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Insights into Hepatoblastoma Relapse from the German HB99 Trial and the Liver Tumour Registry

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Abbreviations

5FU	5-Fluoruracil
AFP	Alpha-Fetoprotein
B	Brain
C	Combined Disease (Liver + Metastatic)
Ca	Carboplatin
CE	Carboplatin + Etoposide
Chemo/CTx	Chemotherapy
Cis	Cisplatin
CHIC	Children's Hepatic Tumours International Collaboration
CNS	Central Nervous System
COG	Children's Oncology Group (USA)
C5VD	Cisplatin + 5-FU + Vincristine + Doxorubicin
D	Death
DFS	Disease Free Survival
Dox	Doxorubicin
E	Etoposide
E+	Extrahepatic Disease
EFS	Event Free Survival
EFS_{dia}	Event Free Survival from initial diagnosis to first relapse
EFS_{rel1}	Event Free Survival from date of relapse to date of next event
F+	Multifocal Disease
FR	Further Relapse
FU	Follow-Up
GPOH	German Society for Paediatric Oncology and Haematology
HB	Hepatoblastoma
HB-99 / H	Hepatoblastoma 99 Study
HB-ET	Hepatoblastoma with epithelial components only
HB-ET (DD HCC)	Hepatoblastoma of epithelial type but with possible differential diagnosis as hepatocellular carcinoma
HB-MT	Hepatoblastoma with mixed epithelial and mesenchymal components
HCN-NOS	Hepatocellular Neoplasm – Not otherwise specified
HCC	Hepatocellular Carcinoma
HD CE	High Dose Carboplatin+Etoposide
HR	High Risk
I	Ifosfamide
IPA	Ifosfamide + Cisplatin + Adriamycin (Doxorubicin)
Ir	Irinotecan
JCCG/ JPLT	Japanese Children's Cancer Group
L	Local (Liver) Disease
LMU	Ludwig-Maximillians-University
LTR	Liver Tumour Registry
Lu	Lung
M	Metastatic Disease
M+	Metastatic Disease (Ref Roebuck)
MD	Missing Data
Me	Melphalen
Mets	Metastases
Op	Operation
OS	Overall Survival

OS_{dia}	Overall Survival from diagnosis
$OS_{rel\ 1}$	Overall Survival from first relapse
P	Peritoneal
P+	Portal Vein Invasion
PLADO	Cisplatin + Doxorubicin
Super PLADO	Cisplatin + Doxorubicin + Carboplatin
PRETEXT	Pre Treatment Extent of Disease
Rem	Remission
S	Sorafenib
SCUD	Small Cell Undifferentiated Disease
SIOPEL	International Childhood Liver Tumours Strategy Group
SR	Standard Risk
Surg	Surgery
TLCT	Transitional Liver Cell Tumour
Tx	Transplant
V+	Vena-Cava (VC) +/- Liver Vein Invasion
VCR	Vincristine
VI	Vincristine+Irinotecan
WDF	Well Defined Foetal Histology

1. Introduction

1.1 Hepatoblastoma

Hepatoblastoma (HB) although rare in absolute terms (1.2-1.5 people per million per year, [Hafberg et al. 2019]) is the commonest primary malignant liver tumour in infants and small children [Stocker 2001]. Long-term survival in HB patients is usually possible when the tumour can be resected completely. However, complete removal of the tumour is possible in <50% at diagnosis [Meyers at al. 2014, Raney 1997]. Hence, the poor patient survival rates of around 30% in the 1970s, when surgical resection alone was the primary treatment option available. Since this time there has been a significant improvement in the outcome for HB patients as a result of a number of factors. The most notable of these are the introduction and subsequent refinement of chemotherapy treatment, the improvements in surgical techniques including the introduction of liver transplants, the increasing accuracy in histopathological HB identification [Czauderna et al. 2014], and advances in the quality of radiological imaging. Studies by the 4 main paediatric liver tumour study groups, which are the German Society for Paediatric Oncology and Haematology (GPOH), the European International Childhood Liver Tumours Strategy Group (SIOPEL), the Children's Oncology Group (COG) and the Japanese Children's Cancer Group (JCCG), were instrumental in this process.

One of the most significant changes in the treatment of HB was the introduction of chemotherapy. Evidence that HB was chemoresponsive (allowing eradication of residual disease post-resection) began to emerge in the early 1970s. In the years that followed it became increasingly common to use adjuvant chemotherapy to treat both resectable and non-resectable HB patients (e.g. [Evans et al. 1982]). The observation of the reduction in tumour size achieved via the use of chemotherapy in those patients with initially inoperable tumours led to the trial of neo-adjuvant chemotherapy for those patients whose tumours were deemed to be unresectable at the time of initial diagnosis (e.g. [von Schweinitz et al. 1997]). The success of this approach actually resulted in one of the main liver tumour research groups (SIOPEL) recommending pre-operative chemotherapy for all HB patients [Czauderna et al. 2014].

As the timing of chemotherapy treatment with relation to that of tumour resection became more defined over this period, so did the type of chemotherapy treatment used. Evidence from numerous studies led to the establishment of the central role of platinum-based chemotherapy in the treatment of HB. Thus, for example, the INT-0098 trial by Ortego et al. between 1989 und 1992 compared the efficacy of 2 cisplatin-based treatments and suggested that the cisplatin might in fact be the principal anti-tumour agent in the 2 regimens used [Ortego et al. 2000]. Since this point, all of the large trials have had at least one treatment arm containing a platinum-based chemotherapeutic agent, usually

cisplatin. The standard treatment in Germany from 1999-2008 was a combination of ifosfamide, cisplatin and doxorubicin (IPA) for standard risk (SR) patients or a combination of carboplatin and etoposide (CE) for high risk (HR) patients as part of the HB-99 trial. This trial ultimately achieved a 3-year overall survival (OS) rate of 94% for the SR patients and 64% for the HR patients [Häberle et al. 2019]. This was then adapted to cisplatin monotherapy (CDDP-M) for SR patients and a combination of cisplatin, carboplatin and doxorubicin (Super PLADO) for HR patients following the results of the SIOPEL 3 and 4 trials [Perilongo et al. 2009, Zsiros et al. 2010, Zsiros et al. 2013] which had 3-year OS rates of 95% (SIOPEL 3) for those SR patients and 69% (SIOPEL 3)/ 83% (SIOPEL 4) for the HR patients. The variation in treatment as a result of the patients' prognostic outcome at diagnosis as seen in these 2 trials (i.e. SR versus HR) resulted from observations from the 70`s onwards, that generalised chemotherapy treatments led to better outcomes in certain patient groups as compared to others. For example, in the SIOPEL 1 trial, the intention was that all patients would receive a standard amount of chemotherapy treatment irrespective of the initial disease stage. The 5-year OS for all patients in this study was 75%, but the 5-year OS for stage I patients was 100% as compared to 57% for stage IV patients [Brown et al. 2000]). Hence over time studies started to trial the use of prognostic-group directed treatment. Initially, this more patient specific treatment was only employed to reduce treatments given to those patients with very good prognoses such as in the INT-0098 trial [Ortego et al. 2000]. Here, patients were divided into 1 of 4 main groups based on surgical staging criteria and then further divided into subgroups based on tumour histology. Patients who were classified as having stage I disease with pure well-defined foetal histology received less chemotherapy than was used to treat all the other HB-patients in the trial. As this group of patients still achieved a 5-year overall survival (OS) of 100% [Ortego et al. 2000], this indicated that these patients had previously been overtreated and required less chemotherapy than other HB patients with more extensive disease and different histological findings.

Eventually, all of the major studies started to routinely classify patients into a minimum of 2 different treatment groups (e.g. SR versus HR) based on a variety of prognostic factors (e.g. JPLT-1 [Sasaki et al. 2002], SIOPEL 2 [Perilongo et al. 2004], HB94 [Fuchs et al. 2002], SIOPEL 3 ([Perilongo et al. 2009], [Zsiros et al. 2010]), P9645 ([Malogolowkin et al. 2011], [Katzenstein et al. 2009], [Malogolowkin et al. 2006]), JPLT-2 [Hishiki et al. 2011], HB99 [Häberle and von Schweinitz 2012], and AHEP0731 [Malogolowkin et al. 2012]). One of the most commonly used prognostic factors was, and still is, the surgical resectability/extent of the tumour at diagnosis. The two main methods that have been used to evaluate this are a surgery-based Evans-style staging system and the radiology-based PRETEXT staging system. The Evans staging system stratifies patients into different groups based on the pre-chemotherapy extent of the tumour and the extent of the initial surgical resection achieved [Evans et al. 1982]. The PRETEXT staging system (pre-treatment extent of disease) consists of a PRETEXT value

and a number of associated annotation factors [Brown et al. 2000]. This system provides a measure of pre-treatment tumour distribution both within and external to the liver. A PRETEXT value from I-IV is assigned according to the number and distribution of involved liver segments on radiological scans. Thus, for example PRETEXT I tumours affect only one of four anatomically defined non-central sectors whilst PRETEXT IV tumours affect all four. The annotation factors described by Brown et al. in 2000 included "V" as a measure of vena cava/hepatic vein involvement, "P" as a measure of portal vein involvement, "E" as a measure of extrahepatic involvement via direct spread, and "M" as a measure of distant metastatic involvement. Unfortunately, as the staging systems used differed between the 4 major study groups comparison of results between trials has historically proved difficult. In order to solve this issue, which is significant given the comparative rarity of HB, there has been an increase in international cooperation in more recent years. Hence, the creation of the Children's Hepatic Tumours International Collaboration (CHIC) with the aim of validating old/identifying new prognostic factors and combining these to produce a staging system which could be used in all future hepatoblastoma trials. This group examined data obtained from 8 of the largest multicentre HB trials over the previous 25 years in order to identify the most significant prognostic factors for HB patients [Czauderna et al. 2016]. They then used this information to create the CHIC classification system [Meyers et al. 2017]. This system uses the PRETEXT grade, patient age, the alpha-fetoprotein level, the original PRETEXT annotation factors V, P, E and M, and the newer PRETEXT annotation factors R (tumour rupture or intraperitoneal haemorrhage) and F (multifocal/unifocal) to split patients into 4 different risk categories from very low risk to high risk. This staging system, which has been altered only slightly since its inception with the introduction of modifications to the PRETEXT annotation factors V and P ([Roebuck et al. 2006], [Towbin et al. 2017]), is now being used along with histological information to direct treatment decisions within the current Paediatric Hepatic International Liver Tumour Trial (PHITT). This is the first trial where all the major research groups are collaborating and using the same risk classification system to group patients into treatment groups. This has allowed the splitting of patients into multiple more patient-specific treatment groups and it is likely that there will be further developments in this direction over the coming years (e.g. as a result of the inclusion of biological information into the classification system).

Another significant development leading to increased survival times of HB patients was an improvement in surgical techniques and the introduction of liver transplants for the treatment of HB patients with locally unresectable HB and either no metastases or treatable metastases. Liver transplants were first attempted following the introduction of chemotherapy as it was felt that the chemotherapy would reduce the risk from micrometastatic disease and increase the ease of surgical resection as a consequence of tumour shrinkage and indeed it quickly became clear that shorter-term

outcomes were better for the affected group of patients following a liver transplant. Thus, in the study by Otte et al. the overall survival at 10 years post-liver transplant was 66% for all transplant patients and 85% for those patients who had received a primary liver transplant. Of note however was the poorer outcome, 40% 10-year post-transplant survival, for those patients who had a "rescue" transplant following a previous surgical intervention (e.g. resection, transplant) [Otte et al. 2004]. This finding led to a push for primary transplants not just for those patients who had unresectable tumours but also for patients where the viability of resection was unclear. Newer data have however questioned this finding as their results suggested that rescue liver transplants in fact do not appear to have a significantly worse prognosis [e.g. Fuchs et al. 2017] whereas patients who have had liver transplants often have a poor long-term prognosis [de Ville de Goyet et al. 2021].

Improvements in outcome have also been achieved over this time due to increasing refinements in the histopathological diagnosis of HB and the introduction of an internationally recognised standardised classification system [López-Terrada et al. 2014] for these often heterogeneous tumours. The importance of histopathological findings in HB patients has been clear for some time with regards to the identification of a subtype of HB tumours that consist solely of a well-defined foetal histology. The sole presence of this histology has been used as a signal to reduce the amount of chemotherapy treatment given within the lowest risk group as previously mentioned for the INT-0098 trial [Ortego et al. 2000] among others. More recently, introduction of new techniques have helped more accurately classify tumour entities which have historically been identified as HB tumours, but which are now thought to be separate tumour subgroups based on their histopathological (e.g. immuno-histochemical) features. These entities often have different survival outcomes as compared to those of the HB tumour group. One example of this is a subset of small cell undifferentiated tumours, which were previously identified as hepatoblastomas with a poorer prognosis than might be expected from their other tumour characteristics [Czauderna et al. 2014]. This subset has now been reclassified, on the basis of presence/absence of specific immunohistochemistry markers/genetic alterations, as malignant rhabdoid tumours of the liver [Vokuhl et al. 2016] as opposed to hepatoblastomas. Another example is the group of tumours where it is difficult to distinguish on histological findings alone whether they are HB or HCC tumours. These were initially classified as transitional liver cell tumours in 2002 [Prokurat et al. 2002] and then as Hepatocellular Neoplasm NOS (not otherwise specified, HCN-NOS) in 2014 [López-Terrada et al. 2014]. The importance of provision of clinical information (e.g. patient age) to help with the correct classification of these tumours was highlighted during the 2014 symposium. These tumours often have a worse outcome than would be expected based on their risk classification. Some of these tumours would have previously been classified as HB tumours.

The overall result of all of this treatment refinement is the continued improvement in patient outcomes to >80% ([Czauderna et al. 2014], [Erdmann et al. 2019]). Thus, for example, the HB99 study from the GPOH reported a 3-year overall survival (OS) for SR patients of 94% vs 65% for HR patients and a 3-year events free survival (EFS) for SR patients of 92% vs 51% for HR patients [Häberle et al. 2019]. The SR and HR classifications took into account both the COG-staging system (based on the Evans staging system) und the PRETEXT system. Indeed, the outcomes for patients with limited localised disease have improved to such an extent that more recent studies, including the GPOH HB99 study mentioned above [Häberle et al. 2019], have actually begun trialling further reductions in chemotherapy given so as to minimise the long-term treatment-induced toxicities. Further examples of this include the P9645 study [Malogolowkin et al. 2011] and the AHEP0731 study (ongoing – verbal report), which found that surgical resection alone was sufficient for Evans stage I HB with well-defined foetal (WDF) histology, the SIOPEL-2 [Perilongo et al. 2004] and 3 [Perilongo et al. 2009] studies, which found that cisplatin alone (i.e. without doxorubicin) was sufficient for treatment of "standard risk" patients, and the AHEP0731 study, which demonstrated a maintenance of excellent outcome (EFS > 90 %, verbal report Howard Katzenstein) despite reduction in number of treatment cycles (from 4 to 2) given to "low risk" patients.

The outcome for patients with extensive local disease and/or metastatic disease has also improved [Czauderna et al. 2014] but is still not as good as that for the lower risk patients. The 3-year EFS and OS for patients with high risk hepatoblastoma was 76% and 83% respectively in the SIOPEL 4 study [Zsiros et al. 2013]. Studies have been (JPLT-1 and -2; HB 94 and HB 99 (GPOH); SIOPEL-2, -3 and 4; P9645) and still are (AHEP0731, PHITT) looking at time-based intensification of chemotherapy and the use of novel agents (e.g. etoposide with carboplatin (HB 94, HB 99), irinotecan with vincristine (AHEP0731)) in combination with more radical surgery in an attempt to further improve prognosis. Some patients have been found to respond well to intensified pre-operative chemotherapy regimens whilst others do not (SIOPEL 4) suggesting a role for further stratification of patients according to response with subsequent associated treatment modification within this high risk group.

1.2 Relapse

Despite these advances however, the outcomes for those patients who relapse after an initial remission remains poor (3 year EFS 34%, 3 year OS 43% - [Semeraro et al. 2013]). Research in this area has been slow, as collection of sufficient information for such an infrequent event in such a rare condition ideally requires multinational pooling of data over many years in order for sufficiently robust predictions/recommendations to become identifiable for this subset of HB patients. This kind of

international collaboration has only started recently and has been complicated as a consequence of the different staging and subsequent treatment methods adopted by the different study groups.
At present, there have only been a relatively small number of articles published which provide insight into the factors that influence occurrence and outcomes in relapse patients. Many of these have been single centre studies based on results from only a few patients with the outcomes from progression and relapse patients often being analysed together, such as in the recent article from Hou et al. 2021, or case reports. The 2 larger studies of note in this area are based on results from the SIOPEL 1-3 cohort ([Semeraro et al. 2013]: 59 relapse patients alone) and results from patients enrolled in multiple COG phase I and II studies ([Trobaugh-Lotrario et al. 2016]: approx. 71 relapse/progression patients). Current treatment of relapse involves a combination of surgery and chemotherapy, ideally with different agents to those used during the initial therapy ([Venkatramani et al. 2012], [Trobaugh-Lotrario and Feusner 2012]).

1.3 Aim of Dissertation

The aim of this work was firstly to improve our understanding of recurrent HB. Areas of particular interest include:

(1) validation of previous observations with regards to characteristics of patients who are likely to relapse and identification of further potential risk factors,
(2) increased knowledge of the time course of relapsed HB,
(3) deeper insight into the best treatment options for these patients,
(4) guidance as to the role of liver transplant/pulmonary metastasectomy versus more conservative treatment options in those patients who are chemo responsive,
(5) re-evaluation of the outcomes for relapse patients in-light of improvements that have been implemented over recent years.

Although the small number of subjects in this paper limit the statistical analysis of this data, the patients analysed do at least represent a relatively homogenous data set.

Secondly, a further aim was to increase the available published data on relapsed HB in order to enable consolidation of data from multiple studies as was seen in the CHIC collaboration. The increased patient numbers resulting from this process should allow statistical analysis of this data and evidence-based improvements in outcomes for this patient group in the future.

2. Methods

2.1 Participants

This retrospective data review included those patients from the HB99 Study ([Häberle and von Schweinitz 2012], [Häberle et al. 2019]) and the Liver Tumour Register (LTR) who had had a tumour relapse, as evidenced on imaging, after complete remission (CR). The HB99 study prospectively recruited patients from the GPOH centres in Germany, Austria and Switzerland between 1.1.1999 and 31.12.2008. The LTR started recruiting patients on the 17.1.2011 from the GPOH centres in Germany, Austria and Switzerland. Recruitment is still ongoing. Patients who were entered into the LTR between 17.01.2011 and 30.08.2019 were included in this study.

2.2. Ethics Approval

Informed consent for data collection was obtained from either the patient and/or the parents as appropriate at the time of recruitment into either the HB99 study or the LTR. EK approval for the HB99 study was granted on the 9.5.2000 by the Children`s University Hospital in Basel and on the 9.6.2000 by the Rheinische-Friedrich-Wilhelms-University in Bonn. The EK approval for the LTR was granted on the 17.1.2011 by the LMU in Munich.

2.3 Study Method

The HB99 study was a national clinical trial that prospectively recruited hepatoblastoma patients between 1.1.1999 and 31.12.2008. The methods used in the HB99 study have previously been described in the 2019 paper from Häberle et al. The LTR is a national register that has been prospectively recruiting patients from 17.1.2011 and is still ongoing. After attainment of consent, information is collected at the site and then sent to the Ludwig-Maximillian-University (LMU) in Munich where it is entered onto a database. Clinical data were extracted retrospectively from the aforementioned data sets and any missing data were subsequently requested from the involved treatment centres.

The treatment that the patients received at primary diagnosis varied with regards to the standard treatments used at the time of occurrence of this initial treatment and the risk stratification of the patients at that time. Thus, patients were stratified as either standard risk (SR) or high-risk (HR) using the PRETEXT system as it was used for the HB-99 patients ([Häberle et al. 2019], [Roebuck et al. 2006]) and the LTR patients who have been included in this work. SR patients were those who had tumour confined to ≤ 3 segments of the liver (PRETEXT I-III) and no additional prognostic factors (i.e. AFP >100 ng/ml, no metastases (M-), no extrahepatic tumours (E-), no vessel involvement (V-, P-), unifocal tumours (F-), no tumour rupture (R-)). All other patients were classified as HR patients. This system is

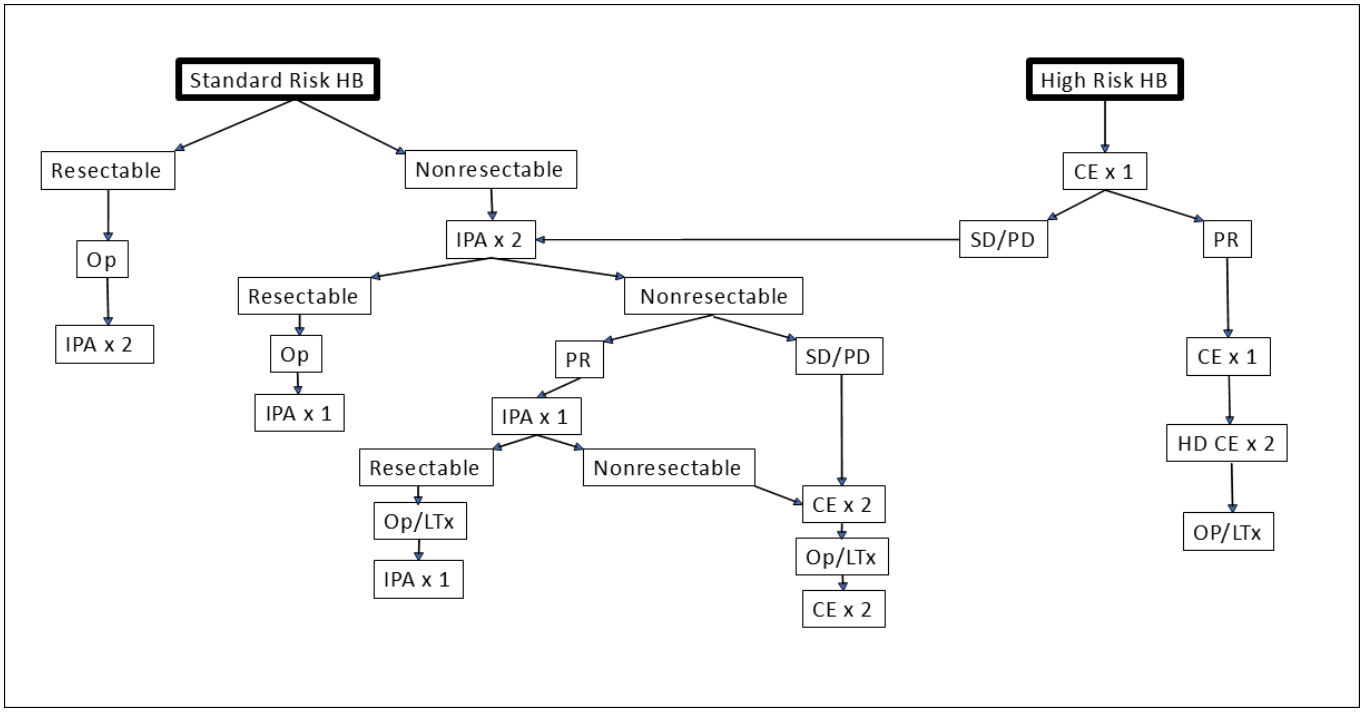
similar to that used by the SIOPEL group at the same time and thus allows comparison of this data with that from the Semeraro et al. 2013 relapse paper.

The standard chemotherapy treatment on the HB99 trial was as follows [Häberle et al. 2019]:

(1) SR-patients received ifosfamide, cisplatin and doxorubicin (IPA)

(2) HR-patients initially received 1-2 cycles of carboplatin and etoposide at the conventional dose. Responders went on to receive high-dose (HD) carboplatin and etoposide followed by an autologous stem cell transplantation. Non-responders went on to receive IPA instead.

Fig. 1: HB99 Chemotherapy Plan as per Häberle et al. 2003: I=ifosfamide, P=cisplatin, A=Adriamycin (doxorubicin), Op=operation, PR=partial response, LTx=liver transplant, SD=stable disease, PD=progressive disease, C=carboplatin, E=etoposide, HD=high dose.

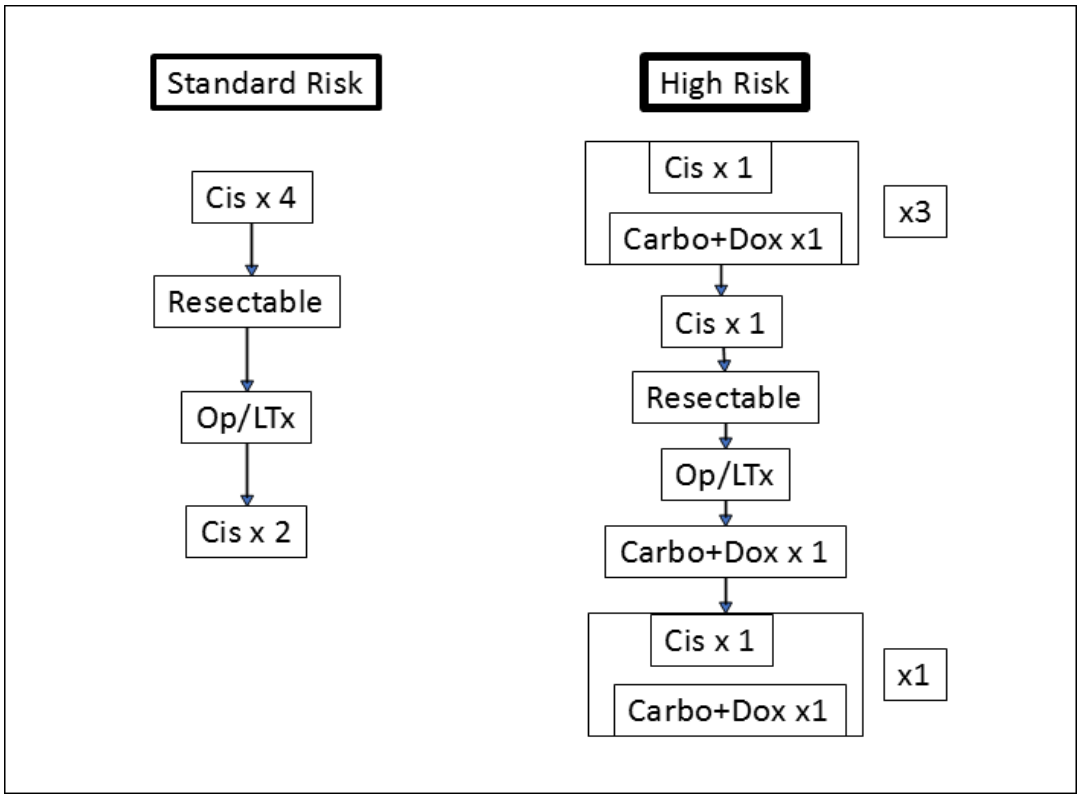


The standard chemotherapy given to patients whose data were collected in the LTR was based on the applicable German Guidelines at the time of patient treatment. For the majority of patients in the LTR this followed a SIOPEL 3-based protocol:

(1) SR patients received Cisplatin monotherapy [Perilongo et al. 2009]

(2) HR patients received Cisplatin alternating with Carboplatin and Doxorubicin [Zsiros et al. 2010]

Fig. 2: SIOPEL 3 Chemotherapy Plans as per Perilongo et al. 2009 (SR HB) and Zsiros et al. 2010 (HR HB): Cis=cisplatin, Dox=doxorubicin, Op=operation, LTx=liver transplant, Carbo=carboplatin.



Tumours were resected during or post-chemotherapy. Lung metastases (mets) were resected if still visible on the CT scan after neoadjuvant chemotherapy. Unresectable liver tumors were treated via liver transplantation, after surgical excision of radiologically identified palpable lung mets where applicable. The extent of tumour resection was classified according to available data (surgery +/- histology reports). R0 resections are those where there was a complete resection (histology), R1 resections were microscopically incomplete, R2 resections were macroscopically incomplete, R3 resections were biopsies only. A primary liver transplantation was the first surgical liver tumour treatment. Transplantations after a first tumour resection were rescue transplantations.

Routine examinations were timetabled to be performed during follow-up after the achievement of complete remission. These were planned to include AFP measurements, abdominal ultrasound scans, and chest x-rays. The frequency of these examinations reduced over time (e.g. 1^{st}-2^{nd} year: 3 monthly, 3^{rd} year: 6 monthly, 4^{th} – 9^{th} year: yearly).

Overall survival (OS_{dia}) from diagnosis is the time between date of diagnosis and date of death from any cause or the date of last follow up (FU). Event free survival from initial diagnosis (EFS_{dia}) is the time

from the date of diagnosis to the date of first relapse. OS from first relapse (OS_{rel1}) is the interval from date of first relapse to date of death from any cause or to date of the last FU. EFS from first relapse (EFS_{rel1}) is the time from the date of diagnosis of the 1st relapse to the date of the next relapse, start of progression, death, or the date of last FU, whichever occurred first. Complete remission was defined as successful completion of surgical/chemotherapeutic treatment combined with at least one normal AFP (for age) result.

Relapse was defined as new tumour on imaging, with or without an increase in AFP, after previous achievement of a complete remission. An AFP rise without radiological findings was not counted as a relapse. Patients with progressive disease were excluded from the study. Progression was defined as the continued growth of tumour or failure of AFP to normalise after attempted tumour resection or during therapy in patients who were never resected.

2.4 Statistical Analysis

Statistical analysis was performed using SPSS Version 26. Kaplan-Meier-Survival Plots were used to calculate OS and EFS figures [Kaplan and Meier 1958]. The use of statistical analysis in this paper was however limited owing to the small number of patients included in this study.

3. Results

3.1 Relapse Rate

Of the 142 patients who were included in the HB99 study (1.1.1999-31.12.2008) and the 220 HB patients included from the LTR (17.1.2011–30.8.2019) at the time of our analysis, 12 (8.5%) and 13 (5.9%) patients respectively were identified as having a relapse according to our relapse criteria. The HB99 rate is comparable with the combined relapse rate reported for the SIOPEL 1-3 studies, which was 8.4% [Semeraro et al. 2013]. The LTR rate is slightly lower and this difference will be considered later in more detail in the discussion section. The overall relapse rate was 6.9% (25/362).

Histological diagnosis in the patients selected was confirmed in 21 out of 25 patients by means of a local and central GPOH (German Society for Paediatric Oncology and Haematology) pathology review. In 4/25 patients only the occurrence of a local pathology review could be confirmed with the available data.

3.2. Relapse Patient Characteristics and Initial Treatment

Table 1 provides an overview of the clinical characteristics of the 25 relapse patients and their initial treatment.

Table 1. Overview of Clinical Characteristics of the 25 identified patients and their initial therapy. This information has been provided for the group as a whole and also for subsets of patients according to the location of their relapse. A combined relapse is one that occurred both in and outside of the liver. Figures from the HB 99 trial have been provided for comparison where available. These figures are not yet available for the LTR.

	All relapses (25)	**Liver Relapse (6, 24%)**	**Metastatic Relapse (17, 68%)**	**Combined Relapse (2, 8%)**	**All HB99 patients (142)**
Median Age and range (months)	24 (3-158)	72,5 (10-158)	18 (3-75)	78.5 (40-117)	16
Gender (M/F)	19/6 (3.2/1)	5/1	12/5	2/0	98/44 (2.2/1)
PRETEXT					
I	1(4%)	0	1 (6%)	0	4 (3%)
II	5 (20%)	1 (17%)	3 (18%)	1 (50%)	48 (34%)
III	10 (40%)	1 (17%)	8 (47%)	1 (50%)	70 (49%)
IV	9 (36%)	4 (67%)	5 (29%)	0	20 (14%)
Risk Group					
SR	5(20%)	0	4 (24%)	1 (50%)	85 (60%)
HR	20(80%)	6 (100%)	13 (76%)	1 (50%)	57(40%)
M+	10 (40%)	1 (17%)	9 (53%)	0	29 [(2a)] (21%)
V+	4 (16%)	0	4 (24%)	0	9 (6%)
P+	6 (24%)	2 (33%)	4 (24%)	0	12 (9%)
E+	2 (8%)	0	2 (12%)	0	5[(2b)] (4%)
R+	3 (12%)	1 (17%)	1 (6%)	1(50%)	8[(2c)] (7%)

F+	8 (32%)	4 (67%)	4 (24%)	0	35[(2d)] (25%)
Central Histology	21 (84%)	5 (83%)	14 (82%)	2(100%)	-
Missing data for central hist.	4 (16%)	1(17%)	3 (18%)	0	-
AFP at Diagnosis					
<100 ng/ml	2 (8%)	0	2 (12%)	0	10 (7%)
>100 ng/ml	23 (92%)	6(100%)	15(88%)	2(100%)	132 (93%)
Initial Chemo					-
IPA	4	1(17%)	2(12%)	1(50%)	-
CE	0	0	0	0	-
CE + HD CE	3	1(17%)	2(12%)	0	-
Mix IPA/CE/HDCE	3	1(17%)	2(12%)	0	-
Other HB99	2[(1a)]	0	2(12%)	0	-
CIS	2	0	1(6%)	1(50%)	-
Super PLADO	5	3(50%)	2(12%)	0	-
C5V	1	0	1(6%)	0	-
Other LTR	5[(1a)]	0	5(29%)	0	
Extent of Initial Liver Surgery (incl. Tx)					
R0	17(68%)	4(67%)	11(65%)	2(100%)	113 (80%)
≥R1	8(32%)	2(33%)	6(35%)	0	17(12%)
Not operated	0	0	0	0	12 (8%)
Primary Transplant	2(8%)				5(4%)
R1	1	1(17%)	0	0	-
Tumour Leak	1	0	1(6%)	0	-
Later Transplant but before relapse	1	0	1(6%)	0	-
Lung Mets at diagnosis – Op	7	0	7(41%) (1 op but no resection)	0	-
Lung Mets at diagnosis - No OP	3	1(17%)	2(12%)	0	-
Lung Mets during Treatment –All had Op	3	0	3(18%)	0	-
Time from 1st Liver OP to next Chemo					-
< 21 Days	8	3 (50%)	3(18%)	2 (100%)	-
≥21 Days	17	3 (50%)	14 (82%)	0	
When Relapse (from Rem) –					-
< 6m	12	2(33%)	9(53%)	1(50%)	-
≥6m-<12m	2	0	2 (12%)	0	-
≥12-<18m	6	3(50%)	3(18%)	0	-
≥18-<24m	1	1(17%)	0	0	-
≥24m	4	0	3(18%)	1(50%)	-

[(1a)] See Table 2

[(2a)] Data missing: 1 patient

[(2b)] Data missing: 7 patients

[(2c)] Data missing: 20 patients

[(2d)] Data missing: 1 patient

3.2.1 Median Age

The median age at initial diagnosis for all of the relapse patients was 24 months (3m-158m) as opposed to a median age at initial diagnosis of all HB 99 patients of 16 months [Häberle et al. 2016] and of all SIOPEL 1-3 patients of 17.2 months [Semeraro et al. 2013]. The median age of the local relapse patients (6/25 patients) at initial diagnosis was 72.5 months (10m-158m) as compared to 18 months (3m-75m) for the metastatic relapse patients (SIOPEL 1-3: 84 vs 21, [Semeraro et al. 2013]) and 78.5months (40m-117m) for the combined relapse patients (2/25 patients) (SIOPEL 1-3: 9m, [Semeraro et al. 2013]).

3.2.2 Gender

The male/female ratio (M/F) was 19/6 (3.2M/1F) amongst our relapse patients as opposed to 42/17 (2.5M/1F) for the SIOPEL relapse patients [Semeraro et al. 2013]. The M/F ratio for all patients on the HB99 trial was 98/44 (2.2M/1F) [Häberle et al. 2019].

3.2.3 PRETEXT Grade at Primary Diagnosis

The PRETEXT grade [Roebuck et al. 2006] at primary diagnosis was assigned based on completed questionnaires, radiological reports (CT/MRI), and surgical observations (where applicable). There was 1 PRETEXT I patient (4%), 5 PRETEXT II patients (20%), 10 PRETEXT III patients (40%) and 9 PRETEXT IV patients (36%). This was similar to the results in the Semeraro paper from 2013 (PRETEXT I 3%, PRETEXT II 26%, PRETEXT III 41% and PRETEXT IV 29%) as in both cases the majority of the patients are in group III or IV. In comparison in the Häberle et al. HB99 paper, which includes all HB patients from the HB99 study, the majority of patients were in groups II and III (3% PRETEXT I, 34% PRETEXT II, 49% PRETEXT III, 14% PRETEXT IV).

The 4 local relapse patients who were PRETEXT IV all had multifocal tumours (F+) at diagnosis. Only one of these patients had a transplant during the initial treatment (219 LTR). This patient was however also P+ at diagnosis and the transplant operation was graded as R1 due to portal vein invasion.

3.2.4 Risk Group at Diagnosis

5/25 (20%) relapse patients were classified as standard risk (SR) and 20/25 (80%) of relapse patients were classified as high risk (HR). The proportion of HR patients was also greater than that of the SR patients in the SIOPEL 1-3 relapse study (55% vs 45%). In comparison, there were fewer HR patients than SR patients in the HB99 study ([Häberle et al. 2019]: 40% vs 60%) which looked at all HB patients and not just those who had relapsed.

3.2.5 Additional Risk Factors at Diagnosis

The percentage of relapse patients with risk factors at initial diagnosis appears higher than the percentage seen when considering all the patients involved in the HB99 Trial (M+: 40% vs 21%, V+: 16% vs 6%; P+: 24% vs 9%, E+: 8% vs 4%, R+: 12% vs 7%, F+: 32% vs 25%). This pattern was also observed in the SIOPEL trial [Semeraro et al. 2013] for metastases (29% vs 18%) and for multifocal tumours (36% vs 17%) but not so noticeably for vascular involvement (15% vs 11%). This classification was made from the available data (site questionnaires, radiological reports, histological reports, surgical reports) according to the Häberle et al. 2019/Roebuck et al. 2006 papers. There was unfortunately no central radiology review of all the involved scans thus weakening the potential quality of this data.

3.2.6 AFP at Primary Diagnosis

The majority (23/25, 92%) of relapse patients had an AFP >100 ng/ml at initial diagnosis. 1 patient (126 HB 99) had an AFP <5ng/ml and 1 patient (128 HB 99) had an AFP of 33ng/ml. The same was true when one considered the AFP of all the patients included in the HB 99 trial and the SIOPEL 1-3 trials. Patient 126 had a local histology report, which described the tumour as being an epithelial foetal HB. Patient 128 had local and central histology reports, which described the tumour as being undifferentiated small cell HB. INI1 staining was not mentioned in either histology report.

3.2.7 Initial Chemotherapy

The 1 standard risk patient with recurrence on the HB99 trial received IPA (ifosfamide, cisplatin and adriamycin) alone as per protocol. 3 HR patients HB99 also received IPA alone. 3 HR HB 99 patients with later recurrence received CE (carboplatin and etoposide) followed by HD CE (high-dose CE). 3 HR HB99 patients with later recurrence received a combination of CE, HD CE and IPA. The remaining 2 HR HB99 patients with recurrence had other treatment combinations (see **Table 2**).

Only 2 of the 4 SR recurrence patients in the LTR received cisplatin monotherapy alone. The remaining 2 patients received cisplatin plus a combination of other therapies (1 =C5V, 1 = cisplatin, carboplatin, doxorubicin, etoposide, vincristine, irinotecan). Of the 9 HR patients with later recurrence, 5 received a combination of cisplatin, carboplatin and doxorubicin. The other 4 patients received combinations including one or more of the following in addition to cisplatin, carboplatin and doxorubin: etoposide, HD carboplatin and etoposide, ifosfamide, irinotecan, vincristine, and sorafenib.

Table 2: Initial Cumulative Chemotherapy

Cis=cisplatin, Ca=carboplatin, Dox=doxorubicin, I=ifosfamide, E=etoposide, Me=melphalen, Ir=irinotecan, 5FU=5-Fluoruracil, VCR=vincristine, S=sorafenib, MD=Missing Data, M=Metastatic Relapse, L=Local Relapse, C=Combined Relapse, H=HB99 study, LTR=Liver Tumour Register. Median cumulative dose of cisplatin 400mg/m^2 (Range: 100mg/m2-594mg/m^2). Median cumulative dose of carboplatin was 2500mg/m^2 (Range: 550mg/m2-8475mg/m^2). Median cumulative dose of doxorubicin was 240mg/m^2 (Range: 30-413mg/m^2).

Patient Number	Cis (mg/m^2)	Ca (mg/m^2)	Dox (mg/m^2)	I (mg/m^2)	E (mg/m^2)	Me (mg/m^2)	Ir (mg/m^2)	5FU (mg/m^2)	VCR (mg/m^2)	S (mg/m^2)
186(H) (M)	300		180	8991						
187(H) (C)	200		240	6000						
58(H) (L)	100	5600	60	2100	4800					
78(H) ((M)		2200			5300	240				
81(H) (M)		6000			5000					
100(H) (M)	MD	MD	MD	MD	MD		MD			
113(H) (M)	200	5600	30	6000	4800					
126(H) (M)	MD	MD	MD	MD	MD					
128(H) (M)	300		180	8991						
137(H) (L)		4850			3925					
193(H) (M)		4920			4800					
246(H) (L)	500		300	15000						
4(LTR) (M)	594							3600	25.5	
124(LTR) (M)	480									
149(LTR) (M)	405	2706	240		396		1002		12	
434(LTR) (M)	495	1503	120				751.5		4.5	
489(LTR) (C)	480									
55(LTR) (M)	567	8475	240	36000	6000		500		6	
68(LTR) (M)	500	1250	240	9000	200		250		3	
132(LTR) (L)	590	1020	300							
182(LTR) (M)	307.5	550	412.5							
213(LTR) (L)	400	2500	150							
219(LTR) (L)	290	1800	290							
292(LTR) (M)	400	2500	150							
473(L) (M)	372.8	1500	400				500		6	8236

3.2.8 Operations during Initial Therapy

23 of the 25 patients had a partial hepatectomy and 2 patients (both from the LTR) had a primary liver transplant. 17 of the 23 patients had a R0 resection (1 of these patients had a tumour rupture and resection at the time of diagnosis), 6 had R1/R2 resections. One of these R1/R2 patients (149) had a subsequent liver transplant following the initial resection. Of the 2 primary liver transplants, one was linked with tumour spillage (68), one was deemed to be an R1 resection (219).

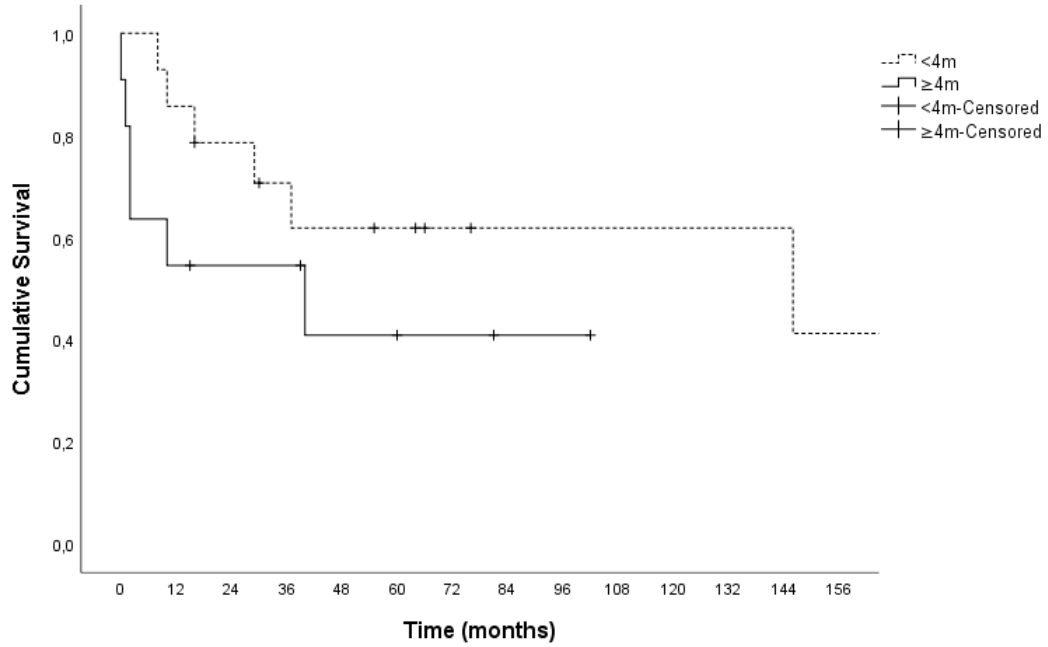
Of the 10 patients with mets at diagnosis, all had lung mets. In 6 of these patients, the lungs mets were surgically resected prior to remission. 1 patient had a sternotomy but no resection. One patient had recurrence of lung mets following initial resection and underwent a further operation. In 3 of these 10 patients with lung mets an operation was not performed. 3 patients developed lung mets during the initial treatment phase. All 3 of these patients had resection of their lung mets.

3.2.9 Median Time from Initial Diagnosis to Liver Resection

The median time from initial diagnosis to the initial liver resection was 3.5m (0-6m) (3.7 months HB99 (0-6m), 3.4 months LTR (1-5m)).

Of the 3/25 patients who were operated on in <2 months, none are still alive. Of the 6 patients operated from 2-<3 months post diagnosis, 3 (50%) were still alive at the last FU, none of these had had further relapses. Of the 5 patients operated on from 3-< 4 months post diagnosis, 5 (100%) were still alive at last FU with one being treated for a 2nd relapse. Of the 8 patients operated on from 4-< 5 months post initial diagnosis, 4 were still alive at last FU (50%), one of whom has been treated for further relapses. Of the 2 patients treated from 5-<6 months, 1 was still alive at last FU (50%) and has not had a further relapse. The 1 patient operated on greater than 6 months post-diagnosis is dead.

Figure 3: Overall-survival from relapse according to how much time passed between initial diagnosis and the first liver operation. Patients who were operated on <4 months after their initial diagnosis appear to have a similar/slightly better prognosis than those who were operated on ≥4 months after their diagnosis. There was no significant effect of this difference on overall survival time (p=0.217, Log-Rank (Mantel-Cox)). In the table, the figures in brackets are those who had their last FU prior to 156 months, the figure not in the brackets includes these patients and those patients we know to be alive at 156 months.



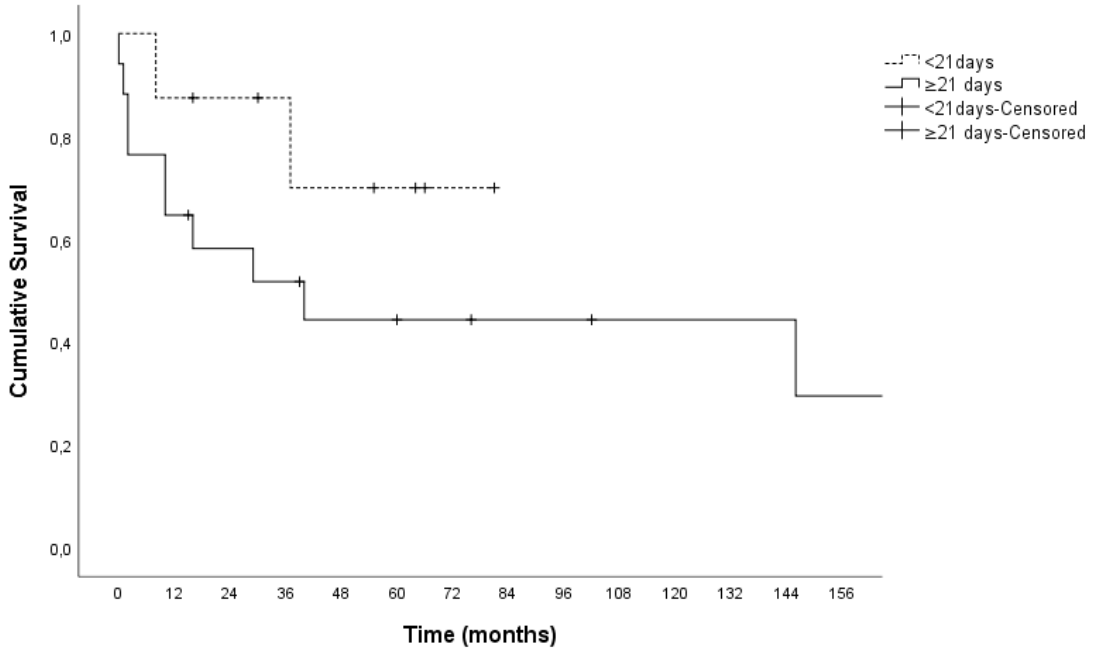
Months	0	12	24	36	48	60	72	84	96	108	120	132	144	156
<4months	14	12	11(1)	10(2)	9(2)	9(3)	9(5)	9(6)	9(6)	9(6)	9(6)	9(6)	9(6)	8(6)
≥4months	11	6	6(1)	6(1)	5(2)	5(2)	5(3)	5(4)	5(4)	5(5)	5(5)	5(5)	5(5)	5(5)

Patients who were operated on <4 months after their initial diagnosis appear to have a similar/slightly better prognosis than those who were operated on ≥4 months after their diagnosis. There was no significant effect of this difference on overall survival time (p=0.217, Log-Rank (Mantel-Cox)).

3.2.10 Time between Initial Liver Operation and Next Chemotherapy Block

The majority of patients had a delay of ≥21 days (17/25 (68%) between their initial liver operation and their next chemotherapy treatment. 10/17 (59%) of those who had a delay of ≥21 days are dead as compared to 2/8 (25%) who had <21 days. 10/12 (83%) of the HB-99 patients had a delay of ≥21 days as compared to 7/13 (54%) of the LTR patients.

Figure 4: Overall-survival from relapse according to how much time passed between initial liver operation and the subsequent chemotherapy block. Those patients who had a <21 days delay appeared to have a better chance of survival. There was however no significant effect of this difference on overall survival time (p=0.207, Log-Rank (Mantel-Cox)). In the table, the figures in brackets are those who had their last FU prior to 156 months, the figure not in the brackets includes these patients and those patients we know to be alive at 156 months.



Months	0	12	24	36	48	60	72	84	96	108	120	132	148	156
<21 days	8	7	7(1)	7(2)	6(2)	6(3)	6(5)	6(6)	6(6)	6(6)	6(6)	6(6)	6(6)	6(6)
≥21 days	17	11	10(1)	9(1)	8(2)	8(2)	8(3)	8(4)	8(4)	8(5)	8(5)	8(5)	7(5)	7(5)

Those patients who had a <21 days delay between their operation and their next chemotherapy appeared to have a better chance of survival. There was however no significant effect of this difference on overall survival time (p=0.207, Log-Rank (Mantel-Cox)).

3.2.11 Tumour Histology

The majority of the patients had had a central pathology review. In 4 cases however, there were only local reports available in our records even though the central reviews may also have been performed. The commonest histology was a hepatoblastoma with epithelial components only (11/25, 44%). Further analysis was not possible due to the limited information available on the histology reports.

Table 3: Histology of the tumours at initial diagnosis

HB-MT: hepatoblastoma with mixed epithelial and mesenchymal components, HB-ET: hepatoblastoma with epithelial components only, HB-MD: identified as hepatoblastoma only as other information is missing, HB-ET (DD HCC): hepatoblastoma of epithelial type but with possible differential diagnosis as hepatocellular carcinoma, SCUD: small cell undifferentiated hepatoblastoma, HCC: hepatocellular carcinoma. **HB99 patients' numbers are in bold font.**

HB Type	Central Path – Biopsy	Central Path-Op	Local Path-Biopsy	Local Path-Op
HB-MT		55, 292, 4, 182, 434, 66, **81**		
HB-ET	489, 132, 219, 149, 473[3], **100**	124, **78, 246**[2]	**126**	**137**
HB-MD				**113, 186**
HB-ET (DD HCC)	**58, 187**			
SCUD	213[4]	**128**[5]		
HCC	**193**[1]			

[1] 193 – Response to therapy suggested HB rather than HCC, thus this tumour was classified as HB. The central pathology result at the time of the operation reported a hepatoblastoma which was principally mesenchymal (osteoid) in differentiation.

[2]246 – Local pathology reported HB with macrotrabecular growth.

[3] 473 – Local pathology reported HCC.

[4]213 – Central pathology indicates the presence of epithelial and SCUD components. INI1-expression was positive thus this was not a rhabdoid tumour.

[5]128 –No information as to INI1-expression is available here.

3.2.12 Median Time from Initial Diagnosis to Relapse

The median time from initial diagnosis to first relapse was 19 months (7-45 months) for the patients from the HB99 study and 12 months (5-66 months) for the patients from the LTR. This may reflect an improvement in radiological techniques and recognition of warning signals (slowly increasing AFP) over

time. For the 2 groups combined the median time from initial diagnosis to first relapse was 13 months (5-66 months). In the SIOPEL 1-3 analysis, the median time from initial diagnosis to first relapse was 12 months (4-115 months)) [Semeraro et al. 2013].

12/25 (48%) patients relapsed within less than 12 months of initial diagnosis. Only 5 relapsed in ≥24 months (20%) after initial diagnosis. A late relapse (>36 months) was seen in 2 patients (8%, vs 10% in SIOPEL trial). One of these patients had a metastatic relapse (18 months old at initial diagnosis) and the other a combined relapse (117 months old at initial diagnosis). The patient with the combined relapse had a slowly rising AFP for over 2 years before the mets were detected on ultrasound examination. The patient with the metastatic relapse had a rise in AFP to 23.4ng/ml about 36 months after remission which then increased to 2176ng/ml at the next check-up 6 months later.

The median interval from diagnosis till relapse was longer for those patients with local relapse (20 months, 7-26 months) and combined relapse (25 months, 5-45m), as compared to 12 months for those with metastatic relapse (7-66 months). In comparison, the median interval to local relapse was shorter (10 months) as compared to metastatic relapse (20 months) in the SIOPEL relapse study [Semeraro et al. 2013].

3.2.13 Median Time from Remission to First Relapse

The median time from remission to first relapse was 14 months (1-44 months) for the patients from the HB99 study and 5 months (1-48 months) for the patients from the LTR. For the 2 groups combined the median time from remission to first relapse was 7 months (1-48 months).

14 (56%) patients relapsed within less than 12 months of remission. 4 (16%) relapsed ≥24 months after remission. A late relapse (>36 months after remission) was seen in the same 2 patients as mentioned above.

The median interval till relapse was longer for those patients with local relapse (14 months, 2-20 months) and combined relapse (22m, 1m-44m), as compared to those with metastatic relapse (6 m, 1-48 m).

3.3 Relapse

An overview of the characteristics of the relapse in the 25 patients who relapsed and information about their relapse therapy is provided in **Table 4**.

Table 4: Overview of the characteristics of the relapse in the 25 identified patients and information about their relapse therapy.

	All relapses (25)	**Local Relapse (6, 24%)**	**Metastatic Relapse (17, 68%)**	**Combined Relapse (2, 8%)**	**5 J EFS_{rel1}/OS_{rel1} (%)**
Relapse Location					
Liver	6	6	0	0	67/67
Lung	11	0	11	0	73/81
Peritoneum	4	0	4	0	0/0
CNS	2	0	2	0	0/≤50[a]
Combined	2	0	0	2	0/0
AFP at Relapse (ng/ml)					
≤100	10 (40%)	0	9	1	40/50
>100	14 (56%)	6	7	1	57/59
MD	1 (4%)	0	1	0	-
Chemotherapy					
IPA	2	0	2	0	-
CE	1	0	1	0	-
CE + HD CE	1	0	1	0	-
Mix IPA/CE	1	0	1	0	-
C5VD	1	0	1	0	-
ICE/VI	1	0	1	0	-
VI	3	1	2	0	-
CE/VI	1	1	0	0	-
Super PLADO/VI	1	0	1	0	-
Other	10	3	5	2	-
No Chemo	3	1	2	0	-
Time from start of last Initial Chemo to Chemo for Relapse					
≤ 12 months	11	4	6	1	46/53
>12 months	11	1	9	1	64/69
No 2nd Chemo	3	1	2	0	-
Time from OP to next Chemo					
≤ 21 Days	10	2	8	0	70/80
>21 Days	8	2	5	1	33/39

Initial OP Lung (7) – Relapse Location	7	0	7 (5 lung)	0	-
Initial Mets no OP (3) – Relapse Location	3	1	2 (1 lung)	0	-
Lung Mets dev. during initial Tr. (3)– Relapse Location	3	0	3(2 lung)	0	-
Extent of Relapse Surgery (1st OP)					
R0	15	5	10	0	73/78
≥R1	6	1	5	0	0/0
Data missing/unclear	1	0	0	1	-
No Op in Relapse	3	0	2	1	-
Transplant in relapse	3	3	0	0	-
How many achieved 2nd CR	16	5	11	0	
HB99	8	3	5	0	-
LTR	8	2	6	0	-
Surgery Alone	0	0	0	0	-
Chemo Alone	1	0	1	0	-
Both - Surgery First	6	0	6	0	-
Both – Chemo First	9	5	4	0	-

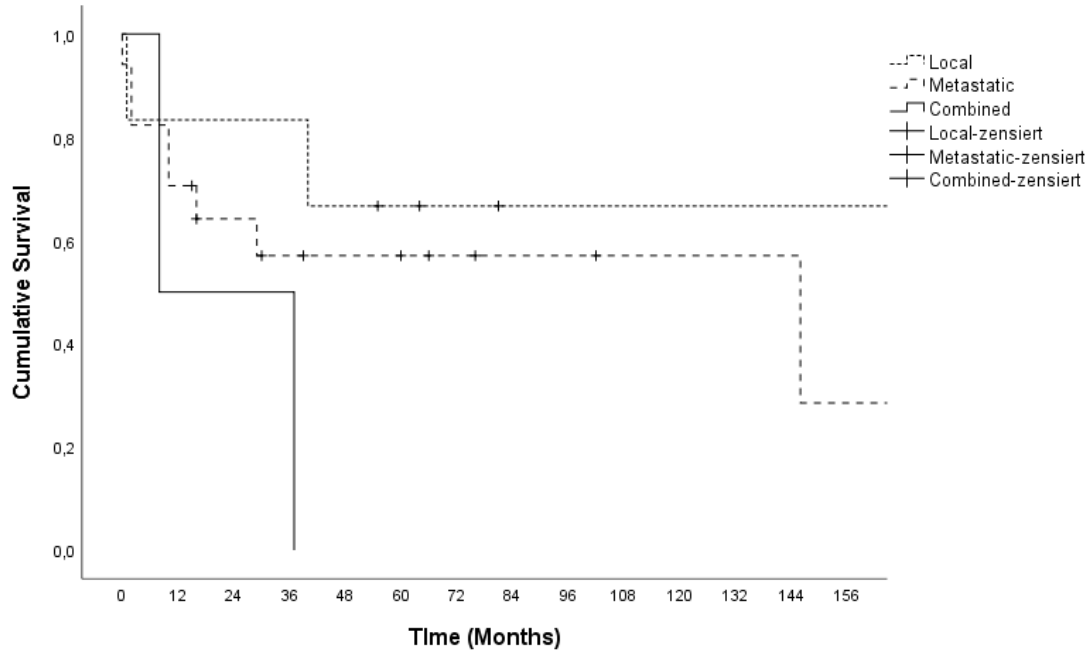
[a]**:** Second patient had last FU at 16 months and was having treatment for relapse at this time.

3.3.1 Location of Initial Relapse

2% of patients from the HB99 study and the LTR experienced a local relapse (6/362). 5% of patients had a metastatic relapse (17/362). 1% of patients had a combined relapse (2/362). This is similar to the rates in the Semeraro et al. 2013 study (3% vs 5% vs 1%).

The initial relapse occurred in the liver in 6/25 patients (24%), was metastatic in 17/25 patients (68%) and was both in 2/25 patients (8%). This is again similar to the SIOPEL cohort results (36% vs 55% vs 9%). Of the 17 patients with mets: 11/17 (65%) had lung mets, 4/17 (24%) had peritoneal mets, 2/17 (12%) had cerebral mets. In the SIOPEL study these figures were 84% vs 13% vs 3%. Of the 2 patients with combined relapse: one had tumour in the liver and in the peritoneum, the other had mets at multiple sites including liver, lungs, and peritoneum.

Figure 5: Overall-survival time from relapse as related to area of initial relapse. Patients who had a local relapse appeared to have a longer OS than patients with metastatic disease or combined disease at relapse. These findings were however not significant (Log Rank (Mantel-Cox), p=0.232). In the table, the figures in brackets are those who had their last FU prior to 156 months, the figure not in the brackets includes these patients and those patients we know to be alive at 156 months.

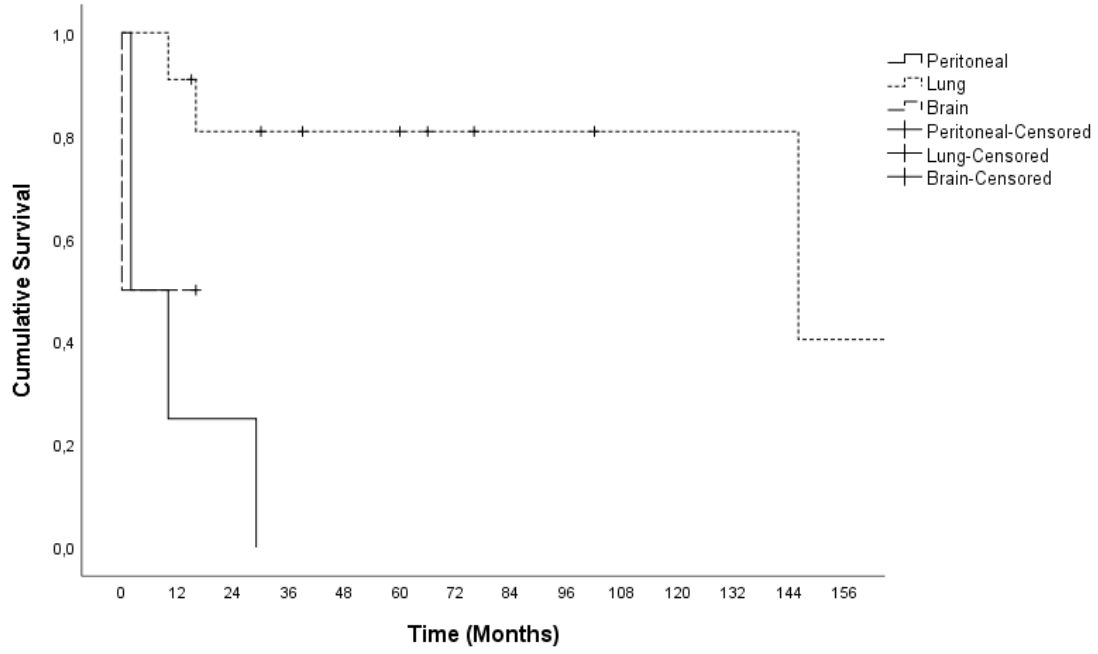


Months	0	12	24	36	48	60	72	84	96	108	120	132	144	156
Local	6	5	5	5	4	4(1)	4(2)	4(3)	4(3)	4(3)	4(3)	4(3)	4(3)	4(3)
Metastatic	17	12	11(2)	10(3)	10(4)	10(5)	10(6)	10(7)	10(7)	10(8)	10(8)	10(8)	10(8)	9(8)
Combined	2	1	1	1	0	0	0	0	0	0	0	0	0	0

Patients who had a local relapse appeared to have a better OS than patients with metastatic disease or combined disease at relapse. These findings were however not significant (Log Rank (Mantel-Cox), p=0.232). Those patients with combined relapse appeared to have the worst prognosis.

Of the 17 patients with metastatic relapse, 11 had lung mets, 4 had peritoneal mets and 2 had brain mets. 8/11 (73%) of the patients with lungs mets were alive at last FU. None of the patients with peritoneal mets were alive at last FU. 1/2 (50%) patients with brain mets were alive at last FU however this patient had had a further relapse in the lungs and is in ongoing treatment at last contact (16 months after relapse).

Figure 6: Overall-survival time from relapse according to location of metastases at initial relapse. Those patients who had lung mets appeared to have a better prognosis from relapse than those who had mets in other locations. There was a statistically significant difference in overall survival from relapse between those who had peritoneal relapses, brain relapses and pulmonary-based relapses (Log Rank (Mantel-Cox), p=0.008). In the table, the figures in brackets are those who had their last FU prior to 156 months, the figure not in the brackets includes these patients and those patients we know to be alive at 156 months.



Months	0	12	24	36	48	60	72	84	96	108	120	132	144	156
Peritoneal	4	1	1	0	0	0	0	0	0	0	0	0	0	0
Lung	11	10	9(1)	9(2)	9(3)	9(3)	9(5)	9(6)	9(6)	9(7)	9(7)	9(7)	9(7)	8(7)
Brain	2	1	1(1)	1(1)	1(1)	1(1)	1(1)	1(1)	1(1)	1(1)	1(1)	1(1)	1(1)	1(1)

Those patients who had lung mets appeared to have a better prognosis from relapse than those who had mets in other locations. There was a statistically significant difference in overall survival from relapse between those who had peritoneal relapses, brain relapses and pulmonary-based relapses (Log Rank (Mantel-Cox), p=0.008).

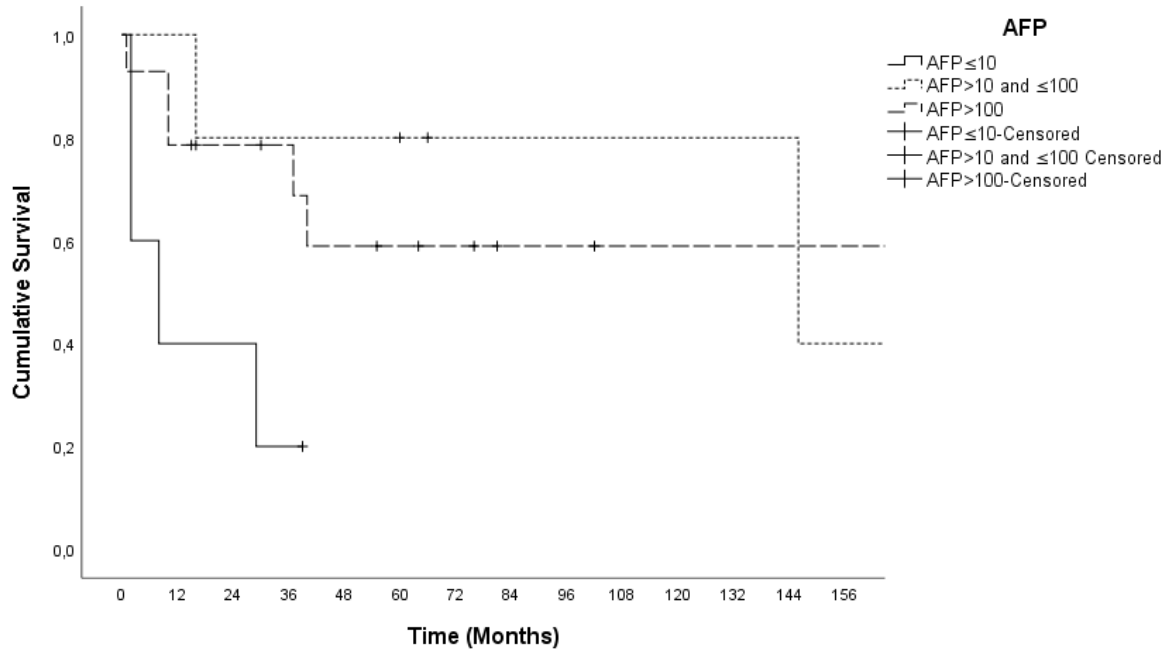
3.3.2 AFP at Time of Relapse

5/25 (20%) patients had an AFP <10 ng/ml at relapse. 5/25 (20%) patients had an AFP between 10 and 100ng/ml. 14/25 (56%) had an AFP between 101 and 1 million ng/ml. The AFP units were missing for 1 patient. The median AFP level at relapse was 174 ng/ml (5-119674 ng/ml) as compared to 185 ng/ml in the SIOPEL 1-3 paper [Semeraro et al. 2013]. The 2 patients who had had an AFP of <100 ng/ml at

diagnosis also had an AFP < 100 ng/ml at relapse. Both of these patients with consistently low AFPs ultimately died (126, 128). The patient with an AFP <10 ng/ml at diagnosis died after the first relapse. The patient with an initial AFP of 33 ng/ml died after a number of subsequent relapses.

In 2/25 patients (186, 187) the AFP level was >100ng/ml for more than 2 months prior to the radiological confirmation of recurrence. No AFP measurements were available in the 2 months pre-relapse for 1 patient (55). This patient was a late relapse and had 6 months between an AFP measurement of 24.8ng/ml and the next AFP measurement of 2176ng/ml.

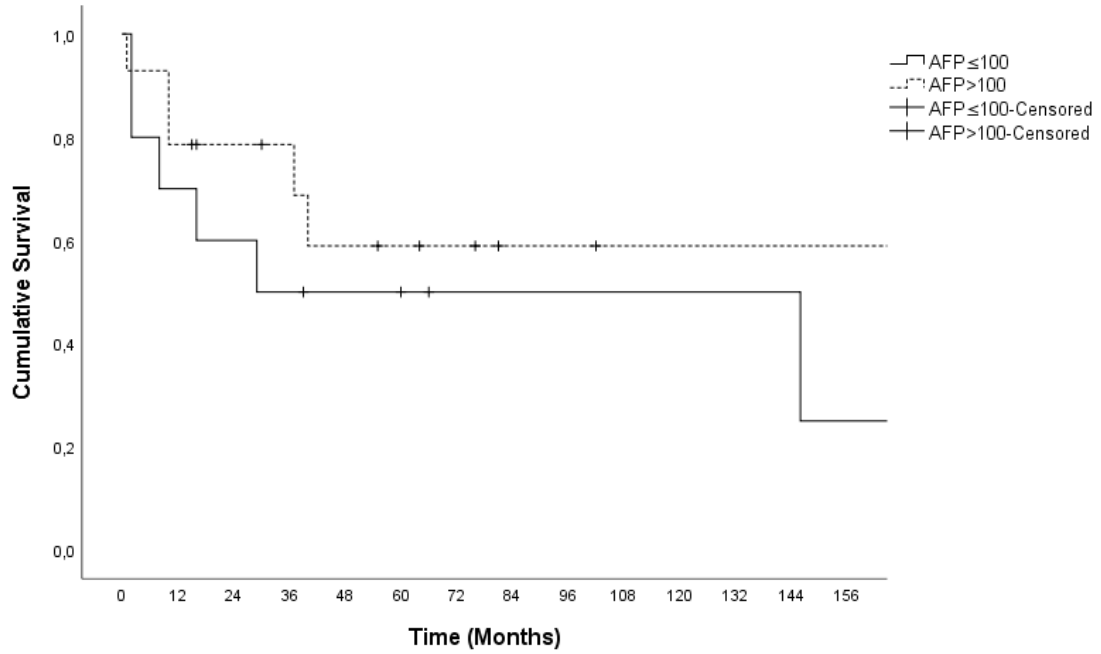
Figure 7a: Overall-survival time from relapse as related to AFP at time of initial relapse. Patients who had an AFP of ≤ 10ng/ml at the time of relapse had a much lower survival rate than those with a higher AFP. These findings were significant (Log Rank (Mantel-Cox), p=0.033). In the table, the figures in brackets are those who had their last FU prior to 156 months, the figure not in the brackets includes these patients and those patients we know to be alive at 156 months.



Months	0	12	24	36	48	60	72	84	96	108	120	132	144	156
AFP≤10	5	2	2	1	1(1)	1(1)	1(1)	1(1)	1(1)	1(1)	1(1)	1(1)	1(1)	1(1)
AFP >10-≤100	5	5	4	4	4	4(1)	4(2)	4(2)	4(2)	4(2)	4(2)	4(2)	4(2)	3(2)
AFP>100	14	11	11(2)	11(3)	9(3)	9(4)	9(5)	9(7)	9(7)	9(8)	9(8)	9(8)	9(8)	9(8)

Patients who had an AFP of ≤ 10ng/ml at the time of relapse had a much lower survival rate than those with a higher AFP. These findings were significant (Log Rank (Mantel-Cox), p=0.033).

Figure 7b: Overall-survival time from relapse as related to AFP at time of initial relapse. Patients who had an AFP of ≤100ng/ml at the time of relapse appeared to have a slightly lower survival rate to those with a higher AFP. No significant difference was found (Log Rank (Mantel-Cox), p=0.379). In the table, the figures in brackets are those who had their last FU prior to 156 months, the figure not in the brackets includes these patients and those patients we know to be alive at 156 months.



Months	0	12	24	36	48	60	72	84	96	108	120	132	144	156
AFP≤100	10	7	6	5	5(1)	5(2)	5(3)	5(3)	5(3)	5(3)	5(3)	5(3)	5(3)	4(3)
AFP>100	14	11	11(2)	11(3)	9(3)	9(4)	9(5)	9(7)	9(7)	9(8)	9(8)	9(8)	9(8)	9(8)

Patients who had an AFP of ≤100ng/ml at the time of relapse appeared to have a slightly lower survival rate to those with a higher AFP. No significant difference was found (Log Rank (Mantel-Cox), p=0.379).

3.3.3 Treatment of First Relapse

Most patients (20/25) were treated with chemotherapy and surgical resection. 2 of these patients had palliative chemo (78 (HB99), 126 (HB99)) only.

2/25 patients had chemo alone:

(1) disseminated HB (489 LTR; OS_{rel1}=8m(Died), EFS_{rel1}=2m).

(2) lung mets - Remission with chemo alone (193 HB99; OS_{rel1}=146m (Died), EFS_{rel1}=80m).

2/25 patients had surgical resection alone:

(1) 1 patient (100 HB99). Lung mets diagnosed days after peritoneal operation (R0) and therefore the decision was made to stop further treatment.

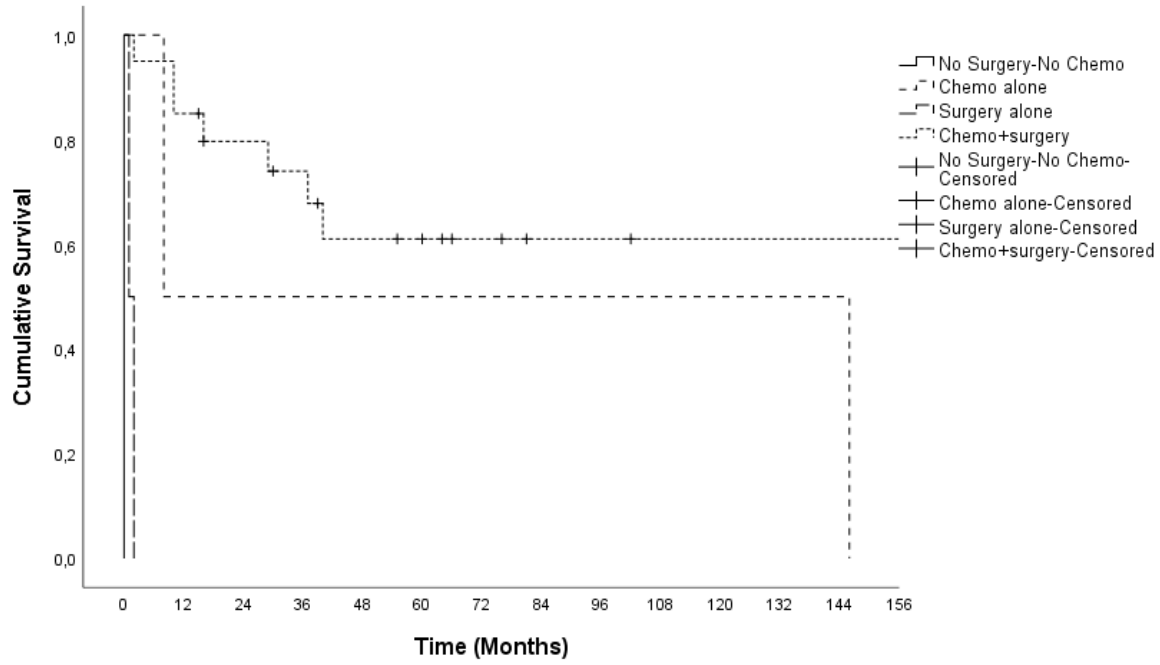
(2) 1 patient had a 2nd liver transplant (R1) but no chemo as this patient died shortly after transplant from heart failure (219 LTR).

1/25 patients had no chemo and no surgery:

(1) 1 patient (4 LTR) died the day after the cerebral mets were first detected. (Central histology review at time of initial resection: Mixed epithelial and mesenchymal HB).

Of the 22 patients operated on for relapse, 6 had a local relapse (2 had an initial transplant post-relapse, 1 had a delayed transplant post-relapse), 15 had metastatic lesions (10 lung, 4 abdominal, 1 brain) and 1 had both local and abdominal lesions.

Figure 8: Overall-survival time from relapse according to treatment received at initial relapse. Patients who had both chemotherapy and surgery appeared to do better than those who did not. Owing to the small number of patients in 3 of the 4 groups however a detailed statistical analysis of this area was not possible. In the table, the figures in brackets are those who had their last FU prior to 156 months, the figure not in the brackets includes these patients and those patients we know to be alive at 156 months.



Months	0	12	24	36	48	60	72	84	96	108	120	132	144	156
No Tr	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Surg.	2	0	0	0	0	0	0	0	0	0	0	0	0	0
CHx	2	1	1	1	1	1	1	1	1	1	1	1	1	0
Surg.+CHx	20	17	16(2)	15(3)	13(4)	13(6)	13(8)	13(10)	13(10)	13(11)	13(11)	13(11)	13(11)	13(11)

Patients who had both chemotherapy and surgery did better than those who did not. Owing to the small number of patients in 3 of the 4 groups however a detailed analysis of this area was not possible.

15/20 (75%) patients who had both chemotherapy and surgery achieved a 2nd complete remission, 13/20 (65%) of these patients were still alive at last FU. 0/2 patients who had surgery alone achieved a 2nd complete remission. 1/2 patients who had chemo alone achieved a 2nd complete remission. This patient later relapsed and died. 0/1 patients who had no chemo and no surgery achieved a 2nd complete remission.

Of the 20/25 patients who had chemotherapy and surgical resection, 9 had surgery before chemo and 11 after chemo had started. 6/9 (67%) patients who had surgery first achieved a 2nd remission, 5/9 (56%) were still alive at last FU. 9/11 (82%) patients who had chemo first achieved a 2nd complete remission, 8/11 (73%) of these patients were still alive at last FU.

3.3.3a Surgery First

Of the 9/20 patients who had an operation before chemo 6 had an initial lung operation (5 R0, 1 R1) and 3 had an initial peritoneal operation (one of which was exploratory only, one was associated with a tumour tear, one of which revealed peritoneal carcinosis). For more information on outcome of these patients see **Table 5**.

Table 5: Outcome for those 9 patients who had an operation before chemo. The OS_{rel1} and the EFS_{rel1} figures are given in months. D=Death, H=HB99, LTR=Liver Tumour Register, M=Metastatic, L=Liver, C=Combined, Y=Yes.

Patient No.	Initial Relapse OP Grade	Relapse Location	Further ops during 1st relapse?	On-going Remission	Remission and further relapse(s)	Death before remission
78(H) (M)	≥R1 Carcinosis	Peritoneal	No			Y EFS_{rel1}=3 OS_{rel1}=10
128(H) (M)	≥R1 Tumour Tear	Peritoneal	No		Y EFS_{rel1}=18 OS_{rel1}=29 D	
126(H) (M)	≥R1 Exploratory	Peritoneal	No			Y EFS_{rel1}=2 OS_{rel1}=2
81(H) (M)	R0	Lung	No	Y $EFS_{rel1=}$102 OS_{rel1}=102		
124(LTR) (M)	R0	Lung	No	Y EFS_{rel1}=66 OS_{rel1}=66		
434(LTR) (M)	R0	Lung	No	Y EFS_{rel1}=30 OS_{rel1}=30		
292(LTR) (M)	R0	Lung	No	Y EFS_{rel1}=60 OS_{rel1}=60		
68 (LTR) (M)	R0	Lung	Yes – Lung		Y EFS_{rel1}=14 OS_{rel1}=39	
182 (LTR) (M)	R1	Lung	Yes – Lung, Liver			Y EFS_{rel1}=4 OS_{rel1}=16

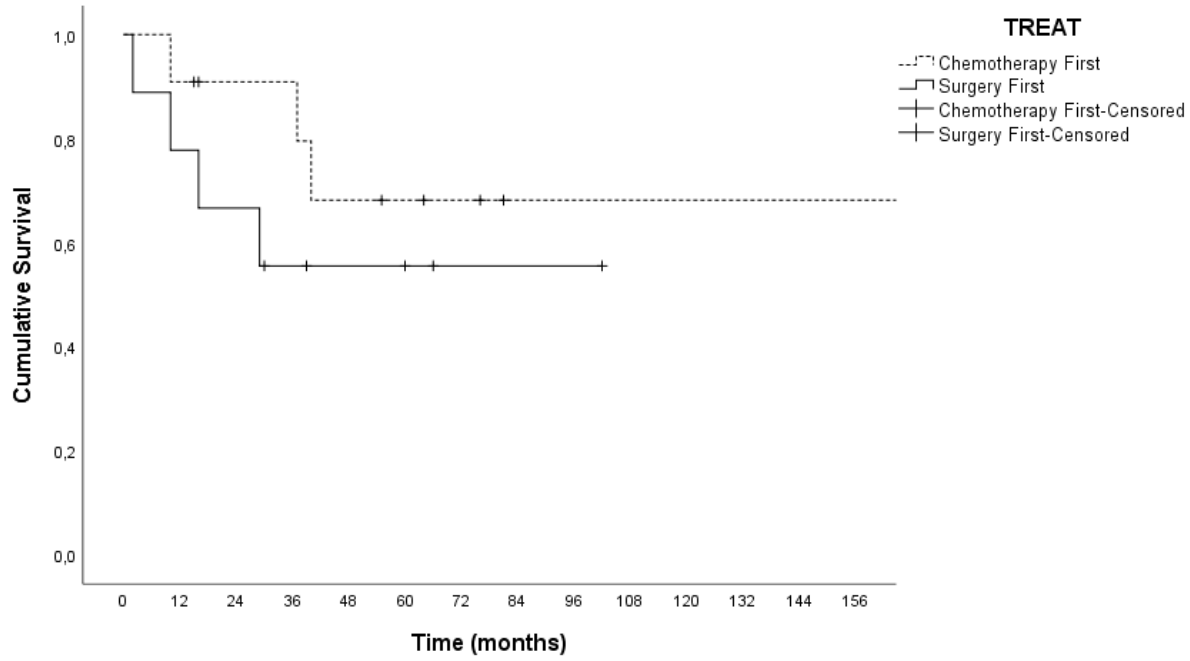
3.3.3b Chemotherapy First

Of the 11/20 patients who had chemo before the operation: 6 had an initial liver operation (5 R0, 1 MD), 4 had an initial lung operation (4 R0), and 1 had a craniotomy (R1). For more information on outcome see **Table 6.**

Table 6: Outcome for those 11 patients who had chemo before their operation. The OS_{rel1} and the EFS_{rel1} figures are given in months. D=Death, H=HB99, LTR=Liver Tumour Register, M=Metastatic, L=Liver, C=Combined, Y=Yes.

Patient No.	Initial Relapse OP Grade	Relapse Location	Further ops during 1st relapse?	On-going Remission	Remission and further relapse(s)	Death before remission
187(H) (C)	MD	Liver/ Omentum	Y-Liver			Y EFS_{rel1}=8 OS_{rel1}=37
58(H) (L)	R0	Liver	No		Y EFS_{rel1}=13 OS_{rel1}=40 D	
137(H) (L)	R0	Liver	No	Y EFS_{rel1}=186 OS_{rel1}=186		
246(H) (L)	R0	Liver	Y – Liver Tx	Y EFS_{rel1}=64 OS_{rel1}=64		
132(LTR) (L)	R0	Liver Tx	No	Y EFS_{rel1}=55 OS_{rel1}=55		
213(LTR) (L)	R0	Liver	No	Y EFS_{rel1}=81 OS_{rel1}=81		
113(H) (M)	R0	Lung	No	Y EFS_{rel1}=171 OS_{rel1}=171		
186(H) (M)	R0	Lung	No	Y EFS_{rel1}=76 OS_{rel1}=76		
149(LTR) (M)	R0	Lung	No			Y EFS_{rel1}=2 OS_{rel1}=10
55(LTR) (M)	R0	Lung	No	Y EFS_{rel1}=15 OS_{rel1}=15		
473(LTR) (M)	R1	Brian	No		Y EFS_{rel1}=12 OS_{rel1}=16	

Figure 9: Overall-survival time from relapse according to order of treatment received at initial relapse. Those patients who had chemo first appeared to have a better overall survival from relapse than those who had surgery first. This was not however a statistically significant difference (Log Rank (Mantel-Cox), p=0.344). In the table, the figures in brackets are those who had their last FU prior to 156 months, the figure not in the brackets includes these patients and those patients we know to be alive at 156 months.



Months	0	12	24	36	48	60	72	84	96	108	120	132	144	156
CHx 1st	11	10	10(2)	10(2)	8(2)	8(3)	8(4)	8(6)	8(6)	8(6)	8(6)	8(6)	8(6)	8(6)
Surg 1st	9	7	6	5(1)	5(2)	5(3)	5(4)	5(4)	5(4)	5(5)	5(5)	5(5)	5(5)	5(5)

Those patients who had chemo first appeared to have a better overall survival from relapse than those who had surgery first (see **Fig. 9**). This observation is probably at least partly due to the poorer outcome of the patients with peritoneal mets who were predominately surgically treated initially (see **Fig. 6**).

3.3.4 Chemotherapy Used to Treat First Relapse

A multitude of different chemotherapy regimens were used to treat the relapse patients. The commonest single treatment was vincristine + irinotecan (VI) with 3/22 patients, all of whom were from the LTR. The next commonest treatment was ifosfamide, cisplatin and doxorubicin (IPA) with 2/22 patients (both of whom were from the HB 99 study). All the other patients had combinations of a number of different agents. 17/22 received one or more doses of cisplatin and/or carboplatin. The lowest number of different agents given was 0, the most was 7 (1 patient). Treatment used was

changed due to side effects, lack of response, or progression despite treatment. For more information with regards to treatments given please see the cumulative chemotherapy listed in **Table 7**.

Table 7: Cumulative Chemotherapy given during treatment for first relapse (Cis=cisplatin, Ca=carboplatin, Dox=doxorubicin, I=ifosfamide, E=etoposide, Me=melphalen, Ir=irinotecan, 5FU=5 fluouracil, VCR=vincristine, MD=Missing Data, H=HB99, L=Local (liver), M=Metastatic, Lu=Lung, P=Peritoneum, B=Brain, C=Combined, FU=in follow-Up, Tx=Transplant, Rem=Remission, D=Death, FR=Further Relapse)

Patient. No.	Cis mg/m2	Ca mg/m2	Dox mg/m2	I mg/m2	E mg/m2	Me mg/m2	Ir mg/m2	5FU mg/m2	VCR mg/m2	No chemo	Other	Extent Initial Relapse OP	EFS_{rel1}/ OS_{rel1} (m)
186(H) (M-Lu) Rem	200	1200	120	6000	1360							R0 (Lu)	76/76
187(H) (M-C) D		6540		15300	5650						a	≥R1 (L + P)	8/37
58(H) (L) FR- D			60								b	R0 (L)	13/40
78(H) (M –P- Palliative) D											c	≥R1 (P)	3/10
81(H) (M-Lu) Rem	200		120	6000								R0 (Lu)	102/ 102
100(H) (M-P) D										X		R0 (P)	0/2
113(H) (M-Lu) Rem	100					140	800				d	R0 (Lu)	171/ 171
126(H) (M-P) Palliative CTX D	MD		MD	MD								≥R1 Exploratory peritoneal OP	2/2
128(H) (M-P) FR-D		5600			4800							≥R1 (P - Capsule torn)	18/29
137(H) (L) Rem	160	252	146	9000								R0 (L)	186/ 186
193(H) (M-Lu) FR -D		800	120	6000								No Op L	80/ 146
246(H) (L) Rem		800			400		260				e	R0 (L) Then Tx (R0)	64/64
4(LTR) (M-B) D										X		No Op (B)	0/0
124(LTR) (M-Lu) Rem		2000	240									R0 (Lu)	66/66
149(LTR) (M-Lu) D		804			396							R0 (Lu)	2/10
434(LTR) (M-Lu) Rem	495		240					2400	18			R0 (Lu)	30/30
489(LTR) (M-C) D		2500	240	6000	300			1200	3		f	No Op (Disseminated)	2/8
55(LTR) (M-Lu) Rem		2400		36000	1200		1500		18			R0 (Lu)	15/15
68(LTR)							2250		15			R0	14/39

(M-Lu) FR – in FU							(oral)					(Lu)	
132(LTR) (L) Rem							750		4.5			R0 (Tx)	55/55
182(LTR) (M-Lu) D	60	187	60				20		1.5			R1 (Lu)	4/16
213(LTR) (L) Rem		3200			1600		250		1.5			R0 (L)	81/81
219(LTR) (L) D										X		MD (Tx)	1/1
292(LTR) (M-Lu) Rem							1000		12			R0 (Lung)	60/60
473(LTR) (M-B) FR – In FU		3600			2400	180	100		1,5			R1 (Brain)	12/16

a: Bevacizumab, sorafenib, sunitinib

b: Topetecan 2.25mg/m^2

c: Trofosfamid 600mg + idarubicin (MD)

d: Idarubicin 14mg/m^2 + busulphan 96mg/m^2

e: Bevacizumab 440mg absolute (326mg/m2)

f: Imatinib (200mg/d. MD re no. of days).

18/22 patients were given at least one new agent in comparison to the initial treatment. Only 4 patients (55 L, 68 L, 149 L, 126L) were given only agents that had been used previously and here they had used multiple agents in the initial treatment.

3.3.4a Doxorubicin

Of the 7 patients who did not have doxorubicin during the initial treatment, 5/7 had it as part of their relapse treatment. Of the remaining patients, 1 had no chemo for relapse and the other received only palliative chemotherapy. 3/5 of these patients are still in complete remission. Of the 2 who died, one had chemotherapy only for their lung met relapse and subsequently had a late 2nd liver/omental mets relapse, the other patient had disseminated disease at relapse.

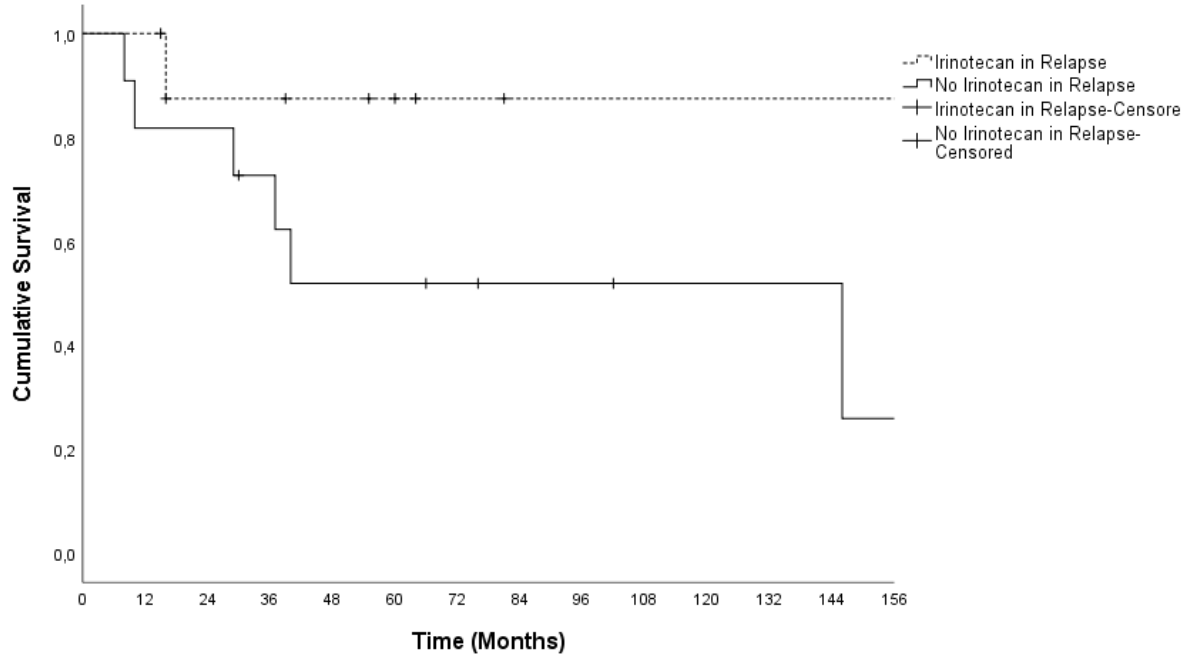
3.3.4b Carboplatin

Of the 7 patients who did not have carboplatin during their initial treatment, 6/7 had it as part of their relapse treatment. The remaining patient had no chemo for relapse. 3/6 of these patients are still in complete remission. The 3 that died all had metastatic disease with peritoneal involvement while those that survived had liver or lung mets.

3.3.4c Irinotecan

6 of the 19 patients who did not receive irinotecan as part of their initial treatment were given it as part of their relapse treatment (2 HB99, 4 LTR). 9/22 patients in total received irinotecan as part of their relapse treatment. 5/9 had lung mets, 3/9 had liver mets and 1/9 had brain mets. 8/9 (89%) of these patients were still alive at the last follow-up. Of these 2 have had further relapses (1 patient with lung mets, one patient with brain mets). The patient who died had lung mets and only had 20mg/m^2 of post-op irinotecan in total before progression to further mets. 13/22 patients had no irinotecan in their relapse treatment (2 of whom had had it during their initial treatment). 2/13 received palliative chemo only. 5/11 (45%) patients who received non-palliative relapse chemo treatment, excluding irinotecan, were still alive at last follow-up. Of these 11, 2 initially had liver mets, 6 had lung mets, 1 had peritoneal mets and 2 had mets at multiple sites. 1 patient with liver mets and 4 patients with lung mets survived.

Figure 10: Overall-survival time from relapse for those patients who were treated with irinotecan during relapse and those who were not. Those patients who did not receive irinotecan during follow-up appeared to have a poorer OS from relapse than those who did. The 2 patients who received palliative chemo have been removed from this analysis. This difference was not statistically significant (Log Rank (Mantel-Cox), p=0.118). In the table, the figures in brackets are those who had their last FU prior to 156 months, the figure not in the brackets includes these patients and those patients we know to be alive at 156 months.



Months	0	12	24	36	48	60	72	84	96	108	120	132	144	156
Irino	9	9	8(2)	8(2)	8(3)	8(4)	8(6)	8(7)	8(7)	8(7)	8(7)	8(7)	8(7)	8(7)
No Irino	11	9	9	8(1)	6(1)	6(1)	6(2)	6(3)	6(3)	6(4)	6(4)	6(4)	6(4)	5(4)

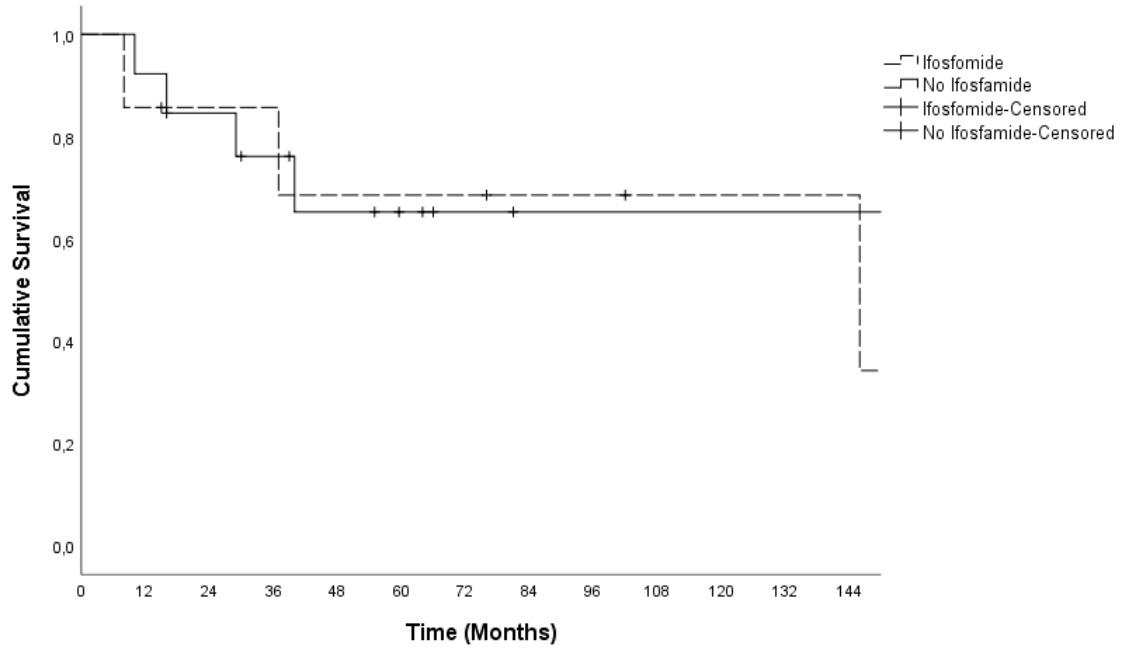
Those patients who did not receive irinotecan during follow-up appeared to have a poorer OS from relapse than those who did.

3.3.4d Ifosfamide

4 of the 15 (27%) patients who did not receive ifosfamide as part of their initial treatment were given it as part of their relapse treatment, (3 HB99, 1 LTR). In total 8/22 (36%) patients received ifosfamide as part of their relapse treatment. 1 of these patients received palliative chemo only. 50% (4/8, 2 of whom received it only in relapse) of these patients were still alive without further relapse at the last follow-up. Of the 4 patients who survived 3 had lung mets and 1 had liver mets. Of the 4 patients who died 2 had combined disease, 1 peritoneal mets and 1 lung mets (no op). 9/14 (64%) patients who did

not receive ifosfamide were still alive at last follow-up. 1 of these 14 patients received palliative chemo only. 2 of these patients have had at least one further relapse.

Figure 11: Overall-survival time from relapse for those patients who were treated with ifosfamide during relapse and those who were not. There was no statistically significant difference with regards to overall survival between those patients who received ifosfamide during their relapse treatment and those who did not (Log Rank (Mantel-Cox), p=0.815). The 2 patients who received palliative chemo have been removed from this analysis. In the table, the figures in brackets are those who had their last FU prior to 156 months, the figure not in the brackets includes these patients and those patients we know to be alive at 156 months.



Months	0	12	24	36	48	60	72	84	96	108	120	132	144	156
Ifo	7	6	6(1)	6(1)	5(1)	5(1)	5(1)	5(2)	5(2)	5(3)	5(3)	5(3)	5(3)	4(3)
No Ifo	13	12	11(1)	10(2)	9(3)	9(4)	9(7)	9(8)	9(8)	9(8)	9(8)	9(8)	9(8)	9(8)

There was no statistically significant difference with regards to overall survival time from relapse between those patients who received ifosfamide during their relapse treatment and those who did not (Log Rank (Mantel-Cox), p=0.815).

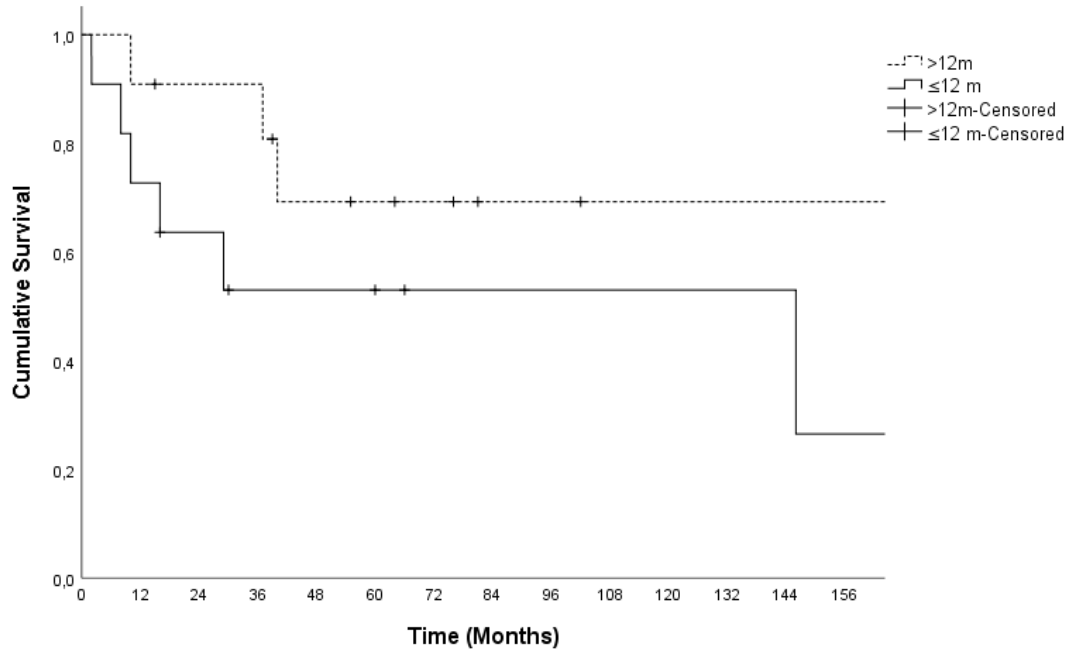
3.3.5 Time from Start of Last Chemo Block in the Initial Therapy to Start of the First Chemo Block of the Relapse Therapy

All patients had >21 days between the start of their last chemotherapy block of their initial therapy and the start of their relapse chemotherapy programme. 1 patient was on olaparib between the remission and the relapse. 3 patients had no chemotherapy treatment in relapse.

The median time between start of the last chemotherapy block in the initial therapy and the start of the relapse chemotherapy was 12 months (range: 1-48). 11/22 patients had > 12 months as compared to 11/22 patients who had ≤ 12 months. Of the 11 patients with >12 months, 3 (27%) patients have subsequently died (1 after a further relapse) and one has had a further relapse but is still alive. Of the 11 patients with ≤ 12 months, 6 (55%) patients have subsequently died (2 after further relapses) and 1 has had a further relapse but is still alive.

The >12 months group consisted of 4 local relapses, 5 lung relapses, 1 peritoneal relapse and 1 combined relapse. The ≤ 12 months group consisted of 1 local relapse, 6 lung relapses, 2 peritoneal relapses, 1 combined relapse and 1 brain relapse.

Figure 12: Overall-survival time from relapse for those patients whose relapse chemo started ≤ 12 months from the start of the last block of their initial chemotherapy as compared to those whose relapse chemo started >12 months after the start of the last block of their initial chemotherapy. Those patients whose relapse chemotherapy started >12 months after the start of the last block of their initial chemotherapy appear to have a better OS from relapse than those whose started ≤ 12 months after the start of their last block of chemo. This difference was not statistically significant (Log Rank (Mantel-Cox), p=0.189). In the table, the figures in brackets are those who had their last FU prior to 156 months, the figure not in the brackets includes these patients and those patients we know to be alive at 156 months.



Months	0	12	24	36	48	60	72	84	96	108	120	132	144	156
≤ 12 m	11	8	7(1)	6(2)	6(2)	6(2)	6(4)	6(4)	6(4)	6(4)	6(4)	6(4)	6(4)	5(4)
>12 m	11	10	10(1)	10(1)	8(2)	8(3)	8(4)	8(6)	8(6)	8(7)	8(7)	8(7)	8(7)	8(7)

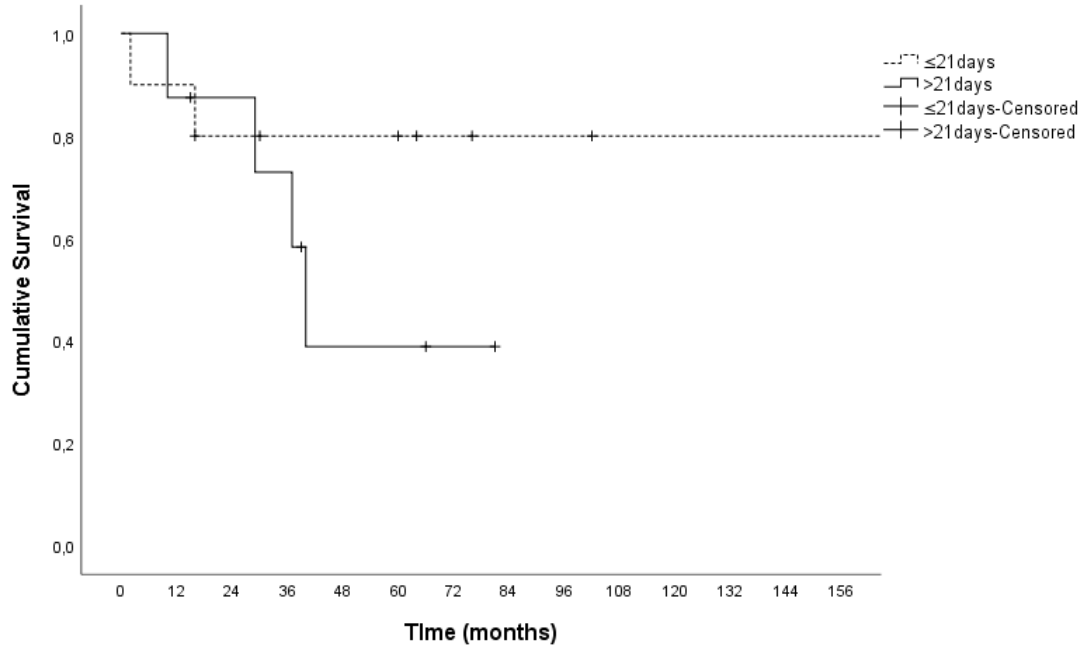
Those patients whose relapse chemotherapy started >12 months after the start of the last block of their initial chemotherapy appear to have a better OS from relapse than those whose started ≤ 12 months after the start of their last block of chemo. This difference was not statistically significant (Log Rank (Mantel-Cox), p=0.189).

3.3.6 Time from First OP Post-Relapse to Next Chemo

10/22 patients had ≤ 21 days and 8/22 patients had >21 days between their first OP post-relapse and their next chemo. 4 patients had an operation that was not followed by chemotherapy (2 x transplant, one of whom died of heart failure shortly after the transplant; 2 had rapid progression after the OP). 3 patients were not operated on during relapse (see above).

7/10 (70%) patients whose chemo started ≤21 days after their relapse OP were still in remission at last FU, 2 have died and 1 is still being treated (relapse/progression). Of the 8 patients whose chemo started >21 days after their relapse OP 3/8 (38%) were still in remission at last FU. 2/8 died without achieving remission and 3/8 had further treatment for relapse/progression, with 2 from these 3 subsequently dying.

Figure 13: Overall-survival time from relapse for those patients whose next block of relapse chemo started ≤ 21 days after their initial relapse operation as compared to those whose next block of relapse chemo started >21 days after their initial relapse operation. Those patients whose next block of relapse chemotherapy started >21 days after their initial relapse operation appear to have a worse OS from relapse than those whose next block of chemo started ≤ 21 days after their initial relapse operation. This difference was not statistically significant (Log Rank (Mantel-Cox), p=0.234). In the table, the figures in brackets are those who had their last FU prior to 156 months, the figure not in the brackets includes these patients and those patients we know to be alive at 156 months.



Months	0	12	24	36	48	60	72	84	96	108	120	132	144	156
≤ 21 days	10	9	8(1)	8(2)	8(2)	8(2)	8(4)	8(5)	8(5)	8(6)	8(6)	8(6)	8(6)	8(6)
>21 days	8	7	7(1)	6(1)	4(2)	4(2)	4(3)	4(4)	4(4)	4(4)	4(4)	4(4)	4(4)	4(4)

Those patients whose next block of relapse chemotherapy started >21 days after their initial relapse operation appear to have a worse OS from relapse than those whose next block of chemo started ≤ 21 days after their initial relapse operation. This difference was not statistically significant (Log Rank (Mantel-Cox), p=0.234).

3.3.7 Lung Mets at Relapse

10 patients presented at diagnosis with definite lungs mets. 6/10 of the initial lung met patients had a lung met resection during the initial treatment. Of the remaining 4/10 patients: 1 patient had an operation but after examination no tissue was removed and 3 patients had lung mets that were treated with chemotherapy only (see Table 1).

4/6 (67%) patients who were operated on during the initial treatment had a subsequent lung relapse following remission. Of the 4 patients treated with chemotherapy only, 2 (50%) went on to have lung mets at relapse (this includes the patient who had a thoracotomy but no resection as no mets were palpable).

3 patients developed lung mets during the initial treatment (one of whom (473 LTR) was reported as having possible mets at diagnosis). All 3 of those patients who developed lung mets had these mets operated on. 2/3 (67%) went on to relapse with lung mets.

Of the 5 patients who had lung mets at diagnosis or developed them during the initial treament who didn't relapse with lung mets, 3 had been operated and had had chemo, 2 had only had chemo.

3.3.8 Extent of Relapse Surgery

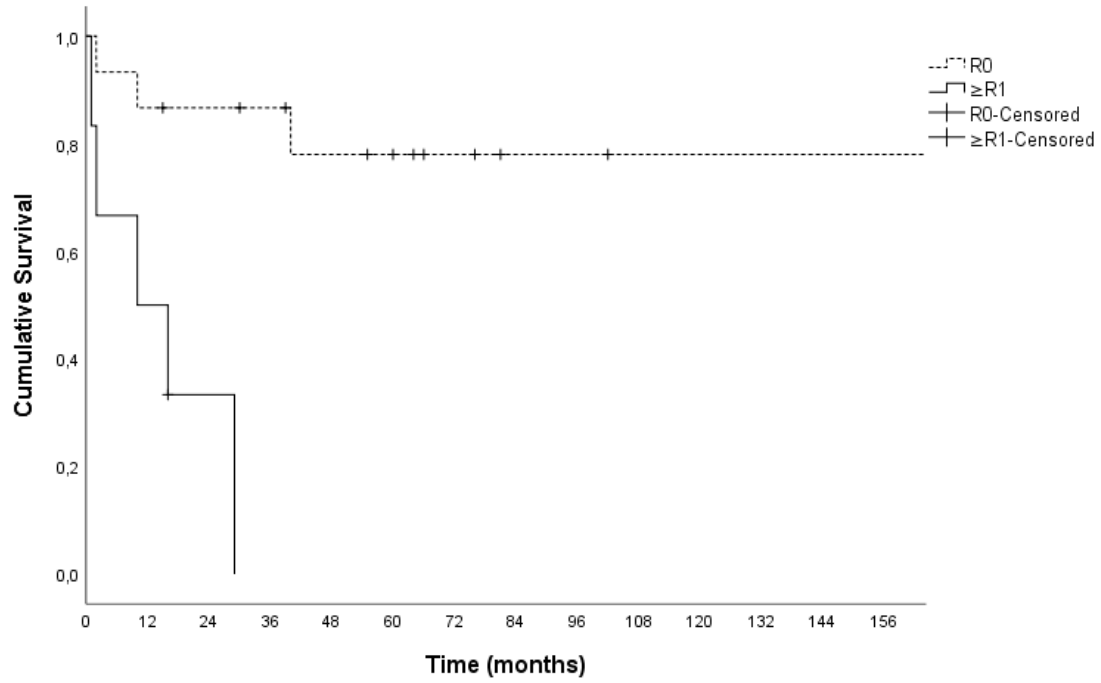
15 of the 22 relapse patients who had surgery had initial relapse operations (5 liver, 9 lung, 1 peritoneal) which were classed as R0. 6 of the 22 patients were classified as ≥R1. 3 of these patients had R1 (1 lung, 1 brain, 1 liver) operations, 3 of the 22 patients were classified as ≥ R1 as the extent of the resection in these cases was difficult to accurately define. 1 of these patients had peritoneal carcinosis, 1 had a biopsy only of a peritoneal abscess which was later found to be malignant and 1 was classified surgically as R0 but histologically as unclear owing to a tumour tear. For 1 of the 22 patients there was insufficient data to classify the operation correctly (1 liver+ omentum).

11/15 (73%) patients with a R0 1st relapse operation were still in second remission at last FU (4/5 liver, 7/9 lung, 0/1 peritoneal). 1/15 (1/5 liver) patients relapsed again and subsequently died. 1/15 patients (1/9 lung) relapsed again and is still in follow-up. 2/15 patients (1/9 lung, 1/1 peritoneal) died following

the 1st relapse. Of the 3 R1 relapse operation patients, 1 (lung) died without going into remission, one (brain) went into remission and subsequently relapsed again and is still in treatment, 1 (liver) died as a result of heart failure post liver transplant. All of the 3 patients with ≥ R1 relapse operations died. 2 were declared palliative after their relapse operation, and 1 died following a 2nd relapse. The patient with the missing data died following progression.

4/22 patients went on to have further operations during the first relapse (see **Table 5** and **Table 6**).

Figure 14: Overall-survival time of those patients who had a R0 initial relapse operation as opposed to those who had a ≥R1 relapse operation. Patients who had an R0 relapse operation had a significantly better overall survival than those whose operation was ≥ R1 (Log Rank (Mantel-Cox), p=0.001). In the table, the figures in brackets are those who had their last FU prior to 156 months, the figure not in the brackets includes these patients and those patients we know to be alive at 156 months.



Months	0	12	24	36	48	60	72	84	96	108	120	132	144	156
R0	15	13	13(1)	13(2)	12(3)	12(4)	12(7)	12(9)	12(9)	12(10)	12(10)	12(10)	12(10)	12(10)
≥R1	6	3	2(1)	1(1)	1(1)	1(1)	1(1)	1(1)	1(1)	1(1)	1(1)	1(1)	1(1)	1(1)

Patients who had an R0 relapse operation had a significantly better overall survival than those whose operation was ≥ R1 (Log Rank (Mantel-Cox), p=0.001).

3.3.9 AFP Declines after Treatment in Relapse Patients

11/25 patients had surgery first. 13/25 patients had chemo first. 1 patient had neither chemo nor surgery.

3.3.9a Surgery First

5 of the 11 patients who had surgery first on relapse had a >50% drop in AFP after the operation, 1/11 had a drop of <50%, 1/11 had an increase of >50%, 2/11 had an AFP < 10ng/ml pre-op., by 2/11 patients' data were missing.

Of the 5 patients who had a >50% drop of their AFP following surgery, 4/5 went on to have chemo and 1/5 died of heart failure before chemo could be given (219 LTR). Of the 4 patients who had chemo:

- 2/4 had a further > 50% drop in AFP with the chemo and both went into ongoing remission (81 HB99 had IPA x 2. 434 LTR had C5VD x 2),
- 1/4 had AFP <15 pre- and post-chemo and went into ongoing remission (124 LTR had Carbo+ Dox as the first 2 cycles post-op),
- 1/4 had an AFP that increased with palliative chemo and subsequently died (trofosfamid+idarubicin x 1 + trofosfamid x 1, 78 HB99, peritoneal relapse).

The patient who had a <50% AFP drop with surgery, had a >50% drop in AFP with chemo (VI x 2) and went into remission (292 LTR).

The patient with the increase in AFP following the operation (182 LTR), also had an increase in AFP after 2 rounds of chemo (Cis+Carbo+Dox x1, Cisx1), they subsequently had a drop of AFP <50% after a further operation but ultimately died following disease progression.

Of the 2 patients with AFP < 10ng/ml pre-and post-op, one (126 HB99) had had an explorative laparotomy and subsequently went on to receive palliative chemo (IPA) and died. The other (68 LTR) received chemo (VI x 2) and the AFP stayed <10ng/ml, however this patient has had a number of further relapses.

3.3.9b Chemotherapy First

5 of the 13 patients who had chemotherapy first had a >50% drop in AFP after 1-2 blocks of chemo, 3/13 had a drop <50%, 5/13 had an increase in AFP.

Of the 5 patients who had a >50% drop in their AFP, 4/5 also had an operation and 1/5 had no operation (193H). The patient who was not operated on achieved a 2nd complete remission but later suffered further relapses and died. Of the 4 patients who had operations:

- 2/4 had a >50% post-operative drop in AFP. One of these patients (187 HB99) had omental and liver mets at relapse and he subsequently progressed and died. The other (55LTR) patient is in ongoing remission,
- 1/4 had a <50% post-operative drop in AFP (186 HB99) and is ongoing remission,
- 1/4 had an AFP <15ng/ml pre-and post-operation and is in ongoing remission (213 LTR).

The 5 patients with a > 50% drop in their AFP received the following chemo: (1) 1 x CE then op; (2) 2 x CE; (3) 2 x I + Ca+ Dox; (4) 2 x ICE; (5) 2 x CE.

Of the 3 patients with <50% drop in AFP, all 3 went on to have an operation and they all had a >50% drop in AFP post-op. All 3 were in ongoing remission at last FU. These patients received the following first blocks of chemo pre-op: (1) Ir x 2; (2) IPA x 1; (3) VI x 2.

Of the 5 patients with an increase in AFP:

- 3 had subsequent operations followed by AFP drops of >50%. 1 of these patients is in on-going remission (246 HB 99), 1 went into remission but then had further relapses and died (58 HB 99), and 1 (473 LTR) is currently being treated for a further relapse.
- 1 (disseminated HB at relapse) died without having an operation (489 LTR).
- 1 had a subsequent operation where the AFP dropped by <50% (149 LTR). This patient progressed and died without achieving remission.

These patients received the following first blocks of chemo pre-op: (1) Ir + bevacizumab x 1; (2) topotecan + dox x 1; (3) VI x 1; (4) ICE x1 then Ca +Dox x 1; (5) CE x 1.

3.3.10 Outcomes after Relapse

16/25 (64%) patients achieved a 2nd complete remission (8 HB99, 8 LTR). 1 of these patients was treated with chemotherapy alone, 15 were treated with both surgery and chemotherapy. 52% of patients achieved a second CR in the Semeraro et al. 2013 paper.

Of these 16 patients, 11 (69%) (5 HB99, 6 LTR) were still in a second complete remission at last follow-up with a median follow-up of 66 months (15-186 months) from the date of first relapse (58%, Semeraro et al., median FU 84 months (3-175m)).

5 (3 HB 99, 2 LTR) of the 16 patients (31%) had a further relapse (42%, Semeraro et al.). The median time from first relapse to second relapse was 14 months (range 13 – 80 months) (median 10 months, 4 - 42 months range, Semeraro et al. 2013). Of these 5, 3 have subsequently died (3 HB 99), 1 is being treated for the 2nd relapse and the other is in their 5th remission and is under close observation for a potential further relapse.

3.4 Transplants

Overall, 6 patients had one liver transplant, one patient had 2 transplants. 3 transplants occurred during the initial treatment (68 LTR, 149 LTR, 219 LTR), 1 of which was following an initial conservative liver operation (149 LTR). 3 transplants occurred (246 HB99, 219 LTR, 132 LTR) during the first relapse, 1 after a previous liver transplant (219 LTR) and one after a liver resection for the relapse (246). 2 transplants occurred during a 2nd relapse (193 HB99, 58 HB99), one of whom (58) who had already had a liver resection for the 2nd relapse.

The 2 patients with primary liver transplants during the initial therapy both had an initial diagnosis of PRETEXT IV F+ hepatoblastoma (68 LTR, 219 LTR). One of these patients had a lung relapse (68 LTR) and one had a local relapse (219 LTR). The patient with the rescue transplant during the initial treatment (149 LTR) was initially diagnosed with a PRETEXT III F- hepatoblastoma. This patient had an initial lung relapse.

One of the two patients who had a 1st transplant after the first relapse (132 LTR) was initially diagnosed with a PRETEXT IV F+ hepatoblastoma. The other patient (246 HB99) was initially diagnosed with a PRETEXT II F- hepatoblastoma. Both of these patients had their transplant after local relapses. The patient with the 2nd transplant during their first relapse (219 LTR) has already been discussed above.

One of the 2 patients who had their transplant during their 2nd relapse (193 HB) had a PRETEXT IV F- hepatoblastoma at diagnosis. This patients' first relapse was a metastatic lung relapse. The other patient (58 HB99) who had their transplant during their 2nd relapse had a PRETEXT III F- hepatoblastoma at diagnosis. Their first relapse was local.

Overall, only 2 of these patients remain in remission (132 LTR, 246 HB99), both of whom had their first transplants after the first relapse. One of two (50%) primary transplant patients survived, this patient is under close observation following further lung relapses (68 LTR). The other patient (219 LTR) died due to post-op complications following a second transplant after a liver relapse. The patient who had a rescue transplant during the initial therapy (149 LTR) progressed and died after a lung relapse. Neither of the patients who had a transplant after a 2nd relapse survived. One died following progression (58 HB99 (lung then brain)) and the other died after new mets were found in the liver (193 HB99).

Of the 6 patients who were diagnosed with PRETEXT IV F+ tumours, 2 (219 LTR and 68 LTR) had primary transplants and one (132 LTR) had a transplant after the first relapse. 67% (2/3) of these patients were

still alive at last follow-up. 33% of these patients (132 LTR) was still in ongoing second remission. 100% (3/3) of those who diagnosed with PRETEXT IV F+ tumours who did not have a transplant were still alive at last follow-up (213 LTR, 137 LTR, 473 LTR). 67% (2/3) of these patients (213 LTR, 137 LTR) were still in ongoing second remission.

Of the 8 patients originally diagnosed with F+ disease, 4 had a local relapse and 4 had a metastatic relapse. Two of these patients had a primary transplant, one had a first transplant during the first relapse and one had a second transplant during the first relapse. 67% (2/3) of those who had a transplant(s) were still alive at last follow-up. 80% (4/5) of those who did not have a transplant were still alive at last follow-up.

3.5 Survival

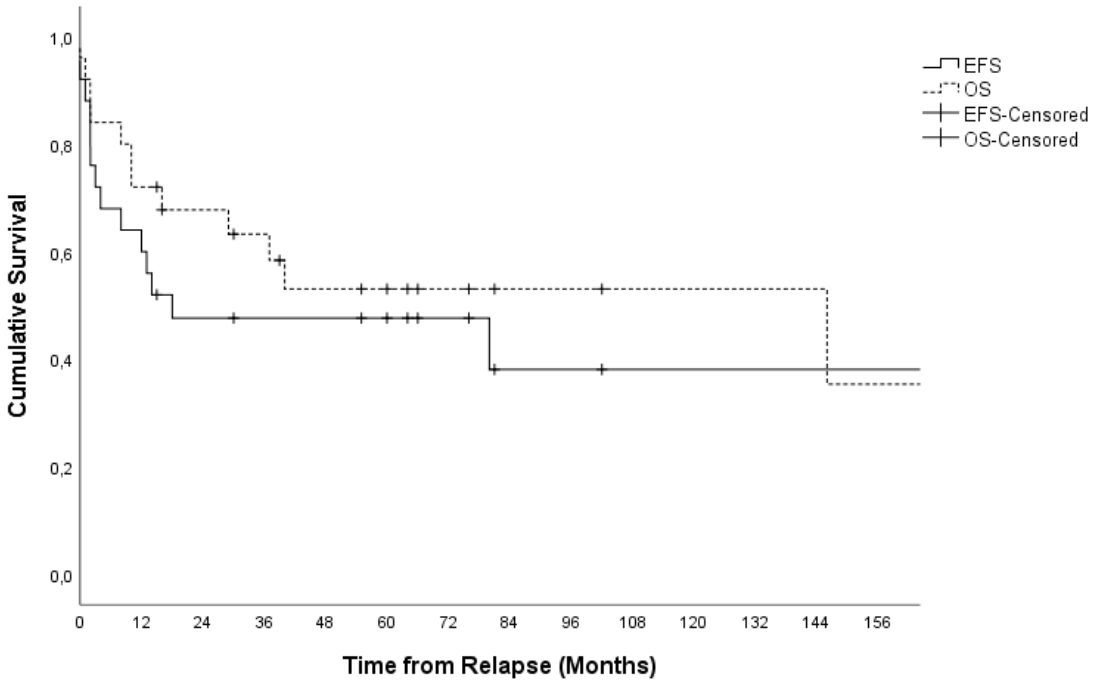
3.5.1 Overall Survival from Relapse

13/25 (52%) of the relapse patients were still alive at their last follow-up (5 HB99, 8 LTR). 4/13 of those patients who were still alive had an initial local relapse, 9/13 of these patients had an initial metastatic relapse. None of these patients had a combined relapse. 12/25 (48%) patients were in remission (11 in second remission, 1 in 5th remission) (39%, [Semeraro et al. 2013]) and 1 is currently being treated for their second relapse (473 LTR). The 12 patients in remission had a median time of 65 months (15-186 months) from first relapse to last follow-up (83 months, [Semeraro et al. 2013]).

Overall, 12/25 (48%) relapse patients died (61%, [Semeraro et al. 2013]). The median survival time from relapse till death for those patients who died was 10 months (0-146 months) ([Semeraro et al. 2013], 12m). 8 died of disease progression after the first relapse. 1 died of complications following liver transplant. 3 died following subsequent relapses.

The 3-year OS from relapse for all the 25 relapse patients was 63% and the 3-year EFS was 48% ([Semeraro et al. 2013], 3y OS 43%, 3y EFS 34%). The 5-year OS from relapse for all the 25 relapse patients was 53% and the 5-year EFS was 48%.

Figure 15: Overall survival (OS_{rel1}) versus event free survival (EFS_{rel1}) from relapse for the HB relapse patients in this study. There was no significant difference between OS and EFS from relapse in this group of patients (Log Rank (Mantel-Cox), p=0.447). In the table, the figures in brackets are those who had their last FU prior to 156 months, the figure not in the brackets includes these patients and those patients we know to be alive at 156 months.



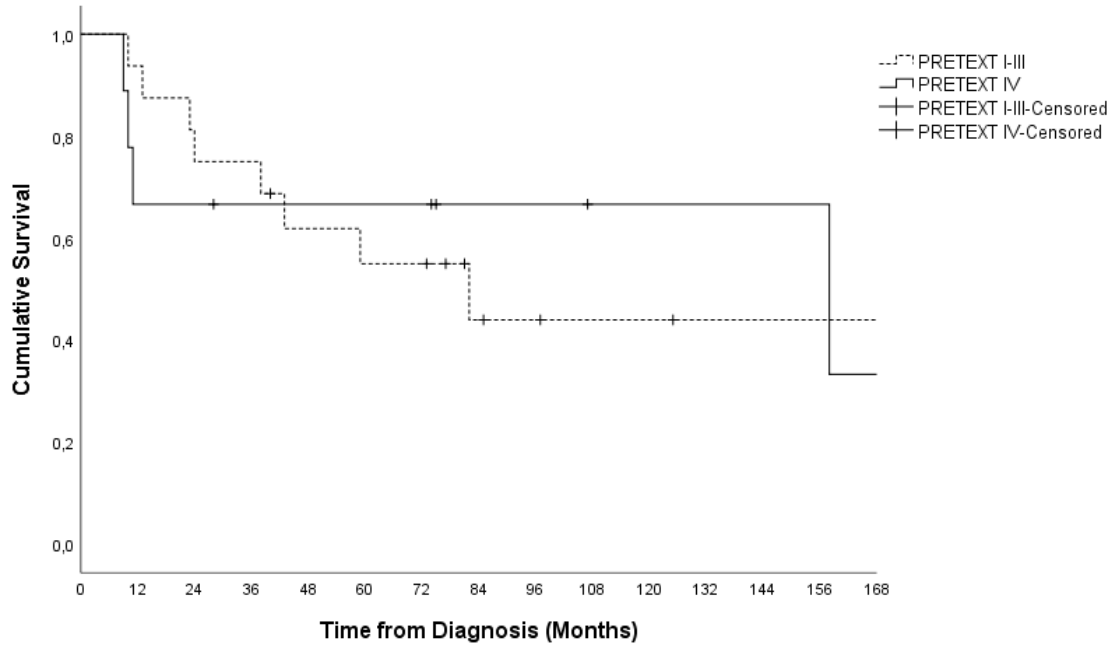
Months	0	12	24	36	48	60	72	84	96	108	120	132	144	156
OS_{rel1}	25	18	17(2)	16(3)	14(4)	14(5)	14(8)	14(10)	14(10)	14(11)	14(11)	14(11)	14(11)	13(11)
EFS_{rel1}	25	15	12(1)	12(2)	12(2)	12(4)	12(6)	11(8)	11(8)	11(9)	11(9)	11(9)	11(9)	11(9)

There was no significant difference between the EFS and the OS from relapse in our group of 25 patients. The EFS_{rel1} appears to drop quicker than the OS_{rel1} but ultimately, they both reach a similar level. The patients who went on to have a further event after the first relapse were usually those who subsequently had a lower chance of survival.

3.5.2 Survival from diagnosis according to PRETEXT classification at time of diagnosis

As seen in **Table 1**, 1 of the 25 relapse patients (4%) was classified as PRETEXT I at diagnosis, 5/25 (20%) as PRETEXT 2, 10/25 (40%) as PRETEXT III and 9/25 (36%) as PRETEXT 4. The one PRETEXT I patient (100%) was still alive at last FU and in 2nd CR. 3/5 (60%) PRETEXT II patients were still alive at last FU, all of whom are in 2nd CR. 4/10 (40%) PRETEXT III patients were still alive at last FU, all of whom were in 2nd CR. 5/9 (56%) PRETEXT IV patients were alive at last FU, 3 of whom were in second CR.

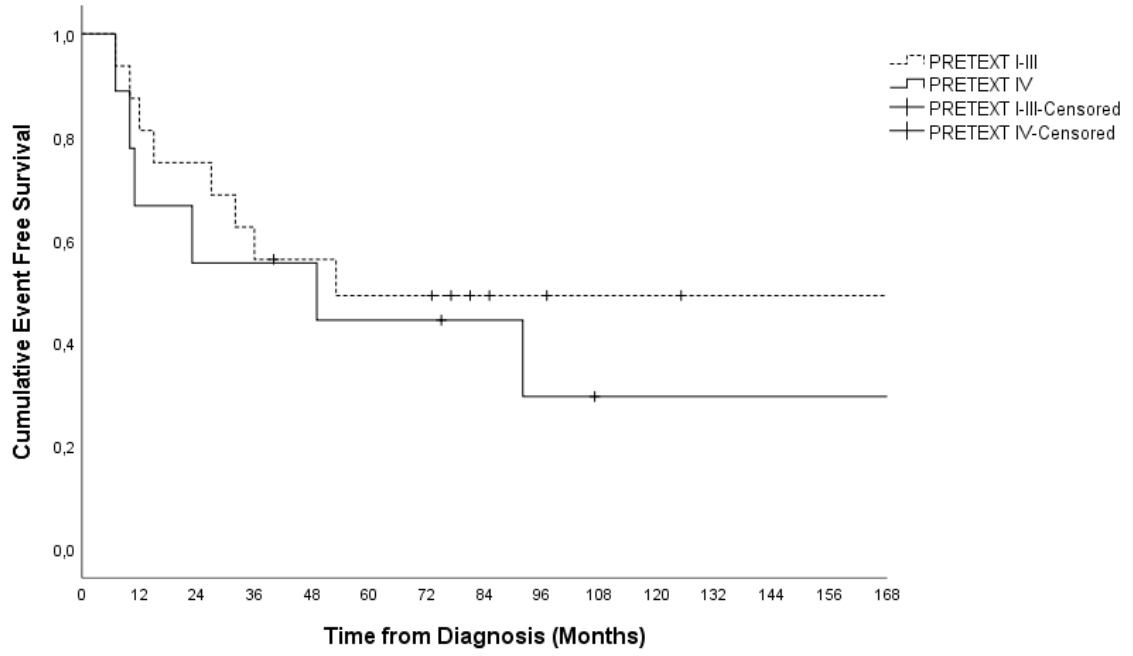
Figure 16: Overall survival (OS_{dia}) from diagnosis for PRETEXT group I-III patients as compared to PRETEXT group IV patients. There was no significant difference in terms of OS from diagnosis between these groups of patients (Log Rank (Mantel-Cox), p=0.866). In the table, the figures in brackets are those who had their last FU prior to 168 months, the figure not in the brackets includes these patients and those patients we know to be alive at 168 months.



Months	0	12	24	36	48	60	72	84	96	108	120	132	144	156	168
PRETEXT I-III	16	15	13	12	10(1)	9(1)	9(1)	8(4)	8(6)	8(6)	8(6)	8(7)	8(7)	8(7)	8(7)
PRETEXT IV	9	6	6	6(1)	6(1)	6(1)	6(1)	6(3)	6(3)	6(4)	6(4)	6(4)	6(4)	6(4)	5(4)

Due to the small number of patients available in our study we combined PRETEXT groups I-III and compared these results with those of group IV. As a result, we were unable to demonstrate a significant difference in the OS of patients from diagnosis based on their PRETEXT group assigned at diagnosis.

Figure 17: Event Free Survival (EFS_{dia}) from diagnosis for PRETEXT group I-III patients as compared to PRETEXT group IV patients. Patients with PRETEXT IV tumours appeared to have a worse outcome than those with PRETEXT I-III tumours. There was no significant difference however in terms of EFS between these groups of patients (Log Rank (Mantel-Cox), p=0.516). In the table, the figures in brackets are those who had their last FU prior to 164 months, the figure not in the brackets includes these patients and those patients we know to be "event free" at 164 months.



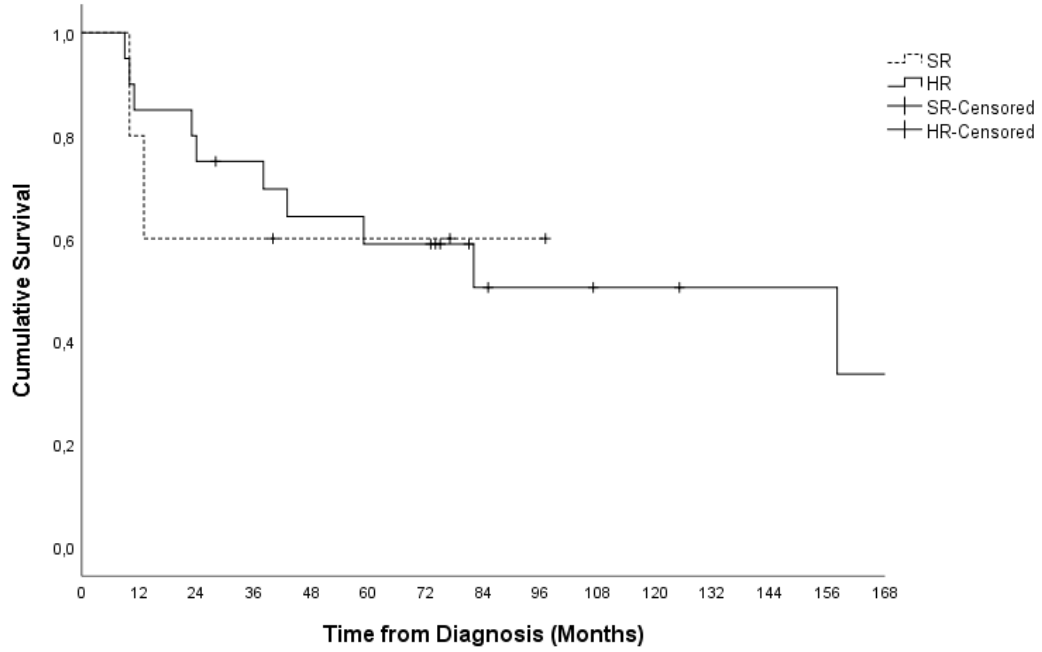
Months	0	12	24	36	48	60	72	84	96	108	120	132	144	156	168
PRETEXT I-III	16	14	12	9	9(1)	8(1)	8(1)	8(4)	8(5)	8(6)	8(6)	8(7)	8(7)	8(7)	8(7)
PRETEXT IV	9	6	5	5	5	4	4	4(1)	3(1)	3(2)	3(2)	3(2)	3(2)	3(2)	3(2)

Patients classified as PRETEXT I-III at diagnosis appear to have a slightly reduced risk of further events following a first relapse as compared to patients classified as PRETEXT IV at diagnosis. This difference was not significant probably as a result of the small sample size in this study.

3.5.3 Survival from diagnosis according to SR versus HR classification at time of diagnosis

As seen in Table 1, 5/25 (20%) of the relapse patients were classified as SR at diagnosis and 20/25 (80%) of the relapse patients were classified as HR at diagnosis. 3/5 (60%) SR patients were still alive at last FU, with all 3 being in 2nd CR. 10/20 (50%) of the HR patients were still alive at last FU, with 8/10 of these patients still in 2nd CR.

Figure 18: Overall survival (OS_{dia}) from diagnosis for SR patients as compared to HR patients. Patients with SR tumours at diagnosis ultimately appeared to have a better survival prognosis than those with HR tumours. There was however no significant difference in terms of OS from diagnosis between these groups of patients (Log Rank (Mantel-Cox), p=0.919). In the table, the figures in brackets are those who had their last FU prior to 168 months, the figure not in the brackets includes these patients and those patients we know to be alive at 168 months.



Months	0	12	24	36	48	60	72	84	96	108	120	132	144	156	168
SR	5	4	3	3	3(1)	3(1)	3(1)	3(2)	3(2)	3(3)	3(3)	3(3)	3(3)	3(3)	3(3)
HR	20	17	16	15(1)	13(1)	12(1)	12(1)	11(5)	11(6)	11(7)	11(7)	11(8)	11(8)	11(8)	10(8)

The patients classified as SR at diagnosis may have a slightly better overall survival from diagnosis than those patients who are classified as HR at diagnosis. This difference was not statistically significant. The number of patients in the study was not sufficient to adequately address this issue.

4. Discussion

4.1. Relapse Rate

Using data obtained retrospectively from the HB99 study and the Liver Tumour Register (LTR) a group of 25 relapse patients (recruited over the periods from 1.1.99-31.12.08 and 17.1.11-30.8.2019) was identified for closer evaluation.

The relapse rate for the 25 patients after complete remission was found to be 6.9% (25/362) (8.5% HB99 and 5.9% LTR). The HB99 rate is comparable with the combined relapse rate reported for the SIOPEL 1-3 studies, which was 8.4% [Semeraro et al. 2013]. The slightly lower rate seen in the LTR patients is likely partly a reflection of the reduced length of follow-up time for those patients recruited into the LTR after their initial diagnosis in 2018/2019. It may also however at least in part be the result of evidence driven changes in patient treatments between the trials (SIOPEL 1-3 1990-2004, HB99 1999-2008, LTR 2011-ongoing; [Häberle et al. 2019]).

This reduction in the relapse rate noted above is consistent with the improvement seen in the 3-year-OS and -EFS figures for relapse patients obtained from our study as compared to those obtained from Semeraro et al. 2013. Thus, the patients in our study were found to have a 3-year-OS and -EFS from relapse of 63% and 48% respectively as compared to 43% and 34% respectively in the Semeraro et al. 2013 study. This finding would further support an effect resulting from improvement in treatment. These changes will be discussed in more detail below.

4.2 Diagnosis and Initial Treatment

4.2.1 Characteristics of Relapse Patients

4.2.1a Age

The patients who relapsed had a slightly higher median age at the time of initial diagnosis (24months) as compared to the results for all HB patients in the SIOPEL 1-3 trials (17.2m, [Semeraro et al. 2013]) and the HB99 trial (16 months, [Häberle et al. 2019]). This finding was even more pronounced among those patients who went on to have a local relapse (72.5m) (84m, [Semeraro et al. 2013]). It is also in line with the previous observations of a higher rate of poorer outcomes in older HB patients and has been discussed in the paper by Häberle et al. 2020. It may be the result of accumulation of a combination of chromosomal, epigenetic, genetic and transcriptional alterations with time in older liver tumour patients ([Vogelstein et al. 2013], [Buendia 2014], [Sumazin et al. 2016]).

The study by Eichenmüller et al. in 2014 suggested that this phenomenon could not be explained on the basis of age-related exomic somatic mutations alone. Of note however is that this study analysed data from classical HB entities separately from HCN-NOS (previously TLCT) entities. This is in contrast

to the situation at the time of the SIOPEL 1-3 trials, the HB-99 trial and the initial years of the LTR. At this point, patients who now would be classified as SCUD and HCN-NOS were still classified as HB patients. The HCN-NOS patients are more likely to be older, have severe disease and relapse. Closer analysis of the relapse patients in our data set revealed a minority of patients who were a number of years older than the majority of the relapse patients (5/25 patients >5 years old). The pathology reports for these 5 patients all contained discussions as to possible HCC and/or macrotrabecular growth patterns. Thus, some of the age difference between the relapse group and the general group may be a reflection of an increased incidence of HCN-NOS patients within the relapse group as compared to the general HB group. Unfortunately, the data available is not sufficient to allow reclassification of all patients retrospectively in order to allow a more accurate comparison of these groups.

4.2.1b Gender

The male/female ratio was 3.2/1 for our relapse patients as compared to 2.2/1 for all of the HB patients in the HB99 Study [Häberle et al. 2019]. The reason for this slightly higher number of males among the relapse group is unclear but it is similar to the findings in the Semeraro trial (2.5/1 vs 1.5/1).

4.2.1c Risk Classification Status

The initial tumour in relapse patients is more likely to be graded as PRETEXT IV (36% vs 14% in the HB99 study) or HR (80% vs 40% in the HB99 study) and they are more likely to have additional risk factors such as metastases (40% vs 21% in the HB99 study) at initial diagnosis. Therefore, the general assumption that such patients are at a higher risk for relapse from the beginning of their treatment appears to be correct. In addition, this higher risk of relapse was maintained in spite of the intensified chemotherapy that these patients received in comparison to the standard risk patients (e.g. CE+HD CE vs IPA (HB99)), cisplatin + carboplatin + doxorubicin vs cisplatin (LTR)). In the currently ongoing PHITT Trial a proportion of those patients whose metastases persist despite the initial therapy will go on to receive vincristine with irinotecan in addition to cisplatin and doxorubicin. It would be interesting to see if this group of patients suffer fewer relapses than those patients in the other arm of this subgroup who will receive similar treatment to that our patients had previously (CD+CE).

4.2.1d AFP Level

The majority of relapse patients had an AFP of >100ng/ml just like the majority of the hepatoblastoma patients who did not suffer a relapse (92% vs 93% HB-99). Thus, in this small study no marked difference in AFP level at diagnosis was found between those HB patients who went on to relapse and those who did not.

4.2.1e Tumour Histology

The most common histological subtype amongst our patients was an epithelial histology. Of note was the inclusion of certain tumour subtypes within the HB group, which may now no longer be classified as HB-tumours. Thus, there was one tumour with a SCUD histology which may actually have been a rhabdoid tumour [Vokuhl et al. 2016] and there were 4 tumours which may now be classified as HCN-NOS tumours [Lopez-Terrada et al. 2014]. These tumour subtypes tend to be associated with poorer outcomes.

4.2.2 Treatment

4.2.2a Initial Chemotherapy

The patients received a variety of chemotherapy regimens during their initial chemotherapy treatment. All patients received platin (cisplatin or carboplatin), the max. cumulative dose was 594mg/m^2 cisplatin and 8475mg/m^2 carboplatin. 72% (18/25) patients had doxorubicin, the max. cumulative dose was 412.5mg/m^2. Further analysis of these findings is limited due to the small patient number and the spread of agents used. Most of the patients from the HB99 study received CE and/or IPA. Most of the patients from the LTR received cisplatin monotherapy and/or cisplatin + carboplatin + doxorubicin. The number of additional agents used was greater in the LTR patients than in the HB99 patients reflecting the introduction of an increasing number of agents over time and no stringent trial requirements at this time. Nearly half of the relapse patients (11/25, 44%) received additional/alternate chemotherapy agents to those with which the initial treatment began. As changes in chemotherapy often result following poor response to treatment, it seems likely that the existence of chemoresistance during the initial treatment is an identifying feature for those who will go on to relapse in the future.

4.2.2b Operation

All patients underwent liver surgery during their initial treatment. The majority of patients with metastatic disease also had resections of their metastases.

Median Time from Diagnosis to Operation

Patients who had liver operations between 3-<4 months after diagnosis appear to have had better outcomes than those who were operated on before or after this point. This improvement in survival is hinted at in the Kaplan-Meyer analysis performed comparing the results for the group of patients operated on at <4 months versus those operated on at ≥4 months (Fig. 3). This difference was not significant, but this is probably at least in part because of the inclusion of the poorer outcomes from

those operated on in the first few months of this <4 months period. Unfortunately, more detailed group analysis was not possible due to the small number of patients involved.

The poorer outcomes for those with <3 months from diagnosis to operation is partly due to those who presented acutely and were subsequently operated on under suboptimal emergency conditions (<2month group, 2/3 patients were patients with tumour rupture). The worsening of outcomes from ≥4 months between diagnosis and operation may reflect an increased baseline chemoresistance in this group, which is then further compounded by a higher risk of incomplete tumour clearance and further increases in tumour chemoresistance due to longer chemotherapy exposure.

Time from Operation to next Chemo

Most patients (68%) in our study had a delay of ≥21 days between their initial liver operation and their next cycle of chemotherapy. Those patients who had a delay of < 21 days appeared to do better than those who had a delay of ≥21 days although this difference was not significant (Fig. 4). The number of patients who had a delay of ≥21 days between their original liver operation and their next chemotherapy block was reduced amongst the LTR relapse patients as compared to the HB-99 relapse patients (83 % HB-99 vs 54% LTR). This may be due to changes in practice between the 2 studies (HB99: 1999-2008, LTR: 2011-onwards) as a result of publications suggesting that those patients who had a shorter delay appeared to have a better chance of survival [Becker et al. 2015]. Those patients with a shorter delay are believed to have reduced exposure to the increases in hepatocyte growth-factor (HGF) that occur following liver tumour resection [von Schweinitz et al. 2000]. HGF is thought to promote liver tumour cell growth/survival ([von Schweinitz et al. 2000] [Grotegut et al. 2010]). Our results, whilst not statistically significant, appear to provide further support for this proposal.

Extent of Resection

Fewer relapse patients had a R0 resection when compared to all the patients in the HB99 study [Häberle et al. 2019] (68% versus 80%). This is unsurprising given the higher risk classification of the tumours in the relapse patients as compared to the general group. It also highlights a further risk factor for why the relapse patients went on to relapse.

4.3 Median Time to Relapse

4.3.1 Median Time from Initial Diagnosis to First Relapse

The median time from initial diagnosis to first relapse was 13 months (5-66mo) (12m, [Semeraro et al. 2013]). The median time between diagnosis and first relapse was longer for the HB 99 patients (19mo) than it was for the LTR patients (12mo). 1 patient in each group relapsed significantly later than the others (45 months HB99 vs 66 months LTR) so this difference can't be explained by a few very late

relapses in the HB 99 group. A review of the LTR patients with relapses who were diagnosed with HB and registered in the LTR before the end of this study but weren't included in this study because they hadn't yet relapsed did not lead to a change in these results even though this was performed 27 months after the data for the study was collected. Thus, an exclusion of patients with longer relapse times due to data collection timing also does not explain this difference. An analysis of the mean time to relapse revealed a mean time to relapse of 19 months for the HB patients and of 18 months for the LTR patients. Therefore, there must be more patients with a shorter relapse time in the LTR group. The reason for this difference appears to be a quicker investigative response to even small increases in AFP and an improvement in the radiological diagnostics between the time of the HB 99 study and the LTR, thus leading to quicker diagnoses and a shorter relapse time. There were 2 cases in the HB99 study where there was an extremely long gap between the AFP increasing and the radiological diagnosis of the tumour (18 months (peritoneal tumour), 30 months (AFP fluctuant and very slowly rising over this time). Similar gaps were not seen in the data provided for the LTR (6 months was the longest gap here).

As mentioned above there were a number of late relapses within the group with 5 (20%) patients relapsing ≥24 months after diagnosis, 2 of whom (8%) relapsed ≥36 months after initial diagnosis. This once again confirms the need for prolonged follow-up for HB patients as noted in a number of previous research papers ([Häberle et al. 2019], [Semeraro et al. 2013]). There are insufficient patient numbers to comment on the characteristics of those patients who relapse late but part of the cause may be late detection and not just late relapse. Factors leading to later detection of relapse in our study included radiological detection difficulties (e.g. peritoneal tumour), consistently low AFPs, and slowly progressive relapses with subsequent diagnostic uncertainty (e.g. one patient had a small AFP increase, after several years in remission, which was detected 6 months before the next follow-up check was performed). This last case shows the importance of not underestimating an increase in AFP even when the last treatment was several years previously.

Patients with local relapse relapsed later after diagnosis than those with metastatic relapse (median time 20m vs 12m) in our trial in contrast to the results in the SIOPEL trial (10m local vs 20m metastatic). All the local relapse patients had increases in AFP, thus this longer relapse time can't be attributed to a lack of tumour marker signal leading to a delay in diagnosis. Both groups had patients where there was a delay between AFP increase and radiological diagnosis of relapse. Removal of these patients from the analysis reduces but does not eliminate this finding. Reasons for this finding are unclear and given the disagreement with the findings from Semeraro et al. would first need to be further investigated within a larger group of patients to confirm the validity of these findings. Of note however, is the finding from Semeraro et al. that 5/6 of their late relapses (>36 months after diagnosis) were localised in the liver, this appears to correlate with our data that local relapses occur later.

4.3.2 Median Time from Remission to First Relapse

The median time from remission to first relapse was 7 months (1-48 months). The median time between remission and first relapse was longer for the HB 99 patients (14 months) than it was for the LTR patients (5 months). These results are similar to those already discussed above for median time between diagnosis and remission and as noted above probably reflect improvements in radiological techniques and recognition of warning signals (slowly increasing AFP). The median time interval till relapse was, as above, longer for patients with local relapse and combined relapse than it was for patients with metastatic relapse.

4.4. Relapse

4.4.1 Relapse Location

The majority of relapses were metastatic (68%) with the majority of metastases occurring in the lung (65%) as was the case in the Semeraro et al. 2013 paper. There was no statistically significant difference in survival between local, metastatic and combined relapses, although those patients with combined relapses appeared to do significantly worse than the other groups (Fig. 5).

A more detailed examination of the metastatic relapse location revealed that non-pulmonary metastatic relapses had a statistically significant reduction in overall survival (Log Rank (Mantel-Cox), p=0.008)) as compared to pulmonary mets. Thus, 1/2 (50%) patients with brain mets and 3/3 (100%) patients with peritoneal mets ultimately died as opposed to 3/11 (27%) with lung mets (Fig. 6).

A majority (4/6, 67%) of the patients with local relapse had had multifocal liver disease at diagnosis. This raises the question of whether these patients should have had a transplant rather than a resection as their initial operation. However, without information as to how many patients with multifocal disease who had a resection did not relapse, no further inference can be drawn here. It is however worth noting that both of the multifocal patients (68 LTR, 219 LTR) who had a primary liver transplant during the initial treatment went on to have a recurrence (one liver, one lung). Neither of these patients had R0 transplant operations. Thus, transplant operations do not always prevent recurrence and often do lead to considerable long-term complications and costs.

53% of those with metastatic relapse had had lung mets at initial diagnosis. A significant minority (40%) of those patients with metastatic lung disease at initial diagnosis did not have their lung mets resected and were thus treated with chemotherapy alone. Were these the patients who relapsed with lung mets and would these patients have benefited from lung resections during their initial therapy? This matter will be discussed further below.

4.4.2 AFP at Relapse

In most patients, the relapse was associated with a rise in AFP. The median AFP level at relapse was 174ng/ml (185ng/ml, [Semeraro et al. 2013]). Interestingly, despite this association with a rise in AFP there were still 10/25 patients (40%) at relapse who had AFPs of ≤100ng/ml. These patients appear to have a worse outcome (although not statistically significant in our small study) than those with an AFP of >100ng/ml at relapse (Fig. 7a and 7b). Thus, our study suggests that even small increases in AFP (≤100ng/ml) are important and should lead to further investigations. Unfortunately, we do not as yet have data from our studies with regards to the frequency of small AFP fluctuations (10ng/ml-100ng/ml) for patients in remission who do not go on to relapse. Further work needs to be done here. New studies with regards to the potential of measuring the AFP-L3 fraction may provide another potential means of more accurately identifying those patients with slightly raised AFPs who will relapse from those who won't [Kawahara et al. 2021]. This could prove to be extremely useful if large numbers of non-relapses are also found to have AFPs in this range.

There have been discussions about the use of potentially harmful radiological scans (CT/CXR) in the long-term follow-up of HB patients given the low relapse rate/good outcomes achieved in the majority of these patients. The use of regular AFP measurements has been proposed as an alternative surveillance technique for detection of recurrence [Rojas et al. 2014]. Implementation of AFP measurements alone would have led to delayed diagnosis in at least 5 (20%) of our patients who had an AFP of < 10ng/ml at relapse. In these patients the relapse was diagnosed purely on the radiological findings (1*MRT, 2*CT, 1 CT & MRT, 1 MD). A further 5 of the patients may or may not have had a delayed diagnosis depending on the cut-off AFP value for additional screening. These patients had an AFP of >10ng/ml but less than <100ng/ml. One of these was picked up by radiology during the EOT phase, 4 of them had very small increases which were followed by radiological screening which may well have been part of the normal surveillance procedures and not specifically ordered as a result of the small changes in the AFP. The use of a monitoring system combining AFP levels above 10ng/ml following remission with a risk classification-based imaging strategy (e.g. PRETEXT III-IV) would however have picked up all of our relapse patients. Thus, **both** regular AFP measurements and long-term follow-up imaging are essential screening elements for the high-risk patients whereas only AFP measurements alone may be sufficient for lower risk patients. Larger studies are needed to determine if this is indeed the case. The role of ultra-sound scans in the screening is not clear from our data as information as to whether ultrasound scans were performed prior to the CT/MRI scans at relapse was not available for the majority of our patients.

Consistently low AFPs (at diagnosis and independently at relapse) were associated with a poor prognosis (2 patients, both of whom died). This agrees with the CHIC finding that patients with AFP

levels of <100 ng/ml at diagnosis tend to have a worse outcome [Czauderna et al. 2016]. More recent analysis of the CHIC data, which excluded HB tumours that have subsequently been reclassified into the rhabdoid tumour group with SMARC-B1 (INI1) mutations, suggests that patients with an AFP of <100ng/ml at diagnosis actually would not be expected to have a worse prognosis [Trobaugh-Lotrario et al. 2023]. Interestingly, our patient who had an initial AFP of 33ng/ml was actually diagnosed with SCUD HB in 2003. Unfortunately, there is no record of INI1-staining in this case, thus this may indeed have been what was classified as a SCUD HB tumour but is now graded as a malignant rhabdoid tumour [Vokuhl et al. 2016]. This potentially explains the poorer outcome in this patient than might be expected according to the new CHIC data.

4.4.3. Relapse Treatment

4.4.3a Surgery, chemotherapy or both?

The majority of first relapses were treated with a combination of surgery and chemotherapy (80%). Those who had only surgery or neither surgery nor chemotherapy tended to have a very poor prognosis. Those who had only chemotherapy also appeared to have a worse outcome than those who had both surgery and chemo but better than those who did not have chemo (Fig. 8). Owing to the small patient numbers involved no conclusions can however be drawn here.

4.4.3b Surgery First or Chemotherapy First?

9 patients had an operation first. 11 patients had chemo first. 4/9 patients (44%) with the operation first and 7/11 (64%) with the chemotherapy first were still in complete 2nd remission at their last follow-up. Thus, it would appear that one should only operate prior to chemo at relapse diagnosis so as to confirm the relapse histologically with resections at this point being reserved for cases where an R0 resection really is possible. All other patients should receive chemotherapy first. Although our data seems to suggest that chemotherapy first leads to better outcomes this difference was not statistically significant (Fig. 9). Indeed, the poorer outcome in the surgery first group was in part a reflection of the occurrence of 3 of the 4 peritoneal-based relapses in this group. These patients were found to have a statistically worse prognosis than those with lung mets.

4.4.3c Relapse Chemotherapy and outcomes

The relapse patients received a variety of chemotherapy regimens for their relapse treatment, with all patients who were given chemotherapy receiving at least 2 different agents and with no more than 3 patients all receiving exactly the same types of chemotherapy. This is probably partially as a consequence of the variety of chemotherapy agents used during the initial treatment and also partially due to the lack of guidelines regarding treatments to use in relapse patients. Once again, the majority

of patients (17/22, 77%) received either cisplatin or carboplatin. The majority of patients (19/22, 86%) were given at least one new agent in comparison to their initial treatment [Venkatramani et al. 2012]. Owing to the numbers involved and the variety of agents used, it was not possible to analyse if one treatment regime was better than another in relapse or if adding new agents led to an improvement in outcomes.

Doxorubicin

100% (5/5) of patients who received non-palliative chemotherapy at relapse, and who had not had doxorubicin during the initial treatment, were given doxorubicin (with other agents) at relapse. The OS rate of this group was 60%. This OS rate appears to be better than the OS rate recorded for all relapse patients by Semeraro et al. 2013 (3-year OS 43%) from the SIOPEL trials where not all such patients appear to have had doxorubicin as they did in our trials. A potential benefit of doxorubicin in anthracycline-naïve relapse patients has been noted by a number of authors including Malogolowkin et al. in 2008 and Trobaugh-Lotrario and Feusner in 2012. Our results would seem to provide further evidence for this benefit.

Carboplatin

100% (6/6) of those patients who received non-palliative chemotherapy at relapse, and who had not had carboplatin during the initial treatment, were given carboplatin (with other agents) at relapse. The OS rate of this group was 50%. This is in line with findings from Fuchs et al. in 1999. This OS rate is similar to the OS recorded by Semeraro et al. 2013 (3 year OS 43%), who also gave a significant number of patients carboplatin in relapse. The patients who did not survive in our study all had metastatic disease including peritoneal mets.

Irinotecan

32% (6/19) of those patients who received chemotherapy at relapse, and who had not had irinotecan during the initial treatment, were given irinotecan (with other agents) at relapse. 9/22 relapse patients in total received irinotecan (with other agents) as part of their chemotherapy relapse treatment. The OS rate of this group was 89% (8/9). The OS rate of the group who did not have irinotecan was 45% (5/11, 2 patients not included as they were palliative only). Those patients who received irinotecan as part of their relapse treatment appeared to have a better prognosis than those who did not, although this difference was not significant (Fig 10). If one compares the outcomes for only the liver and lung met patients and excludes the patient who only received 20mg/m^2 prior to progression then 100% (8/8 patients) who received irinotecan survived as compared to 63% (5/8 patients) who did not. There has been a number of articles ([Zsiros et al. 2012], [Trobaugh-Lotrario and Feusner 2012], [Zhang et al.

2015]) which suggest a benefit of irinotecan in relapse patients and this study would also seem to confirm that view.

Ifosfamide

27% (4/15) of those patients who received chemotherapy at relapse, and who had not had ifosfamide during the initial treatment, were given ifofosfamide (with other agents) at relapse. 57% (4/7 patients) who received non-palliative chemotherapy including ifosfamide were still alive at last follow-up. 69% (9/13 patients) of those who received non-palliative chemotherapy excluding ifosfamide were alive at last follow-up. Those patients who received ifosfamide appear to have a similar/slightly worse outcome than those who did not (Fig.11). One possible explanation for the apparent lack of improvement with ifosfamide is the greater effect of the agents that were used instead of ifosfamide in those patients who did not receive this medication (e.g. doxorubin, irinotecan, cisplatin, carboplatin). Many of these other medications have been shown to have a greater effect than ifosfamide on hepatoblastoma in in-vivo animal studies such as that from Fuchs et al. in 1998 where treatment with doxorubin and cisplatin were found to cause greater tumour volume reduction than treatment with ifosfamide, carboplatin or etoposide.

Time from last chemotherapy block in the initial treatment to start of the first chemotherapy block in the relapse treatment

The median time between the start of the last chemotherapy block and the start of the relapse chemo was 12 months. Those who relapsed in ≤12 months appeared to have a worse overall survival than those who relapsed after this point (Fig. 12). This difference was not however statistically significant and is probably at least in part a reflection of the greater number of metastatic relapses in this group. It may also be that the shorter relapse time is associated with the more aggressive tumours and/or greater chemotherapy resistance and therefore the poorer outcome in this group.

Time from first operation post-relapse to the next chemotherapy

Patients who had ≤21 days between their relapse OP and their next chemo appear to have an improved prognosis compared to those who had >21 days (see Figure 13). This corresponds to the findings in the initial therapy and provides further weight for the argument that the time from operation to restarting chemotherapy is important. This is thought to be the result of increasing chemotherapeutic resistance (e.g. via HGF/c-Met) with time in the regenerating liver ([Schweinitz et al. 2000], [Becker et al. 2015], [Grotegut et al. 2010]). On the basis of these findings, we would recommend that postoperative chemotherapy commences within 21 days of tumour resection.

4.4.4 Lung Mets at Relapse

67% of those who had lung met resection during the initial treatment went on to have a lung relapse as compared to 50% of those who had lungs mets but who did not have a resection. The risk of relapsing again with lung mets after being treated with chemotherapy alone for lung mets therefore surprisingly appears to be less than after being treated with chemotherapy and surgery.

Those patients who were not operated on are probably the patients whose mets responded well to chemotherapy. Thus, if the lung mets respond to chemotherapy then an additional surgical resection may not be necessary to reduce the risk of further lung relapses. Those patients who had chemotherapy and surgery are likely to be those whose mets were less chemo-responsive and these patients appear to have a higher risk of further lung relapses. Surgery still plays an important role in the treatment of these patients. Due to the small numbers of patients involved the predictive value of our results are limited. They are however similar to other findings in the literature such as those of the Zsiros et al. 2013 paper.

4.4.5 Extent of Relapse Surgery

The majority of relapse patients had an R0 relapse operation (68%) and the majority of these R0 patients were still alive at last follow-up (80%) and still in second complete remission (73%). Of those patients who did not achieve an R0 relapse operation, the majority of them (83%) died, the remaining patient has had further relapses. Thus, an initial R0 operation in relapse in our study led to a significantly better ($p>0.001$) overall survival than a ≥ R1 relapse operation (see Fig. 14). The majority of these R0 operations were in patients with liver or lung mets.

4.4.6 Prognostic Value of AFP Changes

4.4.6a Rapid AFP Declines Following Treatment

In those patients who had surgery first at relapse, 67% (6/9 patients, excluding the 2 MD patients) had a drop in AFP shortly after surgery. Of these 67% (4/6 patients) were still alive at last follow-up as compared to 33% (3/9 patients) of the patients who had either no change or an increase in AFP. All the patients who had a drop in AFP in response to surgery and in response to the next 2 cycles of chemotherapy (3/3) were still alive at last follow-up.

In those patients who had chemotherapy first at relapse, 62% (8/13) of the patients had a drop in AFP after 1-2 cycles of chemotherapy. Of these 75% (6/8) were still alive at last follow-up as compared to 40% (2/5) of those patients who had either no change or an increase in AFP. The majority of patients who had a drop in AFP in response to chemo and in response to the subsequent operation (83%, 5/6) were still alive at last-FU.

Rapid declines in AFP following treatment of relapse patients appears to be associated with an improved prognosis. This has also been found to be the case during the initial treatment of hepatoblastoma patients ([Koh et al. 2011], [Nguyen at al. 2018]). Rapid declines would be expected in those patients who have complete tumour resection and/or tumours which are sensitive to chemotherapy. It is therefore unsurprising that these patients tend to have a better prognosis than those patients whose AFP does not drop so rapidly/at all.

4.4.6b AFP Constant or Increasing in the Weeks Following Treatment

Unchanging or rising AFP levels shortly after relapse surgery and/or chemo appear (see above), as would be expected, to be associated with poorer patient outcomes. These findings are unsurprising as an increasing AFP after surgery/chemotherapy suggests the ongoing presence of residual vital tumour despite chemotherapy (risk of chemoresistance) or surgery (incomplete resection or further mets). A low/unchanging AFP could indicate the presence of undifferentiated tumour cells, which also tend to have a poorer prognosis/ greater chemoresistance [Marin et al. 2019]. The presence/development of chemoresistance is a significant problem in the treatment of hepatoblastoma. The underlying mechanisms responsible for this vary from patient-to-patient and in response to treatment received [Marin et. al 2019]. The current on-going PHITT-Trial aims to shed more light on this complicated area by analysis of patients clinical data combined with their associated gene expression patterns. The ultimate aim being patient-specific chemotherapy treatment regimens. Increasing AFP levels or consistently low AFP levels despite treatment appear to continue to be a prognostic marker of poorer outcome for relapse patients as they are for patients during initial treatment ([Koh et al. 2011], [Nguyen at al. 2018]).

4.4.7 Outcome after Relapse

64% (16/25) of patients achieved a 2nd complete remission. 94% of these patients were treated with both surgery and chemotherapy during relapse. 69% (11/16) of these patients were still in complete 2nd remission at last follow-up (median follow-up 66m). These results appear slightly better than those seen in the SIOPEL 1-3 trials where 52% of patients achieved a second complete remission [Semeraro et al. 2013]. They are however similar to the results from the SIOPEL 4 trial HR arm where 60% of HR patients (not SR and HR like our patients) who went on to relapse then achieved a second complete remission [Zsiros et al. 2013]. This apparent improvement may be a result of treatment changes over time in our group (1999-2018) in comparison to the SIOPEL 1-3 group (1990-2004) and the SIOPEL 4 group (2005-2009). These changes include improvements in management of chemoresistance (alternative agents in relapse, intensification of chemotherapy regimens, reducing post-op treatment delays) and improvements in diagnosis (e.g. radiology) and surgical management of relapse patients.

Patients who went on to have further relapses (31%, 5/16) had a poor prognosis with only 2/5 (40%) still alive at last follow-up. Both of these patients have had further relapses and only one of these patients is currently in remission.

4.5 Liver Transplant

7/25 relapse patients had a liver transplant (one of whom also had a 2nd transplant). 3 transplants occurred prior to relapse. 3 transplants occurred after the first relapse. 2 transplants occurred after a second relapse. Only 2/7 (29%) of those patients who had a transplant were still in ongoing remission at last follow-up. These were both patients who had a transplant after the first relapse. The majority of our patients who received a transplant are either dead (57%, 4/7) or have gone on to have further relapses (1/7). 3 of these post-transplant progressions/relapses occurred in the lungs, 2 occurred in the liver.

The 2 primary transplants were for patients with PRETEXT IV F+ tumours. One of these patients had a local relapse and one had a metastatic relapse. 50% (1/2) of these patients was still alive at last follow-up. The other 4 patients who were PRETEXT IV F+ did not receive primary transplants. 100% (4/4) of these patients were still alive at last follow-up. 3 of these patients had local relapses, one had a metastatic relapse. One of these patients had a transplant after the first relapse.

Of the 8 patients originally diagnosed with F+ disease, 4 had a local relapse and 4 had a metastatic relapse. Two of these patients had a primary transplant, one had a first transplant during the first relapse and one had a second transplant during the first relapse. 67% (2/3) of those who had a transplant(s) were still alive at last follow-up. 80% (4/5) of those who did not have a transplant were still alive at last follow-up.

These findings would seem to agree with the findings from Fahy et al. 2019 that not all patients with multifocal hepatoblastoma need to have a liver transplant.

4.6 Overall Survival from Relapse

52% (13/25) of the relapse patients were alive at their last follow-up. Those with a local relapse (67%, 4/6), or a metastatic relapse (53%, 9/17) appeared to be more likely to survive than those who had a combined relapse (0/2).

For those who died the median time from relapse to death was 10 months. Most (67%, 8/12) died because of progression following the first relapse.

The 3 year OS and EFS from relapse were 63% and 48% respectively (Semeraro et al. 43% and 34%) (Fig. 15). Although our results suggest that the patients in the lower risk groups at diagnosis may have

a better prognosis, the OS and/or EFS from diagnosis did not differ significantly with staging (Fig. 18) or PRETEXT group at diagnosis (Fig. 16,17). This is most likely due to the small numbers of patients, especially in the lower risk groups.

5. Conclusions

From the 362 patients with HB recruited into the HB99 study and the Liver Tumour Register a total of 25 relapse patients (recruited over the periods from 1.1.99-31.12.08 and 17.1.11-30.8.2019) were identified for closer evaluation. This evaluation led to the following conclusions:

1. Only a minority of HB patients' relapse (6.9%). These are more likely to be patients who are older at diagnosis, male, classified as PRETEXT III-IV and/or high risk, and those with additional risk factors at diagnosis.
2. The majority of HB patients' relapse < 24 months after remission but some do relapse after 36 months and long-term follow-up is therefore important. The latest relapse amongst our patients occurred 48 months after first remission.
3. Both imaging and AFP results are important in the follow up of HB patients in their first complete remission since 40% (10/25) of the patients had an AFP below 100ng/ml, with 20% (5/20) having a normal AFP of below 10ng/ml, at the time of relapse. However, AFP results may prove to be sufficient for monitoring PRETEXT I-II patients, with an AFP of >10ng/ml being a signal for the need for closer follow-up and the use of imaging such as ultrasound, x-rays, MRI or CT scans.
4. HB relapse patients with the following features seem to have a worse prognosis: combined relapses, AFP <10ng/ml at relapse diagnosis, non-pulmonary mets, starting chemo >21 days after the relapse operation, inability to achieve a R0 resection, and failure of AFP to normalise rapidly with relapse treatment.
5. Relapse patients treated with surgery and chemotherapy and the addition of a novel chemotherapeutic agent (to the specific patient) such as doxorubicin or irinotecan seem to have a better outcome.
6. Not all patients with multifocal tumours appear to need to have a liver tumour transplant in order to achieve a good outcome.
7. Those patients whose lung metastases have successfully been treated with chemotherapy during the initial treatment do not appear to also need to have surgical resection of the affected areas to achieve a good outcome.
8. The 3y OS from relapse for this group of 25 relapse patients was 63% and the 3y EFS was 48%. Thus, there is a good chance that HB patients will achieve a second remission despite a first relapse. However, patients who suffer further relapses have a much poorer prognosis.

6. Zusammenfassung

Aus einer Kohorte von 362 Patienten mit Hepatoblastom (HB) aus der HB-99-Studie und dem Lebertumorregister trat in einem Zeitraum von knapp 20 Jahren (1.1.1999 - 30.08.2019) bei nur 25 Kindern (6,9 %) ein Rezidiv auf. Dabei handelt es sich um Patienten, die bei Diagnose des malignen Lebertumors älter und/oder männlich und/oder als PRETEXT III-IV und/oder als Hochrisikopatienten mit zusätzlichen Risikofaktoren eingestuft waren.

Bei den meisten Patienten trat das Rezidiv innerhalb von 24 Monate nach erster Remission auf. Es gab aber auch Patienten, bei denen das Rezidiv erst nach 36 Monaten auftrat, weshalb eine langfristige Nachbeobachtung wichtig ist. Der letzte Rückfall bei unseren Patienten trat 48 Monate nach erster Remission auf.

Sowohl bildgebende Verfahren als auch AFP-Ergebnisse sind für die Nachsorge von HB-Patienten in erster Remission wichtig, da 40 % (10/25) der Patienten zum Zeitpunkt des Rezidivs ein AFP unter 100ng/ml und 20 % (5/20) ein normales AFP von unter 10 ng/ml aufwiesen. Ein AFP-Wert von >10ng/ml ist ein Signal für eine eingehende Kontrolluntersuchung und den Einsatz von bildgebenden Verfahren wie Ultraschall, Röntgen, MRT oder CT. Im Gegensatz zu Hochrisikopatienten könnten sich AFP-Kontrollen alleine als ausreichend für die Überwachung von Niedrigrisiko Patienten in erster Remission erweisen. Um dieses Ergebnis zu validieren, sind jedoch Studien mit größerer Fallzahl notwendig.

HB-Rezidivpatienten mit den folgenden Merkmalen scheinen eine schlechtere Prognose zu haben: kombinierte Rezidive, AFP <10ng/ml bei der Rezidivdiagnose, nicht-pulmonale Metastasen, Beginn der Chemotherapie >21 Tage nach der Rezidivoperation, keine R0-Resektion und Versagen einer raschen Normalisierung des AFP bei der Rezidivbehandlung.

Rezidivpatienten, die mit Operation und Chemotherapie behandelt wurden und bei denen zusätzlich ein neues Chemotherapeutikum für den jeweiligen Patienten wie Doxorubicin oder Irinotecan eingesetzt wurde, scheinen ein besseres Ergebnis zu erzielen als Patienten, die nur eine oder keine dieser Behandlungsoptionen erhalten.

Nicht alle Patienten mit multifokalen Tumoren scheinen eine Lebertumortransplantation zu benötigen, um ein gutes Ergebnis zu erzielen.

Bei Patienten, deren Lungenmetastasen bei der Erstbehandlung erfolgreich mit Chemotherapie behandelt wurden, scheint eine chirurgische Resektion der betroffenen Bereiche nicht erforderlich zu sein, um ein Rezidiv zu vermeiden.

Das 3-Jahres-OS ab Rezidiv für diese 25 Rezidivpatienten lag bei 63 %, das 3-Jahres-EFS bei 48 %. Es besteht somit eine gute Chance, dass HB-Patienten trotz eines ersten Rückfalls eine zweite anhaltende Remission erreichen. Patienten, die weitere Rückfälle erleiden, haben schlechte Prognose.

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8. Appendix

8.1 Affidavit

LMU LUDWIG-MAXIMILIANS-UNIVERSITÄT MÜNCHEN

Promotionsbüro
Medizinische Fakultät

MMRS

Eidesstattliche Versicherung

Maxwell, Rebecca Ann

Name, Vorname

Ich erkläre hiermit an Eides statt,

dass ich die vorliegende Dissertation mit dem Titel

Insights into Hepatoblastoma Relapse from the German HB99 Trial and the Liver Tumor Registry

selbständig verfasst, mich außer der angegebenen keiner weiteren Hilfsmittel bedient und alle Erkenntnisse, die aus dem Schrifttum ganz oder annähernd übernommen sind, als solche kenntlich gemacht und nach ihrer Herkunft unter Bezeichnung der Fundstelle einzeln nachgewiesen habe.

Ich erkläre des Weiteren, dass die hier vorgelegte Dissertation nicht in gleicher oder in ähnlicher Form bei einer anderen Stelle zur Erlangung eines akademischen Grades eingereicht wurde.

München, 12.04.2023

Ort, Datum

R. Maxwell

Unterschrift Doktorandin bzw. Doktorand

8.2 Acknowledgements

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8.3 Publication List

- Boekstegers A, Schmidt H, Kurzay M, Vallée T, Jung E, Dubinski I, **Maxwell R**, Schmid I. Cortisol response in children with cancer and fever during chemotherapy: A prospective, observational study using randon serum cortisol levels. Cancer Med 2023 Feb; doi: 10.1002/cam4.5667. Online ahead of print.
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