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Advances in the surgical treatment of liver cancer

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SUMMARY Liver resection is the standard curative treatment for liver cancer. Advances in surgical techniques over the last 30 years, including the preoperative assessment of the future liver remnant, have improved the safety of liver resection. In addition, advances in nonsurgical multidisciplinary treatment have increased the opportunities for tumor downstaging. Consequently, the indications for resection of more advanced liver cancer have expanded. Laparoscopic and robot-assisted liver resections have also gradually become more widespread. These techniques should be performed in stages, depending on the difficulty of the procedure. Advances in preoperative simulation and intraoperative navigation technology may have also lowered the threshold for their performance and may have promoted their widespread use. New insights and experiences gained from laparoscopic surgery may be applicable in open surgery. Liver transplantation, which is usually indicated for patients with poor liver function, has also become safer with advances in perioperative management. The indications for liver transplantation in liver cancer are also expanding. Although the coronavirus disease 2019 pandemic has forced the postponement of liver resection and transplantation procedures, liver surgeons should appropriately tailor the surgical plan to the individual patient as part of multidisciplinary treatment. This review may provide an entry point for future clinical research by identifying currently unresolved issues regarding liver cancer, and particularly hepatocellular carcinoma.

Keywords liver cancer, hepatocellular carcinoma, resection, transplantation, COVID-19

1. Introduction

Hepatocellular carcinoma (HCC) is one of the most intractable cancers and is the fifth most common carcinoma worldwide. Moreover, it is the second most common cause of cancer-related deaths. Annually, 854,000 new cases are diagnosed, and 810,000 deaths occur. HCC accounts for approximately 90% of primary liver cancers. Regionally, incidence increases with age, peaking in the 70s. The number of HCC cases is increasing worldwide with population growth and aging, increasing 75% from 1990 to 2015. Medications for hepatitis viruses, a major cause of HCC, have also improved. However, HCC due to chronic liver damage caused by nonalcoholic fatty liver disease is on the rise (*1*). Surgery is the main form of treatment for HCC. The prognosis after surgery is 60-80%, which is better than that for unresectable HCC. HCC with distant metastasis is not an indication for surgery. Conversely, the indications for resectability differ in different countries and facilities. Surgery is usually indicated for patients with a tumor diameter of ≥ 3 cm and 3 or fewer tumors. In practice, liver resection (LR) can be performed in patients who exceed the aforementioned tumor criteria. Ablation is reported to have similar results for early-stage HCC. With advances in interventional radiology and systemic therapy, the opportunities for curative resection of initially unresectable HCC are increasing after down-staging. Liver transplantation (LT) can be performed in patients with a resectable tumor but is contraindicated for LR because of poor liver function such as Child-Pugh class C cirrhosis. LT has improved with advances in perioperative management, and the indications for LT for HCC are expanding.

This review outlines the advances in LR for liver cancer, and particularly HCC, over the past 30 years. It also aims to provide an entry point for future clinical research by identifying currently unresolved issues.

2. Reduction of intraoperative blood loss

2.1. Occlusion of inflow

Managing blood loss during LR affects both short- and long-term prognosis. Therefore, various efforts have been made to reduce blood loss. The Pringle maneuver

reduces the blood inflow during hepatic dissection by simultaneously clamping the hepatoduodenal ligament. The procedure was originally performed in 1908 to control liver hemorrhage caused by trauma (*2*). Until the 1970s, however, occlusion of blood inflow was not widely used; it was considered to be contraindicated because the impaired liver is vulnerable to anoxia, and inflow blood deprivation contributes to postoperative liver failure. Makuuchi *et al*. (*3*) devised the hemiliver vascular occlusion method in 1987 to preserve liver function and reduce blood loss. Some surgeons in the 1980s considered the Pringle maneuver unnecessary. Thus, randomized control trials (RCTs) were conducted in the 1990s to confirm its usefulness. Man *et al*. (*4*) compared groups treated with (n = 50) and without (n = 50) the Pringle maneuver. The safety and efficacy of the Pringle maneuver were established with a lower bleeding rate per hepatic dissection area and a faster dissection rate. Intermittent clamping was used in that RCT. In 1998, Belghiti *et al*. (*5*) compared the Pringle maneuver using intermittent versus continuous clamping. Although the amount of blood loss during parenchymal dissection was significantly greater in the intermittent clamping group (n = 44) than in the continuous clamping group (n = 42), the incidence of postoperative hepatic dysfunction was significantly higher in the continuous clamping group. Both major postoperative liver failure (4/42, 9.5%) and surgery-related death (2/42, 4.8%) were noted only in the continuous clamping group. Moreover, intermittent clamping is better tolerated and remains a mainstay of the Pringle maneuver. In the intermittent clamping group in the RCT, clamping was performed for 15 min and the released for 5 min. Although the maximum continuous ischemic time was approximately 120 min, intermittent clamping did not cause hepatic failure in the normal liver, even after a cumulative clamping time of 322 min (*6*). The Pringle maneuver is now routinely performed, resulting in decreased blood loss during LR and improved surgical outcomes. Nevertheless, caution should be exercised in patients with HCC, as they often have pre-existing hepatic impairment. To minimize the effect on the remnant liver, selective clamping specifically of the blood inflow to the resected side may be effective.

2.2. Clamping the outflow

Partial or complete clamping of the hepatic vein or inferior vena cava is an effective technique for controlling bleeding from the hepatic vein. The total hepatic vascular exclusion (THVE) technique is the complete occlusion of blood inflow and outflow in the liver and was reported by Heaney *et al*. in 1966 (*7*). In 1974, Fortner *et al*. (*8*) reported a THVE technique performed under cooled perfusion of the liver. In 2015, Azoulay *et al*. (*9*) reported a 19.5% 90-day mortality rate in 77 patients who underwent LR using standard THVE with hypothermic portal perfusion and venovenous bypass, and they further recommended improvements to the method and patient selection. The necessity of cooling has also long been debated. In 1978, Huguet *et al*. (*10*) reported a THVE method involving cooling at room temperature. They also published a 25-case series in 1992, which indicated that an extracorporeal perfusion system is not necessary for at least 90 min of THVE for a healthy liver (*11*). Regardless of whether it is performed with or without cooling, the indications for THVE are limited to uncontrolled bleeding, large tumors, or the presence of a tumor thrombus in the hepatic vein or the inferior vena cava. Given the time and effort required for clamping, it is usually not necessary in hepatic resection. In fact, a systematic review of four RCTs by Rahbari *et al*. (*12*) found no benefit in performing hepatic vein clamping to reduce intraoperative blood loss.

2.3. Controlled low central venous pressure

Multiple RCTs have indicated that keeping the central venous pressure low during parenchymal dissection can reduce bleeding. Liu *et al*. (*13*) analyzed 18 RCTs involving 1,285 patients. That systematic review noted a 312-mL reduction in blood loss, a 59% reduction in patients requiring blood transfusions, and a significantly lower alanine transaminase level in the first 5 days after surgery in the low central venous pressure group than in the control group. Liu *et al*. also noted no significant differences in postoperative complications between the groups. Central venous pressure can be reduced in several ways, including reducing intraoperative infusions, phlebotomy, decreasing the tidal volume as part of ventilator management, and partial clamping of the inferior vena cava.

2.4. Hanging maneuver

In right hepatectomy, the basic procedure is to mobilize the liver before transection. However, the procedure may be difficult for large tumors or tumors involving the diaphragm. In such cases, the anterior approach is useful and should thus precede liver transection before mobilization. Belghiti *et al*. (*14*) introduced the hanging maneuver, in which the liver is taped between the dorsal side of the liver parenchyma and the ventral aspect of the inferior vena cava before hepatic transection. The advantages of this procedure are the easily understandable direction of transection, monitoring of the positions of the inferior vena cava and middle hepatic vein, an improved surgical field as a result of traction on the tape, and assessment of the effectiveness of compression hemostasis. Procedures to reduce blood loss during liver resection are summarized in Table 1.

3. Dealing with the insufficient future liver remnant

Table 1. Summary of procedures to reduce blood loss during liver resection

Procedures	Author	Year	Type of study	Results
Hemihepatic vascular occlusion	Makuuchi M, *et al*. (*3*)	1987	Historical cohort	Reduced intraoperative blood loss and postoperative hyperbilirubinemia
Pringle maneuver	Man K, *et al*. (*4*)	1997	RCT	Resulted in less blood loss, less blood transfused, and a shorter liver transection time
Intermittent Pringle maneuver	Belghiti J, *et al*. (*5*)	1999	RCT	Associated with an intraoperative blood loss comparable to that noted after continuous clamping, but with less severe parenchymal injury, especially in patients with an underlying liver disease.
Hepatic vascular exclusion	Rahbari N, *et al*. (*12*)	2009	Meta-analysis	Did not offer any benefit in terms of outcomes for patients undergoing hepatic resection compared to portal triad clamping alone.
Low central venous pressure	Liu TS, *et al*. (*13*)	2021	Meta-analysis	Reduced blood loss during liver resection, blood transfusions, and the number of patients requiring transfusion
Hanging maneuver	Belghiti J, *et al*. (*14*)	2001	Case series	Offered several advantages: *i*) smaller transection plane from the anterior surface of the liver to the anterior surface of the IVC, *ii*) upward traction on the tape pulls the liver up and allows better exposure, hemostasis of the transection surface, and protection of the IVC, and *iii*) applying leftward traction on the tape allows access to the transection plane, allowing safe isolation of the trunk of the middle hepatic vein.

RCT, randomized control trial; IVC, inferior vena cava.

3.1. Portal vein embolization

Postoperative liver failure is a fatal complication. If major LR is indicated but the future liver remnant is small, multi-step treatment is required. In patients with a small future remnant liver, portal vein embolization (PVE) is performed to promote the enlargement of the residual liver by embolizing the portal venous branch near the tumor before surgery, as first reported by Makuuchi *et al*. in 1982 in a Japanese population (*15-17*). The two types of percutaneous transhepatic approaches to PVE are the ipsilateral approach, in which embolization is performed from the side of the liver where the tumor is located, and the contralateral approach, in which embolization is performed from the side of the liver without a tumor. Although the ipsilateral approach is ideal to minimize the impact on the remnant liver, appropriate approaches should be selected depending on the circumstances of the case. Trans-ileocecal embolization can also be performed in open surgery. Before the widespread use of interventional radiology, ligation of the portal vein was performed in open surgery. However, PVE is superior given its minimal invasiveness and lower complication rate (*18,19*). PVE also results in greater hypertrophy than ligation (*20*). This may be because portal vein ligation produces more central occlusion of the portal blood flow, whereas PVE results in more peripheral occlusion. A study summarizing 319 cases at a single facility suggested that PVE is an effective technique for avoiding postoperative liver failure (*21*). The median waiting time from embolization to LR was 24 days. Nevertheless, 20% of patients were unable to undergo LR after embolization. This was mainly due to tumor progression, not the rate of hypertrophy. Hence, attempts are being made to shorten the waiting period. The rate of liver hypertrophy per week, referred to as the kinetic growth rate, may be more closely correlated with postoperative liver failure than the rate of hypertrophy of the remnant liver in colorectal liver metastases (*22*). This suggests that calculation of the liver volume alone is not sufficient to evaluate remnant liver function.

3.2. Sequential trans-arterial chemoembolization and portal vein embolization

PVE alone may not be sufficient to enlarge the future remnant liver, and especially in HCC. This may be the result of the following possible causes: (*i*) The background liver is often impaired or cirrhotic and may have already regenerated; (*ii*) a compensatory increase in arterial blood flow to the embolized liver may promote tumor progression; and (*iii*) if the HCC has an arterioportal shunt, PVE alone may be insufficient for embolization. Therefore, a sequential strategy was proposed in which transarterial chemoembolization (TACE) was performed before PVE. In 2004, Aoki

et al. (*23*) reported 22 ± 4% hypertrophy of the non-embolized liver within 2 weeks of TACE+PVE in a case series of 17 patients. Moreover, the cumulative overall 5-year survival rate was 55.6%. In 2006, Ogata *et al*. (*24*) retrospectively compared TACE + PVE (n = 18) and PVE (n = 18) and noted a significantly better rate of liver hypertrophy in the TACE + PVE group. In addition, complete tumor necrosis after resection was achieved in 15 of 18 patients in the TACE+PVE group compared to 1 of 18 patients in the PVE group, and the 5-year recurrence-free survival (RFS) rate was significantly better in the TACE + PVE group. Conversely, this technique is theoretically contraindicated in patients with extensive portal thrombus or severe portal hypertension and after choledochojejunostomy (*25*).

3.3. Two-stage hepatectomy

Two-stage hepatectomy was proposed by Adam *et al*. (*26*) for multiple tumors in both lobes in 2000. Minor hepatectomy of the remnant liver is performed in the first stage, followed by major hepatectomy, often accompanied by PVE, in the second stage. Today, in hybrid operating rooms with interventional radiology capabilities, LR and PVE can be performed in the same operating room in a single stage. This contributes to a shorter waiting period for second-stage surgery (*27*).

3.4. Associating liver partition and portal vein ligation for staged hepatectomy

The associating liver partition and portal vein ligation for staged hepatectomy (ALPPS) procedure has further increased the rate of hypertrophy of the remnant liver by performing hepatic transection with PVE or ligation during the first stage of two-stage surgery. The goal is to complete the resection before tumor progression occurs (*28*). The novel ALPPS technique ignited excitement in the hepatobiliary surgery community because ALPPS challenged the idea of unresectability and it extended the limits of liver surgery. Moreover, liver hypertrophy of up to 80% was induced in a shorter time than PVE or ligation. Nonetheless, the ALPPS technique raised serious concerns due to the high morbidity and mortality (up to 40% and 15%, respectively) related to postoperative liver failure and bile leakage. Identifying the risk factors associated with ALPPS has opened up a new dimension in the field of ALPPS surgery to improve surgical outcomes through careful patient selection. The benefit of the ALPPS technique is enhanced when performed on young patients with a borderline future remnant liver. Technical modifications of ALPPS, such as middle hepatic vein preservation, surgical management of the hepatoduodenal ligament, the anterior approach, and partial ALPPS, may improve its performance. Few studies have noted the theoretical survival benefits of ALPPS (*29*). An RCT comparing ALPPS and two-stage hepatectomy in colorectal cancer liver metastasis noted a better rate of resection with ALPPS (*30*). In a 2021 study, long-term follow-up data indicated that the ALPPS group had a better prognosis than the two-stage hepatectomy group (*31*). However, a letter to the editor identified problems with the study design, an insufficient follow-up, and relatively poor results in the two-stage group compared to those in previous studies. In addition, although the indications and effectiveness of ALPPS have not yet been determined, it is performed not only in colorectal cancer liver metastasis but also in bile duct cancer and HCC in clinical practice (*32*).

3.5. Liver venous deprivation

In 2009, Hwang *et al*. (*33*) reported subsequent right hepatic vein embolization in 12 patients who had undergone right PVE for right liver resection. In 2020, Laurent *et al*. (*34*) evaluated the effects of simultaneous radiological portohepatic vein embolization before hepatectomy and reported a significantly better rate of postembolization hypertrophy of 61% in the portohepatic vein embolization group compared to 29% in the PVE alone group. A similar study confirmed the significantly greater kinetic growth rate in portohepatic vein embolization compared to PVE alone (*35*). Thus, portohepatic vein embolization is considered a safer procedure than ALPPS, but no clinical studies have directly compared it to ALPPS. Initial experiences with procedures to deal with the insufficient future liver remnant are summarized in Table 2.

4. Anatomic resection *vs*. non-anatomic resection

Since intrahepatic micrometastases can develop from HCC via the portal vein, anatomical resection depending on the distribution of the tumor-bearing portal vein should be performed to eradicate the tumor. Although left and right hepatectomies are anatomical resections, Makuuchi *et al*. (*36*) presented a case series of 57 patients who underwent systemic subsegmentectomy using intraoperative ultrasonography in 1985. This type of resection was performed in accordance with Couinaud's subsegmental boundaries, and Makuuchi *et al*. found that even patients with impaired liver function can safely undergo resection and that the resection is highly curative oncologically. In 2005, the same group reported long-term outcomes in 210 patients with solitary HCC (*37*). Multivariate analysis indicated that anatomical resection contributed to a risk reduction in both overall survival (OS) and RFS (hazard ratios [HR]: 0.57 and 0.65, respectively). In 2016, a study that performed propensity score matching indicated that anatomical resection contributed to prolonged RFS and decreased local recurrence in Child–Pugh

Table 2. Initial experiences with procedures to deal with an insufficient future liver remnant

Procedures	Author	Year	Number of patients	Results
PVE	Makuuchi M, *et al*. (*15*)	1982	14	Liver resection was performed after portal vein embolization and postoperative deaths were not noted.
Sequential TACE and PVE	Aoki T, *et al*. (*23*)	2004	17	Radical liver resection was completed in 88.2% of HCC cases. The 5-year overall and disease-free survival rates after curative resection were 55.6% and 46.7%, respectively
Two-stage hepatectomy	Adam R, *et al*. (*26*)	2000	13	Median survival was 31 months from the second hepatectomy in patients with colorectal liver metastases.
ALPPS	Schnitzbauer A, *et al*. (*28*)	2012	25	After a median waiting period of 9 days (range = 5-28 days), the median volume of the left lateral lobe increased 74% (range = 21-192%). Mortality was 12.0%.
Liver venous deprivation	Hwang S, *et al*. (*33*)	2009	12	Future liver remnant volume increased 14.2 ± 4.9% after PVE and 27.6 ± 8.6% after hepatic vein embolization. There were no serious adverse events.

PVE, portal vein embolization; TACE, transarterial chemoembolization, ALPPS, associating liver partition and portal vein ligation for staged hepatectomy

class A patients with a solitary HCC smaller than 5 cm. Liu *et al*. (*38*) performed a systematic review of 14 studies involving 9,444 patients. The anatomical resection group (n = 4260) had a significantly better 5-year OS (odds ratio [OR]: 1.19; $P < 0.001$) and RFS (OR: 1.26; $P < 0.001$) than the nonanatomical resection group. Anatomical resection was also associated with a longer operating time (mean difference: 47.08; $P < 0.001$), greater blood loss (mean difference: 169.29; $P = 0.001$), and wider surgical margins (mean difference: 1.35; $P = 0.04$). There were no significant differences in the rate of blood transfusions (OR: 1.16; $P = 0.65$) or postoperative complications (OR: 1.24, $P = 0.18$). However, most of the studies were from Asian countries such as China, Japan, and South Korea.

A Japanese multicenter RCT conducted in 2021 noted no significant difference in RFS between the LR and radiofrequency ablation groups in HCCs with a diameter < 3 cm and in those with three or fewer tumors (*39*). In this RCT, 69 of 150 patients (46%) underwent anatomical resection, but the prognostic impact of the technique was not studied.

5. Hepatectomy for highly advanced cancer

5.1. Portal vein tumor thrombus

Portal vein tumor thrombus (PVTT) is a common occurrence and a primary obstacle in the treatment of HCC with a high rate of recurrence and poor prognosis. No global consensus has been reached and no standard guidelines have been established regarding the management of HCC with PVTT. In Western countries, sorafenib and lenvatinib are the recommended first-line treatment options for HCC with PVTT, which is now regarded as Barcelona Clinic Liver Cancer Stage C, regardless of the type of PVTT. Relatively favorable results of hepatic resection have been reported in Asian populations. Kokudo *et al*. (*40*) used propensity score matching to compare 2,093 patients with PVTT in Japan who underwent LR and 4,381 who were treated otherwise. Results indicated that the median survival in the surgical group was significantly longer than that in the nonsurgical group (2.87 *vs.* 1.10 years; $P < 0.001$) with Child–Pugh class A disease. Further subgroup analysis indicated that LR could result in survival benefits as long as the PVTT is limited to the first-order branch of the portal vein (Vp1-Vp3). However, the benefit was not significant in patients whose PVTT affected the main trunk or contralateral branch (Vp4). A similar study in a Chinese population reported that as long as the PVTT was confined to the first-order branch of the portal vein, the patient may be eligible for resection (*41*). Resection most commonly precedes hepatic dissection. While emerging studies have suggested that the elimination of PVTT first may improve surgical outcomes, no conclusions have been reached with regard to better approaches.

5.2. Vascular resection and reconstruction

Hepatic resection with vascular resection and reconstruction is challenging. A limited number of high-level facilities are offering it because surgery with curative intent is currently the only treatment that can prolong long-term survival in advanced hepatic

malignancy. Various studies on the hepatic artery, the portal vein, the hepatic vein, and the inferior vena cava have been published. If a direct anastomosis is not possible, a patch or graft should be placed on the defect. Depending on the facility and circumstances, the materials used for reconstruction include autologous materials or a homograft, xenogenous materials, and synthetic materials. In other words, material selection must consider the vessel diameter, the size of the defect, the risk of infection, the availability of anticoagulation therapy, operating time, and cost, among other factors. Naturally, familiarity with variations in anatomy is essential. Many vascular reconstruction techniques have been adapted from experience with LT. Moreover, approaches that involve a total hepatectomy for tumor resection as ex situ LR have been reported. Still, clinical questions remain, such as whether anticoagulation is needed after reconstruction and the steps to perform it, if needed (*42*).

6. Minimally invasive hepatectomy

6.1. Laparoscopic hepatectomy

Laparoscopic hepatectomy was reported in the 1990s based on the approaches used for other organs and is now widely performed for HCC and other diseases because of its established safety and efficacy. Hendi *et al*. (*43*) conducted a systematic review of 23 studies that involved 1,363 patients with HCC who underwent laparoscopic hepatectomy, of which 364 (27%) underwent major hepatectomies. Blood transfusions were required in only 4.9% of patients. Only 2 (0.21%) postoperative deaths were noted, and the overall morbidity was 9.9%. Tumor recurrence occurred within 6-25 months. The 1-year, 3-year, and 5-year RFS rates were 71.9-99%, 50.3-91.2%, and 19-82%, respectively. The 1-year, 3-year, and 5-year OS rates were 88-100%, 73.4-94.5%, and 52.6-94.5% respectively.

6.2. Laparoscopic donor hepatectomy

Laparoscopic hepatectomy has been adapted to donor LR for living donor LT at some facilities because of its improved safety. Gao *et al*. (*44*) reported that a laparoscopic donor hepatectomy group (n = 633) had a longer operating time than an open living donor hepatectomy group (n = 1368) but shorter postoperative hospitalization, less blood loss, and fewer complications.

6.3. Robotic hepatectomy

Robot-assisted LR has recently been introduced at some facilities as a minimally invasive procedure. Because of the use of highly movable arms, robotic surgery is considered easier to perform than laparoscopic surgery. The aim is to achieve better aesthetic outcomes, less pain and morbidity, and better quality of life without compromising safety. As with laparoscopic surgery, there are efforts to expand the indications for donor LR (*45*). However, understandably, publication bias exists, the surgical team needs to be experienced, and indications should be carefully determined. This is especially true for living donors, the safety of whom is important.

7. Simulation and navigation

7.1. Simulation using three-dimensional imaging

In addition to conventional preoperative imaging techniques, such as computed tomography and magnetic resonance imaging, three-dimensional imaging is also important in LR. The technology emerged in the 2000s as a method of virtually reconstructing anatomy and simulating surgery. Using specialized software, a more accurate calculation of the volume of the liver in sub-areas and even smaller units is now possible, as well as the estimation of the area of congestion in hepatic vein resection by calculating the venous return area. using A three-dimensional printer has also been used in attempts to create a three-dimensional model to confirm the surgical plan. These visualization techniques show potential as educational tools for physicians and medical students to facilitate their understanding of surgery and may be useful in the preoperative explanation of the surgical plan to patients. Challenges include the cost of implementation and the difficulty of fully simulating the actual surgery because of liver deformation during dissection (*46*).

7.2. Advances in intraoperative ultrasound

Similar to the techniques for preoperative simulation, intraoperative navigation techniques are also evolving. Intraoperative ultrasonography was first used in LR in the 1980s. Contrast-enhanced intraoperative ultrasonography was established in the 2010s to differentiate HCCs and identify new HCCs or colorectal liver metastases during surgery (*47,48*). The clinical applications of real-time virtual sonography, a technique that links preoperative computed tomography and magnetic resonance images with intraoperative ultrasound images, have become apparent in recent years (*49*).

7.3. Fluorescence imaging

The use of fluorescence imaging technology has advanced over the last few decades. Although various fluorescent agents are available, indocyanine green is the most commonly preferred, and especially in LR. Indocyanine green is used to evaluate liver function before LR, especially in Asian countries. During hepatectomy, fluorescence imaging in the near-infrared spectrum begins with the depiction of the biliary tract

as a result of the uptake of indocyanine green into the biliary tract. This modality has been used to identify tumors, such as HCCs, and regional boundaries (*50*). Capturing fluorescence intraoperatively in real time is now possible using the Medical Imaging Projection System (*51*). It can accommodate liver deformities during surgical manipulation. Image-guided technology is considered especially important in laparoscopic and robotic surgeries, where palpation is not possible as it is in open surgery (*52*).

7.4. Augmented reality

A new technology that can be used in surgery creates three-dimensional stereoscopic images preoperatively and it projects them onto the actual surgical field intraoperatively as augmented reality. Augmented reality attempts to see through the tumor and vascular structures inside the liver. At present, this technology is only used to examine the position of port insertion in laparoscopic or robotic surgery and the puncture position in ablation; its use in actual clinical practice is still limited. It may be useful at ensuring an appropriate margin from the tumor and avoiding unnecessary damage to the vasculature. Novel techniques may not necessarily be needed by already skilled liver surgeons but may be beneficial for less experienced ones (*53*).

8. Evaluation of difficulty in hepatectomy

With the advent and widespread use of laparoscopic hepatectomy, surgical safety has become an issue. Attempts have been made to objectively classify the difficulty of hepatectomy. Ban *et al*. (*54*) scored surgical difficulty on a 10-point scale depending on tumor characteristics and the surgical procedure in 90 cases at three facilities in Japan and found that surgical difficulty was correlated with operating time and blood loss. Kawaguchi *et al*. (*55*) analyzed the rate of laparotomy conversion as an endpoint in 452 cases at a single French facility. Notably, resection of the posterosuperior segments is more difficult than that of the anterolateral segments, even with a limited hepatectomy, and the results agree with those from actual clinical practice. The classification of surgical difficulty is also applicable to open surgery (*56*) and is thus a by-product of the development of laparoscopic surgery. While it is not an advancement in surgical techniques or equipment, it should serve as a valuable reference in surgical education.

9. Role of surgery in multimodal treatment

9.1. Adjuvant therapy after hepatic resection

No standard adjuvant therapy after hepatic resection for HCC has been established. Several RCTs involving postoperative TACE were conducted in the 1990s, but consistent results were not obtained due to differences in patient characteristics (*57*). In 2006, an RCT involving oral uracil-tegafur noted no significant difference in both RFS and OS between the uracil-tegafur group (n = 79) and the control group (n = 80) (*58*). A phase 3 international multicenter trial (STORM trial) in 2015 found no significant difference in median RFS, with 33.3 months in the sorafenib group (n = 556) versus 33.7 months in the placebo group (n = 558) (*59*). An ancillary study examined differences in biomarkers but failed to find a group of patients who benefited from sorafenib (*60*). Ke *et al*. (*61*) conducted a meta-analysis of 1,333 patients in 12 studies to assess whether adjuvant hepatic artery infusion chemotherapy improved long-term prognosis. They found that both the OS rate and RFS rate in the adjuvant hepatic artery infusion chemotherapy group were better than those in the surgery alone group (HR = 0.56, 95% confidence interval (CI) = 0.41-0.77, $P < 0.001$; HR = 0.66, 95% CI = 0.55-0.78, $P < 0.001$, respectively). Moreover, they found that adjuvant hepatic artery infusion was particularly effective in patients with microvascular and macrovascular invasion. However, further studies are needed to determine the effects of adjuvant treatment.

9.2. Conversion surgery

Conversion surgery remains controversial in HCC treated with TACE, transarterial radioembolization with yttrium-90 microspheres, radiotherapy, systemic therapies, and combinations of multimodality treatment approaches. In recent years, hepatectomy has been performed to attain a radical cure and improve the prognosis for initially unresectable HCC (*62*). Sorafenib and lenvatinib have been commonly used as first-line therapies, followed by atezolizumab, a recently developed programmed death ligand-1 monoclonal antibody, and bevacizumab, an anti-vascular endothelial growth factor monoclonal antibody. The median survival time has gradually improved to over 1.5 years (*63*). In a study by Shindoh *et al*. (*64*) 16 patients with advanced HCC were treated with lenvatinib after surgical intervention, including 9 patients undergoing curative LR. The conversion rate for curative resection was 8.4%. Such studies are expected to increase in the future.

9.3. Y-90 radioembolization

Radioembolization is a form of hepatic arterial therapy that provides high-dose brachytherapy by delivering yttrium 90 beta-emitting beads to the tumor. Conversion surgery after treatment with yttrium-90 radioembolization has also been reported (*65*). In a meta-analysis of 276 patients from 16 studies on yttrium-90 radioembolization, the 90-day mortality

rate was 3.0% (95% CI 0.3-7.4%). The median time to resection after yttrium-90 radioembolization was 2.0-12.5 months in various studies. In all of the studies where resection was performed 8 or more months after yttrium-90 radioembolization, the 30-day mortality rate was 0%. A meta-analysis of grade 3 morbidity or higher overall revealed a rate of 26% (95% CI 16-37%). A meta-analysis yielded a pooled conversion rate of 11% (95% CI 5-17%). An interval of 8 months from Y-90 radioembolization to surgery may reduce mortality.

10. Indications for LT

Since Starzl (*66*) performed the first LT in 1963, transplantation has mainly been for patients with end-stage liver failure. However, since Mazzaferro *et al*. (*67*) proposed the Milan criteria in 1996, LT has been performed as a curative treatment for malignant tumors. They reported that patients with a solitary HCC with a diameter ≤ 5 cm or those with ≤ 3 tumors with a diameter ≤ 3 cm had a 4-year survival rate of 85% and RFS of 92% (n = 35). Currently, efforts are being made to further expand the indications for LT. Tumor characteristics, including serum alpha-fetoprotein, the presence of microvascular invasion, tumor grade or differentiation, and largest tumor size, are among the most important predictors of recurrence after LT (*68*). Bridging therapy to downstage the tumor before LT is also proposed. A study in 2020 found that atezolizumab plus bevacizumab resulted in a better progression-free survival than sorafenib (*69*). Immune checkpoint inhibitors may increase the risk of rejection, and debate has arisen regarding their impact on the perioperative period in LT and optimal immunosuppressant protocols.

11. COVID-19 pandemic

The coronavirus disease 2019 (COVID-19) pandemic has become a global health emergency that has also caused profound changes in the treatment of cancer. Liver cancer is no exception and requires prioritization since it is not a condition for which treatment can be postponed. However, the more invasive the procedure, the more likely it is to require postoperative intensive care units, ventilators, and blood transfusions, which may be affected by COVID-19 protocols. A general agreement has been made to delay non-urgent treatment for localized HCC by 8-12 weeks if oncological outcomes are unlikely to be affected. The tumor doubling time for patients with large tumors with alpha-fetoprotein of less than 20 ng/mL and non-viral cirrhosis is approximately 33 weeks. For incidental liver lesions <1 cm, imaging studies and liver biopsy can be delayed. If surgery cannot be delayed, other local treatments should be considered. For HCCs with a diameter < 3 cm and < 3 tumors, ablation can produce results comparable to surgery (*39*). For larger tumors, TACE may be considered as a bridging treatment until resection. Data from two international reporting registries indicated a high mortality rate of 39.8% in patients positive for COVID-19 with chronic liver disease. In symptomatic patients positive for COVID-19, treatment of COVID-19 should be a priority. In asymptomatic patients who are COVID-19-positive, surgery can be postponed reasonably until the patient is negative. The major guidelines are in favor of a temporary suspension of elective living donor LT due to lower priority for patients near the lower end of the Milan criteria, patients with compensated cirrhotic HCC, and patients who respond well to bridging therapy. The use of immunosuppressants after LT should follow the usual protocol. The impact of COVID-19 on posttransplant patients is unknown. With limited human and financial resources, a stratified risk model should be used for triage and prioritization (*70*).

12. Conclusion

This study has outlined the advances in surgical treatments for liver cancer. Over the last 30 years, the safety of hepatectomy has improved, and efforts have been made to further reduce the amount of bleeding. For HCC, anatomical resection along Couinaud's subsegmental boundary may increase oncological curability depending on the tumor's characteristics. PVE, two-stage hepatectomy, and ALPPS have been proposed for instances of a small future remnant liver. With advances in surgery, perioperative management, other local treatments, and systemic therapy, indications for LR and LT are expanding. However, appropriate patient selection is important to achieving long-term outcomes. Nevertheless, surgical equipment has made marked advances. Laparoscopic and robot-assisted hepatectomy have also become popular options due to their minimal invasiveness. Preoperative simulation and intraoperative navigation may help to reduce the experience gap between skilled and new surgeons and practitioners. the importance of a multidisciplinary approach tailored to each patient has only increased during the COVID-19 pandemic. Thus, liver surgeons should work as part of a multidisciplinary team.

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Review

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Treatment of biliary tract carcinoma over the last 30 years

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SUMMARY Surgical resection could offer the only chance of a long-term cure for biliary tract carcinoma. However, only a small percentage of these patients can undergo surgery based on the progression of the disease. Most patients with biliary tract carcinoma receive palliative chemotherapy. Until 2010, patients with unresectable biliary tract carcinoma received fluorouracil (5-FU), gemcitabine (GEM), and cisplatin (CDDP)-based chemotherapies. The ABC-02 study established GEM with CDDP as the first-line therapy for patients with unresectable biliary tract carcinoma, and phase III studies indicated that several combinations of anti-cancer drugs such as GEM with S-1 benefited patients. In contrast, clinical studies on targeted therapy dosages for biliary tract carcinoma in the 2010s failed to corroborate the advantages of administering cancer treatment with or without other anticancer drugs. Due to the easy access to cancer panels, precision medicines (such as ivosidenib for *IDH1* mutations, pemigatinib for *FGFR2* fusions, and entrectinib and larotrectinib for *NTRK* fusions) were recently found to be effective in the treatment of patients with these genetic alterations. Moreover, many clinical studies on immune checkpoint inhibitors for advanced biliary tract carcinoma are currently underway and could provide more effective treatment options in the near future.

Keywords biliary tract carcinoma, chemotherapy, anti-cancer drug, targeted therapy, precision medicine, immune checkpoint inhibitor

1. Introduction

Biliary tract carcinoma refers to a group of malignancies of the biliary epithelium. Based on anatomical origin, biliary tract carcinoma is classified into the following categories: intrahepatic cholangiocarcinoma, perihilar cholangiocarcinoma, distal cholangiocarcinoma, gallbladder carcinoma, and ampullary cancer (*1*). Pathologically, most of these tumors are adenocarcinoma (*2*). Surgical resection with negative margins and porta hepatis lymphadenectomy is the standard of care and offers the only chance of a long-term cure (*3*).

However, only a few patients with biliary tract carcinoma are eligible for curative surgery because of metastasis to distant sites and lymph nodes and direct invasion of the major vessels (*4*). Moreover, even patients who undergo curative resection have poor outcomes due to the high rate of tumor recurrence (*1*). Therefore, the development of non-surgical treatment options is a pressing issue for patients with biliary tract carcinoma.

Chemotherapy is performed using a drug or a combination of drugs and is a palliative treatment option for patients with advanced disease. Anti-cancer drugs such as fluorouracil (5-FU), gemcitabine (GEM), and cisplatin (CDDP) are cytotoxic; they kill tumor cells by inhibiting the division of rapidly growing cells, yet they simultaneously affect normal cells that have fast proliferation rates. However, targeted therapies are cytostatic and use monoclonal antibodies or small molecule inhibitors that act on specific molecular targets that are associated with cancer to induce the death of tumor cells via apoptosis and stimulation of the immune system. When used in combination with anticancer drugs, targeted therapies deliver anticancer drugs to cancer cells, consequently minimizing undesirable adverse reactions (*5,6*).

The current review has focused on the 30-year history of chemotherapy for advanced biliary tract carcinoma, including anticancer drugs, targeted therapies, precision medicine, and immunotherapies. Here, a systemic review of the literature was conducted to estimate the level of evidence supporting the use of a chemotherapy regimen for patients with advanced biliary tract carcinoma.

2. First-line chemotherapy

Patients with advanced biliary tract carcinoma receive

Table 1. Chemotherapy for biliary tract carcinoma (phase III and randomized comparative phase II trials) (> 80 patients)

Author	Trial	Patient No.	Year	Regimen	Primary end point	Remarks	*Ref.*
First-line chemotherapy							
Glimelius		90	1996	5-FU with/without etoposide *vs*. BSC	NA	including pancreatic cancer	(*10*)
Valle	ABC-02	410	2010	GEM/CDDP *vs*. GEM	OS		(*7*)
Okusaka		84	2010	GEM/CDDP *vs*. GEM	1-year OS		(*24*)
Sharma		82	2010	GEMOX *vs*. BSC *vs*. FUFA	OS	Gallbladder carcinoma	(*28*)
Kim		224	2019	XELOX *vs*. GEMOX	6-mo PFS	Not inferior	(*30*)
Morizane	JCOG1113	354	2019	GEM/CDDP *vs*. GEM/S-1	OS	Not inferior	(*31*)
Second-line chemotherapy							
Lamarca	ABC-06	162	2021	FOLFOX *vs*. BSC	OS		(*36*)
Adjuvant chemotherapy							
Primrose	BILCAP	447	2019	Capecitabine *vs*. Observation	OS		(*41*)

GEM, gemcitabine; CDDP, cisplatin; OS, overall survival; BSC; best supportive care; FUFA, 5-FU plus folinic acid; XELOX, capecitabine plus oxaliplatin; GEMOX, gemcitabine plus oxaliplatin; FOLFOX, 5-FU plus oxaliplatin; PFS, progression-free survival.

chemotherapy as the main treatment when surgical resection is not an option. However, randomized control trials involving large cohorts were not conducted until 2010, when the ABC-02 study proved that combination chemotherapy using GEM and CDDP was associated with longer patient survival (*7*). It remains one of the options for first-line treatment of unresectable biliary tract carcinoma (Table 1).

2.1. Fluorouracil-based chemotherapy

In the late 1980s and 1990s, 5-FU-based chemotherapy yielded modest results in patients with unresectable biliary tract carcinoma (*8-11*). In a prospective randomized Eastern Cooperative Oncology Group (ECOG) study, 53 patients with advanced gallbladder cancer and 34 with advanced bile duct cancer were treated with oral 5-FU-based chemotherapy (oral 5-FU alone or oral 5-FU with streptozotocin or oral 5-FU with methyl-CCNU), and about 10% of patients had an objective response (*9*). In the late 1990s, a small-scale randomized study indicated that chemotherapy (5-FU with/without etoposide) was effective for patients with unresectable biliary tract or pancreatic cancer compared to best supportive care (median overall survival [OS] time, 6.0 months *vs*. 2.5 months) (*10*). The overall response rate to 5-FU modulated with leucovorin was 32%, indicating that the regimen could lead to prolonged patient survival (*8*). A phase II trial indicated that a regimen of 5-FU, doxorubicin, and mitomycin C was also effective, and a partial response was achieved in 31% of patients with advanced or recurrent biliary tract carcinoma (*12*). Besides 5-FU, single agents, such as CDDP and mitomycin C, do not have significant antitumor activity against biliary tract carcinoma (*13,14*).

2.2. Gemcitabine alone

GEM is a nucleotide analog with biological activity against a broad spectrum of solid tumors such as pancreatic, breast, and lung cancers (*15*). It has remarkable efficacy against advanced biliary tract carcinoma and is now considered to be a key drug to treat these neoplasms (*16*). Several phase II studies with GEM alone (a dosing regimen of 1,000-2,200 mg/m^2, GEM administered over 30 min weekly for two or three weeks with a week of rest) were reported in the early 2000s (*17-20*). These trials had a response rate ranging from 12 to 36% within an acceptable level of toxicities and median OS of 7.2 to 11.5 months.

2.3. Gemcitabine in combination with platinum compounds

Later, phase II trials using GEM in combination with other agents were reported. In the early 2000s, the median OS of patients with advanced biliary tract carcinoma receiving GEM with a 5-FU infusion along with intravenous infusion of leucovorin ranged from 4.7 to 9.7 months (*21,22*). In the late 2000s, many phase II studies that included > 30 patients by arm assessed a combined regimen of GEM and CDDP (GEM/CDDP) (*23*). The administered dosage was 1,000 or 1,250 mg/m^2 and 20−80 mg/m^2, respectively. In a meta-analysis of 16 studies using the GEM and CDDP combination, the median OS was 9.8 months (range: 5.0−15.2 months).

In 2010, the multicentric phase III ABC-02 study established GEM (1,000 mg mg/m^2) with CDDP (25 mg mg/m^2) as the standard of first-line therapy for patients with unresectable biliary tract carcinoma, and it continues to be standard first-line chemotherapy (*7*). GEM with CDDP resulted in a significant survival advantage as chemotherapy for advanced biliary tract carcinoma; patients who were treated with GEM/CDDP lived longer than those treated with GEM alone in terms of OS (median: 11.7 *vs*. 8.1 months, $P < 0.001$) and progression-free survival (PFS) (8.0 *vs*. 5.0 months, $P < 0.001$). The effectiveness of this regimen was reproducibly demonstrated in a randomized phase

II study in Japan (median OS: 11.2 months *vs*. 7.7 months) (*24*).

In the late 2010s, GEM plus nab-paclitaxel became a standard treatment regimen for advanced biliary tract carcinoma (*25,26*). The median OS and PFS of 74 patients who received intravenous nab-P and GEM were 12.4 and 7.7 months, respectively (*26*). Moreover, a better PFS (median, 11.8 months) and OS (19.2 months) were indicated in a phase II study using nab-paclitaxel in addition to GEM/CDDP for 62 patients with advanced biliary tract carcinoma (*27*).

Oxaliplatin is a third-generation platinum compound that causes much less nausea, vomiting, and renal toxicity, but it has a high rate of peripheral neuropathy compared to high-dose CDDP. Besides the GEM/CDDP regimen, phase II studies using GEM with oxaliplatin (GEMOX) for advanced biliary tract carcinoma were reported in the late 2000s (*23*). A meta-analysis of data of the 14 GEMOX group indicated that median OS was 10 months (range: 8.8−11 months), suggesting that GEMOX could be considered as a standard equivalent to GEM/CDDP. A study by Sharma *et al*. was the only phase III study to find that GEMOX helped to prolong OS in patients with advanced gallbladder carcinoma compared to those receiving best supportive care (median OS: 9.5 *vs*. 4.5 months, $P = 0.039$) in 2010 (*28*).

Capecitabine is an oral fluoropyrimidine prodrug that exhibits preferential conversion to 5-FU in tumor tissue. Capecitabine plus oxaliplatin (XELOX) has also displayed modest activity against biliary tract carcinoma (*29,30*). In a 2019 phase III study, the median OS was 10.4 months for the GEMOX group and 10.6 months for the XELOX group ($P = 0.131$), and the median PFS was 5.3 months and 5.8 months ($P = 0.171$), respectively (*30*). Grade 3 to 4 adverse events did not differ significantly between the two groups. However, the XELOX group had a significantly lower frequency of hospital visits due to the oral administration of capecitabine. The aforementioned randomized trial indicated that XELOX was not significantly inferior to GEMOX in terms of the 6-month PFS rate.

2.4. GEM in combination with S-1

S-1 is an oral dihydropyrimidine dehydrogenase inhibitory fluoropyrimidine based on biochemical modulation of 5-FU, and it results in a high 5-FU concentration in the blood for a long duration. In 2019, the JCOG1113 study indicated that GEM plus S-1 (GEM/S-1) was not inferior in treating advanced biliary tract carcinoma, and it had an acceptable toxicity profile compared to GEM/CDDP in a phase III study (median OS, 15.1 months *vs*. 13.4 months; median PFS, 6.8 months *vs*. 5.8 months) (*31*). That study was the first to provide positive results for advanced biliary tract carcinoma since the ABC-02 study. Unlike GEM/CDDP, the GEM/S-1 regimen does not require hydration; therefore, it became a convenient standard option for patients with advanced biliary tract carcinoma. Moreover, the TG 1308 study, a phase II trial using a modified GEM/S-1 regimen, noted a moderate efficacy (median OS, 12.7 months, and median PFS, 5.4 months) with a favorable safety profile in patients with advanced biliary tract carcinoma in 2020 (*32*).

3. Second-line chemotherapy

Available evidence from the phase III ABC-02 and JCOG1113 studies indicated that GEM/CDDP and GEM/S-1 are the standard first-line chemotherapy regimens for advanced biliary tract carcinoma (*7,31*). After standard first-line chemotherapies, however, there is little available evidence to propose second-line chemotherapy for the disease.

In the mid-2010s, multicentric retrospective studies using various types of regimens indicated that the OS and RFS of patients receiving second-line chemotherapy after first-line chemotherapy with GEM and platinum (GEM/CDDP or GEMOX) were 6.5−6.7 months and 1.9−3.2 months, respectively (*33-35*). The heterogeneous patient populations, small sample sizes, and lack of phase III trials were responsible for the absence of a standard second-line chemotherapy beyond the failure of GEM/CDDP treatment at this point.

In 2021, the ABC-06 phase III study indicated that 5-FU plus oxaliplatin (FOLFOX) chemotherapy could improve OS for patients with advanced biliary tract carcinoma after progression to first-line GEM/CDDP (*36*). A total of 162 patients were enrolled in that study, and the survival of patients receiving second-line FOLFOX chemotherapy (every 2 weeks for a maximum of 12 cycles) was significantly longer than that of the best supportive care group (median OS, 6.2 months *vs*. 5.3 months, $P = 0.031$), with a clinically meaningful increase in PFS (median, 4.0 months) and objective response (4.9%). That said, a higher rate of grade 3−5 adverse events was reported in the FOLFOX group (69.1% *vs*. 51.8%).

Phase II studies have evaluated the efficacy and safety of modified 5-FU plus oxaliplatin and irinotecan (FOLFIRINOX) as a second-line treatment for patients who failed to respond to GEM-based treatment for advanced biliary tract carcinoma. These studies indicated that the objective response rate was 10−26% with no complete response and that the median OS and PFS were 6.2−13.2 months and 2.8−6.7 months, respectively (*37-39*). FOLFIRINOX could be considered as an option for salvage treatment in these patients if long-term administration of modified FOLFIRINOX with toxicity management is possible.

Besides anticancer drugs, targeted therapies and precision medicine have been examined as a second-line treatment for patients with advanced biliary tract carcinoma (described below).

Table 2. Targeted therapy for biliary tract carcinoma (> 100 patients)

Author	Trial	Patient No.	Year	Regimen	Primary end point	Remarks	*Ref.*
Phase III trial							
Lee		268	2012	GEMOX/erlotinib *vs*. GEMOX	PFS		(*54*)
Abou-Alfa	ClarlDHy	185	2020	ivosidenib *vs*. placebo	PFS	IDH1 mutation	(*59*)
Phase II trial							
Bibeau	FIGHT-202	107	2022	pemigatinib (single arm)	NA	FGFR2 fusions	(*62*)
Tumor-agnostic therapy							
Hong		154	2020	larotrectinib (single arm)	NA	NTRK fusions	(*71*)
Demetri		121	2022	entrectinib (single arm)	NA	NTRK fusions	(*72*)

PFS, progression-free survival; GEMOX, gemcitabine plus oxaliplatin.

4. Adjuvant chemotherapy

Surgical resection is the only curative treatment for patients with biliary tract carcinoma, but these patients experience tumor recurrence at a high rate even after complete resection (*1*). Therefore, the efficacy of adjuvant therapy for biliary tract carcinoma should be verified (*40*).

Three phase III trials on adjuvant chemotherapy were conducted in the late 2010s. The phase III BILCAP study in 2019 compared oral capecitabine with observation as an adjuvant therapy in patients with biliary tract carcinoma after curative resection, and it provided evidence that capecitabine could improve the OS of these patients. Although the OS primary endpoint analyzed in the intention-to-treat analysis did not reach statistical significance (median OS: 51.1 months *vs*. 36.4 months; $P = 0.097$), the adjusted median OS was 53 months in the capecitabine group and 36 months in the observation group according to the per-protocol analysis ($P = 0.028$). Recurrence-free survival (RFS) of patients in the capecitabine group was also significantly longer than that of patients in the observation group (median RFS: 24.4 months *vs*. 17.5 months; $P = 0.033$) (*41*).

Alternatively, adjuvant GEMOX provided no benefit to patients undergoing curative resection for biliary tract carcinoma. In a phase III trial reported in 2019, both OS (median, 75.8 months *vs*. 50.8 months; $P = 0.74$) and RFS (30.4 months *vs*. 18.5 months; $P = 0.48$) did not differ significantly between the GEMOX group and the surveillance group (*42*).

Given that GEM/CDDP has been the standard first-line treatment for patients with unresectable biliary tract carcinoma as indicated in the ABC-02 trial (*7*), GEM/CDDP should be effective as adjuvant chemotherapy as well. A non-randomized small cohort phase II study indicated the promising survival of patients undergoing curative resection for biliary tract carcinoma (*43*). Moreover, a multicenter, open-label, randomized phase III trial on the efficacy of adjuvant GEM/CDDP is underway (*44*).

5. Targeted therapy

Targeted therapy is a type of personalized medical therapy that is designed to block specific molecules involved in the growth and spread of cancer cells. Interfering with a specific biochemical pathway kills cancer cells or keeps them from developing, growing, and spreading. Targeted therapy may cause less harm to normal cells and may cause fewer adverse reactions than other types of cancer treatment (Table 2).

5.1. Phase II trials using targeted therapy for biliary tract carcinoma

Phase II trials using targeted therapy for biliary tract carcinoma were reported from the late 2000s to the 2010s, but most of them failed to demonstrate the benefit of targeted therapies in cancer treatment with or without other anticancer drugs.

Lapatinib is an inhibitor of epidermal growth factor receptors (EGFRs) 1 and 2 and was administered to 17 patients with advanced biliary tract carcinoma (*45*). However, the response rate was 0%, indicating that treatment with lapatinib was not effective against biliary tract carcinoma. The addition of other molecularly targeted therapies to anticancer drugs did not enhance the activity of chemotherapy in patients with advanced biliary tract carcinoma. The phase II, randomized NCT00552149 study indicated that OS was 11.0 months in the GEMOX plus cetuximab group (cetuximab is an EGFR antagonist) and 12.4 months in the GEMOX alone group, and PFS was 6.1 months and 4.0 months, respectively (*46*). Sorafenib is a multi-kinase inhibitor drug and is the first drug that has demonstrated effectiveness against advanced hepatocellular carcinoma (*47*). First-line GEM plus sorafenib was evaluated in a double-blind phase II study (NCT00661830), but the addition of sorafenib to GEM did not result in improved efficacy in patients with advanced biliary tract carcinoma (median OS: 8.4 months [GEM plus sorafenib] *vs*. 11.2 months [GEM alone]; median PFS: 3.0 months *vs*. 4.9 months) (*48*). In a single-arm phase II study in 2018, the addition of a humanized anti-vascular endothelial growth factor (VEGF) monoclonal antibody, bevacizumab, to GEM/capecitabine did not improve outcomes for patients with advanced biliary tract carcinoma compared to

historical controls (response rate: 24%; median OS: 10.2 months; median PFS: 8.1 months) (*49*).

5.2. Erlotinib for biliary tract carcinoma

Erlotinib is an oral EGFR tyrosine-kinase inhibitor, and its most common and severe toxicity is a skin rash. The drug was approved for patients with various types of cancer such as pancreatic (*50*) and colorectal cancers (*51*), and in the late 2000s, phase II trials using erlotinib alone (*52*) or in combination with bevacizumab (*53*) indicated that the median OS and PFS in patients with advanced biliary tract carcinoma were 7.5−9.9 months and 2.6−4.4 months, respectively.

In the NCT01149122 phase III study in 2012, patients with advanced biliary tract carcinoma were assigned to receive either GEMOX or GEMOX plus erlotinib (*54*). This study noted no significant difference in either RFS (median: 4.2 months *vs*. 5.8 months, $P = 0.087$) or OS (9.5 months *vs*. 9.5 months, $P = 0.611$) between the GEMOX alone and GEMOX plus erlotinib groups. However, a subgroup analysis based on primary origin indicated the additional effect of erlotinib on PFS in patients with advanced cholangiocarcinoma (median: 3.0 months *vs*. 5.9 months, $P = 0.049$).

6. Precision medicine

With recent advances in biological technologies, high-throughput genome sequencing has been used to elucidate the genetic basis of many types of cancer. To date, next-generation sequencing (NGS) technologies have identified molecular targets, and genome-based drugs have been used clinically (*55,56*).

The US Food and Drug Administration (FDA) approved ivosidenib (for patients with *IDH1* mutation) and pemigatinib (for patients with *FGFR2* fusions/rearrangements or alterations) for patients with biliary tract carcinoma as a second-line chemotherapy. Both of these were well-tolerated and resulted in a favorable OS benefit.

6.1. Ivosidenib for biliary tract carcinoma with *IDH1* mutation

The Cancer Genome Atlas (TCGA) study that analyzed 38 intrahepatic cholangiocarcinoma samples found the *IDH1* mutation in seven samples (18.4%) of intrahepatic cholangiocarcinoma (*57*). Ivosidenib is a small molecule inhibitor of mutated *IDH1* that decreases the abnormal production of oncometabolite 2-hydroxyglutarate and that contributes to the differentiation of malignant cells (*58*).

The phase III randomized clinical ClarlDHy trial involved 187 patients with biliary tract carcinoma harboring the *IDH1* mutation who had disease progression after prior treatments (*59,60*). These patients were randomly assigned (2:1) to receive ivosidenib or a matched placebo. The PFS of the ivosidenib group (median, 2.7 months) was significantly longer than that of the placebo group (1.4 months, $P < 0.001$) (*59*). However, OS did not differ significantly between the two groups (median: 10.3 months *vs*. 7.5 months; $P = 0.09$). When adjusted for crossover, however, the median OS of the placebo group (5.1 months) was significantly shorter than that of the ivosidenib group ($P < 0.001$).

6.2. Pemigatinib to treat biliary tract carcinoma with *FGFR2* aberrations

In the TCGA study, RNA-seq data revealed that expressed *FGFR2* fusion/rearrangements were involved in the pathogenesis of cholangiocarcinoma. Pemigatinib is an oral *FGFR1*, *2*, *3* inhibitor that was first approved as a targeted treatment for biliary tract carcinoma by the US FDA in 2020.

The FIGHT-202 study – a multicenter, open-label, phase II study – included patients who had received first- or second-line systemic therapy for advanced biliary tract carcinoma. This study indicated that an objective response was achieved in 38 (35.5%) of 107 patients with *FGFR2* fusions/rearrangements treated with pemigatinib; a complete response was achieved in 3 (2.8%), a partial response was achieved in 35 (32.7), and 50 (46.7%) had stable disease (*61*). A follow-on study involved the same cohort was published two years later and it indicated that the median PFS was 7.0 months for patients with *FGFR2* fusions/rearrangements ($n = 65$) who received second-line pemigatinib during the trial (*62*). The phase III FIGHT-302 study comparing the efficacy of first-line pemigatinib *vs*. GEM/CDDP in patients with biliary tract carcinoma with *FGFR2* fusions/rearrangements is ongoing (*63*).

7. Tumor-agnostic treatment

Due to the direct detection of gene fusion using the NGS approach, *NTRK* fusion assessment has recently become a standard part of management for patients with diverse types of advanced cancers (*64*), although the frequency of *NTRK* fusions in biliary tract carcinoma is estimated to be 0.25−3.6% (*65,66*). Gene fusions involving *NTRK1*, *NTRK2*, or *NTRK3* were found in a broad range of pediatric and adult malignancies (*67,68*), leading to the expression of chimeric rearrangements in tropomyosin receptor kinases (*TRKs*). Entrectinib and larotrectinib are inhibitors of *TRKA*, *B*, and *C*, and have been shown to have prominent anti-tumor activity against oncogenic *NTRK* gene fusion-positive solid tumors including biliary tract carcinoma (*69,70*).

In 2020, a pooled study of larotrectinib for *TRK* fusion-positive advanced solid tumors (NAVIGATE), including biliary tract carcinoma, indicated that an objective response was achieved in 121 (79.0%) of 153

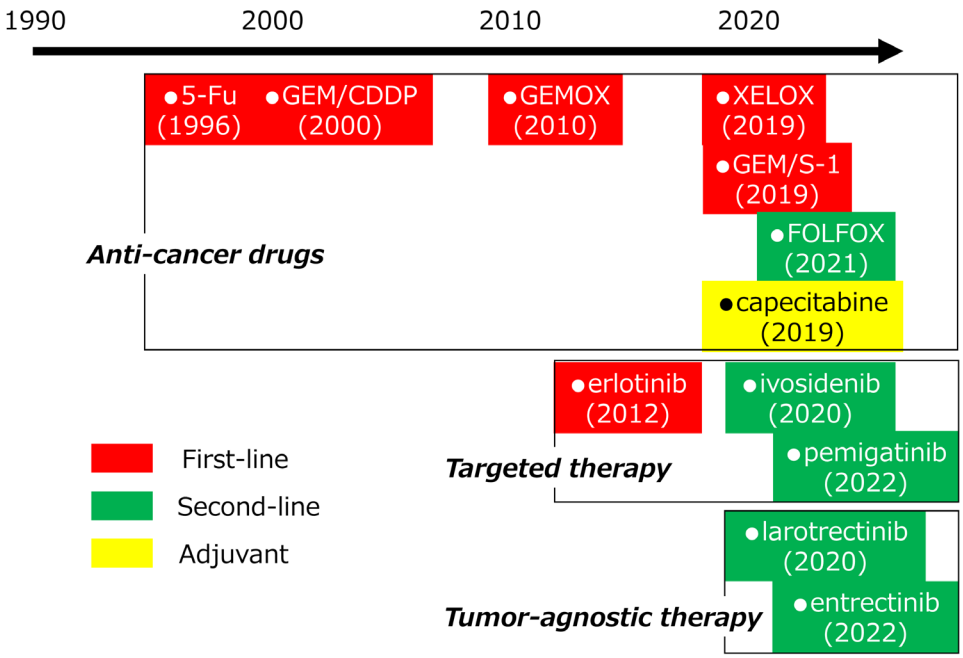

Figure 1. The history of treatment for biliary tract carcinoma.

patients while a complete response was achieved in 24 (15.6%) (*71*). Moreover, integrated analysis using the datasets of three ongoing clinical trials of entrectinib (ALKA-372-001 [phase I], STARTRK-1 [phase I], and STARTRK-2 [phase II]) was performed in 2022. This pre-specified analysis of 121 adult patients with advanced *NTRK* fusion-positive solid tumors included 1 patient with biliary tract carcinoma. An objective response was achieved in 74 patients (61.2%), including a complete response in 19 (15.7%), a partial response in 55 (45.5%), and stable disease in 13 (10.7%). At the data cut-off, OS and PFS were 33.8 months and 13.8 months, respectively (*72*).

8. Immunotherapy

Checkpoint inhibitors are monoclonal antibodies targeting the cytotoxic T lymphocyte antigen 4 or PD-1/PD-L1 immune checkpoint pathways, which block a signaling pathway that prevents the activation of T cells from attacking the cancer and enable tumor-reactive T-cells to mount an anticancer immune response (*73*). In 2017, the US FDA approved the anti-PD-1 agent pembrolizumab for the treatment of any type of cancer with microsatellite instability-high (MSI-H) (*74*). However, no studies have indicated the efficacy of immune checkpoint inhibitors for advanced biliary tract carcinoma thus far (*75,76*).

Immunotherapy for biliary tract carcinoma has now been explored and is currently being evaluated in several clinical trials to provide novel and more effective treatment options. A randomized phase II IMbrave 151 study (atezolizumab + GEM/CDDP in combination with or without bevacizumab) is now underway, and that regimen is expected to be effective as a first-line treatment for advanced biliary tract carcinoma (*77*).

9. Future perspectives

After the establishment of a first-line treatment using GEM/CDDP or GEM/S-1 regimen for advanced biliary tract carcinoma, the next era will witness the identification of biomarkers that determine subtypes of patients who are amenable to precision medicine (Figure 1). Due to the easy access to cancer panels, the presence of driver mutations, such as *IDH1*, and fusion events, such as the *FGFR2* and *TRK* genes in biliary tract carcinoma, and MSI-H in all types of solid tumors can easily be determined. Hence, the personalized treatment options for patients with advanced biliary tract carcinoma are steadily increasing. However, such precision medicine is still limited to only a minority of patients receiving treatment for biliary tract carcinoma. However, clinical trials of immune checkpoint inhibitors in combination with or without other anticancer drugs are currently underway, and immunotherapy options for biliary tract carcinoma are a current topic of debate. Data from these clinical trials should lead to more effective treatment options for this immunologically "cold" malignancy.

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Review

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Trends in the surgical treatment for pancreatic cancer in the last 30 years

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SUMMARY Pancreatic cancer has the poorest prognosis among digestive cancers. During the 1990s, the 5-year survival rate of surgical patients with pancreatic cancer was 14% in Japan. However, survival rates have increased to 40% in the 2020s due to the refinement of surgical procedures and the introduction of perioperative chemotherapy. Several pivotal randomized controlled trials have played an indispensable role to establish each standard treatment strategy. Resectability of pancreatic cancer can be classified into resectable, borderline resectable, and unresectable based on the anatomic configuration, and multidisciplinary treatment strategies for each classification have been revised rapidly. Investigation of superior perioperative adjuvant treatments for resectable and borderline resectable pancreatic cancer and the establishment of optimal conversion surgery for unresectable pancreatic cancer are the progressive subjects.

Keywords pancreatic cancer, multidisciplinary treatment, resectability, perioperative adjuvant therapy, conversion surgery

1. Introduction

Pancreatic cancer (PC) is known to have the poorest prognosis among all digestive cancers. Although surgical resection is the only feasible treatment to cure this disease, only 15-20% of PC cases are resectable at the time of the first diagnosis, while 30-40% are locally advanced cases and 50-60% are distant metastatic cases (*1*). The latter two cohorts are initially unresectable.

In the 1990s, a Japanese nationwide survey showed that the overall 5-year survival rate in patients undergoing radical resection for PC was 14% (*2*). Nowadays, the 5-year survival rate of resectable PC has increased to 40% (*3*) owing to the gradual refinement of surgical procedures and the subsequent introduction of perioperative chemotherapy. In this chapter, we review the pivotal surgical approaches that have contributed to the advancement of multidisciplinary treatment for PC.

2. Limitations of extended resection for PC

During the 1990s, there was no effective chemotherapy for PC in Japan. Hence, radical pancreatectomy combined with extended lymphadenectomy, including the paraaortic lymph nodes and nerve plexus dissection around major peripancreatic arteries, were performed for PC to eradicate cancer cells completely and to improve patient survival (*4-8*). This concept was originally advocated by Fortner who had originally started radical resection for PC in the 1970s (*9,10*). However, the short- and long-term survival rates of patients with PC were far from satisfactory, fomenting controversy regarding the advantages and disadvantages of radical pancreatectomy combined with extensive nodal and/or nerve dissection and controversy because aggressive dissection was associated with increased morbidities.

To resolve the above clinical question, randomized clinical trials (RCTs) were then performed to reveal the prognostic superiority of extended radical pancreatectomy against standard pancreatectomy for PC. A total of five RCTs on the extent of dissection during pancreatectomy were conducted between 1991 and 2009 (Table 1) (*11-15*). Results showed no significant difference in the overall survival (OS) between the extended and standard lymphadenectomy groups in the five RCTs, *i.e.*, none of the RCTs revealed any prognostic advantage of extended lymphadenectomy against standard lymphadenectomy during pancreatectomy for PC. With respect to surgical complications, no significant differences were found in the incidence of surgical morbidity and mortality between the two groups, except for the series performed in Johns Hopkins Hospital, in

Table 1. The results of 5 RCTs comparing standard and extended pancreatectomy

Author	Year	Procedure of extended resection	Number Extended *vs.* Standard	Median OS (months)	Morbidity and Mortality
Pedrazzoli *et al*. (*11*)	1991-1994	Lymphadenectomy	41 *vs*. 40	500 days *vs*. 355 days NS	Morbidity: NS Mortality: 4.8% *vs*. 5%, NS
Yeo *et al*. (*12*)	1996-2001	Lymphadenectomy Distal gastrectomy	148 *vs*. 146	20 *vs*. 21 NS	Morbidity: 49% *vs*. 29%, $p = 0.01$ Mortality: 2% *vs*. 4%, $p = 0.30$
Farnell *et al*. (*13*)	1997-2003	Lymphadenectomy	39 *vs*. 40	19 *vs*. 26, $p = 0.32$	Morbidity: NS Mortality: 3% *vs*. 0%, NS
Nimura *et al*. (*14*)	2000-2003	Lymphadenectomy	50 *vs*. 51	13.8 *vs*. 19.9 $p = 0.119$	Morbidity: 22% *vs*. 20%, NS Mortality: 2% *vs*. 0%, NS
Jang *et al*. (*15*)	2006-2009	Lymphadenectomy Nerve plexus, Ganglion	86 *vs*. 83	18.0 *vs*. 19.0 $p = 0.401$	Morbidity: 43% *vs*. 32.5%, $p = 0.16$ Mortality: 2.3% *vs*. 0%, NS

NS: not significant.

which the morbidity rate was higher in the extended compared to the standard group (49% *vs*. 29%, $p = 0.01$) (*11*). These results suggested no oncological advantage for extended lymphadenectomy in pancreatectomy for PC, and the researchers' concern gradually shifted from radical surgical resection to employing a multidisciplinary treatment for PC.

3. Development of multidisciplinary treatment for PC

3.1.Adjuvant chemotherapy for PC following resection

With regard to adjuvant chemotherapy for PC following resection, several RCTs comparing adjuvant 5-fluorouracil (5-FU) based chemotherapy with surgery alone were conducted in the 1990s. In a trial of adjuvant 5-FU plus mitomycin treatment *vs*. surgery alone, the 5-year survival rate was 11.5% in the adjuvant 5-FU plus mitomycin group and 18.0% in the surgery alone group, showing no significant difference (*16*). Similarly, another trial of adjuvant 5-FU plus cisplatin *vs*. surgery alone revealed that the 5-year survival rate was 11.5% in the adjuvant 5-FU plus cisplatin group and 18.0% in the surgery alone group, also showing no significant difference (*17*).

In 1997, the prognostic superiority of gemcitabine (GEM) treatment over 5-FU for unresectable (UR) PC was reported (*18*). This result was followed by clinical trials administering GEM as an adjuvant setting for resectable PC (*19*). In 2007, a trial of adjuvant GEM *vs*. surgery alone (CONKO-001) conducted in Germany showed a significant increase in the recurrence-free survival in the adjuvant GEM group (median, 13.4 months *vs*. 6.7 months, $p < 0.001$) and a significant increase in OS in the adjuvant GEM group during follow-up (22.8 months *vs*. 20.2 months, $p = 0.01$) (*20*). In a Japanese trial of GEM *vs*. surgery alone (JSAP-02 trial), no significant difference was found in the OS between the two groups (median, 22.3 months *vs*. 18.4 months, $p = 0.19$), but the disease-free survival (DFS) was significantly longer in the GEM group (median, 11.4 months *vs*. 5.0 months, $p = 0.01$) (*21*). Since the announcement of these positive results, adjuvant GEM therapy has become the standard therapy for resectable PC in Japan at the beginning of the 2010s.

Meanwhile, several RCTs of adjuvant chemotherapies were conducted in comparison with adjuvant GEM therapy for resectable PC (Table 2) (*3,22-25*). In the ESPAC-4 trial, the OS in the adjuvant GEM + Capecitabine (Cape) group was significantly improved compared with the adjuvant GEM group (28.0 months *vs*. 25.5 months, $p = 0.032$) (*23*). Based on these findings, the ASCO, NCCN, and ESMO guidelines started to recommend GEM + Cape as the standard adjuvant therapy for resectable PC. In Japan, the JASPAC-01 trial revealed that the OS in adjuvant S-1 groups was significantly improved compared to adjuvant GEM group (25.5 months *vs*. 46.5 months, $p < 0.0001$) (*3*). As a result, the Japanese guidelines recommended S-1 as the standard adjuvant therapy for resectable PC (*26*).

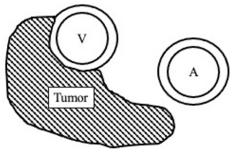
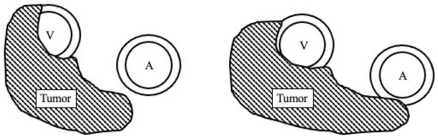
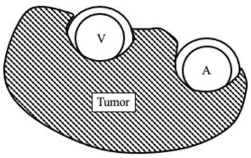
Since 2011, the modified FOLFIRINOX (mFFX) therapy has become one of the leading regimens for UR PC with distant metastasis (*27*). This regimen has also been utilized in adjuvant therapy for resectable PC. The PRODIGE24-ACCORD24 and CCTG PA6 trials revealed that the DFS (21.6 months *vs*. 12.8 months, $p < 0.0001$) and OS (54.4 months *vs*. 35.0 months, $p = 0.003$) were significantly prolonged in the mFFX group compared to the GEM group. As a result, the NCCN and ESMO guidelines recommended adjuvant mFFX for resectable PC (*24*). GEM + nab-paclitaxel therapy (GnP) has been another leading regimen for unresectable PC since 2013 (*28*). A trial of adjuvant GEM *vs*. GnP was conducted in the United States, whose results were reported at ASCO 2019 annual meeting (*25*). In an interim analysis, the OS was significantly improved in the GnP group compared to the GEM group (40.5 months *vs*. 36.2 months, $p = 0.045$). Further studies on adjuvant therapy are expected to improve the outcomes of resectable PC in the future.

Table 2. The results of RCTs comparing with GEM in adjuvant chemotherapy

Author	Year	Regimen	Number	Primary endpoint	DFS				OS			
					Months	HR	95%CI	*p*-value	Months	HR	95%CI	*p*-value
Moore *et al*.	2007	GEM	217	DFS	11.4	0.94	0.76-1.15	0.26	26.5	-	-	0.61
CONKO-005 (*22*)		GEM+Elro	219		11.4				24.5			
Neoptolemos *et al*.	2017	GEM	366	OS	13.1	0.86	0.73-1.02	0.082	25.5	0.82	0.68-0.98	0.032
ESPAC-04 (*23*)		GEM+Cape	354		13.9				28.0			
Uesaka *et al*.	2016	GEM	193	OS	11.3	0.60	0.47-0.76	< 0.001	25.5	0.57	0.44-0.72	< 0.001
JASPAC 01 (*3*)		S-1	192		22.9				46.5			
Conroy *et al*.	2018	GEM	246	DFS	12.8	0.58	0.46-0.73	< 0.001	35.0	0.64	0.48-0.86	0.003
PRODIGE24 (*24*)		mFOLFIRINOX	247		21.6				54.4			
Tempero *et al*.	2019	GEM	434	DFS	18.8	0.88	0.73-1.06	0.182	36.2	0.82	0.68-1.00	0.045
APACT (*25*)	in ASCO	GnP	432		19.4				40.5			

Cape: capecitabine, CI: confidence interval, DFS: disease-free survival, Erlo: erlotinib, GEM: gemcitabine, GnP: gemcitabine plus nab-paclitaxel, HR: hazard ratio, mFOLFIRINOX: modified FOLFIRINOX, OS: overall survival, RCT: randomized control trial.

Table 3. International consensus of classification of resectability in pancreatic cancer based on anatomical definition using CT imaging

Resectable (R)	SMV/PV: no tumor contact or unilateral narrowing; SMA, CA, CHA: no tumor contact.	
Borderline resectable (BR)		
BR-PV SMV/PV involvement alone	SMV/PV ▪ Tumor contact 180°or greater; ▪ Bilateral narrowing/occlusion, not exceeding the inferior border of the duodenum; SMA, CA, CHA: no tumor contact/invasion;	
BR-A Artery involvement	SMA, CA: tumor contact of less than 180°without showing deformity/stenosis; CHA: tumor contact without showing tumor contact of the PHA and/or CA.	
Unresectable (UR)		
Locally advanced (LA)	SMV/PV: bilateral narrowing/occlusion, exceeding the inferior border of the duodenum; SMA, CA: tumor contact / invasion of 180°or more; CHA: tumor contact/invasion showing tumor contact/ invasion of the PHA and/or CA; Ao: tumor contact or invasion .	

3.2.Establishing the definition of resectability for PC

At the beginning of the 2000's, an attempt was made to classify PC into categories according to their resectability. Resectability of PC was first classified in the NCCN guidelines in 2004, and further objective classification based on the anatomical extension on computed tomography (CT) images was proposed by M. D. Anderson Cancer Center (MDACC) in 2006 (*29*). Briefly, all PCs were classified into resectable (R), borderline resectable (BR), and unresectable (UR) based on the local extension and presence or absence of distant metastasis (Table 3). In the 20th meeting of the International Association of Pancreatology in Japan (2016), the International consensus on the classification of BR PC was defined based on anatomical configurations on CT imaging (*30*). Nowadays, the treatment strategy for PC is determined by the resectability status at the time of diagnosis, and a multidisciplinary treatment strategy is a key for successful treatment for PC.

3.3. Neoadjuvant therapy for BR or R PC

In cases of R/BR PC, chemo (radiation) therapy can be performed as neoadjuvant therapy on the assumption

that surgery is to be performed. Possible advantages of neoadjuvant therapy for R/BR PC include the following: 1) it is a more aggressive treatment option compared to adjuvant therapy, 2) has the potential for improved resectability and R0 rate due to tumor shrinkage, 3) can control potential nodal or distant metastases, and 4) can select the ineligibility for radical resection. Many researchers have attempted to clarify the efficacy of neoadjuvant therapy, and several RCTs for R/BR PC have been conducted (Table 4) (*31-34*). Motoi *et al*. in Japan reported that preoperative chemotherapy by GEM plus S-1 for R/BR PC significantly prolonged OS compared to upfront surgery (median, 36.7 months *vs*. 26.7 months, $p = 0.015$) (*34*). However, the remaining three RCTs did not demonstrate the survival superiority of neoadjuvant therapy compared to upfront surgery in the treatment of R/BR PC (*31-33*). Therefore, the true impact of neoadjuvant therapy for R PC still remains controversial. Table 5 shows the ongoing RCTs of neoadjuvant therapy for R PC (*35-39*), and the results of these trials may resolve this controversy in the near future.

Neoadjuvant chemo(radiation) therapy for BR PC was introduced before surgery relatively earlier than for R PC, because it is sometimes difficult to obtain negative margins in upfront surgery for BR PC. In 2008, Katz *et al*. in MDACC classified BR PC into three groups (Type A, B, and C) based on local anatomic factors, tumor factors, and patient factors, and investigated the effect of preoperative chemoradiotherapy on these factors. The authors found that patients who were re-classified as resectable after preoperative chemoradiotherapy had improved survival rates in all three groups (*40*). According to the multi-institutional survey data presented by the Japanese society of pancreatic surgery, the OS of 57 patients among 539 patients with resected BR PC who underwent preoperative treatment was significantly improved compared to the remaining 482 patients who did not (median, 12.1 months *vs*. 23.8 months, $p = 0.023$) (*41*). Nagakawa *et al*. also reported significantly better survival rates in the preoperative treatment group ($n = 297$) than in the non-treatment group ($n = 297$) in a multicenter retrospective study using propensity score matching (median OS, 25.7 months *vs*. 19.0 months, $p = 0.015$) (*42*).

Table 4. RCTs of neoadjuvant therapy for resectable / borderline resectable pancreatic cancer

Author	Year	Country	Resectability	Regimen	Number	Number of resection (%)	R0 resection (%)	Median OS (months)
Golcher *et al*. (*31*)	2015	Germany	R	GEM/Cisplatin+RT Upfront surgery	33 33	19 (58) 23 (70)	52 *vs*. 48 ($p = 0.81$)	17.4 *vs*. 14.4 ($p = 0.96$)
Casadei *et al*. (*32*)	2015	Italy	R	GEM+RT Upfront surgery	18 20	11 (61) 15 (75)	39 *vs*. 25 ($p = 0.49$)	22.4 *vs*. 19.5 ($p = 0.97$)
Versteijine *et al*. (*33*)	2020	Netherlands	R/BR	GEM+RT Upfront surgery	119 127	72 (61) 92 (72)	71 *vs*. 40 ($p < 0.001$)	16.0 *vs*. 14.3 ($p = 0.096$)
Motoi *et al*. (*34*)	2019	Japan	R/BR	GEM+S-1 Upfront surgery	182 180	140 (77) 130 (72)	-	36.7 *vs*. 26.7 ($p = 0.015$)

BR: borderline resectable, R: resectable, RT: radiation therapy.

Table 5. Ongoing RCTs of neoadjutant therapy for resectable pancreatic cancer

Study	Design	Country	Resectability	Regimen	Number	Primary endpoint
NEONAX (*35*)	Phase II	Germany	R	Perioperative GnP (pre 2, post 4) Adjuvant GnP (post 6)	166	DFS at 18 months after randomization
nlTRO (*36*)	Phase II	Italy	R	Nal-IRI + 5-FU/LV + oxaliplatin (pre 3, post 3)	72	R0 resection rate
NorPACT-1 (*37*)	Phase III	Normay	R	Surgery first Preoperative FOLFIRINOX (4)	90	Overall mortality at 1year
PANACHE01-PRODIGE48 (*38*)	Phase II	France	R	FOLFINOX (pre 4, post 8) FOLFOX (pre 4, post 8) Surgery first +Adjuvant (12)	160	OS at 12 months Full therapeutic sequence
Alliance A021806 (*39*)	Phase III	USA/Canada	R	Perioperative FOLFIRINOX (pre 4, post 2) Adjuvant FOLFIRINOX (6)	352	OS

DFS: disease-free survival, GnP: gemcitabine plus nab-paclitaxel, Nal-IRI: nanoliposomal- irinotecan, LV: levofolinate, OS: overall survival, R: resectable, RCT: randomized control trial, 5-FU: 5-fluorouracil.

Table 6. Ongoing trial comparing chemotherapy and chemoradiotherapy for borderline resectable pancreatic cancer

Study	Design	Country	Regimen	Number	Primary endpoint
ALLIANCE NCT02839343	Phase II	USA	FOLFIRINOX FOLFIRINOX + SBRT	112	1.5-yaer OS
PANDAS-PRODIGE44 NCT02676349	Phase II	France	mFOLFIRINOX + Cape-base RT mFOLFIRINOX	92	R0 resection rate
GABANANCE trial	Phase II/III	Japan	GnP S-1 + RT	110	Phase II: R0 resection rate Phase III: OS

Cape: capecitabine, GEM: gemcitabine, GnP: gemcitabine plus nab-paclitaxel, mFOLFIRINOX: modified FOLFIRINOX, OS: overall survival, RT: radiation therapy, 5-FU: 5-fluorouracil.

Recent leading regimens, such as FFX and GnP, have been introduced in neoadjuvant therapy for BR PC. Miyasaka *et al*. reported that the group of neoadjuvant chemotherapy by GnP [median number of chemotherapy courses administered: 3 (1-10)] in patients with BR PC achieved a higher R0 resection rate (100% *vs*. 77%, $p = 0.01$) and better survival rate (2-year survival, 73% *vs*. 25%, $p = 0.03$) compared to the upfront surgery group (*43*). Furthermore, a meta-analysis performed by Janssen *et al*. also reported that preoperative FFX therapy in BR PC was associated with a 67.8% resection rate and 83.9% R0 resection rate, respectively, and the median survival time and progression-free survival time were 22.2 months and 18 months, respectively (*44*).

Jang *et al*. reported the results of a trial comparing neoadjuvant chemoradiotherapy (NACRT) (GEM 400 mg/m2/week + 54 Gy/6 weeks) with upfront surgery for BR PC. Results showed that the NACRT group had a higher R0 resection rate than the upfront surgery group (82% *vs*. 33% $p = 0.01$). NACRT group had a higher R0 resection rate (82.4% *vs*. 33.3%, $p = 0.01$) and a significantly better prognosis (median survival time, 21 months *vs*. 12 months, $p = 0.028$) than the upfront surgery group (*45*). Recently, the results of an RCT (PREPANIC trial) study on R/BR PC in the Netherlands showed remarkable results. The NACRT group (GEM + radiation) for BR PC showed a significantly higher R0 resection rate compared with the upfront surgery group (79% *vs*. 13%, $p < 0.01$) and significantly improved OS (median, 17.6 months *vs*. 13.2 months, $p = 0.029$) and significantly improved OS (median, 17.6 months *vs*. 13.2 months $p = 0.029$) (*46*). The results of ESPAC-5F, which is four arms prospective multicenter randomized phase II trial or upfront surgery compared with neoadjuvant therapy (GEM + Cape or FFX or chemoradiotherapy) in patients with BR-PC were reported at ASO in 2020. In this report, these neoadjuvant therapies had a significant survival benefit compared with upfront surgery (one year survival rate: 77 % *vs*. 40%, $p < 0.001$), however, resection rate and R0 resection rate were not significant differences (resection rate: 55% *vs*. 62%, $p = 0.668$, R0 resection rate: 23% *vs*. 15%, $p = 0.721$) (*47*). Still the optimal neoadjuvant therapy for BR PC remains controversial, and the ongoing RCTs including neoadjuvant chemotherapy and NACRT will be keys to solving this clinical question (Table 6).

3.4. Conversion surgery for initially UR PC

Approximately 30-40% of PCs are unresectable at the time of initial diagnosis due to locally advanced cases, and 50-60% due to the presence of distant metastases, and both groups are classified as initially unresectable, *i.e*., unresectable for locally advanced (UR-LA) and unresectable for metastasis (UR-M).

Systemic chemotherapy with/without radiotherapy is the first-line treatment for UR PC. With the development of novel chemotherapeutic agents, tumor shrinkage and control of distant metastases can be expected in UR PC. Surgical resection of initially UR PC after remission following chemo(radio)therapy is defined as conversion surgery (CS).

4. Multidisciplinary treatment for UR-LA PC

In 2020, FFX and GnP replaced the first-line chemotherapeutic regimen for patients with UR PC. The objective response rates and median OS rates of FFX and GnP were reported to be 31.6% and 23%, and 11.1 months and 8.5 months, respectively (*27,28*). Owing to the good response rates associated with these regimens, CS in patients with good responses has been gradually advanced. A meta-analysis of 13 trials of FFX for UR-LA PC reported that 91 of 325 patients (28%) underwent CS achieving 74% of R0 resection (*48*). Table 7 shows the recent results of CS for UR-LA PC, *i.e*., 20-36% of patients with UR-LA PC underwent CS after chemotherapy or chemoradiotherapy, with a median survival of 24.9-35.5 months (*49-54*). Apparently, these results highlight that optimized patient selection is bound to facilitate favorable R0 resection rates and long-term outcomes while introducing CS after effective chemotherapy in patients with initially UR-LA PC.

5. CS for UR-M PC

Table 7. Conversion surgery for unresectable locally advanced pancreatic cancer

Author	Year	Country	Regimen	Number	Number of resection (%)	R0 resection (%)	MST (months)
Sadot *et al*. (*49*)	2015	USA	FOLFIRINOX	101	31 (31)	55	25
Marthey *et al*. (*50*)	2015	France	FOLFIRINOX	77	28 (36)	-	24.9
Bednar *et al*. (*51*)	2017	USA	Various	92	19 (21)	74	32
Lee *et al*. (*52*)	2018	Korea	FOLFIRINOX	64	15 (23)	73	> 40 (NR)
Gemenetzis *et al*. (*53*)	2019	USA	FOLFIRINOX GEM-base	415	84 (20)	89	35.5
Philip PA *et al*. (*54*)	2020	USA	GnP	107	17 (16)	44	-

GEM: gemcitabine, MST: median survival time, NR: not reached.

5.1. CS seems to be more controversial for UR-M PC than for UR-LA.

There are few reports of CS for PC with synchronous metastases, which included only selected patients and poor prognoses after surgery with an approximately 10-month median OS (*55*). A small number of patients have responded remarkably well to the novel chemotherapy approach, and metastatic tumors are no longer detectable in imaging studies. Frigerio *et al*. reported that among 535 patients with UR PC with liver metastases undergoing CS, 24 patients (4.5%) with resolution of liver metastases on imaging and decreased CA19-9 levels after chemotherapy had a favorable prognosis (median OS, 56 months) (*56*). Wright *et al*. reported that among 1147 patients of UR-M PC, 23 (2.0%) patients underwent surgical resection of the primary tumor with or without metastasectomy (liver, $n = 16$; lung, $n = 6$; peritoneum, $n = 2$) after a favorable response to systematic chemotherapy. The median surgical and diagnostic OS were 18.2 and 34.1 months, respectively (*57*). Satoi *et al*. reported CS for UR-M PC with only peritoneal dissemination or positive peritoneal washing cytology. The authors treated patients with intravenous and intraperitoneal paclitaxel with S-1 before CS. The OS in eight (24.2%) of 33 patients who underwent CS was significantly higher compared to nonsurgical patients (median, 27.8 months *vs*. 14.2 months, $p = 0.0038$) (*58*). The number of patients with UR-M PC who could expect a good prognosis after CS is significantly limited, however, CS is likely to improve patient survival. To date, previous reports on CS are retrospective and involve significant bias. In addition, these reports included patient who were resected and responded well enough to chemotherapy to be considered candidates for CS, and continued chemotherapy may provide a similar prognosis. Therefore, to prove the efficacy of CS for UR PC, it is necessary to demonstrate that CS is more effective than continued chemotherapy in patients who have responded to chemotherapy and are deemed resectable. Currently, a retrospective study is being planned, mainly in Asia, to retrospectively compare patients with UR-LA or UR-M PC who have objectively responded to chemotherapy by FFX or GnP with patients who underwent CS and continued chemotherapy.

5.2. Criteria for going to CS

The optimal criteria for converting to adjuvant surgery after systemic chemotherapy with/without local radiation therapy remain unclear. As for the timing, in a retrospective multicenter study involving 97 patients with UR-LA PC in Japan, CS was more beneficial in patients with more than eight months of preoperative therapy compared to patients with less than eight months (*59*). However, this study was conducted before the introduction of FFX and GnP. Recently, Gementzis *et al*. reported that 116 (28%) of 461 patients with UR-LA PC who received FFX, GEM-based, or both chemotherapies were deemed eligible for surgery, and 84 (20%) of them were resected. The median duration of chemotherapy in the 84 patients undergoing CS was five months (range: 4-6 months) (*53*). In the Clinical Practice Guideline for Pancreatic Cancer 2019 in Japan, CS is weakly recommended for UL-LA PC (*26*) and is not defined for UR-M PC. The reported morbidity and mortality rates after CS are comparable with those after conventional pancreatectomy, and the reported survival rate of patients undergoing CS is better than patients with only chemotherapy. However, CS for UR-LA PC is technically demanding and associated with both resection and reconstruction of the portal vein, but also dissection from the superior mesenteric arteries or hepatic arteries. Thus, CS for UR PC should be performed in highly skilled institutions.

6. Conclusion

Surgical treatment results of PC have improved along with the refinement of surgical procedures and chemo/chemoradiation therapy advancements. However, many clinical questions pertaining to the optimal treatment regimen, preoperative treatment duration, and surgical resection criteria remain unresolved. The results of the ongoing prospective studies are bound to provide answers to these questions.

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Mini-Review

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Liver transplantation for patients with hepatocellular carcinoma: Its current status and advances

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SUMMARY Liver transplantation is one of the best treatment options for selected patients with hepatocellular carcinoma (HCC). The Milan criteria (a single tumor with a maximum size of 5 cm or two or three tumors with a maximum size of 3 cm without evidence of vascular or extrahepatic involvement or metastasis) are one of the most common criteria to select patients with HCC for transplantation, though they are considered too restrictive. A moderate expansion of the criteria has been found to yield comparable recurrence-free survival rates. HCC will recur in approximately 10% of patients, and mostly within the first 2 years after transplantation. The preoperative level of alpha-fetoprotein, macrovascular invasion, tumor size, and tumor number are prognostic factors for recurrence. Recurrence of HCC after transplantation results in a poor prognosis.

Keywords liver transplantation, Milan criteria, hepatocellular carcinoma

1. Introduction

Liver transplantation has been one of the standard treatment options for patients with early-stage hepatocellular carcinoma (HCC) (*1*). Liver transplantation would be an ideal treatment for HCC since it can treat both the tumor and the damaged liver in the background, providing a higher chance of a cure than other treatments.

Currently, liver transplantation to treat HCC represents approximately15% of all liver transplants (*2*). Since liver transplantation is an excellent treatment option for HCC, the number of candidates exceeds that of available donors (*3*). A more advanced tumor is presumed to result in a poorer outcome. Patients who would receive a major survival benefit from liver transplantation need to be selected. Therefore, the selection criteria for candidates are an important topic (*4*).

The current review describes the current status of liver transplantation for HCC. The selection criteria that will result in the maximum recurrence-free survival are described. Immunosuppressor regimens are also reviewed. Finally, the management of HCC recurrence after liver transplantation is described.

2. Selection criteria

In the past, liver transplantation for HCC had poor outcomes (*5*) because of the high incidence of recurrence. Later, Mazzaferro *et al*. (*6*) proposed criteria for the stage of HCC: a single tumor ≤ 5 cm or two or three tumors ≤ 3 cm without major vessel invasion or extrahepatic tumor spread based on imaging studies. When the criteria were met, the 4-year patient survival was 75% and the recurrence-free survival was 83%. The criteria have been adopted for deceased donor liver transplantation and living donor liver transplantation (LDLT) in many centers all over the world. The criteria, however, are believed to be too strict, preventing many patients from undergoing transplantation. The most commonly used extended criteria are shown in Table 1.

Unlike in the West, in East Asian countries (*7*) including Japan, most transplants have been LDLTs (*8*). LDLT is a private issue between patients and their families, and the indications for LDLT in terms of tumor status can be considered on a case-by-case basis Many transplantation centers performing LDLT have adopted expanded criteria (*9*). This might enable more patients to receive transplants without significantly increasing the rate of HCC recurrence.

In Japan, the Japanese Organ Transplantation Act was enacted in 1997 and amended in 2006. However, there are still not enough deceased donor livers. By the end of 2020, 658 deceased donor liver transplantations and 9760 LDLTs have been performed. Of these, 1,747 were performed to treat HCC. LDLT for HCC has a 1-year survival rate of 85%, a 3-year survival rate of 76%, a 5-year survival rate of 71%, a 10-year survival

Table 1 The extended criteria

Criteria & year	Disease-free survival	Disease-free survival	Overall survival
Milan (*6*), 1996	a single tumor ≤ 5 cm or two or three tumors ≤ 3 cm without major vessel invasion or extrahepatic tumor spread	92% at 4 years	85% at 4 years
USCF (*43*) , 2007	a single tumor ≤ 5 cm or 3 tumors ≤ 4.5 cm with total ≤ 8 cm	91% at 5 years	81% at 5 years
Up-to-7 (*44*), 2009	Tumor number and sum of tumor diameter < 7	64% at 5 years	71% at 5 years
Total tumor volume (*45*), 2015	≤ 115 cm^3, AFP ≤ 400 ng/mL	68% at 4 years	78% at 4 years
Extended Toronto (*23*), 2016	Not poorly differentiated, without major vessel invasion or extrahepatic tumor spread	30% at 5 years	68% at 5 years
5-5-500 (*10*), 2019	Tumor number ≤ 5, Tumor size ≤ 5 cm, AFP ≤ 500 ng/mL	73% at 5 years	76% at 5 years

AFP. alpha-fetoprotein

rate of 63%, a 15- year survival rate of 56%, and a 20-year survival rate of 46%.

A recent study of a database (*10*) examined 965 patients who underwent LDLT for HCC between 1990 and 2005. Of those patients, 664 were within the Milan criteria and 301 were outside those criteria. New criteria were proposed (the 5-5-500 rule) consisting of a tumor number ≤ 5, a tumor size ≤ 5 cm in diameter, and a serum alpha-fetoprotein (AFP) level ≤ 500 ng/mL. This enables more candidates (n = 725) to receive a transplant and it results in a 5-year recurrence rate of less than 10%. The insurance system of the Japanese Ministry of Health, Labor, and Welfare has now covered the cost of the transplantation within the 5-5-500 rule for deceased donor liver transplantation and LDLT.

3. Resection and partial liver segment 2-3 transplantation with delayed total hepatectomy (RAPID) procedure or auxiliary partial orthotopic liver transplantation (APOLT)

RAPID is a new but an extrapolated concept of auxiliary partial orthotopic liver transplantation (APOLT), which is a long-used procedure (*11*).

Few patients have undergone RAPID thus far (*12*), and few of those patients had HCC (13). Indications for RAPID are HCC located in the left lobe of the liver, cirrhosis with a low MELD score, and moderate portal hypertension (*14*). A recent case reported by Balci *et al*. (*15*) suggested that RAPID is effective and safe in a patient with a MELD score of 27.

4. Immunosuppression

Patients at high risk of HCC recurrence may benefit from adjustment of immunosuppression. A high level of calcineurin exposure has been found to be related to HCC recurrence after transplantation. One hypothesis that over-exposure to calcineurin inhibitors in the early postoperative period might prevent the immune system from detecting and destroying remaining HCC cells (*16*).

The mammalian target of rapamycin (mTOR) inhibitors (sirolimus and everolimus) are considered to have anti-neoplastic effects on HCC (*17*). Their use might be related to lower rate of HCC recurrence (*18*). A prospective, randomized, open-label, international multicenter trial was conducted with 525 transplant recipients with HCC (*19*). Results indicated that sirolimus was associated with a statistically better recurrence-free survival at 3 and 5 years after liver transplantation. An analysis (*20*) of the Scientific Registry of Transplant Recipients database indicated a survival benefit of immunosuppression regimens that included sirolimus. A prospective study (*21*), however, revealed that everolimus had no significant effect on HCC recurrence. The effectiveness of mTOR inhibitors on the recurrence of HCC has yet to be confirmed.

5. HCC recurrence

With careful patient selection, the rate of HCC recurrence ranges between 10-20% (*22-24*). Most recurrence occurs within 2 years of transplantation (*25*). The average time to recurrence ranges from 16 to 18 months (*26-28*). The sites of extrahepatic lesions (around 60%) include the lungs, bone, the peritoneum, and lymph nodes, followed by the liver (around 30%) (*29,30*).

6. Treatment and prognosis

Survival time from the diagnosis of recurrence ranges between 10 and 12 months (*26,27*). Treatment of lesions is largely the same as that for the patients who have not undergone transplantation (*27,31,32*). If technically feasible, the most effective radical treatment for recurrence is resection of the lesion (*33*). Ablation therapy can be indicated if the lesion is small and limited to the liver. The 1-year survival rate after radical

resection to treat recurrence is 94% and the 2-year survival rate is 53% (*34*).

If radical treatment is not feasible, trans-arterial chemotherapy with sorafenib or lenvatinib will be considered. Patients who received sorafenib or lenvatinib and who tolerated it well had a survival of 20 months (*34*). Immune checkpoint inhibitors (nivolumab and pembrolizumab) are now being tried as a second-line therapy for recurrence (*34,35*). Their effectiveness has yet to be confirmed.

Conversion to sirolimus is one therapy (*36*). In one study (*36*), patients receiving sirolimus from the moment that recurrence was diagnosed had a survival of 12 months while those receiving unmodified immunosuppressants had a survival of 8 months. Patients receiving symptomatic treatment did not survive 1 year (*27*).

7. Predictors of recurrence

Tumor factors are the most relevant to tumor recurrence. They are followed by preoperative levels of the tumor markers AFP and DCP, which are used to exclude patients from eligibility or the waiting list.

Models of the risk of recurrence are useful at devising protocols for postoperative screening. The RETREAT score (*37*) consists of 3 variables: microvascular invasion, preoperative level of AFP, and the sum of the largest viable tumor diameter and the number of viable tumors on explant. Scores range from 0 to 5 or higher. A higher score indicates a higher risk of recurrence; the possibility of recurrence is < 3% with a score of 0 and > 75% with a score of 5 or higher.

The post-MORAL score (*38*) consists of four independent predictors of recurrence: grade 4, vascular invasion, size > 3 cm, and number > 3 (all are postoperative). A study by UCLA (*27*) revealed that predictors of mortality following recurrence included the model for end-stage liver disease at transplantation > 23, time to recurrence, > 3 recurrent nodules, the maximum size of the recurrent lesion, bone recurrence, the AFP level at recurrence, donor serum sodium, and the pretransplant recipient neutrophil-lymphocyte ratio. Sapisochin *et al*. (*32*) identified three significant predictors: the lack of the potential for radical treatment (resection or thermos-ablation), an AFP level at the time of diagnosis > 100 ng/mL and early recurrence within a year after transplantation. Those predictors were confirmed by multicenter data (*29*).

8. Surveillance of recurrence

Postoperative screening will allow early detection of recurrence. It can help to identify patients who are eligible for radical treatment. Screening is related to an increased chance of survival (*25,30,31*). Although there are no universal protocols to screen for recurrence (*29,31*), some experts recommend performing computed tomography of the abdomen and chest with contrast medium, bone scintigraphy, and measuring the AFP level every 3-6 months for 2-3 years. Thereafter, the test interval can be prolonged to 6-12 months (*25,32,39*). Postoperative screening should be performed for each patient (*25*).

9. Perspectives for the future

The appearance of direct-acting antivirals (DAAs) to treat hepatitis C virus (HCV) has improved the prognosis for patients with HCV. A recent study (*40*) indicated that approximately 20% of cirrhotic patients infected with HCV with or without HCC might be delisted because of improved liver function after therapy. The appearance of DAAs should decrease patients undergoing liver transplantation for HCV and HCC.

In contrast, the increase of non-alcoholic steatohepatitis (NASH) has led to an increase in the number of liver transplants for NASH with or without HCC (*41*). The number of liver transplants for NASH with HCC is expected to fill "the vacancy" left by the decrease in the number of transplants for HCV with HCC (*42*).

10. Conclusion

Liver transplantation is an established treatment for patients with early-stage HCC. However, the shortage of available organs necessitates the adoption of criteria to ensure the optimal use of donor organs. In the LDLT setting, some restrictive criteria are needed from an ethical point of view to ensure a satisfactory recurrence-free survival after liver transplantation.

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Review

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Liver transplantation in China: Achievements over the past 30 years and prospects for the future

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SUMMARY Over the last three decades, liver transplantation (LT) in China has made breakthroughs from scratch. Now, new techniques are being continuously incorporated. However, LT in China differs from that in other countries due to cultural differences and the disease burden. The advances made in and the current issues with LT in China need to be summarized. Living donor LT (LDLT) has developed dramatically in China over the last 30 years, with the goal of increasing transplant opportunities and dealing with the shortage of donors. Western candidate selection criteria clearly are not appropriate for Chinese patients. Thus, the current authors reviewed the literature, and this review has focused on the topics of technological advancements in LDLT and Chinese candidate selection. The Milan criteria in wide use emphasize tumor morphology rather than pathology or biomarkers. α-fetoprotein (AFP) and pathology were incorporated as predictors for the first time in the Hangzhou criteria. Moreover, Xu *et al*. divided the Hangzhou criteria into type A (tumor size ≤ 8 cm or tumor size > 8 cm but AFP ≤ 100 ng/mL) and type B (tumor size > 8 cm but AFP between 100 and 400 ng/mL), with type B serving as a relative contraindication in the event of a liver donor shortage. In addition, surgeons in Chengdu and Shanghai have the ability to perform a laparoscopic hepatectomy for right and left lobe donors, respectively. China has established a complete LT system, including recipient criteria suitable for Chinese people, a fair donor allocation center, a transplant quality monitoring platform, and mature deceased donor or living donor LT techniques.

Keywords hepatocellular carcinoma; living donor liver transplantation; Hangzhou criteria; donation after cardiac death; donation after brain death

1. Introduction

Primary liver cancer is the seventh most common malignant tumor according to the World Health Organization and the fourth leading cause of cancer-related mortality. It has soared to the second leading cause of cancer-related mortality in China, after lung cancer. In 2020, the number of cases surpassed 410,000, with more than 390,000 deaths, placing a huge burden on China's health system (*1*). Hepatocellular carcinoma (HCC), intrahepatic cholangiocarcinoma (ICC), and other uncommon liver cancers are types of primary liver cancer. HCC accounts for a sizable chunk of the total therein. The main etiological factors for HCC are liver cirrhosis, hepatitis, and aflatoxins, while the high incidence of HCC in China is attributed to the high prevalence of the hepatitis B virus. HCC treatment options include hepatectomy, trans arterial chemoembolization (TACE), radiofrequency ablation (RFA), liver transplantation (LT), and conservative therapy. However, only LT can eliminate the tumor and underlying liver disease at the same time. Accordingly, LT is the treatment of choice for end-stage liver disease and early-stage HCC (*2*).

China has seen remarkable progress in orthotopic LT since 1977, when it was first performed on the Chinese mainland. According to the China liver Transplant Registry (CTLR), LT cases in China account for more than a third of all LT cases worldwide (*3*). As of June 2015, a total of 29,360 cases of LT were performed, about 50% of which were performed to treat HCC (*2*). With economic and technological advances, LT in China is no longer constrained by the procedure but rather by a scarcity of donors and a high rate of postoperative recurrence. Under such conditions, efforts are being made to address the issue of a donor shortage and to improve the prognosis for transplant patients. The legal framework for government oversight in 2007 was the initial step to regular organ transplantation. However, several ethical and legal issues remained. The pilot

program for organ donation after cardiac death (DCD) in 2012 represented a milestone in Chinese organ transplantation (*4*). At the same time, general surgery specialists have successfully devised Chinese transplant indications such as the Shanghai Fudan Criteria, the Hangzhou Criteria, and the West China Criteria with the goal of expanding the transplant indications of the Milan criteria without diminishing prognosis.

In 2002, surgeons at West China Hospital performed the first adult-to-adult LDLT in mainland China, further resolving the problem of the liver donor shortage at the surgical level (*5*). In 2013, physicians at West China Hospital studied 290 living donors from 2002 to 2012, focusing on reasons why donor hepatectomy was 'not feasible' (*6*). There were two main reasons for the failure of the operation in the 5 donors, namely poor liver quality and inappropriate biliary anatomy. All 5 donors recovered without complications and the long-term follow-up was good, indicating that China has achieved a low rate of 'no go' donor hepatectomy and that abandonment of surgery had no effect on short-term and long-term outcomes. Laparoscopic donor hepatectomy started late in mainland China, and until 2014 only a few transplant centers had performed this procedure. A prospective case-matched study confirmed the advantage of these minimally invasive approaches in reducing the duration of hospitalization and administration of analgesics, but the total cost of hospitalization was significantly higher (*7*). In fact, previous studies in mainland China tend to favor LDLT and laparoscopic donor harvesting. Therefore, the current review aims to describe the progress of LT in China over the past 30 years by describing the selection of Chinese recipients, with a special focus on the achievements of and issues with LDLT and laparoscopic donor hepatectomy in mainland China.

2. Advances in standardization of LT procedures

LT in China has gone through three stages over the past 30 years. The first stage is from the initial LT (1977) to 2005. During this period, various transplant centers came to the fore but there was no platform to assess and control the quality of LT. With the establishment of the CTLR in 2005 and the formulation of a legal framework for government oversight of LT in 2007, all transplant centers were instructed to upload data and accept inspections by the Ministry of Health of China in 2008. Afterwards, the number of transplant centers plummeted, but the quality of surgery was better. The increasing number of LT operations rely on a sufficient number of liver donors. Influenced by the traditional Confucianist view that a corpse should be intact, resistance to organ donation still exists. The Chinese Organ Transplant Response System (COTRS) was created in 2012 to change unethical practices, combat illicit organ trading, and to end transplantation tourism in order to make the procedure more open and efficient (*4*). When voluntary organ donation became the main source of organ donation marks the beginning of the second phase. In 2015, the Chinese Government declared voluntary organ donation to be the sole legal type of organ donation, ushering in a new age of organ transplantation in the country. By December 2021, there were 37 842 organ donors and 113,294 donated organs (*8*).

3. Selection criteria for treatment of HCC

The first appearance of LT was in the context of treating unresectable HCC. Because of its high recurrence rate, HCC was later deemed a contraindication for LT. In 1996, Mazzaferro *et al*. presented the first liver transplant selection criteria on HCC, the Milan criteria (*9*). Later, sets of criteria were proposed by various experts in order to broaden the Milan criteria's strict requirements for the number and size of tumors, including the Pittsburgh criteria (*10*), the Navarro criteria (*11*), and the University of California San Francisco (UCSF) criteria (*12*). The Milan criteria and UCSF criteria are the criteria that are most widely used internationally. Chinese patients diagnosed with HCC often do not meet the Milan criteria due to the high incidence of HBV, and these guidelines are too strict for them, so many patients with HCC who might benefit from the procedure are excluded. Therefore, Chinese experts put forward criteria for choosing Chinese patients. The following is a summary of those criteria.

3.1. Chengdu (West China) criteria

Patients who meet the Milan criteria can also undergo liver resection in China, with the same prognosis as LT. Due to high costs and long waiting times, LT was only seen as an adjunct to liver resection for a period (*13*). Yan *et al*. (*13*) reported in 2005 that LT can provide a satisfactory prognosis for patients with large HCC outside the Milan criteria. Thus, they defined LT indications as follows: 1) Small liver cancer and resectable liver cancer with severe liver cirrhosis or hepatic insufficiency, 2) Unresectable large liver cancer without main portal vein tumor thrombus (PVTT) or distant metastasis, and 3) Main PVTT should be regarded as a contraindication. Yan *et al*. studied 112 patients from February 1999 to February 2005 and found that those with unresectable large liver cancer can still have a good survival rate after LT, with the exception of those with main PVTT. If a single tumor was larger than 10 cm or numerous cancers were still limited to the hemi-liver, the 3-year survival rate was as high as 77%. Patients with a tumor that has progressed to the entire liver without extrahepatic metastasis had a 2-year survival rate of 73.8%. Patients with main PVTT, in contrast, had a 1-year survival rate of only 20%. The

Chengdu criteria provide a new treatment option for unresectable liver cancer, but they do not specify the size and number of the tumors. The Chengdu criteria were preliminary criteria, and they are rarely mentioned in subsequent studies.

3.2. Shanghai Fudan criteria

In 2006, Fan *et al*. put forward new criteria for China based on the UCSF criteria (*14*). The Shanghai Fudan criteria are as follows: 1) The tumor has not invaded the blood vessels or lymph nodes, 2) The tumor size for patients with a single tumor must not surpass 9 cm in diameter, and 3) The number of tumors in a patient with numerous tumors should not exceed 3. Each one must be no larger than 5 cm in diameter. The tumor's overall diameter must not surpass 9 cm. Compared to patients who failed to meet the criteria, those who met the criteria had an advantage in terms of their overall survival rate (OS) and tumor-free survival rate (TFSR) (OS&TFSR: Log rank $p < 0.001$). There was no discernible difference between patients who met the Milan criteria and those who met the Shanghai Fudan criteria but exceeded the Milan criteria (OS: $p = 0.429$; TFSR: $p = 0.952$). Thus, the Shanghai Fudan criteria have further expanded the indications for LT without diminishing prognosis.

3.3. Hangzhou criteria and new techniques

In 2008, Zheng *et al*. proposed new criteria for LT, the Hangzhou criteria (*15*). The Hangzhou criteria are as follows: 1) The tumor has not invaded the blood vessels or lymph nodes and 2) The total diameter of the tumor cannot exceed 8 cm or more than 8 cm, AFP is less than 400 ng/mL, and the cancer is well- or moderately differentiated. Further research indicated that AFP ≤ 100 ng/mL and a tumor burden ≤ 8cm were two independent prognostic factors, so the Hangzhou criteria were stratified into two types (*16*) (Table 1). Type A confers a better prognosis than type B and suggests that a patient may be an optimal candidate for LT while type B can be regarded as a relative contraindication due to the shortage of liver donors. The Hangzhou criteria included AFP and pathology as evaluation for the first time, leading to a new model for LT recipient selection. Later, in 2018, Fan *et al*. and Mazzaferro *et al*. established a competing risk model for analysis using the aforementioned factors such as AFP and tumor size and number (*17*). Nowadays, as an alternative to doctors' experience, artificial intelligence has been used to guide the selection of patients with HCC. When the patient's clinical test data and imaging data are entered into the gradient boosting decision tree (GBDT) algorithm, the system will output a series of results including diagnosis, treatment recommendations, and survival and relapse data. The system has been verified, indicating that recipient selection for LT will be fairer, more accurate, and more efficient in the future.

Table 1. Subgroups according to the Hangzhou criteria

Item	Type A		Type B
Tumor size (cm)	≤ 8	> 8	> 8
>AFP (ng/mL)	N.A.	≤ 100	100 ~ 400

4. Living donor LT (LDLT)

From the early years to present, HCC remains the main indication for LT in China. The success of LT depends on whether there are sufficient donors, which is the most important issue in organ donation worldwide, and the same holds true in China. Back in 2004, professors cited LDLT as a critical way to deal with the donor shortage in China (*18*). In mainland China, LT gradually emerged in the 1990s and West China Hospital successfully performed the first adult-to-adult LDLT in mainland China only in 2002 (*5*). Deputy Minister of Health Huang Jiefu said, "Following the first LDLT at West China Hospital, Tianjin, Beijing, Shanghai, and many other places have also performed LDLT, and China's LT entered a period of rapid development especially after 2006".

Ensuring the donor's safety and postoperative quality of life is the doctor's first priority. As early as 2013, donor hepatectomy in China has been validated as low-risk and highly efficient, and even the abandonment of the procedure did not diminish the donor's prognosis (*6*). However, there are several issues to be mindful of. The biggest problem is the accuracy of preoperative liver quality assessment. As previously mentioned, donation was abandoned in 5 candidates of 290 donors; 2 were attributed to worsening liver condition (massive cirrhotic nodule and serve steatosis, respectively) and 1 was due to small residual liver volume (*6*). After the first 35 cases, Chinese experts replaced the risk of hemorrhage due to biopsy with a comprehensive evaluation of 3 aspects: body mass index (BMI), hepatitis virus infection, and a related history of drinking or smoking. How can serious steatosis be predicted without a biopsy? A simple formula containing the BMI and computed tomography (CT) data appears to solve the problem [HMS = 47.7 + 1.48BMI – 1.14CT] (*19*). A point worth noting is that the model appears to be unable to reliably predict hepatic macrovesicular steatosis < 5% in a candidate. When calculating the residual liver volume, Chinese experts referred to both CT data and the Chengdu formula [SLV(mL) = 11.5 × BW(kg) + 334 (SLV: standard liver volume; BW: body weight)]. The Chengdu formula has proven to be reliable in LDLT (*20*). In 2015, a preoperative non-invasive model for evaluation of liver fibrosis in donor livers was proposed (*21*). The current manner of assessing remnant liver

volume is based on graft size, while the quality of the liver is another factor that affects 'functional size'. Both approaches were used in a candidate for whom donation was abandoned due to insufficient postoperative liver volume, but the 'margin of error' resulted in an eventual miscalculation. As the experts say: 'This is unpredictable and unexpected but it infrequently occurs in LDLT'.

Graft size is a crucial factor in ensuring the success of LDLT, but the importance of good venous drainage of the anterior sector of the right hemiliver has been recognized. If middle hepatic vein (MHV) tributaries from these segments are ligated and the MHV is not included in the liver graft, venous congestion of Couinaud's segments V and VIII of the right hemiliver graft is common (*22*). After portal vein reperfusion, the effects of a compromised venous outflow may be evident in some circumstances. Segments V and VIII can become swollen and turgid and have a dusky discoloration. Although a graft without the MHV is prone to a disorder in hepatic segment V & VIII blood return, extended donor hepatectomy potentially increases the risk for donors, and especially for those with hepatic steatosis, hepatitis, or of advanced age (*23*). Because hepatitis and cirrhosis are so common in China, 'borderline donors' who are positive for the hepatitis B core antibody (HBcAb) but negative for hepatitis B surface antigen (HBsAg) must be used (*6*). Back in 2005, Yan *et al*. reported the first 13 cases of LDLT without the MHV in grafts in mainland China (*24*). A 3D technique was used to preoperatively reconstruct the structure of the hepatic vein and to assess the remnant liver volume, and the branches of the right inferior hepatic vein and MHV > 5 mm are preserved. In the aforementioned study, the inferior right hepatic vein (IRHV) was reconstructed in 5 patients, and 1 or 2 thick branches of the MHV were reconstructed *via* an autologous saphenous vein bypass in 5 patients, ensuring that hepatic venous drainage was sufficient after reperfusion and ensuring the transplanted liver's survival and function. Moreover, Yan *et al*. enhanced the procedure in two ways. After excising the right hepatic vein (RHV) stump and expanding the right hepatic vein opening downward to the recipient's inferior vena cava (IVC), they directly anastomosed the RHV of the graft with the opening of the RHV of the recipient's IVC, without retaining the RHV remnant, preventing the compression and distortion caused by the existence of the remnant blood vessel between the right liver and the IVC and effectively ensuring RHV return. In the second enhancement, when the MHV branch is bypassed, the autologous saphenous vein is anastomosed with the branch opening of the MHV in the preservation container to reduce the anastomosis time on the operating table. Utilizing these surgical improvements, the same research group reported on 160 cases of consecutive living donor right hepatectomy between 2002 to 2008 (*25*). They used the Clavien grading system to define and grade the severity of donor complications; all donated livers were right lobe grafts without the MHV and all IRHVs > 5 mm in diameter were preserved for subsequent anastomosis to the recipient IVC. The occurrence of complications was as follows: A Grade 1 complication involving any deviation from the normal postoperative course without the requirement for medication and intervention (whether local therapy or surgery) was noted in 18.1% (29/160). A Grade 2 or 3 complication requiring medication or intervention was noted in 14.4% (23/160). No life-threatening complications or deaths occurred, validating the ability of the Chinese surgical approach to ensure donor safety.

Small-for-size syndrome (SFSS) is another problem with LDLT due to insufficient donor liver volume. Thus, right-lobe hepatectomy is often required to obtain a graft with adequate liver volume. How is surgery performed when the only available donor has an insufficient right lobe? In 2006, a Chinese group successfully implemented an adult-to-adult (A-A) LDLT combined with a cadaveric split left lateral segment (*26*). The patient received a right lobe without the MHV from a living donor and a left lateral segment from a cadaveric donor. The right lobe with the MHV from the cadaveric donor was transplanted into another patient. The advantage of this approach is that it maximizes the use of a cadaveric donor and it reduces the requirement for the graft size harvested from a donor, therefore protecting the donor's safety. Moreover, this approach theoretically results in a satisfactory prognosis since the right lobe and the left lateral segment can be implanted orthotopically in their original position. However, experts have suggested that this technique should not be considered as a standard treatment and that it should only be performed in unusual circumstances. If a patient has SFSS, selective transplenic artery embolization may be a solution (*27*). A case report indicated that after receiving a small-for-size right lobe from a living donor, a liver graft recipient showed clinical signs of protracted cholestasis and intractable ascites. A computed tomography scan revealed congestion in liver segments V and VIII, and both Doppler ultrasonography and vena cava angiography revealed a lack of patency of the anastomosis between V5/V8 and the internal vena cava, indicating blocked outflow of the segment V and VIII anastomosis. The Chinese approach can rapidly reduce the portal venous flow rate, thereby decreasing serum total bilirubin and eliminating ascites. Accordingly, selective splenic artery embolization is a technically simple procedure for the treatment of portal overperfusion injury in SFSS.

5. Laparoscopic donor hepatectomy

LDLT, a procedure without any health benefits but a risk of death for living donors, poses potential ethical dilemmas. The close relationship between a donor and recipient motivates the donor to save the recipient's life regardless of the cost. The most serious concern

with LDLT is donor safety. A point worth noting is that donor complications can still occur (*28*) and even result in death (*29*). Even without serious complications, the large permanent abdominal scar following standard open surgery results in emotional and physical stress for some living donors, and particularly young women, possibly leading to hesitancy in undergoing donor hepatectomy. A recent meta-analysis reported that laparoscopic surgery was associated with a shorter duration of hospitalization, less blood loss, fewer postoperative complications, and a longer operating time than open surgery (*30*). Minimally invasive donor hepatectomy (MIDH) including laparoscopy-assisted hepatectomy, total laparoscopy hepatectomy, and indocyanine green fluorescence (ICG) image-guided total laparoscopic hepatectomy are becoming the main approaches of the future. A study reported that MIDH was superior to open donor hepatectomy (ODH) in terms of blood loss, the duration of hospitalization, and overall complications without compromising liver function (*31*). However, the study in question did not perform a subgroup analysis based on the type of MIDH (laparoscopy-assisted or total laparoscopy). A larger graft is known to be riskier for living donors than a smaller one. According to an analysis of donor hepatectomy in Japan, the morbidity risk generally increased as the hepatectomy mass increased from left lateral section donation (8.2%) to left lobe donation (12.0%) and then to right lobe donation (19.0%). Right lobe donors suffered a significantly higher rate of complications than lateral segment and left lobe donors. ($p < 0.0001$, and $p < 0.0001$, respectively) (*32*) In 2002, the world's first left hepatic lobectomy (resection of segments II and III) was performed laparoscopically to save a child 1 year of age who had billary atresia (*33*). Studies in greater numbers of patients in several experienced hospitals have validated laparoscopic left lateral sectionectomy (L-LLS), which is now regarded as the standard treatment for adult-to-pediatric donation (*33-35*). However, there is no consensus on left lobe or right graft procurement in adult-to-adult LDLT (*36*). Laparoscopy-assisted donor hepatectomy (LADH) requires more from the surgical team, which must be familiar with both living donor hepatectomy and laparoscopic liver surgery. Surgeons were concerned that LADH would have to converted to an open approach. A study of a large series of 66 cases reported that 2 eventually had to be converted to open donation in the interest of donor safety (*37*). Owing to these technical difficulties, LADH in China was initially performed as right lobe MIDH, in which the hands were introduced in the abdomen while still maintaining the pneumoperitoneum. A meta-analysis indicated that LADH is associated with less intraoperative blood loss, less analgesic use, and fewer postoperative complications but a similar duration of hospitalization and increased operating time (*38*). In 2016, the first purely laparoscopic right hemihepatectomy in a living donor was performed domestically, further reducing the length of the incision (*39*) (Figure 1).

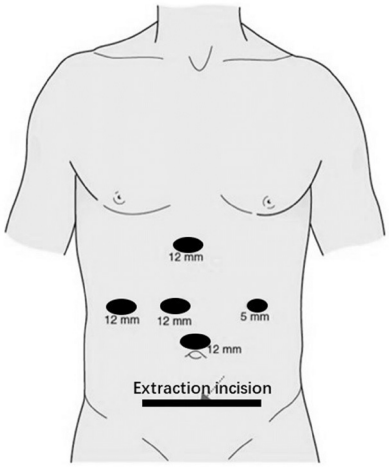

Figure 1. Trocar placement for total laparoscopic right hemihepatectomy in a living donor.

5.1. L-LLS

Interestingly, a left lateral graft was the first living donor liver graft to be harvested conventionally (*40*) and laparoscopically (*33*). The left lateral section accounts for 15-30% of total liver volume, so postoperative liver failure is unlikely to occur. Hence, laparoscopic procedures for donor hepatectomy involving a left lateral section donation are the least contentious (*41*). At present, a consensus has been reached on the feasibility and safety of pure laparoscopic sectionectomy (*42*). A liver incision on the left side of the falciform ligament, which is a well-defined surface landmark where the vertical section of the left portal vein is located, is the standardized laparoscopic procedure. The arterial inflow, biliary drainage, and portal venous branches of each segment and subsegment of the left lateral section converge intra-parenchymally within the Glissonian sheath on the left side of the falciform ligament, so all pedicles to segments 2 and 3 will be divided by transecting along the left side of the falciform ligament (*43*). In 2020, Chinese surgeons reported the first case of single-port L-LLS, and they achieved satisfactory cosmetic results (*44*) (Figure 2). The bifurcation and dividing point of the bile duct were determined using intraoperative ICG fluorescence cholangiography. However, the feasibility of advanced manipulation is based on the simple anatomy in the patient. Thus, an experienced surgical team should carefully identify donors and recipients.

5.2. Laparoscopic right hepatectomy (LRH)

Right liver grafts have the ability to meet the metabolic demands of a larger recipient, so right lobe hepatectomy

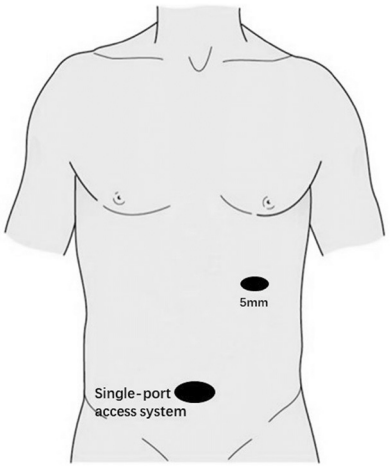

Figure 2. Trocar placement for single-port laparoscopic left lateral sectionectomy in a living donor.

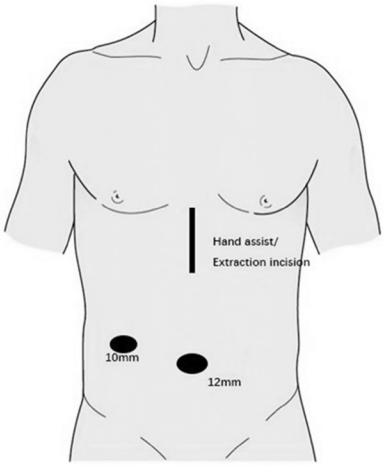

Figure 3. Trocar placement for hand-assisted laparoscopic right hemihepatectomy in a living donor.

is more common (*45*). Due to technical difficulties, LRH was initially performed as laparoscopy-assisted right hepatectomy (LARH) in which hands were introduced into the abdomen through an upper midline incision (Figure 3). The hilar dissection and parenchymal transection were done openly, while only the right lobe mobilization was done with hand-assisted laparoscopy. In 2014, a prospective study indicated that LARH was successfully performed in 25 Chinese patients; none had to be converted to conventional open surgery (*7*). Based on experience performing LARH on patients with a benign tumor, the amount of fat tissue in the abdomen rendered laparoscopic mobilization of the right liver lobe technically problematic in some overweight individuals due to an inadequate surgical field. Under such circumstances, a 5-cm midline epigastric extraction incision, a 12-mm umbilical port, and a 10-mm right lateral subcostal port appear to be insufficient to complete the procedure. Thus, several technical modifications were made. First, for some overweight donors (BMI > 25 kg/m^2), the surgeon should install a laparoscopic retractor to clear the surgical field by adding an additional 12-mm right lateral subcostal port in the right midaxillary line. Second, if access to the retrohepatic IVC after dissection of the right hepatic ligaments is problematic, the remaining laparoscopic surgery, which includes dissection of the short hepatic veins and posterior vena cava ligament, is performed under direct view through the upper middle incision.

A preliminary comparative study in China reported that purely laparoscopic right hepatectomy (PLRH) was associated with less blood loss, fewer postoperative complications, and a shorter duration of postoperative hospitalization but also higher postoperative ALT and AST compared to LARH and open right hepatectomy (ORH) (*46*). That study confirmed the feasibility and safety of PLRH, but it also indicated that PLRH must be performed in highly specialized centers with adequate postoperative monitoring and support. A point worth noting is that LRH results in a larger liver graft with multiple bile duct openings. This makes recipient intracorporeal suturing more challenging and results in more bile leakage. Despite quality preoperative magnetic resonance cholangiopancreatography (MRCP) and real-time ICG fluorescence cholangiography, surgeons may still be hesitant to determine the accurate bile duct dividing point and they may prefer to shift to the right side. When dividing the bile duct, experts replace the intracorporeal suturing with two clips at the remnant side. Two clips occupy space, so the dividing point of the bile duct may have been shifted more to the right than intended (*47*). To compensate for the constraints caused by the significantly shorter bile duct and portal vein resulting from the use of twin clips and a stapler, highly experienced and talented surgeons are required. In conclusion more time is needed to transition from a hybrid to a purely laparoscopic approach.

5.3. Laparoscopic left hepatectomy (LLH)

The harvesting of a left lobe graft is restricted due to its relatively small volume compared to the right lobe. In 2021, a study reported on 285 patients in a Shanghai cohort who underwent left lobe LDLT (*48*). Results confirmed that LLH could be performed as safety as open surgery. In an innovative approach, the surgical team combined ICG fluorescence imaging with laparoscopic donor liver harvesting because of the unique staining features of ICG. Laparoscopy with ICG fluorescence can theoretically reduce intraoperative blood loss and reduce the likelihood of post-operative biliary complications, as indicated by the aforementioned study. Hence, LLH with or without ICG should be considered as a valuable adjunct when

unsatisfactory donor conditions are encountered.

6. Prospects for the future

LT requires multidisciplinary cooperation, so the development of LT is a sign of the development of comprehensive medical prowess. Deceased donors account for a substantial portion of organ donations, but brain death was not been adopted as a standard until now. The Chinese Ministry of Health published criteria and operational requirements for brain death in 2003 (*49,50*), but they have not been promoted in a long time. There seems to be no end to the debate on ethical issues in this area. A point that should be stressed is that many countries have established a complete legal framework for brain death, so China should promptly catch up with the rest of the world. Donation after brain death (DBD) has irreplaceable advantages since DBD can maintain blood flow even after "death", thereby resulting in better liver function. In addition, the liver comes from a deceased donor and can be split in situ, which can reduce cold ischemia time compared to in vitro splitting. Hence, DBD should be legalized and implemented as soon as possible.

For numerous reasons, LDLT is being investigated as a possible replacement to DDLT. First, living donors represent a flexible source of donors and thereby minimize waiting time, the high rate of dropouts, and deaths during the waiting period. Second, better graft function will be achieved as a result of an optimized preoperative plan and shorter warm and cold ischemia times. Third, LDLT involving relatives results in immunological benefits and therefore reduces incidents of rejection because of the genetic compatibility between the donor and the recipient. LDLT is known to have a comparable survival rate to DDLT. However, the rate of recurrence for the two treatment modalities remains a subject of controversy. A study in Canada indicated that LDLT had a worse DFS according to a quantitative analysis of non-randomized studies (*51*). Several other studies have yielded similar results (*52,53*). This phenomenon was thought to be due to the transplantation of more advanced HCC or "fast-tracking" to transplant. Patients undergoing LDLT consistently tended to fall outside the Milan criteria. A study in Guangzhou indicated that LDLT does not compromise patient survival or promote the recurrence of HCC in comparison to DDLT, and especially for patients meeting Milan criteria (*54*). Intent-to-treat (ITT)-OS was measured from the time of registry for transplantation. According to one study, LDLT was linked to a superior 5-year ITT-OS (*55*). Notably, LDLT is sometimes utilized as a salvage procedure in individuals in whom all other treatments have failed. The aforementioned study was based on ITT principle to avoid this selection bias. Hence, LDLT should receive more emphasis and receive the same attention as DDLT.

Today, surgeons in China are capable of performing every type of LDLT and laparoscopic donor hepatectomy. Chinese doctors have improved the techniques to suit Chinese patients. Owing to the current donor shortage, LDLT should be actively promoted.

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Original Article

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Genetic features of TP53 mutation and its downstream FOXA1 in prostate cancer

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SUMMARY Metastasis is the most lethal form of prostate cancer, and finding new therapeutic targets remains a major clinical challenge. TP53 mutation has been identified to be involved in tumor progression and metastasis. Nevertheless, direct evidence of the role of TP53 mutation in prostate cancer metastasis and its underlying mechanism remain obscure. Herein, TP53 was found to be the most mutated gene in prostate cancer, and missense mutations were the primary mutation type based on bioinformatics data analysis. Subsequently, TP53 rs12947788 mutation site was significant in prostate cancer, and correlated with metastasis and tumor-node-metastasis (TNM) stage. Furthermore, forkhead box A1 (FOXA1), a target of TP53, was highly expressed in prostate cancer tissue, especially in TP53-mutant patients. It was also associated with patients' Gleason scores and nodal metastasis. Knockdown of FOXA1 suppressed the migration in prostate cancer cells *in vitro*. Our findings indicate that targeting TP53 mutation and FOXA1 might be a promising therapeutic target for prostate cancer metastasis.

Keywords TP53 mutation, FOXA1, bioinformatics, prostate cancer, metastasis

1. Introduction

Prostate cancer is the second malignant tumor worldwide and the fifth leading cause of cancer mortality in men (*1*). Metastasis is the most lethal form of prostate cancer, and it has a poor overall survival of only 30% at 5 years (*2*). Androgen deprivation therapy is the most common because prostate cancer cells are highly sensitive to the androgen pathway. However, relapse is inevitable. A previous study has revealed that 10-20% of patients with prostate cancer metastasis develop castration resistance within 5 years, which leads to rapid progression. Unfortunately, although the treatment strategies including enhanced hormonal or chemohormonal therapy are used in this setting, more organs show metastases because of the inconsistent efficacy. Meanwhile, the median survival time is approximately 14 months (range 9-30), which markedly increases the mortality burden of patients (*3-5*). More recently, evidence supports that targeting gene therapies holds great promise for the treatment of prostate cancer. However, sensitivity is low since therapeutic genes are lacking, limiting its clinical application. Therefore, finding new treatments for metastasis remains a major clinical challenge. Elucidating the underlying mechanisms of prostate cancer metastasis is imperative for developing novel therapeutic strategies for prostate cancer.

Deregulation of some genes are involved in prostate cancer progression from localized to metastatic disease, and control of genetic stability is frequently lost. TP53 on human chromosome 17, encoding a 53 kDa protein (also called cellular tumor antigen p53), plays a pivotal role in several tumors progression (*6,7*). Importantly, p53 exerts various effects through regulating downstream genes in prostate cancer metastatic cascade. Zhan Yang *et al* find that p53/RBM25-mediated circAMOTL1L-miR-193a-5p-protocadherin-α regulatory axis contributes to regulate epithelial to mesenchymal transition in prostate cancer metastatic progression (*8*). Results from Qiji Li *et al* reveal that wild-type p53 directly interacts with Frizzled8 (FZD8) promoter, participating in bone metastasis in prostate cancer by Wnt/β-catenin signaling (*9*). These results give us a hint that TP53 plays an essential in prostate cancer. In fact, TP53 is prone to

a gene mutation in approximately half of malignant tumors, such as colon, lung, liver, breast, skin, and bladder, which shows that TP53 mutation contributes to tumor initiation and malignant progression (*10*). Interestingly, the clinical significance of TP53 status in prostate cancer has been and continues to be a hot topic. Previous studies demonstrate that TP53 mutation frequency is about 10% in primary prostate cancer but up to 50% in metastases, which is associated with poor overall survival and progression-free survival (*11*). Prostate cancer patients with ctDNA TP53 mutation in plasma have extremely rapid disease recurrence, and are associated with a significantly shorter metastasis-free survival (*12*). This drives us to explore the underlying mechanism of TP53 mutations in prostate cancer. Mutant TP53 attenuates wild-type p53 functions, developing worse clinical outcomes (*13*). Thus, reactivation of TP53 function represents an attractive therapeutic strategy for suppressing prostate cancer metastasis. However, only a few studies have investigated the effect of TP53 mutation on prostate cancer metastasis.

Forkhead box A1 (FOXA1, a member of the FOX family) is a well-studied pioneer factor and involved in embryonic development and disease progression (*14*). It is a crucial transcription factor in the occurrence and development of lung cancer and breast cancer (*15*). Interestingly, the role of FOXA1 in prostate cancer is still controversial. Study demonstrates that FOXA1 promotes prostate cancer angiogenesis (*16*). Whereas J Kim *et al* report that FOXA1 exhibits tumor-suppressing function and inhibits prostate cancer neuroendocrine differentiation (*17*). A previous study has revealed nuclear co-localization of mutant TP53 and FOXA1 *in vivo*, and mutant TP53 regulates FOXA1 expression directly at FOXA1 promoter, which is involved in pancreatic ductal adenocarcinoma metastasis (*18*). In prostate cancer, FOXA1 is a driver of onset and progression. It reprograms the androgen receptor binding to chromatin and regulates genes associated with cell cycle and epithelial to mesenchymal transition (*19*). Despite these previous findings, our understanding of the role of FOXA1 involvement in prostate cancer metastasis remains incomplete, and it needs further to be elucidated.

In this study, bioinformatics data analysis was employed to illuminate the role of TP53 mutation in prostate cancer metastasis. Subsequently, TP53 mutation and FOXA1 expression were detected in clinical specimens by Sanger sequencing and RT-qPCR, respectively. The relationship of TP53 mutation with FOXA1 expression was analyzed, and the associations of both with clinical characteristics in prostate cancer were also evaluated using multiple online analysis tools. FOXA1 expression were detected in prostate cancer tissues and cells. Further, the effects of FOXA1 knockdown in prostate cancer cells on migration were investigated.

2. Materials and Methods

2.1. Clinical samples

Fifty-six prostate cancer tissues were collected and embedded in paraffin in the Department of Urology, Qilu Hospital of Shandong University. Meanwhile, the paired normal adjacent tissues from ten of them were also collected. Ages ranged from 45 to 84 years old, and the median age was 68. No patients had been treated with chemotherapy or radiotherapy before surgery. Tumor-node-metastasis (TNM) staging was according to the 8th edition of the American Joint Committee on Cancer (AJCC). Data on demographic and clinicopathological parameters were also recorded, including age, history of smoking and alcohol intake, metastasis, differentiation, TNM stage, and Gleason score (Table 1). This study was approved by the Ethics Committee on Scientific Research of Shandong University Qilu Hospital (KYLL-2019-258).

2.2. DNA extraction

DNA was extracted using a paraffin-embedded tissue DNA extraction kit (Tiangen Biochemical Technology Co., Ltd., DP331-02) according to instructions. The concentration and purity were detected by Onedrop OD-1000+ spectrophotometer detector.

2.3. Sanger sequencing

A PCR amplification instrument was utilized to amplify the target fragment of TP53. Amplification cycle conditions were as follows: 95°C for 5 min followed by 40 cycles of 95°C for 1 min, 53°C for 1 min, 72°C for 1 min, and a final elongation at 72°C for 10 min. The samples were purified using a Cycle Pure Kit (D6492-02, Omega Biotek, USA), sequenced with Big Dye Terminator v3.1 kit (Thermo Fisher Scientific, USA), then purified. Finally, sequencing analysis was performed by ABI 3500 gene sequencer.

2.4. RNA extraction and RT-qPCR

Total RNAs were isolated from prostate cancer tissues and cells using TRIzol reagent (Invitrogen, USA), then reversely transcribed into cDNA using PrimeScript TM RT reagent kit (Takara, Japan). Real-time quantitative PCR was assessed by SYBR Green qPCR Master Mix (Thermo Fisher Scientific). Data were normalized to Glyceraldehyde-3-phosphate dehydrogenase (GAPDH), and relative gene expression was calculated by the $2^{-\Delta\Delta Ct}$ method. Primers were shown in Table 2.

2.5. ICGC and cBioportal

Mutated genes in prostate cancer were analyzed by

Table 1. TP53 mutation and clinicopathology in prostate cancer

Parameters	Number of patients	Mutation		
		No	Yes	*P* value
Age				
≤ 68 years	30	10	20	0.397
> 68 years	26	6	20	
History of smoking and alcohol intake				
No	37	11	26	0.789
Yes	19	5	14	
Metastases				
Without	34	13	21	0.047
Present	22	3	19	
Differentiation				
Moderate	25	10	15	0.089
Poor	31	6	25	
TNM stage				
II	42	15	27	0.040
III+IV	14	1	13	
Gleason score				
≤ 7	25	10	15	0.089
> 7	31	6	25	

Table 2. RT-qPCR detection of specific primer sequences for gene expression

Gene	Forward	Reverse
TP53	5'-CAGCACATGACGGAGGTTGT-3'	5'-TCATCCAAATACTCCACACGC-3'
FOXA1	5'-CTACTACGCAGACACGCAGG-3'	5'-CCGCTCGTAGTCATGGTGTT-3'
GAPDH	5'-CGCTCTCTGCTCCTCCTGTTC-3'	5'-ATCCGTTGACTCCGACCTTCAC-3'

the International Cancer Genome Consortium (ICGC) database (*https://dcc.icgc.org/*), which was used to store raw data (*20*). Prostate cancer somatic mutation data were downloaded from the cBio cancer genomics portal platform (cBioportal, *http://www.cbioportal.org/*). cBioportal is a comprehensive open network platform that integrates data mining, data integration, and visualization functions. It includes tumor genome data, the main data types with DNA copy number changes, somatic mutations, DNA methylation, mRNA and microRNA expression, and so on (*21*).

2.6. TCGA database

The Cancer Genome Atlas (TCGA) database (*https://portal.gdc.com*) was used to download the expression of FOXA1 in prostate cancer TP53 mutant (n = 56), TP53 nonmutant (n = 436) and normal (n = 52). Corresponding clinical information for prostate cancer was also obtained.

2.7. UALCAN database

FOXA1 expression in prostate adenocarcinoma (PRAD) based on TP53 mutation status, nodal metastasis status and patients' Gleason score was analyzed through the University of ALabama at Birmingham CANcer (UALCAN) database, noted below (*22*). *http://ualcan.path.uab.edu/cgi-bin/TCGAExResultNew2.pl?genenam=FOXA1&ctype=PRAD*

2.8. TIMER database

Tumor IMmune Estimation Resource (TIMER, (*https://cistrome.shinyapps.io/timer/*) database provides three main analysis modules: Immune, Exploration, and Estimation (*23*). FOXA1 expression in pan-cancer tissues was obtained and analyzed through the TIMER database.

2.9. Cancer Cell Line Encyclopedia dataset

Cancer Cell Line Encyclopedia (CCLE) is a tumor genomics research project led by the Broad Institute. It collects and sorts out the omics data of cell lines (*24*). FOXA1 expression in prostate cancer cell lines was analyzed by the CCLE dataset (*https://portals.broadinstitute.org/ccle/page?gene=FOXA1*).

2.10. Immunohistochemistry

Paraffin-embedded prostate cancer tissues and its paired normal adjacent tissues were deparaffinized and endogenous peroxidase activity was blocked using 3% hydrogen peroxide. After antigen retrieval, tissues were incubated with primary antibody anti-FOXA1 (1:200, HUABIO, Hangzhou HuaAn Biotchnology CO., Ltd, China) at 4°C overnight, followed by the secondary

Table 3. Sequence used for FOXA1-siRNA

siRNA	Sense(5'-3')	Antisense(5'-3')
FOXA1#1	GGAUGUUAGGAACUGUGAATT	UUCACAGUUCCUAACAUCCTT
FOXA1#2	GGACUUCAAGGCAUACGAATT	UUCGUAUGCCUUGAAGUCCTT
FOXA1#3	CCGGCAACAUGUUCGAGAATT	UUCUCGAACAUGUUGCCGGTT
Negative Control	UUCUCCGAACGUGUCACGUTT	ACGUGACACGUUCGGAGAATT

antibody (Cat: PV-9001, ZSGB-BIO, Beijing, China) at 37°C for 30min. Staining was observed with DAB (Cat: ZLI-9019, ZSGB-BIO, Beijing, China). Meanwhile, DP260 Autostainer (Dakewe Biotech Co., Ltd., Beijing, China) was used for hematoxylin and eosin (H&E) staining, according to the manufacturer's instructions.

2.11. Prostate cancer cell lines and cell culture

Two prostate cancer cell lines (DU145 and PC3) were purchased from the Chinese Academy of Sciences Cell Bank (China). Cells were grown in RPMI 1640 medium (Gibco, USA) containing 10% fetal bovine serum (FBS, Invigentech^{TM} USA), and cultured at 37°C in 5% CO_2.

2.12. Small interference RNA (siRNA) transfection

SiRNA targeting FOXA1 and stable negative control were designed and synthesized by Shanghai Generay Biotech Co., Ltd (Shanghai, China). Prostate cancer cells (2×10^5/mL) were seeded in 6-well plates for 24 h. After 70% confluence, cells were transfected with FOXA1-siRNA (100 nmol/L) using Lipofectamine 3000 Transfection Kit (Invitrogen, USA), according to the manufacturer's instructions. Sequences were shown in Table 3.

2.13. Cell migration

Cell migration assays were conducted using transwell chambers. Prostate cancer cells were transfected with FOXA1-siRNA and suspended in 200 μL serum-free medium. Then cells were seeded into the upper chamber of 24-well plate, and the lower chamber was covered with 600 μL medium containing 10% FBS. After incubation for 24 h, cotton swabs were used to remove the cells remaining on the upper membrane. Migrated cells were fixed with 4% paraformaldehyde and stained with 0.1% crystal violet. Finally, cells were photographed under microscope (IX81, OLYMPUS).

2.14. Statistical analyses

Data were presented as mean±standard deviation (SD). Student's *t*-test or one-way analysis of variance (ANOVA) was used for comparing differences between groups. Statistical analyses were performed using SPSS 25.0 software. The Pearson's chi-square test was utilized to evaluate statistical significance between the clinical variables and mutational profile. $P < 0.05$ was considered statistically significant.

3. Results

3.1. TP53 was the main mutated gene in prostate cancer

ICGC analysis showed that TP53 was the main mutated gene in prostate cancer (Figure 1A). Furthermore, calibration frequency of TP53 in 19 prostate cancer-related studies was analyzed through cBioportal database, and mutational information in TP53 was described (mutation and missense) (Figure 1B). The distribution of TP53 mutation was detected by exome sequencing, and results were illustrated on cBioportal database. It also showed that TP53 mutation mainly included missense variant, frameshift variant and stop gained, of which missense mutation was the most common (Figure 1C). The highly conserved sites of TP53 point mutation were R175H, R245H, R248H, R249H, R273H, and R282H (Figure 1D).

3.2. TP53 correlated with prostate cancer metastasis and TNM stage

Notably, TP53 expression differed among different mutation types and copy-number alterations in the cBioportal database (Figure 2A and B). Furthermore, 56 prostate cancer tissues were collected and detected by Sanger sequencing. Heterozygous mutation was found at TP53 rs12947788 site, and the rate was 71.4% (40/56). TP53 rs12947788 mutation was significantly associated with metastasis ($p = 0.047$) and TNM stage ($p = 0.040$), but not with age, history of smoking and alcohol intake, differentiation, or Gleason score (Table 1). These findings revealed that TP53 mutation might be involved in the occurrence and metastasis of prostate cancer.

3.3. TP53 mutation correlated with FOXA1

In a previous study, p53 participated pancreatic cancer metastasis by interacting FOXA1 (*18*). Herein, FOXA1 expression in prostate cancer based on TP53 mutation status was analyzed through UALCAN database. FOXA1 expression in prostate cancer tissues was significantly higher than in normal tissues (Figure 3A). Meanwhile, the level of FOXA1 in TP53-mutant patients was higher than that in TP53-nonmutant patients, which was verified in the TCGA database (Figure 3B). Data

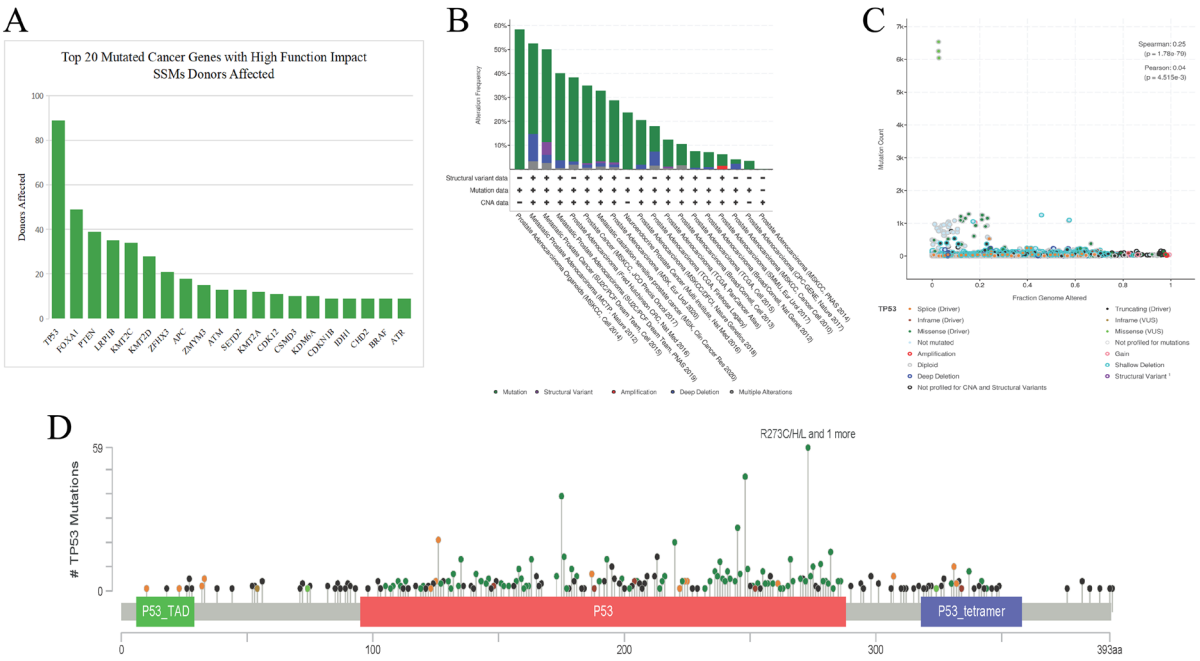

Figure 1. TP53 was the most mutated gene in prostate cancer. (A) Distribution of the mutated genes in prostate cancer from ICGC database. **(B)** Calibration frequency of TP53 in 19 prostate cancer-related studies analyzed by cBioportal database. **(C)** Types of TP53 mutation and its distribution **(D)** in prostate cancer investigated *via* cBioportal database.

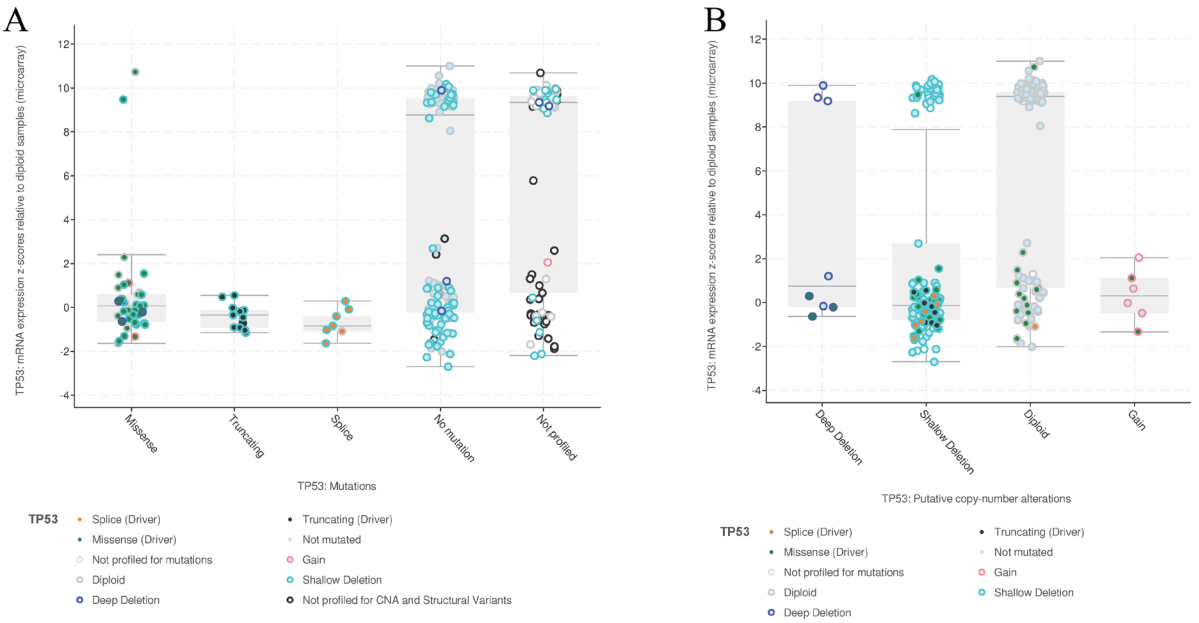

Figure 2. Effect of TP53 mutation and copy number alternation on its expression. Effect of TP53 mutation types **(A)** and copy number alterations **(B)** on mRNA expression from cBioportal database.

from our clinical samples showed that TP53 mutation frequency was 71.4% (Figure 3C). A similar trend with an online database was also investigated (Figure 3D). FOXA1 expression was positively correlated with TP53 mutation, suggesting that TP53 mutation might promote prostate cancer metastasis by regulating FOXA1.

3.4. FOXA1 was up-regulated in prostate cancer and associated with metastasis

FOXA1 expression in pan-cancer tissues and cell lines was analyzed by the TIMER database and CCLE database, respectively. Results illustrated that FOXA1 in tumor tissues was higher than that in normal tissues, especially in prostate cancer (Figure 4A). As well, FOXA1 was more highly expressed in prostate cancer cells than in other cells (Figure 4B). Immunohistochemical experiments from our clinical specimen demonstrated that the level of FOXA1 protein

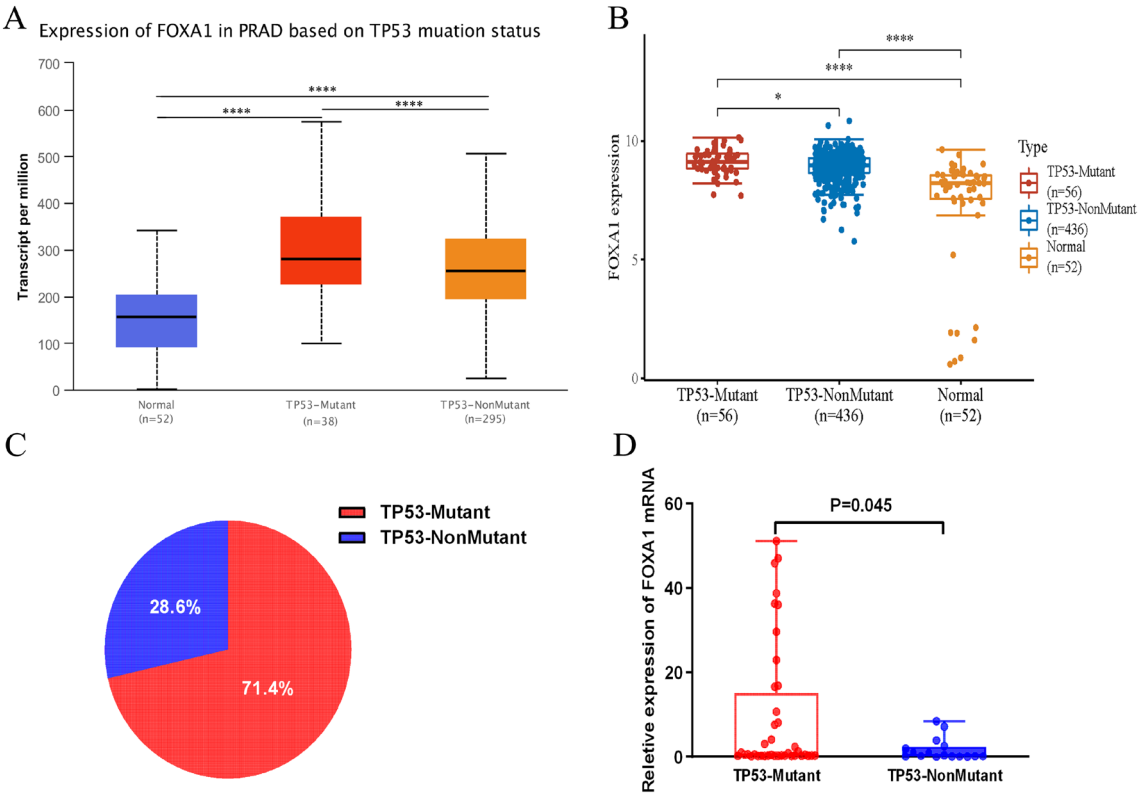

Figure 3. TP53 mutation correlated with FOXA1 expression. (A) FOXA1 expression in prostate cancer based on TP53 mutation status through UALCAN database. **(B)** Gene expression in FOXA1 and TP53 mutation in prostate cancer from TCGA database. **(C)** TP53 mutation was detected by Sanger sequencing and frequency was calculated. **(D)** FOXA1 expression was detected by RT-qPCR in TP53 wildtype (WT) and TP53 mutation (MUT) prostate cancer tissues. *, $P < 0.05$; ****, $P < 0.0001$.

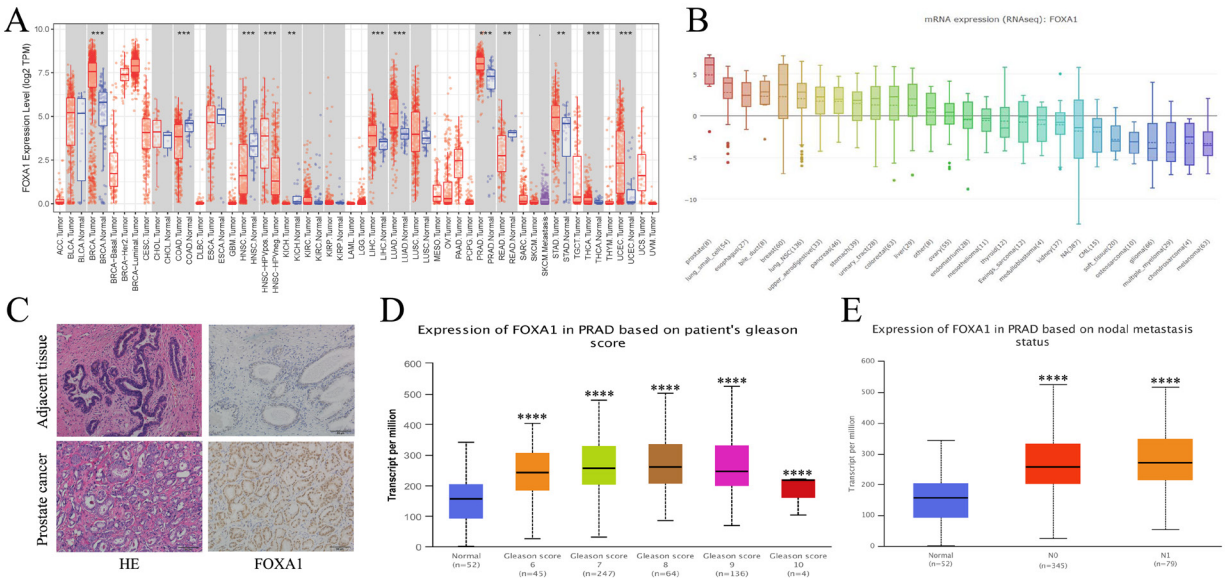

Figure 4. FOXA1 was up-regulated in prostate cancer and associated with tumor metastasis. (A) FOXA1 expression in pan-cancer tissues was assessed through TIMER database. *, $P < 0.05$; **, $P < 0.01$, ***, $P < 0.001$. **(B)** FOXA1 expression in cells from the CCLE database. **(C)** FOXA1 protein level in prostate cancer tissues and its paired normal adjacent tissue by immunohistochemistry (scare bar: 50 μm). **(D)** FOXA1 expression in prostate cancer based on patients' Gleason scores was analyzed by UALCAN database. ****, $P < 0.0001$. **(E)** FOXA1 expression in prostate cancer based on nodal metastasis status by UALCAN database. ****, $P < 0.0001$. N1: 1 to 3 axillary lymph node.

in prostate cancer tissues was significantly higher than in their paired normal adjacent tissues (Figure 4C).

Subsequently, FOXA1 expression in prostate cancer based on patients' Gleason scores and nodal metastasis status was further explored by UALCAN database. FOXA1 expression in prostate cancer with different Gleason scores was higher than in the normal group (Figure 4D). FOXA1 expression was significantly up-

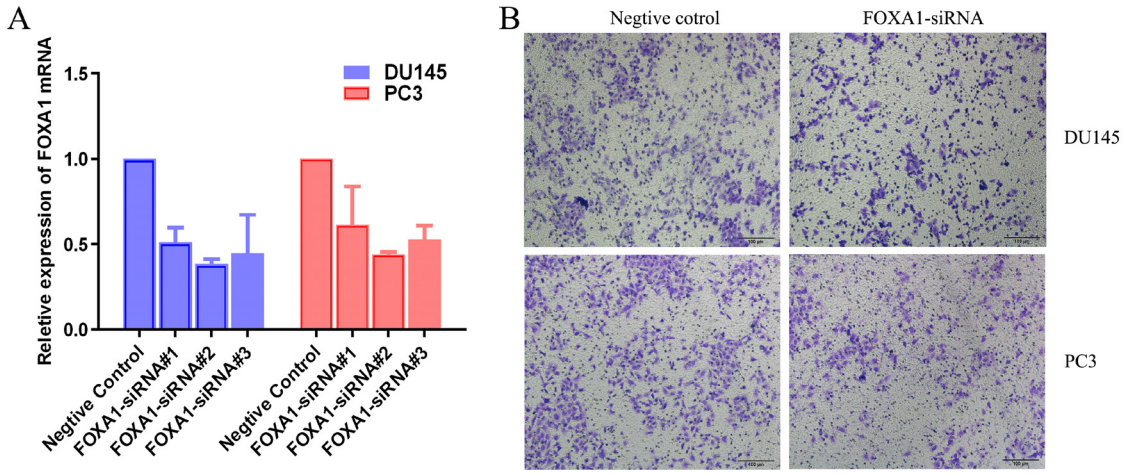

Figure 5. Effect of FOXA1 on the migration of prostate cancer cells *in vitro*. (A) Transfection efficiency of FOXA1-siRNA in prostate cancer cells (DU145 and PC3) detected by RT-qPCR. **(B)** Transfection with FOXA1-siRNA significantly inhibited migration of DU145 and PC3 cells (scare bar: 100 μm).

regulated in prostate cancer lymph node metastases compared with the normal group (Figure 4E).

3.5. Knockdown of FOXA1 inhibited migration of prostate cancer cells *in vitro*

To explore the role of FOXA1 in regulating prostate cancer metastasis, knockdown of FOXA1 by siRNA was performed in prostate cancer cells (DU145 and PC3). The interference efficiency was evaluated using RT-qPCR. Results showed that FOXA1-siRNA#2 was the highest and used in the following experiments (Figure 5A). As reflected by transwell migration, transfection with FOXA1 siRNA could effectively inhibit migration of prostate cancer cells (Figure 5B), which indicated that up-regulation of FOXA1 promoted cell metastasis.

4. Discussion

Prostate cancer is the most frequently diagnosed cancer that seriously affects men's health (*25*). The incidence and mortality rates are closely related to the age (*26*). It is reported that prostate cancer often involves lymph node and/or bone sites metastasis, which causes most cancer-related deaths (*27*).

TP53, a tumor suppressor gene, is frequently altered in various cancers including prostate cancer (*28*). In this study, TP53 was confirmed to be the main gene in prostate cancer with high mutation frequency *via* ICGC and cBioportal databases. It has many mutation sites and types, and missense mutations are dominant. The relationship between gene mutation and tumor development is a complex biological process. TP53 mutations often occur in the central DNA-binding domain such as R249H and R273H and have oncogenic action. The interaction between mutant p53 and most regulatory molecules including p63 and microRNAs affects the stability of those molecules and the crucial molecular pathways involved in invasion and metastasis through regulating Zinc finger E-box binding homeobox 1 (ZEB1) and zinc finger protein 652 (ZNF652) (*10*). Different TP53 mutation types have different effects on TP53 expression, but missense mutation can make TP53 dysfunctional, while nonsense mutation may result in TP53 function loss. Data of exome sequencing from cBioportal database showed that the distribution of TP53 mutations in prostate cancer was very scattered.

As a third-generation genetic marker, a single nucleotide polymorphism (SNP) reflects the genetic differences between individuals, which have provided unique insights into the basis of cancer genetic susceptibility (*29*). Interestingly, men with gene mutations are at an increased risk of metastatic cancer, which has prompted further studies in the field. One study showed that TP53 rs1042522 polymorphism increased the susceptibility of malignant bone tumors (*30*). TP53 Arg72Pro (SNP rs1042522) was significantly associated with the risk of non-Hodgkin lymphoma (*31*). However, the functional link among TP53 polymorphism, causation of biological behavior and prognosis in prostate cancer remains elusive. In the present study, TP53 was found to have mutations in 19 prostate cancer-related studies through the cBioportal database. Our findings from clinical specimens using Sanger sequencing revealed that the rate of TP53 muation was 71.4%, and heterozygous mutation site was at rs12947788.

Prostate cancer is prone to lymphatic spread to locoregional lymph nodes, bone marrow stroma predominantly in the axial skeleton, even distant visceral sites. This is the most lethal form of prostate cancer. Because the mechanism is poorly understood, there is no effective treatment for prostate cancer. Further analysis showed that TP53 mutation was significantly associated with metastasis and TNM stage. It was consistent with a previous report that TP53 mutations could enhance

early prognostication of prostate cancer progression (*32*). Deletion of wild-type p53 promoted prostate cancer cells metastasis to bones by regulating the C-X-C chemokine receptor type 4/ C-X-C motif chemokine 12 (CXCR4/ CXCL12) activity (*33*). This suggested that elucidating the downstream mechanism of TP53 mutation would help us find a promising therapeutic strategy.

FOXA1 is a pioneer transcription factor and essential for various type of tumor progression, including liver, bladder, prostate, and lung cancer (*34*). Several studies have shown that FOXA1 is a potential prognostic biomarker in prostate cancer (*35,36*) and has been implied to promote androgen-dependent prostate cancer growth (*37*). This suggests that FOXA1 might be a novel therapeutic strategy for prostate cancer. A previous study has revealed that targeting FOXA1-mediated transforming growth factor-beta (TGF-β) signaling can effectively suppress castration-resistant prostate cancer progression (*38*). Multiple pro-angiogenic factors induced by FOXA1 can promote prostate cancer angiogenesis (*16*). However, the mechanism of FOXA1 in regulating prostate cancer metastasis still remains unclear.

Our results demonstrated that FOXA1 expression was high in prostate cancer patients and cells, and significantly up-regulated in Gleason score and lymph node metastases. This may provide a strategy for assigning risk in combination with FOXA1 and Gleason scores. Furthermore, the level of FOXA1 in TP53-mutant patients was higher than in TP53-nonmutant patients. This funding was verified by data from our clinical specimen and UALCAN and TCGA database. Previous research has shown that GATA binding protein 3 (GATA3) mutations can disrupt localization of estrogen receptor-alpha (ER-α) and FOXA1 in breast cancer (*39*). In this study, TP53 mutations may lead to aberrant transcription factor localization and change in FOXA1 downstream transcriptional networks. Based on these results, we speculated that TP53 mutation and FOXA1 might functionally converge in modulating prostate cancer tumorigenesis and metastasis. However, the current study provides no evidence regarding the underlying molecular mechanism by which TP53 mutations may regulate FOXA1 in prostate cancer metastasis.

To further clarify this issue, the clinical significance of FOXA1 in normal and cancerous tissues from prostate cancer, as well as the function of FOXA1 in the regulation of tumor cell migration *in vitro* were investigated. Results showed that FOXA1 knockdown might inhibit prostate cancer cell migration. This may be related to some pathways, such as the repression of TGF-β signaling, androgen receptor pathway. Future research will investigate these mechanisms further.

In summary, our study illustrated that TP53 was the mutation gene with high frequency in prostate cancer and rs12947788 which were the main sites. FOXA1 was highly expressed in prostate cancer, especially in TP53-mutant patients, and was highly associated with Gleason scores and metastasis. Moreover, we confirmed that FOXA1 was significantly up-regulated in prostate cancer tissues, and knockdown of FOXA1 significantly suppressed migration in prostate cancer cells. This suggested that TP53 and FOXA1 might be promising therapeutic targets for inhibiting prostate cancer metastasis. However, the limitations of this study still exist, including its retrospective nature and relatively few patients. Future work will look to verify these results in multicenter studies with larger sample sizes.

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Brief Report

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Needs of cancer patients during the SARS-CoV-2 Omicron lockdown: A population-based survey in Shanghai, China

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SUMMARY The aim of this study was to investigate the medical and healthcare needs of cancer patients during the Shanghai lockdown due to the SARS-CoV-2 Omicron pandemic. From April 15 to April 21, 2022, 4,195 cancer patients from every district in Shanghai were surveyed using quota sampling via an online platform. The questionnaire consisted of three main parts: demographic and sociological data, disease diagnosis, and different dimensions of patients' needs. Correlation analysis was used to examine the relationship between participants' need scores in each dimension, and generalized linear regression models were used to analyze the factors influencing patients' need scores. The mean age of participants was 63.23 years (SD: 7.43 years), with more female than male participants (80.38% *vs*. 19.62%). Among participants, the three leading groups of patients were those with breast cancer (39.02%), colorectal cancer (12.82%), or tracheal and bronchial lung cancer (10.23%). Social support, dietary/nutritional support, and psychological counselling ranked as the top three needs of cancer patients. In addition, vaccination against SARS-CoV-2 may reduce psychological anxiety in cancer patients. Compared to participants who had never received the SARS-CoV-2 vaccine, participants who had received one, two, or three doses of the vaccine were respectively 36% (odds ratio (OR): 0.64, 95% confidence interval (CI): 0.56-0.73), 38% (OR: 0.62, 95% CI: 0.59-0.54), and 37% (OR: 0.63, 95% CI: 0.60-0.66) less likely to have an increased need for psychological counseling. In light of constraints on offline medical resources for cancer patients during the lockdown, the current authors have begun to re-examine the universal accessibility and spread of telemedicine in the future. In addition, immune barriers can be established for cancer patients and vaccination guidelines for different disease stages, tumor types, and treatment regimens can be explored in detail.

Keywords Shanghai lockdown, SARS-CoV-2 Omicron, cancer patients, needs

1. Introduction

The SARS-CoV-2/COVID-19 Omicron variant was first identified in November 2021 in Botswana and South Africa (*1*). Although immunological and clinical data did not provide definitive evidence, the omicron variant displayed early signs of high transmissibility, reduced severity, and immune escape, potentially increasing the difficulty of controlling the pandemic (*2,3*). In late February 2022, a wave of omicron BA.2 infection rapidly appeared in Shanghai, China. Shanghai is one of the most important international economic, financial, trade, and shipping centers in China, with a resident population of more than 25 million. According to the Shanghai Municipal Health Commission, from February 26 to May 31, 2022, 58,000 cases were reported, and 588 people died with or from the omicron variant of SARS-CoV-2 (*4*). To reduce the spread of the pandemic, Shanghai imposed a lockdown with movement restrictions, social distancing, and home confinement starting April 1, 2022.

Cancer patients endured multiple challenges in terms of infection risk, prognostic outcomes, and tumor recurrence during the COVID-19 pandemic (*5*). Huang *et al*. (*6*) reported that the 30-day mortality was higher in COVID-19 patients with cancer and that patients with both cancer and cardiovascular disease (CVD) have significantly increased Pro-BNP and D-Dimer levels. Dai *et al*. (*7*) provided evidence that COVID-19 patients with cancer had a higher risk for all severe outcomes. Patients with hematologic cancer, lung cancer, or with metastatic cancer (stage IV) had the highest frequency of severe events. In addition, delays in early tumor screening, detection, monitoring of recurrence, and treatment may potentially have a negative impact on the outcomes for cancer patients during the COVID-19 pandemic. An observational/modeling study reported

that delays in surgery for incident cancers of 3-6 months would decrease life-years gained by said surgery by 19% and 43%, respectively, and by 26% and 59% when considering resource-adjusted life-years gained (*5,8*).

Shanghai had 79,000 new cancer cases and 490,000 existing patients in 2021, with an overall prevalence of 3% (*9*). The number of patients is correlated with the healthcare resources required by the patient. Unfortunately, the strict lockdown in Shanghai disrupted the normal life of the public, and medical resources were overwhelmed by patients with the Omicron variant of SARS-CoV-2. Since some cancer patients are elderly and there is no immune barrier, they may face many difficulties in such dire situations. Formulating supportive care strategies for cancer patients will be on the agenda as soon as it is feasible (*10,11*). However, few studies have surveyed the needs of cancer patients and few have provided valid evidence on related topics.

To investigate the medical and healthcare needs of cancer patients during the Shanghai lockdown, 4,195 cancer patients from every district in Shanghai were surveyed using a quota sample. The hope is that this study will provide evidence to support the formulation of scientific plans for public health emergencies in megacities in the future. As the pandemic rages around the world, further analysis of the impact of COVID-19 on cancer patient needs and healthcare delivery systems will be essential in order to better tailor the management of cancer patients and minimize disruptions to cancer care.

2. Materials and Methods

2.1. Study design and data collection

From April 15 to April 21, 2022, cancer patients in 16 districts in Shanghai were surveyed with the help of volunteers from the Shanghai Cancer Rehabilitation Club during the Shanghai lockdown. Quota sampling, which improves the representativeness of a sample by determining the sample size of various (tiers) units and randomly selecting samples within the quota, was used. Surveyors were recruited and trained in each district of Shanghai. Three hundred questionnaires were distributed to each district in Shanghai *via* an online platform, and the quality of data was managed by filtering IPs, time limits, *etc*. After all the questionnaires were returned by the surveyors, members of the research team checked them again. A total of 4,900 questionnaires were distributed in this study, 4,221 were returned, 5 invalid questionnaires were excluded, and 4,195 questionnaires were finally included in the statistical analysis, for a valid response rate of 99.4%.

Inclusion criteria for study participants were: *i*) adults over the age of 18 who have been diagnosed with cancer; *ii*) in the stable or convalescent stage but not in the acute stage; *iii*) and residing in Shanghai for the last three months.

This study was approved by the ethics committee of the Shanghai Health and Health Development Research Center (Shanghai Institute of Medical Science and Technology Information), approval no.: SHDRC2022005. All participants provided written informed consent. The details of the questionnaire can be obtained by contacting the corresponding author.

2.2 Questionnaire

The questionnaire on the needs of cancer patients during public health emergencies used in this study was designed by the research team based on the literature and advice from relevant experts (*12-14*). The questionnaire has three parts: *i*) demographic and sociological data, including age, gender, and level of education; *ii*) disease diagnosis, including disease diagnosis, staging, and the treatment plan; and *iii*) patient needs in 9 dimensions, namely outpatient and emergency medical care, drug supply, nursing care, online medical care, COVID-19 infection concerns, dietary/nutritional support, approval to visit a medical facility for treatment, and psychological counseling. Since different numbers of questions needed to be designed in accordance with the specifics of each dimension to reflect the patient's actual situation, weighted factor scoring was used to evaluate the need score in each dimension, and the total need in each dimension was given a score of 3 points. The magnitude of the score reflects the degree of participant need.

2.3 Statistical analyses

Quantitative data (such as need scores) with a normal distribution were expressed as the mean ± standard deviation (SD). Qualitative data (such as gender and marital status) were expressed as a value or percentage. A correlation analysis was performed using a nonparametric rank sum test on the need scores of different categories of patients. Dichotomous variables (such as gender) were analyzed using a *t*-test; three or more categories were analyzed using variance analysis or a nonparametric test.

Cancer patients' need scores in different dimensions served as the dependent variable, and a set of variables served as independent variables based on a review of the results of multiple studies and previous univariate analyses. Multivariate linear regression analysis was performed using a generalized linear regression model.

All statistical analyses in this study were performed using the software IBM SPSS Statistics 21.0 and R Studio 4.0.2, and $p < 0.05$ was considered statistically significant.

3. Results and Discussion

Table 1 summarizes the characteristics of study participants by gender. A total of 4,195 participants were

included in this study. The mean age of participants was 63.23 years (SD: 7.43 years). There were more female than male participants (80.38% *vs*.19.62%). Among participants, the three leading groups of patients were those with breast cancer (39.02%), colorectal cancer (12.82%), or tracheal and bronchial lung cancer (10.23%). Fewer patients had metastatic cancer than primary cancer (82.26% *vs*.5.50%). Detailed participant disease information is shown in Table S1 (*http://www.biosciencetrends.com/action/getSupplementalData.php?ID=101*).

The high number of female participants in this study may have led to bias, but the types of cancer in the study participants include 25 types commonly classified by site, and the needs they reflect are representative, so the rigor of the results may not have been seriously affected.

Figure 1 shows information on the physical health status of the study participants, which mainly includes disease stage, treatment regimen, and the number of doses of the COVID-19 vaccine. Patients in the early stages of disease (stage I and stage II) accounted for more than half of the total participants. More than a quarter of the patients used Chinese medicine in their recovery, and 17% did not require treatment now. Worryingly, 54% of survey participants were never vaccinated with the COVID-19 vaccine, and only 25% of survey participants completed the full three-dose vaccination.

In Shanghai, the overall vaccination rate for the entire population is over 90%, but the rate for the elderly is 62% and only 38% have received a booster

Table 1. Demographic and sociological information on study participants

Characteristic	Males	Females	All participants
No. of participants	823	3,372	4,195
Age (years)			
18-44	11 (1.3)	54 (1.6)	65 (1.5)
45-59	108 (13.1)	993 (29.4)	1,101 (26.2)
60-74	625 (75.9)	2,201 (65.3)	2,826 (67.4)
> 75	79 (9.6)	124 (3.7)	203 (4.8)
Marital status			
Married	750 (91.1)	2,886 (85.6)	3,636 (86.7)
Single/widowed	73 (8.9)	486 (14.4)	559 (13.3)
Level of education			
< 9 years	341 (41.4)	1,592 (47.2)	1,933 (46.1)
9-12 years	302 (36.7)	1,343 (39.8)	1,645 (39.2)
> 12 years	180 (21.9)	437 (13.0)	617 (14.7)
Employment status			
Employed	39 (4.7)	123 (3.6)	162 (3.9)
Retired	727 (88.3)	2,926 (86.8)	3,653 (87.1)
Unemployed	57 (6.9)	323 (9.6)	380 (9.1)
Physical activity			
Extremely active	254 (30.9)	581 (17.2)	835 (19.9)
Highly active	147 (17.9)	597 (17.7)	744 (17.7)
Moderately active	336 (40.8)	1,415 (42)	1,751 (41.7)
Sedentary	86 (10.4)	779 (23.1)	865 (20.6)
Average monthly income (RMB)			
< 3,000	187 (22.7)	790 (23.4)	977 (23.3)
3,001-6,000	450 (54.7)	1,926 (57.1)	2,376 (56.6)
6,001- 9,000	129 (15.7)	448 (13.3)	577 (13.8)
> 9,000	57 (6.9)	208 (6.2)	265 (6.3)
Medical insurance			
Basic medical insurance	272 (33.0)	1,195 (35.4)	1,467 (35.0)
Employee medical insurance	526 (63.9)	2,010 (59.6)	2,536 (60.5)
Commercial medical insurance	20 (2.4)	161 (4.8)	181 (4.3)
None	5 (0.6)	6 (0.2)	11 (0.3)
Tumor status			
Primary tumor	689 (83.7)	2,762 (81.9)	3,451 (82.3)
Metastatic tumor	53 (6.4)	178 (5.3)	231 (5.5)
Not sure	81 (9.8)	432 (12.8)	513 (12.2)

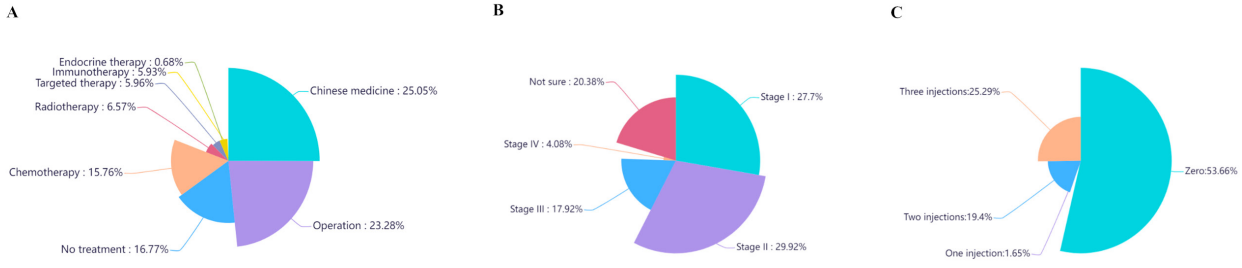

Figure 1. The current status of (A) participant's treatment, (B) disease stage, and (C) COVID-19 vaccination.

Table 2. The different dimensions of study participants' need scores and the top two needs for each dimension score

No.	Dimension	Entry	Mean	SD
1	Social support		0.58	0.47
		Financial support	0.57	0.45
		Volunteer services	0.49	0,37
2	Dietary/nutritional support		0.48	0.36
		Purchase of nutritious meals	0.52	0.38
		Need nutritional guidance	0.48	0.29
3	Psychological counseling		0.39	0.31
		Anxiety and depression	0.42	0.32
		Panic	0.40	0.36
4	Outpatient and emergency medical care		0.33	0.20
		Make appointments with doctors	0.43	0.22
		Ambulance	0.32	0.30
5	COVID-19 infection concerns		0.32	0.49
		Risk of infection	0.41	0.34
		Temporary hospital closure	0.30	0.27
6	Drug supply		0.18	0.43
		Logistical interruptions	0.22	0.41
		Purchasing restrictions	0.13	0.43
7	Approved to visit a medical facility for treatment		0.18	0.44
		Complicated pass procedures	0.20	0.33
		Public transportation/travel suspended	0.17	0.36
8	Online medical treatment		0.15	0.14
		Risk of misdiagnosis, missed diagnosis	0.17	0.16
		Inability to operate	0.10	0.41
9	Nursing care		0.14	0.10
		Purchase of medical devices (e.g., PICC tubes)	0.15	0.18
		Interrupted continuity of care	0.13	0.22

(*11*). Cancer patients have lower vaccination rates compared to the general population, but vaccine hesitancy in this susceptible population is influenced by multiple factors. Di Noia *et al*. (*15*) found that the most common reasons for vaccine refusal were fear of adverse events related to the vaccine (48%), negative interactions with concomitant antineoplastic therapy (27%), and fear of allergic reactions (11%). These concerns, along with the lack of guidance from oncologists and information about the safety and efficacy of COVID-19 vaccines and the inability of primary care physicians to meet patients' counseling needs, are the most common factors associated with cancer patients' vaccine hesitancy (*16-18*).

As shown in Table 2, the greatest need for supportive patient care in each dimension was the social support dimension (mean (SD): 0.58 (0.47)), followed by dietary/nutritional support (mean (SD):0.48 (0.36)) and psychological counseling (mean (SD): 0.39 (0.31)). Outpatient emergencies (mean (SD): 0.33 (0.20)) were the most prevalent of the three dimensions of medical care, and participants had a significantly greater need for doctor appointments (mean (SD): 0.43 (0.22)). Table S2 (*http://www.biosciencetrends.com/action/getSupplementalData.php?ID=101*) shows the correlation between participants' need scores in each dimension.

Social support is a multidimensional concept that may be defined as "the aid – the supply of tangible or intangible resources – individuals gain from their network members" (*19*). The current results indicated that social support was a top need of cancer patients during the Shanghai lockdown. Numerous studies corroborate this finding. A longitudinal study conducted in Germany reported that social support factors were strongly associated with all quality of life indicators. Compared to 0-3 social contacts per week, ten or more social contacts were associated with a 70% reduction in the risk of more depressive symptoms, a 39% reduction in the risk of more anxiety symptoms, while increasing the chance of increased well-being by 73% (*20*). In Australia, a national coalition – Ending Loneliness Together – has been established to bring together researchers and care providers, and this interdisciplinary collaboration between social science and clinical medicine is important for public issues such as the lack of social support that existed prior to the COVID-19 pandemic and now (*21*).

The current results indicated that participants had less of a need for online medical care (mean (SD): 0.15 (0.14)). One possible reason was that online hospitals could not meet the needs of cancer patients (Table 2). In 2018, China began to develop an "online medical/healthcare" system, providing online services such as medical appointments, follow-up of chronic conditions, and telemedicine. The global COVID-19 pandemic has occasioned a transition from "face-to-face" to "online and offline" healthcare. As of June 2021, China has more than 1,600 online hospitals, and healthcare locations are expanding from hospitals to cover prevention, treatment, and rehabilitation (*22*). However, the development of online medicine faces issues such as the scope of physician practice, the use of telemedicine tools, and reimbursement of expenses (*23*). The current COVID-19 pandemic is again providing a reminder of the importance of using telehealth to deliver care, and especially as a means of reducing the risk of cross-contamination caused by close contacts (*24*). Findings from a cohort study

Table 3. Results of univariate regression analysis of study participants' need scores

Groups	Need score								
	Outpatient and emergency medical care	Drug supply	Nursing care	Online medical treatment	COVID-19 infection concerns	Dietary/nutritional support	Approved to visit a medical facility for treatment	Psychological counseling	Social support
Gender									
Males	0.35	0.18	0.14	0.17	0.25	0.48	0.25	0.30	0.49
Females	0.32	0.18	0.14	0.15	0.34*	0.46	0.16*	0.41*	0.46*
Age (years)									
18-44	0.48	0.28	0.18	0.20	0.34	0.61	0.25	0.48	0.51
45-59	0.33	0.17	0.15	0.13	0.34	0.45	0.14	0.40	0.49
60-74	0.33	0.18	0.13	0.16	0.32	0.47	0.19	0.39	0.45
> 75	0.29	0.16	0.18	0.21*	0.22*	0.40*	0.16*	0.31	0.44
Marital status									
Married	0.32	0.18	0.13	0.15	0.31	0.46	0.18	0.38	0.45
Unmarried/widowed	0.37*	0.19	0.18*	0.16	0.36*	0.50	0.18	0.46*	0.53*
Education level									
< 9 years	0.28	0.17	0.14	0.14	0.33	0.47	0.17	0.35	0.45
9-12 years	0.34	0.19	0.13	0.15	0.31	0.45	0.16	0.41	0.48
> 12 years	0.45*	0.20	0.16	0.20*	0.31	0.49	0.24*	0.45*	0.49
Working status									
Employed	0.39	0.19	0.15	0.22	0.28	0.47	0.21	0.43	0.44
Retired	0.33	0.18	0.13	0.15	0.32	0.46	0.18	0.39	0.46
Unemployed	0.29	0.13	0.17	0.10*	0.34	0.53	0.16	0.37	0.54*
Physical activity									
Sedentary	0.37*	0.23*	0.17*	0.18*	0.33	0.48	0.21	0.44*	0.49
Extremely active	0.28	0.14	0.14	0.14	0.34	0.48	0.18	0.36	0.46
Highly active	0.28	0.18	0.13	0.13	0.31	0.46	0.16	0.37	0.43
Moderately active	0.36	0.17	0.13	0.15	0.31	0.46	0.17	0.39	0.47
Average monthly income (RMB)									
< 3,000	0.29	0.17	0.15	0.15	0.34	0.53	0.17	0.36	0.51
3,001-6,000	0.35	0.19	0.14	0.15	0.32	0.46	0.19	0.39	0.47
6,001-9,000	0.31	0.16	0.12	0.16	0.31	0.42	0.15	0.41	0.36
> 9,000	0.35	0.17	0.13	0.14	0.28	0.37*	0.20	0.41	0.41*

*p-value < 0.05

Table 4. Results of generalized linear regression models for multifactor analysis of study participants' need scores

Groups	OR (95% CI)								
	Outpatient and emergency medical care	Drug supply	Nursing care	Online medical treatment	COVID-19 infection concerns	Dietary/ nutritional support	Approved to visit a medical facility for treatment	Psychological counseling	Social support
Age									
18-44	Reference								
45-59	0.92 (0.79-1.08)	0.90 (0.80-1.00)	0.99 (0.90-1.09)	0.97 (0.88-1.08)	0.99 (0.87-1.13)	0.90 (0.79-1.02)	0.94 (0.84-1.05)	0.96 (0.83-1.12)	0.87 (0.77-0.98)*
60-74	0.90 (0.77- 1.06)	0.90 (0.81-1.02)	0.98 (0.90-1.08)	1.01 (0.91-1.13)	0.98 (0.86-1.12)	0.93 (0.82-1.06)	0.99 (0.88-1.11)	0.97 (0.84-1.13)	0.84 (0.74-0.95)*
> 75	0.84 (0.70-1.00)*	0.88 (0.78- 1.00)	1.03 (0.92-1.15)	1.05 (0.93-1.19)	0.90 (0.78-1.04)	0.85 (0.74-0.99)*	0.93 (0.82-1.06)	0.90 (0.76-1.06)	0.83 (0.72-0.95)*
Gender									
Males	Reference								
Females	0.97 (0.92-1.02)	0.99 (0.95-1.02)	0.99 (0.96-1.02)	0.99 (0.96-1.02)	1.08 (1.04-1.13)*	0.99 (0.95-1.03)	0.91 (0.89-0.95)*	1.12 (1.07-1.17)	0.96 (0.92-1.00)*
Marital status									
Married	Reference								
Single/widowed	1.02 (0.97-1.08)	1.00 (0.96-1.04)	1.03 (1.00-1.07)	1.00 (0.96-1.03)	1.03 (0.98-1.07)	1.04 (1.00-1.08)	0.99 (0.95-1.03)	1.03 (0.98-1.08)	1.01 (0.97-1.05)
Education level									
< 9 years	Reference								
9-12 years	1.04 (0.99-1.08)	1.00 (0.97-1.03)	0.99 (0.96-1.01)	0.99 (0.96-1.01)	0.98 (0.95-1.02)	0.99 (0.96-1.03)	0.98 (0.95-1.01)	1.01 (0.97-1.05)	1.01 (0.98-1.04)
> 12 years	1.15 (1.08-1.22)*	1.01 (0.97-1.06)	1.01 (0.97-1.05)	1.03 (0.99-1.07)	1.02 (0.97-1.07)	1.06 (1.01-1.12)*	1.06 (1.01-1.11)*	1.08 (1.03-1.15)*	1.03 (0.98-1.08)
Working status									
Employed	Reference								
Retired	1.00 (0.90-1.11)	1.02 (0.94-1.09)	0.99 (0.93-1.06)	0.92 (0.86-0.99)*	1.04 (0.95-1.13)	1.01 (0.92-1.09)	0.97 (0.90-1.05)	0.97 (0.89-1.07)	1.05 (0.97-1.13)
Unemployed	0.97 (0.87-1.09)	0.95 (0.88-1.04)	1.03 (0.96-1.11)	0.88 (0.82-0.95)*	1.04 (0.94-1.14)	1.04 (0.95-1.14)	0.96 (0.89-1.05)	0.96 (0.86-1.06)	1.04 (0.95-1.14)
Physical activity									
Sedentary	Reference								
Extremely active	0.95 (0.90-1.01)	0.91 (0.88-0.96)*	0.96 (0.92-1.00)*	0.95 (0.91-1.00)*	1.02 (0.97-1.07)	0.98 (0.93-1.03)	0.95 (0.91-1.00)*	0.98 (0.92-1.04)	1.06 (1.01-1.12)*
Highly active	0.94 (0.89-1.00)	0.95 (0.91-0.99)*	0.95 (0.92-0.99)*	0.95 (0.91-0.99)*	0.99 (0.94-1.04)	0.97 (0.93-1.02)	0.95 (0.91-1.00)*	0.97 (0.92-1.03)	1.05 (1.00-1.11)*
Moderately active	0.99 (0.94-1.04)	0.94 (0.90-0.97)*	0.95 (0.92-0.98)*	0.97 (0.93-1.00)*	0.99 (0.95-1.03)	0.97 (0.94-1.01)	0.95 (0.92-0.99)*	0.98 (0.93-1.02)	1.04 (1.00-1.07)*
Average monthly income (RMB)									
< 3,000	Reference								
3,001-6,000	1.03 (0.98-1.07)	0.99 (0.95-1.02)	1.00 (0.97-1.03)	0.97 (0.94-1.01)	0.99 (0.95-1.03)	0.93 (0.90-0.97)*	1.00 (0.97,1.04)	1.00 (0.95-1.04)	1.04 (1.01-1.08)*
6,001-9,000	0.95 (0.89-1.02)	0.95 (0.90-1.00)*	0.97 (0.93-1.01)	0.97 (0.93-1.02)	0.98 (0.93-1.04)	0.88 (0.84-0.93)*	0.94 (0.90,0.99)*	1.02 (0.96-1.08)	1.06 (1.00-1.12)*
> 9,000	0.99 (0.91-1.08)	0.96 (0.90-1.03)	0.98 (0.93-1.04)	0.95 (0.90-1.01)	0.95 (0.89-1.02)	0.84 (0.79-0.90)*	0.99 (0.93,1.06)	1.02 (0.94-1.11)	1.04 (0.97-1.11)
Disease staging									
Stage I	Reference								
Stage II	1.01 (0.96-1.05)	1.01 (0.97-1.04)	1.01 (0.98-1.03)	1.00 (0.97-1.03)	1.03 (0.99-1.07)	1.00 (0.97-1.04)	1.01 (0.97-1.04)	1.01 (0.96-1.05)	1.00 (0.97-1.04)
Stage III	1.07 (1.01-1.13)*	1.06 (1.02-1.09)*	1.02 (0.99-1.06)	1.02 (0.99-1.06)	1.03 (0.99-1.08)	1.07 (1.02-1.12)*	1.05 (1.01-1.09)*	1.08 (1.02-1.14)*	1.02 (0.97-1.06)
Stage IV	1.13 (1.02-1.23)*	1.15 (1.07-1.23)*	1.21 (1.14-1.28)*	1.07 (1.01-1.14)*	0.98 (0.90-1.06)	1.07 (1.00-1.16)	1.03 (0.96-1.11)	1.09 (1.00-1.20)	0.91 (0.85-0.99)*
Not sure	1.01 (0.96-1.06)	1.03 (0.99-1.07)	1.05 (1.02-1.08)*	1.02 (0.98-1.05)	1.00 (0.96-1.05)	1.04 (0.99-1.08)	1.02 (0.99-1.06)	1.00 (0.95-1.05)	0.97 (0.93-1.01)
COVID-19 Vaccine									
Zero	Reference								
One injection	0.88 (0.76-1.02)	1.04 (0.93-1.15)	0.94 (0.86-1.03)	0.95 (0.86-1.04)	0.94 (0.84-1.05)	1.01 (0.90-1.14)	1.03 (0.92-1.14)	0.64 (0.56-0.73)*	1.00(0.90-1.12)
Two injections	0.92 (0.88-0.97)*	0.93 (0.90-0.96)*	0.97 (0.94-1.00)*	0.97 (0.93-1.00)*	0.93 (0.90-0.97)*	1.09 (1.05-1.14)*	1.00 (0.96-1.03)	0.62 (0.59-0.64)*	1.04 (1.00-1.07)*
Three injections	0.90 (0.85-0.93)*	0.90 (0.88-0.93)*	0.95 (0.92-0.98)*	0.93 (0.90-0.96)*	0.89 (0.85-0.91)*	1.08 (1.04-1.12)*	0.96 (0.93-1.00)*	0.63 (0.60-0.66)*	1.06 (1.03-1.11)*

*p-value < 0.05

support the value proposition of virtual care (the delivery of telehealth *via* information and communication technology), as it minimized disruptions to patient care during the COVID-19 pandemic (*25*). A scoping review, which identified and included 66 studies, reported that digital solutions can be integrated into routine supportive care in oncology practice to provide improved patient-centered care (*26*). Telehealth visits are appropriate if the primary reason for a cancer patient's visit is to follow up on adherence to oral medications, survival, genetic counseling, support services, or education (*27*). With the help of the 5G network and artificial intelligence, the adoption of telemedicine needs to be expanded and the accessibility of online healthcare in China needs to be improved.

Tables 3 and 4 respectively show the results of univariate and multivariate regression analysis. Disease stage is a factor influencing cancer patients' need scores, for example, participants in stage III of disease were 1.07 times more likely to have an increased need for outpatient medical care than patients in the early stages of disease (stage I). In addition, an increase in the number of patients receiving a COVID-19 vaccine was associated with less need for medical care. Participants who had received one, two, or three doses of a COVID-19 vaccine were 36% (OR: 0.64, 95% CI: 0.56-0.73), 38% (OR: 0.62, 95% CI: 0.59-0.54), and 37% (OR: 0.63, 95% CI: 0.60-0.66) less likely to have an increased need for psychological counseling, respectively, compared to participants who had never received a COVID-19 vaccine.

A study of 1,129 breast cancer patients at a cancer center in Taiwan yielded results similar to the current findings, and the study reported that patients with stage II, III, or IV breast cancer had significantly fewer nutritional needs than patients with stage I cancer (*28*). A study in Italy confirmed the dynamic nature of cancer patients' needs, emphasizing that individual unmet needs differ significantly in different stages (*29*). However, previous studies have paid less attention to comparing changes in the needs of cancer patients due to epidemic lockdowns, and there is still insufficient evidence from real-time population studies.

The current findings provide evidence that vaccination with a COVID-19 vaccine reduced the psychological needs of cancer patients during the Shanghai lockdown, but the relevant evidence is still mixed. Like the current study, a Polish study of 1,696 participants reported that COVID-19 vaccination reduced the level of anxiety about being infected and anxiety due to COVID-19 (*30*). Another study in the United States found that vaccinated participants were 15% less likely to be anxious (adjusted odds ratio [AOR]: 0.85, 95% CI: 0.83-0.90) and 17% less likely to be depressed (AOR: 0.83, 95% CI: 0.79-0.85) compared to those who were not vaccinated (31). In contrast, Voss *et al*. noted that state anxiety levels did not differ significantly before, during, and after vaccination. Although anxiety levels tended to decrease after vaccine approval, the decrease was not significant (*32*). Vaccination against COVID-19 is a key step in establishing a universal immune barrier (*33,34*), and its unique role in the psychological domain also warrants examination in depth.

In conclusion, the needs of a large number of cancer patients cannot be ignored while fighting the COVID-19 epidemic. Telemedicine should allow the practice of patient-centered care and provide greater convenience and accessibility. More findings based on quality evidence can facilitate vaccine development and clinical trials while drafting more detailed guidelines for vaccinating cancer patients to build an immune barrier.

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Treating patients infected with the SARS-CoV-2 Omicron variant with a traditional Chinese medicine, Shufeng Jiedu capsule

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SUMMARY Patients infected with the Omicron variant of SARS-CoV-2 mainly develop mild COVID-19, manifesting as upper respiratory symptoms, fatigue, and fever. Shufeng Jiedu capsule (SFJDC), a traditional Chinese medicine indicated for treatment of upper respiratory infections in China, was tested for its efficacy and safety in treatment of an Omicron infection at a mobile cabin hospital in response to an outbreak of COVID-19 in Shanghai, China in April 2022. In this open-label, randomized controlled trial, patients in the control group received best supportive care, while those in the test group received additional SFJDC therapy for 7 days. SFJDC markedly alleviated patients' symptoms including a sore throat, coughing, fatigue, and a fever after 7 days of treatment. The virus negative time was significantly shorter in the SFJDC treatment group, but there were no obvious differences in the virus negative rate between the two groups at the end of the 7-day follow-up. These results suggest that patients with the Omicron infection may benefit from SFJDC treatment. Double-blind, randomized controlled trials are warranted to comprehensively evaluate the efficacy and safety of SFJDC in a large cohort study in the future.

Keywords Shufeng Jiedu capsule, Omicron, COVID-19, fatigue, fever, cough

The coronavirus disease 2019 (COVID-19) has been a global epidemic for over two years, and multiple mutations of the SARS-CoV-2 virus have occurred (*1*). The Omicron variant was first identified in Africa in November 2021 and is believed to be a very highly transmissible variant (*2*). Analysis of the genomic sequences of Omicron has revealed a number of non-synonymous mutations, several of which have been proven to be associated with transmissibility, disease severity, and immune escape (*3*). Omicron had been reported to have lower replication competence in lung parenchyma compared to earlier variants and to lead to less severe illness in patients (*4,5*). Upper respiratory infection symptoms such as a sore throat and coughing are reported to be the predominant clinical manifestations of Omicron (*6,7*).

Antivirals that are effective at treating an Omicron infection are limited and scarce. Traditional Chinese medicines (TCM) such as Shufeng Jiedu capsule (SFJDC) and Lianhua Qingwen capsule have been recommended to treat COVID-19 patients with a fever and fatigue by the Guidance for Coronavirus Disease 2019: Prevention, Control, Diagnosis and Management in China since the COVID-19 outbreak in 2020. SFJDC is indicated to treat an acute upper respiratory infection and it has displayed efficacy in alleviating a fever, fatigue, and coughing in patients with COVID-19 in previous studies (*8,9*). SFJDC consists of eight medicinal herbs: Rhizoma Polygoni Cuspidati (Huzhang), Fructus Forsythiae (Lianqiao), Radix Isatidis (Banlangen), Radix Bupleuri (Chaihu), Herba Patriniae (Baijiangcao), Herba Verbenae (Mabiancao), Rhizoma Phragmitis (Lugen), and Radix Glycyrrhizae (Gancao). SFJDC was reported to reduce the virus load, reduce the inflammatory factors IL-6, IL-10, TNF-α, and IFN-γ, and increase the number of CD4+ and CD8+ cells in a mouse model of infection with the HCoV-229E species of coronavirus (*8*). A meta-analysis of active ingredients in SFJDC found that SFJDC has the potential to suppress SARS-CoV-2 and regulate immunomodulatory and anti-inflammatory targets via multiple pathways (*10,11*).

In January 2022, Omicron was first identified in Shanghai and subsequently spread in the community.

As an emergency response to the COVID-19 outbreak in April, mobile cabin hospitals were built in Shanghai to provide a safe treatment site for patients with mild COVID-19 symptoms and to provide an effective isolation area to prevent the spread of SARS-CoV-2. From April 2, 2022 to May 1, 2022, an open-label, randomized controlled trial (RCT) was initiated to evaluate the efficacy and safety of SFJDC in patients infected with the Omicron variant in a mobile cabin hospital. Patients in the control group received best supportive care while patients in the test group received additional SFJDC treatment (0.52 g per capsule, 4 capsules at a time, *t.i.d.*) for 7 days. In this study, efficacy was evaluated based on 1) recovery from symptoms including a sore throat, coughing, fatigue, and a fever and 2) RT-PCR measurements of COVID-19 viral RNA. Safety was evaluated via adverse event monitoring. The outcomes of this clinical trial are reported here.

Of the 415 patients screened, 240 patients who met the enrollment criteria (Supplementary Table S1 and Supplementary Figure S1, *http://www.biosciencetrends.com/action/getSupplementalData.php?ID=102*) were included in this study. All of the patients had mild COVID-19 with symptoms mainly including a sore throat, coughing, fatigue, and a fever. Patients were randomized into the SFJDC treatment group or the control group at a ratio of 1:1 (120 patients in each group). A major protocol violation occurred with 3 patients in each group, so they were therefore excluded from the final analyses. Primary baseline demographic and clinical features of the patients are shown in Supplementary Table S2 (*http://www.biosciencetrends.com/action/getSupplementalData.php?ID=102*). There are no significant differences between two groups in terms of age, gender, clinical symptoms, duration from symptom onset to hospitalization, and vaccination status.

Results indicated that all of the symptoms including a sore throat, coughing, fatigue, and a fever were eliminated in 98 patients in the SFJDC treatment group (recovery rate: 83.8%) and 82 patients in the control group (recovery rate: 70.1%) after 7 days of treatment ($p < 0.05$) (Figure 1A). In addition, recovery time from all symptoms was significantly shorter in the SFJDC treatment group compared to the control group (4.9 days *vs*. 5.9 days, $p < 0.001$) (Figure 1B). Recovery time from a single symptom such as coughing (5.4 days *vs*. 6.5 days, $p < 0.001$), fatigue (4.2 days *vs*. 5.4 days, $p < 0.001$), and a sore throat (4.2 days *vs*. 6.1 days, $p < 0.001$) was also shorter in the SFJDC treatment group than in the control group (Figure 1C). Of the eligible patients, 61.1% (143) developed transient signs of a fever at the onset of the disease before admission to the hospital. During the 7-day treatment period, only a small percentage of patients (18 (15.4%) in the treatment group, and 20 (17.1%) in the control group)

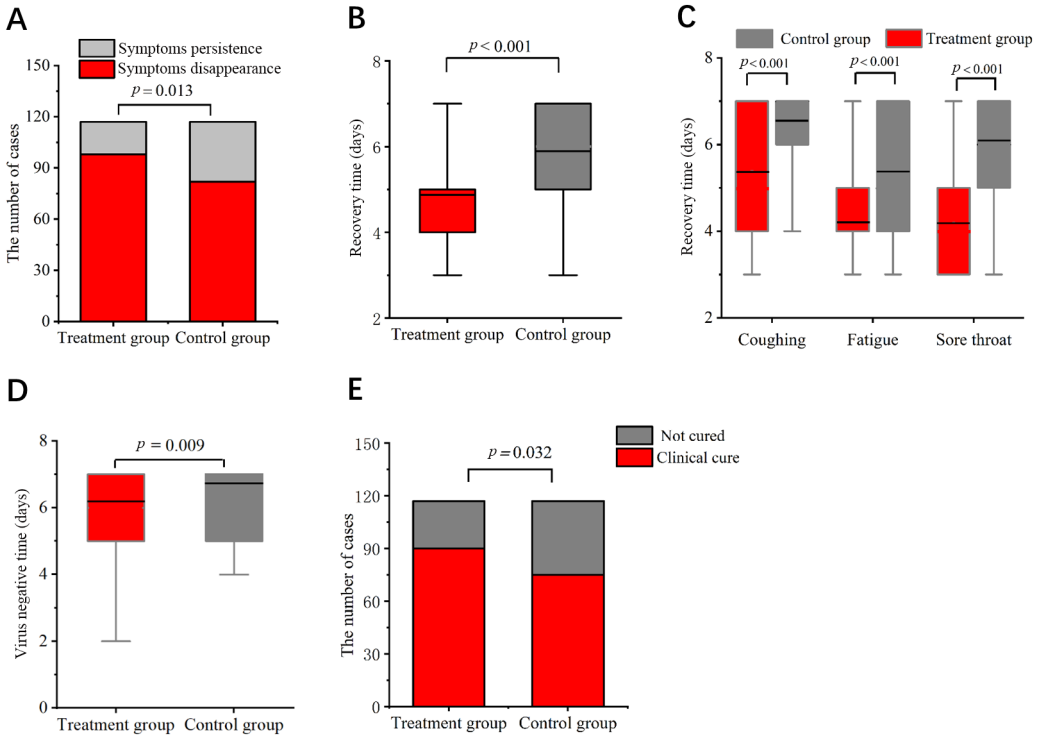

Figure 1. The efficacy of SFJDC in the treatment of patients infected with Omicron. Patients in the control group received best supportive care while patients in the treatment group received additional SFJDC treatment (0.52 g per capsule, 4 capsules at a time, t.i.d.) for 7 consecutive days. The number of patients without disease symptoms **(A)**, recovery time from all disease symptoms **(B)**, recovery time from a single disease symptom **(C)**, virus negative time **(D)**, and the number of clinically cured cases **(E)** were analyzed in each group and compared between the two groups.

exhibited a persistent fever. After 7 days of treatment, all of the patients in the treatment group had a normal temperature while 3 patients in the control group still exhibited a persistent fever. Fever duration did not differ significantly between the two groups (2.0 days *vs*. 2.7 days, $p > 0.05$).

Real-time PCR was used to measure virological outcomes in this study. The SFJDC group tested negative for the virus more quickly than the control group (6.2 days *vs*. 6.7 days, $p = 0.012$) (Figure 1D). However, there were no obvious differences in the virus negative rate between the two groups at the end of the 7-day follow-up ($p > 0.05$). In addition, the clinical cure rate was analyzed. It was defined as follows: 1) a normal temperature for longer than 3 days; 2) disappearance of symptoms (coughing, fatigue, or a sore throat); 3) no abnormalities in chest CT images; and 4) virus negative in two consecutive PCR tests (at an interval of at least 24 h). Results indicated that more patients in the SFJDC treatment group were clinically cured and that the clinical cure rate was significantly higher in the treatment group than in the control group (76.9% *vs*. 64.1%, $p = 0.032$) (Figure 1E). Few patients (0 patients in the treatment group *vs*. 3 patients in the control group) had disease progression, and no significant differences in progression were noted (0.0% *vs*. 2.6%, $p > 0.05$).

During the 7 days of treatment, adverse events such as gastrointestinal discomfort, loss of appetite, and headache were recorded in both groups. No serious adverse events were noted in this study (Supplementary Table S3, *http://www.biosciencetrends.com/action/getSupplementalData.php?ID=102*). There were no significant differences in the occurrence of adverse events between the two groups ($p > 0.05$).

This study had several limitations. A blinded or placebo-controlled design was not implemented due to the urgency of the disease outbreak and the timeliness of treatment. In addition, limited resources at the mobile cabin hospital precluded performing some laboratory tests (such as tests of liver and kidney function) in a timely and comprehensive manner. A multi-center study with a detailed safety evaluation would help to shed more light upon the clinical value of and potential adverse reactions to SFJDC in the treatment of COVID-19.

In summary, this study indicated that SFJDC is capable of alleviating a sore throat, coughing, fatigue and a fever in patients infected with Omicron. The virus negative time was significantly shorter in the SFJDC treatment group, suggesting that SFJDC may inhibit the virus replication. Double-blind, randomized controlled trials are warranted to comprehensively evaluate the efficacy and safety of SFJDC in a large cohort study in the future.

Ethical considerations: The First Affiliated Hospital of Anhui University of Chinese Medicine dispatched a medical team to a mobile hospital in Shanghai for medical support, and this clinical trial was initiated by the First Hospital. The Ethics Committee of the First Affiliated Hospital of Anhui University of Chinese Medicine approved the protocol used in this study (2022AH-18). All patients consented to participate in this study, and informed consent was obtained in writing from each adult patient.

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Letter to the Editor

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How should designated COVID-19 hospitals in megacities implement a precise management strategy in response to Omicron?

Jing Cao, Min Wen, Yirong Shi, Ting Huang, Yunlan Yi, Youfeng Su, Xiaohui Liu, Yanling Chao, Hongzhou Lu*

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SUMMARY As a new variant of COVID-19 with varied mutations, Omicron is more transmissible, more rapidly contagious, and has a greater risk of reinfection. Given those facts, a precise manage strategy needs to be formulated and implemented in designated megacities. Here, the precise COVID-19 prevention and control strategy for a designated hospital in Shenzhen, China is summarized, including implementation of a two-wing "On duty/On standby" approach based on busy and calm periods, an identification, classification, and grading system for the occupational exposure risks of medical staff, classification of patient transmission risks, separate admission, and an innovative treatment (nasal irrigation). The strategy has enabled the efficient and orderly integration of resources, it has resulted in zero infections among medical staff even during the peak hours of the pandemic at the hospital (1,930 patients admitted to both wings in a single day), and it has significantly reduced the initial period of no virus detection when patients infected with Omicron received saline nasal irrigation ($P < 0.001$). This strategy has provided evidence of precise prevention and control in a hospital, infection control, and efficient patient treatment in an era when Omicron is widespread.

Keywords COVID-19, precise prevention and control strategy, transmission risk, medical staff

To the Editor,

The World Health Organization officially designated Omicron as a new variant of the 2019 Coronavirus Disease (COVID-19, caused by SARS-CoV-2) on November 26, 2021 (*1*). Based on the number of people infected and the proportion of severe cases, this strain is less virulent than previous strains. However, there may be a sudden increase in the number of people infected due to Omicron's high transmissibility and immune escape capacity, as well as a higher absolute number of hospitalizations and deaths compared to the Delta variant, thus, posing a significant burden on the healthcare system (*2*). Designated COVID-19 hospitals need to formulate a precise strategy to manage the epidemic.

The designated hospital for COVID-19 treatment in Shenzhen has implemented a two-wing "On duty/On standby" approach based on busy and calm periods. The specific operation of the two wings is described in detail in a previous study (*3*). The designated hospital has gone through two "On duty/On standby" shifts since the outbreak of the epidemic in Shenzhen in January 2020. This period included peak hours on March 17, 2022, at 7:00 AM, when a total of 1,930 patients infected with the Omicron variant were admitted to the hospital simultaneously, with 980 admitted to the isolation wing and 950 admitted to the original wing. The "On duty/On standby" shifts allow for the efficient and orderly integration of resources as well as accurate prevention and control measures.

Since the outbreak of COVID 19, China has made significant achievements in pandemic control. Two of the most effective measures in response to Omicron variant are a "dynamic zero COVID-19 policy" and "precise management." Regular staff rotations can protect the physical and mental wellbeing of medical personnel to a significant degree. At the same time, however, it has a negative effect on ward management and the consistency of hospital infection prevention and control. Use of a checklist provides defined procedures for vital steps such as hospital infection prevention and control and ward management, and it directs all staff to complete various tasks in accordance with the checklist, including diagnosis and treatment paths, environmental management, equipment management, and guidance on terminal disinfection. Using a checklist-based approach helps expedite the familiarization of rotating personnel with the surroundings and workflow.

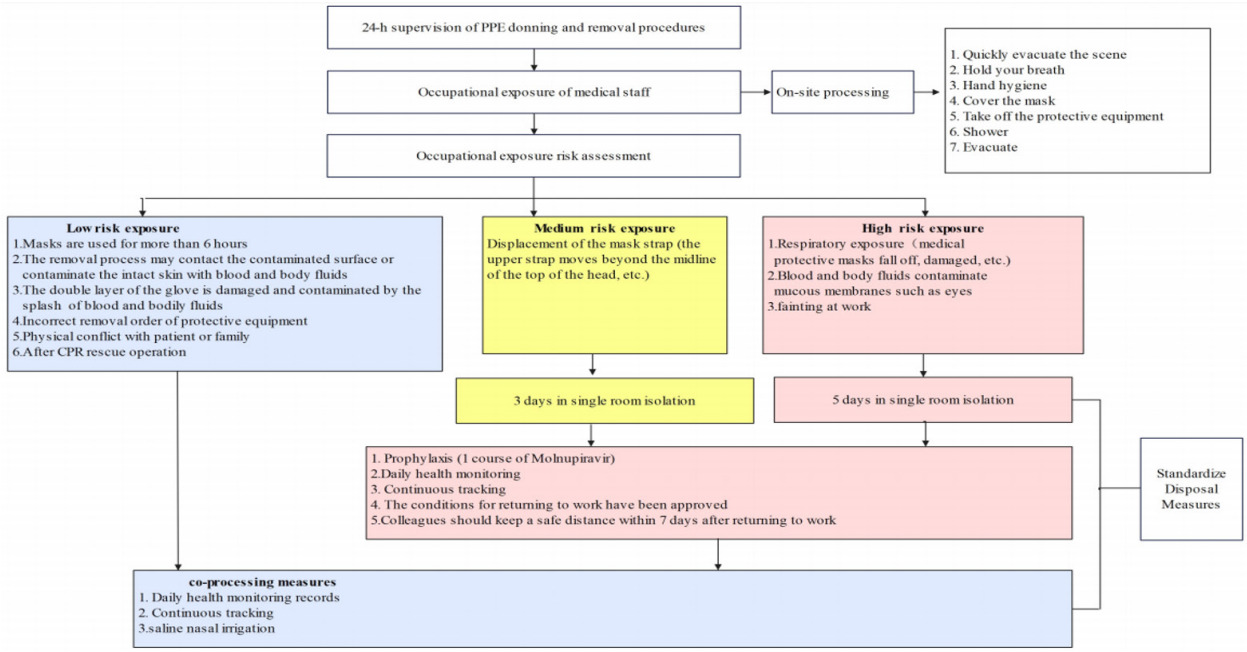

Figure 1. Guidelines for the classification of the occupational exposure of medical staff.

Omicron variant strains are more transmissible, spread more quickly, and have a higher risk of reinfection (*4*). Occupational exposure risks for medical personnel are identified, graded, and managed in the designated hospital. Key links to occupational exposure hazards are identified and appropriate procedures are devised to reduce the infection risk for medical staff and to achieve zero infections through 24-hour supervision of the wear and removal of personal protective equipment (*5-6*). Grades and classifications of occupational exposure risks for medical staff are shown in Figure 1.

The duration of the Omicron infection is significantly shorter, and patients with an infection for longer than 15 days are considered to be far less of a danger. The cycle threshold (Ct) is the minimum number of PCR cycles required to detect SARS-CoV-2 viral RNA. Higher Ct values are associated with less viral replication (*7*). Patients admitted to Shenzhen's designated hospital for COVID-19 treatment were assigned to various wards based on the course of their disease and their Ct value. If the course of disease exceeds 15 days or the Ct value is 30, which indicates that infectivity is decreasing, then they are admitted to the original wing; otherwise, they are admitted to the isolation wing.

Since a relatively large number of children have been infected with the Omicron strain, accurate classification and treatment are critical. According to the American Academy of Pediatrics and the Children's Medical Association, the proportion of children under the age of 18 infected with an Omicron strain was as high as 19% as of February 24, 2022, with children from different continents accounting for 1.4% of inpatients (*8*).

Children under the age of 14 account for 20% of patients with COVID-19 admitted to the designated

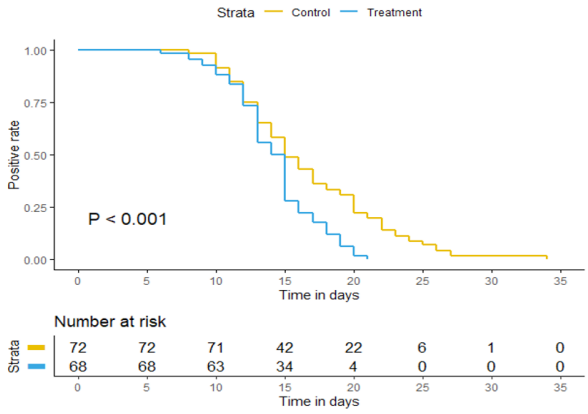

Figure 2. Survival curve analysis of the first negative conversion time of the two groups of patients.

hospital, with 50.4% being under the age of 6 (the youngest patient: one month old). Special precautions are taken for children infected with an Omicron strain: (*i*) The personal protection level is raised from grade 2 to grade 3; (*ii*) Treatment and care are centralized and performed by 2 persons; (*iii*) When cuddling children and providing care, the child is placed near the return air outlet while the caregiver is on the opposite side; (*iv*) Children who are able to walk are hugged for less time; (*v*) There is a special nursing assistant to act as a 24-hour escort for children who are able to walk; (*vi*) A child-friendly environment is created to comfort patients; and (*vii*) When bathing a child, a caregiver should try to avoid soaking personal protective equipment.

Studies have indicated that the pulmonary virulence of Omicron is lower, resembling that of an upper respiratory virus that is especially susceptible to nasal mucosa (*9*), and they have confirmed that patients

frequently exhibit five cold-like symptoms, including a runny nose, headaches, fatigue, sneezing, and a sore throat.

Nasal irrigation with normal saline is recommended by the designated COVID-19 hospital in Shenzhen as an innovative measure to prevent occupational exposure and to treat patients in the early stages of infection. The initial period of no virus detection for patients infected with Omicron was significantly reduced with saline nasal irrigation ($P < 0.001$), according to a study conducted at this facility (Figure 2).

In this era of the Omicron variant, an integrated two-wing "On duty/On standby" approach based on busy and calm periods has been implemented, and it includes identification, grading, and classification of occupational exposure risks, classification of patient transmission risks, separate admissions, and use of a "checklist" in isolation wards. This strategy can help to achieve precise prevention and control of infectious diseases.

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Conflict of Interest: The authors have no conflicts of interest to disclose.

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