



# A Contemporary Approach to Diagnosis and Treatment of Combined Hepatocellular-Cholangiocarcinoma

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## Abstract

**Purpose of Review** To provide updates on terminology, epidemiology, diagnosis, and treatment of combined hepatocellular-cholangiocarcinoma (cHCC-CCA).

**Recent Findings** cHCC-CCAs are tumors that in the same nodule contain a variable degree of HCC and CCA components with a transition zone. cHCC-CCAs develop in cirrhotic and non-cirrhotic livers like and is associated with poor outcomes. Mutations in *TP53*, *TERT* promoter, and *ARID1A* are the most common genetic aberrations in cHCC-CCA. Fusion gene *PTMS-AP1G1* is unique for cHCC-CCA. A biopsy is required for diagnosis. Surgical resection remains treatment of choice, while liver transplantation for early cHCC-CCA is associated with favorable outcomes. Gemcitabine-based therapy shows benefits for advanced cHCC-CCA.

**Summary** cHCC-CCAs are a heterogeneous group of primary liver cancers with unique biological behavior. Multicenter studies are required for a molecular analysis to inform novel therapeutic approaches, and understand epidemiology and benefits of liver transplantation, liver-directed, and targeted therapies for this rare aggressive cancer.

**Keywords** Primary liver cancer · Mixed liver cancer · Genetic aberrations · Liver transplantation · Loco-regional therapy

## Abbreviations

cHCC-CCA	Combined hepatocellular-cholangiocarcinoma
iCCA	Intrahepatic cholangiocarcinoma
WHO	World Health Organization
MRI	Magnetic resonance imaging
PECAR	Prognostic estimation of cHCC-CCA after resection
LN	Lymph node

## Introduction

Combined hepatocellular-cholangiocarcinoma (cHCC-CCA) is a rare primary liver cancer (PLC) which has been increasingly recognized as a neoplasm with a unique biological behavior. Its epidemiology, radiological and histological characteristics, and clinical behavior are distinct from classical HCC and intrahepatic CCA (iCCA). While cHCC-CCA was first described more than 100 years ago [1] due to lack of use of unified terminology and diagnostic criteria, its true incidence and prevalence are not well known and were likely underestimated in the past. It had been reported to account for 0.4–14.2% of all PLCs. Lately, increased awareness of the unique biological behavior of cHCC-CCA has resulted in increased attention and number of studies dedicated to this cancer.

To improve cHCC-CCA diagnosis, treatment, and research approaches, an international group of pathologists, clinicians, and radiologists offered a new consensus document on cHCC-CCA [2••]. Additionally, the World Health Organization (WHO) updated the definition of cHCC-CCA in their 5th, most recent edition [3••]. According to the international

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consensus and WHO, cHCC-CCA is now defined as a cancer with simultaneous presence of both hepatocytic and cholangiocytic cytologies and architecture within the same tumor [2, 3]. These tumors often have transitional features and various degree of cellular differentiation and proportion of each cell subtype making this group very heterogenous. Routine histopathology with hematoxylin and eosin staining (Fig. 1) is sufficient to make the cHCC-CCA diagnosis. Specialized immunostains are no longer required for the cHCC-CCA diagnosis. While it has been recommended that pathologists document presence of stem cell phenotype [2, 4], cHCC-HCCs are no longer subclassified on classical and stem cell-like subtypes. Further subclassification on typical, intermediate, and cholangiocellular types also is no longer recommended. Moreover, the latter is currently termed cholangiolocarcinoma and is considered a subtype of intrahepatic CCA (iCCA) [4–6]. Finally, cHCC-CCA should be distinguished from collision tumors, when HCC and iCCA develop separately in the same liver but meet to form one nodule, and from multifocal HCC and iCCA. This review will focus specifically on cHCC-CCA.

## Epidemiology

The incidence of cHCC-CCA is increasing worldwide [7, 8, 9]. Its prevalence varies depending on the geographic region, risk factors within a population, and means of diagnosis [2, 10, 11]. In fact, most combined tumors are diagnosed postoperatively [6], and are thought to represent 1–4.7% of all resected liver cancers [12, 13]. In Western countries, cHCC-CC is more often

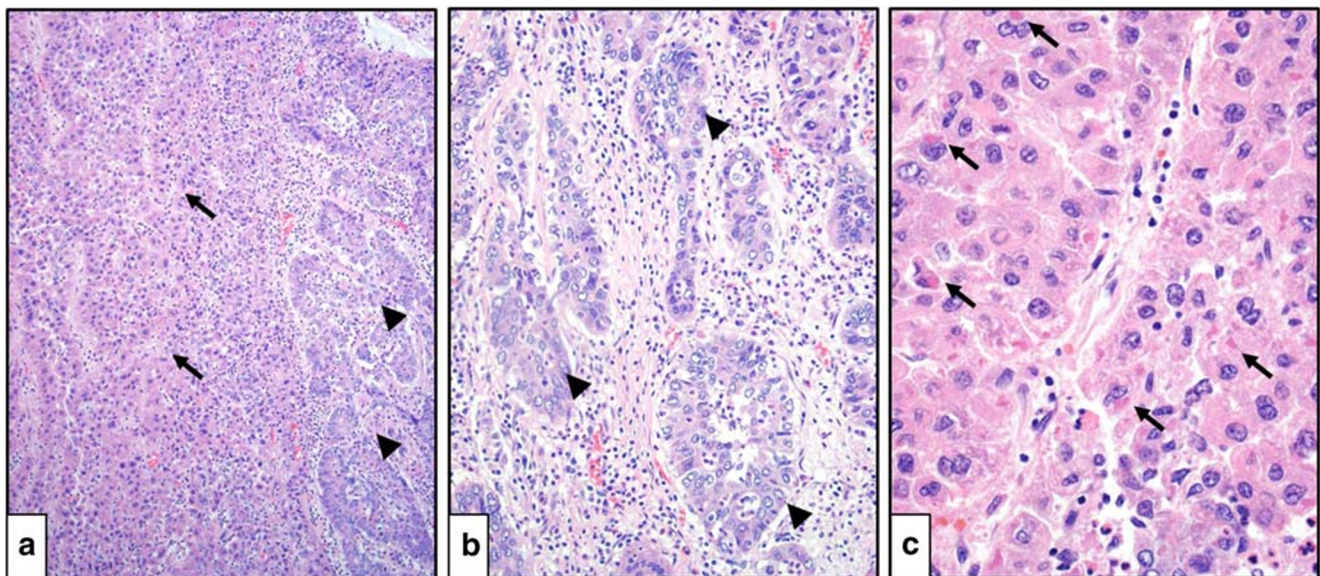
diagnosed before 60 years of age [7, 8]. In Asia, it accounts for 0.25–0.70% of PLCs [14, 15] with the mean age at diagnosis of 67 years old [14, 15]. Association of cHCC-CCA with sex has not been well established. Some reports from Asia indicate male predominance [7, 8, 14–16], while reports from Western countries do not support this sex difference [2, 16].

cHCC-CCA can be diagnosed in both cirrhotic and non-cirrhotic livers. This is unlike HCC, which is more often associated with cirrhosis [17], and iCCA, which is most often diagnosed de novo [18]. In Western countries, only 15% of patients diagnosed with cHCC-CCA have history of viral hepatitis B or C, and do not have cirrhosis. In some Asian studies, etiological factors were reported to be similar between HCC and cHCC-CCA [19, 20], while in other studies from Asia, a history of cirrhosis and viral hepatitis among patients with cHCC-CCA reported to be less common than in patients with HCC, but more frequent than in iCCA patients [11, 21]. Prevalence of cHCC-CCA in patients with non-alcoholic fatty liver- and alcohol-related liver diseases is not well defined but warrants investigation. Overall, the epidemiological profile of cHCC-CCA is likely intermediate between HCC and iCCA (Fig. 2) [22].

## Diagnosis

### Clinical Presentation

A mass lesion in patients undergoing surveillance for liver cancer or in symptomatic patients with or without known liver disease is the most common clinical presentation of cHCC-



**Fig. 1** Combined hepatocellular and cholangiocellular carcinoma (hematoxylin and eosin staining). **a** Hepatocellular (arrows) and cholangiocellular (arrowheads) components are present in the same tumor ( $\times 100$  magnification). **b** A cholangiocellular tumor component

demonstrates distinct tubular structures with lumens (arrowheads;  $\times 200$  magnification). **c** A hepatocellular tumor component demonstrates Mallory-Denk bodies (arrows;  $\times 400$  magnification)

**Clinical characteristics:**

- Very rare primary liver cancer diagnosed in 7<sup>th</sup> decade of life.
- Occurs in cirrhotic and non-cirrhotic livers.

**Radiological characteristics:**

- HCC-like arterial enhancement with washout.
- iCCA-like peripheral ring-enhancement with progressive centropetal arterial enhancement alternating with hypovascular areas; capsule retraction; and biliary dilatation.
- Mixed pattern with both HCC and iCCA characteristics in the same nodule.

**Histological features:**

- HCC-like: Mallory-Denk hyaline bodies, alpha-1 antitrypsin granules, albumin, pseudoglandular trabecular arrangement, and bilirubin in canaliculi.
- CCA-like: mucin producing cells CK7/CK19 positive cells forming glandular structures within prominent desmoplastic stroma.
- Transitional cell zone.

**Molecular alterations:**

- Frequent mutations in *TP53*, *TERT* promoter, and *ARID1A*.
- Unique *PTMS-AP1G1* gene fusion.

**Treatment:**

- Treatment of choice - surgical resection.
- Emerging role for liver transplantation.
- Undetermined role for loco-regional therapies.
- Systemic therapy with a combination of gemcitabine with platinum-based agents for advanced cancer.

**Fig. 2** Characteristics of cHCC-CCA

CCA [10, 19, 23, 24]. Fatigue, weight loss, abdominal pain, new jaundice, night sweats, or worsening of portal hypertension can be observed. Owing to their transitional nature, combined tumors can cause symptoms secondary to portal vein tumor invasion [25] or with biliary obstruction. Enlarged due to metastases, lymph nodes (LN) had been reported in 27–73% of cHCC-CCs [15]. The wide range of reported frequency of metastases can be due to differences in diagnostic criteria for combined cancers between studies leading to misclassification. History of risk factors for liver disease, including obesity, alcohol misuse, intravenous drug use, or immigration from areas with high prevalence of viral hepatitis, can prompt abdominal imaging. The average tumor size at diagnosis is 5.3 cm [26]. However, patients who undergo cHCC-CCA resection and have cirrhosis tend to have smaller, asymptomatic cancers as compared to patients without prior history of liver disease likely due to earlier diagnosis during active cancer surveillance [27].

### Imaging Studies

Presence of both hepatocellular and cholangiocellular components makes diagnosis of combined cancer based on imaging studies challenging as these components have a different degree of vascularization and fibrosis among other factors influencing radiological appearance (Fig. 2) [28]. Not surprisingly, the majority of patients undergoing surgery based on radiological appearance alone are often misdiagnosed with either HCC or iCCA preoperatively [24]. Abdominal ultrasound is commonly used for cancer surveillance and can reveal a hypoechoic mass with a central hyperechoic focus or a heterogeneous hypoechoic mass, which requires further characterization with dynamic contrast-enhanced abdominal imaging such as computed tomography or magnetic resonance imaging (MRI).

Radiological characteristics of cHCC-CCA depend on the predominant histological features of HCC or CCA. Classical HCC demonstrates a homogenous diffuse enhancement during the arterial phase, a washout on the delayed venous phase, and pseudocapsule. However, owing to the cholangiocellular cancer component, cHCC-CCAs can also exhibit a peripheral ring-like enhancement with progressive centripetal enhancement due to presence of the fibrotic stroma [6, 15, 29–31]. Biliary dilatation and capsular retraction can be observed similar with iCCA [32]. The retrospective analysis of resection or post liver transplantation samples of patients with cHCC-CCA had shown that tumors with > 50% HCC histology are more radiologically similar to HCC with arterial enhancement and washout, while lesions with > 50% iCCA histology demonstrate delayed enhancement like iCCA [33]. Another analysis has reported that masses showing a mixed pattern with progressive enhancement, arterial enhancement with or without washout, and hypovascular areas all in one lesion have a 48% sensitivity and 81% specificity for diagnosis of cHCC-CCA

[34]. The MRI can additionally demonstrate intralesional fat, hypointensity on T1-weighted images, and hyperintensity on T2-weighted images due to hepatocellular cancer part [35].

The Liver Imaging Reporting and Data System (LI-RADS) was developed to standardize radiological approach to liver lesion classification and facilitate HCC diagnosis in patients with cirrhosis [36••]. It grades lesions from LR-1 (definitely benign) to LR-5 (definitely HCC). cHCC-CCA lesion is reported as LR-malignant (LR-M) as it does not meet HCC criteria but appears as a malignant mass [37, 38]. Accurate preoperative diagnosis of cHCC-CCA is imperative because the treatment approach and outcomes differ from that of other PLCs. Thus, the frequency of lymph node metastases in cHCC-CCA is high which mandates LN dissection during surgery as in iCCA [28]. Serological markers and ultimately an imaging-guided mass biopsy should aid the correct diagnosis.

### Histopathological Diagnosis

When a suspicious liver mass is associated with elevation of CA 19-9 characteristic for CCA and AFP typical for HCC, cHCC-CCA should be ruled out and warrants a mass biopsy. At a cutoff value of 20 ng/mL, AFP can be lower in cHCC-CC as compared to HCC [19, 24]. Histologically, cHCC-CCA is confirmed by unequivocal histological areas of HCC and CCA in the same tumor nodule [39, 40]. The hepatocellular component can demonstrate Mallory-Denk hyaline bodies, alpha-1 antitrypsin granules, albumin, pseudoglandular trabecular arrangement, and presence of bilirubin in neoplastic canaliculi lined by the well-differentiated liver cells (Fig. 1). Nodule boundaries may contain cells with eosinophilic cytoplasm [41]. A cholangiocellular cancer component is often characterized by gland-like structures composed of mucin-producing cells positive for biliary differentiation markers CK7 and CK19, and by a prominent desmoplastic stroma. A periductal infiltration or intraductal growth can also be observed [6].

There is no minimum amount of HCC and CCA components that qualify patients for combined tumor diagnosis and each component can be independently well or poorly differentiated [16, 23]. As PLCs often arise in the cirrhotic livers, histopathological features of underlying liver disorder such as alcoholic and non-alcoholic fatty liver disease or viral hepatitis-related liver changes can be present [16, 42].

There is a differentiation of cHCC-CCA from other rare types of PLCs for prognostication. Thus, recently re-classified as a subtype of iCCA intermediate cell PLC is more common in females and associated with larger tumor size and less fibrosis compared to cHCC-CCA. The latter can have less inflammation in surrounding tissue in comparison to cholangiolocarcinoma [43]. In turn, the cholangiolocarcinomas are often smaller tumors with a lower differentiation grade than cHCC-CCA making them more aggressive [25, 39].



## Molecular Pathogenesis

Genetic profile of cHCC-CCA appears to be distinct from this of HCC and CCA. Thus, a recent study of 133 patients from Asia demonstrated a higher frequency of *TP53* mutations (49%) in cHCC-CCA as compared to HCC and CCA [44•], which was supported by other studies [5, 16, 45]. Mutations in *CTNNB1* and *KRAS*, which are frequently observed in HCC and CCA, respectively, are rare in cHCC-CCA (6% and 0%, respectively) [44•]. A unique cHCC-CCA fusion gene *PTMS-AP1G1* had been reported in 11.7% of cases and might be clinically relevant. Overexpression of *NESTIN* in cHCC-CCA had been reported in an association with poor prognosis [44•]. Study of 53 cHCC-CCA patients from Japan had demonstrated that mutations in the *TERT* promoter (31.3% of cases) are mainly observed in females, in previously treated tumors, and are associated with hepatitis B and poorer outcomes [4•]. In the same study, mutations in *ARID1A* (13.2% of cases) were more frequently observed in alcoholic liver disease-associated cHCC-CCA and in smaller tumors [4•]. Mutations in *IDH1/2*, which are frequent in iCCAs, were reported in 11.8% of patients with cHCC-CCA. However, they were not predictive of clinical outcomes [4•]. Highly targetable *FGFR* aberrations, which are also common in iCCA, are infrequent in combined cancers [4•]. An analysis of combined tumors in 18 US patients showed that tumors with high prevalence of stem cells and *SALL4* positivity are enriched for progenitor-like signatures, demonstrate activated *MYC* and *IGF* pathways, and are associated with poorer outcomes [5].

## Staging and Outcome Predictors

Several staging systems have been developed for HCC and CCA [46–49]. According to the 8th edition of the American Joint Committee on Cancer (AJCC) cancer staging manual, cHCC-CCA is staged the same as iCCA [50•]. However, these staging systems cannot be directly applied to combined cancers due to their transitional nature. A risk prediction preoperative model and a prognostic estimation of cHCC-CCA after resection (PECAR) systems have been proposed for outcome prediction [44•, 51]. Risk predictors in the former model include age; presence of anti-HBc and tumor in vein; levels of AFP, CEA, and blood urea nitrogen; and red blood cell count [44•, 52]. Predictors of recurrence in the PECAR system include male sex, elevated GGT, presence of the macrovascular invasion, and hilar LN metastases. These models were not validated externally.

## Treatment

### Surgical Approach

Surgical resection with dissection of hilar LNs is a treatment of choice for patients with compensated liver function and

without distant metastases. Underlying liver function, degree of portal hypertension, volume of a potential liver remnant, and patient age influence patient surgical candidacy. Portal vein embolization for preoperative enhancement of the future liver remnant can be considered. Despite curative intent of the resection, the 5-year survival rate is still suboptimal at approximately 30% [20] with a 5-year post-surgical resection recurrence rate of 78%. A resection margin > 10 mm (R0 resection) is associated with a longer disease-free survival [44•, 53]. One- and 3-year survival rates in cHCC-CCA have been reported at 81.9% and 47.0%, respectively [11]. cHCC-CCA patients more often have poorly differentiated tumor (29.2%) as compared with HCC and iCCA (10.3% and 17.2%, respectively,  $P < 0.001$ ), which might explain a shorter median overall survival of 7.9 months versus 10.8 months in HCC and 8.2 months in iCCA ( $P < 0.001$ ) [12]. The post-resection stage-specific survival for cHCC-CCA patients has been reported to be similar with those with HCC [12].

### Role for Liver Transplantation

Liver transplantation is a well-established treatment approach associated with improved survival in patients with HCC, who meet Milan criteria [44•, 54]. iCCA is not a widely accepted indication for liver transplantation, while there is an accumulating body of evidence for favorable outcomes in patients with early (< 2 cm) iCCA [55•, 56], and some patients with more advanced iCCA after neoadjuvant chemotherapy [57]. Notably, the majority of small iCCAs and cHCC-CCAs are diagnosed post-transplantation incidentally, with 0.48–2.7% of cHCC-CCA found in the explanted livers [39, 44•, 52].

Some studies, including those using adjuvant chemotherapy [28, 44•], have reported the 1-, 3-, and 5-year cHCC-CCA survival rate post-transplantation of 79%, 66%, and 16%, respectively [44•]. Another study directly comparing outcomes for liver transplantation for cHCC-CCA, iCCA, and HCC had reported the 5-year survival rates of 40%, 47%, and 62%, respectively [44•]. Tumors > 2 cm, presence of concurrent HCC, LN metastases, and portal vein invasion were associated with poorer prognosis after liver transplantation [44•].

Data from transplant centers show comparable outcomes of the surgical resection and liver transplantation for cHCC-CCA with the 1-, 3-, and 5-year survival rates after resection at 71%, 46%, and 42.1%, respectively, and after transplantation at 89%, 48%, and 50%, respectively [44•]. Taking into a consideration a scarce pool of donor organs, resection should be considered a first-line treatment for localized to the liver cHCC-CCA in acceptable surgical candidates. The role of neoadjuvant or adjuvant therapy is not known.

## Loco-Regional Therapies

Extrapolating from experience with HCC and iCCA [13, 25], inoperable relatively small cHCC-CCs (< 4 cm) can potentially be treated with image-guided ablation. Radiation therapy can be used when a tumor location precludes safe ablation, and the tumor cannot be visualized on an imaging study, or in patients with suboptimal liver function. An unpublished experience with stereotactic radiation therapy (SBRT) for cHCC-CC from our center is highly favorable. A paucity of data on outcomes of ablation and radiation therapy in cHCC-CC is likely due to rarity of these tumors and difficulties with diagnosis.

Degree of cHCC-CCA arterialization is determined by HCC component and can predict a potential response to transarterial catheter-based therapies. cHCC-CCAs treated with transarterial therapies versus resection were reported to be larger (mean size of 8.9 cm versus 5.8 cm, respectively) and more often were associated with LN metastases (33% versus 8%, respectively) [8, 16, 35]. The partial response rate to transarterial chemoembolization, radioembolization, and hepatic arterial infusional chemotherapy has been reported at 20%, 50%, and 66%, respectively [35]. The median progression-free and overall survival after catheter-based therapies has been reported at 8.3 and 16 months, respectively [8, 16]. Thus, catheter-based transarterial therapies might have utility for well-vascularized large or multifocal inoperable cHCC-CCAs in patients with acceptable liver function. Overall, more data are needed to demonstrate effectiveness of loco-regional therapies for cHCC-CCA.

## Systemic Therapy

There are no standardized recommendations on systemic agents for cHCC-CCA. Most centers use a combination of gemcitabine with platinum-based regimens [58], which can be justified by the data from several recent retrospective studies. One had compared outcomes in 36 patients treated with gemcitabine/cisplatin, gemcitabine/5-fluorouracil, sorafenib, and other regimens, including S-1 alone or in a combination with gemcitabine, or gemcitabine alone [44•, 59]. The median overall survival with gemcitabine/cisplatin, gemcitabine/5-fluorouracil, sorafenib, and “other regimens” was 11.9, 10.2, 3.5, and 8.1 months, respectively, and there was no difference in a progression-free survival between groups [44•]. Thus, sorafenib use was associated with an inferior overall survival (hazard ratio 15.83,  $P = 0.006$ ) as compared with gemcitabine-based regimens. Sorafenib inferiority for treatment of cHCC-CCA also was demonstrated in a study of 123 patients treated with either gemcitabine alone or in combination with 5-fluorouracil or platinum-based agents, sorafenib alone, or “other drugs” [8, 44•]. The overall survival after treatment with gemcitabine combinations versus sorafenib was 11.5 and 9.6 months, respectively. The progression-free

survival was also lower in the sorafenib group (4.8 months) as compared with the gemcitabine combination with platinum-based agents (8 months) or the gemcitabine combination with 5-fluorouracil (6.6 months) groups [44•].

Based on clinical trials, molecular targeted therapies are effective for HCC [60] and iCCA [61]. The data on targeted therapies use in cHCC-CCA are very limited. One case review has shown that bevacizumab, which had been recently approved by the Federal Drug Administration as a first-line agent in a combination with atezolizumab for advanced HCC [60, 62], is associated with improved outcomes in cHCC-CCA patients when used in a combination with gemcitabine-based therapy [59•]. Immunotherapy alone or in combination with other systemic agents and liver-directed modalities had been shown to improve outcomes in HCC [63]. Its utility for cHCC-CCA is not known.

## Conclusions

cHCC-CCAs are a distinct group of PLCs with unique genetic landscape and aggressive biological behavior. Due to its rarity and non-uniform use of cHCC-CCA terminology and diagnostic criteria, its epidemiology and best therapeutic approaches to treat it are not well established. Histological tumor evaluation is required for diagnosis of combined cancers and can be based on a routine hematoxylin and eosin staining. Patient outcomes with cHCC-CCA appear to be worse than with HCC but better when compared with iCCA. Surgical resection is the treatment of choice for early stage cHCC-CCA confined to the liver. The role of liver transplantation for cHCC-CCA is emerging, while the role of liver-directed therapies and adjuvant and neoadjuvant therapies is unknown. Catheter-based therapy can be beneficial for patients with highly vascularized tumors. Limited retrospective studies support use of a combination of gemcitabine with platinum-based chemotherapeutic agents until data for other regimens are available. Studies investigating the role of molecular targeted therapies in cHCC-CCA are highly desirable but might be difficult to conduct due to rarity of this cancer. Consistent use of proper cHCC-CCA terminology and multicenter collaborations will promote better understanding of this rare cancer epidemiology and genetics to inform potentially actionable targets and facilitate clinical trials.

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## Compliance with Ethical Standards

**Conflict of Interest** Olga Raevskaya, Henry Appelman, and Natalita Razumilava declare that they have no conflict of interest.

**Human and Animal Rights and Informed Consent** This article does not contain any studies with human or animal subjects performed by any of the authors.

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- Of importance
- Of major importance

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