–1383 (2001).
- 43 Hopfner M. Targeting the epidermal growth factor receptor by gefitinib for treatment of hepatocellular carcinoma. *J. Hepatol.* 41(6), 1008–1016 (2004).
- 44 Philip PA. Phase II study of Erlotinib (OSI-774) in patients with advanced hepatocellular cancer. *J. Clin. Oncol.* 23(27), 6657–6663 (2005).
- 45 Tang SH, Yang DH, Huang W *et al.* Phase II study of Erlotinib (OSI-774) in patients with advanced hepatocellular cancer. *Clin. Cancer Res.* 12(27), 4171–4177 (2006).
- 46 Tovar V, Alsinet C, Villanueva A *et al.* IGF activation in a molecular subclass of hepatocellular carcinoma and preclinical efficacy of IGF-1R blockade. *J. Hepatol.* 52(4), 550–559 (2011).
- 47 Coulouarn C, Factor VM, Thorgeirsson SS. Transforming growth factor-beta gene expression signature in mouse hepatocytes predicts clinical outcome in human cancer. *Hepatology* 47(6), 2059–2067 (2008).
- 48 Elsharkawy AM, Mann DA. Nuclear factor-kappaB and the hepatic inflammation-fibrosis-cancer axis. *Hepatology* 46(2), 590–597 (2007).
- 49 Sun B, Karin M. NF-kappaB signaling, liver disease and hepatoprotective agents. *Oncogene* 27(48), 6228–6244 (2008).
- 50 Poon RT, Ho JW, Tong CS, Lau C, Ng IO, Fan ST. Prognostic significance of serum vascular endothelial growth factor and endostatin in patients with hepatocellular carcinoma. *Br. J. Surg.* 91(10), 1354–1360 (2004).
- 51 Rosmorduc O, Housset C. Hypoxia: a link between fibrogenesis, angiogenesis, and carcinogenesis in liver disease. *Semin. Liver Dis.* 30(3), 258–270 (2010).
- 52 Lachenmayer A, Hoshida Y, Llovet JM. Hippo tumor suppressor pathway: novel implications for the treatment of hepatocellular carcinoma. *Gastroenterology* 139(2), 692–694 (2010).
- 53 Avruch J, Zhou D, Fitamant J, Bardeesy N. Mst1/2 signalling to Yap: gatekeeper for liver size and tumour development. *Br. J. Cancer* 104(1), 24–32 (2011).
- 54 Sanger Institute Catalogue Of Somatic Mutations In Cancer. www.sanger.ac.uk
- 55 Bamford S, Dawson E, Forbes S *et al.* The COSMIC (Catalogue of Somatic Mutations in Cancer) database and website. *Br. J. Cancer* 91, 355–358 (2004).
- 56 Boyault S, Rickman DS, de Reynies A *et al.* Transcriptome classification of HCC is related to gene alterations and to new therapeutic targets. *Hepatology* 45(1), 42 (2007).
- 57 Hoshida Y, Nijman SM, Kobayashi M *et al.* Integrative transcriptome analysis reveals common molecular sub-classes of human hepatocellular carcinoma. *Cancer Res.* 69(18), 7385 (2009).
- 58 Wehbe H, Henson R, Meng F *et al.* Interleukin-6 contributes to growth in cholangiocarcinoma cells by aberrant promoter methylation and gene expression. *Cancer Res.* 66(21), 10517–10524 (2006).
- 59 Andersen JB, Thorgeirsson SS. Genetic profiling of intrahepatic cholangiocarcinoma. *Curr. Opin. Gastroenterol.* 28(3), 266–272 (2012).
- 60 Ross JS, Wang K, Gay L *et al.* New routes to targeted therapy of intrahepatic cholangiocarcinomas revealed by next-generation sequencing. *Oncologist* 19(3), 235–242 (2014).
- 61 Tannapfel A, Benicke M, Katalinic A *et al.* Frequency of p16 (INK4A) alterations and *K-ras* mutations in intrahepatic cholangiocarcinoma of the liver. *Gut* 47(5), 721–727 (2000).
- 62 Momoi H, Itoh T, Nozaki Y *et al.* Microsatellite instability and alternative genetic pathway in intrahepatic cholangiocarcinoma. *J. Hepatol.* 35(2), 235–244 (2001).
- 63 Terada T, Ashida K, Endo K *et al.* c-erbB-2 protein is expressed in hepatolithiasis and cholangiocarcinoma. *Histopathology* 33(4), 325–331 (1998).
- 64 Terada T, Nakanuma Y, Sirica AE. Immunohistochemical demonstration of MET overexpression in human intrahepatic cholangiocarcinoma and in hepatolithiasis. *Hum. Pathol.* 29(2), 175–180 (1998).
- 65 Tannapfel A, Sommerer F, Benicke M *et al.* Mutations of the *BRAF* gene in cholangiocarcinoma but not in hepatocellular carcinoma. *Gut* 52(5), 706–712 (2003).
- 66 Sia D, Hoshida Y, Villanueva A *et al.* Integrative molecular analysis of intrahepatic cholangiocarcinoma reveals 2 classes that have different outcomes. *Gastroenterology* 144(4), 829–840 (2013).

- Shows a subset of patients with cholangiocarcinoma have tumors with gene rearrangements of FGFR2 offering novel targeted therapy.
- 67 Miller G, Socci ND, Dhall D *et al.* Genome wide analysis and clinical correlation of chromosomal and transcriptional mutations in cancers of the biliary tract. *J. Exp. Clin. Cancer Res.* 28, 62 (2009).
- 68 Gatalica Z, Millis S, Chen S *et al.* Integrating molecular profiling into cancer treatment decision making: experience with over 35,000 cases. *J. Clin. Oncol.* 31(Suppl.), Abstract 11001 (2013)
- 69 Saha SK, Parachoniak CA, Ghanta KS *et al.* Mutant IDH inhibits HNF-4 α to block hepatocyte differentiation and promote biliary cancer. *Nature* 513(7516), 110–114 (2014).
- Offers a nice model system to explore the molecular mechanisms involved in cholangiocarcinoma with possible areas for targeted therapy.
- 70 Schonleben F, Qiu W, Allendorf JD *et al.* Molecular analysis of *PIK3CA*, *BRAF*, and *RAS* oncogenes in periampullary and ampullary adenomas and carcinomas. *J. Gastrointest Surg.* 13(8), 1510–1516 (2009).
- 71 Andersen JB, Spee B, Blechacz BR *et al.* Genomic and genetic characterization of cholangiocarcinoma identifies therapeutic targets for tyrosine kinase inhibitors. *Gastroenterology* 142(4), 1021–1031.e15 (2012).
- 72 Goldenberg D, Rosenbaum E, Argani P *et al.* The V599E BRAF mutation is uncommon in biliary tract cancers. *Mod. Pathol.* 17(11), 1386–1391 (2004)
- 73 Loaiza-Bonilla A, Clayton E, Furth E, O'Hara M, Morrisette J. Dramatic response to dabrafenib and trametinib combination in a BRAF V600E-mutated cholangiocarcinoma: implementation of a molecular tumour board and next-generation sequencing for personalized medicine. *Ecancermedicalscience* 8, 479 (2014).
- Shows how the use of genomic data allowed discovery of treatment options not previously considered.
- 74 Woo HG, Lee JH, Yoon JH *et al.* Identification of a cholangiocarcinoma-like gene expression trait in hepatocellular carcinoma. *Cancer Res.* 70(8), 3034 (2010).
- 75 Schwaederle M, Parker BA, Schwab RB *et al.* Molecular Tumor Board: The University of California San Diego Moores Cancer Center experience. *Oncologist* 19(6), 631–636 (2014).
- 76 Nakazawa K, Dobashi Y, Suzuki S, Fujii H, Takeda Y, Ooi A. Amplification and overexpression of c-erbB-2, epidermal growth factor receptor, and c-met in biliary tract cancers. *J. Pathol.* 206(3), 356–365 (2005).
- 77 Wu YM, Su F, Kalyana-Sundaram S *et al.* Identification of targetable *FGFR* gene fusions in diverse cancers. *Cancer Discov.* 3(6), 636–647 (2013).
- 78 Arai Y, Totoki Y, Hosoda F *et al.* Fibroblast growth factor receptor 2 tyrosine kinase fusions define a unique molecular subtype of cholangiocarcinoma. *Hepatology* 59(4), 1427–1434 (2014).
- 79 Ettrich TJ, Seufferlein T. Regorafenib. *Recent Results Cancer Res.* 201, 185–196 (2014).
- 80 Gu TL, Deng X, Huang F *et al.* Survey of tyrosine kinase signaling reveals ROS kinase fusions in human cholangiocarcinoma. *PLoS ONE* 6(1), e15640 (2011).
- 81 Scheffler M, Schultheis A, Teixido C *et al.* *ROS1* rearrangements in lung adenocarcinoma: prognostic impact, therapeutic options and genetic variability. *Oncotarget* 6(12), 10577–10585 (2015).
- 82 TCGA Research Network. <http://cancergenome.nih.gov>
- 83 ClinicalTrials Database. www.clinicaltrials.gov
- 84 Dowlati A, Nethery D, Kern JA. Combined inhibition of epidermal growth factor receptor and JAK/STAT pathways results in greater growth inhibition *in vitro* than single agent therapy. *Mol. Cancer Ther.* 3(4), 459–463 (2004).
- 85 Vaclavicek A, Bermejo JL, Schmutzler RK *et al.* Polymorphisms in the Janus kinase 2 (*JAK*)/signal transducer and activator of transcription (*STAT*) genes: putative association of the *STAT* gene region with familial breast cancer. *Endocr. Relat. Cancer* 14(2), 267–277 (2007).
- 86 Hoeller D, Hecker CM, Dikic I. Ubiquitin and ubiquitin-like proteins in cancer pathogenesis. *Nat. Rev. Cancer* 6(10), 776–788 (2006).
- 87 Abrams J, Conley B, Mooney M *et al.* National Cancer Institute's precision medicine initiatives for the new National Clinical Trials Network. 2014 ASCO Educational Book. <http://meetinglibrary.asco.org/sites/meetinglibrary.asco.org/files/edbook/144/pdf/zds00114000071.pdf>
- 88 De Mattos-Arruda L, Rodon J. Pilot studies for personalized cancer medicine: focusing on the patient for treatment selection. *Oncologist* 18(11), 1180–1188 (2013).
- 89 International Cancer Genome Consortium. International network of cancer genome projects. *Nature* 464(7291), 993 (2010)
- 90 Marquardt JU, Andersen JB. Next-generation sequencing: application in liver cancer-past, present and future? *Biology (Basel)* 1(2), 383–394 (2012).
- 91 Tripathy D, Harnden K, Blackwell K, Robson M. Next generation sequencing and tumor mutation profiling: are we ready for routine use in the oncology clinic? *BMC Med.* 12(1), 140 (2014).
- Lays some ground work for understanding molecular data generation and utility.
- 92 Dikic I, Crossetto N, Calatroni S, Bernasconi P. Targeting ubiquitin in cancers. *Eur. J. Cancer* 42(18), 3095–3102 (2006).