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Review

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Review

Liver cancer: Approaching a personalized care

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Summary

The knowledge and understanding of all aspects of liver cancer [this including hepatocellular carcinoma (HCC) and intrahepatic cholangiocarcinoma (iCCA)] have experienced a major improvement in the last decades. New laboratory technologies have identified several molecular abnormalities that, at the very end, should provide an accurate stratification and optimal treatment of patients diagnosed with liver cancer. The seminal discovery of the *TP53* hotspot mutation [1,2] was an initial landmark step for the future classification and treatment decision using conventional clinical criteria blended with molecular data. At the same time, the development of ultrasound, computed tomography (CT) and magnetic resonance (MR) has been instrumental for earlier diagnosis, accurate staging and treatment advances. Several treatment options with proven survival benefit if properly applied are now available. Major highlights include: i) acceptance of liver transplantation for HCC if within the Milan criteria [3], ii) recognition of ablation as a potentially curative option [4,5], iii) proof of benefit of chemoembolization (TACE), [6] and iv) incorporation of sorafenib as an effective systemic therapy [7]. These options are part of the widely endorsed BCLC staging and treatment model (Fig. 1) [8,9]. This is clinically useful and it will certainly keep evolving to accommodate new scientific evidence.

This review summarises the data which are the basis for the current recommendations for clinical practice, while simultaneously exposes the areas where more research is needed to fulfil the still unmet needs (Table 1).

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Epidemiology

Liver cancer (including HCC and iCCA) is the 2nd cause of cancer related death [10] and one of the cancers with a still increasing incidence rate [11]. Since risk factors are well known, prevention is an achievable aim. Control of hepatitis B (HBV) and C (HCV) infection, as well as reduction in alcohol consumption would have a huge impact if applied on a large scale. While health plans are implemented to achieve this goal, the epidemic of overweight and metabolic syndrome has emerged as a relevant risk factor [12]. Prospective follow-up data about incidence and specific high-risk individuals in this subset as compared to HBV, HCV or alcohol are still scarce. However, the future reduction in viral related cases because of HBV and HCV control is counterbalanced by the increase in such an etiologic group.

Cancer related death will decrease due to a reduction in exposure to risk factors and because of a higher rate of early diagnosis leading to effective treatment with long term disease free survival. This is the basis to recommend screening for HCC in the population at risk [4,5]. Some restrictions should be in place to make screening cost-effective [13]. Risk should be high enough and modelling studies have placed such cut-offs at an annual rate of 1.5% [14]. Such a figure is exceeded in liver cirrhosis of most etiologies [15,16]. In addition, patients entering screening should be suitable for treatment if they would be diagnosed with HCC. If comorbidities or end-stage liver disease not leading to transplant exist, screening and diagnosis of HCC and its potential treatment will be of no benefit. Finally, diagnosis, accurate and effective options should be available. Unfortunately, an unknown proportion of patients with cirrhosis may not be yet diagnosed, and even so, implementation of screening is usually suboptimal. In the future, the evaluation of the specific risk in an individual patient and prognostic prediction will be refined by molecular profiling of the oncogenic cirrhotic liver and the tumor.

Molecular pathogenesis and signalling pathways

Molecular classification should aid in understanding the biological subclasses and drivers of cancer and optimize benefits from molecular therapies and enrich trial populations [5]. From the biological standpoint, different HCC classes have been characterized including a Wnt subclass (25% of cases; enriched with *CTNNB1* mutations and HCV etiology), a proliferation class (with two subclasses: S1-TGF-beta and S2-EpCAM positive) and an inflammation/interferon class [17–20]. The proliferation subclass accounts for 50% of cases and is enriched with tumors derived from progenitor cells (e.g., "EpCAM"2) and tends to have worse

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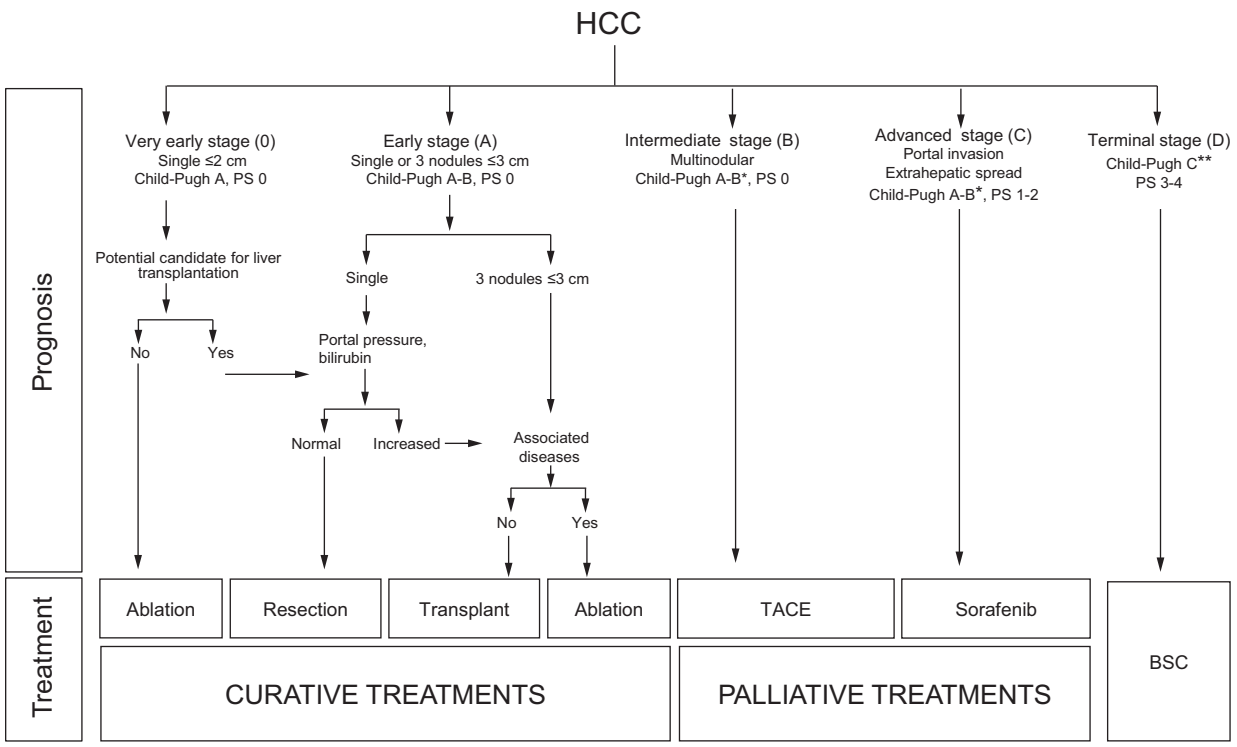

Fig. 1. BCLC staging and treatment strategy [as per Semin Liver Dis. 2014 Nov;34(4):444–55]. The figure represents the first approach to the evaluation of the patients with expected prognosis and initial treatment option to be considered. As shown, the upper part of the scheme defines prognosis according to the relevant clinical and tumor related parameters. Bottom part depicts the decision process to select a treatment option for first consideration. As in all recommendations, final treatment indication should take into account a detailed evaluation of additional characteristics (age, comorbidities) of the patients that imply a personalized decision making. *Note that Child-Pugh classification is not sensitive to accurately identify those patients with advanced liver failure that would deserve liver transplant consideration. Some patients fitting into Child-Pugh B, and even A, may present a poor prognosis because of clinical events not captured by such system, i.e. spontaneous bacterial peritonitis, recurrent variceal bleeding, refractory ascites with or without hepatorenal syndrome, recurrent encephalopathy, severe malnutrition. **Patients with end-stage cirrhosis due to heavily impaired liver function (Child-Pugh C or earlier stages with predictors of poor prognosis, high MELD score) should be considered for liver transplantation. In them, HCC may become a contraindication if exceeding the enlistment criteria.

prognosis. Nonetheless, no molecular subclass has been reported to respond to specific targeted therapy [5].

Several prognostic mRNA-based molecular signatures from tumor or non-tumoral adjacent tissue have been reported [21,22]. Signatures identifying progenitor cell-like and/or a cholangiocyte profile (EPCAM signature3, CK19 signature [22]) display worse prognosis. Similarly, a 5-gene score signature (*TAF9*, *RAN*, *RAMP3*, *KRT19*, and *HN1* genes) predicted overall survival in four independent cohorts of Caucasian and Asian patients [23]. In parallel, gene expression profiling of adjacent non-tumoral liver tissue has highlighted the importance of tumor microenvironment in HCC prognosis. The poor prognosis with 186-gene signature was associated with both survival after resection and survival, HCC occurrence and decompensation in cirrhotic HCV patients without tumors [24,25]. Molecular profiling together with assessment or major clinical predictors of risk of HCC and death (degree of portal hypertension, concomitant treatments during follow-up, sustained alcohol intake or coffee consumption) and comorbidities will permit a more personalised approach.

Oncogenic drivers and tumor suppressors

High-resolution analysis of molecular alterations in human malignancies has allowed for the identification of new drivers, which are ideal targets for treatments in some solid malignancies (lung, breast or melanoma). Recent studies have provided a broad picture of the mutational profile in HCC and identified an average of 30–40 mutations per tumor, among which 6–8 are considered drivers [26,27]. Main mutations are in the promoter region of *TERT*, *TP53*, *CTNNB1*, *ARIDA1A*, and Axin 1 (Table 2). Deep-sequencing studies confirmed *TP53* and *CTNNB1* are frequently mutated. Mutations in these genes are mutually exclusive – an indication that they could act as drivers of tumor progression. In addition, these studies discovered novel mutations in genes involved in the chromatin remodelling pathway (*ARID1A* and *ARID2*), in ubiquitination (*KEAP1*), RAS/MAPK signalling (*RPS6KA3*) and oxidative stress (*NFE2L2*) and JAK1 in 9% of HBV-related HCC. Genes commonly mutated in other solid tumors such as *EGFR*, *PIK3CA* or *KRAS* are rarely mutated in HCC (<5% of cases, Table 2 [26,27]). Several chromosomal alterations have been recurrently identified.

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Table 1. Major unmet needs in the field of HCC.

1.	Detection of the population at risk for HCC in the community in order to recruit them into early HCC detection plans.
2.	Stratification of the risk for HCC to distinguish those at significant risk from those with minimal or no risk (blending conventional clinical assessment and molecular profile).
3.	Development of biomarkers to detect liver cancer development prior to image recognition.
4.	Development of minimally invasive imaging techniques to detect and characterise liver nodules prior to their over malignant transformation.
5.	Validation of organ specific contrasts for accurate diagnosis of nodules detected during screening.
6.	Development and prospective validation of a molecular classification of liver cancer that would allow refined prognosis prediction and optimised treatment selection.
7.	Identification and prospective validation of biomarkers to recognise therapeutic targets that would indicate the benefit of a specific systemic treatment.
8.	Development of functional imaging criteria for the recognition of response and treatment failure in systemic therapy.
9.	Development and validation of HCC specific criteria for the definition of objective response and disease progression that would reliably predict significant therapeutic activity and survival benefit.
10.	Design of randomised trials to evaluate adjuvant options after curative treatment, combined/sequential treatment approaches and novel therapeutic options.
11.	Elucidate the molecular nature of mixed hepato-cholangio tumors and their specific prognosis and treatment approach.

Table 2. Landscape of the most prevalent mutations and high-level gene amplifications in human HCC (modified from Llovet *et al.*, Cancer Cell 2014;22).

Gene	Pathways/gene functions involved	Estimated frequency based on deep-sequencing studies (%)
Driver genes frequently mutated in HCC		
TERT promoter	Telomere stability	60
TP53	Genome integrity	20-30
CTNNB1	WNT signalling	15-25
ARID1A, ARID2	Chromatin remodelling	10-16
TTN	Chromosome segregation	4-10
NFE2L2	Oxidative stress	6-10
JAK1	JAK/STAT signalling	0-9
Oncogenes/tumor suppressors rarely mutated in HCC		
IDH1, IDH2	NAPDH metabolism	<5
EGFR	Growth factor signalling	<5
KRAS, NRAS	RAS/MAPK signalling	<5
PIK3CA	AKT signalling	<5
PTEN	AKT signalling	<5
Oncogenes contained in high-level amplifications in HCC		
FGF19	FGF signalling	5-10
CCND1	Cell cycle	5-10
VEGFA	HGF signalling/angiogenesis	7-10

These include; (i) high level amplifications at 5–10% prevalence containing oncogenes in 11q13 (Cyclin D1 and *FGF19*) and 6p21 (*VEGFA*)2, TERT focal amplification [28] and homozygous deletion of *CDKN2A*; [28] and (ii) common amplifications containing Myc (8q gain) and Met genes (focal gains 7q31). No oncogenic addiction loop for any driver has been defined in HCC.

Signalling pathways

Hepatocarcinogenesis is a complex multistep process where multiple signalling cascades are altered. This leads to a heterogeneous biological portrait. Several signalling pathways are implicated:

Table 3. Molecular abnormalities and potential therapeutic agents.

Altered pathway/function	Genes involved	Somatic mutations (reported mutations)	Targeted therapy
Telomere stability	*TERT* promoter	~60%	Vx-001 (immunotherapy)
	TERT	11%	BIBR1532 (telomerase inhibitor) GRN163L (antisense nucleotides) telomelysin (gene therapy)
TP53/cell cycle control	*TP53*	20-30%	Adenovirus p53 construct (gene therapy, phase I) RG7112 (inhibition of p53-MDM2 interaction, phase I)
	c-myc		
	CDKN2A	7-10%	
	ATM	4-5%	
	RB1	3-10%	
	IRF2	1-5%	
	CCND1		
	CCNE1	4-5%	
	CDKN1A	1-4%	
Wnt/β-catenin signaling	*CTNNB1*	9-41%	
	AXIN1	4-15%	
	APC	2-3%	
Chromatin remodeling	*ARID1A*	10-17%	
	ARID1B	2-7%	
	HDAC family members		Resminostat, vorinostat, belinostat (pan-HDAC inhibitors)
	ARID2	5-9%	
	MLL3	4-13%	
	MLL	1-6%	
	MLL2	1-6%	
PI3K/Akt/mTOR pathway	*RPS6KA3*	2-10%	Everolimus, termsirolimus, (mTOR inhibitor)
	PIK3CA	<5%	
	PTEN	4-5%	
JAK/STAT signaling	*JAK1*	0-9%	Baricitinib and AZD1480 (JAK inhibitors)
VEGF signaling	*VEGFA*		Bevacizumab (monoclonal antibody against VEGFA) Ramucirumab (monoclonal antibody against VEGFR2, a VEGFA receptor) Cabozantinib (dual VEGFA/c-MET TKI) Brivanib (VEGFR2 and FGFR TKI) Sorafenib, regorafenib, sunitinib, linifanib, lenvantinib, axitinib (multi TKIs)
FGF signaling	*FGF19*		BGJ398 (pan FGFR inhibitors) FGFR4 inhibitors Brivanib (VEGFR2 and FGFR TKI)
	FGFR4	1%	
	FGFR2	1%	
	FGF5	1%	
IGF signaling	*IGF2R*		MEDI-573 (monoclonal antibody against IGF1/IGF2) Cixutumumab, BIIB022, dalotuzumab (monoclonal antibodies anti-IGF1R)
	IGF1R		
RAS/RAF/MEK/ERK pathway	*KRAS/NRAS*	2%	Sorafenib (multi TKIs) Vemurafenib (BRAF inhibitor)
	BRAF	<5%	
MET signaling	*c-MET*		Selumetinib (MEK/ERK inhibitor) Refametinib (MEK inhibitor) Sorafenib (multi TKIs) Cabozantinib (dual VEGFA/c-MET TKI) Tivantinib (c-Met inhibitor)
PDG signaling	*PDGFRA*	2%	Sorafenib, regorafenib, linifanib, orantinib, sunitinib (multi TKIs)
EGF signaling	*EGFR*	<5%	Cetuximab (monoclonal antibody against EGFR) Erlotinib and gefitinib (EGFR TKIs)
Proteasome system	*UBE3C*	1-16%	Bortezomib (proteasome inhibitor)

1) Vascular endothelial growth factor (VEGF) signalling is the cornerstone of angiogenesis in HCC. High level amplifications have been identified in 7–10% of cases [17,29]. VEGFR signalling can be targeted by the monoclonal antibody bevacizumab directed against VEGF-A ligand or by ramucirumab targeting the VEGFR-2, or by inhibiting the intracellular tyrosine kinase by small molecules such as sorafenib. Other activated angiogenic pathways are Ang2 and FGF signalling. In a retrospective analysis, VEGFA amplified tumors have been suggested to be more responsive to sorafenib [29].

2) Ras MAPK signalling is activated in half of early and almost all advanced HCCs [30,31]. Activation results from upstream signalling by EGF, IGF, and MET activation, and from the epigenetic silencing of tumor suppressors such as *NORE1A*, and *RASSF1A15*. Mutations of K-Ras are infrequent (<5%). Sorafenib and regorafenib have shown partial cascade blockage.
3) The PI3K/PTEN/Akt/mTOR pathway controls cell proliferation, cell cycle and apoptosis, and is activated by various RTKs such as EGFR or IGFR and inactivated by PTEN [32,33]. It is activated in 40–50% of HCCs [32].
4) Dysregulation of the c-MET receptor and its ligand HGF, critical for hepatocyte regeneration after liver injury, are common events [18,30]. MET activation occurs in 50% of advanced HCC, but activating mutations or amplifications represent less than 5% of cases [34].
5) Insulin-like growth factor receptor (IGFR) signalling is activated in 20% of cases through; a) allelic loss affecting the tumor suppressor IGF2R; b) overexpression of the oncogenic ligand IGF2; and c) deregulation of the IGF binding proteins IGFBP2 and IGFBP3 [35].
6) Wnt/β-Catenin pathway is crucial for hepatocarcinogenesis [36,37]. Around half of HCCs have activation of the Wnt signalling pathway, either as a result of β-catenin mutation, or overexpression of Frizzled receptors or inactivation of E-cadherin [36,37].

Additional pathways and their role in targeted therapy such as the extrinsic/intrinsic apoptotic pathway, Hedgehog signalling, JAK/STAT signalling, TGF-β signalling, Notch pathway, Ubiquitinin-Proteasome pathway, nuclear factor-κB signalling, EGFR signalling, cell cycle control, and the role of the tumor microenvironment have to be further defined (Table 3). Similarly, the potential role of recently described oncoMIRs relevant to hepatocarcinogenesis as molecular targets should be confirmed by clinical investigations.

Screening, diagnosis and staging

Screening for HCC in the population at risk should be based in ultrasound examination every 6 months [4,5,38]. Adding tumor marker determination provides no benefit. Alpha-fetoprotein (AFP) is a predictor of advanced disease and poor prognosis. Hence, even if some cases could be detected through AFP, these would not likely belong to early stage [39,40].

The goal of screening is to detect solitary tumors ⩽20 mm, when the likelihood of vascular invasion or intrahepatic spread is low and curative treatment is highly likely [41]. Nodules <10 mm within a cirrhotic liver are frequently not malignant and accurate diagnosis is extremely challenging by biopsy or imaging techniques. Thus, active diagnostic approach is initiated when nodule size exceeds 10 mm (Table 4). If such a nodule presents increased contrast uptake in the arterial phase, followed by contrast washout in the venous/delayed phases of dynamic imaging (CT, MR) the diagnosis is established without need of biopsy confirmation [5,38,42–44]. Organ specific contrasts await proper evaluation and their routine use is not currently endorsed (Table 4) [43]. If the pattern is not this specific one, diagnosis should be based from biopsy, that is mandatory to diagnose iCCA [39]. AFP is again of limited use as it may increase both in HCC and in iCCA [4], and positron emission tomography (PET) has no value for diagnosis.

Table 4. Diagnostic criteria for HCC.

• Tumor biopsy
• Imaging criteria (only for patients at high risk for HCC (EASL, AASLD): recognition of a nodule >10 mm with increased contrast uptake (“washin”) followed by reduced contrast uptake (“washout) in venous/delayed phases at dynamic imaging at CT or MR (AASLD, EASL, LIRADS)
• Organ specific contrast have not been validated for diagnosis
• AFP and other tumor markers are not recommended to set HCC diagnosis.

Evaluation of the patients to estimate prognosis should take into account tumor burden, liver function and general health status. Presence of cancer related symptoms (assessed through the performance status test or the Karnofsky index [45]) is associated to poor outcome. Evaluation of liver function should not simply be based on Child-Pugh as this does not allow proper stratification. Parameters such as episodes of encephalopathy, renal failure, bacterial peritonitis, hyponatremia and others indicate end-stage liver disease in need of transplant evaluation irrespective of Child-Pugh A or B class [46,47]. MELD has also limited discrimination capacity if liver function is not at end-stage [48]. Indeed, if liver function would prime liver transplant evaluation in the absence of HCC, the presence of HCC may just become a contraindication for it and thus, such patients should be classified as end-stage [9,49].

Resection, transplantation and ablation: Signs of progress in the never ending debate

Years ago the competition between resection, transplantation and ablation was fuelled by the absence of robust data. Now, we have a large set of informative studies about the benefits of each option and its long term results in survival. It needs to be stressed that the endpoint of treatment is to provide the longest survival with the less impaired quality of life. Thereby, goals of tumor removal or lower recurrence risk if survival is not modified should not be the driver to favor one option. As a consequence, the debate should take into account the outcome that each option is able to provide in different profiles of patients as per tumor burden and liver function [50].

If patients present hepatic decompensation, the expected outcome offered by liver transplantation, if the Milan criteria are not exceeded, is clearly superior to surgery and ablation. Indeed, surgical resection should be considered contraindicated in such instance, and even ablation may not have a positive impact, as the impaired liver function already determines a dismal outcome. Accordingly, the debate affects patients with compensated liver disease and among them, those with solitary HCC smaller than 2 cm or up to 3 cm at most. Survival of multifocal HCC within Milan criteria is still optimal (>70% at 5 years) after transplant, while resection and ablation may initially be effective, but HCC recurrence will reduce long term survival (50% at 4–5 years). In that profile, TACE could become a competitive option as proper selection and technique may provide similar survival [51–54]. Prospective trials with adequate sample size and design are needed to deliver an evidence-based recommendation.

Large tumors are not well served by ablation. Long lasting complete response in HCC >3 cm is less frequent and recurrence rate is high [55]. Contrarily, resection may be successful and this is why tumor size does not constitute a contraindication by itself [5,8,42]. Increased size parallels risk of vascular invasion and dissemination as reflected by satellites or additional nodules [56,57]. However, some patients may develop expansive tumors and not suffer dissemination. If liver function is preserved resection may provide optimal results. Tumors larger than 5 cm are not within the recommended transplant criteria, but this is due to the lack of enough donors. If the availability of organs would be unlimited, the selection criteria would be surely expanded. New criteria may take into account parameters such as AFP, total tumor burden/volume and/or response to treatment even if baseline stage would be beyond the criteria [58–62]. Prospective research needs to be finished in order to validate all approaches [63]. Indeed, because of the shortage of donors, there is a delay between transplant indication and the procedure. During this time, HCC may progress and prime exclusion from the list. Priority policies are applied in most transplant programs. They may leave solitary tumors <2–3 cm without priority and hence, no real chance of transplantation unless liver failure primes it. Thus, only large and/or progressing HCC get priority. Since this profile is associated to a more advanced disease, priority may bring a reduction in post transplant outcomes. The risk of inconsistencies in liver graft allocation for HCC may be limited by benefit consideration, intended as the difference in outcome with or without transplant when alternative treatments are applicable [64,65]. Several attempts of allocation on the net benefit in survival are likely to be developed, as the utility of transplant on the sole basis of absolute survival may not serve equally the large population of end-stage liver diseases without cancer (Table 5).

In patients without hepatic decompensation the survival after resection and ablation is influenced by the existence of clinically significant portal hypertension [66–68]. In the absence of portal hypertension the 5-year survival exceeds 70% and when it is present it is significantly reduced. If survival for very early HCC is similar for resection and ablation, what are the benefits of resection? In large tumors, a safety margin may benefit, but this is also highly debated. In HCC <3 cm both ablation and resection may provide a safety margin, but in HCC <2 cm the risk of satellites is low and margin benefit may not exist. Resection allows pathology inspection. If microvascular invasion or satellites are detected, the risk of recurrence is high [69]. Some authors propose enlistment because of risk (priority based in imaging prior to surgery) [70,71]. This may be a relevant benefit from surgery. Finally, tumor location and need of extensive liver resection are also involved in treatment selection (Fig. 2). All these variables confound the picture and explain why trials in this setting are challenging and likely will never be strong enough to inform a robust decision in individual patients [72].

Table 5. The conflict between urgency, utility and benefit when developing priority policies in patients considered for liver transplantation (adapted from Bruix *et al.* Gut 2014:1–12).

Model	Definition
Urgency	Focused on pre-transplant risk of dying: patients with worse outcome on the waiting list are given higher priority for transplantation (based on Child-Pugh or MELD score)
Utility	Based on maximization of post-transplant outcome. Takes into account donor and recipient characteristics: mainly used for HCC since the MELD score poorly predicts post-transplant outcome in HCC, due to the absence of donor factors and lack of predicting progression while waiting
Benefit	Calculated by subtracting to the survival achieved with LT the survival obtained without LT. The benefit approach ranks patients according to the net survival benefit that they would derive from transplantation and maximize the lifetime gained through transplantation. If applied to HCC without adjustments may prioritize patients at highest risk of progression, higher recurrence rate and lower survival

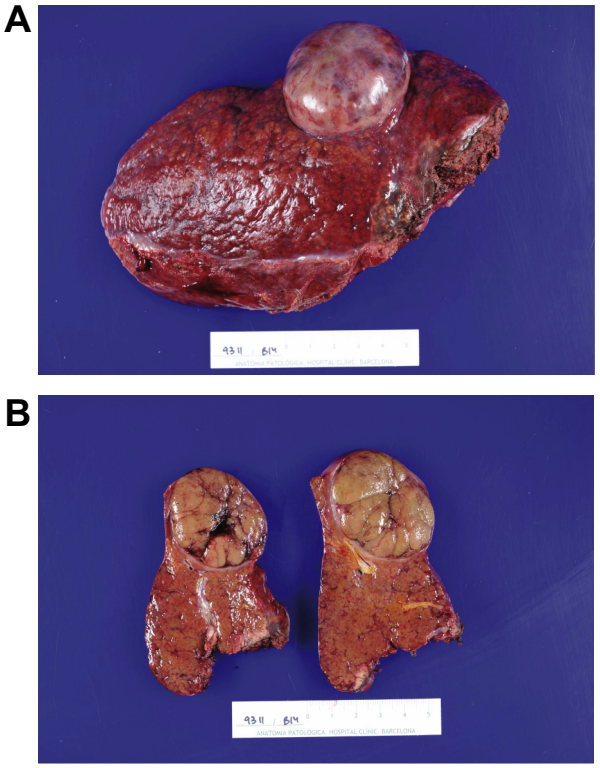

Fig. 2. Personalised decision making in a patient with HCC. (A) Macroscopic view of a resected HCC in a patient with cirrhosis due to HCV infection. Diagnosis was based on imaging techniques and its size was between 3 and 4 cm. Liver function was preserved, but there was clinically significant portal hypertension (hepatic venous pressure gradient = 11 mmHg). Liver transplant was contraindicated because of comorbidities and its location protruding in the liver surface precluded safe ablation (direct access without a protective rim of non-tumoral liver is associated to increased risk of bleeding and peritoneal seeding). In addition, size >30 mm is a predictor of incomplete ablation. Because of these considerations it was decided to recommend surgical resection through laparoscopy. (B) Partition of the HCC shows capsule formation and no macroscopic satellites. It is possible to differentiate the separate tumor areas and even some minute intratumoral nodules. There is a necrotic haemorrhagic area in the central part. This macroscopic heterogeneity (also identified by different differentiation degrees across the nodule) predicts a heterogeneous molecular profile if assessed by any of the currently available technologies. Increased proliferation markers will be present everywhere, but tumor needle biopsy will be at risk to fail to accurately inform about the tumor biology profile.

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The availability of effective treatments for HCV infection will have an impact in the future. The number of patients reaching end-stage cirrhosis in need of transplant may decrease and the problems related to reinfection of the graft will be controlled. Thereby, the demand of organs for end-stage cirrhosis may decrease and allow an expansion of the criteria for HCC patients. Furthermore, if HCV is cured, the risk of recurrence due to *de novo* tumors in the cirrhotic liver may no longer be a major concern after a first resection/ablation. The data of the potential impact of HCV eradication with prior treatments [73] and in HBV patients treated with antivirals reinforce this hope and suggest that in the future the advantage of transplant to prevent oncogenic risk may not be a valid concept. Only recurrence due to dissemination will still be a major problem. All trials using different agents to reduce recurrence risk have failed [74]. This is an area where active research is needed.

Locoregional approach

Locoregional treatments are widely used in intermediate-stage HCC. They include ablation, conventional TACE (cTACE), TACE with drug-eluting beads (DEB-TACE), transarterial radioembolization (TARE), hepatic arterial infusion chemotherapy (HAIC) and external radiotherapy.

The role of ablation as compared to surgery has been discussed above. Radiofrequency (RFA) is the first line technique, but its failure rate increases in HCC >3 cm [75,76]. Microwave ablation is a promising option that may successfully ablate larger tumors, but long term data are needed. Same applies to high intensity focused ultrasound.

To overcome the current limitations, several approaches have been investigated. Intravenous administration of ThermoDox®, a heat-activated formulation of liposomal doxorubicin, delivers higher concentrations of anti-cancer drugs directly to the periphery of RFA ablation zone, which are most commonly responsible for post-treatment tumor recurrence [77]. Unfortunately, a phase III trial comparing ThermoDox® plus RFA with RFA alone has been negative [78]. Combination of RFA with TACE has also been evaluated, but despite suggestions of improved efficacy, robust evidence is lacking in patients beyond the optimal profile for RFA [78–80].

Transarterial treatment was initiated in the 1970s. First approach was simply bland embolization aiming to obstruct the arterial blood flow [81,82]. Studies combining selective arterial obstruction with anti-cancer drug injection (TACE) showed that TACE improves survival of patients with HCC [6,83,84]. Cumulative meta-analysis comparing its efficacy against supportive care has placed TACE as the 1st line treatment option for patients with BCLC intermediate-stage HCC [6]. However, there is still no standardized protocol for TACE in terms of treatment schedule, type and dosage of anti-cancer drug. Moreover, the uneven size of embolic material when using gelatin sponge is another limiting factor to predict its therapeutic efficacy [85]. TACE is associated with transient post-embolization syndrome in most cases, but severe events such as hepatic decompensation, gastrointestinal bleeding and abscess are infrequent [86].

The use of spheres that slowly release chemotherapy while also obstructing arterial blood supply (DEB-TACE) has improved tolerance and enabled standardization. DEB-TACE uses anthracycline-loaded beads rather than the conventional lipiodol-anthracycline emulsion [87,88]. The sustained release of anti-cancer drugs primes a lower systemic drug exposure with higher drug concentration within the tumor. As a result, while maintaining treatment efficacy the systemic adverse events due to chemotherapy are reduced [86,88]. The most relevant issue in TACE is to follow the recommendations in guidelines about when to start and when to interrupt. TACE is effective in HCC patients with compensated liver disease and without cancer related symptoms, vascular invasion or extrahepatic spread. TACE should induce major necrosis and may be repeated upon disease progression. However, if TACE fails to induce response or the criteria to start are no longer present at the time of progression (liver failure, symptomatic disease, vascular invasion/spread) TACE should not be repeated. Applying the recommendations the median survival of TACE treated patients should exceed 3 years. Lower figures indicate inadequate selection or suboptimal treatment application.

The hypoxic tumor microenvironment resulting from arterial embolization induces release of pro-angiogenic factors, such as VEGF and platelet-derived growth factor (PDGF), which adversely affect the prognosis of patients. This provides the rationale to combine TACE with sorafenib, since sorafenib inhibits the action of pro-angiogenic factors [89]. While the combination is safe, data from phase II studies have not shown a clinically significant benefit. Thus, adjuvant treatments to TACE are still needed [90,91].

TARE has shown significant activity. Its application requires a complex setting and this may limit its widespread use. TARE acts by delivery of glass or resin spheres loaded with a radiation agent such as Yttrium-90 or Holmim-166 that are pure β-emitters [92,93]. TARE has less severe short term adverse events compared to TACE [94]. However, actinic damage may appear months after treatment and this demands a careful evaluation of the amount of radiation required to treat the tumor and gauge the risk associated with large, multifocal HCC affecting both lobes. Several heterogeneous study populations show survival rates similar to TACE and sorafenib, particularly in patients with advanced-stage HCC with portal vein thrombosis [95,96]. Ongoing randomized controlled trials comparing TARE with TACE or sorafenib should define its value.

HAIC consists of the selective infusion of chemotherapy into the tumor-feeding hepatic artery through a chemoport. It provides localized delivery of a high dose of anti-cancer drugs, expecting that the first-pass effect in the liver, would reduce systemic concentration and prevent drug related adverse effects. There is no standardized protocol for HAIC, and despite its wide use in some settings, there is no proof of survival benefit [97–99].

Focal high-dose external radiation therapy, including stereotactic body radiation therapy, can be used to treat locally advanced HCC with or without portal vein thrombosis [100,101]. Better tumor targeting may avoid actinic damage of the non-tumoral liver and surrounding structures. It may be used alone or in combination with other treatments, but in the absence of prospective randomised trials assessing its value, no robust recommendation is feasible. Selective radiation of early stage HCC may be a niche competing with percutaneous options [102,103].

Systemic treatment

Absence of proven survival benefit has been the rule in the evaluation of a large number of systemic agents [8]. Conventional

chemotherapy, either alone or in combination, administered intravenously or intra-arterially, or following different regimes, never reached positive results [8]. The last failure affected the trial comparing FOLFOX4 (fluorouracil, leucovorin, and oxaliplatin) *vs.* doxorubicin in an Asia based trial [103]. The primary analysis of the study showed no difference in survival and the suggestion of a positive outcome based in an extended follow-up period does not have scientific strength. Indeed, it could be argued that the use of doxorubicin in the control arm in a population with high HBV carriage may have impaired their survival. Therapies such as antiestrogens, antiandrogens, vitamin D derivatives or interferon have also failed to offer any benefit [8].

This dismal status changed with the success of sorafenib [7]. This agent is part of a large group of novel agents that target specific molecular mechanisms related to cancer development and progression. Because of the hypervascular nature of HCC, a major activity has been focused on angiogenesis pathways but several other targets have been identified and tested (Table 2). Unfortunately, none has beaten the benefits of sorafenib (Table 6), a multi-kinase inhibitor that reduces tumor-cell proliferation and tumour angiogenesis. It acts by inhibiting the serine-threonine kinases Raf-1 and B-Raf and the receptor tyrosine kinase activity of VEGF receptors (VEGFRs 1-3) and PDGF, among others. Despite the absence of a significant response rate according to conventional RECIST criteria, it was envisioned that the efficacy could come from a delay in tumor progression that would ultimately translate into improved survival. This was demonstrated in the pivotal trial testing sorafenib *vs.* placebo [7], and the simultaneous trial run in the Asia-pacific region [104]. In both instances the survival expectancy was improved by 30% after a significant delay in tumor progression. Again, it was shown that the response rate according to conventional criteria was marginal, thus dismissing this parameter as a valuable tool to unequivocally detect therapeutic activity of novel agents.

The survival benefit at advanced stages is still modest and hope was placed in the potential efficacy of other therapeutic agents with similar profiles, but with heterogeneous potency against specific targets, and also agents affecting other pathways. Unfortunately, none of those tested so far has offered survival benefit. Table 4 displays the results of the seminal sorafenib trials and those obtained with sunitinib [105], brivanib [106], linifanib [108] and the combination of sorafenib with erlotinib [109] in first line *vs.* sorafenib, as well as the data in second line testing brivanib [110], everolimus [111] and ramucirumab [112] (Table 6). While these failures are disappointing, dissection of their results have provided meaningful insight to elaborate around the reasons for such negative outcomes. The first argument may be that the drugs are not effective enough for all comers or that their effectiveness is counterbalanced by the lack of safety that would turn into treatment related death, such might be the case of sunitinib [106]. The second argument would affect the design of the trials. The definition of the target population is key to have an informative trial and the characterization of the patient stage and their prognostic predictors may have changed in recent years. The evolution of the path of care of patients with liver cancer has primed an earlier diagnosis with indication of locoregional and systemic treatment at a less advanced stage in which the prognostic predictors have to be refined. Pattern of progression after treatment was already known to be a prognostic predictor after resection, ablation and TACE, and it has now been shown to be relevant under sorafenib treatment [113]. Thus, trial design should not only consider the usual parameters (tumor burden, liver function), but also progression pattern. Specific adverse events related to sorafenib intake (i.e. dermatology reactions) are associated to a slower time to progression and improved survival [114]. Hence, trials in second line should also consider this clinical event to avoid a further risk of bias. Finally, if targeted therapy is aimed to act on specific targets it would make sense to select patients according to the recognition of the molecular pathway to be modulated. This enrichment policy is sound but the challenge is how to properly profile the biomarker status. HCCs present a marked heterogeneity within the same nodule (Fig. 2) and across nodules making a single biopsy highly unlikely to provide an accurate profiling of the tumor as per current technologies. In order to advance in knowledge it is recommended that all therapeutic research trials should collect tumor tissue to allow the investigation of the correlation between the molecular profile and the outcome of the patients. Research in peripheral blood sampling (“liquid biopsy”) is the next technological challenge [115].

All these considerations have to be taken into account when entering clinical evaluation of new drugs. Some of them share in part the mechanisms of action of drugs previously tested but with specific differences in potency and molecular targets. Regorafenib, lenvatinib, cabozantinib and tivantinib are among the currently being evaluated in phase III for regulatory approval. The tivantinib trial is the only one enriched according to molecular profile. The randomised phase II study testing tivantinib

Table 6. Randomized phase III clinical trials completed in HCC in first and second line (2007–2014) (Modified from Reig *et al.* Best Pract Res Clin Gastroenterol. 2014;28(5):921–35).

Drug in study	Author	Year	Randomized drugs	n	TTP (mo)	*p* value	OS (mo)	*p* value
Sorafenib	Llovet *et al.*, [7]	2008	Sorafenib *vs.* placebo	299/303	5.5 *vs.* 2.8	<0.001	10.7 *vs.* 7.9	<0.001
	Cheng *et al.*, [104]	2009		150/76	2.8 *vs.* 1.4	<0.001	6.5 *vs.* 4.2	0.01
Sorafenib plus erlotinib	Zhu *et al.*, [108]	2012	Sorafenib + erlotinib *vs.* sorafenib	362/358	3.2 *vs.* 4	n.s.	9.5 *vs.* 8.5	n.s.
Linifanib	Cainap *et al.*, [107]	2012	Linifanib *vs.* sorafenib	514/521	5.4 *vs.* 4	0.001	9.1 *vs.* 9.8	n.s.
Sunitinib	Cheng *et al.*, [105]	2013	Sunitinib *vs.* sorafenib	530/544	3.6 *vs.* 3.6	n.s.	7.9 *vs.* 10.2	n.s.
Brivanib	Johnson *et al.*, [106]	2013	Brivanib *vs.* sorafenib	577/578	4.2 *vs.* 4.1	n.s.	9.5 *vs.* 9.9	n.s.
FOLFOX-4	Qin *et al.*, [103]	2013	FOLFOX-4 *vs.* doxorrubicin	184/187	2.9 *vs.* 1.8	n.s.	6.4 *vs.* 4.9	n.s.
Brivanib	Llovet *et al.*, [109]	2013	Brivanib *vs.* placebo	263/132	4.2 *vs.* 2.7	0.001	9.4 *vs.* 8.2	n.s.
Everolimus	Zhu *et al.*, [110]	2014	Everolimus *vs.* placebo	362/184	2.9 *vs.* 2.6	n.s.	7.6 *vs.* 7.3	n.s.
Ramicirumab	Zhu *et al.* [111]	2014	Ramicirumab *vs.* placebo	283/282	3.5 *vs.* 2.6	<0.0001	9.2 *vs.* 7.6	n.s.

n.s., not significant; TTP, time to progression; OS, overall survival.

showed that the therapeutic benefit was observed only in patients with c-met positivity by immunostaining and this provided the rationale for such design [116]. Others tackle mechanisms that are sharply different or complementary. These include acting on methylation status or aiming to reactivate immune cancer surveillance [117], thus paralleling the success in melanoma. Other studies are testing chemotherapy in combination with sorafenib or specific chemotherapy formulations to increase the therapeutic activity. Results of all these efforts are eagerly awaited.

Intrahepatic cholangiocarcinoma (iCCA) and mixed HCC-iCCA

Liver cancers may contain features of both HCC and iCCA; these malignancies are referred to as mixed HCC-iCCA using World Health Organization (WHO) criteria [118]. In mixed tumors, the presence of cholangiocarcinoma elements are often confirmed by positive cytokeratin 19 (CK19) staining. If one assumes that CK19 positivity defines a mixed tumor, then its incidence is approximately 11% [119]. The WHO criteria and many authors have been reluctant to call CK19 positive HCC mixed tumors, preferring terms such as HCC with biliary/hepatic progenitor cell markers [119]. However, one cannot infer cell lineage from morphology, as transformed mature hepatocytes are plastic and may assume a CCA phenotype [120]. Hence the definition of mixed HCC-iCCA lesions likely will continue to evolve.

CCA accounts for approximately 15% of all hepatobiliary malignancies [121]. Three anatomic subtypes of CCA can be defined including intrahepatic (iCCA), perihilar (pCCA), and distal (dCCA) cholangiocarcinoma [121]. Although the overall incidence of CCA has been increasing, the subtype of CCA responsible for this increased incidence, intrahepatic *vs.* perihilar, has been debated due to the frequent misclassification of pCCA as iCCA. However, at least one study has implicated an increase of iCCA as responsible for this secular trend [122]. Although time-honored risk factors for CCA have been identified, most cases are sporadic and without known risk factors [121]. More relevant to this review of HCC, there is a marked overlap between the risk factors for HCC and iCCA including cirrhosis, chronic hepatitis B and C, obesity, diabetes and alcohol [123]. Thus, in chronic parenchymal liver disease the presence of a mass lesion cannot be a priori diagnosed as HCC without a consideration of iCCA.

Molecular pathways

A whole-exome sequencing of liver fluke-related CCA tumors identified 206 somatic mutations in 187 genes [124]. Mutations were identified in known cancer-associated genes including *TP53*, *KRAS*, and *SMAD4*, and in newly implicated genes such as *MLL3*, *RNF43*, *PEG3*, and *ROBO2*. These latter genes are involved in deactivation of histone modifiers, activation of G-proteins, and loss of genomic stability [124]. A whole-exome sequencing study in non-fluke related CCA described inactivating mutations in multiple chromatin-remodelling genes (including *BAP1*, *ARID1A*, and *PBRM1*) [125]. Thus, the carcinogenesis process appears to be different depending upon etiology. A transcriptome profiling study found not only *KRAS* mutations as noted above, but also increased levels of EGFR and HER2 signalling [126]. A single-nucleotide polymorphism array, gene expression profile and mutation analysis of iCCA specimens reported two subsets of iCCA, an inflammation and a proliferation class [20]. Oncogenic ROS1 fusions proteins have also been reported in iCCA [127]. Thus, iCCA is also quite genetically diverse and heterogeneous.

Interestingly, isocitrate dehydrogenase 1 and 2 (*IDH1* and *IDH2*) mutations are found in approximately 20% of iCCA tumor specimens [128]. Mutant IDH block liver progenitor cells from undergoing hepatocyte differentiation in experimental models. Combined *IDH-KRAS* mutations drive the expansion of liver progenitor cells and induce progression to metastatic iCCA [129]. The epigenetic changes associated with these mutations likely drive their oncogenic effects; IDH mutations may be amenable to therapeutic targeting [130]. Another mutation common in CCA are fusions of the *FGFR* gene [131–133], which can be targeted with current small molecule inhibitors such as BGJ398 or ponatinib [134].

Staging of iCCA

Biopsy sets diagnosis and CT/MRI define tumor burden. Mass forming iCCA as small as 1 cm may be detected by fluorodeoxyglucose PET (FDG-PET), but iCCA are frequently PET negative and current guidelines do not endorse PET for iCCA staging [121]. Regional lymph node metastases are common in iCCA, and endoscopic ultrasound with fine needle aspirates (FNA) is an approach to diagnose such metastases [135], but it has not been rigorously assessed in regards to clinical decision-making and outcome analysis.

Overview of management of iCCA

Recent guidelines endorse the AJCC/UICCA (7th edition) staging system [121] and support surgical resection as the treatment of choice for iCCA, particularly for patients with single intrahepatic nodules and no dissemination. Conversely, patients with intrahepatic metastases, vascular invasion or lymph node metastases should not undergo resection. There are no established first-line local-regional therapeutic options for patients with non-resectable iCCA, and randomized controlled trials are recommended. Cisplatin and gemcitabine is a systemic therapy practice standard for iCCA in patients with ECOG performance status 0 or 1 [136], but the data are too limited to make this an established standard of care.

Liver transplantation for iCCA is highly controversial. The reports on liver transplantation for iCCA are difficult to summarize given the non-standardized selection criteria, small number of patients, and disparate neoadjuvant and adjuvant treatment protocols [121]. In most transplant centers, iCCA is considered a contraindication given the high recurrence rates. However, this practice has recently been challenged. "Very early" iCCA $\leqslant$2 cm in diameter in cirrhotic patients may be able to undergo liver transplantation without recurrence [137]. Further data will be necessary to support these findings as well as to clarify if the prognosis of a mixed tumor is worse or not than for HCC [138–141].

In summary, while decades ago liver cancer was a field with grim perspectives and with limited clinical and research activity, it has evolved into a highly competitive field where advancements emerge at a constant rate. Success in preventive approaches to eliminate risk factors, better understanding of the molecular mechanism and refined treatment will further expand the current status and ultimately exclude this neoplasm from the top 5 cancer killers.

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Conflict of interest

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