Technische Universität München

Fakultät für Medizin

Comparison of histological tumour response in patients with hepatocellular carcinoma after transarterial chemoembolization, stereotactic body radiation, or combined therapy

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Vollständiger Abdruck der von der Fakultät für Medizin der Technischen Universität München zur Erlangung des akademischen Grades einer Doktorin der Medizin (Dr. med.) genehmigten Dissertation.

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Die Dissertation wurde am 11.06.2021 bei der Technischen Universität München eingereicht und durch die Fakultät für Medizin am 12.10.2021 angenommen

"Once you start studying medicine
you never get through with it"

Dr. Charlie Mayo

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Abstract

Background and Aims:
Hepatocellular carcinoma (HCC) is one of the most frequent malignant diseases and shows an increasing incidence over the recent years. HCC occurs mostly in patients with liver cirrhosis and different multimodal treatments are used dependent on the respective tumour stage. The BCLC classification is the established staging system for HCC, paying reference to liver function, size and number of tumour nodules. Surgical resection is first line treatment in patients with early HCC. However, surgery is often not possible, due to limited liver function or localization of tumour nodules. In a subset of these cases with limited size and number of tumours, liver transplantation has been demonstrated to be a curative treatment option. Because of organ shortage there are long waiting periods for transplant candidates. In order to minimize the risk of waiting list drop-out due to tumour progression, locoregional bridging therapies such as transarterial chemoembolization (TACE), ablation, or stereotactic body radiation (SBRT) have been established. However, tumour progression still occurs in some cases – resulting in wait list dropouts if tumour size and number exceed a certain limit. Therefore, efficient bridging therapies are urgently needed.

Methods:
We conducted a multi-centre retrospective trial of 27 patients treated either with a combination therapy of TACE and SBRT, TACE only, or SBRT only. To identify differences regarding tumour response, histopathological examination of explanted livers was conducted.

Results:
14 patients with confirmed HCC received TACE only, 9 a combination therapy of TACE and SBRT, and 4 SBRT only. In 9 patients, no residual tumour burden was found in liver explants. Strikingly, 8 of these patients had received the combination therapy of TACE and SBRT. A significantly higher number of complete therapy responses was observed in the TACE and SBRT combination compared to the other therapy groups (TACE + SBRT 8/9, 88.89 %, and SBRT only 1/4, 25 %, respectively, p-value < 0.001).

Conclusion:

Our data suggests that a combination therapy of TACE and SBRT seems to be superior to TACE or SBRT only. Although our results are promising, further studies are necessary to evaluate the influence of the combination therapy of TACE and SBRT on tumour recurrence or waiting list removals due to tumour progression.

Zusammenfassung

Hintergrund und Zielsetzung:
Das hepatozelluläre Karzinom (HCC) zählt zu den häufigsten bösartigen Tumorerkrankungen und weist über die letzten Jahre eine steigende Inzidenz auf. Es tritt meist bei Patienten mit Leberzirrhose auf und wird mit unterschiedlichen multimodalen Therapien abhängig vom Tumorstadium behandelt. Die BCLC-Klassifikation ist ein etabliertes Stagingsystem, welches Leberfunktion, sowie Größe und Anzahl der Tumore berücksichtigt. Im frühen Stadium ist die Resektion die Therapie der Wahl. Diese ist aufgrund der kompromittierten Leberfunktion bei Leberzirrhose oder aufgrund der Tumorlage jedoch oft nicht möglich. Wenn Tumorgröße und -anzahl eine bestimmte Grenze nicht überschreiten, kann die Lebertransplantation in diesen Fällen einen kurativen Therapieansatz bieten. Aufgrund des herrschenden Spenderorganmangels kommt es jedoch zu langen Wartezeiten bis zur Transplantation. Um das Risiko für einen Tumorprogress und damit für eine Ablistung von der Lebertransplantationswarteliste zu verringern, werden als sogenannte Überbrückungstherapie lokoregionäre Verfahren wie die Tumorablation, transarterielle Chemoembolisation (TACE) oder stereotaktische Strahlentherapie (SBRT) angewandt. Dennoch kommt es in einigen Fällen zu einen Tumorprogress, der zu einer Ablistung von der Warteliste führt, wenn Tumorgröße und Anzahl ein bestimmtes Limit überschreiten. Aus diesem Grund sind effektive Therapien zur Überbrückung der Zeit bis Transplantation dringend erforderlich.

Methodik:
Es wurde eine retrospektive, multizentrische Analyse von 27 Patienten durchgeführt, welche entweder mit einer Kombinationstherapie aus TACE und SBRT, oder TACE oder SBRT alleine behandelt wurden. Um die Unterschiede in Bezug auf das Therapieansprechen zu identifizieren, wurden die histopathologischen Befunde der Explantatlebern analysiert.

Ergebnisse:
Insgesamt erhielten 14 Patienten mit gesichertem HCC eine TACE, neun erhielten eine Kombinationstherapie aus TACE und SBRT und vier Patienten eine SBRT. Insgesamt ließen sich bei neun Patienten keine vitalen Tumoranteile in der Explantatleber mehr nachweisen. Von

diesen neun waren acht in der Gruppe die eine Kombinationstherapie aus TACE und SBRT erhalten hatten. Es wurde somit eine signifikant höheres komplettes Therapieansprechen bei der Kombinationstherapie im Vergleich zu den anderen Gruppen gezeigt (TACE + SBRT 8/9, 88.89%, SBRT 1/4, 25%; p-Wert <0.001).

Fazit:

Unsere Daten legen nahe, dass seine Kombinationstherapie aus TACE und SBRT einer alleinigen TACE oder SBRT überlegen ist. Ob dieser Therapieansatz auch zu einer Reduktion der Ablistungen aufgrund von Tumorprogress oder zu weniger Tumorrezidiven führt, muss in weiteren Studien untersucht werden.

Abbreviations

AFP	Alpha-fetoprotein
APHE	arterial phase hyperenhancement
BCLC	Barcelona Clinic Liver Cancer
BED	Biologically effective dose
cm	Centimetre
CT	Computed tomography
DEB	Drug eluting beats
DNA	Deoxyribonucleic acid
EMA	European Medicines Agency
f.e.	For example
Gy	Gray
HCC	Hepatocellular carcinoma
HBV	Hepatitis B
HCV	Hepatitis C
INR	International normalized ratio
MC	Milan criteria
MELD	Model of End Stage Liver Disease
mm	Millimetre
MR or MRI	Magnetic Resonance Tomography
mRECIST	Modified Response Evaluation Criteria in Solid Tumours
NAFLD	Non-alcoholic fatty liver disease
NASH	Non-alcoholic steatohepatitis
ng/ml	Nanograms per Milliliter
PBC	Primary biliary cirrhosis
PD-L1	Programmed death-ligand 1
RFA	Radiofrequency ablation
SBRT	Stereotactic body radiation
SD	Standard deviation
SIRT	Selective internal radiation therapy

TACE	Transarterial chemoembolization
TKI	Tyrosine kinase inhibitor
VEGF	Vascular endothelial growth factor

Introduction

General introduction and Epidemiology

Liver cancer is the sixth most common cause of cancer and the third leading cause of cancer deaths globally. 75-85% of the patients with liver cancer suffer from hepatocellular carcinoma (HCC) (Sung et al., 2021). Incidences vary between 2/100.000 to 86/100.000 depending on the global region (Figure 1) (GLOBOCAN, 2020). It is more common among men than in women, and a higher incidence is observed in eastern Asian as well as sub-Saharan countries due to higher rates of chronic hepatotropic viral infections, particularly hepatitis B (HBV) and hepatitis C (HCV) (Wild CP & Stewart, 2020).

In Germany, the incidence is approximately 7.2/100.000 in men and 1.9/100.000 in women. Given in absolute numbers, more than 5700 cases of HCC were diagnosed in 2014. The median age at time of diagnosis is lower in men (71 years) than in women (74 years) and survival rates remain poor with 5-year survival rate of approximately 16% (Schönfeld & Kraywinkel, 2018).

Though therapies regarding viral infections are improving, incidence rates are on the rise (Wild CP & Stewart, 2020).

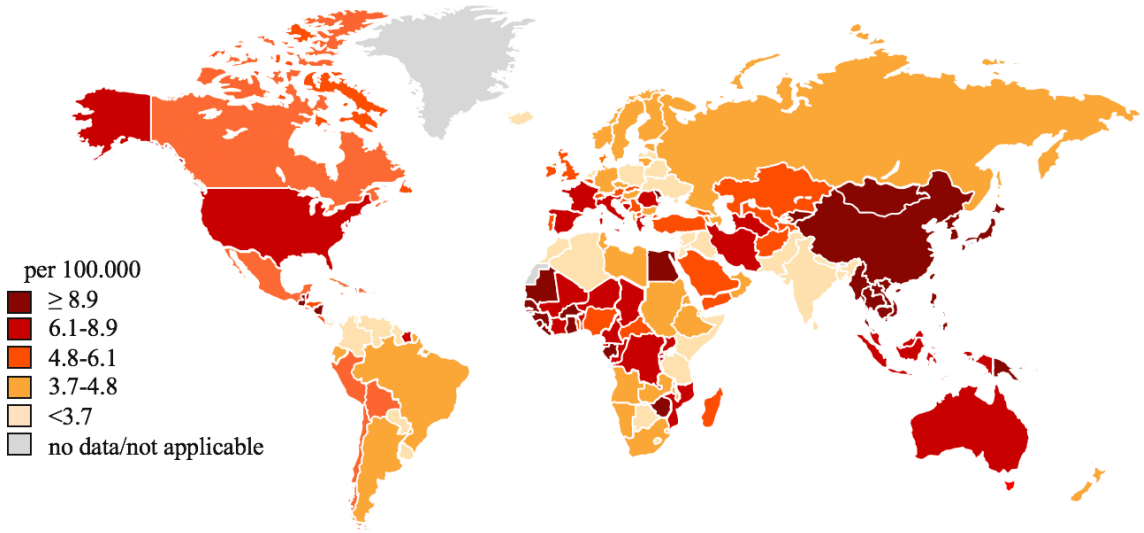

Figure 1: *Estimated age standardized incidence rates (World) in 2020, liver, both sexes, all ages (adapted from (GLOBOCAN, 2020))*

Risk factors for HCC development are underlying liver diseases, especially viral infections, excessive alcohol consumption, non-alcoholic fatty liver disease (NAFLD), and non-alcoholic steatohepatitis (NASH). Chronic viral hepatitis infections (HBV and HCV) are the most common causes of liver tumours worldwide, they are associated with around 80% of all HCCs. Another major risk factor is exposure to aflatoxin b, a mycotoxin produced by the aspergillum fungus. It contributes to the high HCC incidence in sub-Saharan Africa mostly in HBV-infected patients (Ryerson et al., 2016; Scalera & Tarantino, 2014; Wang et al., 2002). A very important risk factor with increasing numbers of patients especially in western countries is NAFLD/NASH. Because of lifestyle changes more and more people suffer from obesity, becoming one of the biggest problems in the 21st century. Diabetes and obesity are too known to increase the risk of HCC. Since many of the patients with NAFLD/NASH suffer from both obesity and diabetes, the risk for HCC in this group increased dramatically (European Association for the Study of the Liver, 2016; Kanwal et al., 2018).

The risk to develop HCC correlates with time, aetiology, and activity of hepatitis. HCC evolves by malignant transformation of hepatocytes. These are triggered by cirrhosis where chronic inflammation, cell death and compensatory proliferation believed to lead to genetic errors and mutations. Generally, molecular pathogenesis of HCC is very heterogeneous. Point mutations (such as in TP53 due to aflatoxin), alterations in signalling pathways (activation of PI3K/Akt or Wnt/β-catenin pathways) or cell cycle checkpoints (such as the p16/Rb checkpoint limiting cell proliferation in response to telomere shortening), DNA damage, oncogene activation, and changes in the tumour cell environment are known changes that occur during HCC development (Couri & Pillai, 2019; Rao et al., 2017).

Diagnosis

Diagnosis mainly is established through imaging. Due to high pre-test probability in cirrhotic patients and exclusive vascularisation there are typical diagnostic algorithms that allow for diagnosis of HCC without obtaining a sample for histopathology (Park et al., 2014) (Figure 2).

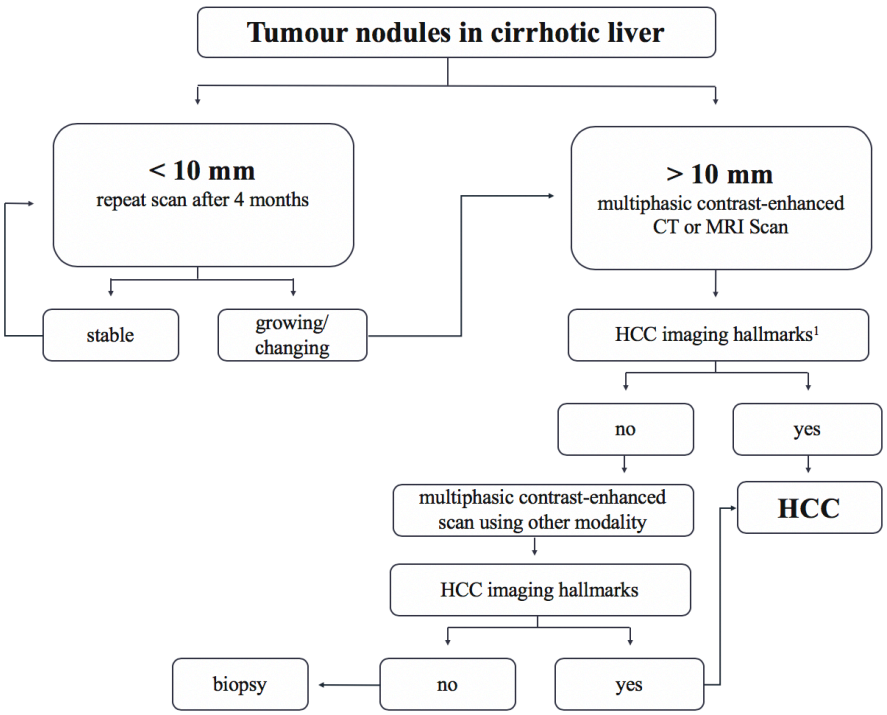

Figure 2: *Diagnostic algorithm for tumour lesions in cirrhotic livers.[1]arterial phase hyperenhancement and portal venous phase wash out. (adapted from (European Association for the Study of the Liver, 2018))*

Non-invasive diagnosis was accepted in 2001 using contrast enhanced scanning techniques (Bruix et al., 2001). In the arterial contrast phase, hyperperfusion of HCC lesions compared to the surrounding liver can be observed, while a wash-out of contrast agent is typical in portal and late venous phases (Figure 3). As sensitivity drops dramatically with smaller size of the lesion, diagnosis can only be made in lesions >9 mm and two different imaging modalities are required for tumours between 10 and 20 mm to establish diagnosis. In cases where these criteria are not fulfilled and in patients without cirrhosis, histopathological examination of liver bioptic tissue

remains the gold standard for diagnosis of HCC (Bruix et al., 2001; European Association for the Study of the Liver, 2018).

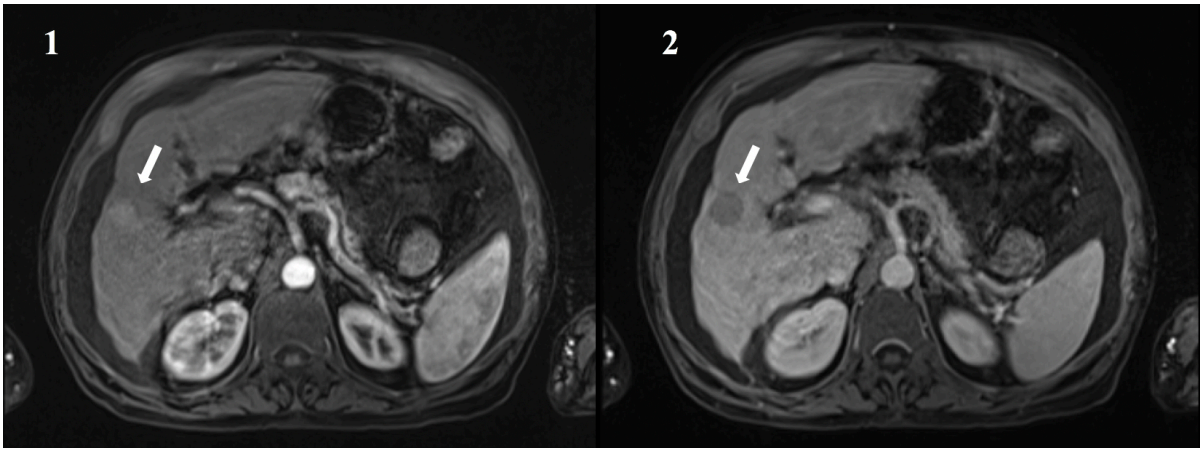

Figure 3: *Contrast enhanced MR scan of a patient suffering from HCC in cirrhotic liver. 1 arterial phase, 2 portal venous phase (Internal Medicine 2, Technical University of Munich, Klinikum rechts der Isar, 2021)*

A detailed algorithm for diagnosis of liver nodules in cirrhotic livers dependent on size of tumour lesions and contrast enhanced imaging criteria (MRI and/or CT scans) is established in international guidelines (Figure 2) (European Association for the Study of the Liver, 2018; Heimbach et al., 2018; Marrero et al., 2018).

Histopathological findings

As diagnosis in patients with cirrhosis is often established by imaging, only few patients undergo liver biopsy and in many of the cases grading and other histopathological aspects are not known (Di Tommaso et al., 2019). Histopathological findings in HCC vary to a great extent. The major growth pattern are trabecular, acinar/pseudoglandular, or compact pattern. With few exceptions – such as fibrolamellar HCC – no correlation between HCC growth pattern and prognosis has been identified. Grading consists of three categories; well, moderately, and poorly differentiated HCC, respectively (G1-G3), and seems to be a major predictor regarding prognosis (Rastogi, 2018). A further predictor of poor prognosis is presence of microvascular invasion and multifocal occurrence (Heimbach et al., 2018). Predictors of therapeutic response – such as PD-L1

expression as a predictor for response to immune checkpoint inhibitors – seem to be of limited value in HCC (Finn et al., 2020).

Alpha-fetoprotein (AFP)

AFP is a glycoprotein produced by immature liver cells. Therefore, adults usually have low levels of AFP (<10 ng/ml). Elevated levels of AFP are found in patients with HCC (75%). Yet, specificity remains low as levels might be moderately elevated in chronic liver diseases (Johnson, 1999). High AFP-levels are a predictor of poor prognosis. On the other hand, patients with unresectable HCC and APF levels > 400 ng/ml could benefit from systemic therapy with ramucirumab – making AFP the first biomarker for prediction of therapeutic response (Zhu et al., 2019).

Prevention and Surveillance

Prevention of the disease is possible through lifestyle modification and prevention of viral infections in order to stop the progress of the undergoing liver disease (Ascha et al., 2010; Chang et al., 1997; Vandenbulcke et al., 2016). Awareness about the risks of alcohol and obesity, vaccination against HBV and antiviral therapy are essential factors. Other agents, like f. e. coffee, seem to have anticarcinogenic effects too (Saab et al., 2014). In patients with high risk for the development of HCC, regular tumour surveillance is recommended. In patients with liver cirrhosis as well as in selected patients with HBV (according to the PAGE-B classes, predicting the risk of developing HCC) or NASH, an ultrasound of the liver should be performed at least every six months (European Association for the Study of the Liver, 2018; Papatheodoridis et al., 2016).

Staging and Treatment:

The Barcelona Clinic Liver Cancer (BCLC) staging system is well established and recommended by international guidelines to allocate appropriate treatment options (Figure 4). The most

important prognostic factors in this classification are number and size of tumour nodules, presence of vascular invasion or extrahepatic metastasis, as well as liver function (European Association for the Study of the Liver, 2018). Liver function is stratified according to the Child-Pugh-Score. Patients with liver cirrhosis can reach a possible score between 5 and fifteen, depending on serum bilirubin, serum albumin, INR, presence and severity of ascites and/or hepatic encephalopathy. These factors lead to a classification in three stages: A (5 to 6 points), B (7 to 9 points) and C (10 to 15 points), from best to worst (Child & Turcotte, 1964; Pugh et al., 1973). Importantly, severely impaired liver function (Child-Pugh C) usually precludes any tumour-directed treatment options as survival is dependent on liver function and not on tumour progression. An exception to this is treatment by liver transplantation, as liver function is supposed to be restored after transplantation.

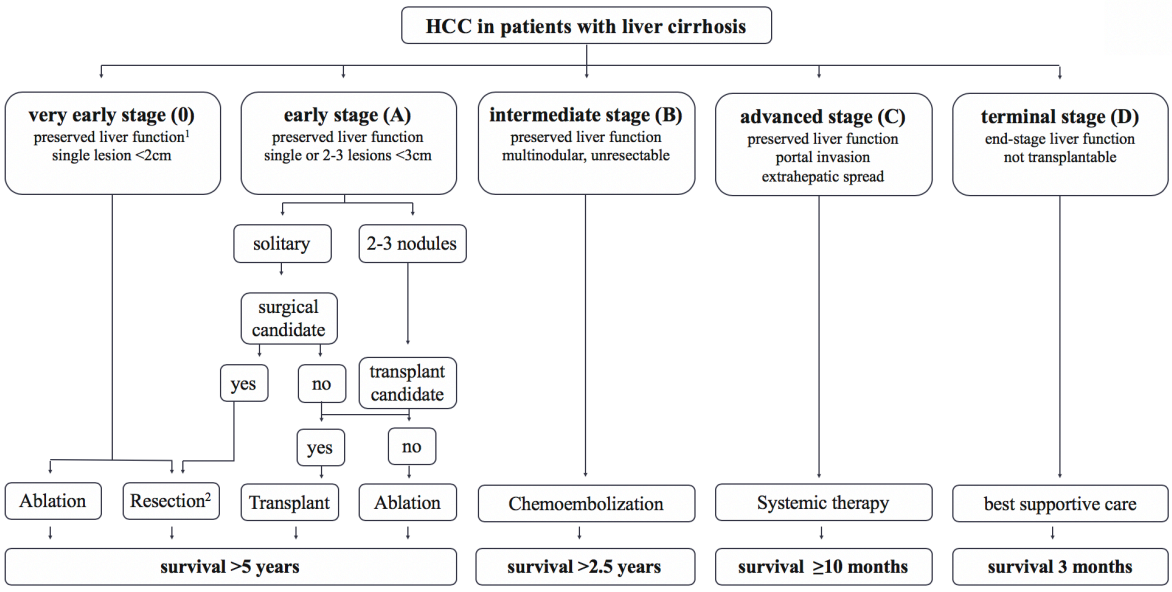

Figure 4: *Modified BCLC staging system and treatment strategy.[1]Referring to Child-Pugh Classification; [2]Only in patients with liver cirrhosis Child-Pugh A, no portal hypertension and sufficient liver volume (adapted from (European Association for the Study of the Liver, 2018))*

Additionally, histopathological differentiation and microvascular invasion as well as high AFP are important prognosis factors. (Lauwers et al., 2002; Ma et al., 2013)

Treatment

Due to concomitant cirrhosis, treatment options are often limited. However, very early and early stages HCC (BCLC stage 0 and A) can be treated curatively. 5-year survival is good with about 50-70% and median survival is estimated to > 36 months (Forner et al., 2010). In BCLC A stage liver cancer, recommended therapies are resection, ablation, or liver transplantation. At intermediary stages, median survival after two years is approximately 50% (Cabibbo et al., 2010). Therapeutic options consist of chemoablation or less commonly selective internal radiotherapy (SIRT) in a selected number of cases (European Association for the Study of the Liver, 2018). Regarding advanced HCC, outcome is less favourable. Advanced HCC is defined by the presence of metastasis or vascular invasion, and median survival is expected to be > 10 months while 5-year survival is negligibly low (Forner et al., 2018). Until 2007 there was no therapeutic treatment options in advanced HCC. Since then Sorafenib, and several other oral tyrosine kinase inhibitors (TKI) showed improved survival outcomes in first- and second-line therapy (Abou-Alfa et al., 2018; Bruix et al., 2017; Rimassa & Santoro, 2009). Most recently, combination immunotherapies yielded promising results and are expected to further improve survival in the upcoming years (Finn et al., 2020). However, in patients with end-stage HCC there still do not exist any relevant therapeutic options and median survival is about 11% at one year. Treatment consists of best supportive care and control of symptoms (European Association for the Study of the Liver, 2018).

Assessment of tumour response

Treatment response in malignant diseases is typically assessed by radiology (Figure 2). In addition to a decrease in tumour size, the reduction or loss of arterial hyperperfusion can signify tumour response in hepatocellular carcinoma. In addition to general changes in tumour size by Response Evaluation Criteria in Solid Tumours (RECIST), treatment outcome in HCC is measured by modified RECIST (mRECIST). The criteria were generated in order to objectify and therefore improve radiological diagnosis. In case of HCC, all tumour lesions detected by contrast enhanced CT or MRI scan should be measured at baseline and a maximum of two intrahepatic target lesions should be selected by size and suitability. The target lesions must show HCC-typical findings, including arterial enhancement. Response rates depend on follow up imaging.

The complete disappearance of arterial enhancement in all target lesions is defined as complete response (Figure 5), a decrease of at least 30% in diameters of contrast enhanced target lesions is defined as partial response. An increase of at least 20% in diameters of arterial enhancement in all target lesions is defined as progressive disease. All other cases are defined as stable disease. Additionally, the occurrence of new lesions with at least 1 cm size or an increase of non-target lesions is defined as progressive disease as well, as long as they show the typical diagnostic pattern. In case of new lesions >1 cm without arterial enhancement, diagnosis of new lesions can be made, if they show at least 1 cm growth. Overall patient response is defined as the combination of target lesions, non-target lesions and new lesions (Lencioni & Llovet, 2010).

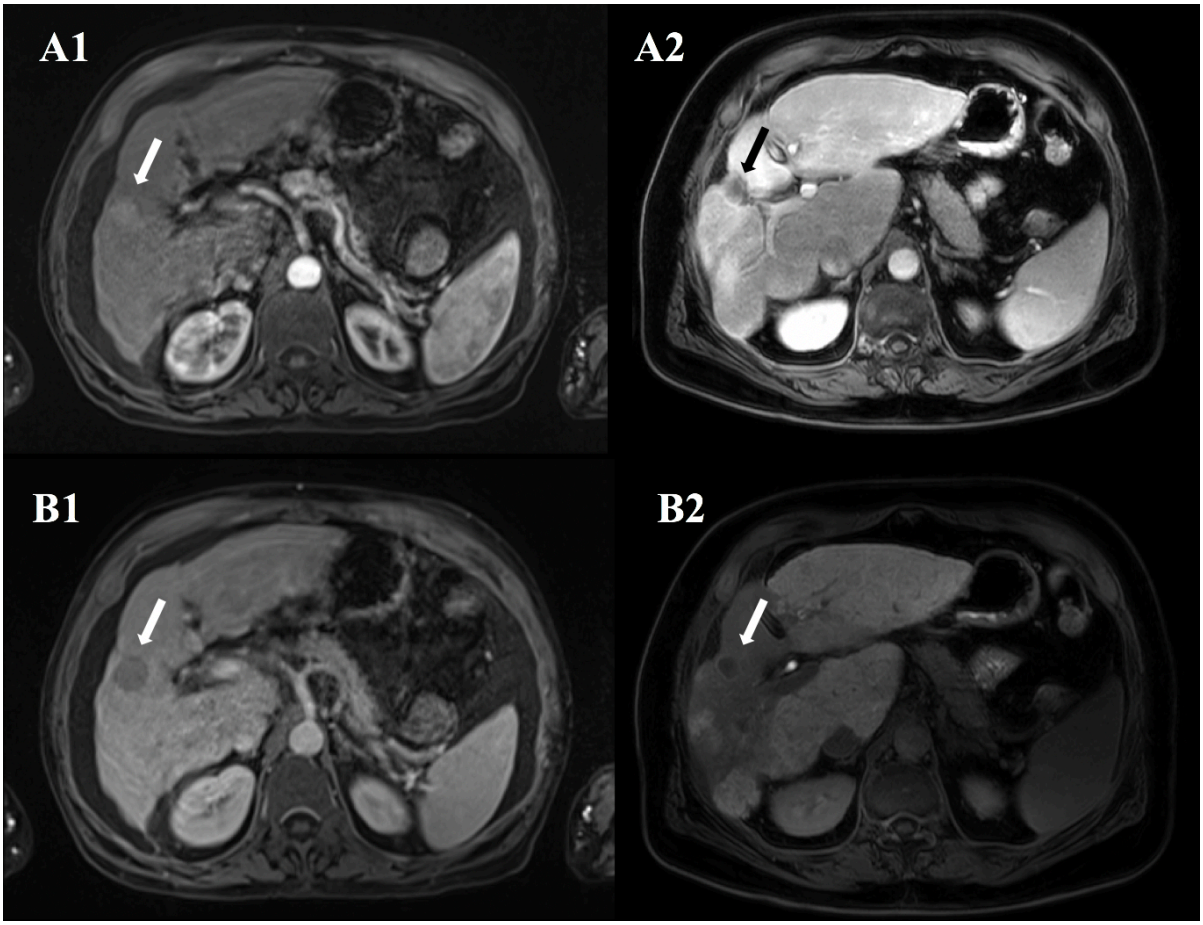

Figure 5: *Contrast enhanced MR scan before (A) and after (B) combination therapy with TACE and SBRT in arterial (1) and portal venous (2) phase with complete disappearance of arterial enhancement, defined as a total radiological response (Internal, Medicine 2, Technical University of Munich, Klinikum rechts der Isar, 2021).*

Resection

Whenever possible, resection is the treatment of choice. Resection volumes differ due to size and localization of tumour nodules. Prognostic is liver function after surgery. In patients without cirrhosis a post-operative liver volume of about 20-30% seems to be enough (Guglielmi et al., 2012). In cirrhotic patients, peri- and postoperative morbidity and mortality remains high. Liver function, portal hypertension, and performance status of the patient should be taken into account before considering resection (Roayaie et al., 2015).
Furthermore, recurrence rates after resection remain high with up to 70% after 2 years. Chan et al. analysed the data of 3903 patients, showing recurrence free survival after resection varies widely and depends on risk factors such as sex, tumour size, and AFP levels (Chan et al., 2018).

Liver transplantation

In case surgical treatment is not possible due to location of the tumour and/or the residual liver function, liver transplantation can be considered in selected cases. Due to limited availability of donor organs there are several mandatory requirements regarding liver transplantation in HCC patients in Germany: HCC has to be classified as early stage (BCLC A) and Milan criteria (MC) have to be fulfilled. Furthermore, the patient must not have any contraindications for solid organ transplantation such as for example concomitant severe heart disease, diagnosis of a secondary malignancy or severely reduced performance status. A relative exclusion criteria is age, that closely relates to performance status and presence of relevant medical preconditions and often limits transplantation in older patients (Bundesärztekammer, 2019) .

First performed in the 1960ies (Starzl et al., 1968), liver transplantation remains a major and demanding operation, mostly for patients with acute liver failure and end-stage liver diseases and requiring long postoperative stay and lifelong medical surveillance and immunosuppressive therapy. Nevertheless, long-term outcome and quality of life after transplantation is good with a 5-year survival of about 80% in Western countries (Samuel & Coilly, 2018). In HCC, the major advantage of liver transplantation is that the underlying pre-cancerous condition – namely liver cirrhosis – is treated as well. Recurrence rates are low and 5-year survival is not different from patients that were transplanted for reasons other than HCC (Clavien et al., 2012).

Initially, however, recurrence rates after liver transplantation for HCC were very high, mostly due to transplantation of more advanced tumours (Yoo et al., 2003). To identify patients with a low risk of recurrence – and therefore a high benefit from transplantation – several trials have investigated predictors of tumour recurrence. Based on the results of these trials, several criteria have been established in order to estimate the risk of recurrence.

In Germany, the MC (introduced in 1996) are widely accepted to select patients that will benefit from liver transplantation. The MC are based on following restrictions: one single tumour lesion <5 cm; up to three tumour lesions <3 cm, no extrahepatic manifestations, no vascular invasion (Mazzaferro et al., 1996). Guidelines for transplantation differ slightly in different countries. For example, downstaging to MC is allowed in the US, but not Germany (Bundesärztekammer, 2019). Even in patients outside MC, survival rates are good dependent on tumour number and size, AFP levels and response to treatment.

Other classification systems that investigated the extension of MC also showed good survival after liver transplantation though recurrence rated are mostly slightly higher than in patients within MC. One of the more widely used criteria are the UCSF criteria, that are based on the following restriction: one single tumour lesion up to 6.5 cm; up to 3 tumour lesions up to 4.5 cm and total diameter up to 8 cm (Herrero et al., 2008; Ito et al., 2007; Kollmann et al., 2017; Yao et al., 2007) and the up-to-seven criteria. Mazzaferro et al. showed in 2009 that patients with HCC beyond MC had a good 5-year overall survival of 71.2% if they fell into the up-to-seven criteria, which are defined as seven as the sum of the number of tumours and the size of the largest tumour in cm. None of them had microvascular invasion (Mazzaferro et al., 2009).

Recently, Mazzaferro et al. performed a multicentre trial with 45 patients with HCC beyond MC, but without macrovascular invasion or extrahepatic metastases. Patients received downstaging with different treatment modalities (locoregional, surgical or even systemic therapies) and all patients with partial or complete responses according to mRECIST criteria were randomized into two groups. In one group, liver transplantation was performed, the control group underwent locoregional and systemic treatment upon tumour progression. 5-year tumour free survival in the transplantation group was 76.8% versus 18.3% in the control group. All patients had preserved liver function (up to Child-Pugh B7) (Mazzaferro et al., 2020).

According to Eurotransplant, an organisation for distribution of solid organ transplants in central Europe, about 20% of liver transplantations were performed due to liver cancer (Jochmans et al., 2017) with rising numbers over the recent years (Kwong et al., 2020). Because of organ shortage, liver transplants are allocated according to priority scores that differ between countries. An established concept in Germany is the Model of End Stage Liver Disease (MELD) score. Introduced in 2002, the scoring system is based on the following blood levels: INR, creatinine, bilirubin. Values range between 6 and 40 and higher scores indicating worse liver function will get higher priority on the waiting list. To appreciate the survival rates of different diseases that are less dependent on reduced liver function, there are exceptions for special patient cohorts (SE, standard exceptions). For example, patients with HCC receive additional scoring points (SE-MELD) every three months increasing the likelihood for transplantation even with preserved liver function (Bundesärztekammer, 2019). This system is designed to ensure that transplantation is performed before tumour progression will lead to dropout from the waiting list.

Ablation

In patients with single lesions up to 3 cm size, thermal ablation with radio frequency or microwave probes is an alternative to surgical resection. In small tumours, it is a curative treatment option with recurrence rates similar to those after resection. The probe is inserted percutaneously and heated to 60 – 100 degrees centigrade by either high frequency alternating current (radio frequency ablation, RFA) or microwaves (MWA). The procedure leads to a death of the tumour cells. 5-year survival rates are about 70% in lesions < 2cm. However, heat application often less efficient at the tumour border, especially in larger lesions, resulting in an increased risk of incomplete ablation. Additionally, in patients with subcapsular HCC close to the diaphragm or close to the liver hilum, the risk of complications due to damage of adjacent organs or vessels remains high (Cho et al., 2010; European Association for the Study of the Liver, 2018; Wells et al., 2015).

Transarterial Chemoembolization (TACE)

In Patients with intermediate stage HCC, defined be multifocal tumour lesions exceeding MC but without vascular invasion, TACE is recommended as a first line palliative treatment option (European Association for the Study of the Liver, 2018). TACE is an interventional angiographic method in which application of chemotherapy and embolization of the tumour by intraarterial infusion into the hepatic artery is performed (Figure 6).

Developed more than 30 years ago, nowadays mainly two different types of TACE differing in the use of embolization agent are performed. Conventional TACE and drug eluting beads (DEB) TACE (de Baere et al., 2016; Nakamura et al., 1989; Yamada et al., 1983). Depending on the catheter position, TACE can be performed selective and superselective. In case of conventional TACE, embolization is performed by infusion of Lipiodol. Lipiodol not only leads to a temporary obstruction of the blood vessels but can also be used in diagnostic pattern as well (Valls et al., 1996). In combination with local chemotherapy (mostly anthracyclines like epirubicin or doxorubicin), cytotoxic effects and hypoperfusion often achieve good local control of tumour growth. DEB-TACE uses beads that are loaded with chemotherapeutic drugs (mostly doxorubicin) to release them gradually. DEB-TACE is supposed to lead to a better drug releasing capacity and less systemic side effects. Yet comparing both methods, data remain controversial and up to now both procedures remain equal (de Baere et al., 2016; Melchiorre et al., 2018; Song & Kim, 2017).

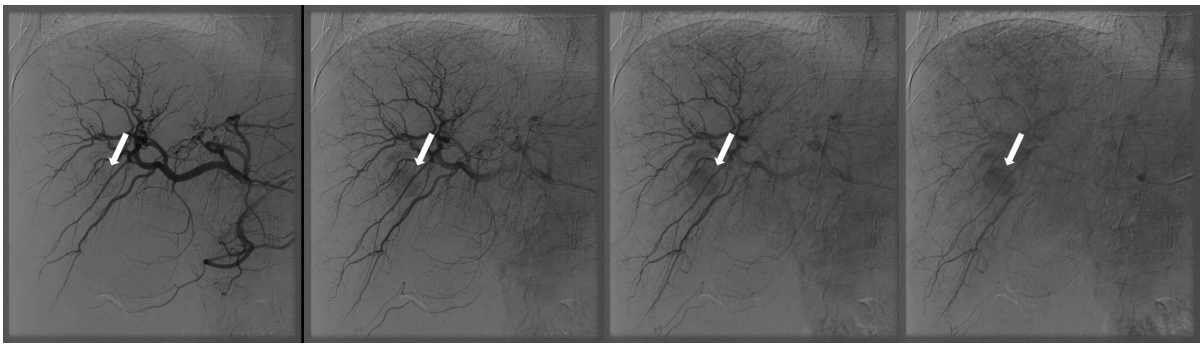

Figure 6: *Angiographic imaging displaying the malignant tumour nodule before embolization (Internal Medicine 2, Technical University of Munich, Klinikum rechts der Isar, 2021).*

TACE treatment can efficiently stop tumour cell growth, as HCCs are mainly supplied via arterial perfusion. Generally, this technique has few side effects and can be done in all patients with sufficient liver function (bilirubin < 2 mg/dl and tumour burden < 50%). Median survival after TACE in is about 40 – 50 months and – in conventional TACE – treatment cycles can be repeated several times (Agopian et al., 2018). Given these promising results, TACE is a widely used treatment to control tumour growth in patients on the waiting list for liver transplantation as outlined below.

Selective internal radiation therapy (SIRT)

SIRT is a local treatment option for advanced HCC that can be used in selected patients where TACE is not feasible due to high tumour burden. It is performed by an angiographic application of radioactive microspheres into hepatic arteries in order to stop local tumour growth. Cohort studies reporting long-term outcomes showed a median survival time of 16.9 to 17.2 months (European Association for the Study of the Liver, 2018; Hilgard et al., 2010; Mazzaferro et al., 2013; Salem et al., 2010; Sangro et al., 2011). In selected cases, SIRT can also be used as bridging to liver transplantation as long as tumour size still remains within the respective guidelines (Salem et al., 2016).

Stereotactic body radiation therapy (SBRT)

SBRT is a palliative treatment option with few side effects for patients not suitable for resection or other local treatment options. Contrary to conventional radiation treatment, the dose of radiation is partitioned in few fractions leading to a high biologically effective dose (BED) in targeted tumour tissue (Fuss & Thomas, 2004) (Figure 7). In study from 2006, Mendez Romero et al. investigated the outcome of 25 patients with liver metastasis or HCC receiving SBRT. 8 patients had HCC, 14 colorectal cancer, 1 breast cancer, 1 carcinoid, 1 lung cancer. All patients were not eligible for other treatments and had not more than 3 lesions, with a maximum size of 7 cm. Local control rates at 1 year were 94%, at two years 82%, whereas 3 patients suffered from acute toxicity grade 3 and one patient with liver cirrhosis and HCC from liver failure (acute

toxicity grade 5) (Mendez Romero et al., 2006). Based on these results, SBRT has been increasingly used in the treatment of HCC. Rim et al. performed a meta-analysis of 32 observational studies including1950 HCC patients, showing a pooled 1-year local control rate of 86.7% and a 1-year overall survival of 72.6% with significant differences regarding tumour size (Rim et al., 2019). However, large randomized controlled trials comparing SBRT to other locoregional treatment options are still missing. Due to lack of data, SBRT does not play a role in international guidelines and therefore remains an alternative treatment option reserved for individualized treatments (Bauschke et al., 2020; Huo & Eslick, 2015; Jacob et al., 2015; Jun et al., 2018; Sapisochin et al., 2017).

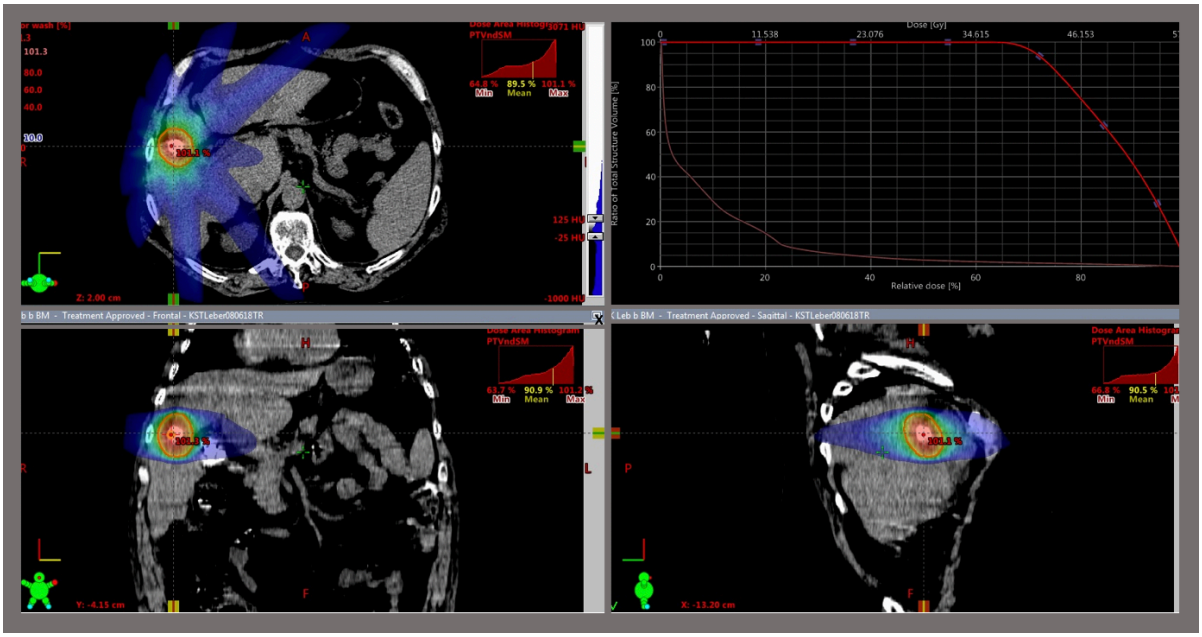

Figure 7: SBRT relative dose for HCC treatment in one patient with HCC and liver cirrhosis (Department of Radiation Oncology, Technical University of Munich, Klinikum rechts der Isar, 2021).

However, promising data show good tumour control with this therapeutic option.

Combination therapy of TACE and SBRT

To improve therapeutic efficiency, combination therapies with different locoregional treatment approaches are used in selected patients. However, there is a lack of prospective randomized trials and available data are mostly limited to retrospective analyses.

The combination of TACE and SBRT is technically feasible and there exist several retrospective studies evaluating this therapy in patients with HCC in comparison to a single treatment modality.

In a meta-analysis of 980 patients comparing a combination therapy of SBRT and TACE versus SBRT alone, a higher complete response rate as well as a better overall survival in patients who received combination therapy was observed. Importantly, there were no statistically significant differences in adverse events (Zhao et al., 2019). In comparison to TACE, the combination therapy of TACE and SBRT shows good local control rates even in cohorts with large HCC lesions. However, the effects on progression-free and overall survival are heterogenous, especially in lager tumours. In small HCC, however, the results seem to be excellent. In an analysis of patients with solitary HCC ≤ 3 cm receiving TACE alone (n=38) or combination therapy of TACE with SBRT (n=39), 96 % of the patients of the combination group showed a complete response (versus 3% of the TACE group). Disease free survival was high with 16 months (vs. 4 months in TACE group) (Honda et al., 2013). The advantage of a combination therapy can be also seen in studies that included patients with larger tumours. A study that also included patients with tumours > 3 cm evaluated data from 199 patients, of whom 85 underwent a combination therapy of TACE and SBRT and 114 received TACE alone. Tumour lesions were up to 5 cm in size and a maximum of 3 tumour lesions were treated. After one year, patients in the combination group showed a significantly higher local control rate (91% *vs*. 70%) and progression free survival (57% *vs*. 42%) than the patients with TACE alone. Though overall survival did not differ. Regarding toxicity there were no statistically significant differences as well (increase in liver enzymes 9% vs. 6%, worsening of liver function 9% vs. 5%) (Jun et al., 2018). A further retrospective multicentre analysis of 147 HCC patients who received either TACE (n = 98) or a combination therapy of TACE and SBRT (n=49) that also included large tumours (median size of the largest tumour 9.5 cm for TAE + SBRT and 10.1 cm for TACE, respectively) showed that survival rates as well as radiological disease control rates were better in the combination group whereas severe toxicity was uncommon in both cohorts (Wong et al., 2019). In a retrospective analysis of 161 patients with tumour lesions ≥ 3 cm that either received TACE (n=124) or a combination therapy of TACE and SBRT (n=37), local recurrence rates were lower in the combination group (11% vs. 26%) which also translated to a significant increase in overall survival (33 months vs. 20 months) (Jacob et al., 2015). Finally, there exist even

promising data with few side effects in tumor lesions ≥ 10cm. Zhong et al evaluated the data of 72 patients receiving SBRT after incomplete TACE. They showed a median survival of 12.2. months with no severe toxicities documented (Zhong et al., 2014). Even though these studies show a promising efficacy of TACE and SBRT combination treatment, the lack of larger prospective trials has so far impeded implementation into international guidelines.

Systemic treatment

Since HCC is mostly not sensitive to classical chemotherapeutic agents, there have been no relevant treatment options in advanced HCC till the development of targeted tumour therapies (European Association for the Study of the Liver, 2018).

The first substance showing a benefit was the TKI sorafenib that improved median survival by 2.8 months (European Association for the Study of the Liver, 2018; Rimassa & Santoro, 2009). More recently, several tumour targeted therapies – mostly TKI – where approved for the treatment of HCC. In 2018, lenvatinib showed noninferior results in terms of overall survival in comparison to sorafenib (Kudo et al., 2018). Regorafenib and cabozantinib are approved for second and third line therapy, respectively, but due to frequent occurrence of side effects in TKI, therapy if often limited and patients suffer from a lower quality of life (Abou-Alfa et al., 2018; Bruix et al., 2017).

The most relevant change in systemic therapy of HCC was the introduction immunotherapy-based combination therapies. Immunotherapies aim to modulate T-regulatory cell checkpoints to achieve destruction of tumour cells by the patient's own immune system. Few side effects were reported, mainly occurrence of immune-related diseases. Recently, the IMbrave150 trial showed superior progression-free survival and overall survival of the combination of atezolizumab (a PD-L1 antibody) and bevacizumab (a VEGF-antibody) in comparison to sorafenib, leading to approval of the combination therapy by the European Medical Agency (EMA) in December 2020 and replacing sorafenib as first line treatment in patients with advanced HCC (Finn et al., 2020; Roderburg et al., 2020).

Bridging therapies

As HCC is a malignant tumour that progresses over time, patients on the waiting list for liver transplantation are therefore always at risk of waitlist withdrawal. Waiting list dropout rates have been reported as high as 30% without bridging therapies (Mehta et al., 2018). Locoregional therapies are used for the treatment of intermediate stage HCC and are described in detail above. They are also commonly used for bridging to transplantation – however, studies on the benefit of bridging therapies show conflicting results.

TACE remains the most common bridging therapy besides ablation as data reporting benefits in survival (Graziadei et al., 2003). Wong et al, recently showed a good outcome with a 5-year survival rate of 93% in 41 patients treated with TACE prior to liver transplantation. Furthermore, in the same study no waitlist withdrawal due to tumour progression was reported.
However, mean time to transplantation was only 178 days, considerably lower than in Germany where organ shortage hinders early transplantation in most cases. According to Eurotransplant, mean time on the waiting list for liver transplantation is above ten months in Germany regardless of aetiology (Eurotransplant, 2020). At our centre, current mean time on the waiting list for liver transplantation in patients with HCC was 203 days (data of 18 patients with liver transplantation from 2015 to 2020). Though it remains unclear if a longer time to transplantation leads to a higher risk of tumour progression. Palmer et al performed a single centre study, in which they reviewed the data of 376 patients with HCC that underwent liver transplantation. They showed no statistically significant difference in recurrence free survival within a time between HCC diagnosis and diagnosis less or more than 180 days. However, mean time on the waiting list for the group with longer time to transplant was only 129 days – still considerable lower than in Germany (Palmer et al., 2017).

Interestingly, poor response to TACE is a predictor of post-transplant HCC recurrence as well, while good or even complete response indicates a favourable prognosis (Tsochatzis et al., 2013). Otto et al. performed a study with 136 HCC patients receiving TACE prior to liver transplantation. Whereas tumour size within MC at referral was not predictive, treatment response after TACE were highly predictive ($p<0.0001$) (Otto et al., 2013). Similar results were shown in a retrospective analysis of 896 patients with HCC that received liver transplantation in

Korea. 688 patients had bridging therapies (mostly TACE). Interestingly, in tumours within MC, overall survival and recurrence free survival rates were similar with or without bridging therapies, whereas in bigger tumours bridging therapies improved outcome. A better response to bridging therapies was associated with a better long-term outcome as well. Again median time interval from initial diagnosis to transplantation was rather short (9 months) and likely not fully comparable to Germany (S. Lee et al., 2020).

For locoregional bridging therapies other than TACE – especially SBRT – the availability of data from large patient cohorts is limited. Lee et al. evaluated the data of 121 patients that received RFA as bridging therapy, of whom 7.4 % suffered from dropout from the waiting list due to tumour progression. 5-year overall survival after liver transplantation was 75.8% (M. W. Lee et al., 2017). In a small case series with 10 patients (11 tumour nodules with a median tumour size of 34 mm) response to SBRT as bridging therapy was investigated. The treatment was well tolerated with no severe acute toxicities. None of the patients showed tumour progression before liver transplantation or tumour recurrence within 5-years after transplantation. Explant histopathology even showed complete response with no viable tumour signs left in 3 (3/11, 27%) tumour nodules (O'Connor et al., 2012).

The choice of bridging therapy usually depends on size and location of tumour lesions as well as on liver function. Currently, there are no data indicating that one locoregional therapy is superior to the other. A comparison of the efficiency of SBRT to TACE and RFA as bridging methods in a total of 379 patients showed that drop-out rate as well as complications were similar between the groups. 5-year survival at time of transplant was 75% in the SBRT group, 69% in the TACE group and 73% in the RFA group (Sapisochin et al., 2017).

In most of the larger studies on bridging therapies, therapeutic response is defined by radiological findings. However, in post-liver transplantation scenarios we have the unique opportunity to examine explant livers in order to analyse treatment response by histopathology. Rubinstein et al. analysed data from 50 patients or 93 tumour nodules, respectively, after different bridging therapies prior to liver transplantation. They examined explant histopathology in order to detect differences to radiological treatment response. 64% of the tumour nodules had complete

radiological response, whereas only 30% of the nodules had complete tumour necrosis by histopathology. These findings indicate that radiological response might underestimate tumour burden. In total, 12% of patients had no residual HCC by histopathology, most of whom were treated with ablation with our without additional TACE (Rubinstein et al., 2017).

In summary, these and several other studies show some response to locoregional therapies, though tumour growths cannot be halted in all cases (Bhoori et al., 2010; Graziadei et al., 2003; O'Connor et al., 2012). To date it cannot be determined if poor outcome in patients who do not respond to local tumour therapies is due to the underlying – possibly more aggressive – tumour biology or if it is due to the low efficiency of current bridging regiments.

Aim of the study

The aim of our study was to investigate the efficiency of different locoregional therapies as bridging methods to liver transplantation using tumour response by histopathology.

More precisely, we **focused** on the efficiency of a combination of TACE and SBRT *versus* TACE only *versus* SBRT only as bridging therapies to liver transplantation.

The **perspective** was to gain knowledge about histopathological outcome in regard to tumour size, number of tumour nodules, and viable tumour tissue.

Based on the following **questions**:
Are there differences between treatment groups regarding:

- tumour size and number of tumour nodules
- viable tumour tissue
- AFP levels

Material and methods

A multicentre retrospective trial was conducted in order to compare tumour response by histopathology for different treatment options in patients with HCC who meet transplantation criteria and possible liver transplantation. As transplant centres, University Hospital rechts der Isar of TU Munich, University Hospital of Munich, and Hannover Medical School participated in the study.

Protocols for patient analysis were reviewed and approved by the local ethics committee of each participating centre (University Hospital rechts der Isar, 02/19 507/18 S-KK; University Hospital of Munich, 19-147, Hannover Medical School, 940-2011). All patients received treatment as standard of care at the respective transplant centres or at referring hospitals and treatment options were discussed at interdisciplinary tumour conferences. All data were collected within medical treatment. No founding was received and no industry participating in this study. Data was collected retrospectively and patient names were anonymized for analysis.

To assess tumour response in our patient cohort, presence of viable tumour tissue in the explanted organ was used as a surrogate marker for therapeutic response. The absence of vital tumour tissue was defined as complete response.

Subject population

Medical records of all patients with liver cirrhosis and HCC within MC who underwent liver transplantation between 2007 and 2019 (University Hospital rechts der Isar) or 2011 and 2019 (University Hospital of Munich) were reviewed. From Hannover Medical School, data from patients who received TACE and SBRT or SBRT only between 2016 and 2019 prior to liver transplantation were collected. Inclusion criteria were treatment with at least one TACE with or without SBRT or treatment with SBRT only as bridging therapy to liver transplantation. Patients who received additional tumour therapies within the treated tumour nodules, such as resection of individual lesions or ablation, were excluded from our study. Observation period started from time of initial diagnosis through December 2019. All patients suffered from

hepatocellular carcinoma within MC at time of transplantation. After liver transplantation, patients were monitored for tumour relapse.

Recorded data

Of all patients, sex, date of birth, cause of underlying cirrhosis (alcohol, chronic viral hepatitis, or other) where applicable, number and size of tumour nodules at time of diagnosis, maximum number and size of tumour nodules, AFP at time of diagnosis, maximum AFP, and AFP at time of transplantation, BCLC stage, number and date of TACE cycles (if applicable), date and radiation dose of SBRT (if applicable), date of transplantation, age at transplantation and tumour appearance by histopathology in explant livers were recorded.

For diagnosis and monitoring of HCC, either MRI or CT-scans of the liver were performed. Size and numbers of tumour nodules were recorded at least every three months until liver transplantation.

To compare the different dose and fractionation regimens used for SBRT, the biological equivalent dose (BED) of the surrounding isodose was calculated according to the formula BED = nd (1+ d/alphabeta) with n: number of fractions, d: single dose and alpha/beta set to 10).

After transplantation, histopathology of liver explants was analysed for the presence of residual tumour tissue and necrotic tumour tissue. Number and size of tumour nodules as well as grading of any remaining tumour tissue was recorded.

Statistical methods

The study was designed as a retrospective multicentre longitudinal survey.

Our Null hypothesis was: „there is no difference regarding size and number of vital tumour nodules in liver explants in patients receiving TACE or a combination of TACE and SBRT as oncologic treatment prior to liver transplantation“. As alternative hypothesis „there is a

statistical significant difference regarding size and number of vital tumour nodules in liver explants patients receiving TACE or a combination of TACE and SBRT as oncologic treatment prior to liver transplantation“. Additionally, data of patients receiving SBRT only was evaluated, though no statistical analysis was performed due to the low number of patients. The α-error was estimated as $p < 0.05$.

Fisher-Freeman-Halton tests were performed to assess statistical significance. Kruskal-Wallis test as well as Mann-Whitney-U test were used for comparisons of variables between or among groups. Microsoft excel (version 16) and SPSS (version 25) were used. Due to the limited sample size, no multivariate analysis was performed. Two-tailed p-values < 0.05 were considered as statistically significant.

Results

Basic demographic parameters

The total study cohort consisted of 27 patients with HCC. Of these, 14 received TACE only (52 %), 4 SBRT only (15 %), and 9 a combination of TACE and SBRT (33 %). Mean patient age was 60 (SD± 6.43) years, with a range from 48 to 71 years. Within the study cohort, 20 (74 %) patients were male, 7 (26 %) female. All patients suffered from cirrhosis, mostly due to alcohol (11/27; 41 %) or HCV infection (10/27 37 %). Two patients were HBV infected, one patient had concomitant alcohol abuse and HCV infection. Three patients suffered from autoimmune hepatitis. (Table 1, figure 8).

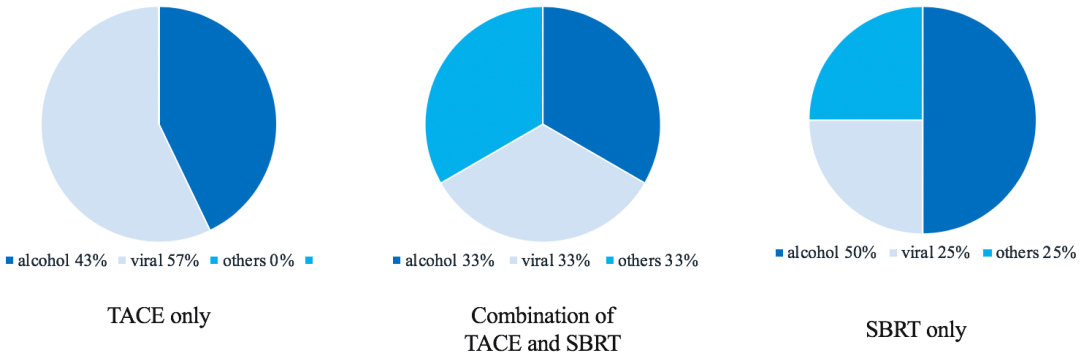

Figure 8: *Cause of cirrhosis according to treatment group*

Number of patients (n=27)		**TACE only (n=14)**	**Combination of TACE and SBRT (n=9)**	**SBRT only (n=4)**	**p-value**
sex					.242
	male	12 (86%)	5 (56%)	3 (75%)	
	female	2 (14%)	4 (44%)	1 (25%)	
age at transplantation in					.936
yrs	mean	59.5	61	59.5	
	SD	± 8.211	± 4.243	± 2.121	
aetiology of cirrhosis					.499
	alcohol	6 (42.9%)	3 (33.3%)	2 (50%)	
	viral	8 (57.1%)	3 (33.3%)	1 (25%)	
	others		3 (33.3%)	1 (25%)	

Table 1: *Basic patient characteristics according to treatment group*

The majority of patients (20/27, 74 %) had a single tumour lesion. With one exception, all patients were classified as BCLC stage A. Only one patient in the TACE only group suffered from BCLC stage 0 HCC (tumour size < 20 mm) and was transplanted for deterioration of liver function. Mean tumour size at time of diagnosis was 29.3 mm (SD ± 9.462 mm, range 12 mm to 49 mm) and median AFP at time of diagnosis was 8.0 ng/ml, with 1st quartile 5.0 ng/ml and 3rd quartile 58.0 ng/ml (range 1.2 to 2515 ng/ml). Mean time interval between SBRT Treatment and transplantation was 214.18 days (SD ± 217.83, range 29 to 786 days), mean time interval between last TACE Treatment and transplantation was 177.44 days (SD ±192.13, range 14 to 805 days) (Table 2, Figures 9 and 10).

Number of patients (n=27)		**TACE only (n=14)**	**Combination of TACE and SBRT (n=9)**	**SBRT only (n=4)**	**p-value**
numbers of TACE treatment cycles	1	5 (36%)	7 (78%)	N/A	.
	2	4 (29%)	2 (22%)		
	3 or more	5 (36%)	0 (0%)		
radiation dose in Gy					.586
	mean	N/A	40.00	36.80	
	SD		± 3.75	± 17.56	
number of tumour lesions					.517
	1	9 (64%)	8 (89%)	3 (75%)	
	2	5 (36%)	1 (11%)	1 (25%)	
tumour size at time of diagnosis					.389
	mean	29.50	27.67	26.67	
	SD	± 7.63	± 9.54	± 14.50	
AFP at time of diagnosis					
	mean	68.51	415.72	9.93	
	SD	± 107.43	± 877.02	± 2.23	
	median	8.05	8	9.85	
	1st and 3rd quartile	5.2 and 84.2	5 and 17.7	8 and 11.85	

Table 2: *Basic treatment and tumour characteristics displayed according to treatment group*

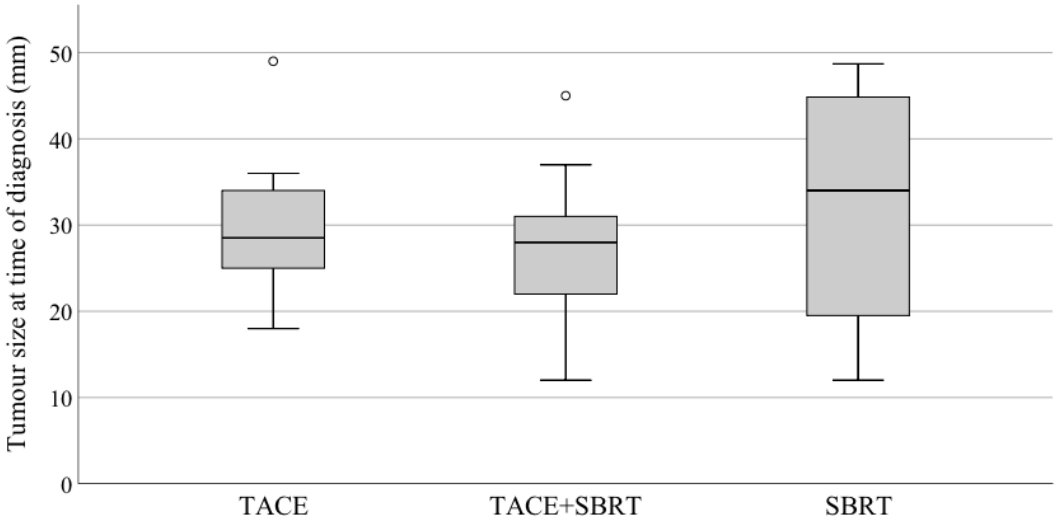

Figure 9: *Box plot showing tumour size referring to the treatment groups. Median is represented by bar, 25-75% confidence interval by box, 10-90% confidence interval by whiskers and outliers by dots.*

Treatment-specific characteristics

TACE only

The TACE only group consisted of 14 patients. 9 patients (64%) had a single tumour lesion. 5 patients had two tumour lesions. Mean tumour size at time of diagnosis was 29.5 mm (SD ± 7.63 mm, ranging from 18 mm to 49 mm), mean AFP at time of diagnosis was 68.51 ng/ml (SD ± 107.43), ranging from 1.2 ng/ml to 338 ng/ml. In 5 patients 1 TACE treatment cycle was performed before transplantation, in 4 patients 2 TACE treatments were performed (ranging from 1 to 5 treatments). One patient in this cohort suffered from an extrahepatic recurrence three years after transplantation.

SBRT only

In the SBRT group, 4 patients were included. Three of them had a single tumour lesion (75%) with a mean tumour size at time point of diagnosis of 26.67 mm (SD ± 14.50 mm, ranging from 12 mm to 48.7 mm). Mean AFP at time of diagnosis was 9.93 ng/ml (SD ± 2.23 ng/ml, ranging from 8 ng/ml to 12 ng/ml). Mean radiation dose was 36.80 Gy (SD ± 17.56 Gy, ranging from 18.9 Gy to 54 Gy. Treatment schemes were individualized for each patient.

Combination of TACE and SBRT

9 patients received a combination of TACE and SBRT. 8 patients (89%) had a single tumour lesion, mean tumour size at time of diagnosis was 27.76 mm (SD ± 9.54, ranging from 12 mm to 45 mm) and mean AFP at time of diagnosis was 415.72 ng/ml (SD ± 877.02, ranging from 3 mm to 2515 mm). 7 patients received one TACE treatment, two patients received 2 treatments. Mean radiation dose was 40.00 Gy (SD ± 3.75 Gy). In 6 patients, radiation dose split into 3 x 12.5 Gy prescribed to the 65% -isodose was applied every other day and in 3 patients 3 x 15 Gy prescribed to the 60%-isodose. Liver transplantation was performed after a median interval of 188 days (range 29 to 786 days) from SBRT treatment. One patient in this cohort suffered from an extrahepatic tumour recurrence 4 months after liver transplantation. Interestingly, this was the only patient with 2 lesions in this group.

There were no statistically significant differences between treatment groups in terms of age, gender, origin of cirrhosis, tumour size and number of tumour lesions, or AFP levels (Tables 1 and 2, figures 6 and 7).

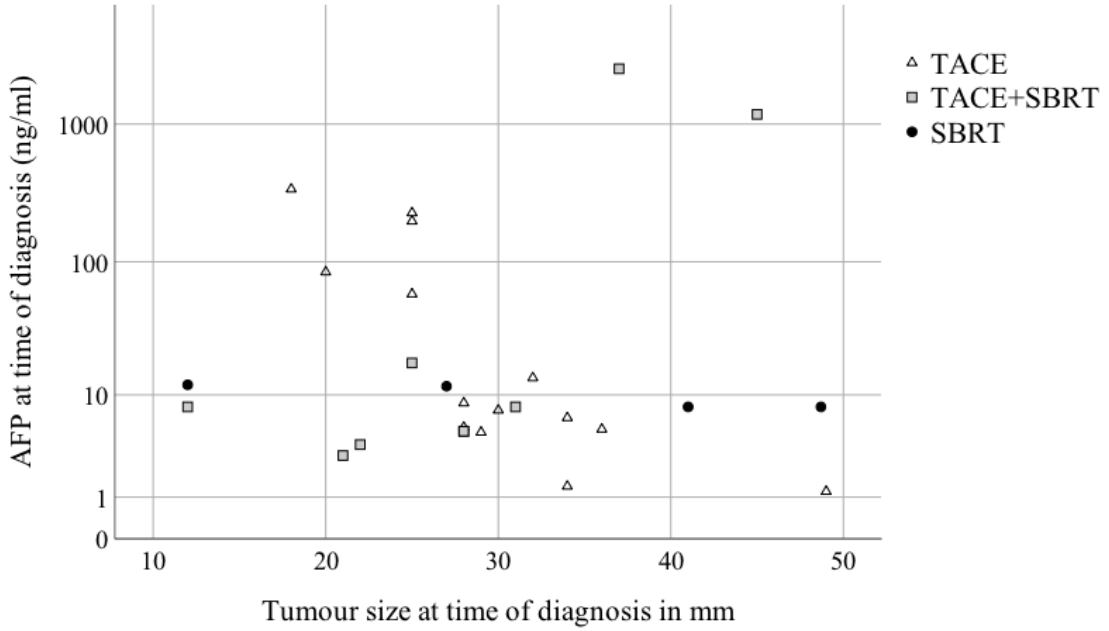

Figure 10: *Scatter chart showing the correlation of AFP and tumour size for each group*

Histopathological findings

In 9 patients (30%), no vital tumour tissue was detected microscopically, which was defined as complete response by histopathology.

The vast majority of patients without residual tumour tissue was in the TACE and SBRT combination group (8/9, 89%). More precisely, 8 out of 9 patients in the TACE and SBRT group had a complete response (89 %), whereas no patient in the TACE only group and one patient in the SBRT only group (1/4, 25 %) no vital tumour tissue was detected in explant livers (p-value <0.001) (Figure 11 and table 3).

In the combination group, the only patient with vital tumour tissue in explanted liver had by far the highest AFP level (2515 ng/ml) and the shortest time interval between SBRT and liver transplantation (29 days). On the other hand, the only patient in the SBRT only group with complete response by histopathology had the smallest tumour in the overall patient cohort (12 mm, BCLC 0) and was transplanted due to impaired liver function.

Though we observed a weak correlation between tumour size and treatment response in the overall patient cohort, the difference was not statistically significant (Figure 12). Even with a limited number of patients, our results show that TACE and SBRT combination therapy results in significantly higher rates of complete histopathological response when compared to individual treatments (TACE or SBRT, respectively) alone.

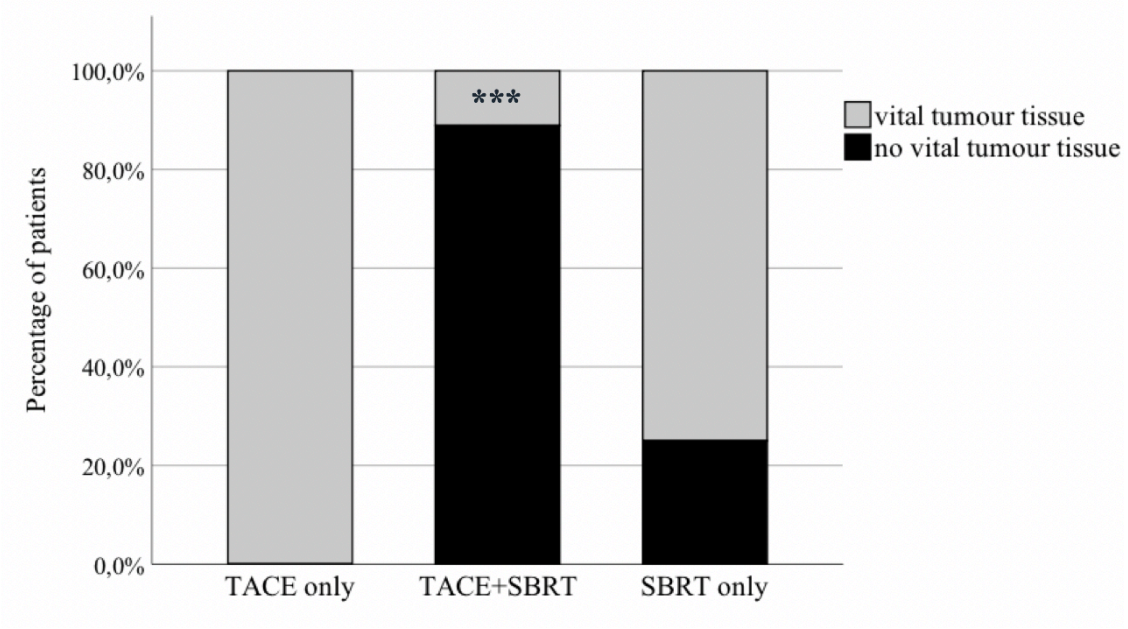

Figure 11: *Bar chart displaying the proportion of vital tumour tissue in treatment groups (*** $p < 0.001$)*

	Total number of patients (n=27)	**TACE only (n=14)**	**Combination of TACE and SBRT (n=9)**	**SBRT only (n=4)**	**p-value**
Complete response	9 (33.33%)	0 (0%)	8 (88.89%)	1 (25%)	**< 0.001**

Table 3: *Treatment response according to treatment groups*

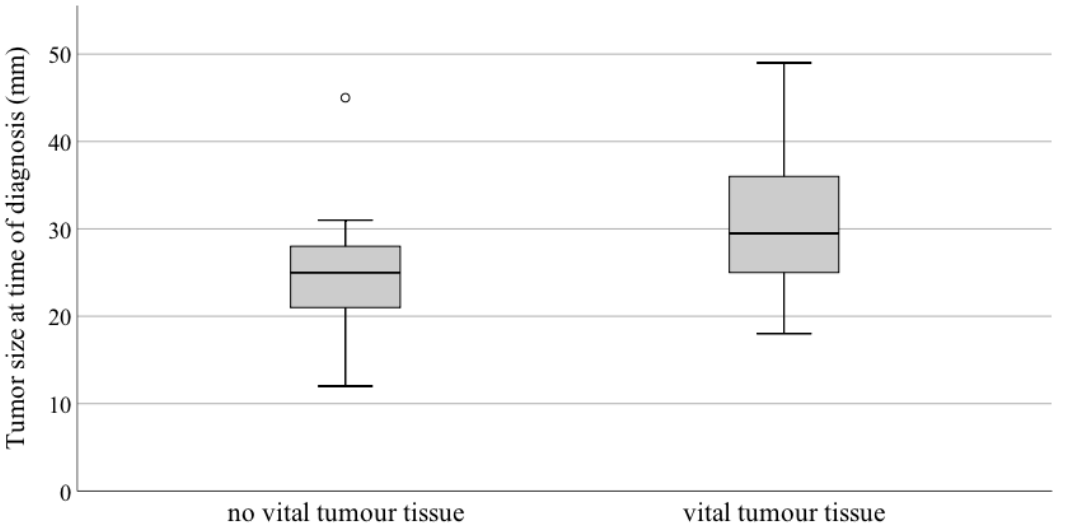

Figure 12: *Box plot showing tumour size in correlation to treatment response. Median is displayed in bar chart, 25-75% confidence interval by box, 10-90% confidence interval by whiskers.*

Recurrence rates

On follow-up, two patients suffered from extrahepatic recurrence after liver transplantation. One patient was in the TACE only group (with vital tumour tissue by explant histology) and one patient was in the TACE and SBRT group (without vital tumour tissue detected in explant liver).

Discussion

Interpretation of data

We compared different bridging therapies in patients with HCC on the waiting list for liver transplantation for histopathological tumour response as a surrogate marker for treatment efficiency. Our patients either received a combination therapy of TACE and SBRT, TACE only or SBRT only. The patient cohort undergoing the combination therapy of TACE and SBRT showed a statistically significant higher rate of complete histopathological response than patients in other groups.

Mechanistically, TACE prior to SBRT might act as a sensitizer for radiation therapy as previously described for other tumour identities, where combined radio-chemotherapy has become the standard of care. For example, in patients with locally advanced anal cancer Bartelink et al. showed in 1997 that simultaneous radiotherapy and chemotherapy leads to improved locoregional control (Bartelink et al., 1997). Additionally, relative tumour hypoxia, which was shown to significantly improve the effect of radiotherapy (Overgaard, 2007), might play a role in HCC after embolization of the tumour feeding blood vessel. Though we did not perform TACE and SBRT simultaneously, the prolonged effect of TACE on sensitization could be explained by embolization (Moding et al., 2016).

Due to organ shortage – especially in countries like Germany – there are long waiting times for transplant candidates. Therefore, patients on the waiting list for liver transplantation have a certain risk for delisting because of tumour progression. Together, in Munich transplant centres two to three patients each year have to be removed due to tumour progression beyond MC. At this stage, curative treatment options are not available any more resulting in a dramatic worsening of prognosis.

Liver transplantation leads to excellent results with a 5-year survival rate of 65-78% and a low recurrence rate of 11-18% if MC are fulfilled. Favourable outcomes can even be achieved with liver transplantation beyond MC, depending on size and number of tumour lesions, AFP and treatment response (5-year survival rate 46%-60%) (Chapman et al., 2008; Clavien et al., 2012; De Giorgio et al., 2010; European Association for the Study of the Liver, 2018; Mazzaferro et al., 2011; Yao, 2008).

Hence, locoregional therapies can be used as bridging to halt tumour growth. Several countries have already implemented response to locoregional therapies into their transplant allocation systems for HCC patients. In the US for example, patients with one lesion up to 8 cm, three lesions less than 5 cm each or five lesions less than 3 cm can be put on waiting list for liver transplantation, if they can be downstaged to MC with locoregional therapies. (Cillo et al., 2015; Marrero et al., 2018; U.S. Department of Health & Human Services, 2017). Regarding the UK, criteria are similar using the Duvoux criteria which also take AFP levels into account. In Duvoux criteria, points are assigned depending on number of tumour nodules (0-3 nodules: 0 points, ≥ 4 nodules: 2 points), largest tumour diameter (≤ 3 cm: 0 points, 3-6 cm: 1 point, >6 cm: 4 points) and AFP levels (≤ 100 ng/ml: 0 points, 100-1000 ng/ml: 2 points, >1000 ng/ml: 3 points). Cut-off for transplantation is set at 2 points. Patients can be down-staged to these criteria if they remain stable within ≥ 6 months from down-staging treatment (Duvoux et al., 2012; NHS Blood and Transplant, 2021)

Though bridging therapies are widely used, currently it is not possible to halt tumour growth in all patients. This might be due to the underlying tumour biology, but might also indicate insufficient treatment. In addition, highly efficient bridging therapies may not only be beneficial for patients within MC in terms of stabilisation of the disease, but may also be useful for down-staging to MC to achieve transplantation in countries where these options are implemented into transplant criteria. Depending on treatment strategy, the therapeutic approach in these patients can change from palliative to curative.

SBRT still remains an individual treatment approach for particular patient groups. Even though studies showed excellent local control of tumour lesions and a good safety profile, current guidelines do not regard SBRT as a primary treatment option due to lack of large randomized trials (Huo & Eslick, 2015; Takeda et al., 2016). The combination of TACE and SBRT has therapeutic benefits with a good safety profile in preliminary trials. A recent retrospective analysis of 49 patients with nonresectable HCC receiving SBRT and TACE and 98 patients treated with TACE only showed that disease control rate, progression free survival, and 3-year overall survival were significantly better in the combination group than in the TACE only group within this palliative setting (Wong et al., 2019). Currently, ongoing prospective, randomized clinical trials are recruiting patients in order to evaluate the combination therapy of TACE and SBRT in comparison to standard therapies in patients with intermediated stage HCC (NCT02513199, NCT03895359, NCT02794337).

With these preliminary results, we expected a better outcome of the combination therapy of TACE and SBRT in comparison to TACE or SBRT only. Still, the percentage of complete response in the combination group was surprisingly high. In only one patient in the SBRT group vital tumour tissue was detected by explant histopathology (TACE and SBRT 88.89% versus TACE only 0%; $p < 0.001$). Of note, this patient had a very high AFP (2515 ng/ml). Despite this marker of poor prognosis – that might even preclude transplantation in some countries – he showed no signs of tumour recurrence more than 4 years after liver transplantation. On the other hand, one patient in the TACE and SBRT combination group suffered from extrahepatic recurrence within short time, despite there was no vital tumour tissue left in explant histology. In this patient, HCC metastasis occurred in the brain less than 6 months after transplantation and might have been present even before transplantation as brain scans are not routinely performed as part of the HCC staging protocol at participating centres in line with international guidelines.

The discrepancy between histopathological findings and clinical outcomes in these two patients shows that further studies on correlation between complete tumour response and long-term recurrence-free survival are needed.

Our data showed that by histopathology, TACE or SBRT alone resulted in lower rates of complete response and might therefore be less efficient as a tumour therapy. Regarding treatment-related therapeutic side effects, no serious side effects in the TACE and SBRT combination therapy group were observed, which corresponds with published literature (Honda et al., 2014; Jang et al., 2020). However, long-term hepatic toxicity plays almost no role in a pre-transplant setting (Lasley et al., 2015; Liu et al., 2013) and a more aggressive tumour therapy might lead to deterioration of liver function in the long run.

Currently, there are two patients with HCC on the waiting list for liver transplantation in our centre who received a combination therapy of TACE and SBRT. Both showed a complete radiological response. They had maintained liver function (Child-Pugh A), only single tumour lesion, BCLC A, with a maximum tumour size of 25 mm, a maximum AFP of 53.8 ng/mL and received one cycle of TACE. One of the patients suffered from liver cirrhosis due to primary biliary cholangitis (PBC) and NAFLD, with a maximum tumour size of 25 mm and a maximum AFP of 4.0 ng/ml. Interestingly, the tumour was not completely embolized by TACE, after additional SBRT (42 Gy) the tumour showed a complete radiological response

with additional decrease in size to 22 mm. By now, more than 17 months after SBRT therapy, there are no vital tumour signs detected. The other patient, suffering from liver cirrhosis due to PBC as well, had a maximum tumour size of 22 mm and a maximum AFP of 53.8 ng/ml. Already after TACE therapy the tumour decreased in size and did not show any arterial hypervascularisation. Up to now, 16 months after additional SBRT therapy, this patient does not show any vital tumour signs. AFP decreased to 3.7 ng/ml. Both patients do not show any clinical or radiological signs of tumour progression after a combination therapy of SBRT and TACE. Up till now, imaging showed complete radiological response. With the presence of concomitant medical preconditions, these patients are set to inactive on the waiting list (non-transplantable) and transplantation will only be considered upon tumour progression.

Our data indicate that the combination therapy of TACE and SBRT might be highly effective in patients with small tumours (BCLC A). The discrepancy to data from other studies that showed tumour progression after combination therapy of TACE and SBRT might be explained by a larger tumour size (up to 30 mm or 40 mm, respectively) (Honda et al., 2014; Takeda et al., 2016). If the combination of TACE und SBRT might even present a curative treatment option in patients with small HCCs though, needs to be evaluated in future studies.

In HCC within MC but not amenable for curative treatment approaches such as resection or ablation, liver transplantation remains the only curative treatment option up to now. Still, there are many patients not suitable for liver transplantation, as due to advanced age and/or accompanying diseases liver transplantation is often not possible. In these patients, the combination therapy of TACE and SBRT could be a promising therapeutic approach to halt tumour growth – even in a primarily palliative setting. Palliative therapy strategies ought to achieve a good tumour control on the one hand and preserve a good quality of life on the other hand. Especially in patients with relevant medical preconditions, the risk of side effects is probably higher. In transplant patients, the combination of TACE and SBRT showed few higher grade side effects, though mostly patients with preserved liver function were evaluated. Whether this treatment approach can be successfully applied in other patient cohorts, needs to be shown in further studies.

While the small sample size could impair validity of this study, our data indicates superior efficiency of the combination of TACE and SBRT over treatment with one single modality.

Limitations

Despite the study was conducted under the intention of precise scientific work, some limitations of this study must be discussed. There could have been a sample bias due to the retrospective design. Furthermore, the sample size was very small, since a small number of patients undergoes liver transplantation in general and an even smaller group does not qualify for ablation therapy that is often used as bridging therapy.

Conclusion and future perspective

A persistent shortage of donor organs for patients with HCC results in prolonged waiting time to liver transplantation and therefore in a higher risk of tumour progression beyond MC. Current bridging strategies aim to stabilize the disease but are not sufficient to delay tumour growth in all patients. More efficient bridging therapies are therefore needed to reduce the number of waiting list removals due to tumour progression. So far only few studies have been published comparing different bridging therapies.

In our small cohort, a high rate of complete tumour response by histopathology suggests that the combination of TACE and SBRT is superior to TACE or SBRT only. If treatment with TACE followed by SBRT can result in a decreased number of waiting list removals due to tumour progression, or in reduced rates of tumour recurrence after transplantation needs to be evaluated prospectively in a larger patient cohort.

Aside from a being a sufficient bridging therapy in patients within MC, the combination of TACE and SBRT might be used as a downstaging method as well. Though downstaging in Germany is not yet possible, other countries that implemented downstaging into their transplant criteria might be able to offer a curative treatment option to a new patient cohort. However, if the combination therapy shows similar response rates in larger tumour outside MC remains to be investigated.

Another group of patients, that could benefit from TACE and SBRT combination therapy, are patients with early HCC (BCLC A) that are not fit for transplant for reasons such as advanced age, accompanying diseases or other reasons. These patients in particular might benefit from low side effects of a treatment modality that is less invasive than ablation or resection.

Acknowledgements

At this point I would like to take the opportunity to thank everybody who was involved - directly or indirectly - into this work.

First and foremost I want to express my sincere gratitude to Priv.-Doz. Dr. Ursula Ehmer who gave me the opportunity to be part of this study and always offered me expert guidance and support. Her continuous feedback was invaluable and without her patient guidance, enthusiastic encouragement and useful critiques this doctoral thesis would not have been possible.

Similarly, I want to express my deepest gratitude to Prof. Andreas Umgelter for his great supervision during the whole thesis as well. He always supported me with useful and constructive suggestions to successfully complete my dissertation.

This project was based on collaborations with University hospital of Munich, Campus Großhadern and Hannover Medical School. I owe special thanks to Dr. Sabine Gerum and Dr. Alexander Philip from university hospital of Munich as well as to Dr. Martha Kirstein from Hannover Medical school.

Furthermore, I thank Bernhard Haller of the institute for Medical Statistics and Epidemiology at Klinikum rechts der Isar of TU Munich for help and critical review of data analysis.

I could not have completed this dissertation without the support and backing of some people, so last but not least I want to thank my family and friends for all the ideas and help they offered to me.

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Annex

Parts of this doctoral thesis were presented as poster at the 28th annual meeting of the German Transplantation Society in Hannover, Germany, October 2019 (Bauer et al. “Comparison of tumor response after TACE with or without SBRT in liver explants from patients with HCC”) and published in World Journal of Gastroenterology, June 2021 (Bauer et al. “High rate of complete histopathological response in hepatocellular carcinoma patients after combined transarterial chemoembolization and stereotactic body radiation therapy”).

Published 06/2021, World Journal of Gastroenterology

World Journal of Gastroenterology

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World J Gastroenterol 2021 June 14; 27(22): 0-0

DOI: 10.3748/wjg.v27.i22.0000

ISSN 1007-9327 (print) ISSN 2219-2840 (online)

ORIGINAL ARTICLE

Retrospective Study

High rate of complete histopathological response in hepatocellular carcinoma patients after combined transarterial chemoembolization and stereotactic body radiation therapy

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Institutional review board statement: This study was reviewed and approved by the local Ethics Committee of each participating center.

Informed consent statement: We performed a retrospective analysis and all data were completely anonymized for analysis and storage. In addition, there was no risk for the subjects as our study was a retrospective analysis of patients treated with standard of care procedures. According to local ethics committees there is no informed consent required given the retrospective study design, anonymous data analysis, and lack of any risks for the subjects in this study.

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

Data sharing statement: The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available as they could compromise the privacy of research participants.

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Abstract

BACKGROUND

Liver transplantation (LT) presents a curative treatment option in patients with early stage hepatocellular carcinoma (HCC) who are not eligible for resection or ablation therapy. Due to a risk of up 30% for waitlist drop-out upon tumor progression, bridging therapies are used to halt tumor growth. Transarterial chemoembolization (TACE) and less commonly stereotactic body radiation therapy (SBRT) or a combination of TACE and SBRT, are used as bridging therapies in LT. However, it remains unclear if one of those treatment options is superior. The analysis of explant livers after transplantation provides the unique opportunity to investigate treatment response by histopathology.

AIM

To analyze histopathological response to a combination of TACE and SBRT in HCC in comparison to TACE or SBRT alone.

METHODS

In this multicenter retrospective study, 27 patients who received liver transplantation for HCC were analyzed. Patients received either TACE or SBRT alone, or a combination of TACE and SBRT as bridging therapy to liver transplantation. Liver explants of all patients who received at least one TACE and/or SBRT were analyzed for the presence of residual vital tumor tissue by histopathology to assess differences in treatment response to bridging therapies. Statistical analysis was performed using Fisher-Freeman-Halton exact test, Kruskal-Wallis and Mann-Whitney-*U* tests.

RESULTS

Fourteen patients received TACE only, four patients SBRT only, and nine patients a combination therapy of TACE and SBRT. There were no significant differences between groups regarding age, sex, etiology of underlying liver disease or number and size of tumor lesions. Strikingly, analysis of liver explants revealed that almost all patients in the TACE and SBRT combination group (8/9, 89%) showed no residual vital tumor tissue by histopathology, whereas TACE or SBRT alone resulted in significantly lower rates of complete histopathological response (0/14, 0% and 1/4, 25%, respectively, P value < 0.001).

CONCLUSION

Our data suggests that a combination of TACE and SBRT increases the rate of complete histopathological response compared to TACE or SBRT alone in bridging to liver transplantation.

Key Words: Hepatocellular carcinoma; Transarterial chemoembolization; Stereotactic body radiation therapy; Bridging therapy; Liver transplantation

Core Tip: In patients with early-stage hepatocellular carcinoma (HCC) who are not eligible for resection or ablation, liver transplantation presents a curative treatment option. To halt tumor growth during waiting time, bridging therapies such as transar-

Manuscript source: Unsolicited manuscript

Specialty type: Gastroenterology and Hepatology

Country/Territory of origin: Germany

Peer-review report's scientific quality classification
Grade A (Excellent): 0
Grade B (Very good): B
Grade C (Good): C, C, C, C, C
Grade D (Fair): 0
Grade E (Poor): 0

Received: February 5, 2021
Peer-review started: February 5, 2021
First decision: March 6, 2021
Revised: March 20, 2021
Accepted: May 19, 2021
Article in press: May 19, 2021
Published online: June 14, 2021

P-Reviewer: Boteon YL, Huang CY, Jeruc J, Yu JI
S-Editor: Wang JL
L-Editor: A
P-Editor: Liu JH

terial chemoembolization (TACE), ablation, and stereotactic body radiation therapy (SBRT) are used prior to liver transplantation. In a multicenter retrospective trial with 27 HCC patients who received either TACE or SBRT alone, or a combination of TACE and SBRT, explant histopathology was analyzed to assess treatment response. Strikingly, almost all patients in the combination group exhibited no residual vital tumor by histopathology, whereas TACE or SBRT alone resulted in significantly lower rates of complete histopathological response.

Citation: Bauer U, Gerum S, Roeder F, Münch S, Combs SE, Philipp AB, De Toni EN, Kirstein MM, Vogel A, Mogler C, Haller B, Neumann J, Braren RF, Makowski MR, Paprottka P, Guba M, Geisler F, Schmid RM, Umgelter A, Ehmer U. High rate of complete histopathological response in hepatocellular carcinoma patients after combined transarterial chemoembolization and stereotactic body radiation therapy. *World J Gastroenterol* 2021; 27(22): 0-0
URL: https://www.wjgnet.com/1007-9327/full/v27/i22/0.htm
DOI: https://dx.doi.org/10.3748/wjg.v27.i22.0000

INTRODUCTION

Hepatocellular carcinoma (HCC) ranks among the leading causes of cancer-associated deaths worldwide. In very early [1 tumor < 2 cm, Barcelona clinic liver cancer (BCLC) 0, United Network for Organ Sharing (UNOS) T1] and early (1 tumor 2-5 cm or 2-3 tumors ≤ 3 cm, BCLC A, UNOS T2) stage HCC, surgical resection or local ablation is the treatment of choice. However, accompanying cirrhosis and tumor location often preclude these curative treatment approaches. Additionally, recurrence rates after resection of early HCC (BCLC A) are high with up to 70% after 2 years[1]. In contrast, liver transplantation (LT) is a curative treatment option not only for the tumor but also for the underlying precancerous condition (*i.e.* liver cirrhosis, chronic HBV infection, non-alcoholic fatty liver disease) with excellent 5-year survival (65%-78%) and low recurrence rates (11%-18%) if Milan criteria (MC, 1 tumor ≤ 5 cm or 3 tumors ≤ 3 cm, without vascular invasion) are fulfilled[2,3]. Acceptable outcomes after LT are even achieved in patients outside MC, though 5-year survival rates are noticeably lower (46%-60%), depending on size and number of tumor lesions, alpha-fetoprotein (AFP), and treatment response[2,4,5]. Therefore, MC are widely accepted to identify HCC patients who will benefit from LT.

Currently about 30%-35% of patients on the waiting list for LT in Europe suffer from HCC[3]. In the US, the percentage is lower, but has been steadily rising over the recent years[6]. Due to organ shortage, waiting periods are long with a high risk for tumor progression and therefore drop-out from the waiting list. Without any bridging therapy, tumor progression beyond MC has been reported in up to 30% of cases[7]. To avoid tumor progression, locoregional therapies are used as bridging to LT and several countries have now implemented response to locoregional therapies into their transplant allocation systems[8,9].

The most commonly used locoregional bridging therapies are transarterial chemoembolization (TACE) and thermal ablation, such as radio frequency ablation (RFA) and microwave ablation (MWA). These therapies are also recommended in current guidelines for the treatment of BCLC A or B stage HCC[3,9]. However, in patients with tumors not suitable for these standard treatment modalities, individual treatment approached such as 90Y radioembolization or stereotactic body radiation therapy (SBRT) have been used to control tumor growth[10-12].

RFA or MWA are established in the treatment of early and very early stage HCC (BCLC A and 0, respectively) as a curative approach or as bridging with excellent long term outcomes after LT[13-16]. However, thermal ablation is not technically feasible in all patients. Tumor location can preclude safe and successful treatment, for example in subcapsular HCC close to the diaphragm or in lesions close to the liver hilum[17]. If these lesions are not amenable for resection, TACE and less commonly alternative treatment options such as SBRT or radioembolization are used to achieve tumor control in a pre-transplant setting[12].

TACE, which also presents the standard of care in patients with intermediate stage HCC, is a widely used bridging therapy that can efficiently halt tumor growth in the pre-transplant setting[2,3,9,18-21]. Even in patients initially outside MC, who achieve

down-staging to fulfill MC after TACE, overall survival rates are comparable to patients that were never outside MC [5,22]. On the other hand, insufficient response to TACE is a predictor of post-transplant HCC recurrence[20]. With longer waiting times due to organ shortage, the risk of tumor progression after treatment with TACE remains a substantial concern.

SBRT is a local ablative treatment option for patients not suitable for resection or thermal ablation. In particular, SBRT can also be applied to tumors close to large blood vessels or wherever tumor location precludes RFA or MWA[17]. Even with excellent local control of tumor lesions and a good safety profile, current guidelines do not regard SBRT as primary treatment option due to a lack of large randomized trials[23-26]. SBRT is therefore mainly performed as an individualized treatment approach in selected cases. In a pre-transplant setting, complete histopathological response after SBRT in 3 of 11 tumor lesions (27%) in a small cohort of 10 patients has been reported[27]. In a larger retrospective analysis of 30 patients treated with SBRT prior to transplantation, drop-out rate (16.7%) and 5-year survival (61%) were not different from patients treated with TACE or RFA[10].

A combination of TACE and SBRT is an alternative local treatment option with therapeutic benefits and a good safety profile, though data from large randomized controlled trials is still missing[28,29]. A recent retrospective analysis of SBRT and TACE (n = 49) compared to TACE alone (n = 98) showed significantly better disease control, progression free survival, and 3-year overall survival for the TACE and SBRT combination group[30]. In a larger study of 199 patients with tumors ≤ 5 cm, combination therapy lead to improved local control rates, but did not have any effect on overall survival[31]. To date, TACE and SBRT combination therapy has been mainly used as a palliative treatment approach and only rarely as bridging to LT. Therefore, data on histopathologic response is limited with only one study reporting on two tumors which showed near complete tumor response after treatment[12]. Three ongoing prospective trials are currently recruiting patients to evaluate TACE and SBRT combination therapy in comparison to TACE (NCT01918683; NCT02513199; NCT03895359).

Given the promising results achieved with TACE and SBRT combination therapy, we aimed to analyze treatment response prior to liver transplantation in patients within MC who could not be treated with resection or ablation and were treated with TACE and SBRT.

MATERIALS AND METHODS

Patients

This multi-center retrospective trial was conducted to specify treatment options that may improve prognosis in patients with HCC and possible LT. Three German transplant centers, University Hospital rechts der Isar, Munich, University Hospital of Munich, and Hannover Medical School participated in the study. Protocols for patient analysis were reviewed and approved by the local ethics committee of each participating center. Decisions for tumor treatment were discussed in a multidisciplinary tumor board. Patients received treatment as standard of care and data were collected retrospectively.

For this study, medical records of all patients with liver cirrhosis and HCC within MC who underwent LT between 2007 and 2019 were retrospectively reviewed. From Hannover Medical School, only patients who received TACE and SBRT or SBRT alone prior to LT were screened. Patients who received at least one TACE with or without SBRT (TACE only: 8 patients at University Hospital rechts der Isar, Munich; 6 patients at University Hospital of Munich; TACE + SBRT: 4 patients at University Hospital rechts der Isar, Munich; 5 patients at University Hospital of Munich), or at least one SBRT alone (2 patients at University Hospital of Munich; 2 patients at Hannover Medical School), were included in our study. Patients who received additional tumor therapies as bridging such as resection of individual lesions, RFA, or MWA were not included into our study since these therapies are established as a curative treatment option. Additionally, data showing excellent response by radiology and histopathology to these therapies is already available[12,32].

Observation period started with initial diagnosis through December 2019. To compare the different dose and fractionation regimens used for SBRT, the biological equivalent dose (BED) of the surrounding isodose was calculated according to the formula BED = nd (1+ d/$alphabeta$) (with n: Number of fractions, d: Single dose and $alpha/beta$ set to 10).

Number and size of HCCs were documented by magnetic resonance imaging or computed tomography scan at the time of diagnosis. Number of treatment cycles, time of treatment, and radiation dose were assessed when applicable. Additionally, age, sex, cause of liver cirrhosis (alcohol, chronic viral hepatitis, other) and serum AFP levels were analyzed. After transplantation, the presence of vital tumor tissue in explant livers was analyzed. Specifically, size and number of any remaining tumor nodules were determined macroscopically and by histopathology in order to identify differences in tumor response. The absence of vital tumor tissue was considered as complete response.

Statistical analysis

The study was designed as a retrospective multicenter longitudinal survey. All data were analyzed using Microsoft Excel (version 16) and SPSS (version 25). Statistics were performed using Fisher-Freeman-Halton test. Due to the limited sample size, no multivariate analysis was performed. Kruskal-Wallis tests as well as Mann-Whitney-*U* tests were used for comparisons of variables between groups, when appropriate. All statistical tests were performed two-sided using a significance level of $\alpha = 5\%$.

RESULTS

The study cohort comprised 27 subjects with HCC of whom 14 received TACE only, four SBRT only, and nine a combination of TACE and SBRT. Within the study cohort, 20 (74%) patients were male, 7 (26%) female. Mean patient age was 60 (SD ± 6) years ranging from 48 to 71 years. All patients suffered from cirrhosis, mostly due to alcohol (11/27; 40%) or hepatitis C (10/27; 37%).

Most patients had a single tumor lesion (20/27; 74%). Of the seven patients with two lesions, one patient received a combination of TACE and SBRT and one patient SBRT only, the others were in the TACE only group. At the time of diagnosis, mean tumor size was 29.3 mm (SD ± 9.5 mm). Median AFP was 8.0 ng/mL, with 1st quartile 5.0 ng/mL and 3rd quartile 58.0 ng/mL (range 1.2 to 2515 ng/mL).

Treatment plans were tailored to each individual patient and varied in number of TACE cycles (median = 2, range 1 to 5) and SBRT radiation dose (range 18.9 to 54 Gy, in 3 to 9 fractions, prescribed to the surrounding isodose) (Table 1). The most common schemes were 3 × 12.5 Gy prescribed to the 65% -isodose and 3 × 15 Gy prescribed to the 60%-isodose delivered every other day. There were no statistically significant differences in age, gender, origin of cirrhosis, tumor size or number of tumor lesions between groups (Table 1, Figure 1). LT was performed after a median interval of 114 d (range 1 to 786 d) from SBRT treatment (Supplementary Figure 1).

Analysis of explant livers by histopathology showed different treatment responses. In 9/27 patients (33%), no vital tumor was detected microscopically, which was considered as complete response (Figure 2A-D, Supplementary Figure 2). Strikingly, for the majority of patients in the TACE and SBRT combination therapy group a complete response was observed (8/9, 89%), compared to none in the TACE only group (0/14, 0%) and only one in the SBRT only (1/4, 25%) group ($P < 0.001$) (Table 2, Figure 2E). When tumor size at the time of initial diagnosis was compared to tumor size in liver explants, treatment with TACE alone led to a stabilization or a decrease in tumor size in the majority of patients, but could not stop tumor growth in all cases. In the combination group, the only sample with vital tumor showed disease stabilization (increase in size < 20%) with most lesions being completely necrotic by histopathology as described above. In the SBRT group, one completely necrotic tumor was observed, but no conclusions on treatment response could be made due to the small sample size (Figure 3).

Of note, the only patient with vital tumor in the TACE and SBRT group had by far the highest AFP level (2515 ng/mL, Figure 4) and the shortest time interval between SBRT and LT (29 d). The only patient with a complete response in the SBRT only group had the smallest tumor in this group (12 mm, BCLC 0), the longest time between SBRT and LT (256 d) and was transplanted due to deterioration of liver function. While there was a weak correlation between tumor size and treatment response in the overall patient cohort, the difference was not statistically significant (Supplementary Figure 3).

On follow-up, two patients suffered from extrahepatic recurrence after LT, of whom one was in the TACE only group (with vital tumor tissue by explant histology) and one patient was in the TACE and SBRT group (no vital tumor detected in explanted liver).

Table 1 Patients characteristics of all patients and separated into treatment groups, *n* (%)

Characteristics	Total number of patients (n^1 = 27)	TACE only (*n* = 14)	Combination of TACE and SBRT (*n* = 9)	SBRT only (*n* = 4)	*P* value
Male/female	20 (74)/7 (26)	12 (86)/2 (14)	5 (56)/4 (44)	3 (75)/1 (25)	
age < 60/≥ 60 yr	13 (48)/14 (52)	6 (43)/8 (57)	5 (56)/4 (44)	2 (50)/2 (50)	
mean age yr ± SD	60 ± 6	59.5 ± 8	61 ± 4	60 ± 2	0.963
Genesis of cirrhosis					
1 alcohol	11 (41)	6 (44)	3 (33)	2 (50)	
2 viral[2]	12 (44)	8 (57)	3 (33)	1 (25)	
3 others[3]	4 (15)	0 (0)	3 (33)	1 (25)	
Numbers of TACE treatment cycle[4]					
1	12 (44)	5 (36)	7 (78)	NA	
2	6 (22)	4 (29)	2 (22)		
3 or more	5 (19)	5 (36)	0 (0)		
mean radiation dose in Gy		NA	40.00 ± 3.75	36.80 ± 17.56	0.586

[1]*n* = 27 is the number of patients included into our study.
[2]Ten patients suffered from hepatitis C virus (HCV), two from hepatitis B virus.
[3]One patient with combination of alcohol and HCV, three patients with autoimmune hepatitis.
[4]No statistical testing due to different therapeutic approaches. Dichotomous variables are presented in number and percentage, continuous variables in mean ± SD. TACE: Transarterial chemoembolization; SBRT: Stereotactic body radiation therapy.

Table 2 Tumor response by treatment (including tumor characteristics), *n* (%)

	Total number of patients (n^1 = 27)	TACE only (*n* = 14)	Combination of TACE and SBRT (*n* = 9)	SBRT only (*n* = 4)	*P* value
Complete response	9 (33.3)	0 (0)	8 (88.89)	1 (25)	< 0.001
Number of tumor lesions					0.517
1	20 (74)	9 (64)	8 (89)	3 (75)	
2	7 (26)	5 (36)	1 (11)	1 (25)	
mean tumor size[2]	29.3 ± 9.46	29.50 ± 7.63	27.67 ± 9.54	26.67 ± 14.50	0.389
BCLC[4]					
0	1 (4)	0 (0)	0 (0)	1 (25)	
A	26 (96)	14 (100)	9 (100)	3 (75)	
Median AFP[3,5]	8.0, 5.0/58.0	8.05, 5.2/84.2	8.0, 5.0/17.7	9.85, 8.0/11.85	

[1]*n* = 27 is the maximum number of patients included into our study.
[2]mean size of largest tumor in mm ± SD at time of diagnosis.
[3]Median AFP in ng/ml with 1st/3rd quartile at time of diagnosis.
[4]No statistical testing due to small sample size.
[5]No statistical testing due to high variation and SD. Patients characteristics of all patients and separated into treatment groups. Dichotomous variables are presented in number and percentage, continuous variables in mean ± SD. AFP: Alpha-fetoprotein; TACE: Transarterial chemoembolization; SBRT: Stereotactic body radiation therapy; BCLC: Barcelona clinic liver cancer.

DISCUSSION

In this study, patients with HCC who received a combination therapy of TACE and SBRT before LT had a significantly higher rate of complete histopathological response than patients who received TACE or SBRT alone.

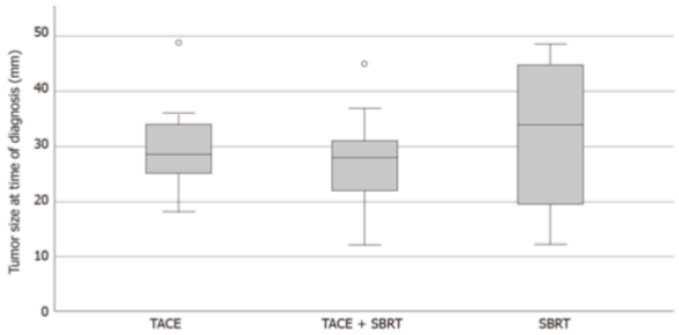

Figure 1 Box plot showing tumor size in each treatment group. Median is represented by bars, 25%-75% percentiles by boxes and outliers by markers. There were no statistically significant differences between groups. TACE: Transarterial chemoembolization; SBRT: Stereotactic body radiation therapy.

Current bridging strategies to LT aim to stabilize the disease but are not sufficient to delay tumor growth in all patients[7]. Thermal ablation and TACE are the most commonly used bridging therapies before LT. However, RFA or MWA are not technically feasible in all patients mostly due to tumor location, and a complete pathologic response to TACE alone is found in less than 35% of patients receiving LT for HCC[21,33]. Disease progression poses a risk in these patients – especially in countries with long waiting times such as Germany, with an average of two to three patients per center removed due to tumor progression each year, revoking any curative treatment option. Based on the outcomes of previous studies indicating improved response after TACE and SBRT combination *vs* TACE alone in HCC[34,35], we used TACE and SBRT combination therapy as an individualized treatment approach in patients at risk for tumor progression beyond MC to achieve long term disease stabilization.

While a better outcome of the combination of TACE and SBRT was expected[28,30], the rate of complete tumor response by histopathology was surprisingly high in our patient cohort. In almost all patients who received combined TACE and SBRT, no residual vital tumor was detected in explant livers (TACE and SBRT 89% *vs* TACE alone 0%; $P < 0.001$). The only patient in the TACE and SBRT group with vital tumor tissue by histopathology had a very high AFP (2515 ng/mL) and was transplanted less than one month after SBRT treatment. On the other hand, one patient with complete response in the SBRT-only group had a lesion < 2 cm, an interval of more than 6 mo between SBRT and LT, and was transplanted for deterioration of liver function.

In the small group of patients treated with SBRT alone (four patients in which chemoembolization was not feasible for anatomical reasons or where treatment decision was made at an external hospital), only one of four patients had no vital tumor by histomorphology. Importantly, we had no indications for differences regarding SBRT schemes between groups in our study cohort (Table 1, Supplementary Figure 4 and 5). However, from a sample size this small and above all a very short time interval between SBRT and LT in three out of four patients in the SBRT only group (Supplementary Figure 1) it cannot be ruled out that SBRT alone might be equally efficient to TACE and SBRT combination therapy. However, recently published data from a cohort of 14 patients showing complete response by histopathology in 23.1% of tumor nodules in liver explants is in line with our data[36] – indicating that complete tumor necrosis is not commonly achieved after SBRT alone.

Importantly, none of our patients showed any higher grade treatment-related toxicities, which is in line with previous analyses[37,38]. While treatment side effects were not prospectively evaluated, there were no reports of deterioration of liver function in the TACE and SBRT group, or other higher-grade side effects observed at our centers. However, our study comprises a relatively small group of patients and almost all patients of our cohort had well-preserved liver function. Therefore, outcomes might have been different in patients with impaired liver function[39,40]. Clearly, long-term hepatic toxicity, which is mostly negligible in a pre-transplant setting, might be limiting in palliative treatment strategies[38].

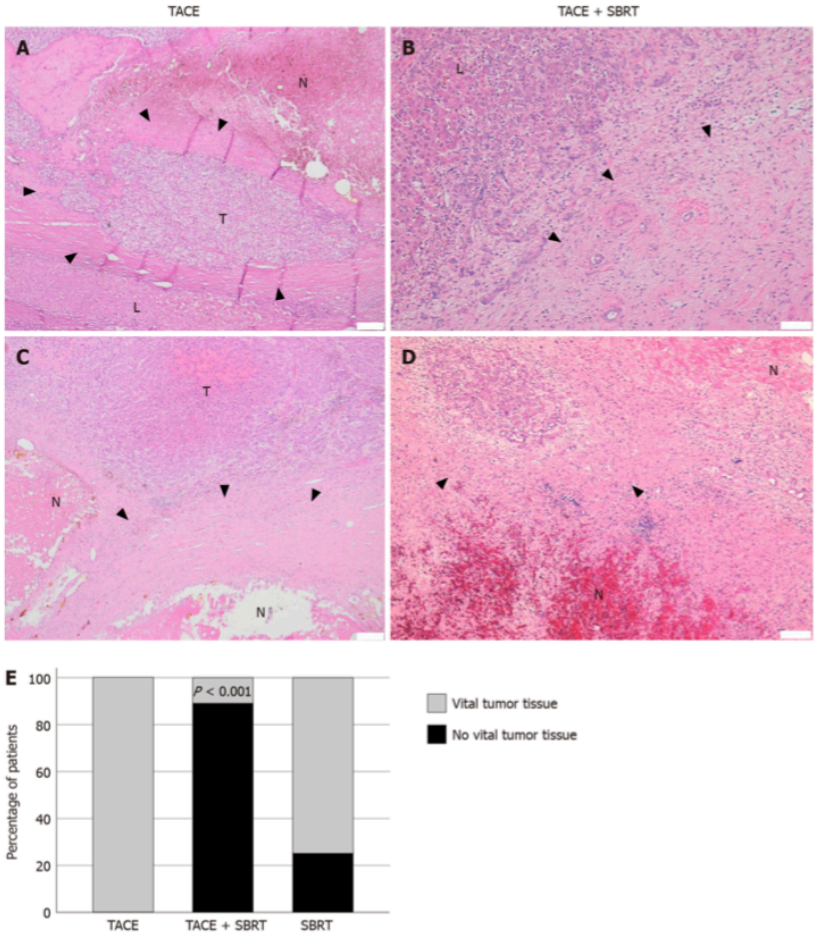

Figure 2 Tumor response by histopathology for each treatment group. A-D: Representative histopathology (Hematoxylin and eosin stain) of tumor lesions in explant livers after transarterial chemoembolization (TACE) (A, C; scale bar 200 μm) or TACE + stereotactic body radiation therapy (SBRT) (B, D; scale bar 100 μm). Samples show necrosis with granulation tissue and organization by connective tissue at the border area (arrowheads) to normal liver. Residual tumor tissue was observed in TACE only samples, while no vital tumor cells could be detected in most patients in the TACE + SBRT group (B, D); E: Bar graph displaying the proportion of vital tumor tissue in each treatment group. Combination therapy with TACE and SBRT leads to a statistically significantly lower number of residual tumor tissue in explant livers ($P < 0.001$). TACE: Transarterial chemoembolization; SBRT: Stereotactic body radiation therapy; N: Necrosis; L: Normal liver; T: Tumor tissue.

Together, data from our study strongly indicate that TACE and SBRT combination therapy might lead to higher rates of complete histopathological tumor response than TACE alone. Nevertheless, this study has some limitations. A sample bias due to the multicenter, retrospective design, large duration of study recruitment and little opportunity to adjust for possible confounders due to small sample size cannot be ruled out. Furthermore, most of the patients with two tumor lesions were in the TACE group, which might have biased the results towards a lower percentage of complete response in this cohort. Additionally, the limited number of patients in this study does not allow to draw any conclusions on tumor recurrence or even overall survival. Our cohort accounts for less than 25% of all patients that were transplanted with HCC in Munich transplant centers as most HCC patients received additional bridging therapies such as thermal ablation or even resection whenever feasible. Therefore, whether these histopathological findings will translate into a survival benefit remains to be investigated prospectively in a larger patient cohort. For example, one patient

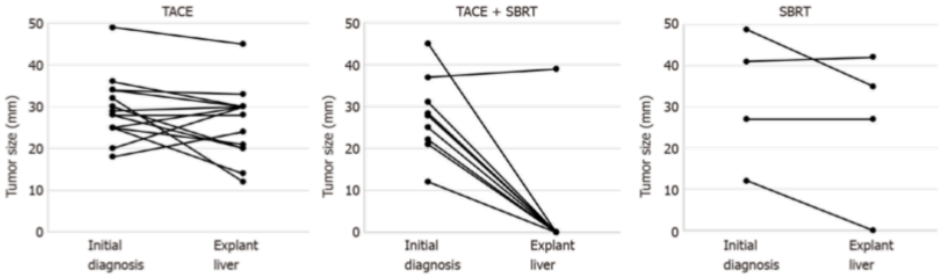

Figure 3 Tumor size at time of diagnosis and in explant histology for each treatment group. Tumor size at initial diagnosis was determined by radiology. When more than one tumor was present, the size of the largest tumor was graphed. In cases where no vital tumor tissue was detected, a size of 0 mm was graphed. TACE: Transarterial chemoembolization; SBRT: Stereotactic body radiation therapy.

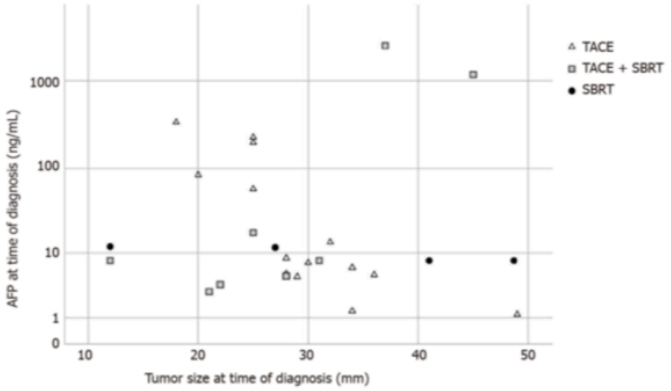

Figure 4 Correlation of alpha-fetoprotein and tumor size for each group. Scatter chart showing the correlation of alpha-fetoprotein in ng / mL and tumor size in mm for each group. AFP: Alpha-fetoprotein; TACE: Transarterial chemoembolization; SBRT: Stereotactic body radiation therapy.

who received TACE and SBRT combination therapy and showed a complete response in the explant liver developed metachronous metastatic disease less than 6 mo after liver transplantation. In this patient, metastases first occurred in the skull that was not routinely screened by standard tumor staging procedures while the patient was on the waiting list. If bone metastases to the skull were already present before completing SBRT bridging therapy before LT cannot be determined retrospectively but is certainly a possibility. The development of extrahepatic metastases therefore remains an eminent risk even with excellent local tumor control, yet it occurs very rarely at this stage. On the other hand, a patient with high AFP (2515 ng/mL) indicating limited prognosis was successfully transplanted after TACE and SBRT combination therapy. Despite only partial response with 20% of vital tumor tissue by histopathology, he shows no signs of tumor recurrence more than 4 years after LT.

More recently, down-staging to MC has been implemented in organ allocation criteria in several countries. In our cohort, a complete response with decrease of tumor size and loss of arterial hyperperfusion was routinely observed in the combination cohort (Figure 5).

Even though our study did not include any patients beyond MC, TACE and SBRT combination therapy might be efficient for down-staging patients to MC to reach requirements for LT. As a sample bias cannot be excluded due to the limited number of patients, retrospective design, and long recruitment time, further studies in a larger patient cohort are needed to confirm high treatment response to TACE and SBRT combination therapy and to clarify if these findings translate into a decreased number of waitlist removals due to tumor progression or into reduced rates of tumor

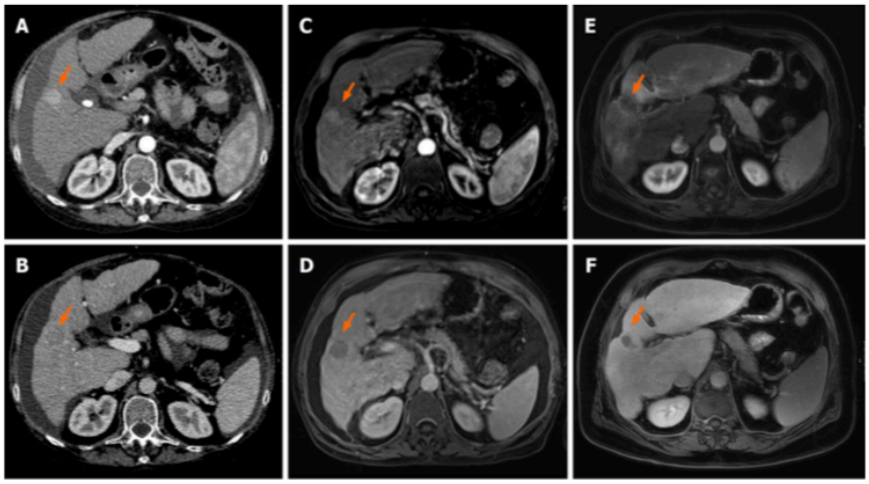

Figure 5 Imaging from before and after combination therapy in one patient in the transarterial chemoembolization + stereotactic body radiation therapy cohort. A, B: Contrast enhanced computed tomography (CT); C-F: Magnetic resonance imaging (MRI). Contrast enhanced CT and MRI, arterial phase (top row) and portal venous phase (bottom row) cross sectional imaging from before (A–D) and after (E, F) treatment. At baseline CT and MRI, a well-defined nodular lesion with typical contrast agent dynamics is noted in the right liver lobe (Arrows). After treatment, typical radiation induced peri-lesional hyperenhancement and no hepatocellular carcinoma-specific contrast agent uptake is noted.

recurrence after liver transplantation.

CONCLUSION

In summary, data from our study shows that patients not eligible for ablation or resection who received TACE and SBRT combination therapy were significantly more likely to have complete histopathological tumor response in explanted livers compared to patients treated with TACE or SBRT only. Whether TACE and SBRT combination therapy results in decreased number of waiting list removals and/or a reduced rate of tumor recurrence after LT needs to be evaluated prospectively in a larger patient cohort. Additionally, future studies will need to show if patients within MC who are not eligible for LT because of old age or relevant co-morbidities could benefit from TACE and SBRT combination therapy if curative resection or ablation is not possible due to tumor location.

ARTICLE HIGHLIGHTS

Research background

In patients with hepatocellular carcinoma (HCC) who are not eligible for resection or ablation therapy, liver transplantation presents a curative treatment option. Due to organ shortage there are long waiting times with the risk of tumor progression. Therefore, efficient bridging therapies are needed.

Research motivation

This study evaluated the treatment response to a combination therapy of transarterial chemoembolization (TACE) and stereotactic body radiation therapy (SBRT) as bridging to liver transplantation.

Research objectives

This study aimed to establish a pathologic response in explant livers after TACE and SBRT.

Research methods

Retrospective multicenter analysis of 27 patients that underwent liver transplantation and received either TACE or SBRT alone or a combination therapy of TACE and SBRT as bridging to liver transplantation.

Research results

About 89% of the patients in the TACE and SBRT combination group had no residual tumor tissue by histopathology, whereas 0% in the TACE only and 25% in the SBRT only group had a complete histopathological response.

Research conclusions

A combination of TACE and SBRT shows superior pathologic response in comparison to TACE or SBRT alone for bridging to liver transplantation in patients with HCC.

Research perspectives

If complete histopathological response in the TACE and SBRT combination group translates into a better progression free and overall survival needs to be evaluated in larger studies.

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