Aus dem Institut für Medizinische Immunologie
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DISSERTATION

“Clinical evaluation of CAP7.1 in therapy refractory biliary tract cancer in a randomized phase II trial”

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von

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List of abbreviations

5FU/FA:	Combination of 5FU and FA
5FU:	Fluoruracil
aBTC:	Advanced Biliary Tract Carcinoma
AoV:	Ampulla of Vater
ARID1A:	AT-rich interactive domain containing protein 1A
AT:	Adjuvant Therapy
BAP1:	BRCA1-Associated Protein 1
biCI:	Bootstrap interquartile CI
BRAF:	V-raf murine sarcoma viral oncogene homolog
BSC:	Basic Supportive Care only therapy
CA 19-9:	Carbohydrate Antigen 19-9
CA-242:	Carbohydrate 242
CAPE:	Capecitabine
CARB:	Carboplatin
CCA:	Cholangiocarcinoma
CI:	95% Confidence Interval
CIS:	Cisplatin
CR:	Complete response
CRA:	Clinical Research Associate
CRF:	Case Report Form
CT:	Computed Tomography
CYFRA 21-1:	Cytokeratin Fragment 21-1
dCCA:	Distal CCA
DCR:	Disease Control Rate
DFS:	Disease Free Survival
DLT:	Dose Limiting Toxicity
eCCA:	Extrahepatic CCA
ECOG:	"Eastern Cooperative Oncology Group" performance status
EGFR:	Epidermal Growth Factor
EMA:	European Medicines Agency
ERCP:	Endoscopic Retrograde Cholangio-Pancreatography
FA:	Folic acid
FDA:	Food and Drug Administration
FGFR2:	Fibroblast Growth Factor Receptor 2
FU:	Fluoropyramidines
GEM/CIS:	Combination of Gemcitabine plus Cisplatin
GEM:	Gemcitabine
GEMOX:	Combination of Gemcitabine plus Oxaliplatin
HBV & HCV:	Hepatitis B and C
HCC:	Hepatocellular Carcinoma
H & E	Hämatoxylin-Eosin-Färbung
iCCA:	Intrahepatic CCA
IDH:	Isocitrat dehydrogenase
IgG 4:	Immunoglobulin G4
IGF-1:	Insulin-like Growth Factor 1
IL-6:	Interleukin 6
ITT:	Intention To Treat
KRAS:	Kirsten rat sarcoma viral oncogene homolog

LFT:	Liver Function Test
LV:	Leucovorin
m:	Months
mmOS:	Median of medians of OS
mmPFS:	Median of medians of PFS
Mac-2BP:	Mac-2 binding protein
MMP-7:	Matrix metalloproteinase-7
MMR:	DNA mismatch repair
mDFS:	Median Disease-Free Survival
mOS:	Median Overall Survival
mPFS:	Median Progression-Free Survival
mRFS:	Median Recurrent-Free Survival
mTTP:	Median Time To Progression
MRCP:	Magnetic Resonance Cholangio-Pancreatography
MRI:	Magnetic Resonance Imaging
MS:	Mean Difference
MSI:	Microsatellite instability
MTD:	Maximum Tolerated Dose
n.p.	Not provided
OR:	Odds Ratio
ORR:	Objective Response Rate
OS:	Overall Survival
OX:	Oxaliplatin
pCCA:	Perihilar CCA
PD:	Progressive disease
PFS:	Progression Free Survival
PP:	Per Protocol
PPV:	Positive Predictive Value
PR:	Partial response
PRR:	Partial response rate
PS:	Performance Status
PSC:	Primary Sclerosing Cholangitis
PTC:	Percutaneous Transhepatic Cholangiography
QoL:	Quality of Life
R0 resection:	Resection without microscopic or macroscopic tumor remnants at margin
R1 resection:	Resection with microscopic tumor remnants at resection margin
R2 resection:	Resection with macroscopic tumor remnants at resection margin
RCT:	Randomized controlled trial
RFS:	Recurrence Free Survival
ROS1:	ROS proto-oncogene 1
RR:	Response Rate
SD:	Stable disease
SDV:	Source Data Verification
TCR:	Tumor Control Rate

TNM:	TNM Classification System of Malignant Tumors
TP53:	Tumour protein p53
TTP:	Time To Progression
ULN:	Upper limit of normal
US:	Ultra Sound = Sonography
VEGF:	Vascular Endothelial Growth Factor

ABSTRACT

Background: Cholangiocarcinoma (CCA) is the most rapidly growing cancer in the West with worst prognosis. Surgery is the only potential cure (cure rate: 2-4%). Thus, 96-98% of patients die within 10 years while most receive chemotherapy at some stage. Life expectancy under Gemcitabine/Cisplatin 1st-line-standard (median-overall-survival [mOS]: 11.7m, median-progression-free-survival [mPFS]: 8.0m) and in 2nd-line (mOS: 5-7m, mPFS: 3m) is limited while no 2nd-line standard exists and superiority over "Best-Supportive-Care" (BSC) is not established. Accordingly, need exists for new more effective therapies especially for patients with end-stage disease.

Methods: CAP7.1 is a novel prodrug converted via carboxylesterases to etoposide. CAP7.1 is able to overcome multi-drug-resistance-1 and deliver 1000-fold higher cytotoxicity in various tumor cell lines compared to etoposide. This is a randomized, controlled, open-label, multicenter, parallel, two-group phase-II study following a group sequential design (O'Brien Fleming) to evaluate CAP7.1's efficacy and safety compared to BSC in adult patients with advanced therapy-refractory CCA after 1st-line-therapy failure. The primary endpoint is disease-control-rate (DCR), while PFS, OS and safety are secondary endpoints.

Results: At first interim analysis the DCR exceeded the study's set target difference of 35% in the per-protocol (PP) analysis (n=18, $DCR_{CAP7.1}$= 56%, DCR_{BSC}= 0%, p=0.014). Median PFS was 3.5m (95% CI: 1.9-6.3m) for CAP7.1 vs 1.2m (95% CI: 0.2-3.7m) for BSC group (p<0.01). Median OS for both groups was around 4.5 month (95% CI: 1.2-15.3m, p=0.37) caused by the switch of BSC patients to CAP7.1 after progression. Patients >=2 CAP7.1 cycles benefited more (mOS: 5.9m [95% CI: 2.5-15.3m]). Estimated 1 year survival was 41%. CAP7.1 hemato-toxicity safety profile was comparable to etoposide and etopophos with the main adverse events being neutropenia (67%), leucopenia (57%), thrombocytopenia (48%), anemia (48%), infections (33%), alopecia (33%), fatigue (23%), nausea (19%), LFT+ (19%), and fever (19%).

Conclusion: Although patient number is small, these are encouraging initial results pointing in one common direction of a significant CAP7.1 effect over BSC especially considering that nearly all other drugs showed little effect so far. Furthermore, the CAP7.1 hemato-toxicity safety profile seems at least as safe as etoposide or etopophos, although a nearly 3-fold equivalent etoposide dose was administered. Most importantly, no organ toxicities were observed which is expected for etoposide at high doses. Thus, CAP7.1 displays an improved, predictable, reversible and manageable safety profile.

ABSTRAKT - DEUTSCH

Einleitung: Das Cholangiokarzinom (CKA) ist der sich am schnellsten verbreitende bösartige Tumor im Westen mit schlechter Prognosen. Heilungchance besteht nur durch Resektion (Heilungsrate: 2-4%). Daher sterben 96-98% der Patienten innerhalb 10 Jahren während die meisten irgendwann eine Chemotherapie erhalten. Sowohl mediane Überlebensdauer (mÜD) als auch mediane progressionsfreie Überlebensdauer (mPFÜ) nach Gemcitabine/Cisplatin-Erstlinientherapiestandard (mÜD=11,7 Monate [m], mPFÜ=8,0m) und Zweitlinientherapie (mÜD=5-7m, mPFÜ=3m) sind begrenzt, während es keinen Zweitlinienstandard gibt sowie keine Chemotherapie Überlegenheit im Vergleich zur einfachen Symptomenkontrolle (SK) gezeigt werden konnte. Daher besteht Bedarf nach neuen, effektiveren Therapien, insbesondere für Endstadium-Patienten.

Methodik: CAP7.1 ist ein neues Vorläufer-Medikament, das hauptsächlich über Carboxylesterasen in Etoposid umgewandelt wird. CAP7.1 ist in der Lage, die Multidrug-Resistenz-1 zu überwinden und eine 1000-fach höhere Zytotoxizität in verschiedenen Tumorzelllinien im Vergleich zu Etoposid zu erreichen. Dies ist eine randomisierte, kontrollierte, offene, multizentrische, zwei Parallel-Gruppen umfassende Phase-II-Studie nach gruppensequentiellem Entwurf (O'Brien Fleming), um die Wirksamkeit und Sicherheit von CAP7.1 im Vergleich zu SK bei erwachsenen Patienten mit fortgeschrittenem, therapierefraktärem CKA nach fehlgeschlagener Erstlinientherapie zu bewerten. Der primäre Endpunkt war die Krankheitskontrollrate (KKR), während mPFÜ, mÜD und Arzneimittelsicherheit sekundäre Endpunkte waren.

Ergebnisse: Bei der ersten Zwischenanalyse übertraf die KKR das Differenz-Ziel der Studie von 35% in der Per-Protokoll Analyse (PP) (n = 18, $KKR_{CAP7.1}$ = 56%, KKR_{SK} = 0%, p = 0,014). Die mPFÜ betrug 3,5m (95% CI: 1,9-6,3m) für CAP7.1 gegenüber 1,2m (95% CI: 0,2-3,7m) für die SK-Gruppe (p<0,01). Die mÜD für beide Gruppen war um 4,5m (95% CI: 1,2-15,3m, p=0,37) bedingt durch den Wechsel von SK-Patienten zu CAP7.1 nach Progression. Patienten mit >=2 CAP7.1-Zyklen profitierten mehr (mÜD: 5,9m [95% CI: 2,5-15,3m]). Die geschätzte 1-Jahres-Überlebensrate betrug 41%. Das hämatotoxische CAP7.1 Sicherheitsprofil war vergleichbar mit Etoposid und Etopophos mit den wichtigsten unerwünschten Ereignissen Neutropenie (67%), Leukopenie (57%), Thrombozytopenie (48%), Anämie (48%), Infektionen (33%), Alopezie (33 %), Müdigkeit (23%), Übelkeit (19%), erhöhte Leberwerte (19%) und Fieber (19%).

Schlussfolgerungen: Trotz kleiner Patientenzahl sind dies ermutigende erste Ergebnisse, die in eine gemeinsame Richtung eines signifikanten CAP7.1-Effekts gegenüber SK hinweisen, insbesondere angesichts der Tatsache, dass alle anderen eingesetzten Medikamente bisher nur wenig Erfolg gezeigt haben. Darüber hinaus scheint das hämatotoxische CAP7.1-Sicherheitsprofil mindestens so sicher zu sein, wie die Sicherheitsprofile von Etoposid oder Etopophos, obwohl eine fast 3-fach höhere Equivalenzdosis von Etoposid verabreicht wurde. Am wichtigsten aber scheint, dass keine Organ-Toxizität beobachtet wurde, die ansonsten für hohe Etoposide Dosierungen erwartet wird. Somit zeigt CAP7.1 ein verbessertes, vorhersehbares, reversibles und kontrollierbares Sicherheitsprofil.

1. INTRODUCTION

1.1 BILIARY TRACT CARCINOMA FACTS

Biliary Tract Carcinoma (BTC) / Cholangiocarcinoma (CCA) is a heterogeneous group of epithelial cell malignancies (90% adenocarcinomas), deriving within the biliary tract usually with pathological features of cholangiocyte differentiation. However, CCA can also originate from hepatocytes[6,7] with mixed hepatocellular-cholangiocellular forms existing (Figure 1).[1,2,3,4,5]

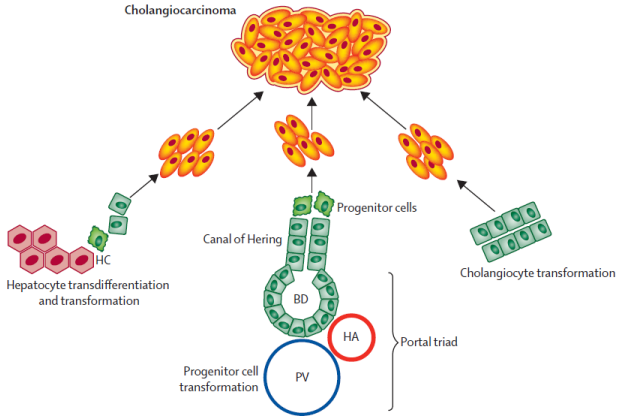


Figure 1 – Potential cells of origin in iCCA. PV = Portal Vein. HA = Hepatic Artery. BD = Bile Duct. HC = Hepatic Cell. (Razumilava, 2014[1]).

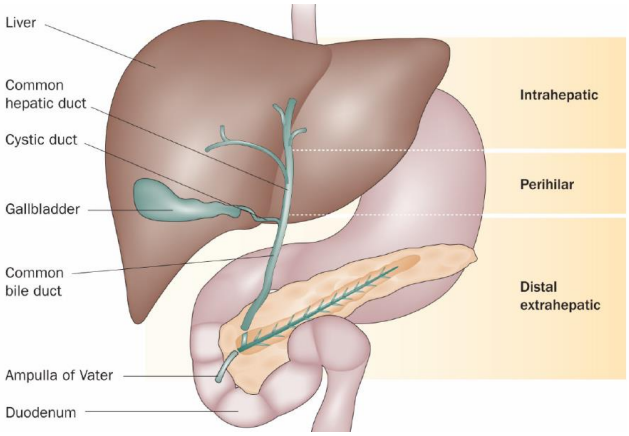


Figure 2 – Cholangiocarcinoma subtypes (Blechacz et al., 2011[9]).

1.1.1 ANATOMICAL CLASSIFICATION

CCA are classified as intrahepatic (iCCA), perihilar (pCCA), and distal (dCCA), the latter two grouped as "extrahepatic" (eCCA) and pCCA also named "Klatskin Tumor" (Figure 2)[1,2,3,4]. The terms "Klatskin" and "extrahepatic", however, are discouraged[2] to avoid misclassification. 50[1]-70%[135] of all CCAs are pCCA, <10[1]-25%[4] iCCA, 20[3,135]-40%[1] dCCA and 5%[3,4] multifocal. Gallbladder carcinomas (GBC) are distinct but often grouped with CCA[135]. [1,2,3,4,5,8,9,135]

1.1.2 EPIDEMIOLOGY

CCA is the 2nd commonest primary liver tumor worldwide after hepatocellular carcinoma (HCC)[3]. Highest incidence rates per 100000 (men: 87.7, women: 36.3) are found along the lower Mekong river in Southeast Asia as the distributional area of Opisthorchis viverrine (liver fluke), a major carcinogen and CCA risk factor[44]. Infections occur via freshwater fish (8 mio infections and 20000 deaths per year in Thailand alone, 2 mio infections in neighbouring countries)[44]. Incidence rates in the Western World are as low as 0.3[2] (Figure 3). Also, annual global iCCA incidence rose rapidly by 5.1/6.9% for women/men[2] and mortality increased by > 3.5%[2] with higher mortality rates in men than women (1.9 vs 1.5)[2] while eCCA incidence decreased[3].

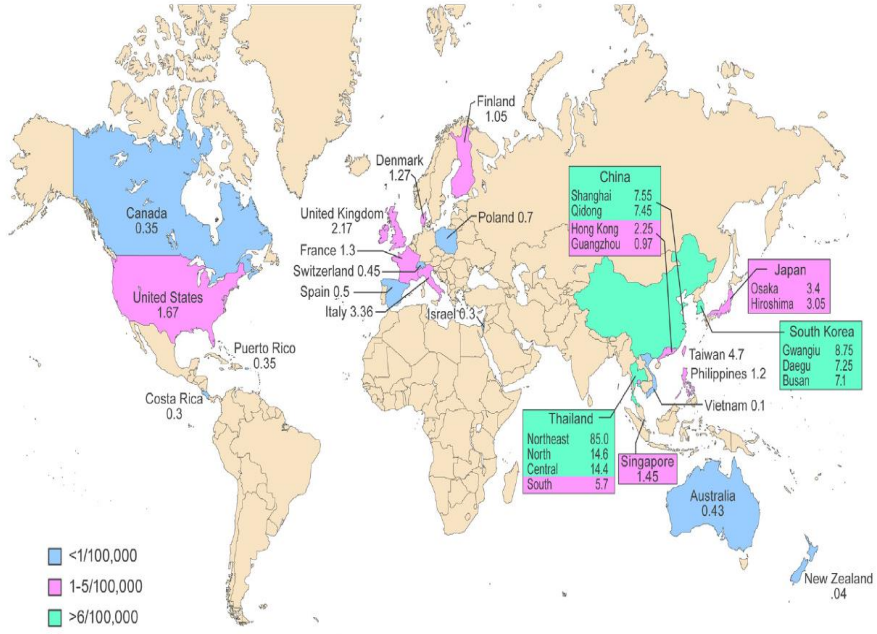


Figure 3 – Incidence of CCA worldwide (Bridgewater et al., 2014[2]).

Relevance for western countries is best shown by US data: While from 2003-2012 mortality rates declined for most cancers and for all cancers combined by 1.5%, liver cancers including iCCA had the highest annual mortality rate increase of all cancers (men: 2.8%, women: 2.2%) while also incidence rates increased sharply[42]. Unfortunately, this report[42] did not distinguish iCCA from other liver cancers. However, 30% of primary hepatic tumors are CCAs[44]. Overall, CCA mortality in the US increased from 1999-2014 by 36% from 2.2 to 3.0/100000 with 2/3 attributable to iCCA[43]. The authors further concluded that lower figures are an under-estimate, that the incidence increase is real, and that "the increased liver cancer mortality […] may be driven by CCA, not HCC"[43]. Similar results exist for UK (Figure 4)[61], Italy (iCCA mortality increase 0.2 to 5.9/mio 1980-2003) and Germany (mortality tripled 1998-2008)[2]. Thus, CCA is the most rapidly growing cancer in the western world.

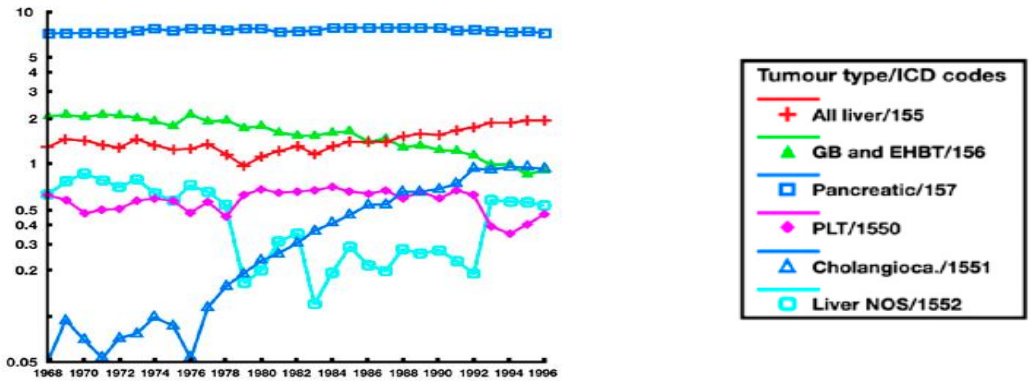


Figure 4 –Age Standardized Mortality Rates / 100000 in UK, log scale, GB = Gallbladder, EHBT = Extrahepatic Biliary Tract, PLT = Primary Liver Tumor, NOS = Not specified as primary or secondary, (Bridgewater, 2016)[61]

1.1.3 Risk factors

While absent in up to 90%[8], following risk factors have been identified accounting for less than 30% of cases: Primary Sclerosing Cholangitis (lifetime risk 5-35%), age >= 65 years (65% of cases), gallstones/hepatolithiasis, bile duct adenoma and biliary papillomatosis, choledochal cysts (lifetime incidence 6-30%), Thorotrast (no longer licensed), liver flukes, chronic typhoid carriage, cirrhosis, chronic HBV & HCV, obesity, diabetes, and alcohol. Suspected risk factors include genetic polymorphisms, smoking, and inflammatory bowel disease.[8]

1.1.4 Diagnosis

The clinical presentation is often nonspecific and insufficient to establish a diagnosis. Thus, diagnosis requires high suspicion and a multidisciplinary approach (clinical, pathology, laboratory, endoscopy and radiology). Imaging and biopsy are the cornerstones of diagnosis. Patients should have a contrast enhanced high resolution CT plus combined MRI/MRCP. ERCP and PTC enable biopsy taking, dilatation and stenting. US is too inaccurate for diagnosis or staging but aids these processes. Biopsy is often required for pathological differentiation and thus is recommended. Immunohistochemistry feature detection helps to further differentiate e.g. iCCA from mixed hepatocellular forms or other carcinomas. However, while positive biopsy establishes the diagnosis, a negative biopsy does not exclude the possibility. Otherwise no specific diagnostic blood tests exist. LFTs may indicate obstruction and nonspecific malignancy markers may be altered in aCCA. CA 19-9 is an established marker for diagnosis and management. High IgG4 indicate IgG4 cholangiopathy which can mimic CCA. CYFRA 21-1 & CA-242 are new serum markers not in routine use with higher specificities than CA 19-9 while other markers still need validation (IGF-1, IL-6, Mac-2BP, trypsinogen, MMP-7, or MUCIN-5AC). TNM staging is important to determine resectability. A separate staging system for all three subtypes only exists since 2009[10] (Appendix 1). Previously, iCCAs were staged with HCCs as “primary liver cancers” while dCCAs and pCCAs were also staged together.[2,3,9] Appendix 2 gives a short overview[1] of contemporary diagnosis and management.[1,2,3,4,5,8,9,10]

1.1.5 Oncological endpoint results interpretation and its limits in CCA research

Due to disease rarity, there is a lack in randomized controlled trials (RCT) and studies comparing treatments to BSC with most evidence coming from retrospective studies. Thus, results presented in this thesis are from retrospective studies unless mentioned otherwise. When interpreting and comparing such results, simple but important epidemiological principals apply, e.g. treatment outcomes of studies vary greatly and are not directly comparable because “differences in

outcome between historical controls and current treatment groups can arise from differences other than drug treatment, including patient selection, improved imaging techniques, or improved supportive care. [RCTs] minimize the effects of these differences by providing a direct outcome comparison"[90] and thus are considered superior evidence. Nevertheless, in the absence of RCTs the only sensible way to draw conclusions from non-randomized studies is to still compare results even if comparability is limited but do it with diligence and care. Accordingly, such interpretation of study results is a main part but also limitation of this thesis. Furthermore, however, the probably two most important endpoints used in the literature are medium overall survival (mOS) as the "gold-standard hard endpoint"[91] of oncology trials and medium progression free survival (mPFS). The FDA defines OS as the "time from randomization to death" and PFS as the "time from randomization until objective tumor progression or death"[90]. While these definitions seem straight forward, there are hidden issues attached, e.g. many authors of retrospective studies may use other time points such as date of first diagnosis, date of first treatment, etc. for OS and PFS calculations. Furthermore, how and at what intervals the "objective tumor response" is measured for PFS determination may also vary. Unfortunately, most authors do not describe their way of OS and PFS calculation while calculation differences may easily lead to differences of weeks to months in the resulting effect sizes between studies which in an environment that is looking for improvements of the same size can become misleading with figures having the same name seeming comparable but not being comparable.

1.2 Current State of the Art Therapy for Biliary Tract Carcinoma (BTC)

1.2.1 The natural course of disease under Best Supportive Care (BSC)

Studies containing a BSC arm are rare while only 2 studies[26,31] exclusively focus on the outcome of the natural course of disease under BSC. Park (2009, n=330)[26] examined the natural course of disease under BSC treatment and reported an overall mOS of 3.9 m (SD 7.8, range 0.2-67.1), i.e. normal distribution assumed, 84.2% die within 1 year with rare cases, however, even surviving up to 5.5 years under BSC only. Other mOS reported for BSC are: 2.5m[80], 3.12m[24], 4.5m[25,82], 4.7m[81], 4.9m[27], and 5.7m[28]. Thus, mOS under BSC seems poor around the 3-6m mark with values of 0.2-10m (CI range of above studies) being a possibility but comparisons between studies being difficult due to pre-treatment and other prognostic factor differences. How important prognostic factor influence is, has just been shown by three studies (PRODIGE-12[69], BILCAP[71], Spolverato[29]) which reported a mRFS of 22m (CI: 13.6-38.3)[69] and 18m (CI: 13-28)[71] after surgery (prognostic factor) and an additional mOS of 8.0m[29] after recurrence adding up to a total mOS of 26-30m after surgery under BSC compared to 3-6m for unresected patients.

Furthermore, there is a selection bias in retrospective studies for non-resectable patients systematically assigning "older, sicker" patients to BSC with a higher risk of premature death and underestimated survival compared to patients deemed "fit for other therapy". For example Yonemoto (2007, n=304)[24] reported 87% of BSC patients being >= 60 years and having significantly less ECOG 0 (20% vs 41-48%) and more ECOG 2 (22% vs 3-13%), 3 & 4 (12% vs 0-2%) patients compared to the chemotherapy group of whom only 28-56% were >= 60 years. For this reason Ji (2015, n=204)[31] investigated the natural course of disease under BSC only for patients with metastatic disease and a good ECOG 0-2 as typically deemed "fit for chemotherapy". Median OS was 7.1m (CI n.p., range: 0.2-46.9 m). Women (OS: 5.60, CI: 3.86-7.34) had a shorter OS than men (OS: 8.3, CI: 6.75-9.85). Furthermore, mOS for the subtypes was significantly different (p=0.015): iCCA: 4.7m, eCCA: 9.7m, GBC: 4.4m, and Ampulla of Vateri (AoV): 11.2m[31]. Results are especially interesting compared to Park (2009)[26] as the only other study investigating the natural course of disease without pre-treatment which, however, included generally "sicker" patients: mOS: 7.1[31] vs 3.9m[26], iCCA: 4.7[31] vs 3.0[26], eCCA: 9.7[31] vs 5.9[26]. Furthermore, local advanced disease had a significantly longer mOS of 13.8m compared to 6.2m for metastatic disease (p=0.001) while also normal baseline CEA and CA 19-9 levels were associated with a significantly longer mOS of 10.6m compared to 5.8/6.0m for increased baseline CEA and CA19-9 levels (p=0.006 and p=0.001) respectively.[31]

In summary survival under BSC seems strongly prognostic factor dependent with an established figure for "patients fit for chemotherapy" being around 7-10m but otherwise mOS ranging from as little as 2.5m (unresected patients) to 30m (resected patients) and some rare cases even surviving up to 37.7[26], 46.9[31] or 67.1[26]m (range extremes above), i.e. 3-5.5 years and thus 3-6 times as long as the established mOS of 11.7m[13] under GEM/CIS standard chemotherapy!

1.2.2 Surgical Treatment Path

Surgery is the only potentially curative therapy and the question of resectability is vital. Furthermore, surgery should only be performed in specialized centers while the decisive long-term survival criterion is R0 resection without lymph node metastases[47]. Even then, however, outcome is limited.[2,3,8,47,53] Important key messages selected from the literature are:

a) The majority of newly diagnosed CCA cases remain unresectable:

According to Ruys (2013)[60], from initially 289 pCCA patients 158 (55%) were unresectable (laparoscopy or imaging results) while 131 (45%) underwent exploration. Of these 83 (64% or

29% of initial cohort) underwent resection of whom 67 (87% or 23% of initial cohort) had R0. Thus 71% of the initial cohort was unresectable while 23% achieved R0 and 6% R1[60]. More generally, 30-40% are typically reported resectable with many procedures being so complex and extensive that only 20-40% progress to surgery while up to 80%[53] remain unresectable.[2,3,8,47,53,54]

b) Long-term survival for resected patients is significantly longer than for unresected patients: Ruys (2013)[60] reported resected patients having a significantly longer mOS than unresected patients (37m vs. 14m, p<0.001) and Waseem (2017, n=242)[45] showed resected patients having better OS than unresected patients independently of additional treatments (Figure 5).

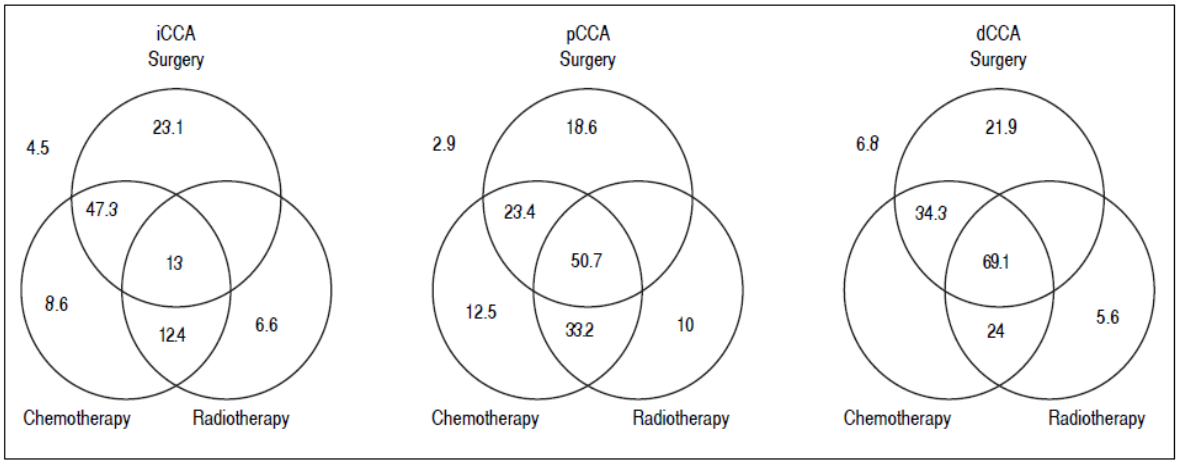


Figure 5 – Median survival in months of CCA patients receiving single modality or multimodality treatment according to Waseem (2017)[45] (figures for patients who received no treatments outside diagram).

Hu (2016, n=814 pCCA)[76] showed superior mOS in the curative intent (26.3m) over the palliative (7.3m; p<=0.001) and no surgery groups (2.6m, p<=0.001) with also a significant advantage of the palliative (7.4m) over the no surgery group (5.5 m, p<=0.001, Figure 6).[76]

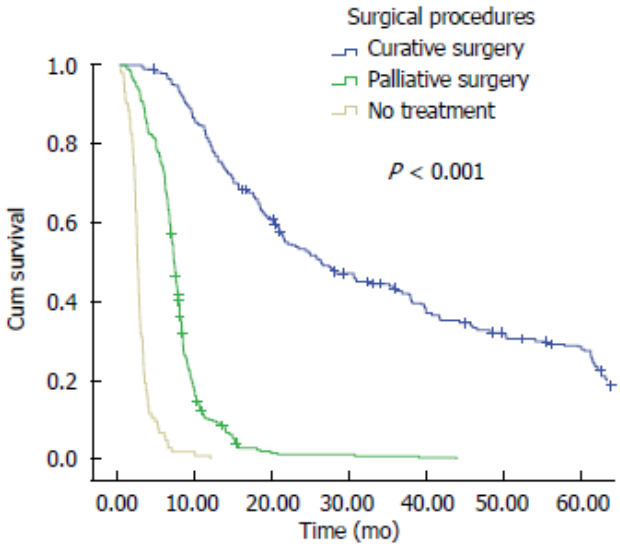


Figure 6 – Long-term survival of 814 pCCA patients according to Hu et al. (2016)[76]

c) Resected patients have a better subsequent therapy outcome than unresected patients

In BT22 (2010, n=83)[14] resected patients had better outcome than unresected patients (GEM/CIS: 16.1m vs. 9.4m, GEM: 12.7 vs. 7.4m)[14]. BILCAP (2017, n=447)[71] reported mOS of 51m for Capecitabine and 36m for BSC for resected patients compared to 11.7m for GEM/CIS[13] or 7-10m[31] for BSC for mainly unresected patients. PRODIGE-12 (2017)[69] also reported mRFS of 30.4m[69] for GEMOX and 22.0m[69] for BSC and Spolverato (2016)[11] mOS after recurrence of 16.8m[11] for chemotherapy and 8.0m[11] for BSC adding up to overall 47.2m for chemotherapy and 30m for BSC for resected patients compared to 11.7m for GEM/CIS[13] or 7-10m[31] for BSC. Thus resected patients have a better outcome under the same therapy than unresected patients and studies should not mix resected and unresected patients to produce meaningful results.

d) There is no difference in long-term survival after surgery between the different anatomical subtypes after adjustment for baseline characteristics:

After adjustment for baseline characteristics, Ercolani (2015, n=479)[54] found no significant differences in long-term survival between subtypes (p=0.127, Table 1, Figure 7).

Table 1 – Survival rates for all anatomical subtypes according to Ercolani (2015)[54]

	All Types	iCCA	pCCA	dCCA
1 Year	78%	85%	70%	83%
3 Year	49%	52%	45%	47%
5 Year	31%	34%	28%	23%

Also Waseem (2017, n=242)[45] found no significant difference in long-term survival between subtypes (Figure 7) even though mOS was higher for dCAA (22m) than for iCAA (13.5m) and pCAA (13.9m).

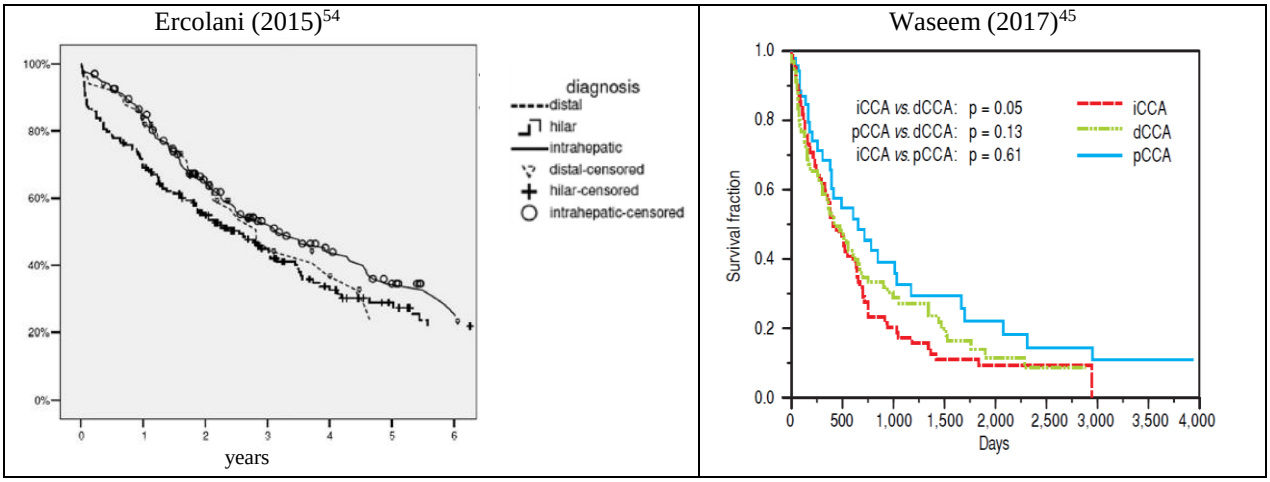


Figure 7 – Survival curves for the different anatomical subtypes after adjustment for different characteristics

Overall the two survival curves look similar with the latter, however, being more curved to the left, which may represent the impact of the additional non-surgical patient group in this study.

These results are important in the current debate as to whether or not anatomical subtypes constitute different tumors. Ercolani[54] concludes that CCA "seem to be the same tumor but depending on site of origin they may show different tendencies to invade bordering structures, which has an impact on the role of surgery and long-term outcome. New biological markers may better clarify the biology and behavior […] based on location"[54]. Therefore, while the subtypes do not act as direct, independent prognostic factors, they may still act as indirect prognostic factors since their typical distribution pattern in terms of other direct prognostic factors may differ in a typical way. Please also see section 1.2.6.3 for molecular genetics tumor profiling.

e) Surgical and other treatment outcomes improve over time:

Yoh (2016)[59] divided 144 iCCA into two periods: Period 1 (1993-2006) and Period 2 (2006-2014). New therapies arrived in 2006. Median OS improved from 21.4 to 57.7m ($p<0.001$), mDFS from 12.2 to 16.6m ($p=0.027$), survival after recurrence from 8.0m to 22.3m ($p<0.001$), and survival with N1 status from 12.4m to 26.0m ($p=0.018$)[59]. Also, the advent of GBC staging was associated with improved survival (Hundal, 2014)[62].

f) Even with curative intent surgery the actual cure rate remains very low:

Using a "cure fraction model", Spolverato (2017, n=576)[55] showed a surgical cure probability of only 14.5% (CI: 8.7 – 23.2) with a time to cure of 9.5 years and a mOS of uncured patients of 21.6m[55]. It is important to emphasize that 14.5% is the cure rate of surgical patients and not CCA in general. The latter, however, can be estimated since 20-40%[1.2.2.a] are initially suitable for surgery with 70%[54,58] still suitable after exploration and 14.5% cured after surgery to be (20/100*70)/100*14.5= 2% to (40/100*70)/100*14.5= 4% while 96-98% die within 10 years. So even with surgery the first 50% die within 2 years (21.6m[55]) while 70% of the other half die within the next 8 years and patients surviving the first 2 years still only having a 30% chance of cure. Only patients without lymph node metastases and a CA 19-9 < 50 U/ml (independent risk factors) have a better cure fraction of 39% after 4.1 years (highest cure fraction and shortest cure time). Furthermore, adjuvant chemotherapy was not a determinant of cure ($p=0.59$).[55]

g) Survival after surgery is strongly prognostic factor driven:

Table 2 & 3 show prognostic factor dependent survival from Hu (2016, n=814)[76] and Wellner (2017, n=2063)[57] with factors changing mOS (26.3m) between -52.9% and +105.7%. Survival thus significantly dependents on prognostic factors even under the same treatment. In addition, Waseem (2017, n=242)[45] reported OS for TNM Stage I, II, III, IV of 23, 25, 14, 4.5m, respectively. Patients with different baseline characteristics thus clearly have different outcomes.

Furthermore, factor dependent 5-year survival ranges from 15.1-65.7%[57] and OS from 12.4-54.1m[76], both mirroring respective survival ranges from the literature[1.2.2.i]. Study result differences thus may reflect underlying prognostic factor rather than outcome differences.[57]

Table 2 – Prognostic factors for OS after surgery based on Hu et al. (2016)[76] (BDR=Hilar bile duct resection)

Prognostic Factor	OS in months (OS) and Percent Decrease (PD) or Percent Increase (PI) from overall mOS of 26.3m				p-value
	OS	PD	OS	PI	
Tumor Size	> 3 cm 14.7m	-44.1%	< 3 cm 35.2m	+33.8%	< 0.001
Surgical Procedure	BDR 20.8m	-20.9%	BDR + hepatectomy 27.6m	+4.9%	0.072
Lymphnodes	N1/2 15.7m	-40.3%	N0 39.9m	+51.7%	< 0.001
Tumor differentiation	Poor 13.5m	-48.7%	Well 54.1m	+105.7%	< 0.001
Resction margin	R1/R2 12.4m	-52.9%	R0 35.2m	+33.8%	< 0.001
Vascular Invasion	Yes 20.9m	-20.5%	No 26.3m	+0.0%	0.009
Caudate lobe resection	No 21.4m	-18.6%	Yes 35.7m	+35.7%	0.040
CA 19-9 > 100 U/L	Yes 23.0m	-12.6%	No 39.7m	+60.0%	0.039
Perneural Infiltration	Yes 20.8m	-20.9%	No 27.3m	+3.8%	0.084
T Stage	T3/4 25.7m	-2.3%	T1/2 27.6m	+4.9%	NS

Table 3 – Influence of prognostic factors on 5 year survival after surgery according to Wellner et al. (2017)[57]

Factor	5 Year Survival		Relative Risk	95% CI	p-value
	Factor present	Factor absent	(RR)		
Factors without influence on survival					
Gender	Male 38.8%	Female 35.0%	0.95	0.68-1.32	0.76
Age	<65 y.o. 35.6%	>65 y.o. 34.4%	1.31	0.82-2.12	0.26
Adjuvant Chemotherapy	34.0%	34.5%	0.71	0.21-2.36	0.57
Factors with influence on survival					
Perineural Invasion	31.3%	65.7%	0.51	0.40-0.64	< 0.00001
Lymphnode metastases	23.7%	47.2%	0.51	0.38-0.70	<0.0001
Negative Resection margins	40.8%	15.1%	2.11	1.36-3.3	<0.001
Tumor differentiation	Well- diff. 54.6%	Not well-diff. 30.8%	1.77	1.39-2.25	<0.00001

h) Survival outcomes after surgery differ significantly in the literature:

There is a confusingly wide range of survival estimates after surgery in the literature (Table 4). Overall, 5-year survival ranges from 13-63% and mOS from 9-69m (<1-5.5 years). Improved survival over time and differences in prognostic factor distribution of study populations may explain this variability. Survival after surgery therefore seems especially prognostic factor dependent with the estimates for 5-year survival being around 30% combined with a survival/cure rate of 14.5%[55] after 10 years[55] and mOS of 13-30m with a closer range of 23-28m (around 2 years) seeming most reasonable for a typical patient and additional therapy mix after resection. However, contemporary mOS may even be as high as 43.5 m (BILCAP[71]).

Table 4 – Overview of studies providing survival estimates for CCA patients after surgery

Study	Study Details	mOS in months	1 year OS rate	3 year OS rate	5 year OS rate
Yamamoto (2011)[48]	Review, 23 studies	12.4-52.9m (range)			17.4-42.9%
Mavros (2014)[50]	Review, 57 studies	28m (range 9-53)			30%
Lubezky (2015)[49]	Review			40-50%	14-63%
Bhardwaj (2015)[56]	Review, 26 studies, pCCA			36-65%	13-40%
Ercolani (2015)[54]	479 mixed subtypes	23m	78%	49%	31%
Yoh (2016)[59]	144 iCCA, mOS improvement over time!	Before 2006: 21.4m After 2006: 57.7m (p<0.001)			
Hu (2016)[76]	814 pCCA	26.3	80%	43%	28%
Wellner (2017)[57]	Review & Meta-Analysis, 23 studies, 2063 dCCA	Stage I: 23m Stage II: 25m All: 13.0-69.1			Estimated around 34-39% from Table X (h) above
Spolverato (2017)[55]	576 pCCA & GBC	22.8m			23.9%
BILCAP (2017)[71]	RCT, 447 mixed subtypes	43.5m (calculated as 51m [Cape] + 36m [BSC]/2=43.5 since n.p.)			

1.2.2.1 Adjuvant Chemo- and Radiotherapy

The rational for adjuvant therapy (AT) are high recurrence and poor survival after surgery[63]. However, there are also safety concerns with patients after hepatectomy not tolerating standard doses of chemotherapy and thus a need for dose reduction limiting efficacy[67]. According to Doherty (2016, review)[64] adjuvant therapy remains controversial and based around retrospective studies, the most influential being a meta-analysis by Horgan (2012, 20 studies)[65] showing only a trend in survival (OR=0.74, p=0.06) with greater benefit for chemotherapy alone (OR=0.39, p<0.001) and radio-chemotherapy (OR=0.61, p=0.049) over radiotherapy alone (OR=0.98, p=0.90). Patients with N1 (OR=0.49, p=0.004) or R1 (OR=0.36, p=0.002) status benefited from AT compared to R0 status (OR=1.26, p=0.20). Thus AT is often recommended for less favorable prognostic factor profile.[64,65] In contrast, Mavros (2014, 57 studies)[50] did not find AT beneficial.

Recent studies continue this controversial debate with some retrospective studies finding some benefit of AT over BSC [(Kim, 2016)[66], (Mizuno, 2017)[67],(Schweitzer, 2017)[68]] while others do not [Wellner (2017)[57], Spolverato (2017)[55]]. Even new insights from eagerly awaited RCTs continue the controversy: PRODIGE-12 (2017, RCT, n=196)[69] reported a not significant difference in mRFS for GEMOX (30.4m, CI: 15.4-45.8) compared to BSC (22.0m, CI: 13.6-38.3) while also the ITT results of BILCAP (2017, RCT, n=447)[71] showed only a non-significant survival trend in mOS for Capecitabine (51m, CI: 35-59) over BSC (36m, CI: 30-45). Further sensitivity analysis adjusting for gender, disease grade, and nodal status, however, then lead to a significant advantage (HR=0.71, CI 0.55-0.92) as did the BILCAP PP analysis which showed significant mOS of 53m for Capecitabine over 36m for BSC (HR=0.75, CI: 0.58-0.97)[71]. This, however, still was not associated with a significant increase in mRFS ($mRFS_{Cape}$: 25m, CI: 19-

37; $mRFS_{BSC}$: 18m, CI: 13-28)[71]. While the BILCAP authors concluded that "Capecitabine improves OS in BTC when used as adjuvant and should become standard of care"[71], the overall result pattern rather seems to be a not quite statistically significant trend in favour for adjuvant chemotherapy making it still highly likely that only certain patient subgroups benefit.

1.2.2.2 Recurrence-Free Survival (RFS) after Resection

Recurrence estimates after resection from different studies are summarized in Table 5.

Table 5 – Overview of studies providing RFS time estimates for CCA patients after surgery

Study	Study Details	mRFS	Recurrence after number of years in %							
			1	2	3	4	5	8	9.5	10
Spolverato (2017)[55]	N=576 pCCA&GBC								85% (77-91)	
Hu (2016)[76]	N=814 pCCA	18.1m	22%		82%		90%			
Koerkamp (2015)[72]	N=306 pCCA	26.0 m (CI: 21-31)		42%			67%	76%		86%
Spolverato (2016)[11]	N=563 iCCA						71%			
Komaya (2017)[75]	N=389 dCCA						54.3			
PRODIGE-12 (2017)[69]	RCT, N=196 mixed	Chemo: 30.4m (CI: 15.4-45.8) BSC: 22.0m (CI: 13.6-38.3)				50-76.9%				
BILCAP (2017)[71]	RCT, N=447 mixed	Chemo: 25m (CI: 19-37) BSC: 18m (CI: 13-28)								
Doussot (2015)[51]	N=188 iCCA	21m (CI: 11.8-30.1)								
REames (2017)[74]	N=1087 iCCA	14-15m								
Lubezky (2015)[49]	Review, iCCA	14-26m								
Miyazaki (2017)[77]		iCCA: 27.6m pCCA: 12.0m dCCA: 15.6m GBC: 22.8m								
Luvira (2016)[73]	N=50 iCCA Thailand	6.3m (CI: 5-10)	84%	95%	97%					

Overall, a probably further by prognostic factors and adjuvant therapy dependent mRFS of 12-30m seems realistic. According to Spolverato (2016)[11] most recurrences happen within 5 years with the first 2 years bearing highest risk (Figure 8) while mRFS is independent of location. Furthermore, Yoh (2016, n=144)[59] showed an impressive improvement in mRFS from 12.2 to 56.6m (p=0.027) from before to after 2006 while Komaya (2017, n=389)[75] showed 5-year RFS depending on prognostic factor number (70.6%, 50.3%, 31.8% and 13.4% for 0, 1, 2, 3 factors present). Factor combinations thus have additive effect. Several prognostic factor models exist[51].

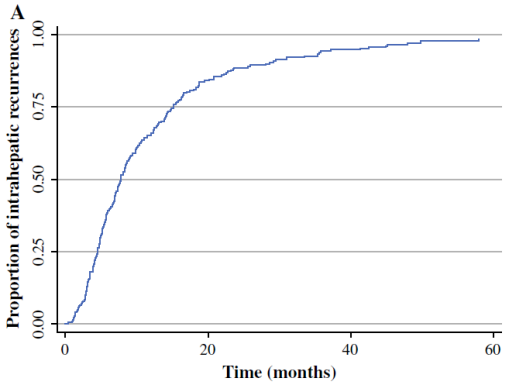


Figure 8 – Five year overall risk of intrahepatic recurrences from Spolverato (2016)[11]

1.2.2.3 Treatment of patients with recurrence and outcome

According to Spolverato (2016, n=563)[11], 47.5% of recurrent cases received therapy while 52.5% received BSC. Of those receiving therapy, 75.8% received repeat liver directed therapy (IAT, repeat resection, ablation only) +/- chemotherapy while only 24.2% received chemotherapy only. This is surprising since chemotherapy is standard while the efficacy of surgery is unknown (Miyazaki, 2017)[77]. Median OS from recurrence was 11.1m (BSC: 8.0m, chemo: 16.8m, liver directed therapy: 18m, p<0.001, Figure 9)[11]. Miyazaki (2017, n=107)[77] also reported better survival after surgery than chemotherapy or BSC with 3- and 5-year survival of 38%, 5.3%, 0% and 19%, 5.3%, 0% (p<0.0001). Miyazaki (2017, 10 studies)[77] also reported mmOS of 26.1m (range 10.0–66.6m) and median 3- and 5-year survival of 51.4% (range 29.0-100.0) and 29% (range 0-51.4%) for repeat surgery. Yoh (2016, n=144)[59] reports a median time to 2nd recurrence for multimodal treatment including surgery of overall 9.6m (range 0.4-173.3) with significantly better survival after 2006 (22.3m) than before (8.1m, p=0.0036). Thus, repeat liver therapy including surgery is a better choice for recurrent BTC after surgery than chemotherapy alone.

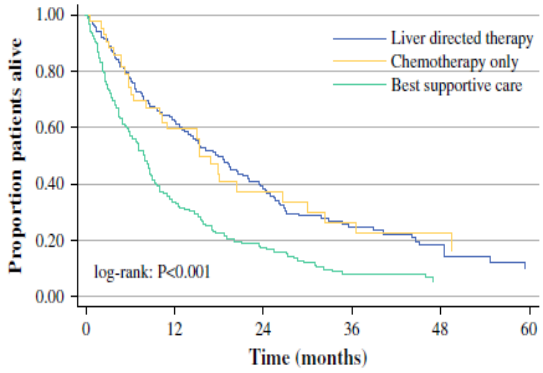


Figure 9 – Five year OS by type of treatment of recurrence - Spolverato (2016) [11]

1.2.3 Systemic Chemotherapies & Biological Therapies

1.2.3.1 First Line Chemotherapy

1.2.3.1.1 Gemcitabine plus Cisplatin as the current 1ST-line therapy standard

Selected key messages from the complex literature regarding 1st line therapy are:

a) Gemcitabine (GEM) plus Cisplatin (CIS) is the currently accepted 1st-line therapy standard:

Historically, GEM became pancreatic cancer standard in the 1990's which sparked interest for use in other hepatobiliary cancers and synergistic effects with CIS lead to GEM/CIS use in lung, pancreatic and bladder cancers[12]. In absence of a 1st line standard due to lack in RCTs, Eckel (2007)[17] analyzed 104 phase II studies and reported a pooled RR to chemotherapy of 22.6% (CI: 21.0-24.2), pooled TCR of 57.3% (CI: 55.3-59.3%), mTTP of 4.1 and mOS of 8.2m with OS

being significantly longer in CCA vs GBC (9.3 vs. 7.2m, p=0.048). Furthermore, cytotoxic drug doublets were superior to monotherapies or poly-chemotherapies with GEM/Platinum overall showing best results so that the authors suggested GEM/CIS or GEMOX as provisional 1st-line standard[17]. However, Eckel's groundbreaking work[17] did not include BSC as a reference point. Subsequently the ABC-01 (2009, n=86)[12] trial evaluated GEM vs GEM/CIS with such success that the study was extended into a phase III trial (ABC-02, n=410)[13] whose results - mOS 11.7m (GEM/CIS, CI: 9.5-14.3) vs. 8.1m (GEM, CI: 7.1-8.7), mPFS 8.0m (GEM/CIS, CI: 6.6-8.6) vs. 5.0m (GEM, CI: 4.0-5.9), DCR 81.4% (GEM/CIS) vs. 71.8% (GEM) – lead to recognition of GEM/CIS as 1st-line standard for locally advanced unresectable and metastatic BTC[32] in 2010[12,13,32]. ABC-2 results were replicated by BT22 (2010, n=83)[14]: mOS 11.2m (GEM/CIS, CI: 9.1-12.5) vs. 7.7m (GEM, CI: 6.1-11.0), mPFS 5.8m (GEM/CIS, CI: 4.1-8.2) vs. 3.7m (GEM, CI: 2.1-5.3), and DCR 68.3% (GEM/CIS, CI: 51.9-81.9%) vs. 50.0% (GEM, CI: 34.2-65.8%) confirming the GEM/CIS standard also for an Asian population. Valle's meta-analysis of both studies (2014, n=493)[15] confirmed significant improvement of GEM/CIS over GEM and concluded that GEM/CIS reduced death/progression risk by about 35% compared to GEM. Further studies supporting these positive ABC-2 results were: ABC-03 (2014, n=62, mOS 11.9m, mPFS 7.4m)[33], Kameda (2013, n=20, OS 13.7m, PFS 6.5m)[35], Yamashita (2015, n=37, OS 14.9m, PFS 7.7m)[36]. However, the latter two had small sample sizes with the 1st only trialing GEM/CIS after GEM failure as 2nd line while not enough English information is available on the 2nd one to evaluate results reliability so that both are just mentioned for completeness. Furthermore, none of all these studies included BSC as a reference point.

Furthermore, Sharma (2010, RCT, n= 81 GBC)[16] published results in parallel to ABC-02 showing superiority of GEMOX (mOS 9,5m, mPFS 8.5m) over 5FU/FA (mOS 4.6m, mPFS 3.5m) or BSC (mOS 4,5m, mPFS 2.8m). These results lead to an acceptance of GEMOX as a standard of care aside GEM/CIS as 1st line therapy. However, GEM/CIS and GEMOX were never conclusively compared until Fiteni (2014, review, 33 studies)[18] arrived at a weighted mmOS of 9.7m for GEM/CIS and 9.5m for GEMOX with GEM/CIS being more toxic. Sensitivity analysis of only the 6 studies using ABC-02 CIS dose revealed an increased mmOS to 11.7m with, however, increased toxicity[18]. Therefore, GEM/CIS with standard CIS dose may exhibit an OS advantage of 2m but at the cost of higher toxicity[18]. In summary GEM/CIS is the currently accepted 1st-line chemotherapy standard for BTC with GEMOX being an alternative.

b) GEM/CIS is safe and effective in patients with biliary tract obstruction and high bilirubin due to luminal disease despite optimal stenting:

Jaundice as expression of biliary tract obstruction defined as bilirubin 1.5 x ULN occurs in 70-84% of patients due to liver metastases or luminal disease. Untreated it leads to complications such as bacterial cholangitis, liver failure, and/or pain and thus impacts not only on QoL but also on toxicity and tolerance of chemotherapy influencing survival directly. Thus, stenting is pivotal. However, even with stenting obstruction can persist and if it does, clinicians often decide against chemotherapy for fear of toxicity and uncertain outcome. Unfortunately, patients with bilirubin >=1.5 x ULN were excluded from the ABC trials so that GEM/CIS standard is not transferable. However, Lamarca (2015, n=33)[23] showed that also these patients benefit considerably from chemotherapy. Overall, bilirubin normalized in 64% during/after chemotherapy and toxicity and outcome (mPFS 6.9m [CI:4.4–9.0], mOS 9.5m [CI:5.7–12.8]) were comparable to ABC-2. A significantly higher DCR was observed in patients whose bilirubin normalized during/after chemotherapy compared to patients whose did not (86% vs. 30%, p=0.004). While baseline bilirubin had no impact on PFS or OS, bilirubin normalization during/after chemotherapy had longer mOS compared to no normalization (11.4m vs. 2.9m, HR=0.49, CI:0.2-1.1, p=0.08). Finally, patients with obstruction related to luminal disease (76%) had better outcome than patients with liver metastases (24%) - mPFS 7.0 vs. 2.6m (p=0.1633), mOS 9.8 vs. 4.4m (HR=0.74, p=0.465). Thus the authors concluded that "for PS 0-1 patients with advanced [BTC] and high bilirubin due to luminal disease despite optimal stenting [GEM/CIS] can be used safely with results similar to those in patients with normal bilirubin".[23]

c) The survival figures established by ABC-02 & BT-22 may represent overestimates:

While superiority of GEM/CIS over GEM is well established (1.2.5.1.1 a), superiority of GEM/CIS over all other therapies should not be viewed as irrevocable with recent work already challenging the status quo mainly by indicating that ABC-02 and BT-22 results may represent overestimates which in turn may challenge GEM/CIS superiority in comparison to other therapies. Table 6 summarizes outcomes of studies supporting ABC-02 findings (1.2.5.1.1.a) vs studies suggesting lower effect sizes. All four reviews (Fiteni (2014)[18], Park (2015)[21], Ulahannan (2015)[22,] Eckel (2014)[92]) supporting similar but lower effect sizes of 9-10m for mOS under GEM/CIS base their results on large sample sizes (771-6337 pooled cases including ABC-02 and/or BT-22) while the remaining 3 retrospective studies also arrive at a similar mOS around 8-10m. Further striking is the difference in mOS CI limits with ABC-02 supporting studies only starting at a lower limit of 9m while studies supporting lower effect sizes have an upper limit at about 11m but start at a much lower limit at around 6m thereby opening up the possibility that

Table 6 – Effect size of the Gemcitabine/Cisplatin combination according to different studies

	Studies supporting ABC-02 effect sizes						Studies supporting lower effect sizes						
	Valle et al. (ABC-02)[13]	Okusaka et al. (BT-22)[14]	Valle et al. (Meta-analysis of ABC-02 & BT-22)[15]	Valle et al. (ABC-03)[33]	Yamashita et al.[36] (abstract only)	Kameda et al.[35]	Woo et al.[37]	Agarwal et al.[32]	Cho et al.[34]	Park et al.[21]	Fiteni et al.[18]	Ulahannan et al.[22]	Eckel et al.[92]
Year	2010	2010	2014	2014	2015	2013	2013	2016	2017	2015	2014	2015	2014
Design	Phase 3 RCT	Phase 2 RCT	Meta-analysis	Phase 2 RCT	Pro-Obs	Retro	Retro-Co	Retro	Retro	Systematic Review	Systematic Review	Systematic Review	Pooled Analysis
Studies	1	1	2	1	1	1	1	1	1	20 (including ABC-01 & 02, BT-22)	18 (including ABC-02, BT-22)	83 (including ABC-02)	161 (all chemotherapy trials 2000-2014)
Patients (Surgically pre-treated)	204 (23.3%)	41 (26.8%)	245 (25.1%)	62 (n.p.)	37 (n.p.)	20 (n.p.)	127 (30.7%)	26 (n.p.)	740 (38%)	912 (n.p.)	771 (n.p.)	1403 (n.p.)	6337 (n.p.)
Treatment	GEM/CIS	GEM/CIS	GEM/CIS	GEM/CIS	GEM/CIS	GEM/CIS after GEM mono-therapy failure	GEM/CIS	GEM/PLATINUM	GEM/CIS	GEM/CIS	GEM/CIS	GEM/PLATINUM [CIS (47%), OX (49%), or CARBO 5%]	GEM/PLATINUM
Median OS	11.7 (CI: 9.5-14.3)	11.2 (CI: 9.1-12.5)	11.6 (CI n.p.)	11.9 (CI: 9.2 – 13.4)	14.9 (CI n.p.)	13.7 (CI: 8.3-19.7)	8.4 (CI: 6.2-10.7)	10.5 (CI: 7.9-18.8)	10.4 (CI: 9.6-11.2)	Range: 4.6-11.7 mmOS*: 9.3 (6.4-12.1)	mmOS: 9.85 (CI: 8.6-11.0, Range: 5.0-15.2) Weighted mmOS: 9.7 (9.0-10.5)	9.5 (Range: 5.0-19.9, Lo-Up-Q: 8.7-10.8)	9.5
Median PFS	8.0 (CI: 6.6-8.6)	5.8 (CI: 4.1-8.2)	8.8 (CI: n.p.)	7.4 (CI: 5.7 – 8.6)	7.7 (CI n.p.)	6.5 (CI: 2.1-6.9)	TTP: 5.6 (CI n.p.)	4.5 (CI: 3.1-8.9)	5.2 (CI: 4.7-5.6)	mmPFS*: 5.8 (4.1-8.2)	mmPFS: 6.3 (CI: 5.8-8.0, Range: 4.0-8.5) Weighted mmPFS: 8.0 (85% CI:8.0-8.0)	4.8 (Range: 3.0-11.0; Lo-Up-Q: 4.0-7.8)	mTTP: 5.3
DCR	81.4% (CI n.p.)	68.3% (CI: 51.9-81.9)	n.p.	n.p.	n.p.	60% (CI n.p.)	67% (CI n.p.)	n.p.	n.p.	45.7-81.4 (CI n.p.)	n.p.	n.p.	mTCR: 63.5%
ORR	n.p.	19.5% (CI: 8.8-34.9)	n.p.	19%	n.p.	15% (CI n.p.)	18.1% (CI n.p.)	n.p.	13% (CI n.p.)	17.1-36.6 (CI n.p.)	n.p.	29.0 (Range: 14.9-50.0; Lo-Up-Q: 21.0-32.0)	n.p.
Assessment	At 12 & 24 weeks	Every 6 weeks	n.p.	12 weekly	n.p.	Week 4-6, 5-13 irregularly	Every 3 cycles	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.

DCR = Disease Control Rate = Complete Response (CR) + Partial Response (PR) + Stable Disease (SD); ORR = Objective Response Rate = Complete Response + Partial Response; n.p. = not provided; Lo-Up-Q = Lower to upper quartile interval; PFS = Progression-Free Survival; OS = Overall Survival; TTP = Time to Progression; GEM + Gemcitabine; CIS = Cisplatin; Ox = Oxaliplatin; Carbo = Carboplatin; RCT = Randomized Clinical Trial; Pro-Obs = Prospective Observational Study; Retro + Retrospective Study; Retro-Co = Retrospective Cohort Study; * This figure was not provided in the original report but was determined by the author of this report from the provided figures in the original report

the true mOS may be as low as half the size suggested by ABC-02. In regards to mPFS even most of the ABC-02 supporting studies have a lower mPFS than ABC-02 while all lower effect sizes supporting studies have a mPFS of around 5-6 m. Finally, also Eckel's new meta-analysis (2014, 161 studies, n=6337)[92] arrived at a lower mTTP of 5.3m and mOS of 9.5m and in addition concluded that "the remarkably high TCR and long OS of the ABC-02 trial could not be reproduced in other trials". In conclusion it is therefore highly likely that the ABC-02 results are overestimates with the true mOS being around 8-10m and mPFS around 5-6m. Consequently, superiority of the GEM/CIS standard compared to other therapies has to be newly evaluated.

d) Other compounds have already been identified that may be more effective than GEM/CIS

According to Ulahannan (2015, 83 studies)[22] GEM/5-FU showed a strong superior trend in OS over GEM/Platinum (mOS 12.5m vs. 9.5m, p=0.047)[22] which was also confirmed by Eckel (2014)[92] who found mOS of 12.5m for the GEM/Platinum/5-FU triplet and mOS of 12.7m for a GEM + targeted therapy combination. Eckel (2014)[92] thus also suggested GEM/Platinum/FU and/or GEM/EGFR targeted therapy as the new standard for patients with a good PS[92].

e) General superiority of GEM/CIS over BSC for all patients is doubtful:

While outcome of GEM/CIS in ABC-02 seems overestimated with real mOS being around 8-10[18,21,22,32,34,37] and mPFS around 5-6m[18,21,22,32,34,37], the outcome for BSC has been underestimated (1.2.1) with new mOS figures from Ji (2015)[31] being as high as 7-10m[31] for patients typically deemed fit for chemotherapy, 30m[(69,71)+29] for resected patients and 8m[29] for post-surgical recurrence. Interestingly, Valle as the main author of ABC-02 in another review in 2010 stated "no adequately powered study has shown conclusively a benefit for chemotherapy compared with BSC alone although three small randomized studies[80,81,82] have suggested an improved survival"[79]. Therefore, hardly any evidence of GEM/CIS superiority over BSC existed right from the start and in the continued absence of RCTs directly comparing GEM/CIS to BSC in an unresected population, the widely assumed superiority of GEM/CIS over BSC for <u>all</u> aCCA seems unsubstantiated with new evidence rather indicating that the so far assumed effect gap is closing thereby creating a possibility that at least certain subgroups may not benefit at all from GEM/CIS compared to BSC.

Additional differences in the study design and populations of ABC-02 and Ji (2015)[31] support this idea of mOS convergence even further. For example, ABC-02 included surgically treated patients while Ji (2015)[31] did not. However, chemotherapy results in resected patients are not comparable/transferable to unresected patients since they investigate mixed effects of surgery +

chemotherapy instead of chemotherapy alone in highly selected patients with favorable prognostic factor profile and generally better outcome (1.2.2.b & c). Furthermore, ABC-02 & BT-22 explicitly excluded patients who in the investigator's opinion had a life expectancy of 3m or less (i.e. the sickest portion of the population) while Ji (2015)[31] did not. Consequently Ji (2015)[31] had also substantially less ECOG 0 (3% vs 32-83%) and more ECOG 2 patients (40% vs. 0-12%) than the other two studies. It thus can reasonably be assumed that real mOS for (Ji, 2015)[31] would be substantially higher than the reported 7-10m if conducted in a population similar to ABC-02 or BT-22 thus challenging the idea of a general GEM/CIS superiority over BSC even further.

In conclusion it seems that the actual general benefit of CIS/GEM over BSC is by far not as large as assumed and at least for certain subgroups may even converge towards zero. While this seems devastating news because it challenges the current status quo of GEM/CIS standard for all aCCA patients regardless of further discrimination, the real situation emerging from the literature is even more complex as will be explained in the next section.

f) Best therapy decisions strongly depend on the individual prognostic factor profile:

Ji (2015)[31] also reported different mOS for subtypes and prognostic factors under BSC which differed significantly from the overall mOS of 7.1m. For example, mOS for GBC under BSC was 4.4m (CI:2.9-5.9)[31]. Similarly, in BT-22[14] mOS for GBC under GEM/CIS of 9.1m (CI:6.9-11.6) was lower than the overall median of 11.2m (CI:9.5-14.3) and even substantially lower than the 13.0m (CI:9.2-n.p.) for non-GBCs. These figures were almost exactly confirmed by other studies[16,82,87]. So while it is difficult to establish an advantage of GEM/CIS over BSC for <u>all</u> subtypes (1.2.6.1.1.e), for GBC GEM/CIS is clearly superior to BSC and should remain 1st-line standard. However, GBC is distinct from other subtypes (1.1.1) so that therapy response may be different. Furthermore, in absence of subtype specific survival data for iCCA under GEM/CIS and due to a similarly low mOS of 4.7m (CI: 3.54-5.87)[31] it seems also reasonable to assume GEM/CIS to remain 1st choice for iCCA.

For the other subtypes, however, the situation is different. With mOS of 9.7 (CI: 6.49-12.41)[31] for eCCA and 11.2m (CI:5.1-17.3)[31] for AoV CCA under BSC survival is similar to the corrected 8-10m figure for GEM/CIS (1.2.6.1.1.c) so that superiority of one therapy over the other cannot be established anymore. Thus, GEM/CIS cannot be recommended as standard therapy for these subtypes, which however, constitute the majority of cases unless the situation is further modified by additional prognostic factors. A range of such prognostic factors has been

identified including locally advanced vs metastatic disease, luminal vs. liver metastases, bilirubin, CEA & CA 19-9 levels, gender, ECOG, RECIST criteria, previous surgery, etc.[14,23,31,34]. Each factor potentially increases or decreases survival under the same therapy (e.g. BSC or GEM/CIS)[14,23,31,34] while the effect of multiple factors is additive[32,75]. The overall effect on survival can be significant (see also 1.2.2.g). For example according to Agarwal (2016, n=26)[32] the presence or absence of three risk factors (PS ≥2, CEA >3, and stage IVb)[32] under GEM/CIS either lower mOS from 10.5[32] to 2.9m[32] (72% decrease) or increase mOS (if absent) to 18m (71% increase) with corresponding figures for BSC still having to be established to arrive at an informed decision. Thus, best therapy decisions strongly depend on the individual prognostic factor profile. Furthermore, in such prognostic factor driven model mOS is no longer an unchangeable, rigid figure onto which a collective therapeutic decision can be based resulting in one best treatment for all. Instead, mOS represents a dynamic, variable entity whose effect size changes within a significant range around the collective overall median (e.g. +/- 75% of median) depending on the presence or absence of individual prognostic factors and their interplay at a particular point in time along the collective patient journey. It is therefore also not necessarily GEM/CIS as the currently established 1st-line standard that needs to be challenged but rather the way how clinicians use a patient's individual prognostic factor profile in combination with available research findings to arrive at a truly evidence based best therapeutic decision for an individual patient.

1.2.3.2 Second Line Chemotherapy

No 2nd-line therapy standard for BTC currently exists and no clear advantage of one regimen over all others has been established[84,85,88]. According to Brieau (2015, n=799)[84] about 33% of mainly 1st-line GEMOX treated patients also received 2nd-line of whom 40% were resected, 68% had ECOG 0-1 and 32% had ECOG 2-3 while 43%[84,85] also received 3rd-line (14% of original cohort). Second line mPFS was 3.2 (CI:2.8-4.0)[84] and mOS was 6.7m (CI:5.6-7.8)[84] with no significant differences between regimes (p_{PFS}=0.31, p_{OS}=0.78)[84]. Fornaro (2015, n=499)[85] arrived at similar figures with mPFS of 3m (CI:2.7-3.4) and mOS of 6.6m (CI:5.1-8.1) as did Lamarca (2014, review, 25 studies)[86] with mPFS of 3.2m (CI:2.7-3.7) and mOS of 7.2m (CI:6.2-8.2). Most frequently used 2nd-line regimens were FOLFIRI + XELIRI[84,85], 5FU or Capacitabine[84,85], GEM/5FU or Capacitabine[85], 5FU/CIS[84], Capecitabine/Mytomicin-C[85], FOLFOX or XELOX[84,85], GEM/CIS or GEMOX[85], Epirubicin/CIS/5FU[85], GEM/Irinotecan[85], GEM[85], other[84,85]. Prognostic factors were CA-19-9, ECOG 0-1, bilirubin level, absence of distant metastases, and disease control during 1st-line therapy[84,85]. Combination therapy was

superior to monotherapy (mOS 7.1m vs. 5.0m, p=0.006)[85]. Furthermore, Doherty (2017, n=382)[83] showed that while "the ABC-02 study used 8 cycles […] as standard with treatment stopped even in the absence of disease progression", patients who had >8 cycles had a significantly longer mPFS of 13.3 vs 4.1m (p<0.001) and mOS of 22.1 vs 9.2m (p<0.001) than patients receiving 2-8 cycles according to ABC-02. Therefore, in 1st-line "the use of continued chemotherapy to disease progression […] is a favorable option".

In summary, general outcome for 2nd-line therapy is poor with mPFS around 3m and mOS around 5-7m. The continued use of 1st -line therapy could prolong survival significantly. It further has also been recognized that as for 1st-line therapy (1.2.5.1.1.e), clear superiority of 2nd-line therapy over BSC has not yet been established. Therefore the results of the ABC-06[96] RCT (n=162, BSC vs Oxaliplatin/5-FU) in 2nd line are eagerly awaited for November 2018.

1.2.3.3 Targeted Therapy, Immunotherapy and beyond

Sahu (2017, review)[89] reports clinical trials with targeted agents (mostly monoclonal antibodies and tyrosine kinase inhibitors against EGFR and VEGF in combination with GEM based regimens) so far only showing marginal to no benefits (mOS range 4.4-12.9m, mPFS range 1.7-9.7m, 15 studies) with the result variability likely being due to the use of different mixed and at molecular/genetic level undifferentiated patient populations. Thus, study results are also not comparable. Similarly, Chong (2016, 25 studies)[95] reports a mPFS range of 1.6-8.8m and mOS range 4.4-15.7m with marginal improvements in mOS around 13.5-15.7m compared to 11.7m (ABC-02) being achieved with [GEM +/- Platinum] + [Cetuximab (3 trials) OR Sorafenib (1 trial) OR Cediranib (1 trial)] combinations while one additional trial (n=31, GEMOX + Panitumumab in KRAS WT) reached mPFS of 10.6 m and mOS of 20.3m. Also, Simone (2017, 13 studies)[93] reports mPFS of 2-8m and mOS of 4.4-20.0m for mostly GEM +/- Platinum + targeted therapy (mainly Bevacizumab, Sorafenib, and Vandetanib) trials. Finally, Eckel (2014, 161 trials, n=6337)[92,89] showed benefits of targeted therapy in combination with GEM-based chemotherapy (mTTP 7.1m, mOS 12.7m) or GEM/Platinum/FU (mTTP 9.0m, mOS 12.5m) over GEM/Platinum (mTTP 5.3m, mOS 9.5m) so that the author also suggests EGFR targeted therapy added to GEM based regimes or a GEM/Platinum/FU to become the new 1st-line standard for patients with good PS[92].

Furthermore, new data supports the idea that anatomical subtypes have different genetic backgrounds (Table 7)[89,93].

Table 7: Genomic aberrations of BTC correlated to anatomical subtypes

Subtype	Genetic Aberration (Simone, 2017)[93]	Molecular Spectrum (Javle, 2016)[94]		
		Genetic Aberration	Pre-valence	Targeted Therapy
iCCA	FGFR2 fusion gene	FGFR2 fusions	10-20%	BGJ398, Ponatinib, JNJ425756493, PRN1371, TAS-120, FGFR anti-bodies and FGFR trap molecules
	IDH1/2 mutations	IDH1/2	22-28%	AG-120, AG-881
		BAP1	15-25%	Histone Deacetylase (HDAC) inhibitors like vorinostat and panobinostat
eCCA		HER2/neu (mutation)	11-20%	Tyrosine Kinase Inhibitors like afatinib, neratinib, and dacomitinib
	PRKACA and PRKACB fusion	PRKACA and PRKACB	9%	Protein Kinase A inhibitors under development
		ARID1A	5-12%	Histone Deacetylase (HDAC) inhibitors like vorinostat and panobinostat
pCCA	KRAS mutations			
GBC	EGFR	EGFR	4-13%	Erlotinib, Cetuximab
		HER2/neu (amplification)	10-15%	Trastuzumab, Lapatinib, Pertuzumab, T-DMI
	ERBB2 and ERBB3	ERBB3	0-12%	Seribantumab (MM-121), Pertuzumab, Trastuzumab, T-DM1
	PTEN (inactivated)	PTEN	0-4%	mTOR inhibitors like Everolimus,AKT inhibitor like MK2206, PI3K inhibitors like BKM120, BYL719 and SF1126
		PIK3CA	6-13%	mTOR inhibitors like Everolimus, AKT inhibitor like MK2206, PI3K inhibitors like BKM120, BYL719 and SF1126
	TSC1 (inactivated)			

However, while a subtype may be associated more frequently with a certain genetic aberration compared to other subtypes overall resulting in a typical probability based genetic aberration profile for each subtype, most if not all aberrations do occur in all subtypes even though at different rates. In addition, the associated rate of occurrence generally is <30% and often even <15% so that for most aberrations about 70-85% of the population or more is not susceptible for targeted therapy which also may be the main reason why targeted therapy has shown so little effect. The only target so far known to be present in a majority of cases seems to be VEGF over expression (54% of iCCA[93,95], 59% of eCCA[93,95], 80% of GBC[93]) while also for KRAS mutation (40% eCCA)[95], FGFR translocations (6-50% of iCCA)[95], TP53 mutations (3-36% iCCA, 45% eCCA)[95], and ARID1A mutations (19-36% of iCCA)[95] at least potentially higher rates have been reported. Thus, there is a need for future studies to preselect patients through genetic profiling if targeted therapy shall be more effective[93].

Impressive new evidence for the existence of 4 distinct genetic clusters (Figure 10) and therefore distinct molecular subtypes comes from Jusakul (2017)[97] who performed a whole-genome and epigenomic analysis of 489 CCA cases from 10 different countries (133 liver fluke positive and 356 liver fluke negative cases). While Cluster 1 comprised mainly liver fluke positive tumours, Cluster 2 was characterized by a fluke positive/negative mix, and Clusters 3 & 4 were mainly liver fluke negative thereby confirming a molecular genetically different signature and indicating different mechanisms of carcinogenesis for fluke positive (extrinsic carcinogen) and negative (intrinsic genetic alteration) tumours. Further statistically significant cluster differences are also listed. The study also disproves the above-mentioned idea that different anatomic subtypes have distinct molecular genetics backgrounds as proposed by other authors since tumours located at

the same anatomical site partially exhibited profound differences in molecular profile while tumours at different anatomical sites also displayed similarities in molecular profile thereby confirming that anatomic site is not a driver of molecular subtypes (1.2.2.e)[97]. Still the majority of Cluster 1 & 2 were pCCA and dCCA while Cluster 3 & 4 were composed almost entirely of iCCA.

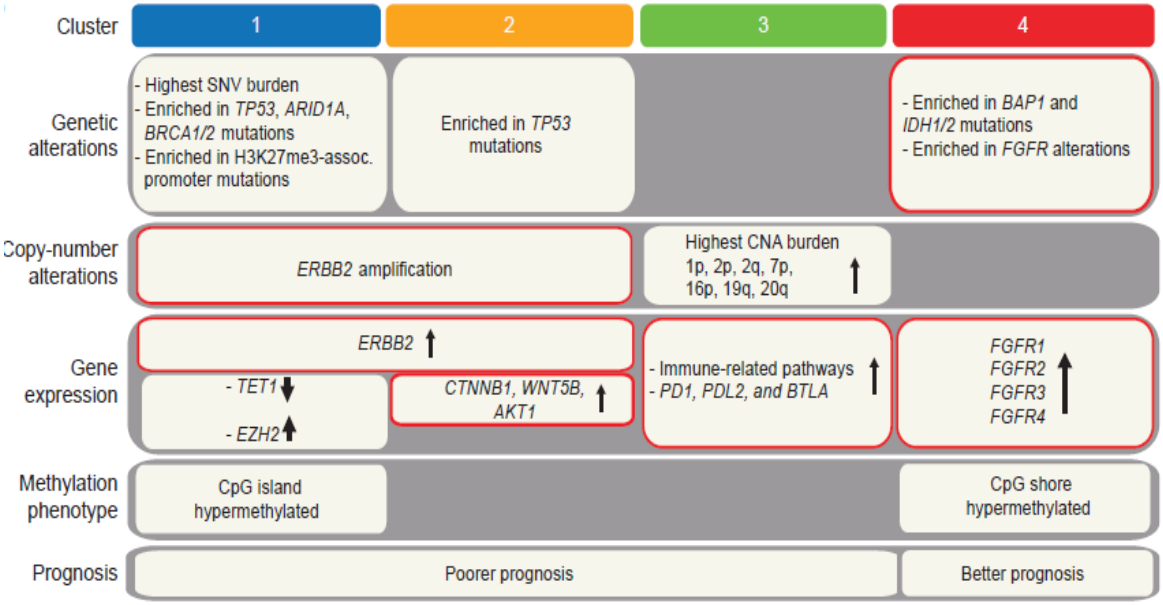


Figure 10 – Distinct genetic CCA clusters as identified by Jusakul (2017)[97]

Furthermore, while the anatomic subtypes do not differ in survival trend (1.2.2.e)[45,54], Cluster 3 & 4 (mainly iCCA) had significantly better survival than Cluster 1 & 2 (mainly pCCA & dCCA, $p<0.001$, Figure 11)[97]. Finally, molecular genetics profiling also highlights distinct targeted therapy opportunities for each cluster with e.g. Cluster 1 & 2 likely to be susceptible for ERBB2/HER2 targeting, Cluster 3 for immunotherapy (e.g. PD-1 inhibitors) and Cluster 4 for IDH inhibitors or FGFR-targeting agents.[97]

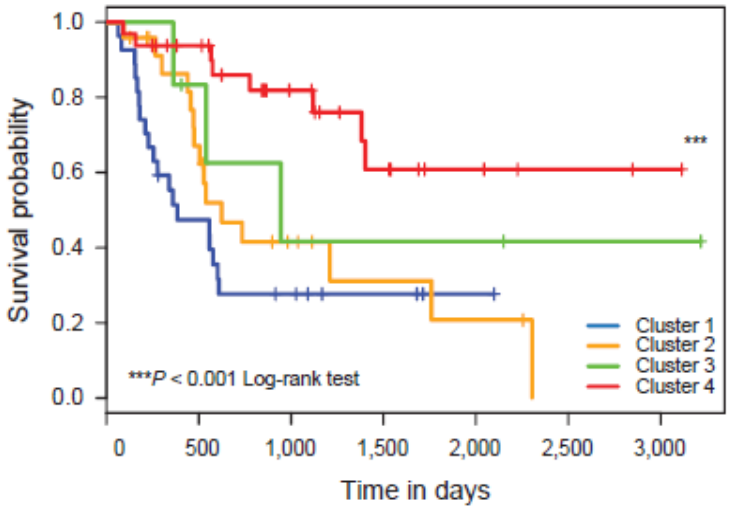


Figure 11 – Differences in survival of the 4 different molecular subtypes/clusters according to Jusakul (2017)[97]

These findings also highlight the potential benefit and limitations of novel PD-1 inhibitors like Pembrolizumab[98,99] in CCA therapy. Following these findings, PD-1 inhibitors would mainly proof beneficial in Cluster 3 and thus only be effective in a small patient subgroup. Also, Walter (2017, n=69)[100] found PD-L1 only on 11.6% of pCCA and dCCA and Fontugne (2017, n=99)[101] on 9% of iCCA and 10% of pCCA tumours while Gani (2016)[103] identified PD-L1 expression in 72% of iCCA tumour fronts. These results seem in line with Jusakul (2017)[97] since Cluster 3 were almost exclusively iCAA patients. It thus seems highly likely that PD-1 inhibitors will only be effective in a small and highly selected patient subset even though a recent case study by Czink (2017)[102] reported on a patient at very progressed eCCA stage who against these odds experienced sustained response to anti-PD-1 therapy despite no PD-L1 expression on tumour cells. However, additional tumour analysis revealed DNA MMR deficiency, microsatellite instability (MSI) and strong HLA class I and II expression which are other predictors of immune checkpoint blockade response[102,104].

1.2.4 Summary and Conclusions

In summary, while being a rare disease, CCA is the most rapidly growing cancer in the western world with one of the worst prognosis. Diagnosis usually happens at advanced stages. Surgery is the only potential cure but only 20-40% progress to surgery while up to 80% need other therapies. Thus only 2-4% of patients initially diagnosed and 14.5% undergoing surgery are cured while 96-98% die within 10 years. Therefore, most patients including resected patients receive chemotherapy at least at some stage before they die. GEM/CIS was first suggested by Eckel[17] in 2007 and confirmed by ABC-02[13] in 2010 as the currently accepted 1st-line chemotherapy standard for unresectable aCCA. However, while ABC-02 arrived at mOS of 11.7m, these results could not be reproduced[92] with the true mOS likely being around 9-10m (1.2.6.1.1.d). Furthermore, with BSC only therapy achieving a general mOS of 7-10m as well, there is no sufficient evidence until today showing that GEM/CIS is superior to BSC only therapy for all patients while existing evidence points towards a rather complex disease reality where best therapy decisions are highly dependent on the individual patient's prognostic factor profile. Also newly developed targeted therapies so far generally only produced marginal to no benefits over standard GEM/CIS with mOS 4.4-12.9m and mPFS 1.6-8.8m for most studies. However, some regimens have already been identified as potentially outperforming GEM/Platinum so that Eckel (2014)[92] suggested GEM/Platinum/FU and/or GEM/EGFR targeted therapy combination to become the new 1st-line standard for patients with good PS[92]. Furthermore, a new profiling study[97] suggests the existence of 4 distinct molecular genetics

clusters with different etiology (liver fluke pos vs. neg), targets for potential therapy, and prognosis (better OS of Clusters 3/4 vs 1/2). The study also showed anatomic site not to be a driver of molecular subtypes and indicated novel PD-1 inhibitors likely to be effective only in a small patient subset (Cluster 3). Otherwise, however, no 2nd-line standard for BTC exists until today and no clear advantage of one regimen over all others is established in 2nd-line with trial results generally being poor (mPFS of 3 and mOS of 5-7m) and a major general weakness once again being the lack of BSC control arms in studies so that superiority over BSC only has not been established.

In conclusion and for all these reasons there is an obvious, urgent and general need for the development of new and more effective therapies for this devastating but very heterogeneous and in terms of incidence and mortality in the Western World rapidly growing cancer in 1st and 2nd-line and this even if these may only prove successful in certain patient subgroups.

2. METHODOLOGY

2.1 GENERAL STUDY RATIONAL

This is a randomized, open-label, multicenter, parallel, two-group phase II study to evaluate the efficacy and safety of CAP7.1 in adult patients with advanced therapy refractory BTC. CAP7.1 is an improved version and prodrug of the well-known cytotoxic agent etoposide.

2.1.1 ETOPOSIDE

Etoposide (VP-16) is a semi-synthetic derivate of podophyllotoxin, a naturally occurring extract of plants in the genus Podophyllum of North America and India[107]. Its anti-mitotic effect was noticed in 1946 but due to its toxicity therapeutic activity in humans could not be reached[108]. During the 1960s and 1970s Sandoz Laboratories synthesized and analyzed various podophyllin derivates targeting for better outcomes. In 1966 etoposide was first synthesized followed by first clinical trials conducted in 1971 and a successful US market launch as VePesid for treatment of testicular cancer in 1983.[109]

Etoposide is a phase specific cytotoxic agent preventing cells from entering mitosis. It causes accumulation of cells in G2 phase and induces cell cycle arrest in metaphase of mitosis only when used in high dosages.[110] Furthermore, etoposide induced single and double stranded DNA breaks while DNA-Protein cross-links were temperature dependent indicating the likely involvement of an enzyme[111] which was identified in 1984 as Topoisomerase-II[112]. Etoposide inhibits Topoisomerase-II via stabilization of the enzyme-DNA cleavable complex during the

catalytic cycle of the enzyme by formation of a covalently bound ternary complex[113]. The crystal structure of a large fragment of Topoisomerase-II complexed to DNA and two drug molecules of etoposide have been described in 2004[114]. By forming the stabilized TOP2cc complex, ligation activity of topoisomerase is inhibited and DNA breaks occur[115] leading to the activation of several damaging molecules (ATM, ChK 1/2, H2AX, p53, RPA) resulting in cell cycle arrest, non-homologue end joining and recombination, and finally cell apoptosis[116].

Etoposide today is mainly used in bladder cancer, brain tumors, cervical cancer, ependyoma, germ cell tumor, gestational trophoblastic neoplasia, head and neck cancer, non-small-cell and small-cell lung cancer, lymphoma, ovarian cancer, prostate cancer, and testicular cancer. Other uses comprise Ewing's sarcoma, hepatoma, Kaposi's sarcoma, acute myeloic and acute lymphocytic leukemia, neuroblastoma, rhabdomyosarcoma, and Wilm's tumor. In combination therapies it is mostly combined with cisplatin, carboplatin, and cyclophosphamide[113,119] at dosages of 50-120 mg/m² for 3-5 days, followed by a treatment free interval of 3-4 weeks, with generally 3-4 cycles[117,118]. Furthermore, it is known to have synergistic antineoplastic activity against testicular, non-small-cell and small-cell lung cancer with some protocols being designed to take advantage of this effect[117].

The main dose limiting side effect of etoposide is myelosuppression[120] (WBC nadir 7-14 days, platelet nadir 9-16, recovery 20 days[117]), particularly seen as neutropenia, but also thrombocytopenia[120]. Other clinically important side effects are type 1 hypersensitivity reaction during i.v. administration (1-3%), fatigue, alopecia (8-66%), anorexia (10-13%), constipation, diarrhea (1-13%), mucositis, nausea and vomiting (31-43%), stomatitis (1-6%), taste alteration, and acute leukemia (2-3-year onset)[117]. However, therapeutic and side effects are both highly schedule dependent with better efficacy and less systemic toxicity observed in prolonged low-dose rather than short high-dose applications and prolonged exposure to low serum levels being the main determinant of cytotoxic efficacy while myelosuppression is highly dose dependent on peak serum levels[121,122,123,124]. Another main limitation of etoposide is the development of multiple-drug-resistances (MDR) through over expression of p-glycoprotein, MRP (multidrug resistance protein) and LRP (lung resistance protein) as well as the forming of specific resistances by alteration of Topoisomerase-II[113,125,126].

2.1.2 Use of Etoposide in BTC

In BTC, etoposide showed promising results as part of combination regimen. Glimelius (1996, n=90)[80] first suggested[130] the advantages of chemotherapy over BSC evaluating an

etoposide/5FU/LV combination against BSC in a mixed population of pancreatic (n=53) and BTC (n=37) patients. For all ($p<0.01$) and pancreatic patients ($p=0.05$) the study showed mOS of 6.0m for chemotherapy which even increased to 6.5m for BTC patients ($p=0.1$) over 2.5m for BSC[80]. This study is also one of the very few indicating benefit of chemotherapy over BSC in general until today.

In addition, Rao (2005, n=54)[131] compared the effects of etoposide/5FU/LV and Epirubicin/CIS/5-FU (ECF) in 1st-line as the only phase III trial before ABC-02. The study included BTC and GBC patients with ECOG 0-2, no prior chemotherapy, bilirubin < 30 mmol/l, and life expectancy > 3m. Due to poor recruitment it did not reach its recruitment target (119 patients) and thus was underpowered to detect significant differences in OS[130,131]. However, there was a trend in favor of the etoposide combination - mOS 12.03m (CI:9.3-14.7) vs 9.02m (CI: 6.46-11.51) for ECF[130,131]. This result is similar to the ABC-02 result of 11.7m (CI: 9.5-14.3) for GEM/CIS in a similar study population with potential existing that the etoposide based combination may actually be superior to GEM/CIS if real mOS for GEM/CIS is around 9-10m (1.2.6.1.1.c). Thus, etoposide based chemotherapy seems a valid alternative option worth trialing especially in second line after GEM/CIS failure.

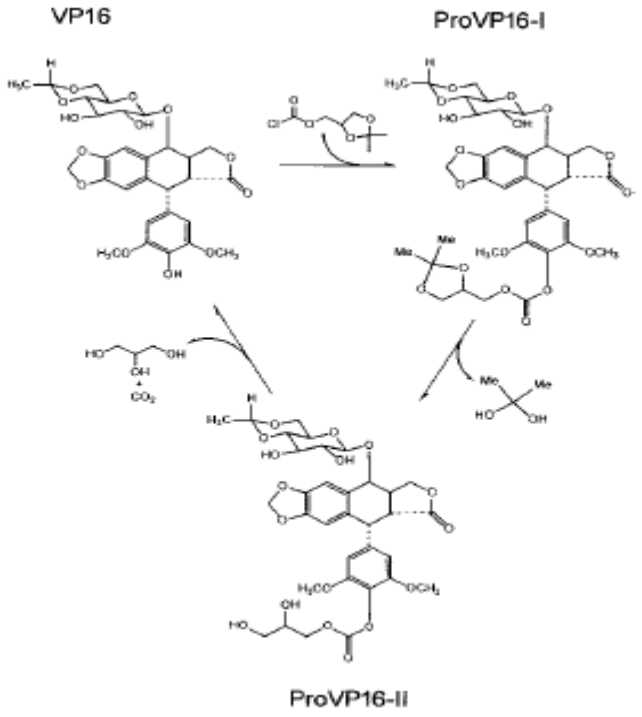
Tepsiri (2005)[132] investigated the expression of genes involved in chemotherapeutic drug resistance in 5 human iCCA lines. TS, DPD, GSTP1, MRP1, MRP2, MRP3 were expressed in all iCCA lines while MDR1 was only detected in one cell line. Four of the 5 cell lines were resistant to etoposide with strong correlations between multi-drug resistance associated protein 3 (MRP3) mRNA expression and the concentration of drug required to inhibit cell proliferation by 50% (IC_{50}) of etoposide ($r=0.98$, $p<0.05$). Furthermore, Cao (1998)[133] found P-glycoprotein (Pgp) in 69-77% and MDR1 mRNA in 52% of all samples of GBC concluding that over expression of these proteins is an important reason for the chemotherapeutic drug resistance of GBC. A comprehensive contemporary overview of chemo resistance in BTC is provided by Marin (2017)[134]. Therefore, it seems that the good results of Rao (2005, n=54)[131] were achieved despite a high degree of multi-drug resistance with improved results potentially to be expected if multi-drug resistance can be overcome.

2.1.3 CAP7.1

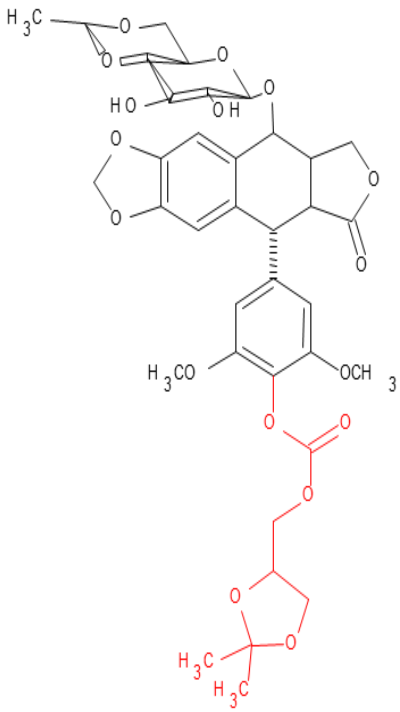
The above described limitations of etoposide demonstrate the need for development of new etoposide analogues like CAP7.1. In 2002 Wrasidlo (2002)[127] first synthesized two new etoposide prodrugs (proVP-16 I & II) by applying a novel hydrolytic activation approach to

etoposide previously effective in lowering toxicity and improving pharmacokinetics of paclitaxel. The authors reported that under acid conditions proVP-16 I converted to proVP-16 II which activated to etoposide and suspected that conversion of both prodrugs to etoposide was facilitated by carboxylesterases (Figure 12). While the latter was confirmed by Schroeder (2003)[129] with the main conversion enzyme identified as Carboxylesterase 2 (CES2) (Utku et al. – unpublished data), proVP-16 I to proVP-16 II conversion was not confirmed.

a) ProVP-16 I (CAP7.1) & II synthesis



b) CAP7.1 Structure



a) The synthesis of proVP-16 I (CAP7.1) involves the reaction of solketal chloroformate and VP-16 (Etoposide). ProVP-16 II is synthesized from proVP-16 I by acid hydrolysis with the elimination of 2,2 dihydroxypropane. The activation of proVP-16 II to VP-16 (Etoposide) occurs with the elimination of glycerol and carbon dioxide. b) Chemical structure of CAP7.1: Addition of a water-soluble ester group removable in the body by Carboxylesterases.

Figure 12 – Synthesis (a) and structure (b) of proVP-16 I (CAP7.1) and proVP-16 II (Schroeder, 2003)[129]

Further in vitro human tumor cell line testing (colon, ovarian, cervical carcinomas, various leukemia and one neuroblastoma cell line) by Schroeder (2003)[129] indicated an up to 1000-fold higher cytotoxicity of both prodrugs over etoposide. In addition, both prodrugs overcame MDR-1 mediated multidrug resistance and also induced apoptosis in vitro while equimolar etoposide was ineffective. Similarly, proVP-16 II showed a dramatic tumor growth reduction and no signs of toxicity in an in vivo multidrug-resistant xenograft T-cell leukemia mice model while equimolar etoposide showed no antitumor response but significant toxicity. Overall, the prodrugs had a 3-fold reduced in vivo systemic toxicity compared to etoposide. In addition, in cells with amplified MDR-1 gene expression a more than 3 log greater efficacy of both prodrugs over etoposide was observed in vitro and in vivo. This profile is likely due to the added ester group enabling CAP7.1 to stay within the cell and bypass or inhibit the MDR pump activity. While

cytotoxic prodrug mechanisms were not understood in detail, additional investigations showed both prodrugs inhibiting topoisomerase II to the same extent as etoposide. Furthermore, prodrugs induced G_2/M phase arrest with complete synchronization previously neither observed nor reported for etoposide. Therefore, additional intracellular targets than isomerase II may be responsible for the increase in cytotoxicity and effectiveness in multi-drug resistant models with decrease in MDR-1-mediated substrate efflux being discussed suggesting significant improvement of prodrug therapy over etoposide. Overall, this study showed proof of concept that these prodrugs may significantly increase the etoposide anti-tumor effect while decreasing toxicity in MDR-1-mediated multidrug resistant relapsed tumor patients, e.g. BTC patients after 1st-line GEM/CIS failure. [127,129]

CES2 as the main CAP7.1 conversion enzyme is present in a wide variety of organs and tissues with highest concentrations in the liver[136,139], gastrointestinal tract[136,139], kidney and bladder[136,139], adrenal cortex[136], bone marrow and immune system[139] and to a lesser degree gallbaldder[139] and lungs138,[139]. Also, CES2 expression was observed in 101 of 154 tumors (66%) and 55 of 60 normal tissues (92%)[137] including HCC and ductal pancreatic adenocarcinoma expressing moderate to intense concentrations of CES2[137]. CES2 protein levels in 13 liver samples varied with a 15-fold range in cytosol and a 3-fold range in microsomes[137]. Thus, there is a wide variety of CES2 expression between different individuals, tissues and tumor types as well as within tumor types suggesting an increased site-specific conversion of CAP7.1 leading to a generally more targeted cytotoxic activity in certain individuals, tissues and tumors and improved side effect profile compared to etoposide.

For all these reasons proVP16 was selected for clinical development under the new name CAP7.1. The first clinical CAP7.1 study (2003) was a compassionate trial in 3 heavily pre-treated children with recurrent stage 4 metastatic neuroblastoma after prior chemotherapy failure (including etoposide) who received Carboplatin+CAP7.1 up to 800 mg/m²/day (unpublished report Gaedicke et al.). The therapy was well tolerated despite higher equivalent etoposide doses in cohort 3 & 4 while toxicity was similar to etoposide mainly limited to hematotoxicity. While one 5 y.o. patient (CAP7.1$_{max}$: 200 mg/m²) progressed and died, a 6 y.o. patient (CAP7.1$_{max}$: 600 mg/m²) had stable disease over 9m and a 12 y.o. patient (CAP7.1$_{max}$: 800 mg/m²) had partial response with stable remission over 2 years.

A first open label, phase I dose escalation trial was performed by Keilholz (2017)[140] in 19 patients with various advanced refractory solid malignancies (median number of previous

treatments 2 [range 1-6] including etoposide in 5 cases) in order to determine the maximum tolerated dose (MTD) and safety profile of CAP7.1. Secondary objectives were pharmacokinetics and early efficacy signs. MTD was defined as the highest dose level below the maximum administered dose at which one of three patients experienced a dose limiting toxicity (DLT). Cap7.1 dose was escalated in cohorts of 3-6 patients using subsequent dose increments of 45, 90, 150, and 200 mg/m^2/day until MTD was reached. CAP7.1 was administered via 1-hour infusions over 5 days every 21 days for a maximum of 6 cycles. Etoposide appeared in plasma rapidly (T_{max} 1.25-2.5h at all dose levels) after CAP7.1 infusion and correlated well with CAP7.1 plasma decline. Furthermore, etoposide maximum concentration (C_{max}) increased with dose in an approximately linear fashion (Figure 13).

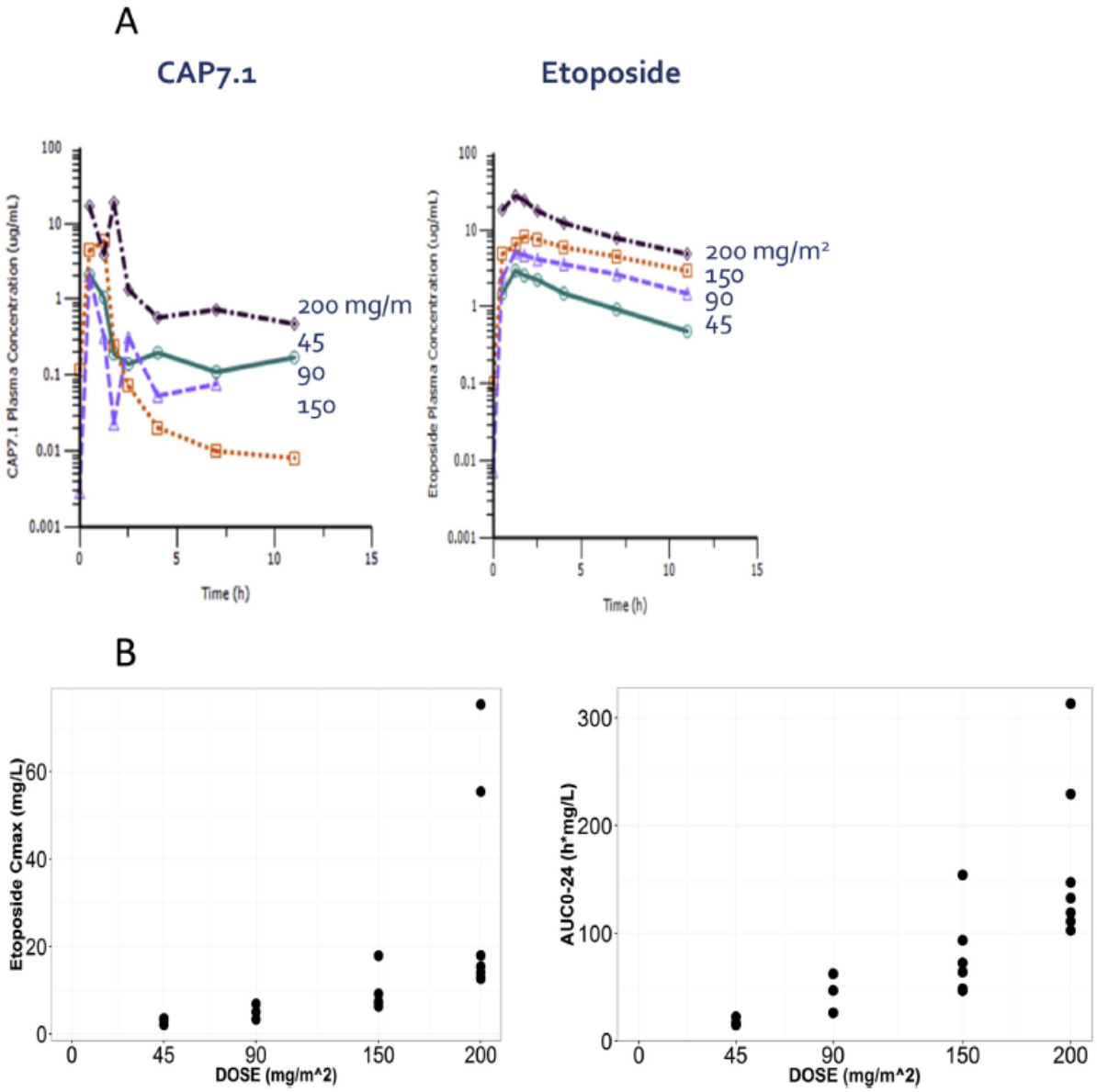


Figure 13 – A) Plasma concentrations of CAP7.1 and etoposide, B) Individual etoposide C_{max} and area under the curve (AUC) values. Etoposide C_{max} and AUC values increased in a approximately linear fashion.[140]

The MTD was 150 mg/m^2/day. However, a manageable side effect profile with only little subjective toxicity but highest efficacy was associated with the 200 mg/m^2/day dose. Most common side effects were fatigue (68.42%), anemia (57.89%), nausea (57.89%), leucopenia (52.63%), pyrexia (52.63%), and alopecia (52.63%). Severe side effects were only observed from a dose of 150 mg/m^2/day onwards with particularly the 200 mg/m^2/day dose associated with a higher number of severe hematotoxic events: leucopenia (100% = 7 patients), neutropenia (85.7% = 6 patients), thrombocytopenia (85.7% = 6 patients), anemia (42.9% = 3 patients), and febrile neutropenia (42.9% = 3 patients). The majority of these events reversed within 7 days. Overall, the safety profile of CAP7.1 was comparable to etoposide and etoposide-phosphate with the main DLT being dose dependent myelotoxicity while unlike etoposide, however, no organ specific toxicity associated with CAP7.1 was observed.[140]

Furthermore, mOS was 6.5m (range 2.5-25.63) while PFS ranged from 1 to 6.5m with a stage IV GBC patient experiencing the longest OS (25.63m) while a BTC patient was amongst the patients with longest PFS (5.4m). Considering the advanced disease stage of patients as well as the likely development of multi-drug resistance of the underlying tumors, these are promising results with some patients reaching a stable disease state up to six months to two years.[140]

2.1.4 Rational for a phase II study, objectives and hypotheses

In summary, etoposide based combination therapies proved successful in BTC therapy already from start[130] with the only phase III trial[131] prior to ABC-02 arriving at results at least comparable but potentially even superior to ABC-02 results as accepted GEM/CIS 1st-line standard. Furthermore, MDR-1 has been shown to be at least partially responsible for the chemotherapeutic drug resistance of CCA and GBC. Finally, a phase I study has shown very encouraging results for one GBC and BTC patient who's OS and PFS were (amongst) the longest of all 19 participants. Thus, CAP7.1 with 3-fold higher dose of etoposide with an improved safety profile compared to etoposide as well as its ability to overcome MDR-1 mediated resistance while being activated predominantly in specific organ and tumor tissues with high CES2 expression including liver and gallbladder tissue seems to have a unique potential to improve the outcome of especially advanced refractory BTC after 1st-line GEM/CIS failure. Thus, the main objective in this multicenter, open-label, controlled, randomized, parallel, two-group clinical phase II study was to assess the anti-tumor activity of CAP7.1 vs BSC only therapy in aCCA patients after CIS based 1st-line therapy failure based on the following primary and secondary endpoints:

a) Primary endpoint: Primary endpoint was the disease control rate (DCR) defined as the sum of all complete remissions (CR), partial remissions (PR) and stable disease states (SD) according to RECIST 1.1[141] over the total number of patients treated with CAP7.1 or BSC (n) after the first tumor response measurement at 2-cycles treatment (8 weeks) after randomization (DCR=[CR + PR + SD]/n). The primary aim was to reach a DCR of 35% as CAP7.1 yielded more than 50% response including stable disease in all solid tumors and even higher response rate in gastrointestinal and lung tumours in the phase I study. This primary aim was a meaningful and robust endpoint due to the very poor prognosis of patients with stage IV refractory BTC facing very short life expectancy after 1st line therapy failure. As DCR represents the control of tumor growth, it serves as a surrogate for improved progression-free and overall survival.

b) Secondary endpoints included:

Progression-free survival (PFS): PFS is defined as the "time from randomization until objective tumor progression or death"[90]. The main hypothesis being that PFS in the CAP7.1 group would be significantly longer than PFS in the BSC group. Furthermore, mPFS is an appropriate surrogate endpoint in a phase II trial for mOS in advanced BTC[91].

Overall survival (OS): OS is defined as the "time from randomization to death"[90], the main hypothesis being that OS in the CAP7.1 group would be significantly longer than OS in the BSC group. However, due to the allowed cross-over of BSC patients to CAP7.1 therapy after progression, this endpoint was likely to be of limited value due to mixing therapy effects.

Safety assessment: Safety assessment of CAP7.1 was another main secondary objective, the main hypothesis being that CAP7.1 is similar or safer than etoposite and/or etopophos.

2.2 Study Design

2.2.1 General Study Design and Inclusion & Exclusion Criteria

In this multicenter, open-label, randomized, parallel, controlled, two-group, clinical phase II study (Figure 14) adult patients with a confirmed diagnosis of BTC and documented disease progression after one line of chemotherapy that included CIS, ECOG 0-2, adequate bone marrow and organ function (Hb>=9 g/dl, neutrophils>=1500/mm^3, platelets>=10000/mm^3, total bilirubin<=5 x ULN, ALT & AST<=2.5 x ULN and <= 5 x ULN for patients with liver involvement, PT-INR and PTT<1.5 x ULN, creatinine clearance>50 ml/min), at least one measurable lesion according to RECIST[141], a high probability of good compliance, and life

expectancy >= 8 weeks were randomized in a 1:1 fashion to either 200 mg/m^2 CAP7.1 daily for 5 days every 28 days for up to 6 cycles (CAP7.1 arm) or BSC only therapy (BSC arm) until disease progression or early discontinuation due to other reasons (e.g. AEs, death). Patients who were pregnant or breast-feeding, had serious concurrent medical conditions potentially affecting compliance or results, had psychological or social conditions not making them a good candidate for trial participation in the investigator's opinion, participated in other clinical trials up to 30 days prior to or during this trial as well as patients who received other anti-cancer therapies were all excluded. BSC patients were allowed to crossover to CAP7.1 after progression. All patients were followed up for survival up to 1,5 years.

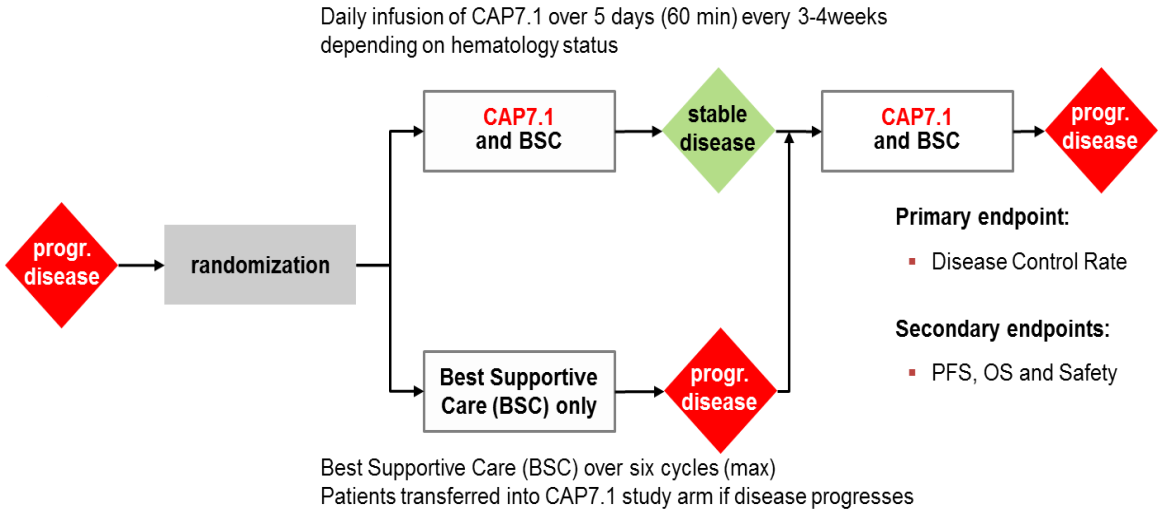


Figure 14 –Study Design

2.2.2 Randomization and Treatment

After written informed consent was obtained, patients were randomized 1:1 to either CAP7.1 or BSC only therapy using a block randomization system. Treatment was required to start within 1 week of randomization and was as follows in the two treatment groups:

a) CAP7.1 Group:

Based on the phase I study[140] results and other preclinical data described above, patients were started on 200 mg/m^2 CAP7.1 daily i.v. for 5 days every 28 days for up to 6 cycles. Therapy was ceased if either disease progression according to RECIST criteria or unacceptable toxicity occurred which could not be managed through dose reductions, the patient wished to discontinue or if otherwise medically warranted. Unacceptable toxicity was defined as:

- Grade 4 neutropenia of any duration if accompanied by febrile neutropenia or sepsis
- Uncomplicated Grade 4 neutropenia lasting more than 7 days
- Grade 3 thrombocytopenia if accompanied by bleeding

- Grade 4 thrombocytopenia of any duration
- any non haematological Grade 3 or higher toxicity (except alopecia) considered related to study drug except for nausea/vomiting or diarrhoea that has not received adequate therapy

Treatment of patients with mid-cycle toxicity without acceptable recovery on day 21 (ANC > 1500, platelets > 100000) was delayed in weekly increments until recovery. Patients without recovery after 2 weeks were discontinued while the dose of recovered patients was reduced to 150 or 110 mg/m^2. Patients not progressing after 6 cycles were able to continue treatment.

b) BSC Group:

These patients received BSC only as per institutional standard (i.e. pain relief, symptom control, biliary drainage, etc.) until progression. Once progressed, however, these patients were allowed to switch to CAP7.1 therapy following the CAP7.1 group guidelines.

2.2.3 Efficacy and Safety Assessments

Informed consent, demographics, medical history, ECG, coagulation screen, and pregnancy test were done at baseline (screening) only. Physical examination, vital signs, and urine were examined at baseline as well as at the beginning of every CAP7.1 cycle (CAP7.1 group) or every 8 weeks (BSC group). ECOG performance status was measured at baseline and then at the start of every 2nd cycle (CAP7.1 group) or every 8 weeks (BSC group). Tumor response was assessed via either CT or MRI not longer than 4 weeks prior to baseline with subsequent measurements after every 2nd cycle (CAP7.1 group) or every 8 weeks (BSC group) according to the RECIST 1.1 criteria. Hematology (WBC with differential: neutrophils, lymphocytes, monocytes, basophils, eosinophils, and platelet count) and biochemistry (sodium, potassium, calcium, phosphorus, urea, creatinine, total protein, albumin, bilirubin, alkaline phosphatase, ALT, AST, glucose, uric acid) were performed on a weekly basis from baseline until 28 days after last CAP7.1 dose (CAP7.1 group) or on a 4-weekly basis (BSC group). Adverse Events (AEs) and concomitant medication were assessed at every patient visit covering the entire study period until 28 days after last study dose intake. Survival follow-ups were done every 3 months after discontinuation for 6 months. Furthermore, tumor tissue samples (biopsies) for determination of CES expression through immune-histochemistry staining, PK samples, tumor markers and circulating tumor cells (CTC) were taken / determined. However, these latter assessments are not part of this thesis with results therefore being presented in the official study report only.

2.3 Statistical Methods

a) Analysis Set Definitions

Three different analysis sets were defined in the study protocol:

- *Full Analysis Set (FAS):* Includes all patients randomized with the statistical analysis of the FAS following the Intention-To-Treat (ITT) principle.
- *Per-Protocol Analysis Set (PP):* Includes all patients without significant protocol deviations/violations and sufficient assessments for primary endpoint determination.
- *Safety Population (SP):* Includes all patients receiving at least one dose of CAP7.1.

b) Statistical study design considerations and sample size

In order to limit the number of patients exposed to CAP7.1 if the therapy in reality proved ineffective, a three-stage group sequential design (two interim and one final analysis) with two treatment groups according to O'Brien-Fleming[142] was chosen. A group sequential design is a type of dynamic, adaptive study design which in contrast to the conventional single stage design allows to stop a study early if efficacy or futility is shown in interim analysis before all "experimental units" (i.e. patients) have been "spent" (i.e. treated)[145,146]. Such design therefore protects patients who so far have not been treated (i.e. "spent") from potential harm if in reality it is highly likely that the treatment under investigation is ineffective while at the same time also protecting patients in the control group from not receiving a superior treatment if in reality it is highly likely that the treatment under investigation is actually more effective than its comparator. In this way these methods can also offer substantial sample size reductions compared to single stage design sample sizes[146]. The O'Brien-Fleming approach is one of the most popular group sequential designs since the significance level at the final analysis is near the overall desired significance level. Drawbacks of the group sequential approach compared to interim statistical testing include the strict requirements that (1) the number of scheduled analyses must be determined prior to the onset of the trial, and (2) there is equal spacing between scheduled analyses with respect to patient accrual. These shortcomings, however, have been overcome by the alpha spending function approach[147].

In this study therefore, for a three-stage sequential design (two interim analyses plus a final analysis) with two-treatment groups, O'Brien-Fleming interim boundaries[142], a 2.5% significance level and a 1-sided test, an unpooled estimate of the variance of the standardized test statistic, 80% power, and the assumption that DCR_{BSC} = 0.1, the maximum total number of patients needed was determined to be 50 while the expected sample size under the alternative hypothesis

was 39.4. The O'Brien-Fleming procedure was used to determine the sample size required, the nominal significance level, and the stopping criterion (sc) to reject the null hypothesis at each of the three analysis stages (1st Interim Analysis: 18 patients, p<=0.00154, sc: 2.96; 2nd Interim Analysis: 34 patients, p<=0.019, sc: 2.09; Final Analysis: 50 patients, p<=0.05, sc: 1.71) with respect to the primary endpoint[142]. At each analysis step all data collected until this point are analysed and the computed test statistics are compared with the corresponding critical values generated from the sequential design in order to stop the study in case of strong efficacy effect. If the study continues to the final stage, the null hypothesis is to be either rejected or accepted.

c) Methods for endpoint analysis

In regards to the primary endpoint, the null and alternative hypotheses were: H_0: $DCR_{CAP7.1} = DCR_{BSC}$ and H_1: $DCR_{CAP7.1} - DCR_{BSC} >= 0.35$. The statistical method to evaluate the effects of CAP7.1 vs BSC with respect to DCR and other rates was the Clopper-Pearson method[143]. Kaplan-Meier plots were used to visualize survival times. For selected parameters, 95% confidence intervals of the mean/median, LS mean, standard error of the LS mean, and 95% confidence intervals of the LS mean were calculated as appropriate. Confidence intervals used a Z-statistic as the critical value. For 95% CI, the critical value was 1.96. The in this thesis presented results correspond with stage one of the interim analyses (O'Brien Fleming).

2.4 Author's own contributions to the conduct of this study

My responsibilities and focus during this thesis were support of patient recruitment, collection of patient data, patient follow-up, monitoring of research sites, maintenance of investigator site files, support of the data management team, quality assurance of data driving from clinical sites and statistical analysis of the interim study data. I supervised individual clinical sites in regards to trial conduct, inspected each research site's work via regular monitoring visits and ensured that clinical sites conducted the trial according to protocol and regulatory requirements. Furthermore, I reviewed all study data from clinical sites and performed "source data verification" (SDV) in order to verify that all in the CRF (Clinical Report Form) documented study data matched the information contained in the patient's "source documents" (i.e. patient's notes, X-ray and lab reports, etc.) according to ICH-GCP. I discussed all identified discrepancies and other issues like protocol deviations, regulatory requirements or investigator site file issues with the investigators and/or study nurses either directly or in the form of queries (written questions). In this way I performed a total of 23 monitoring visits to different clinical research sites during this study, several lasting over 2-3 days. For each of these site visits I wrote a

monitoring visit report (approximately 15-20 pages). Furthermore, I prepared sites for audits and ensured that the Trail Master File at the Sponsor site as well as all Investigator Site Files located at the respective individual clinical research sites were kept up-to-date. In addition, I supported data management and drug safety tasks by conducting systematic central data consistency and plausibility checks, performing a medical review of all medical and safety data, conducting a safety reconciliation of all safety data in collaboration with the drug safety team, and describing the state of the study database and important data deviations for statistical analysis. Finally, I performed the statistical analysis planned for interim analysis (graphs and tables) to evaluate efficacy and safety of CAP7.1 in BTC patient groups as well as on an individual patient base as presented in this thesis. Statistical results were verified by the statistician, Peter Treasure, PhD (Research Associate, Clinical School, & Affiliated Lecturer, Statistical Laboratory, at the University of Cambridge).

3. RESULTS

3.1 PATIENTS AND ANALYSIS-SETS

Table 8 – List of Subjects and Reason of Analysis-Set Exclusion

Site[1]	Subject	Treatment Arm	Analysis Set Membership			CAP7.1 Start Dose (mg)	Reason for Exclusion
			FAS	PP	SP	Start	
A	PAT01	BSC	Yes	Yes	Yes*	200	
A	PAT02	CAP7.1	Yes	Yes	Yes	200	
A	PAT03	CAP7.1	Yes	Yes	Yes	200	
A	PAT04	CAP7.1	Yes	Yes	Yes	150	
A	PAT05	BSC	Yes	No	No	-	PP: Lost to follow-up before 49 days, SP: received no CAP7.1
A	PAT06	BSC	Yes	Yes	Yes*	150	
A	PAT07	CAP7.1	Yes	Yes	Yes	150	
B	PAT08	CAP7.1	Yes	Yes	Yes	200	
C	PAT09	BSC	Yes	Yes	Yes*	200	
D	PAT10	BSC	No	No	No	-	FAS, PP: No baseline CT available, SP: Received no CAP7.1
D	PAT11	CAP7.1	Yes	No	Yes	150	PP: Withdrew because of AE before 2 cycles received
D	PAT12	CAP7.1	Yes	No	Yes	150	PP: Withdrew because of AE before 2 cycles received
D	PAT13	BSC	Yes	Yes	Yes*	150	
D	PAT14	CAP7.1	Yes	Yes	Yes	150	
D	PAT15	CAP7.1	Yes	No	Yes	150	PP: Withdrew because of AE before 2 cycles received
D	PAT16	CAP7.1	Yes	Yes	Yes	150	
D	PAT17	BSC	Yes	Yes	Yes*	150	

Table 8 (continued)

Site	Subject	Treatment Arm	Analysis Set Membership			CAP7.1 Start Dose (mg)	Reason for Exclusion
			FAS	PP	SP	Start	
E	PAT18	BSC	Yes	Yes	Yes*	150	
E	PAT19	CAP7.1	Yes	Yes	Yes	110	
E	PAT20	BSC	Yes	Yes	Yes*	200	
F	PAT21	CAP7.1	Yes	Yes	Yes	200	
F	PAT22	BSC	No	No	No	-	FAS, PP: Consent withdrawn before treatment
F	PAT23	CAP7.1	No	No	No	-	FAS, PP: Screen failure, no treatment
F	PAT24	BSC	Yes	Yes	Yes*	200	
G	PAT25	BSC	Yes	Yes	Yes*	150	
			N=22 BSC:10 CAP: 12	N=18 BSC: 9 CAP: 9	N=21 BSC: 9 CAP: 12		

FAS: Full Analysis Set, PP: Per Protocol Analysis Set, SP: Safety Population, *: BSC patient who switched to CAP7.1 after progression, [1] Sites: A = Berlin, B = Ludwigsburg, C = Freiburg, D = Essen, E = Worms, F = Hannover, G = Leer

To trigger the 1st interim analysis, 18 evaluable patients were required in the Per-Protocol Analysis Set (PP) according to the O'Brien Fleming method at a nominal one-sided threshold of 0.00154 using a Z-test with unpooled variance. At time of analysis, however, the challenge with the PP was that because so many patients in the BSC arm crossed over to the CAP7.1 treatment arm after progression, the PP was not symmetrical with respect to treatment arms. Therefore, it was decided that it was appropriate to use the Full Analysis Set (FAS) in addition. Patients were recruited at 7 different research sites across Germany (Table 8).

3.2 Baseline Characteristics

Patient characteristics at baseline are shown in Table 9-11. Patients in the CAP7.1 group seemed slightly younger than patients in the BSC group with a mean age of 59.4 vs. 65.9 years respectively while gender was balanced and all patients were Caucasians (Table 9).

Table 9 – Demographic Characteristics (FAS)

Demographic Characteristic		CAP7.1 N=12 (55%)	BSC N=10 (45%)	Total N=22 (100%)
Age:	Mean (SD)	59.4 (11.2)	65.9 (8.57)	62.4 (10.4)
	Median	58.0	68.5	64.5
	Range	45-72	52-78	45-78
Age Group:	> 65	5 (42%)	6 (60%)	11 (50%)
	< 65	7 (58%)	4 (40%)	11 (50%)
Gender:	Male	7 (58%)	5 (50%)	12 (55%)
	Female	5 (42%)	5 (50%)	10 (45%)
Race:	White	12 (100%)	10 (100%)	22 (100%)
	Other	0 (0%)	0 (0%)	0 (0%)

Most patients at screening suffered from a moderately to poorly differentiated, extra-hepatic CCA with multiple metastases mainly in the liver and/or lymph nodes which therefore was TNM stage IV at time of screening (Table 10). Metastases were most frequently in the liver, (82%) and/or lymph nodes (77%) followed by peritoneum (46%), lungs (23%) and suprarenal glands (5%). Half of all recruited patients were diagnosed 3-12 months before screening while the other half was diagnosed longer than 12 months before screening. All patients had a good ECOG performance status of 0 or 1 (Table 10).

Table 10 – Disease Characteristics (FAS)

Disease Characteristics		CAP7.1 N=12 (55%)	BSC N=10 (45%)	Total N=22 (100%)
Primary Tumor Site:	Liver	4 (33%)	5 (50%)	9 (41%)
	Lymph Nodes	0 (0%)	1 (10%)	1 (5%)
	Other	8 (67%)	4 (40%)	12 (55%)
Histology:	Adenocarcinoma	5 (42%)	5 (50%)	10 (45%)
	Adenosquamous	3 (25%)	0 (0%)	3 (14%)
	Gallbladder Tumor	1 (8%)	1 (10%)	2 (9%)
	Klatskin Tumor	1 (8%)	0 (0%)	1 (5%)
	Other/Unknown	2 (17%)	4 (40%)	6 (27%)
Histological Grade:	Well differentiated	1 (8%)	0 (0%)	1 (5%)
	Moderately differentiated	4 (33%)	4 (40%)	8 (36%)
	Poorly differentiated	1 (8%)	3 (30%)	4 (18%)
	Unknown	6 (50%)	3 (30%)	9 (41%)
Metastases at Screening:	Yes	10 (83%)	6 (60%)	16 (73%)
	No	2 (17%)	4 (40%)	6 (27%)
Local Recurrence at Screening:	Yes	3 (25%)	3 (30%)	6 (27%)
	No	9 (75%)	7 (70%)	16 (73%)
Time diagnosis to Screening:	< 3 months	0 (0%)	0 (0%)	0 (0%)
	> 3 – 12 months	7 (58%)	4 (40%)	11 (50%)
	> 12 months	5 (42%)	6 (60%)	11 (50%)
ECOG at Screening:	0	4 (33%)	8 (80%)	12 (55%)
	1	8 (67%)	2 (20%)	10 (45%)
Radiological Localization:	Intrahepatic	3 (25%)	3 (30%)	6 (27%)
(at Screening)	Extrahepatic	8 (67%)	4 (40%)	12 (55%)
	Unknown	1 (8%)	3 (30%)	4 (18%)
Radiological Metastases:	Liver	10 (83%)	8 (80%)	18 (82%)
(at Screening)	Lymph nodes	10 (83%)	7 (70%)	17 (77%)
	Peritoneal	7 (58%)	3 (30%)	10 (46%)
	Lung	3 (25%)	2 (20%)	5 (23%)
	Suprarenal	1 (8%)	0 (0%)	1 (5%)
Radiological organ number:	Mean	2.6	2.0	2.3
	Median	2.0	2.0	2.0
	Range	1-4	1-4	1-4

In terms of prior therapy, only about 1/3 of patients was also surgically pre-treated with 75% of these patients being in the BSC group. All patients received prior platin-based chemotherapy, the majority receiving either GEM/CIS or GEMOX 1st-line therapy standard with a median

treatment length of 15.5 (CAP7.1 group) and 27.6 weeks (BSC group) and progression being the main reason for discontinuation of prior 1st-line therapy (Table 11).

Overall, demographic and disease characteristics as well as prior therapy at screening seemed well balanced between the two treatment groups.

Table 11 – Prior Therapy (FAS)

Prior Therapy		CAP7.1 N=12 (55%)	BSC N=10 (45%)	Total N=22 (100%)
Surgery:	Yes	2 (17%)	6 (60%)	8 (36%)
	No	10 (83%)	4 (40%)	14 (64%)
Chemotherapy:	Gemcitabine + Cisplatin	8 (67%)	9 (69%)*	17 (68%)*
	Gemcitabine + Oxaliplatin	3 (25%)	1 (8%)*	4 (16%)*
	Cisplatin alone	1 (8%)	1 (8%)*	2 (8%)*
	Panitumumab	0 (0%)	1 (8%)*	1 (4%)*
	Mitomycin	0 (0%)	1 (8%)*	1 (4%)*
Weeks of prior chemotherapy:	Mean (SD)	23.7 (22.3)	39.5 (28.4)	30.9 (25.9)
	Median	15.5	27.6	25.9
	Range	4.4-82.4	4.1-100.0	4.1-100.0
Reason for Discontinuation:	Progression	10 (83%)	9 (69%)	19 (76%)
	Treatment completed	2 (17%)	2 (15%)	4 (16%)
	Toxicity	0 (0%)	2 (15%)	2 (8%)
* Some BSC patients had more than one prior chemotherapy regime				

3.3 Study Drug Exposure

Overall, the median number of CAP7.1 cycles patients received was 2 with a range of 0.6 (i.e. 3 days) and 7 cycles. The median cumulative CAP7.1 dose patients received was overall 1500 mg with a slightly higher median dose of 1625 mg received in the CAP7.1 group. The median daily dose received was 150 mg CAP7.1 (range 110-200 mg).

3.4 Primary Endpoint Analysis – Disease Control Rate (DCR)

RECIST response data at an individual patient level was extracted from the study database for calculation of the disease control rate as primary study endpoint. In both treatment groups there were no CT scans and thus no proper RECIST assessments available for primary endpoint analysis for 3 patients. In the CAP7.1 group two of these patients died under CAP7.1 therapy so that in absence of a CT scan a progressive disease status was assumed while the third patient was discontinued due to experiencing a cardiac arrest under CAP7.1 therapy which the patient survived with the investigator later clinically confirming new lesions leading also to a PD status in the absence of a CT scan.

In the BSC treatment group progression under BSC treatment was confirmed via ultrasound rather than CT scan for one and via clinical assessment only for another patient while a third patient was lost to follow-up with the RECIST disease status unfortunately remaining unknown. Last but not least also three additional patients in the BSC treatment group died under CAP7.1 therapy after having crossed over to CAP7.1 treatment without a CT scan being available so that in the analysis also a PD status was assumed for these patients.

According to the study protocol, the Per-Protocol Analysis Set (PP) should be used for primary endpoint analysis. The primary endpoint results are summarized in Table 12 (grey area).

Table 12 – Primary Endpoint Analysis - Disease Control Rate

Tumor Response according to RECIST	Per-Protocol Analysis Set (PP) (Database Data)		
	CAP Group on CAP Therapy	BSC Group on BSC Therapy	All on CAP7.1[3] who received >= 2 cycles
Total number of subjects [n]	n=9	n=9	n=15
Complete Response (CR)	0	0	0
Partial Response (PR)	0	0	1
Stable Disease (SD)	5	0	7
Progressive Disease (PD)	4	9	7
RECIST not available [response used for calculation]	0	0	0
Response assessment time point (median [range]) in weeks after randomization (R) or first CAP7.1 dose (C)	*R: 9.3 [6.0-15.4]* *C: 8.0 [4.0-9.6]*	*R: 5.0 [0.9 – 17.3]*	C: 8.0 [4.0-9.6]
Disease Control Rate (DCR) = [CR + PR + SD]/n	5/9 = 55.6%	0/9 = 0%	8/15 = 53.3%
95% Clopper-Pearson Confidence Interval[2] [%]	21.2-86.3%	0.0-33.6%	26.6-78.7%
P-Value (one-tailed)[1]	0.014	[redacted]	
	[redacted]	0.009	
P-Value (two-tailed)[1]	0.029	[redacted]	
	[redacted]	0.009	

[1] Fisher's Exact Test as calculated by http://www.quantpsy.org/fisher/fisher.htm

[2] Clopper-Pearson 95% Confidence Intervals calculated using http://epitools.ausvet.com.au/content.php?page=CIProportion&SampleSize=9&Positive=4&Conf=0.95&Digits=3 and http://www.danielsoper.com/statcalc/calculator.aspx?id=85

[3] This group consists of all CAP patients plus all BSC patients who switched to CAP.

Overall, the disease control rate at about 2 months after randomization for the Per-Protocol Analysis Set was encouraging with a DCR of 56% (CI: 21-86) for the CAP7.1 group compared to 0% (CI: 0-34) for the BSC group (p=0.014). Therefore the study's main objective of demonstrating disease control in >= 35% of CAP7.1 treated patients was achieved. However, even though this difference was clinically relevant, it did not reach the stringent statistical significance level of 0.00154 determined by the O'Brien Flaming method for the first interim

analysis. Therefore, the study was not halted on the grounds of efficacy at this interim stage while, however, these results can still be interpreted as very encouraging.

Furthermore, after pooling the results of CAP7.1 group patients with the results of BSC group patients after initial progression and cross-over to CAP7.1 therapy and comparing these results to the results of BSC group patients under BSC only therapy, DCR results remain similar to the results described above for CAP7.1 treated patients while, however, the one-tailed p values for the difference to BSC patients become even more significant thereby indicating an overall further encouraging and robust trend in positive result development as sample size increases.

Last but not least it seems also noteworthy to mention that one BSC group patient (PAT01) who progressed under BSC after 10 weeks had a partial response after crossing over to CAP7.1 lasting for 23 weeks and thus more than twice the time the patient was stable under BSC only therapy thereby indicating the potential benefit of CAP7.1 for an individual patient.

3.5 Changes in Tumour Burden

3.5.1 Waterfall Plot – Best Objective Response

Figure 15 shows the best objective response measured as the best change in millimetres in the sum of longest diameters of target lesions (tumour burden) from the last assessment before first CAP7.1 administration (CAP7.1 treatment phase) or screening (BSC only treatment phase).

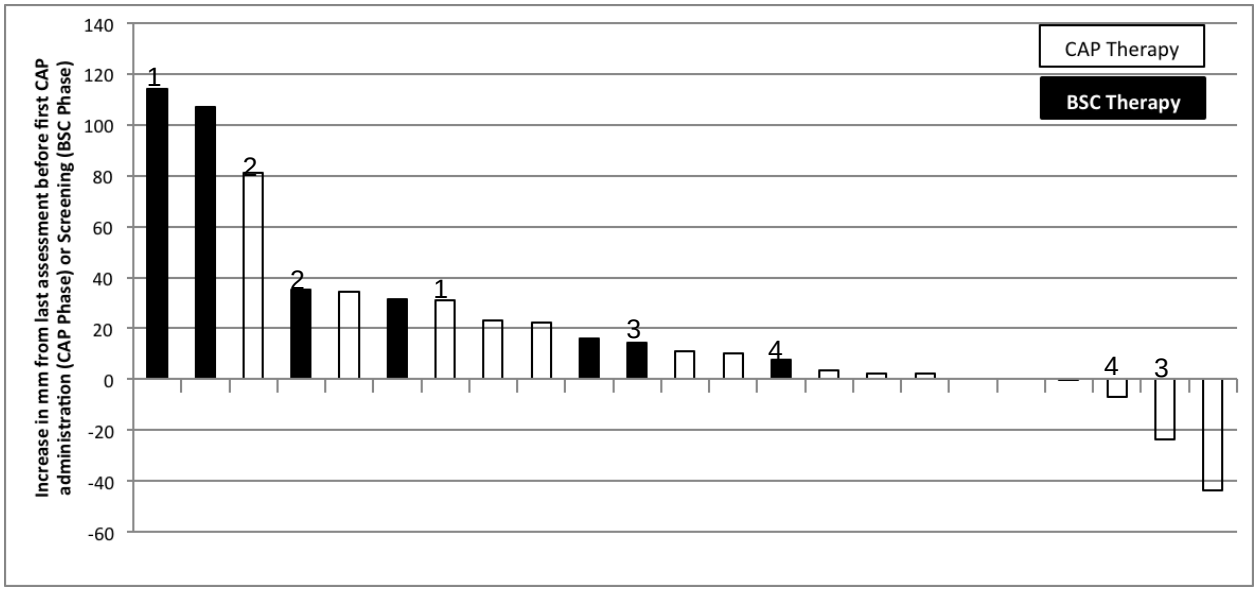


Figure 15– Waterfall Plot of the Best Objective Response (FAS, Local Radiology Assessment, number 1-4: These numbers mark different therapy phases - BSC and CAP 7.1 phase - of the same patient where available)

Overall it is striking to see how the smallest increases in tumour growth as well as all tumour shrinkages were achieved under CAP7.1 therapy while the two biggest increases in tumour growth appeared under BSC therapy only. Furthermore, three BSC group patients who switched to CAP7.1 after initial progression experienced a diminished tumour growth under CAP7.1 compared to BSC only therapy, two of whom even experienced a tumour shrinkage as best response.

3.5.2 Average best Tumour Growth Rate

Furthermore, taking the time component additionally into account by dividing the "best" (i.e. slowest) target lesion diameter growth in millimetres by the number of days between the two measurements and thus arriving at an "average best tumour diameter growth rate per day" reveals that on average tumours grew much slower under CAP7.1 therapy (median diameter growth: 0.05 mm/day) than under BSC only therapy (median diameter growth: 0.55 mm/day) in their "best" (i.e. slowest) growth mode under the respective two therapies (Table 13).

Table 13 – Average "best" (i.e. slowest) Tumour Growth Rate per day in mm of diameter

BSC Treatment		CAP7.1 Treatment	
Patient	Tumour Growth in mm Diameter per Day (RECIST response)	Patient	Tumour Growth in mm Diameter per Day (RECIST response)
PAT06 (BSC - BSC Phase)[1]	0.59 (PD)	PAT06 (BSC - CAP Phase)[1]	0.77 (PD)
PAT18 (BSC - BSC Phase)[2]	2.92 (PD)	PAT18 (BSC - CAP Phase)[2]	0.50 (PD)
PAT01 (BSC - BSC Phase)[3]	0.11 (PD)	PAT01 (BSC -CAP Phase)[3]	-0.15 (PR)
PAT25 (BSC - BSC Phase)[4]	0.15 (PD)	PAT25 (BSC - CAP Phase)[4]	-0.18 (SD)
PAT09 (BSC - BSC Phase)	2.33 (PD)		
PAT13 (BSC)	0.55 (PD)		
PAT17 (BSC - BSC Phase)	0.22 (PD)		
		PAT19 (CAP)	0.44 (PD)
		PAT08 (CAP)	0.30 (PD)
		PAT04 (CAP)	0.20 (PD)
		PAT24 (BSC - CAP Phase)	0.18 (PD)
		PAT03 (CAP)	0.09 (SD)
		PAT14 (CAP)	0.05 (PD)
		PAT16 (CAP)	0.04 (SD)
		PAT07 (CAP)	0.01 (SD)
		PAT02 (CAP)	0.00 (SD)
		PAT20 (BSC - CAP Phase)	0.00 (SD)
		PAT21 (CAP)	0.00 (SD)
Mean (SD)	***0.98 (1.15)***		***0.15 (0.26)***
Median	***0.55***		***0.05***
Range	***0.11 to 2.92***		***-018 to 0.77***

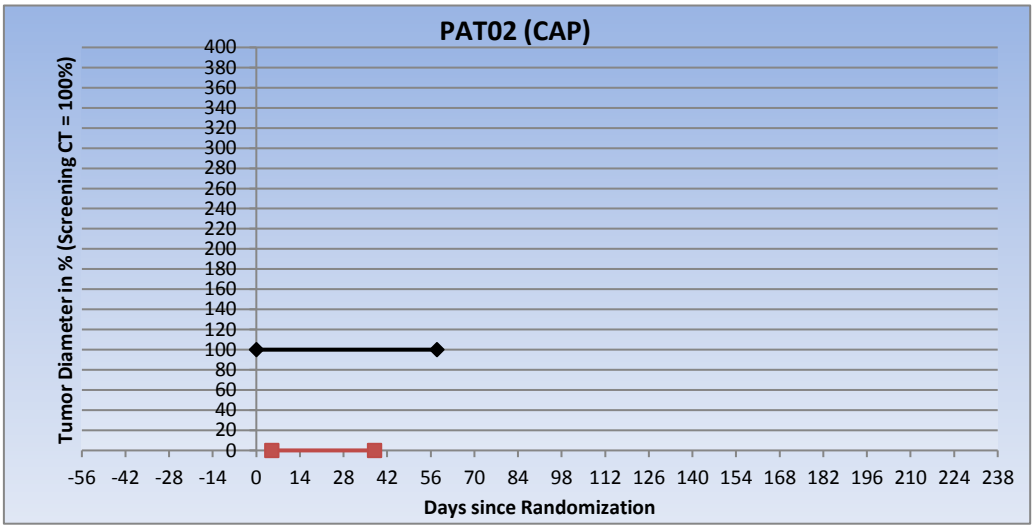
In addition, 62.5% (10/16) of the growth rates under CAP7.1 therapy in general and 70% (7/10) of the growth rates of CAP7.1 group patients are below the slowest growth rate of 0.11 mm/day under BSC only therapy while the two fastest growth rates of 2.92 mm/day and 2.33 mm/day occurred under BSC therapy and were about 3-4 times as high as the highest growth rate under CAP7.1 (0.77 mm/day). Interestingly, all SD measurements had growth rates below 0.10 mm/day while all PD measurements had growth rates above 0.10 mm/day except patient PAT14 who, however, had a PD status due to new lesions rather than target lesion growth. Furthermore, three of the four BSC patients for whom a within patient treatment comparison is available (grey area in Table 13) experienced either a significant reduction in tumour growth (PAT18) or even tumour shrinkage (PAT01, PAT25) under CAP7.1 therapy. Especially the example of PAT18 seems important to note since this patient experienced a significant slow-down in tumour growth under CAP7.1 (0.05 mm/day) compared to BSC (2.92 mm/day) while still only being assessed with a PD under CAP7.1 therapy thereby indicating that CAP7.1 may actually be more effective than otherwise indicated in the primary endpoint analysis based on the RECIST assessment. While these results need to be interpreted with caution due to potential outlier effects in a small sample, they may be seen as another indication for potential CAP7.1 efficacy (Table 13).

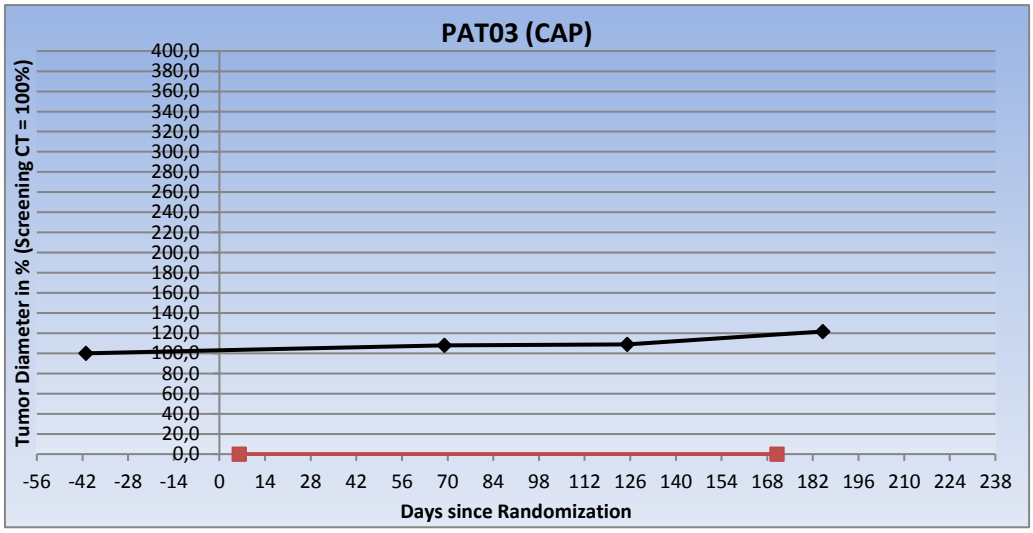
3.5.3 Individual patient tumour response over time

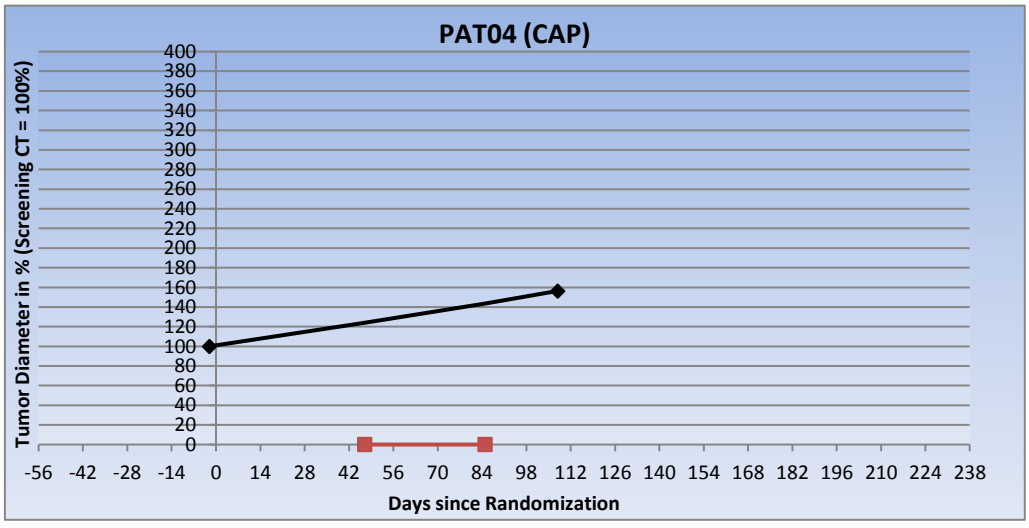
In such devastating heterogeneous and individual prognostic factor driven disease (chapter 1.2) there is merit in reviewing individual tumour burden data (Figure 16). All BSC group patients had increasing tumour burden trajectories during BSC therapy except for PAT20 and PAT24. However, in these two cases progression during BSC therapy was diagnosed via ultrasound (PAT20) or clinically (PAT24) so that one in-between measurement is missing.

Furthermore interesting are BSC patients after progression that experienced a long lasting disease control under CAP7.1. Patient PAT01 experienced a partial response and thus tumour shrinkage from 83 mm to 59 mm (29% reduction) after switch to CAP7.1 lasting for 23 weeks (i.e. 5.4m, 5 cycles). In addition, patient PAT25 experienced a 14% reduction of total tumour diameter under CAP7.1 therapy from 50.9 mm to 43.8 mm and thus achieved a SD status, which was ongoing for 29.4 weeks (i.e. 6.8m, 6 cycles). Finally, patient PAT18 experienced a significant slowdown in tumour growth from 2.92 mm/day under BSC to 0.5 mm/day under CAP7.1 (83% reduction), which lasted at least for 45 days (i.e. 1.5 m) at which point, however, the patient was unfortunately discontinued prematurely while only dying 144 days (i.e. 4.8 m)

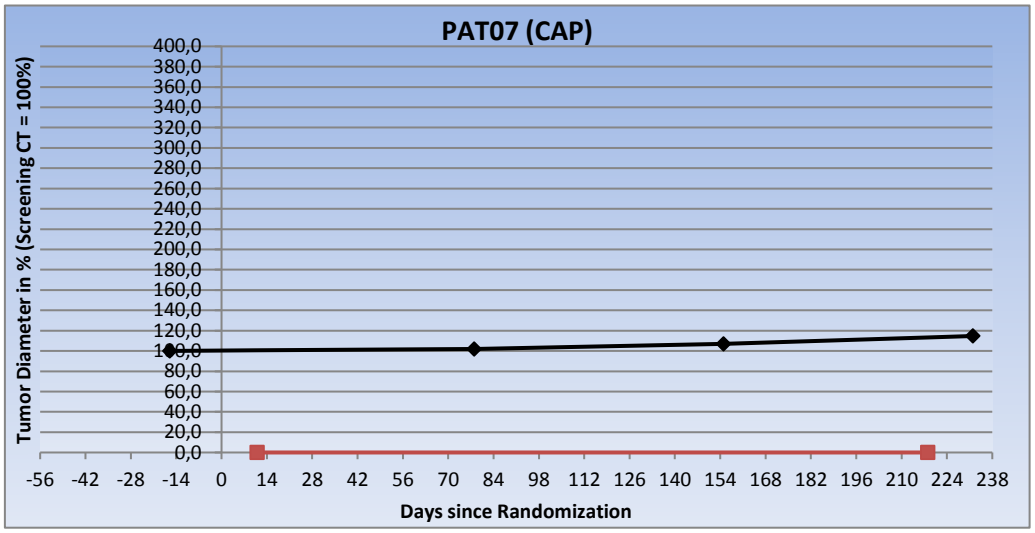
CAP7.1 GROUP

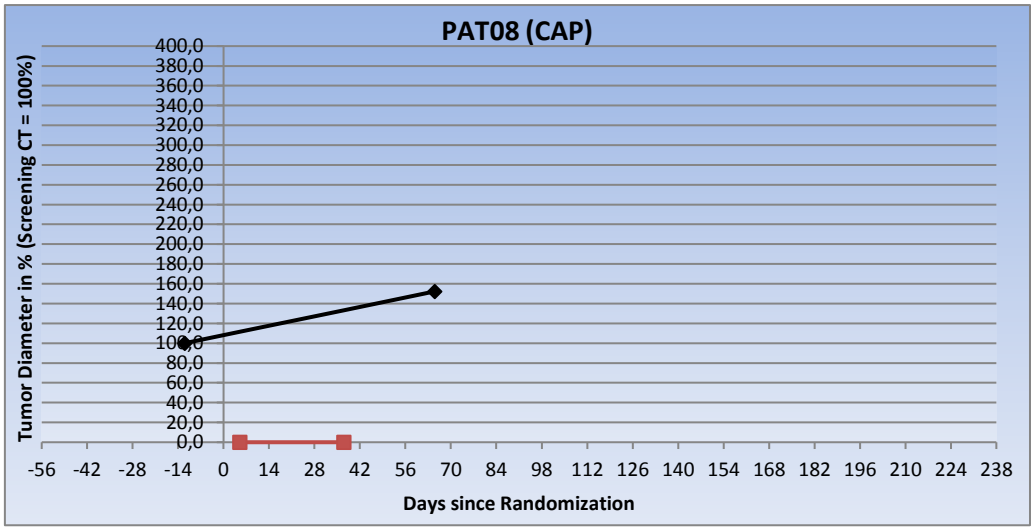

PAT02 (CAP)
Tumor Diameter in % (Screening CT = 100%)
400
380
360
340
320
300
280
260
240
220
200
180
160
140
120
100
80
60
40
20
0
-56 -42 -28 -14 0 14 28 42 56 70 84 98 112 126 140 154 168 182 196 210 224 238
Days since Randomization

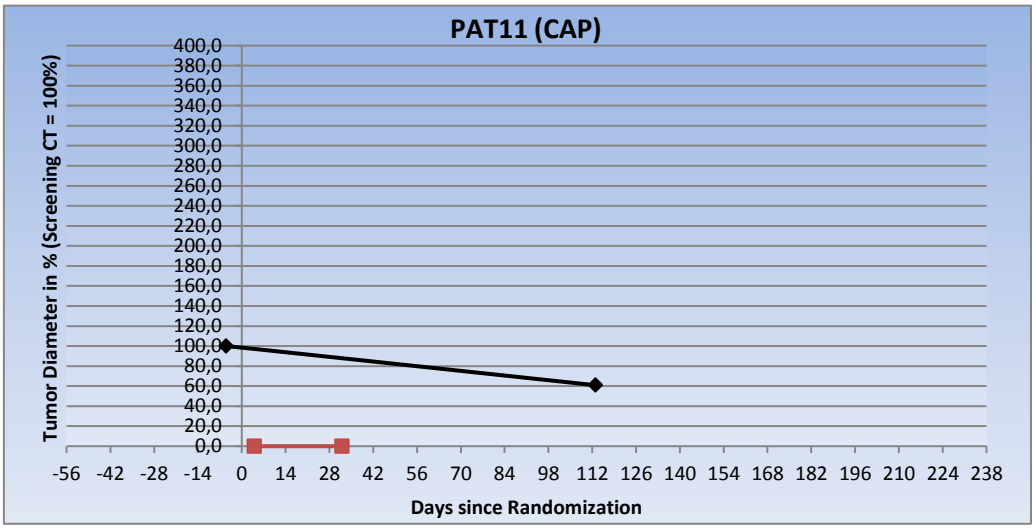

PAT03 (CAP)
Tumor Diameter in % (Screening CT = 100%)
400,0
380,0
360,0
340,0
320,0
300,0
280,0
260,0
240,0
220,0
200,0
180,0
160,0
140,0
120,0
100,0
80,0
60,0
40,0
20,0
0,0
-56 -42 -28 -14 0 14 28 42 56 70 84 98 112 126 140 154 168 182 196 210 224 238
Days since Randomization


PAT04 (CAP)
Tumor Diameter in % (Screening CT = 100%)
400
380
360
340
320
300
280
260
240
220
200
180
160
140
120
100
80
60
40
20
0
-56 -42 -28 -14 0 14 28 42 56 70 84 98 112 126 140 154 168 182 196 210 224 238
Days since Randomization

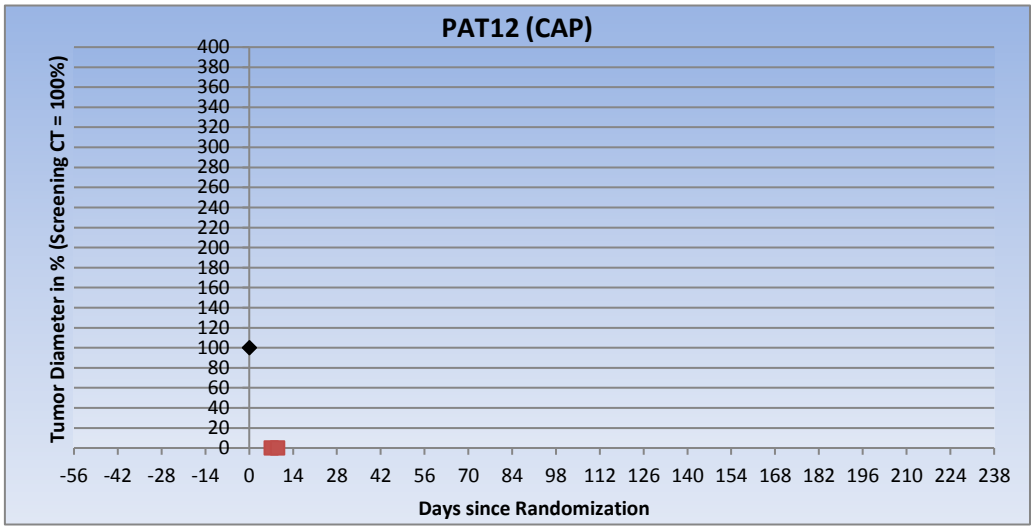
CAP7.1 Group (continued)

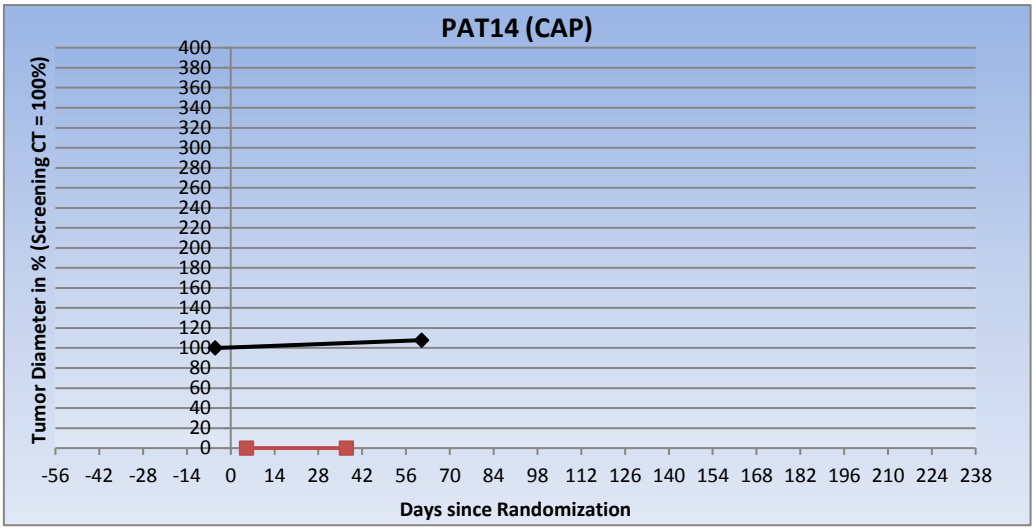


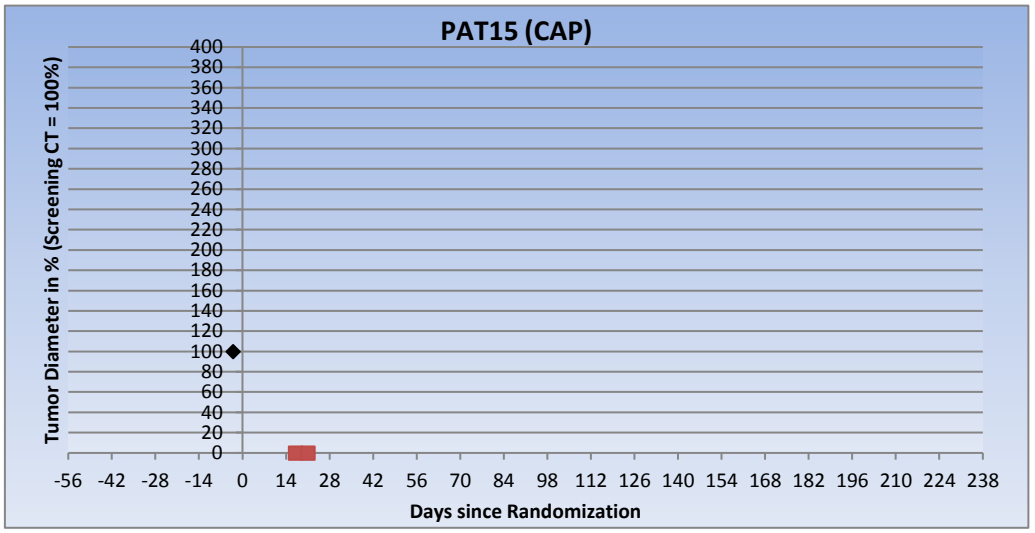




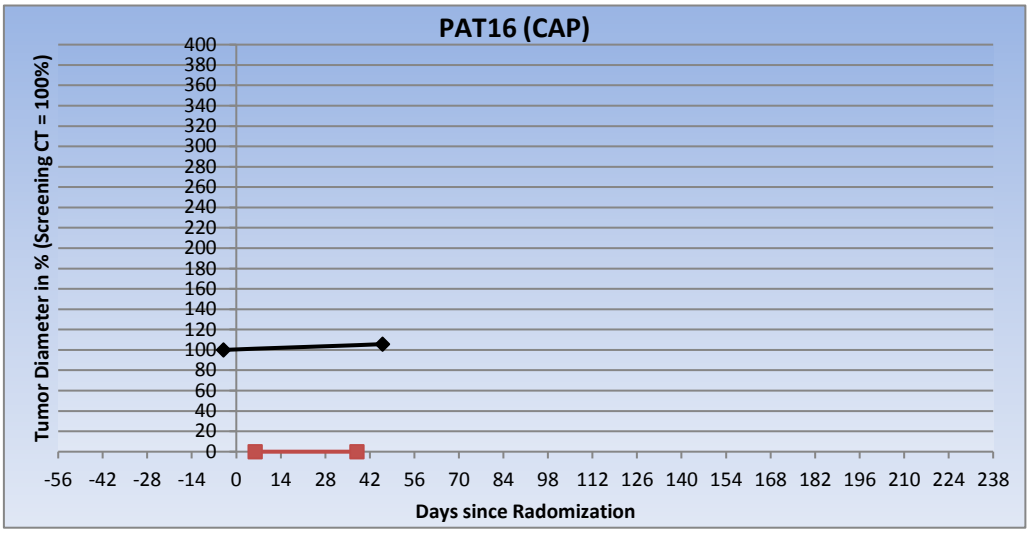
CAP7.1 Group (continued)

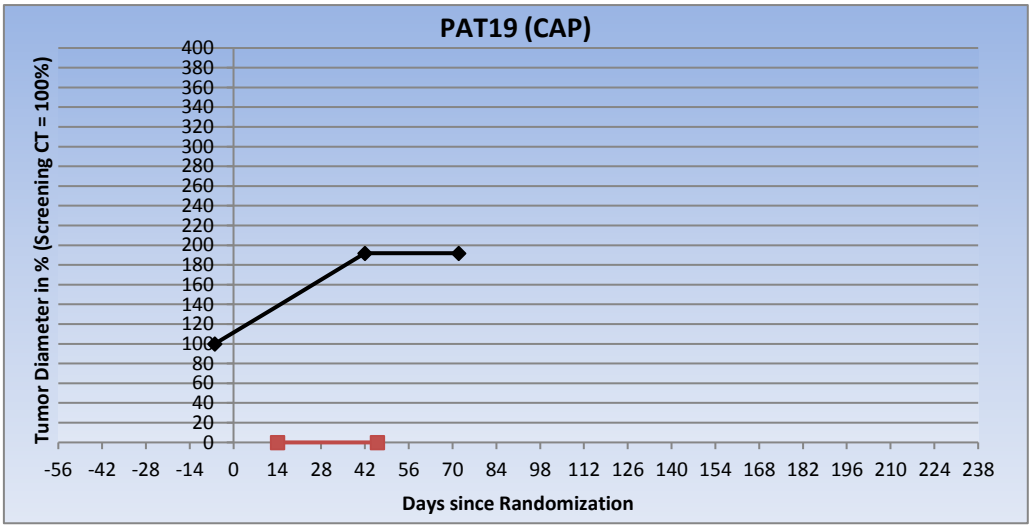


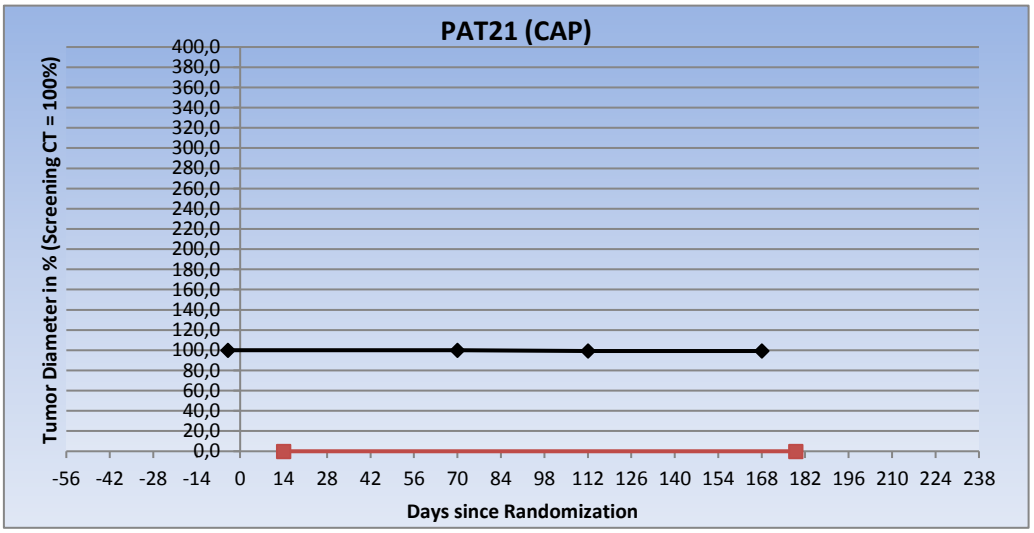




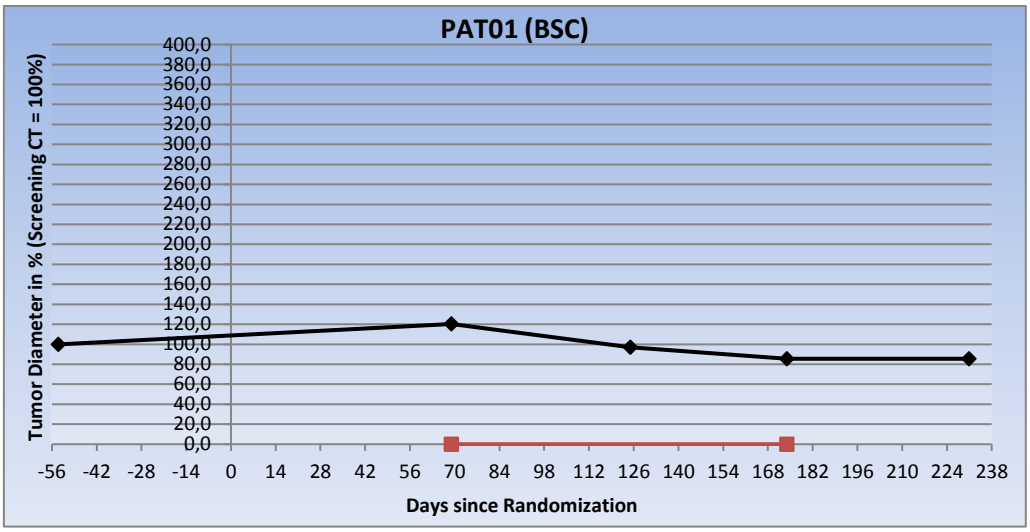
CAP7.1 Group (continued)

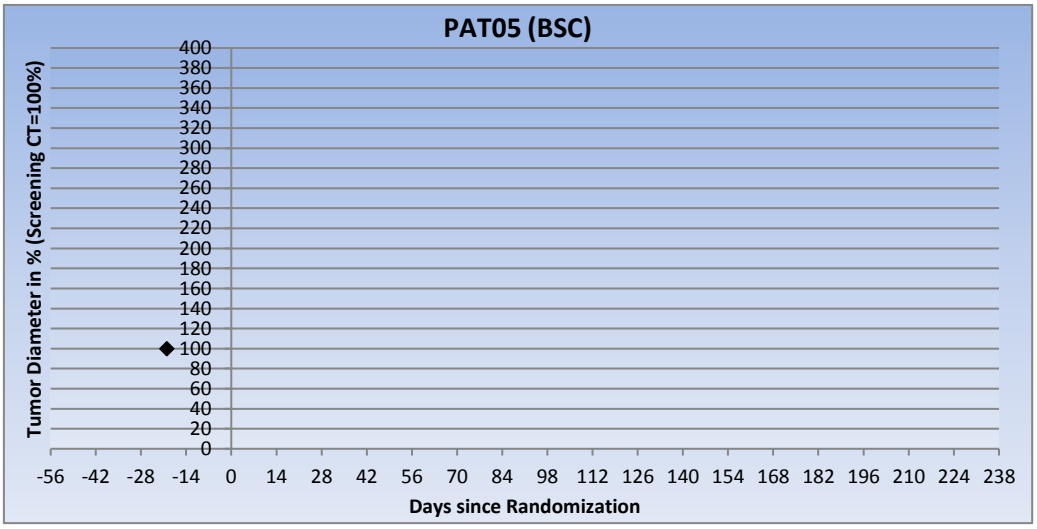


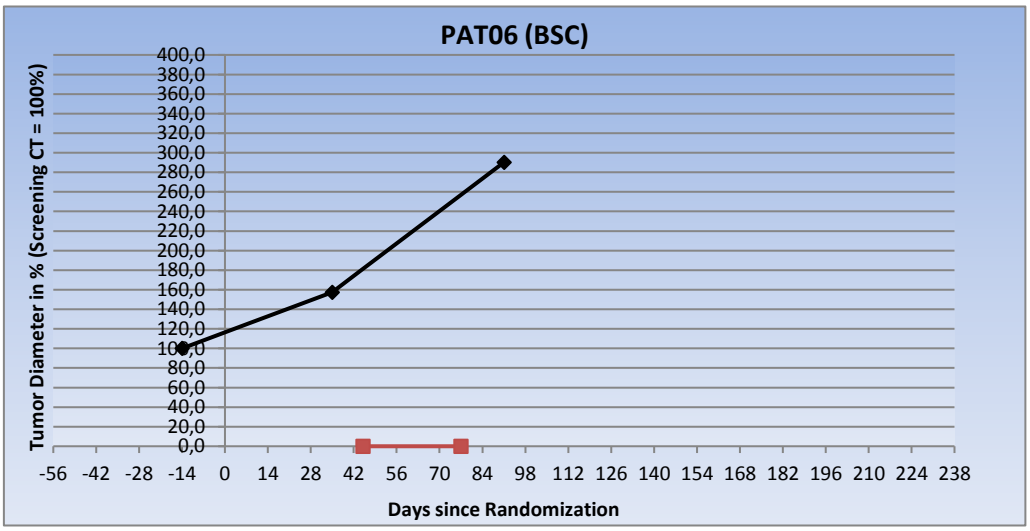




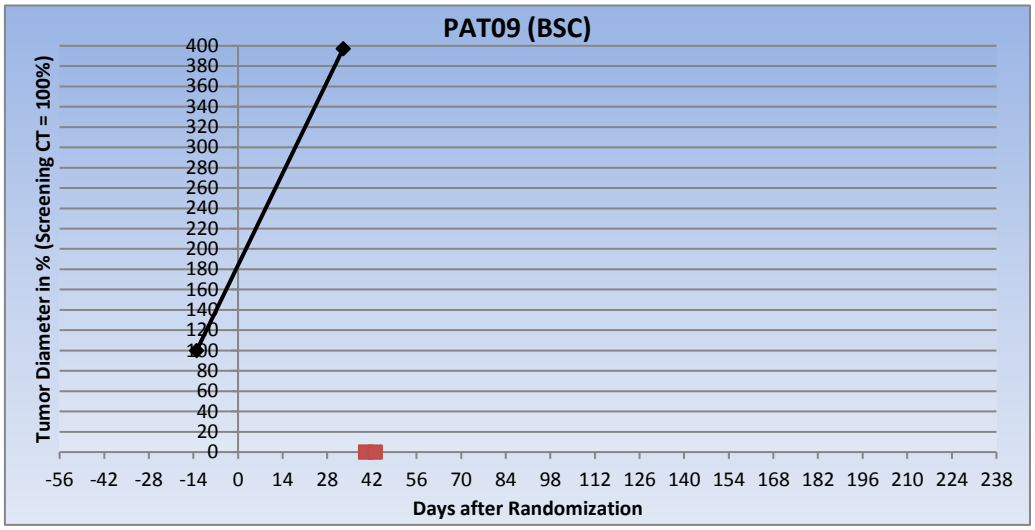
BSC GROUP

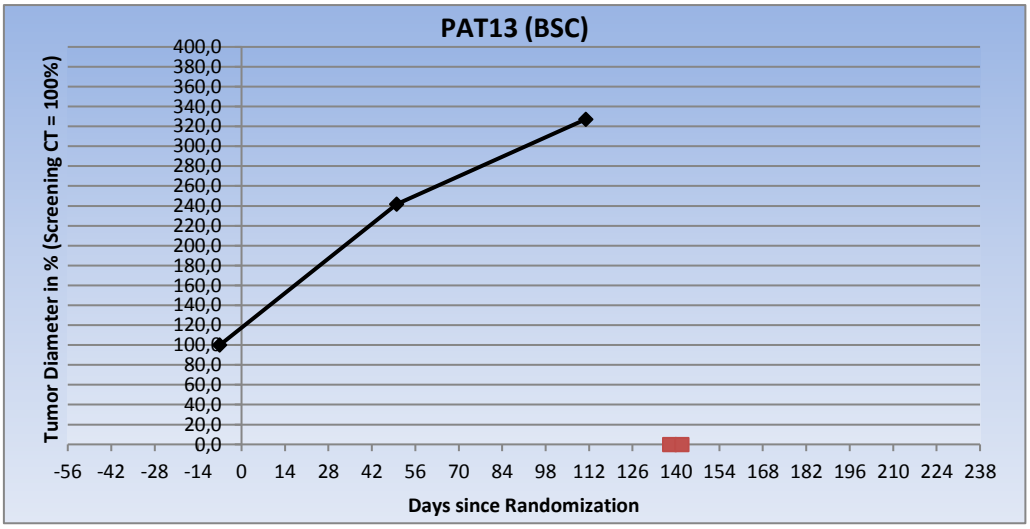

PAT01 (BSC)
Tumor Diameter in % (Screening CT = 100%)
400,0
380,0
360,0
340,0
320,0
300,0
280,0
260,0
240,0
220,0
200,0
180,0
160,0
140,0
120,0
100,0
80,0
60,0
40,0
20,0
0,0
-56 -42 -28 -14 0 14 28 42 56 70 84 98 112 126 140 154 168 182 196 210 224 238
Days since Randomization

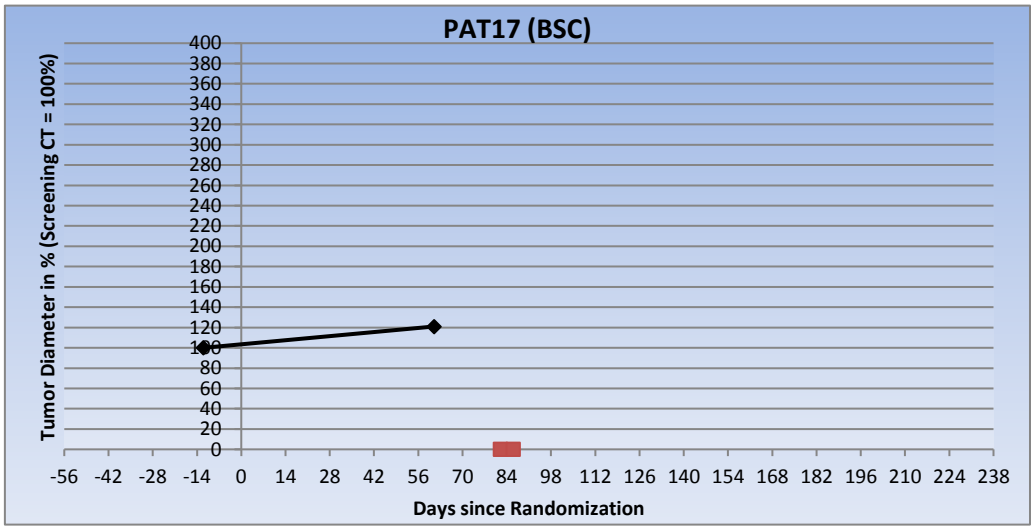

PAT05 (BSC)
Tumor Diameter in % (Screening CT=100%)
400
380
360
340
320
300
280
260
240
220
200
180
160
140
120
100
80
60
40
20
0
-56 -42 -28 -14 0 14 28 42 56 70 84 98 112 126 140 154 168 182 196 210 224 238
Days since Randomization


PAT06 (BSC)
Tumor Diameter in % (Screening CT = 100%)
400,0
380,0
360,0
340,0
320,0
300,0
280,0
260,0
240,0
220,0
200,0
180,0
160,0
140,0
120,0
100,0
80,0
60,0
40,0
20,0
0,0
-56 -42 -28 -14 0 14 28 42 56 70 84 98 112 126 140 154 168 182 196 210 224 238
Days since Randomization

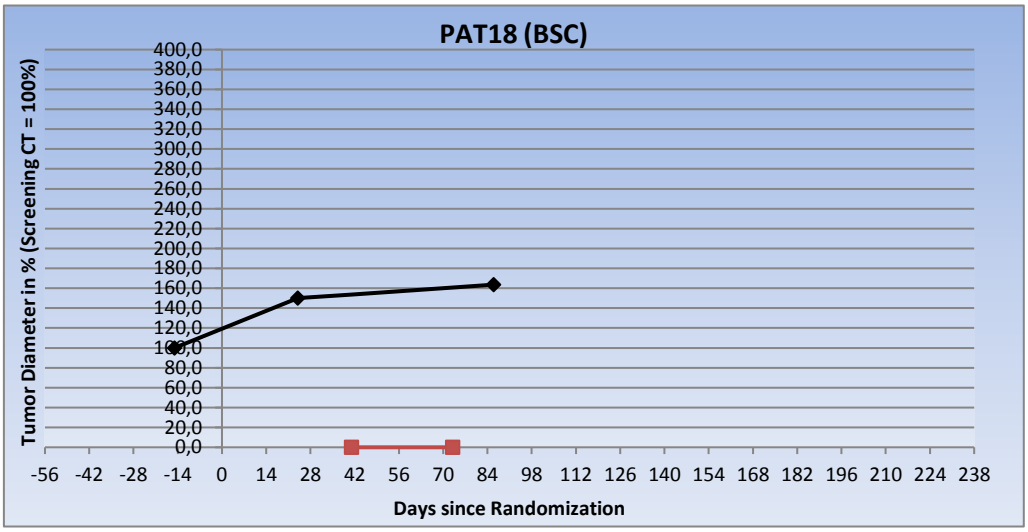
BSC Group (continued)

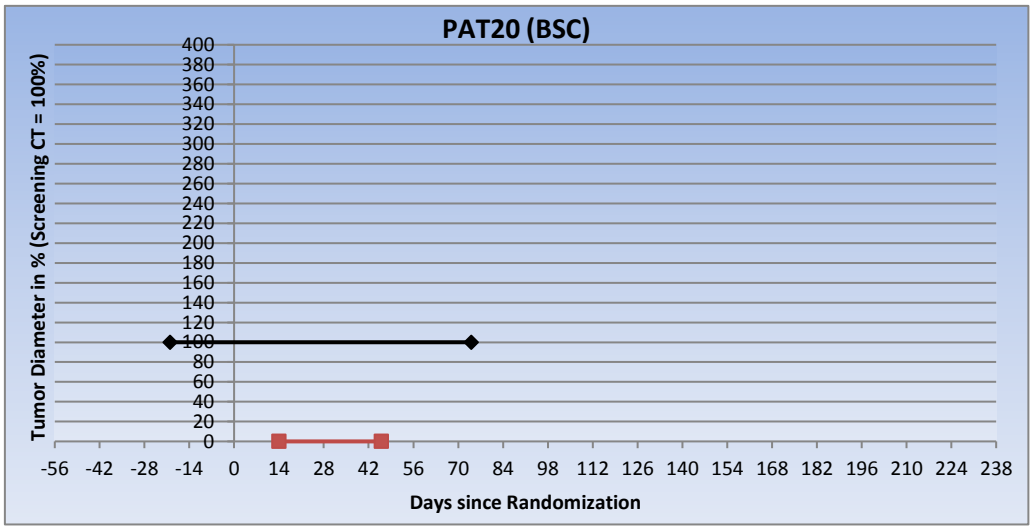


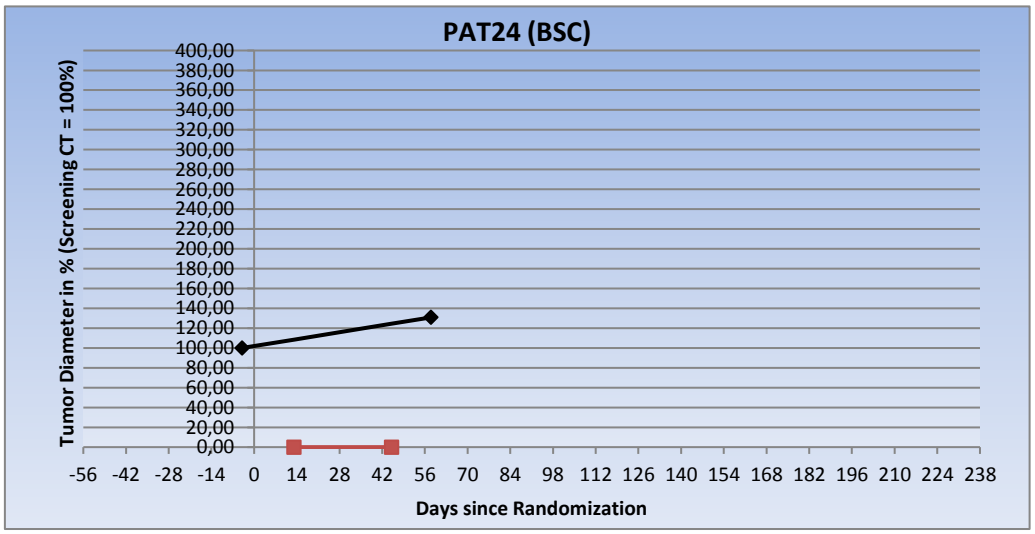




BSC Group (continued)







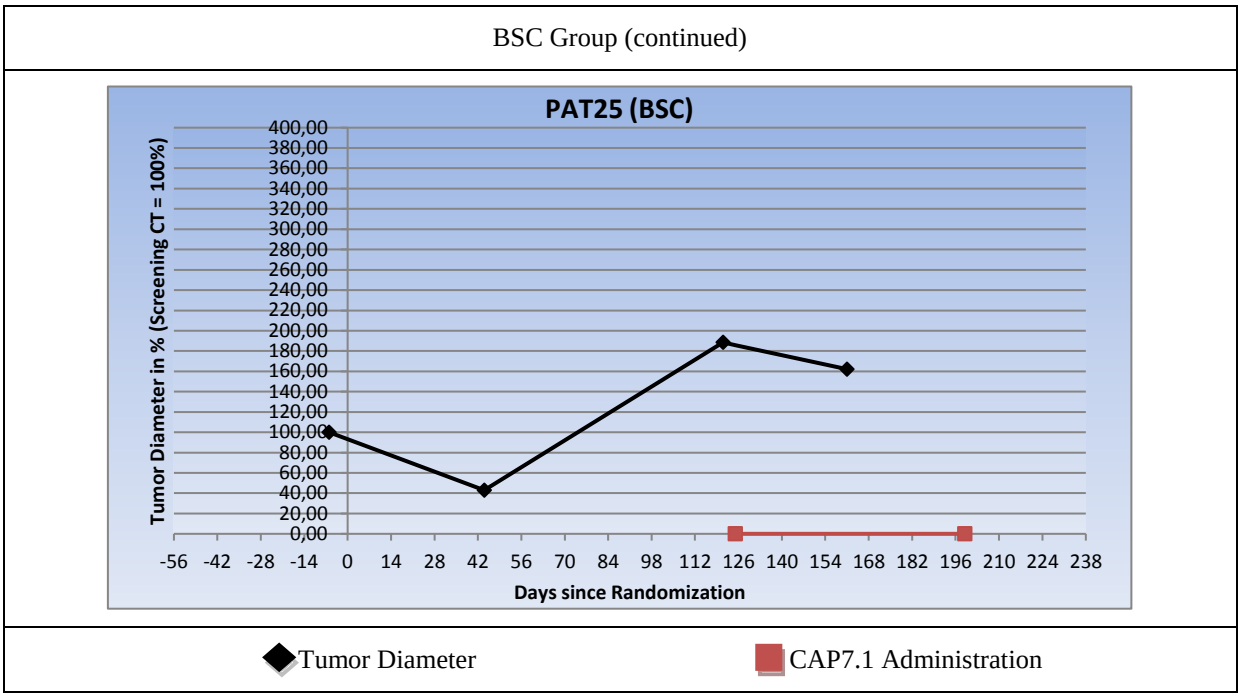


Figure 16 – Individual patient tumour response

after CAP7.1 start without receiving any known additional anticancer therapy. Furthermore, with a rate of 2.92 mm/day under BSC therapy this patient had the highest tumour growth rate of all patients under BSC so that this reduction in growth rate under CAP7.1 therapy seems even more remarkable. Tumour diameter growth actually increased from 0.59 mm/day under BSC therapy to 0.77 mm/day under CAP7.1 (an increase of 30%) for patient PAT06.

Finally, tumour burden trajectories in the CAP7.1 group as a general observation seem on average far more "flat" and thus slower under CAP7.1 than under BSC therapy. Furthermore, especially three of the CAP7.1 group patients (PAT03, PAT07, PAT21) experienced particularly long periods of stable disease of 179 days (i.e. 6m, 6 cycles), 221 days (i.e. 7.4m, 6 cycles), and 154 days (i.e. 5.1m, 7 cycles).

Overall, it therefore seems that tumour growth under CAP7.1 therapy in general is slower compared to BSC only therapy while for especially three BSC group patients (PAT1, PAT18, PAT25) as well as three CAP7.1 group patients (PAT03, PAT07, PAT21) CAP7.1 therapy was particularly beneficial.

3.6 Time to Event Analysis (TTF, PFS, OS)

Even though PFS is the most important oncology trial endpoint besides OS in the literature and in addition also has been suggested as an appropriate surrogate endpoint for OS for phase II trials in advanced BTC patients[91], it is prone to produce rather supportive results in favour of CAP7.1

because withdrawals due to AEs under CAP7.1 therapy as well as loss-to-follow-up events typically happen before progression or death but in PFS analysis are considered only as censored observations rather than actual observed events. Therefore it was decided to perform a "Time-to-Treatment-Failure" (TTF) analysis using the FAS, which considers the earliest of withdrawal, loss-to-follow-up, progression or death as the actual event time point and using the FAS as the most conservative data set in addition to PFS analysis using the PP thereby producing most conservative results. Times for both analyses were calculated only from the start of treatment day rather than day of randomization since in the CAP7.1 group one patient had a significantly delayed CAP7.1 start of 47 days after randomization and thus would have biased results significantly in favour of CAP7.1.

3.6.1 TIME-TO-TREATMENT-FAILURE (TTF)

TTF was defined as the time from therapy start to withdrawal (for any reason), loss-to-follow-up, progression or death, whatever occurred earliest. Even with this conservative analysis a trend in favour of CAP7.1 is observed. The median TTF in the FAS was 61 days for the CAP7.1 treatment group which was almost twice as long as the 34 days for the BSC treatment group with a one-sided p value of 0.041. Therefore even in the most conservative estimate, the advantage of CAP7.1 therapy compared to BSC only therapy was 2 months over 1 month in TTF.

3.6.2 PROGRESSION-FREE-SURVIVAL (PFS)

PFS was defined as the time from start of treatment to treatment-failure/progression with withdrawal and loss-to-follow-up treated as censored observations rather than observed events. Results for the PFS analysis for the PP are summarized in Table 14. The median duration of PFS in the CAP7.1 group was 108 days (3.5 months) compared to 35 days (1 month) in the BSC group with a corresponding one-sided p value < 0.01 indicating statistical significance.

Table 14 – PFS Analysis Results

	Per-Protocol Analysis Set (PP) N=18	
	CAP7.1 Group (n=9)	BSC Group (n=9)
Median [95% CI][1] in days	108 [58-188]	35 [7-111]
Range	58-256	7-121
N (%) censored	1 (10%)	0 (0%)
P-Value[2]	< 0.01	

[1] Confidence interval calculated by Brookmeyer-Crowley method after complementary log-log transformation
[2] One-sided p-value from Log Rank Test

Furthermore, the PFS Kaplan-Meier plot for the PP is shown below (Figures 17).

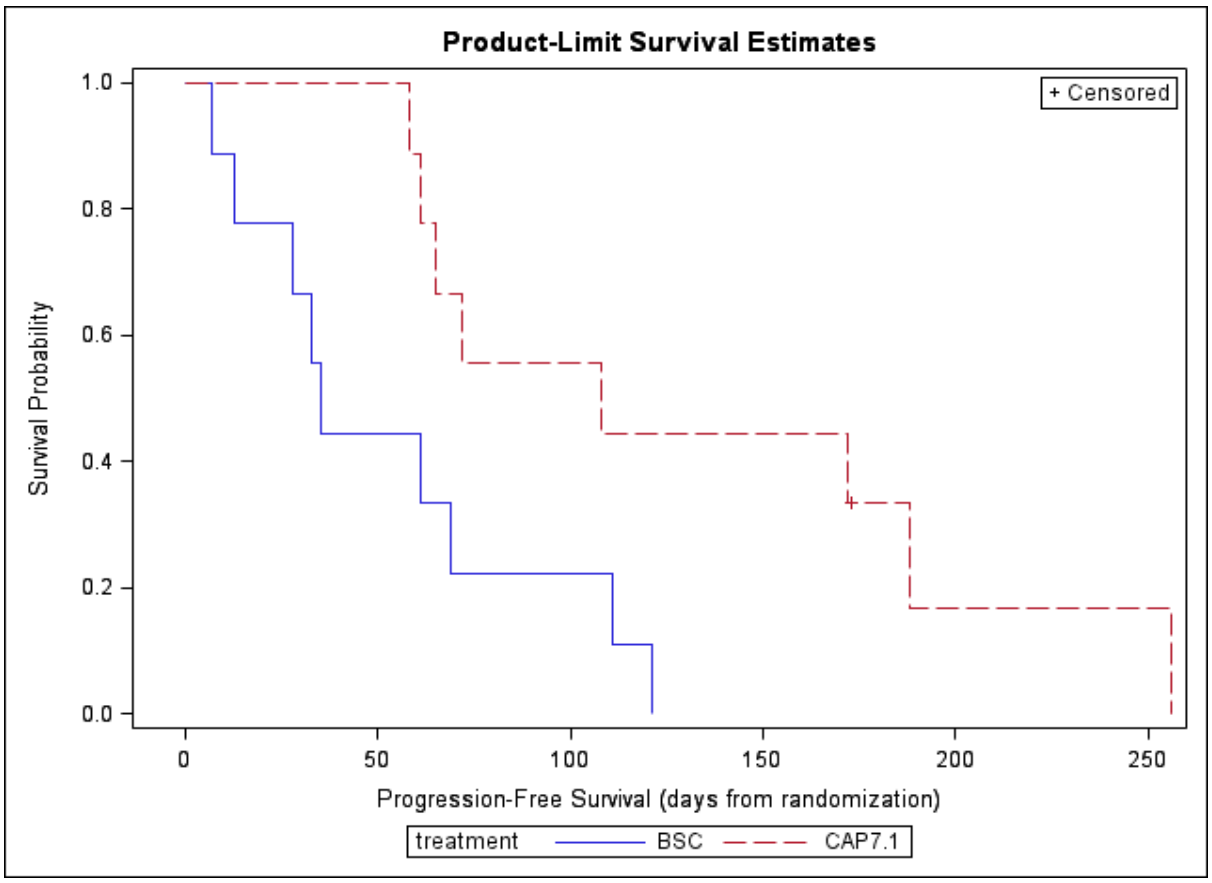


Figure 17 – PFS Kaplan Meier Plot

3.6.3 Overall Survival (OS)

While survival data from the study at the time of interim analysis was not yet mature, this endpoint as a group comparison was likely from the start to be of limited value because of the cross-over of BSC patients to CAP7.1 therapy after progression (2.1.4). At the time of interim analysis 9 of the 10 BSC group patients had crossed over to CAP7.1 therapy after progression while the one BSC patient who did not receive CAP7.1 (PAT05) was lost-to-follow-up. Thus BSC group patients acted as CAP7.1 group patients with a delayed treatment start (BSC phase) who therefore also had an expected similar OS compared to CAP7.1 group patients. This was also confirmed by the study data with CAP7.1 group patients having an mOS of 135 days (i.e. 4.5 m, range 38-467) and BSC group patients who switched to CAP7.1 after progression having a mOS of 140 days (i.e. 4.7 m, range 35-282) with p=0.37 showing the difference clearly not being statistically significant (Table 15). CAP7.1 group patients and BSC group patients after CAP7.1 switch thus benefited equally from CAP7.1 therapy.

Table 15 – OS from start of randomized treatment

	Full Analysis Set (FAS) N=22		Subjects with >= 2 cycles CAP7.1 (CAP7.1 group plus BSC group patients who switched to CAP7.1) N=14
	CAP7.1 Group (n=12)	BSC Group (n=10)	n.a.
Median [95% CI][1] in days	135 [38-467]	140 [35-282]	179 [75-467]
Range	12 to 467	30+ to 375+	58 to 467
N (%) censored	3 (25%)[3]	3 (30%)[4]	5 (36%)
P-Value[2]	0.37		n.a.

[1] Confidence interval calculated by Brookmeyer-Crowley method after complementary log-log transformation
[2] One-sided p-value from Log Rank Test
[3] 2 patients still alive at time of analysis, 1 patient still alive at date of last contact
[4] 2 patients alive at time of analysis, 1 patient (PAT05) lost-to-follow-up
[5] 5 patients all alive at time of analysis

Furthermore, pooling all subjects who received >= 2 cycles of CAP7.1 (CAP7.1 group plus BSC group patients who switched to CAP7.1) into one analysis group (Table 15) reveals a longer mOS of 179 days (i.e. 5.9 months, CI: 2.6-15.3 months) for all such CAP7.1 treated patients with the shortest OS for an individual patient being 58 days (2 months) and the longest survival for an individual patient even being 467 days (i.e. 15.3 months). The latter individual also experienced the longest PFS under CAP7.1 therapy of 182 days (i.e. 6 months).

The Kaplan-Meier plot for these patients is shown in Figure 18. The associated 1-year survival estimate is 41% which seems indeed encouraging for these stage IV patients after 1st-line therapy failure especially when considering that according to Mihalache (2010, n=133)[148] the global 1-year survival under a whole range of therapies including curative and palliative surgery, palliative endoscopic procedures (stents), percutaneous drainage, chemotherapy, and radiotherapy was 22.3% +/- 4.4% with 1-year survival for stage IV patients being even worse (17.1%). Overall these results are encouraging especially considering the advanced stage of disease of this patient group while these OS results also seem in line with and not inferior to the results of other second line therapies (chapter 1.2.6.2).

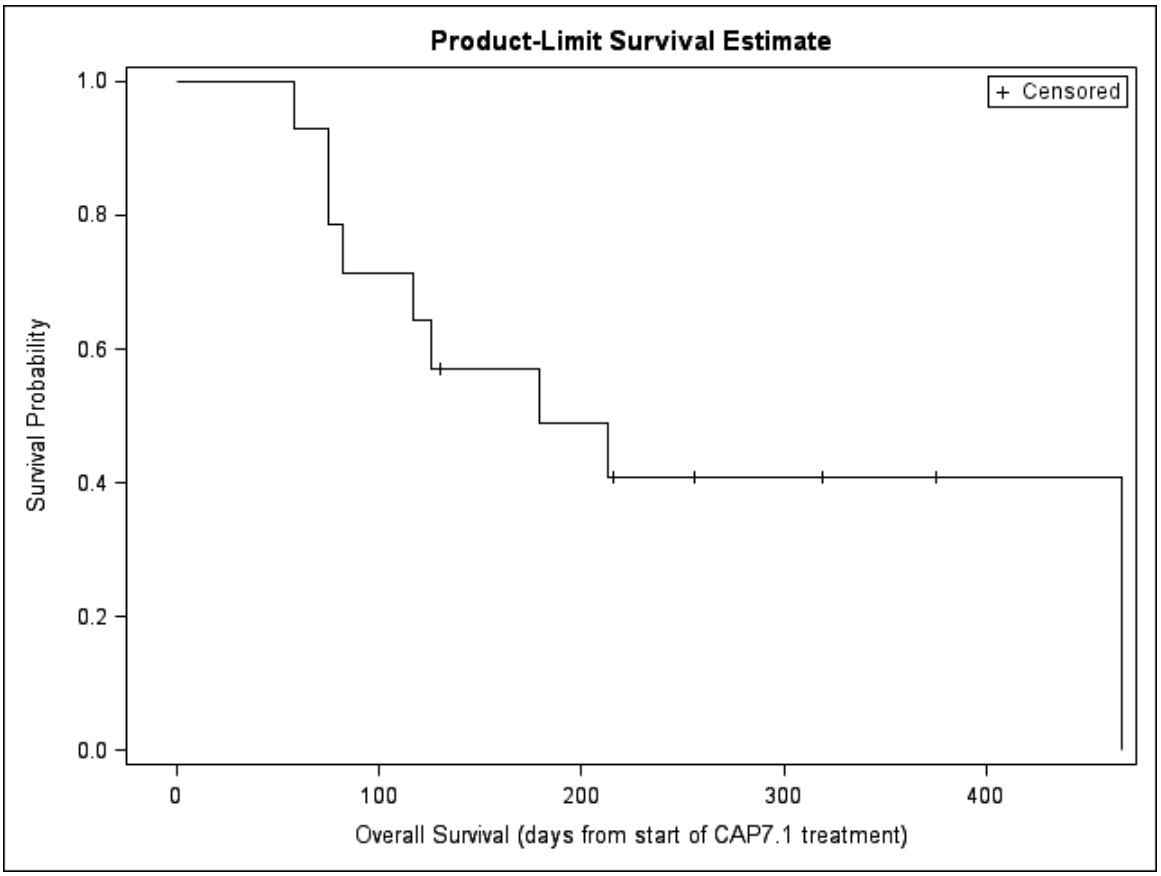


Figure 18 – OS Kaplan Meier Plot for all subjects treated with >= 2 cycles of CAP7.1

3.7 Clinical Safety Analysis

The safety population in this study was defined as all patients who received at least one CAP7.1 dose (2.3.a). At the time of interim analysis, 21 of the 25 in Table 8 (3.1) listed patients had received CAP7.1 and therefore constituted the safety population. Furthermore, Adverse Event (AE) including Serious Adverse Event (SAE) data was collected at every patient visit covering the entire study period from screening until 28 days after last study dose intake (2.2.3). An Adverse Event was defined as: "Any untoward medical occurrence in a patient or clinical trial subject administered an investigational medicinal product which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including any laboratory finding), symptom, or disease temporally associated with the use of an investigational medicinal product, whether or not considered related to the investigational medicinal product". Furthermore, a Serious Adverse Event was defined as: Any Adverse Event, i.e. "any untoward medical occurrence or effect that at any dose results in death or is life-threatening (i.e. the subject is at risk of death at the time of event) or requires hospitalization or requires the prolongation of an already existing hospitalization or results in persistent or significant disability or incapacity or is a congenital abnormality or birth defect". The respective

investigator assessed the causal relationship between every AE and CAP7.1 as either being "certain", "probable", "possible", "unlikely" or "not related".

Overall, at the time of interim analysis there were 311 AEs recorded for the 21 patients of the safety population (SP). Of these 311 AEs 191 (61.4%) had a reasonable causal relationship to CAP7.1 therapy (called "related AEs" from here onwards) while 120 (38.6%) did not (called "unrelated AEs" from here onwards). Of all AEs in the study (related and unrelated) 78.5% (244) were grade 1 & 2 (mild to moderate) while 19.6% (61) were grade 3-5 (severe, life-threatening, or fatal) and for 1.9% (6) the severity grade remained unknown.

The remainder of this analysis will only consider related AEs since only these are of interest in determining the CAP7.1 safety profile. Furthermore, it is important to emphasize that the severity grade describes the severity of an AE at its starting time and as such is not a description of AE outcome, i.e. a "life-threatening" AE was actually life-threatening at the time of onset and subsequently may either have led to death but also may have led to recovery again. Therefore, not all SAEs which lead to death are rated as "fatal" or "life-threatening". Instead, there were 3 SAEs that lead to death and according to the investigator's opinion were "possibly" related to CAP7.1. Two events with death as an outcome (Thrombocytopenia and Bronchitis) might be secondary to hemato-toxic CAP7.1 effects while the third event (hepato-renal syndrome with increased liver enzymes) according to the investigator maybe due to a too fast tumour lyses.

Otherwise, the severity grade distribution of all CAP7.1 related AEs is given in Figure 19. Of 191 related AEs, about 80% (152) were Grade 1 & 2 while about 20% (39) were Grade 3-5.

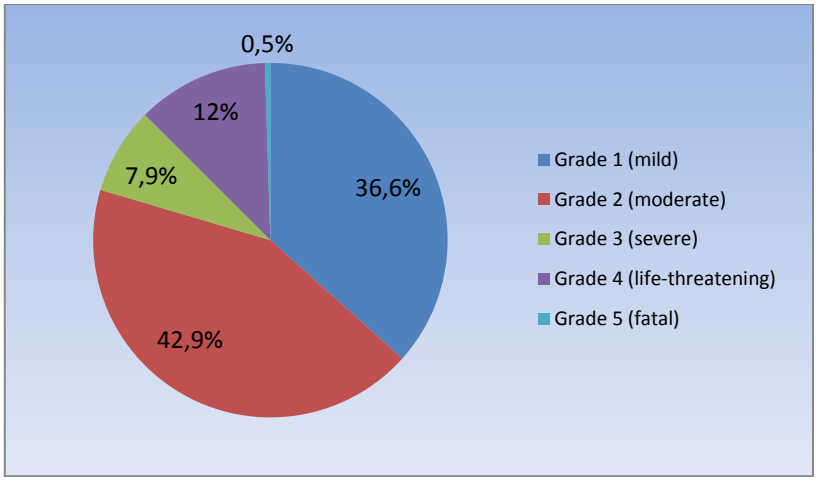


Figure 19 – Severity Grade Distribution of all CAP7.1 related AEs in percent (%)

Looking further into the exact type, frequency and severity grading of the most common (occurrence in >1 patient) and/or most severe (>= Grade 3) AEs reveals that events related to CAP7.1 therapy were almost exclusively hemato-toxic in nature (Figure 20).

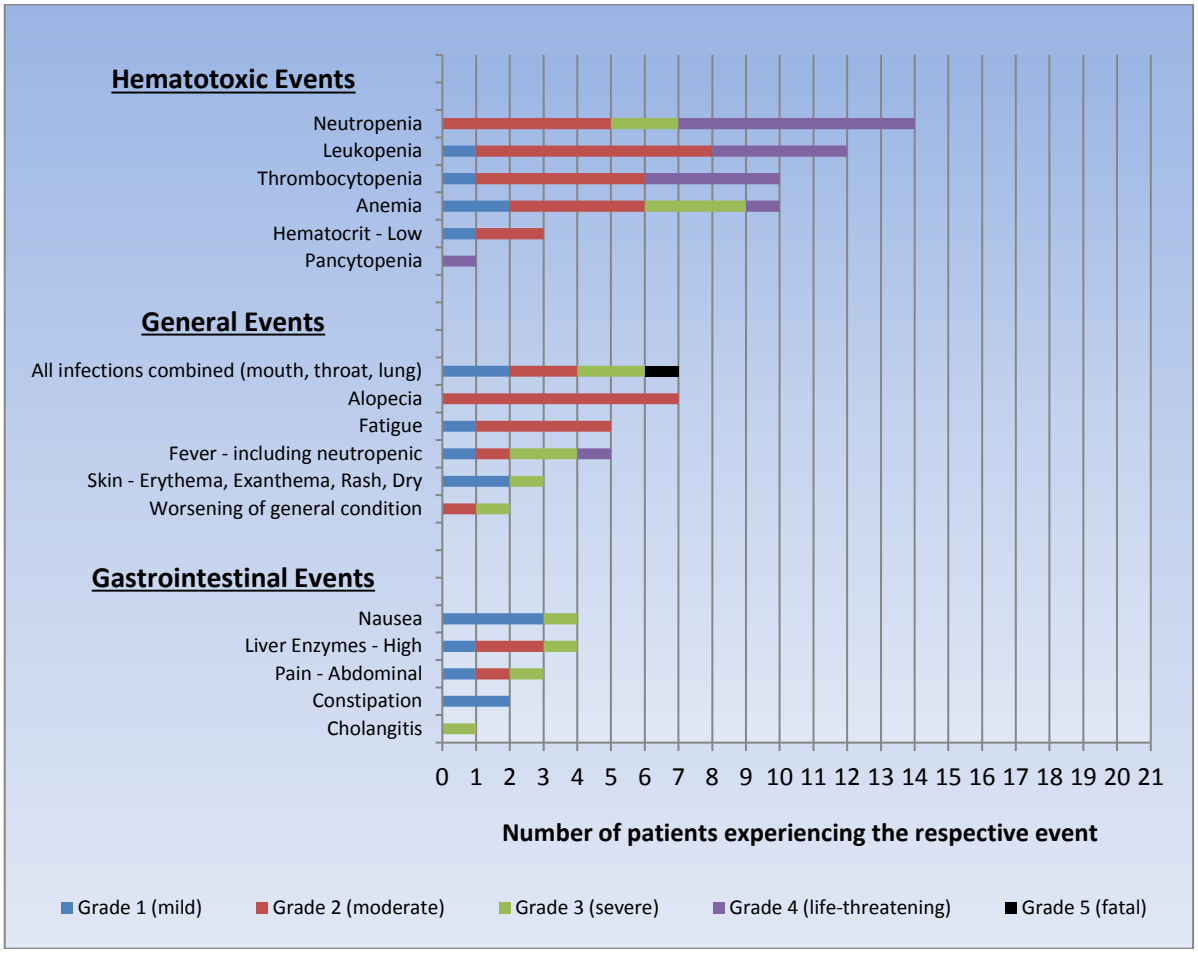


Figure 20 – CAP7.1 related AEs (Safety Population (SP), highest Grade per patient, occurrence at least in 2 patients or Grade at least 3)

The most common and also severe AEs generally occurring under CAP7.1 therapy therefore were neutropenia (67% of patients) followed by leukopenia (57%), thrombocytopenia (48%), anemia (48%), all infections combined (33%), alopecia (33%), fatigue (24%), fever – including neutropenic (24%), nausea (19%), increased liver enzymes (19%), abdominal pain (14%), mycosis mouth (14%), skin rashes (14%), low hematocrit (14%), lung infections (10%), throat infections (10%), constipation (10%), worsening of general condition (10%), cholangitis (5%), neutropenic fever (5%), and pancytopenia (5%).

Comparing these phase II study results with the results of the previous phase I study[140] reveals that the phase II study arrived at higher hemato-toxic event rates but lower gastrointestinal and general event rates for CAP7.1. However, the dose differences between both studies impact for the differences as two low dose cohorts were present in the phase I study. On the other hand

however, the safety profile of the CAP7.1 phase II study at 3-fold higher dose compared to etoposide resembles the hemato-toxic safety profiles of etoposide[149] and etopophos[150] closely (Table 16), but does not show organ toxic effects.

Table 16 – Comparison of safety profiles

Adverse Event	**CAP7.1[140] Phase I Study**	**CAP7.1 Phase II Study**		**Etoposide[149]**		**Etopophos[150]**	
	All Grade	All Grade	Grade 3 & 4	All Grade	Grade 3 & 4	All Grade	Grade 3 & 4
Hematologic Toxicity							
Leukopenia All Grade < 4000/mm^3, Grade 3 & 4 < 1000/mm^3	53% (10/19)	57% (12/21)	19% (4/21)	60-91%	3-17%	91%	17%
Neutropenia All Grade < 2000/mm^3, Grade 3 & 4 < 500/mm^3	21% (4/19)	67% (14/21)	43% (9/21)	-	-	88%	37%
Thrombocytopenia All Grade < 100000/mm^3, Grade 3 & 4 < 50000/mm^3	32% (6/19)	48% (10/21)	19% (4/21)	22-41%	1-20%	23%	9%
Anemia All Grade < 11 g/dl, Grade 3 & 4 < 8 g/dl	58% (11/19)	48% (10/21)	19% (4/21)	0-33%	-	72%	19%
Gastrointestinal Events							
Nausea and/or vomiting	58%-84% (11-16/19)	19% (4/21)	5% (1/21)	31-43%	-	37%	-
Anorexia / Loss of appetite	21% (4/19)	5% (1/21)	0% (0/21)	10-13%	-	16%	-
Constipation	37% (7/19)	10% (2/21)	0% (0/21)	-	-	8%	-
Abdominal Pain	26% (5/19)	14% (3/21)	5% (1/21)	0-2%	-	7%	-
Diarrhea	32% (6/19)	5% (1/21)	0% (0/21)	1-13%	-	6%	-
General Events							
Fatigue / Asthenia / Malaise	68% (13/19)	24% (5/21)	0% (0/21)	-	-	39%	-
Alopecia	53% (10/19)	33% (7/21)	0% (0/21)	8-66%	-	33%	-
Fever & Chills (including febrile neutropenia)	53%-74% (10-14/19)	24% (5/21)	14% (3/21)	-	-	24%	-
Dizziness	21% (4/19)	0% (0/21)	0% (0/21)	-	-	5%	-
CAP7.1 Adverse Event frequency data in percent of patients as documented in the Study Database on 06.11.2015							

To explore the hemato-toxic effects of CAP7.1 in more detail, the specific affected blood parameters of each patient were also individually analysed (Appendix 3). For 16 patients, it was evident that CAP7.1 influenced mainly the neutrophil and WBC counts and to a lesser degree also platelets, lymphocyte, and Hb levels.

Overall, 11 of the 16 patients experienced significant reduction in their neutrophil concentrations to around <=1000/nl while 8 of the same 11 patients in addition also had WBC concentrations below around <=1000/nl. Patient PAT02 is a good example (Figure 21 & 22).

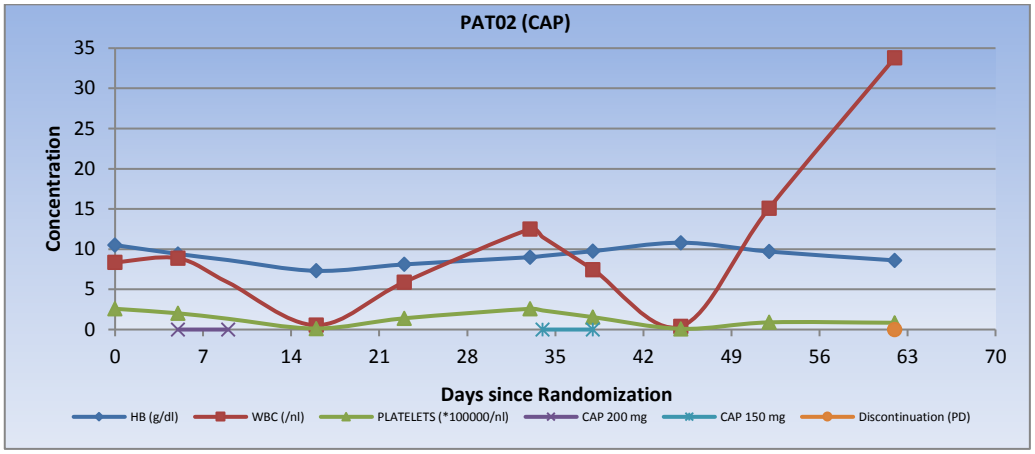


Figure 21 – Typical example of a CAP7.1 dependent reduction in WBC <= 1000 nl (PAT02)

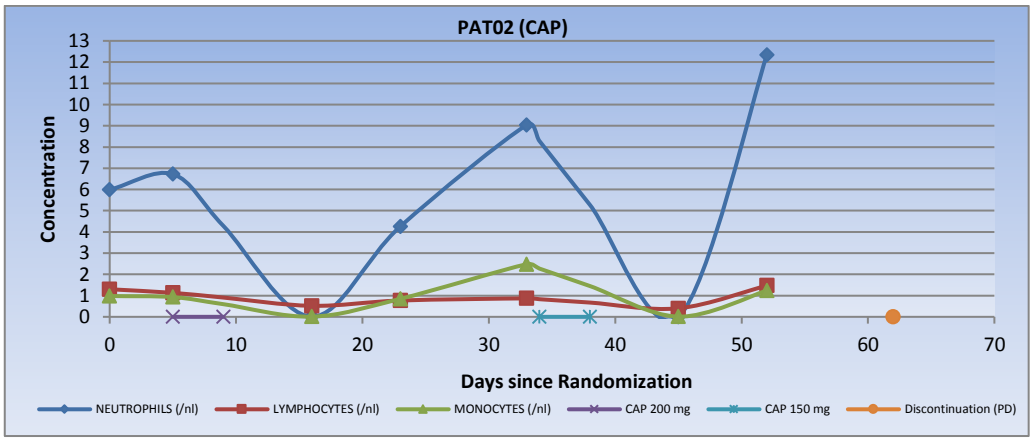


Figure 22 – Typical example of a CAP7.1 dependent reduction in neutrophils <= 1000 nl (PAT02)

The other 5 patients, however, had less dramatic declines in their neutrophil and WBC counts with PAT08 probably being the most stabile example (Figure 23 & 24).

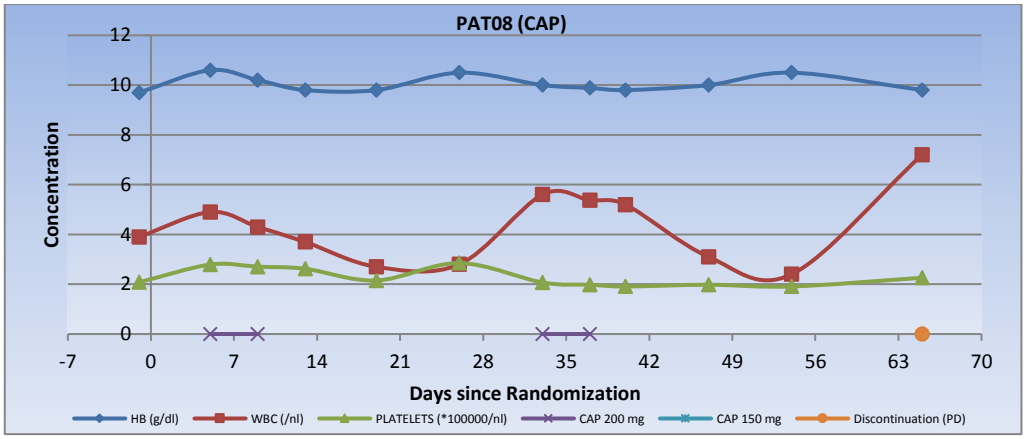


Figure 23 – Example of a relatively stabile WBC count (PAT08)

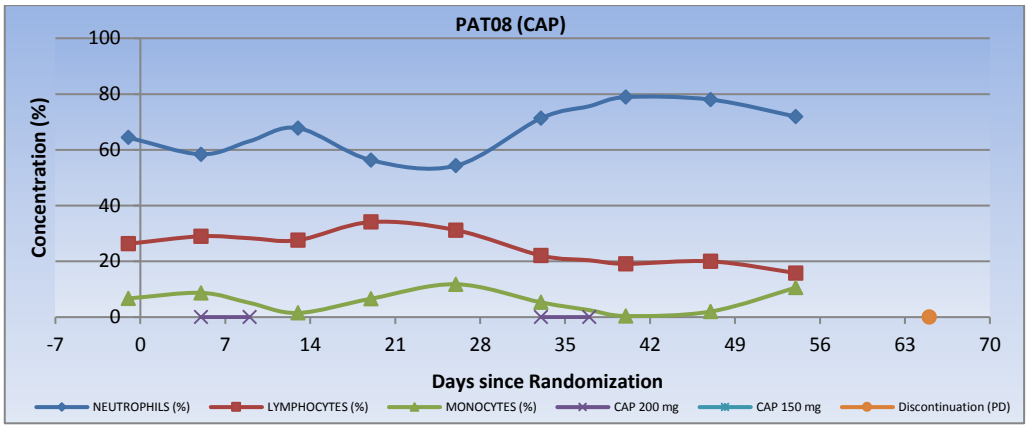


Figure 24 – Example of relatively stable neutrophils (PAT08).
Concentration in % since no absolute concentration per nl available for this patient.

Therefore about 69% (11/16) of patients displayed severe neutropenia and 50% (8/16) had severe leukopenia after CAP7.1 administration according to their haematology results with these percentages almost precisely matching up with the AE data otherwise presented above.

Furthermore, especially for patients who tolerated a high number of CAP7.1 cycles the haematology data shows impressively how blood parameters oscillate from cycle to cycle in a typical "saw tooth" pattern mainly influencing WBC and neutrophils (Figure 25 & 26).

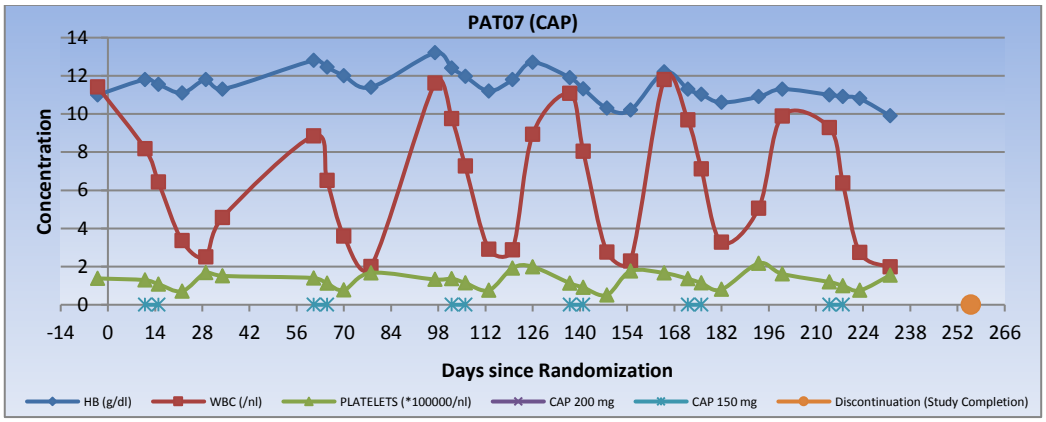


Figure 25 – Example of a typical "saw tooth" pattern (HB, WBC, Platelets) (PAT07)

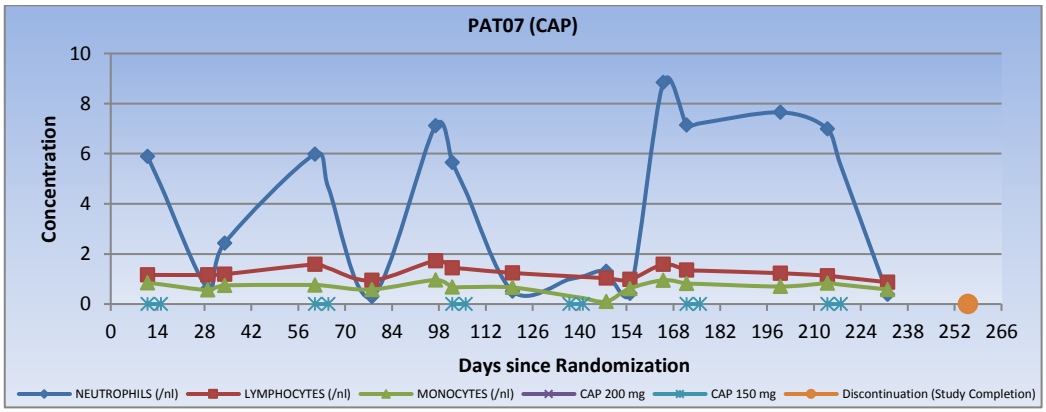


Figure 26 - Example of a typical "saw tooth" pattern (Neutrophils, Lymphocytes, Monocytes) (PAT07)

Last but not least, the graphs of patients PAT21 and PAT24 are interesting. Both patients developed a severe neutropenia after their first dose of CAP7.1 so that the investigator decided to administer Neulasta, a granulocyte-stimulating agent. As a result, neutrophil and WBC concentrations completely reversed avoiding any negative effects on neutrophil and WBC counts during the next CAP7.1 cycles (Figure 27 & 28).

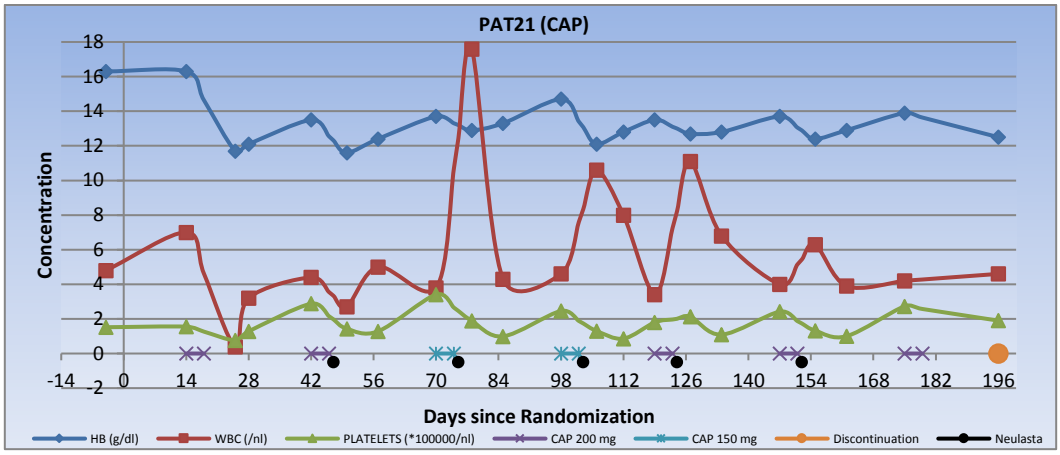


Figure 27 – Effect of Neulasta on WBC (PAT21)

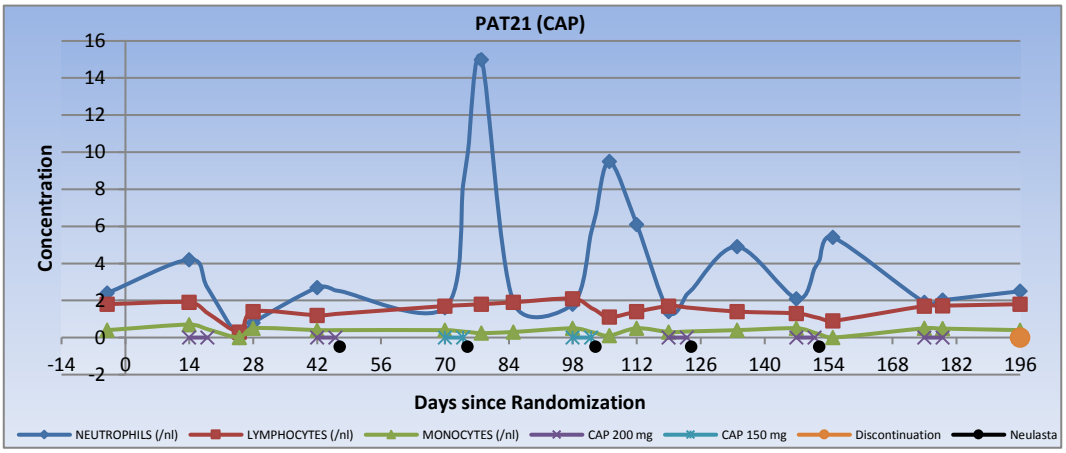


Figure 28 – Effect of Neulasta on Neutrophils (PAT21)

Therefore, administering granulocyte - stimulating agents in parallel to CAP7.1 administration may be a feasible way in the future to avoid severe neutropenias. The haematological data around CAP7.1 administration was incomplete for the following patients for whom a proper assessment of CAP7.1 effects therefore was not possible: PAT09, PAT12, PAT14 (only HB, WBC, and platelets after first CAP7.1 dose available), PAT16, PAT17.

In summary, this phase II study confirmed hematotoxic events in general (neutropenia [67%], leukopenia [57%], thrombocytopenia [48%], anemia [48%]) to be the most frequent side effects of CAP7.1 therapy with neutropenia probably being the most important. However, these

hematotoxic side effects are predictable, reversible and manageable. Other important and common side effects observed were infections (33%, likely secondary to neutropenia), alopecia (33%), fatigue (24%), fever – including neutropenic (24%), nausea (19%), and increased liver enzymes (19%). Furthermore, the high-dose CAP7.1 related hemato-toxicity safety profile is comparable to those of etoposide and etopophos while no organ toxicities were observed.

4. DISCUSSION

4.1 KEY TRIAL RESULTS IN THE CONTEXT OF THE CURRENT LITERATURE

While being a rare disease, CCA is the most rapidly growing cancer in the western world today with one of the worst prognosis. Diagnosis usually happens at advanced stages. Surgery is the only potential cure but only 20-40% progress to surgery while up to 80% are in need of other therapies. Thus only 2-4% of patients initially diagnosed and 14.5% undergoing surgery are cured while 96-98% of patients die within 10 years. Therefore most patients including resected patients receive chemotherapy at least at some stage before they die. While under the currently established GEM/CIS 1st-line chemotherapy standard for advanced and unresectable BTC patients have a maximum mOS of about 11.7m and mPFS of 8.0m, these figures drop to an average mOS of 5-7m and mPFS of 3m for 2nd-line regimes after GEM/CIS failure with no clear advantage of one regimen over all others and therefore no 2nd-line chemotherapy standard existing. Furthermore, 2nd-line therapy studies generally seem rather uncontrolled targeting different mixed general patient populations and disease stages and thus often mixing patients who are at completely different points along the collective patient journey in one treatment group (e.g. mixing resected and unresected patients) while also involving different lines of chemotherapy which by far and large have already been trialed as 1st-line therapy in the past including FOLFIRI/XELIRI, 5FU, Capacitabine, GEM/5FU, 5FU/CIS, Mytomicin-C, FOLFOX, XELOX, GEM/CIS, GEMOX[85], Epirubicin/CIS/5FU, GEM/Irinotecan, GEM, amongst others[84,85]. In addition, also new targeted therapies only showed marginal to no benefit with the one study showing largest effect conducted in a 1st-line setting, however, purposefully also targeting a highly selected KRAS WT subpopulation instead of a general mixed patient population[89,95]. Otherwise, however, also the bulk of targeted therapy studies seem rather uncontrolled using different mixed and at the molecular/genetic level undifferentiated patient populations with many of them in addition trialing new targeted compounds only in the 1st-line setting or even in mixed 1st and 2nd-line patient populations so that results between studies by far and large are not comparable and certainly also are not transferable to the 2nd-line setting.

Furthermore, a major general weakness of most if not all 2nd-line therapy studies is the lack of a BSC control so that superiority over BSC only has generally not been established for any of the trialed 2nd-line regimen. For all these reasons there is an urgent need to develop new and more effective therapies for patients with end stage disease who have relapsed after 1st-line therapy.

While there would be already merit in trialing etoposide itself in 2nd-line therapy on the basis of its successful past use in combination therapies in 1st-line[80, 131] with Glimelius (1996, n=90)[80] even being the original study often cited showing superiority of chemotherapy over BSC only therapy in general, CAP7.1 as a new etoposide prodrug with its higher cytotoxicity and its lower side effect profile compared to etoposide as well as its ability to overcome MDR-1 mediated resistance while being activated and thus concentrated predominantly in specific organ and tumor tissues with high CES2 expression including liver and gallbladder tissue has a unique potential to improve the outcome of especially advanced, chemotherapy resistant refractory BTC patients after 1st-line GEM/CIS failure for whom basically otherwise all established treatment options have been exhausted. Therefore, these patients constitute an unmet medical need at a clearly defined and therefore also clinically relevant point along the collective patient journey. However, published data suggests that only about 15-25% of patients after 1st-line GEM/CIS failure are actually fit enough to receive 2nd-line chemotherapy[86]. One general difficulty, therefore, with this specific target patient population is the fact that it represents a quite highly selective subgroup of the usually in the literature described "mixed general BTC study populations" for whom the exact survival outcome under BSC only therapy, which is the standard of care for this patient group, is not exactly known. However, what is known from the literature is the fact that survival generally decreases with disease stage. For example Waseem (2017, n=242)[45] reported OS for TNM Stage I, II, III, IV of 23, 25, 14, 4.5m respectively after surgical resection while Park (2009, n=330)[26] reported 6.0m (I), 7.4m (II), 4.4m (IIIA), 3.6m (IIIC), and 2.5m (IV) for BSC only treated patients. Furthermore, Agarwal (2016, n=26)[32] described a reduction of mOS from 10.5m to 2.9m for patients under GEM/CIS 1st-line therapy in the presence of three risk factors including disease stage (PS ≥2, CEA >3, and stage IVb) and Glimelius (1996, n=90)[80] arrived at a similar mOS of 2.5m for BSC only treated patients compared to 1st-line chemotherapy. It is thus likely that the survival of the target population of this study, who are basically stage IV patients after 1st-line therapy failure, is lower under BSC only therapy in 2nd-line than the above mentioned 2.9-2.5m estimates under 1st-line therapy and therefore very poor indeed. However, the fact that the target population of this study were the "fit" of these "final unfit/advanced" patients added even further complexity to the situation. As a

direct consequence also the times to progression for these patients under BSC only therapy can only be expected to be considerably shorter than the overall survival times mentioned above but exact times remain unknown. Thus, a randomized two arm design directly comparing CAP7.1 therapy with BSC only therapy for this patient group was chosen in the here described phase II clinical trial in order to have a direct and meaningful comparison of therapy outcomes rather than relying on comparisons from the literature. To the author's knowledge this is the only randomized trial in this specific patient population and indication until today that specifically compares a chemotherapy outcome with BSC only therapy, which therefore is unique.

It is also in view to this assumedly very poor prognosis and short life expectancy of these particular patients with an even shorter time to progression expectancy, that a shorter than usual radiological tumor assessment interval of 8 weeks (rather than 12 weeks as otherwise usual e.g. in ABC-02) was chosen and a primary endpoint objective of 35% disease control rate was felt to represent a clinically relevant outcome for this phase II study and specific patient population. While the treatment groups in regards to baseline characteristics were balanced, this primary clinical outcome objective was actually even exceeded in the PP analysis ($DCR_{CAP7.1}$= 56%, DCR_{BSC}= 0%, p=0.014) thereby indicating a strong trend in favor of CAP7.1 treatment compared to BSC only therapy even though the study did not reach the stringent statistical significance level of 0.00154 determined by the O'Brien Flaming method for the first interim analysis and thus could not be halted on the grounds of efficacy at this stage. Furthermore, the author is also aware that the disease control rate can be a difficult endpoint due to subjectivity of the local radiological reviewers and spacing out of the RECIST assessments. However, for this reason the study protocol also states that a blinded, independent central radiological review of all CT scans will be performed in addition to the local radiologist results presented here in order to assess the robustness of RECIST and PFS results in accordance with EMA guideline (EMA/CHMP/27994/2008/Rev.1). The results of this central radiological review, however, will only be available in the final study report and thus are not part of this thesis.

The above discussed primary endpoint results are further strengthened by the secondary PFS and OS results. Usually OS is the preferred endpoint as the "gold standard" of oncology trials. Unfortunately, due to the cross-over option to CAP7.1 therapy for BSC group patients after initial progression the OS endpoint in this trial is only of limited value since in the end all but one BSC group patient also received CAP7.1 therapy. Thus, a similar OS between the two treatment groups is expected. However, Moriwaki (2016)[91] recently showed that mPFS correlates strongly with mOS in advanced BTC patients indicating that mPFS is indeed an appropriate

surrogate endpoint for mOS in phase II trials. This gives further significance to the PFS results of this study. Overall, these results show a significant difference between the treatment groups with an mPFS of 108 days (CI: 58-188) for CAP7.1 treated patients compared to only 35 days (CI: 7-111) for BSC treated patients ($p < 0.01$). These mPFS times also match well with the life expectancy of less than 2.5m from the literature for this patient group. CAP7.1 therefore prolongs PFS from 1 months in the BSC arm to 3.5 months in the CAP7.1 arm which is a promising initial result considering small patient numbers and the short life expectancy of this particular patient population.

Furthermore, while median OS for both treatment groups was around the 135-140 day mark (i.e. 4.5 months, range 35-467 days) and thus similar between the two treatment groups ($p=0.37$) as expected due to the cross-over of BSC group patients to CAP7.1, patients who actually tolerated at least 2 cycles of CAP7.1 even had a higher median OS of 179 days (CI: 75-467), i.e. 5.9 months (CI: 2.6-15.3m) and thus benefited even more from CAP7.1 which seems impressive compared to the 2.5m mOS estimate derived from the literature. Furthermore, the study arrived at a 1-year survival rate estimate of 41% which is encouraging considering that Mihalache (2010, n=133)[148] arrived at a 1-year survival of 22.3% +/- 4.4% for a mixed treatment population and only 17.1% for stage IV patients.

In summary, results for the three most relevant oncology trial endpoints (DCR, PFS, OS) point toward a common CAP7.1 effect trend over BSC only therapy.

However, since BTC is such heterogeneous and individual risk factor dependent disease as demonstrated in the introduction, there is great merit in examining individual patient level results. Of great interest in this respect are the within patient comparisons (before and after) of the 9 BTC group patients who switched to CAP7.1 after initial progression under BSC therapy. These within patient comparisons of treatment outcome are independent of differences in prognostic factor profiles between individual patients so that observed effects cannot be due to differences in individual prognostic factor profiles which is another very unique trial design feature not usually found in the literature that therefore increases the overall value of this study significantly again.

Individual RECIST assessment results revealed e.g. that five patients in the CAP7.1 group and two patients in the BSC group after initial progression and switch to CAP7.1 therapy achieved a stable disease status with one additional BSC patient responding to CAP7.1 treatment with partial response as best response. This is in strong contrast to the fact that all measured best

responses under BSC only therapy representing exclusively progressive disease (measurements. Therefore, actual durable disease stability (as well as partial response in this study were exclusively observed under CAP7.1 therapy. Furthermore, of the eight SD/PR responses under CAP7.1 therapy, five lasted between five and seven months, one additional response lasted three months and the other two responses lasted approximately two months. These are long individual response times in patients whose median overall survival from the literature otherwise is estimated to be around the 2.5 months mark or less. FDA acknowledges that tumor responses do not necessarily equate with clinical benefit from delay in tumor progression, thus durable SD can be as valuable as PR (Kiba, 2011)[152].

The clinical relevance of SD duration, however, varies for different tumor types and grades. For BTC it is important as the tumors grow rather diffusely within the biliary tract making precise measurements of tumor size and comparisons challenging. Furthermore, some new treatments such as anti-angiogenic or cytostatic / molecular based targeted therapies are designed to stop the development of new tumor blood vessels, predominantly inhibiting tumor growth rather than destroy existing tumor tissue. Thus, they are expected to halt the progress of disease rather than shrinking the tumor. Therefore, in this study observed and partially even relatively long-lasting disease stabilization of a considerable number of individual patients as objective response in this otherwise difficult to treat/relapsed advanced BTC population is interpreted as highly promising initial results.

Furthermore, it needs to be acknowledged that the RECIST definitions of what actually constitutes progressive disease (increase >= +20% of total diameter), stable disease (within -30% to +20% of total diameter), or partial response (decrease > -30% of total diameter) appear quite arbitrary with especially considerable tumor diameter shrinkages of up to -30% not actually being acknowledged as such while also a significant slowdown in tumor growth especially during a progressive disease state is not necessarily acknowledged as a therapy success unless this slowdown also leads to a less than +20% overall diameter growth changing the assessment status then from PD to SD. Another important drawback of the RECIST evaluation system is the fact that it does not consider time as a factor. For example a tumor may grow at a speed of just under 20% in 6 weeks. Therefore, if RECIST assessment is performed every 6 weeks, this patient is assessed as having a stable disease status while in 8 or 12 weekly assessments, the same patient and tumor growth rate would be assessed as progressive disease. For these reasons, additional analysis results such as best objective response (Waterfall Plot, section 3.5.1), average

tumor growth per time unit (section 3.5.2) or individual tumor response graphs (section 3.5.3) provide valuable additional efficacy information.

For example, when looking at the best objective tumour response as described in chapter 3.5.1 it is striking to see that actually the smallest increases in tumour diameter growth as well as all tumour diameter shrinkages were achieved under CAP7.1 therapy while the two biggest increases in tumour diameter growth appeared under BSC therapy only. Furthermore, three patients in the BSC group who switched to CAP7.1 after initial progression experienced a diminished tumour diameter growth under CAP7.1 as best response compared to BSC only therapy. Two of these patients experienced meaningful tumour diameter shrinkage.

Similar results were observed in regards to the average best (i.e. slowest) tumour diameter growth rates over time (chapter 3.5.2) where on average tumours grew much slower under CAP7.1 therapy (median diameter growth: 0.05 mm/day) than under BSC only therapy (median diameter growth: 0.55 mm/day). It could therefore be argued that CAP7.1 slows down tumour diameter growth on average by 91% or by a factor of 11 compared to BSC therapy even though these results need to be interpreted with caution. While these results sound like a very significant effect, it needs to be considered that the theoretical model behind the RECIST assessments is an exponential one where actually the diameter of an otherwise theoretical spherical tumour mass is measured. Overall, the volume of a spherical object is calculated as follows:

$$Volume_{Object} = 4/3 * pi * (diameter/2)^3 \text{ with “pi”= } 3.14^{153}$$

The most striking individual example of a slowed down tumour growth besides the tumour shrinkages in this study is patient PAT18 who experienced a significant slowdown in total tumour diameter growth from 2.92 mm/day under BSC therapy (the highest growth rate of all patients) to 0.5 mm/day under CAP7.1 therapy which represents a 83% reduction in tumour diameter growth rate. The corresponding target lesion measurements of patient PAT18 are: T_0 = 2.28 cm, T_1 = 3.42 cm, T_3 = 3.73 cm and thus the corresponding spherical volume estimates are: $V_{T0} = 4/3 * 3.14 * (2.28/2)^3 = 6.2$ cm^3, $V_{T1} = 4/3 * 3.14 * (3.42/2)^3 = 20.9$ cm^3, $V_{T3} = 4/3 * 3.14 * (3.73/2)^3 = 27.2$ cm^3. Therefore, under BSC only therapy the theoretical tumour mass increased from 6.2 cm^3 to 20.9 cm^3 by 14.7 cm^3 or 237%, while under CAP7.1 the theoretical tumour mass increased further from 20.9 cm^3 to 27.2 cm^3 by 6.3 cm^3 or only 30%. Thus, while CAP7.1 therapy did not stop tumour volume/mass growth completely or reversed growth, tumor growth was slowed down by 237-30=207% compared to BSC only therapy. These findings become more meaningful considering solely advanced last stage BTC patients were treated with CAP7.1.

It has to be emphasized, however, that the calculations performed here are only a theoretical model and simplification of the real situation since they add up the diameters of several target lesions into a single figure calculating the total volume/mass of one main theoretical lesion, which in reality does not exist. However, these calculations demonstrate the main key point, which is: The mathematical relationship between the lesion diameters measured according to the RECIST guidelines and the actual tumour mass of the patient is not linear but exponential. Therefore, even small reductions in lesion diameter growth equate to much larger reductions in tumour mass growth. For this reason, however, even very considerable effects on tumour mass growth may be overlooked if only relying on the RECIST system because like in this example a new compound like CAP7.1 may actually significantly slow down tumour mass growth from e.g. +207% to +30%. However, because diameter growth is above +20% the RECIST assessment still results in a PD. It is therefore possible that while patients in reality benefit from a new therapy like CAP7.1, this benefit does not show up in their RECIST assessment. In this respect it therefore also does not surprise that despite the impressive above described results, patient PAT18 in the interim analysis dataset still had a PD assessment not only under BSC only therapy but also under CAP7.1 therapy. In turn, if therapy subsequently is ceased because of negative RECIST assessment, as happened to patient PAT18, this may impact negatively on the patient's overall survival because an actually effective therapy, which in reality does slow down tumour mass growth considerably, is ceased on false grounds.

In addition, another patient with a reverse tumour growth situation highlighting another potential problem in regards to drawing conclusions from RECIST measurements with a potential to underestimate CAP7.1 effects is patient PAT06. This patient actually experienced a tumour diameter growth of 0.59 mm/day under BSC therapy, which increased further to 0.77 mm/day under CAP7.1 therapy thus representing an increase of about 31% in tumour diameter growth rate. Consequently, the patient also has two PD ratings in his RECIST assessments. However, even though tumour growth has increased by 30% under CAP7.1 therapy so that CAP7.1 therapy seems ineffective at first sight, in reality an alternative situation may exist whereby the increase in tumour diameter growth rate for this patient under BSC only therapy would have actually been much higher than observed due to the progressive nature of end stage disease while CAP7.1 therapy actually and in reality still slowed down tumour growth to the observed level. Therefore, the possibility of a slowing effect on tumour growth of CAP7.1 cannot be excluded on the ground of the observed data. However, even if there is a CAP7.1 effect on tumour growth in

reality, this would not affect the PD RECIST assessment of this patient since the measured tumour diameter growth is 30%.

Last but not least, a potential slowing effect on tumour growth of CAP7.1 can neither be confirmed nor excluded for patients PAT20 and PAT24 since confirmation of progression under BSC therapy was only asserted via ultrasound (PAT20) and clinically (PAT24) so that the in-between RECIST measurement is missing.

In summary, considering the individual within patient tumour responses of the 9 BSC group patients who after progression received CAP7.1 therapy, it becomes clear that 3 of these 9 patients (33%) benefitted from CAP7.1 therapy compared to BSC with two having experienced tumour shrinkages and the third one having experienced the strongest decrease in tumour diameter growth of all other patients. For another three (33%) patients an actual benefit from CAP7.1 therapy can neither be excluded nor confirmed because conclusive data is missing. Of the last three patients, patient PAT09 had the second fastest tumour diameter growth under BSC therapy (2.33 mm/day) and subsequently died very rapidly while not even having received a single cycle of CAP7.1, while patient PAT13 died of a lung infection, and patient PAT17 died of an unexpected acute cardiac event. Therefore at least 33% and potentially more of these patients actually benefitted from CAP7.1 therapy compared to BSC therapy. In the context of this highly heterogeneous and individual risk factor dependent disease it seems once again important to emphasize that these results are intra-individual results which are therefore independent of risk factor differences between patients.

The final secondary objective of this study was to determine and assess the CAP7.1 safety profile. The analysis of conventional safety data collected over the entire course of the study until 28 days after last study drug intake (i.e. AE and SAE reporting) revealed that as was expected, hematotoxic effects (neutropenia [67%], leucopenia [57%], thrombocytopenia [48%], anemia [48%]) and related infections ([33%], lung, throat, mouth) were the main adverse events associated with CAP7.1 intake with all other life-threatening (except patient PAT12 who died from tumor lyses syndrome) and the only fatal event recorded being possibly associated with hematotoxic effects.

Comparing the CAP7.1 safety profile with the official safety profiles of etoposide and etopophos revealed that these profiles are comparable. This is remarkable in so far that as was explained in chapter 2.1.3, CAP7.1 at the used dosages is estimated to deliver an up to 3-fold higher cytotoxic concentration than etoposide. Therefore, non-inferiority of the CAP7.1 safety profile compared

to etoposide and etopophos is an important confirmatory finding indicating that indeed CAP7.1 is a safe option, which might deliver a local cytotoxic tumor effect compared to etoposide or etopophos under a comparable safety profile. Therefore, the clinical use of CAP7.1 is considered to be at least as safe regarding the hematotoxic effects as and superior regarding organ toxicitiy compared to etoposide or etopophos as drugs with existing market approval.

4.2 Trial Limitations

Sample size is a relative limitation of this trial. Quite obviously from a traditional statistical and research point of view it seems often desirable to have an as large sample size as possible. However, in a phase II clinical trial in a rare indication like this one practical considerations and especially patient safety concerns have to prevail and need to be the primary focus. While in a rare disease like BTC it is already practically very difficult to achieve large sample sizes, the main focus when a potentially toxic new investigational product is trialed must always be patient safety. For these two reasons a group sequential design was applied in this study which is a type of dynamic, adaptive study design that allows to stop a study early if efficacy or futility is shown in interim analysis before all "experimental units" (i.e. patients) have been "spent" (i.e. treated)[145,146]. Such design therefore protects patients who so far have not been treated (i.e. "spent") from potential harm if in reality it is highly likely that the treatment under investigation is ineffective or too toxic while at the same time also protecting patients in the control group from not receiving a superior treatment if in reality it is highly likely that the treatment under investigation is actually more effective than its comparator treatment. In this way these methods can also offer substantial sample size reductions compared to a traditional single stage design[146]. The draw-back of such design, however, is that obviously it is very difficult to meet the stringent statistical significance level requirement especially at the time of first interim analysis. Therefore, unless the observed difference in the primary endpoint between the two treatment groups is indeed extreme and thus becomes statistically significant already with a small sample size, such interim analysis is likely and, in this way, also a priori rather expected to produce results that are statistically not significant at this first analysis stage. Consequently, however, the real emphasize in such first interim analysis is most of the time on identifiable strong result trends which justify study continuation until the next stage/interim analysis with larger sample size and a less stringent statistical significance level while if trends are discouraging, it may be time to stop the study on this ground instead. Therefore, when looking at the results of this study, it needs to be understood that the main objective of the first interim analysis is actually only to decide whether to continue or stop the conduct of the study at this point in time and on the basis

of the so far observed data rather than to decide once and for all whether CAP7.1 therapy is actually effective and safe or not as would normally be the goal in a final analysis of a traditionally designed study. Thus, the latter question of CAP7.1 efficacy and safety in this study will also only be definitely answered when the full recruitment target of in this case 50 patients (chapter 2.3) is finally reached unless, however, the observed effects at the respective interim analyses are so extreme, that results become statistically significant already earlier and the study is prematurely halted on this basis. Therefore, however, having identified strong and encouraging trends in this first interim analysis is actually a very encouraging, positive outcome even if statistical significance was not reached because the study was deliberately designed in a way that statistical significance at first interim analysis is extremely difficult and rather unusual and unlikely to be reached.

Another relative limitation is the time interval between RECIST assessments used. Even though an 8 week (every 2nd cycle for CAP7.1 patients) interval is already short compared to other studies in the same indication, for the severely ill patient population of this study with an extremely short life expectancy of sometimes even only weeks rather than months, a six week or even four week interval would probably have been more meaningful from a pure research perspective allowing for more precise measurements in a research environment that is also looking for effects lasting rather for weeks than months. However, best research practices always need to be weighed up against practical concerns and quality of life considerations for this severely ill end stage patient population. Therefore, what may be desirable in terms of research practice may sometimes not be practically or ethically viable and in this study 8 week intervals for different reasons seemed the overall best compromise. Furthermore, however, potential problems with RECIST measurements and their timing were already discussed in the previous section (4.1) using examples from this study as was the need for the conduct of an additional external, independent radiological review according to EMA and FDA guidelines.

Furthermore, missing values constitute a limitation for this study. However, these missing values lead to an underestimation rather than an overestimation of the CAP7.1 effect so that the results presented in this thesis can be considered very conservative and robust. Further RECIST measurements were missing mainly due to adverse events or death or other circumstances not allowing CT scans to be performed.

Another limitation was the CAP7.1 starting dose. According to protocol, the CAP7.1 starting dose was 200 mg/m^2. However, for different reasons only eight patients of the safety population

(SP) were started on 200 mg/m^2 while 12 patients were started on 150 mg/m^2 and one patient was even started on 110 mg/m^2. Therefore, the majority of patients were actually started at a lower dose than 200 mg/m^2, which may have affected efficacy and safety results.

Two other limitations follow from ethical considerations. First of all, even though the study had a randomized design it was open label since it did not seem feasible to conduct a double-blind trial for a cytotoxic drug which potentially therefore may have introduced some bias into the study. Second of all, for ethical reasons as well, patients in the BSC group were allowed to cross-over to CAP7.1 therapy once they progressed under BSC only therapy. This practice, however, on one hand limited the value of the OS analysis since in the end basically also all BSC group patients except for one patient received CAP7.1 after progression. However, on the other hand this unique design feature also made an intra-patient comparison of the two treatments possible which added significant value to the study.

Finally it needs to be mentioned that this phase II clinical trial was designed at a time when GEM/CIS had just been established as the new 1st-line therapy standard for advanced BTC while most of the other more recent findings from the literature as summarized in the introduction of this thesis were still not known/available. The study design therefore also did not consider/address some of the main important literature findings such as the fact that resected patients generally have a better therapy outcome than unresected patients under any additional subsequent therapy. However, as shown in chapter 3.2 (baseline characteristics) about one third of patients were surgically pretreated, 75% of which were in the BSC group. Therefore, this fact can potentially only lead to a further underestimation of the CAP7.1 effect since the majority of surgically pre-treated patients was actually in the BSC group. In addition, there is also a strong general individual prognostic factor dependence of therapy outcome emerging from the current literature so that outcome may in fact be very different for different patient sub-groups. Also this finding can potentially introduce bias to the study if not appropriately considered. However, because both these findings had not yet emerged so clearly from the literature at the time this study was designed, the study was also not designed to enable meaningful relevant subgroup analyses which in turn would have surely also required a larger sample size. Nevertheless, however, because besides the typical between treatment group comparisons this study also provides a within patient comparison of the two treatments for 9 of the BSC group patients, it still provides invaluable information also in the light of these recent literature findings in a very elegant way because these individual within patient comparisons actually are completely independent of prognostic factor profile differences between the patients including also

differences in surgical pre-treatment since their reference point for comparison is the same patient with the same prognostic factor profile no matter how complex this profile otherwise may be. For this reason, the cross-over design of this study is a blessing which further increases the overall validity and quality of the study especially also in the light of all recent literature findings and even if it limits the value of the OS analysis in turn. Furthermore, since Moriwaki (2016)[91] in addition were able to show that mPFS is an appropriate surrogate endpoint for mOS in phase II trials in this indication (see above), also the latter limitation seems not really relevant anymore.

4.3 Overall Summary and Conclusions

CCA is the most rapidly growing cancer in the western world today with one of the worst prognosis and an urgent need for the development of new and more effective therapies. The main objective of this randomized, open-label, multicenter, parallel, controlled, two-group phase II study is the evaluation of the efficacy and safety of CAP7.1, a new etoposide prodrug, in adult patients with advanced therapy refractory BTC. The main primary objective of this study is to demonstrate disease control in more than 35% of patients treated with CAP7.1 using a group sequential study design according to the O'Brien Fleming method. This thesis presents the results of the first interim analysis which was due once data for the first 18 evaluable patients of an overall sample size of 50 patients was available. Unless the interim analysis results are so extreme that efficacy and safety of CAP7.1 can either be clearly established or refuted, the main objective of this first interim analysis was not predominantly to demonstrate clear statistical significant superiority of CAP7.1 therapy over BSC only therapy, but rather to identify meaningful trends in the so far collected data which either justify the continuation or discontinuation of the trial on the grounds of the so far observed data.

Overall, the in this thesis presented interim analysis results identify a very encouraging trend in the study's primary as well as secondary endpoint results in favor of CAP7.1. Treatment groups were generally balanced in terms of baseline characteristics. The disease control rate as the primary study endpoint actually exceeded the study's set target of 35% as a meaningful clinical endpoint in the PP analysis ($DCR_{CAP7.1}$= 56%, DCR_{BSC}= 0%, p=0.014). However, the study did not reach the stringent statistical significance level of 0.00154 as determined by the O'Brien Flaming method for the first interim analysis, i.e. even though these results are very encouraging, they are not extreme enough (p=0.014 > p=0.00154) to simply halt the study already at this stage on the ground of these positive results. Furthermore, also the PFS results show a significant difference between the treatment groups with an mPFS of 108 days (CI: 58-188) for CAP7.1

treated patients compared to only 35 days (CI: 7-111) for BSC treated patients ($p < 0.01$). In addition, median OS for both treatment groups was 4.5 month (135 and 140 days, range 12-467 days) and therefore similar between the two treatment groups ($p=0.37$) as expected due to the allowed switch of BSC group patients to CAP7.1 treatment after initial progression. Patients who tolerated at least 2 cycles of CAP7.1 had an even longer median OS of 5.9 months (179 days, CI: 75-467 days = 2.6-15.3m) and thus benefited even more from CAP7.1 therapy. Furthermore, five patients (24% of all CAP7.1 treated patients, three CAP7.1 group patients and 2 BSC group patients) had RECIST responses (SD/PR), which actually lasted between five and seven months. Furthermore, Moriwaki (2016)[91] showed that mPFS correlates strongly with mOS in advanced BTC patients indicating that mPFS is an appropriate surrogate endpoint for mOS in phase II trials in this indication. Thus the encouraging PFS results are transferable to the OS results thereby indicating that CAP7.1 is likely to significantly also prolong survival compared to BSC only therapy, which is the current standard of care for these severely ill patients. The estimated 1-year survival rate in this study was around 41% which also is a very encouraging result considering the fact that Mihalache (2010, n=133)[148] arrived at a 1-year survival of 22.3% +/- 4.4% for a mixed treatment population and an even lower figure of only 17.1% for stage IV patients who are comparable to the patient population of this trial. Furthermore, it needs to be emphasized that all these results are the most conservative estimates with a possibility existing that results in reality are even more in favor of CAP7.1 as already discussed in section 4.1.

Another indication of CAP7.1 efficacy is its ability to slow down tumor growth per time unit. Overall, CAP7.1 generally seemed to slow down “best” (i.e. slowest) tumor diameter growth according to RECIST target lesion measurements from a median of 0.55 mm/day (range 0.11 to 2.92) under BSC only therapy to a median of 0.05 mm/day (range -0.18 to 0.77) under CAP7.1 therapy, which is a decrease of 91% (factor of 11). This impact can only be more prominent in terms of slowed down tumor mass/volume growth since tumor mass is exponentially related to tumor diameter. In addition, the smallest increases in tumor growth as well as all tumor shrinkages were achieved under CAP7.1 therapy while the two biggest increases in tumor growth appeared under BSC only therapy.

Besides these between patient group comparisons the study also offers the unique additional opportunity of individual within patient comparisons in case of nine individual patients who after progression under BSC switched to CAP7.1 therapy. These results are especially interesting and valuable because they are independent of differences in individual risk factor profile which otherwise may act as a source of bias in the between patient group comparisons.

Overall, three of these patients (33%) benefitted from CAP7.1 therapy compared to BSC with two having experienced tumor shrinkages and the third one having experienced the strongest decrease in tumor diameter growth of all other patients. For another three patients (33%) some benefit from CAP7.1 therapy in terms of tumor growth slowdown can neither be confirmed nor be excluded on the basis of the observed data. These results are an indication of CAP7.1's ability to slow down tumor growth in a number of individual patients compared to BSC only therapy.

However, impressive individual results have not only been observed in the BSC group after switch to CAP7.1 therapy, but also in patients randomized to CAP7.1 arm. Overall, of the eight patients who responded to CAP7.1 therapy with SD/PR according to RECIST, five responses were in the CAP7.1 group while only three responses were in the BSC group. Furthermore, three of the CAP7.1 group patients and two of the BSC group patients had RECIST responses that lasted between five and seven months. These are long individual response times in patients whose mOS from the literature otherwise is estimated to be around 2.5 months.

In regards to the CAP7.1 safety profile, hematotoxic effects (neutropenia [67%], leucopenia [57%], thrombocytopenia [48%], anemia [48%]) and related infections ([33%], lung, throat, mouth) were the main adverse events associated with CAP7.1 therapy. Other common adverse events observed were alopecia (33%), fatigue (24%), fever – including neutropenic fever (24%), nausea (19%), and increased liver enzymes (19%). Overall, the observed CAP7.1 safety profile was comparable to the known hematotoxic safety profiles of etoposide and etopophos, which is remarkable since CAP7.1 at the used dosages is delivering an up to 3-fold higher concentration of etoposide compared to conventional etoposide. Furthermore, severe neutropenias were successfully prevented by the simultaneous administration of Neulasta (a granulocyte stimulating agent) in two patients, which therefore may indicate a way to prevent severe neutropenias as the most frequent and dangerous adverse event of CAP7.1 therapy in the future. Overall, the clinical use of CAP7.1 can be considered to be at least as safe as the use of etoposide or etopophos as drugs with an existing market approval with CAP7.1 outperforming organ toxic side effects of etoposide.

In conclusion, the presented results point into one common direction of a significant CAP7.1 effect over BSC only therapy with CAP7.1 in addition having a safety profile comparable to etoposide and etopophos even though the results at this first interim analysis still did not meet the stringent criterion for statistical significance as provided by the O'Brien Fleming method ($p < 0.00154$) which is an indication that the here observed and reported effect sizes are still not

extreme enough to actually halt the trial on the ground of the so far observed data. Therefore, continuation of the trial until the second interim analysis with 34 evaluable patients and an already far less stringent significance level of < 0.02 according to the O'Brien Fleming method leading to a much higher likelihood of arriving also at statistically significant results is recommended.

4.4 Future outlook in BTC therapy and the potential role of CAP7.1

From the literature (chapter 1.2), following main trends in BTC therapy are currently evident:

1. CCA is the most rapidly growing cancer in the West with one of the worst prognosis.
2. Since 2010 GEM/CIS is the established 1st-line chemotherapy for unresectable aCCA. However, while ABC-02 arrived at mOS of 11.7m, these results could not be reproduced[92] with the true mOS being around 9-10m (1.2.6.1.1.d). In turn, GEM/CIS superiority over BSC and other potential 1st-line therapies is challenged.
3. General superiority of GEM/CIS over BSC for all patients is doubtful. Instead, certain patient subgroups may not benefit from GEM/CIS compared to BSC at all (1.2.6.1.1.e).
4. GEM/5-FU and GEM/Platinum/FU were identified as potentially outperforming GEM/Platinum. Eckel (2014)[92] suggested GEM/Platinum/FU and/or GEM/EGFR targeted therapy to become the new standard for patients with good PS[92].
5. No 2nd-line chemotherapy standard for BTC exists and no clear advantage of one regimen over all others or in fact over BSC only therapy is established with trial results generally being poor (mPFS of 3, mOS of 5-7m). However, continuation of 1st-line therapy until disease progression may be beneficial[83].
6. Also targeted therapies had marginal to no benefits over GEM/CIS with mOS 4.4-12.9m and mPFS 1.6-8.8m for most studies. A majority used a targeted compound in combination with GEM+/-Platinum in 1st-line so that single agent studies and 2nd-line studies are rare with often inferior results. Also most studies used mixed and in terms of target expression unselected populations which may explain poor results. However, Cetuximab or Sorafenib or Cediranib + GEM +/- Platinum have shown advantages over GEM/CIS (mOS 13.5-15.7m) while a single trial (GEMOX+Panitumumab in KRAS WT) reached mPFS 10.6m and mOS 20.3m probably because its study population was highly selected (KRAS WT). Eckel (2014)[92] suggested GEM/EGFR targeted therapy besides GEM/Platinum/FU as new standard for good PS patients[92].
7. A new profiling study[97] suggests the existence of 4 distinct molecular genetics clusters with different aetiology (liver fluke pos vs. neg), targets for potential therapy, and prognosis

(better OS of Clusters 3/4 vs 1/2). It also showed that anatomic site is not a driver of molecular subtypes as suggested by other authors as well as suggesting that novel PD-1 inhibitors may only aid the therapy of a small subset of patients.

8. Combination therapies with GEM alone or GEM/Platinum as backbone seem consistently superior to other single or combination compound option so that it seems desirable to trial new therapy options in combination with and/or against this therapy background.
9. Surgery is one of the strongest positive prognostic factors increasing mOS from 7-10m before surgery to about 30m after surgery under BSC and from 9-10m before surgery to up to 51m after surgery under (adjuvant) chemotherapy. Also resected patients have consistently and significantly better OS than unresected patients independently of additional therapy (1.2.2.b/c). For these reasons resected and unresected patients are very different and not comparable and future studies should not mix both groups or if they do, should provide subgroup analysis to avoid bias and arrive at clinically meaningful results.
10. Survival under all therapies and at all points in time along the collective patient journey is strongly dependent on the individual prognostic factor profile. For example the presence or absence of only one prognostic factor like "previous surgery" can either increase (if present) mOS under GEM/CIS to 16.1m[14] (44% increase) or decrease (if absent) mOS to 9.4m[14] (16% decrease) (1.2.2.c). Similarly the presence or absence of three risk factors (PS ≥2, CEA >3, and stage IVb)[32] under GEM/CIS either lower mOS from 10.5[32] to 2.9m[32] (72% decrease) or increase it (if absent) to 18m (71% increase).

Overall, if these 10 key points are summarized into just one major key point from which all the other key points derive, it would be the extreme heterogeneity of this devastating disease at multiple levels and therefore its strong dependence on individual prognostic factor profile which in turn determines therapy outcome and therefore what the most effective therapy for an individual patient is at any given point along the collective patient journey. Best therapy choices thus are individual prognostic factor profile driven and dependent.

In such prognostic factor driven model, however, mOS is no longer an unchangeable, rigid figure onto which a collective therapeutic decision can be based resulting in one best treatment for all. Instead, mOS represents a dynamic, variable entity whose effect size changes within a high percentage range (e.g. 25-100% and more) of the collective overall median established for a certain treatment depending on the presence or absence of individual prognostic factors and their interplay at a particular point in time along the collective patient journey. Therefore, however, the classical approach of prescribing "one chemotherapy for all patients at all points in time

along the collective patient journey" on the basis of results derived from summary statistics of one large mixed patient study like the ABC-02 trial seems practically rather less meaningful and not too helpful to support best therapy choices for an individual patient whose survival apparently so strongly depends on individual prognostic factors under any therapy. It therefore seems desirable that a far more complex, dynamic and individualized therapeutic decision model is developed for this devastating disease which includes information on how each individual prognostic factor affects the dynamic effect sizes of different therapy options so that for each individual patient at a particular point in time along the collective patient journey under the given individual circumstances an informed decision can be reached as to what the optimal therapy choice actually is. In this sense a paradigm shift is needed in the way how clinicians arrive at therapeutic decisions, away from the so far established "one standard therapy for all" approach to a more individualized medicine that is able to understand that the most effective treatment for two patients with the same diagnosis of "CCA" can in fact be very different according to individual circumstances. It is therefore also not necessarily GEM/CIS as the currently established 1st-line standard that needs to be challenged but rather the way how new research is conducted and newly available research information in regards to the established 1st-line therapy standard, therapy outcome under BSC, individual risk factor influence on survival, and new targeted and other 1st and 2nd-line therapies in general is integrated and used by clinicians in order to arrive at truly evidence based individualized best therapeutic decisions.

In order to arrive at such complex therapeutic decision model, however, a lot of additional research work has yet to be done with a main focus on what is practical and easy to achieve rather than what is best theoretical practice and desirable. So while RCTs are the most desirable level of evidence to base therapeutic decisions upon, reality of this rare and immensely heterogeneous disease makes the conduct of properly powered RCTs for most researchers and purposes simply impossible. Therefore, most new research work also in the future will come mainly from retrospective or small prospective studies. It therefore seems important to streamline these research efforts best at international level in a way that makes results from such studies as useful and comparable as possible despite their shortcomings.

A possibility is to define certain standardized research populations of clinical interest along the collective patient journey so that research results between studies become at least as comparable as possible even without randomization. Furthermore, such standardized research populations need to be clinically meaningful, i.e. they need to represent a typical patient collective at a typical decision point along the collective patient journey. For example, while many studies for

practical reasons enrolled resected and unresected patients and arrived at median survival figures for this patient mix, such study results are clinically meaningless because they are not transferable to any real patient since no "resected unresected" patient exists. Instead, since resected patients generally have better outcomes, study results of such studies are biased for both groups and therefore not transferable in clinical practice to either. Therefore standardized research populations should neither mix unresected and resected patients nor mix patients at completely different points in time along the collective patient journey. In this way research results would become more comparable and also clinically meaningful and research should subsequently then investigate how prognostic factors influence survival under different old and new therapies including BSC and GEM/CIS so that best therapy recommendations can be generated for individual patients with individual prognostic factor profiles at defined points in time along the collective patient journey.

Furthermore, the question whether or not existing or new treatments are actually more effective than BSC is central but needs to be answered for different prognostic patient subgroups separately in order to prevent patients from experiencing severe side effects and other repercussions impacting on their safety and QoL while in reality a treatment is not more effective than BSC, which otherwise should remain the preferable option.

In addition it seems pivotal that at least some properly designed RCTs are conducted in the future comparing GEM/CIS with BSC as well as other GEM based combinations such as GEM/5-FU[22], GEM/Platinum/FU[92] or GEM/EGFR[92] for which already some lower level evidence suggests potential for higher effectiveness or similar effectiveness with a more favorable safety profile. Furthermore due to the importance of proven superiority over BSC, future studies should include a BSC arm wherever possible. Also further studies are needed to identify the factors which in some cases lead to very long survival times of 3-5 years under BSC only and/or chemotherapy.

Finally, before clinical practice for CCA can be "revolutionized" by new "personalized medicine" principals, it is likely that clinical research practices along with regulatory practices and requirements need to change since personalizing medicines in the current legal and regulatory framework seems rather challenging to impossible mainly because personalized approaches do not help to bring new drugs to market.

The above described way of defining standardized research populations of clinical interest along the collective patient journey and thereby standardizing research for smaller patient subgroups is

already one way of potentially "personalizing" research. Furthermore, on the basis of the four distinct molecular genetic clusters identified by Jusakul (2017)[97] an opportunity exists to personalize research by assigning one novel targeted therapy compound to each cluster and investigating GEM/CIS against GEM/CIS plus either all 4 compounds administered together to all patients as a "cocktail" (if toxicity permits) or against GEM/CIS plus one of the four compounds which then needs to be selected personally for every patient depending on molecular genetics cluster membership.

In conclusion, the emerging picture from the currently exploding literature on CCA therapy is one where future systemic CCA therapy will be based mainly on the combination of two separate pillars, one "generalized chemotherapeutic pillar" as a treatment backbone for all patients (currently GEM/CIS as accepted 1st-line standard) and one "individualized targeted therapy pillar" (i.e. newly developed targeted therapies, immunotherapies and/or otherwise identified personalized medicines). Future efforts are likely to be directed towards finding the most effective and safe treatment options and combinations within each treatment pillar as well as between the two pillars for highly selected patient subgroups rather than for all CCA patients at once. At the time this CAP7.1 phase II trial was designed, this overall picture had still not emerged from the literature so that also this trial was designed in a rather traditional way without much initial regard for subgroup analysis. However, it already used a far more homogenous and therefore less mixed aCCA patient population than other trials, which in addition was clearly placed at the very end of the collective patient journey. Furthermore, the trial was randomized and compared CAP7.1 to BSC only as the current therapy standard for this particular patient subgroup as well as offering a within patient comparison of the two treatments for the randomized BSC subgroup of patients whose results therefore is independent of the existing individual prognostic factor profiles. For all these reasons, this phase II clinical trial with its positive results is quite unique and outstanding in that it actually meets identified key criteria for good research practice as identified in the recent literature while it actually was designed and initiated at a time where these conclusions had still not yet been identified so clearly. Thus, these trial results are likely to be clinically more meaningful and transferable to similar patients compared with the results of many other trials.

Furthermore, when looking at the here presented positive trial results and further considering the quite unique properties CAP7.1 has as a CES2 activated prodrug of etoposide, it becomes evident that within the emerging future BTC therapy reality, CAP7.1 is uniquely positioned and suited to improve CCA therapy in several ways and at multiple levels along the collective patient

journey. First and foremost, CAP7.1 is a chemotherapeutic agent, which due to its CES2 dependent activation mechanism is even likely to generally be more active in the liver than other chemotherapeutic agents may be. Therefore, it seems uniquely suited to be trialed as an add-on to the current GEM/CIS chemotherapy standard in 1st-line therapy but also could serve in 2nd-line chemotherapy combinations or as a novel general alternative chemotherapy backbone in 2nd-line therapy after GEM/CIS failure. However, in addition to these general uses as a chemotherapeutic agent in CCA therapy, CAP7.1 through the same CES2 activation mechanism also has properties and thus potential to be used as a targeted/personalized agent in high CES2 expressing tumors/individuals. In this way, CAP7.1 is very unique because unlike other targeted agents whose effectiveness typically depends on the presence or absence of very specific cell mechanisms in an "on/off" fashion (i.e. if mechanisms present, then agent may have an effect but if mechanisms absent, then agent actually cannot have an effect), CAP7.1 as a predominantly chemotherapeutic and therefore still generally cytotoxic agent unlike other targeted agents is likely to actually exert at least some general cytotoxic antitumor effect on all CCA patients/tumors, while in addition potentially being specifically effective in a certain high CES2 expressing subgroup of patients which lends it additional targeted agent properties. Furthermore, the within patient comparison with BSC therapy only in this study was able to show that also a high proportion of individual patients may benefit from CAP7.1 therapy independently of their individual prognostic factor profile. Therefore, CAP7.1 as a rather "hybrid agent" with general cytotoxic as well as additional targeted therapy properties has a unique position and potential to improve therapy outcome of this otherwise so heterogeneous disease in combination with other agents and at multiple treatment levels along the collective patient journey in the future. The continuation of this trial as well as future trials of CAP7.1 in different CCA subpopulations and in combination with different other agents (e.g. GEM/CIS) against standard therapy in exclusively CES2 high expressing tumors/patients is feasible in the future and studies are currently being prepared for the next stage of clinical testing.

APPENDIX 1

Table 3a – TNM Staging Categories for iCCA as an exmple.[52]

	Definition
T-Categories	
T0	No evidence of a primary tumor
Tis	Intramucosal carcinoma / Carcinoma in situ
T1	Solitary tumors without vascular invasion.
T2a/T2b	Solitary tumor with vascular invasion (T2a) or multiple tumors (T2b) with or without vascular invasion.
T3	Tumour(s) perforating the visceral peritoneum or involving the local extra hepatic structures by direct invasion
T4	Tumors with periductal invasion
N-Categories	
NX	Regional lymph nodes not assessable.
N0	No regional lymph node metastases.
N1	Regional lymph node metastases.
M-Categories	
M0	No distant metastases
M1	Distant metastases

Table 3b – TNM Stage Grouping Categories for iCCA as an example.

Stage Grouping	Definition
Stage 0 (Tis, N0, M0)	Intramucosal tumor without any regional lymph node or distant metastases.
Stage I (T1, N0, M0)	Solitary tumor without vascular invasion or any regional lymph node or distant metastases.
Stage II (T2, N0, M0)	Solitary or multiple tumors with vascular invasion but without any regional lymph node or distant metastases.
Stage III (T3, N0, M0)	Adjacent structures invaded but no regional lymph node or distant metastases.
Stage IVa (T4, N0, M0) or (Any T, N1, M0)	Periductal tumor infiltration or regional lymph node metastases but no distant metastases.
Stage IVb (Any T,any N, M1)	Any tumor with distant metastases.

APPENDIX 2

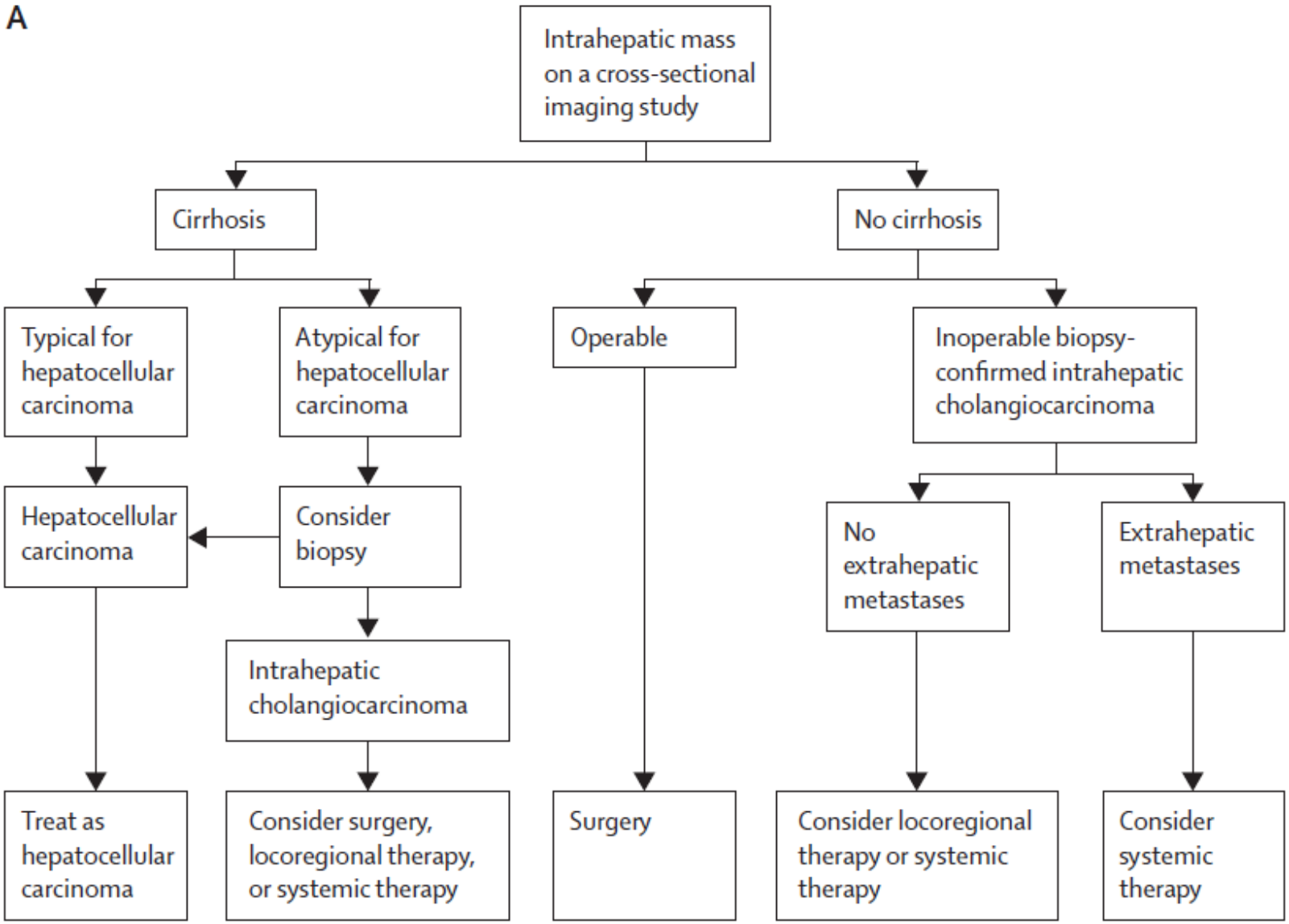


Figure 1 – Approach to diagnosis and management for iCCA taken from Razumilava et al., 2014[1].

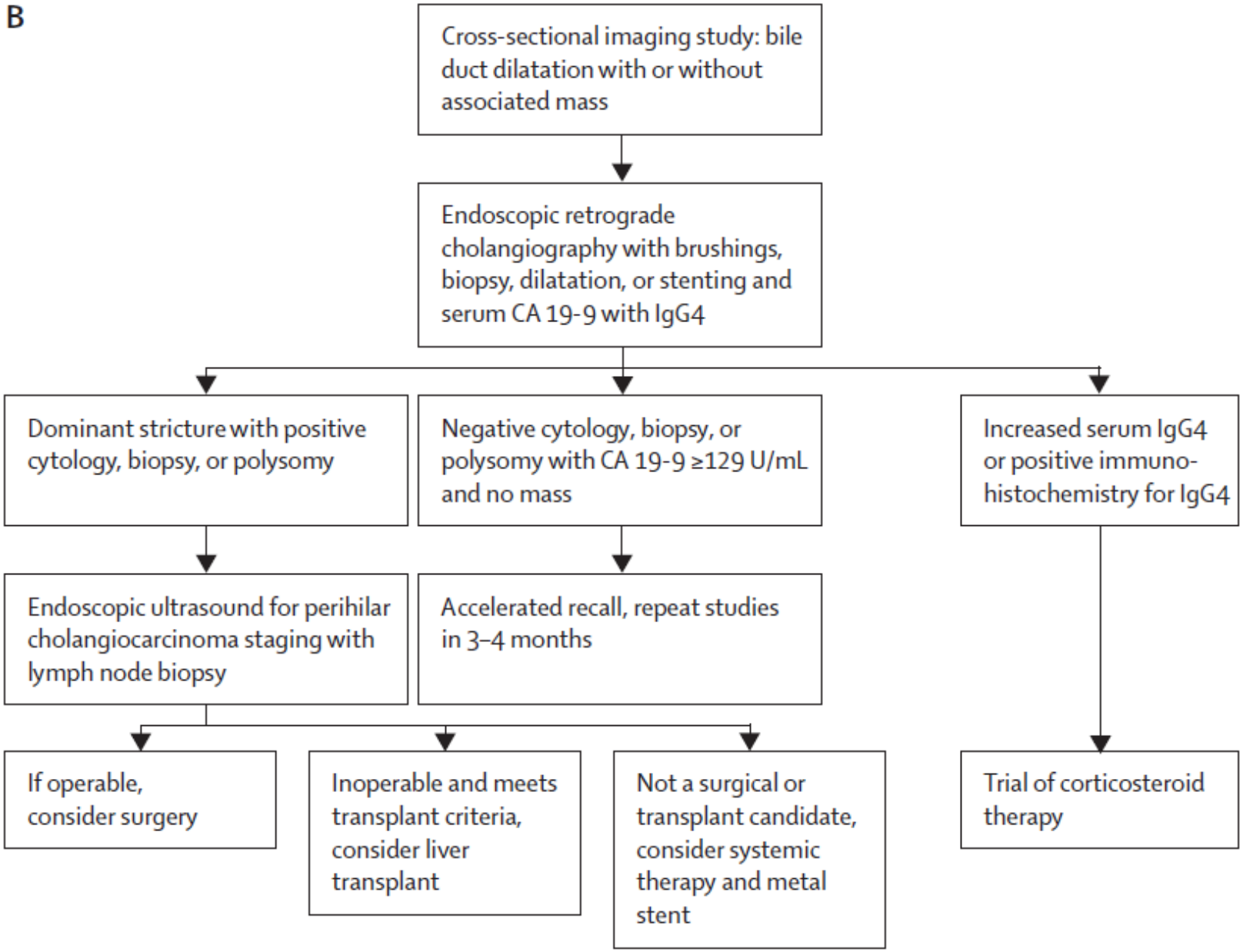
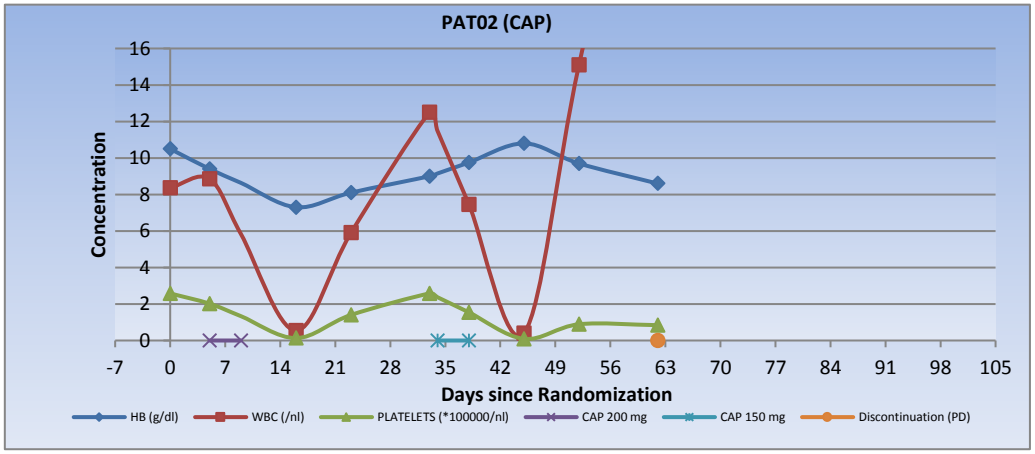


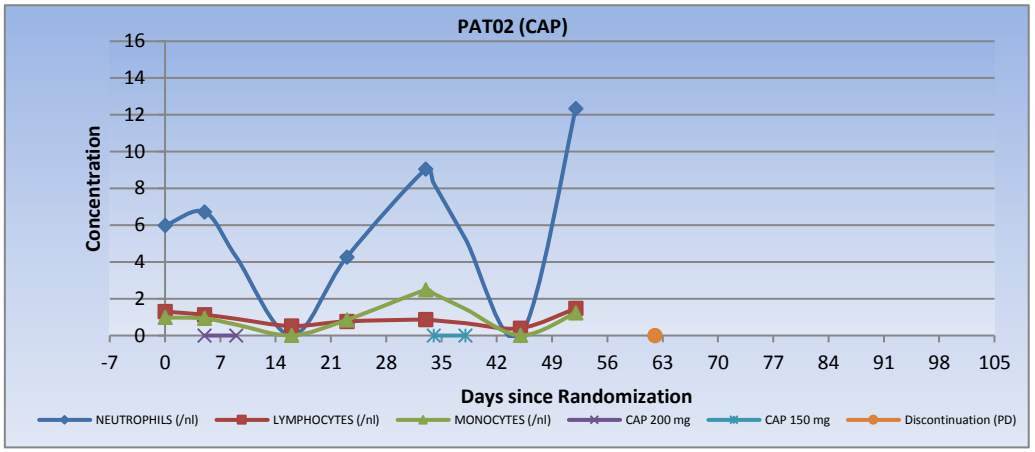
Figure 2 – Approach to diagnosis and management for pCCA taken from Razumilava et al., 2014[1].

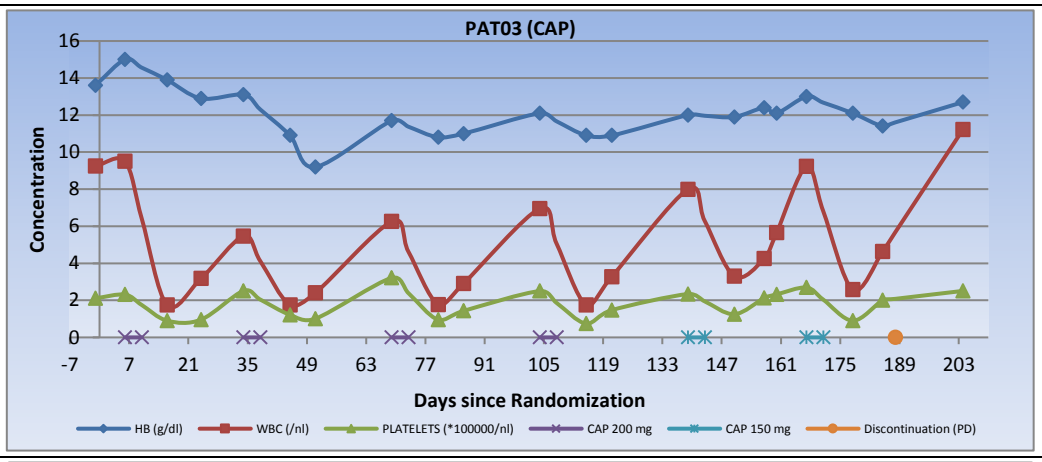
APPENDIX 3

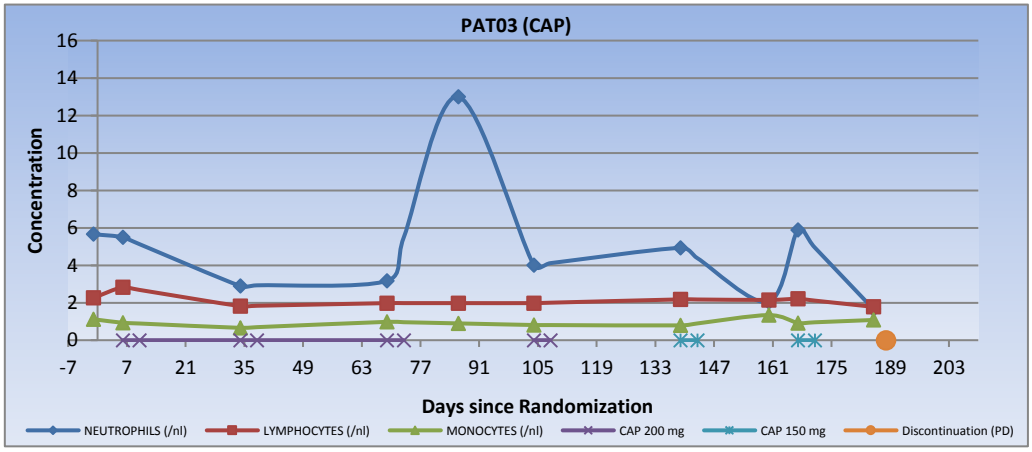
Figure X – Influence of CAP7.1 on blood parameters

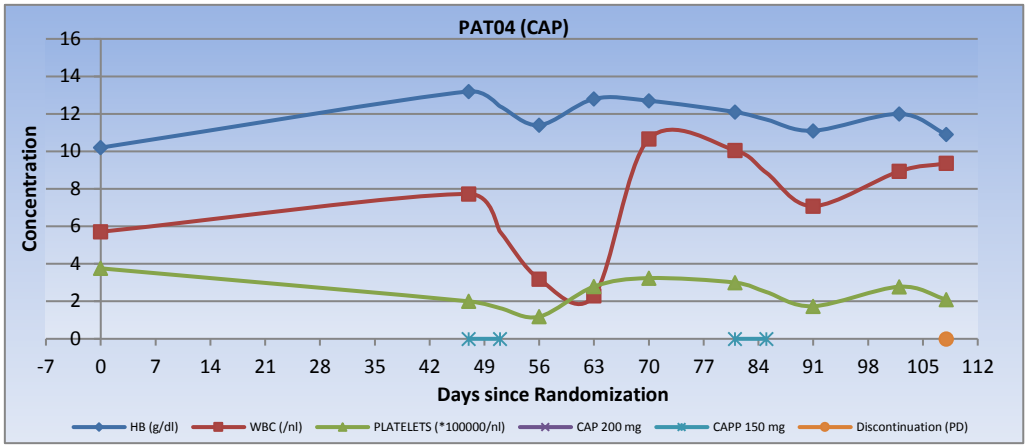
CAP7.1 . Group







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Concentration
Days since Randomization
HB (g/dl)
WBC (/nl)
PLATELETS (*100000/nl)
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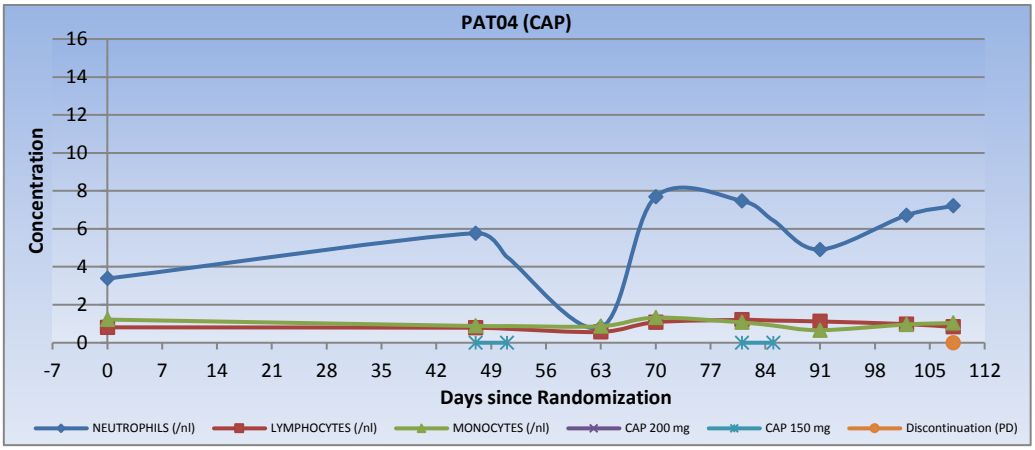
CAPP 150 mg
Discontinuation (PD)


PAT04 (CAP)
Concentration
Days since Randomization
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LYMPHOCYTES (/nl)
MONOCYTES (/nl)
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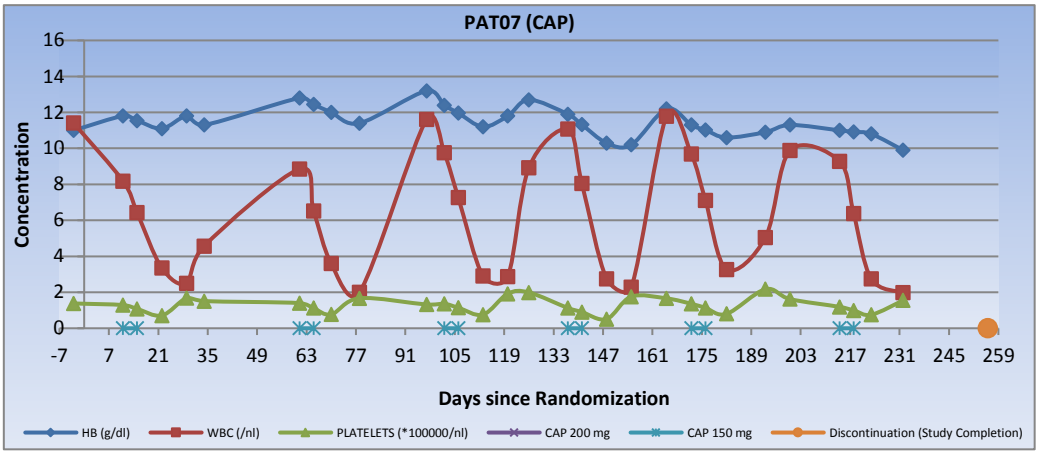
CAP 150 mg
Discontinuation (PD)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Concentration
Days since Randomization
HB (g/dl)
WBC (/nl)
PLATELETS (*100000/nl)
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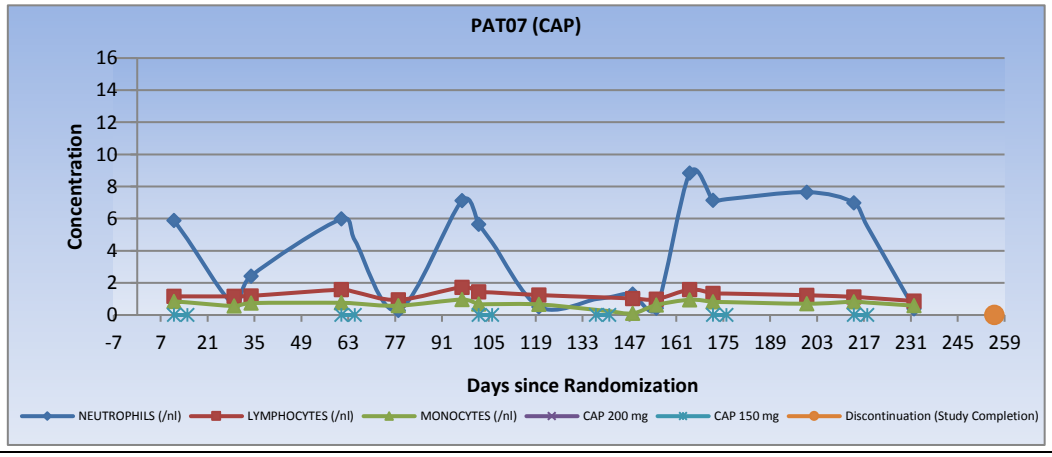
CAP 150 mg
Discontinuation (Study Completion)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Concentration
Days since Randomization
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LYMPHOCYTES (/nl)
MONOCYTES (/nl)
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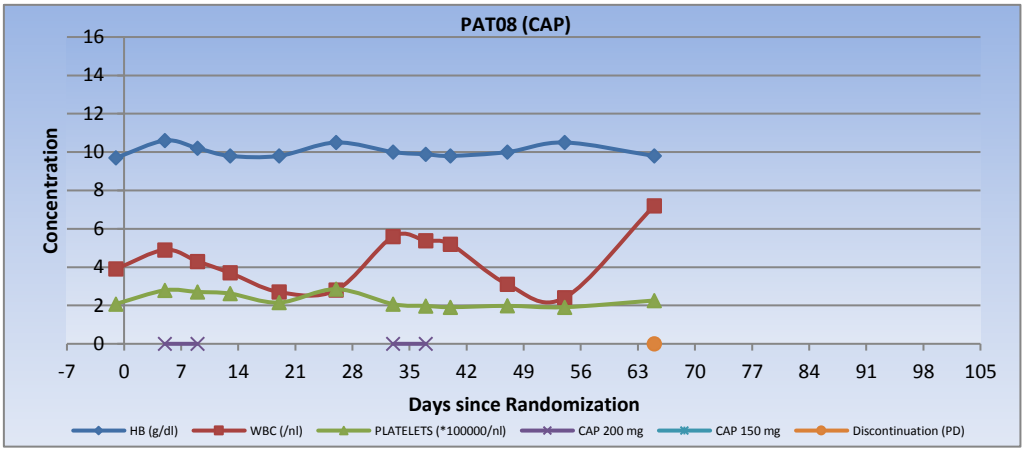
CAP 150 mg
Discontinuation (Study Completion)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Concentration
Days since Randomization
HB (g/dl)
WBC (/nl)
PLATELETS (*100000/nl)
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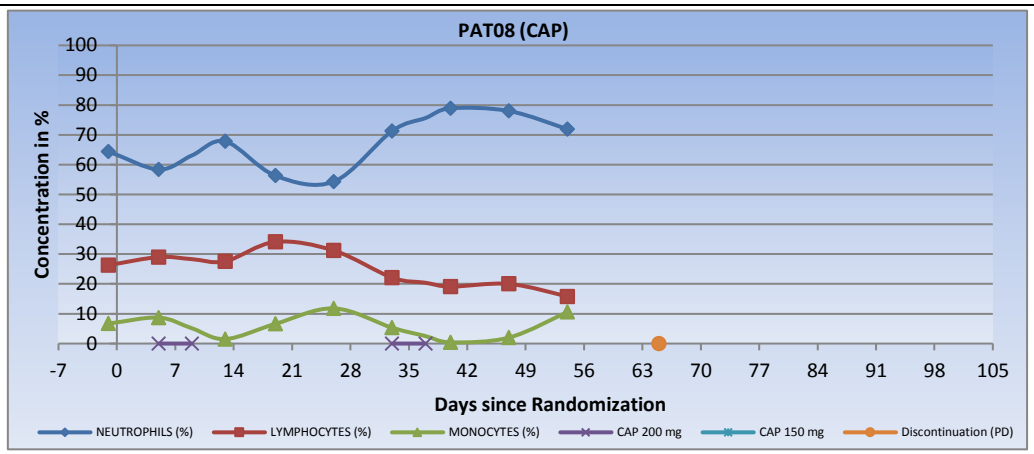
CAP 150 mg
Discontinuation (PD)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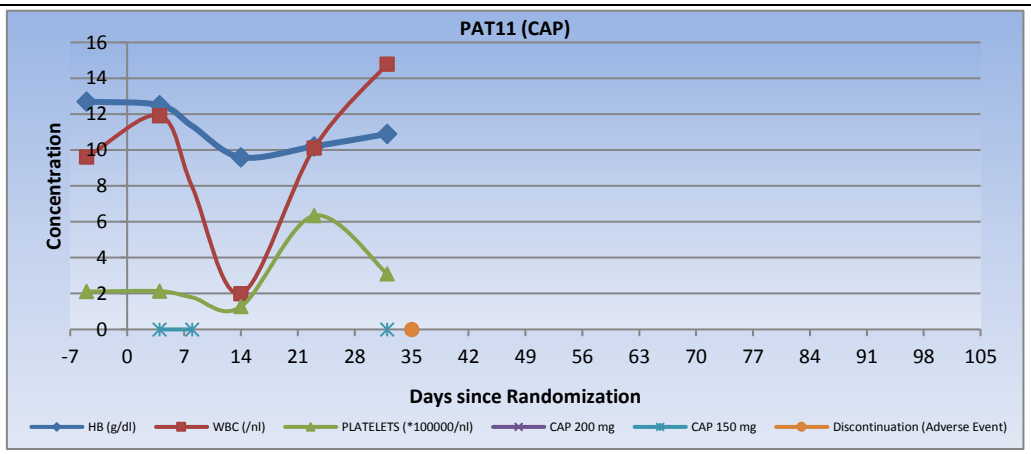
Concentration in %
Days since Randomization
NEUTROPHILS (%)
LYMPHOCYTES (%)
MONOCYTES (%)
CAP 200 mg
CAP 150 mg
Discontinuation (PD)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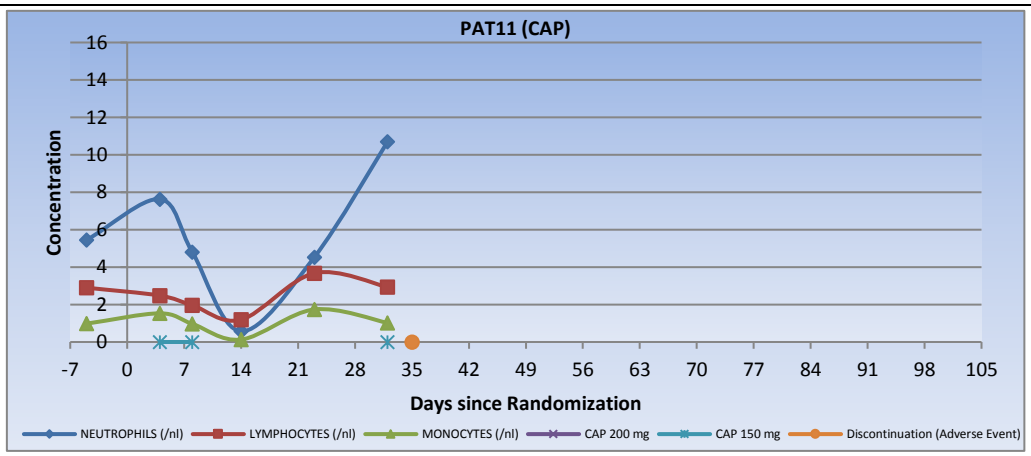
Concentration
Days since Randomization
HB (g/dl)
WBC (/nl)
PLATELETS (*100000/nl)
CAP 200 mg
CAP 150 mg
Discontinuation (Adverse Event)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Concentration
Days since Randomization
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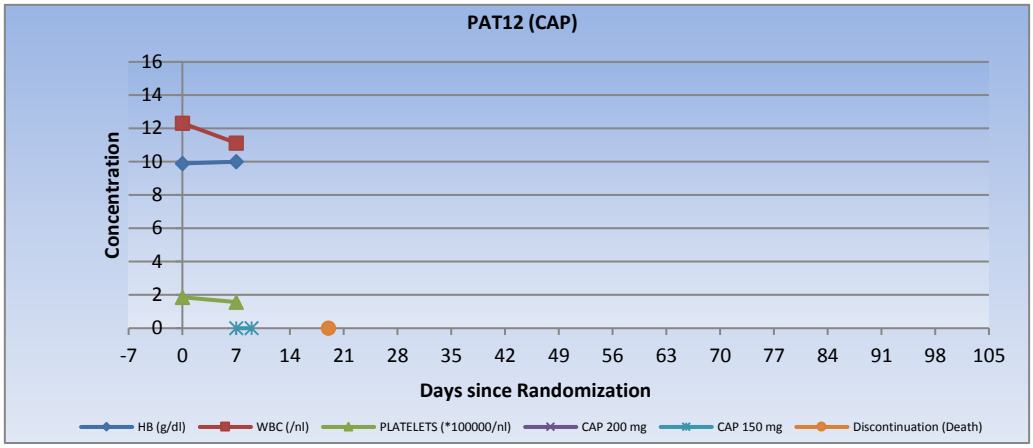
LYMPHOCYTES (/nl)
MONOCYTES (/nl)
CAP 200 mg
CAP 150 mg
Discontinuation (Adverse Event)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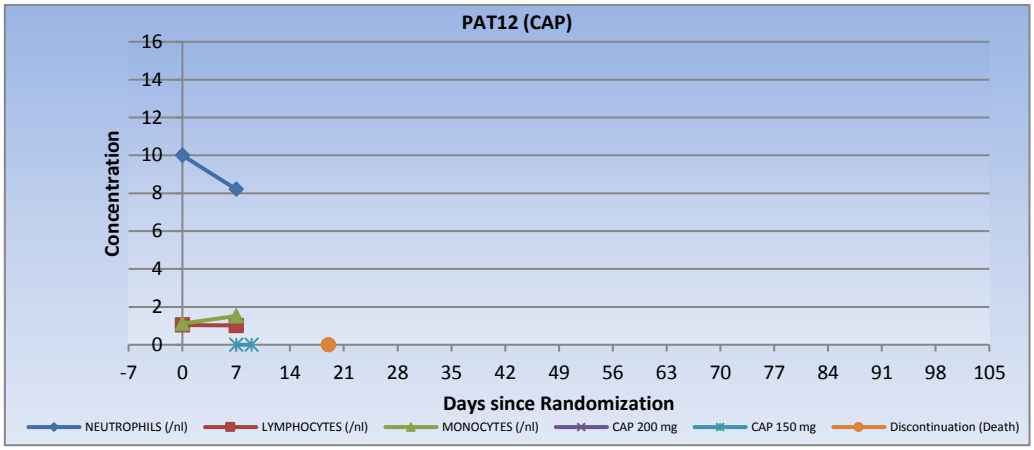
Concentration
Days since Randomization
HB (g/dl)
WBC (/nl)
PLATELETS (*100000/nl)
CAP 200 mg
CAP 150 mg
Discontinuation (Death)


PAT12 (CAP)
Concentration
Days since Randomization
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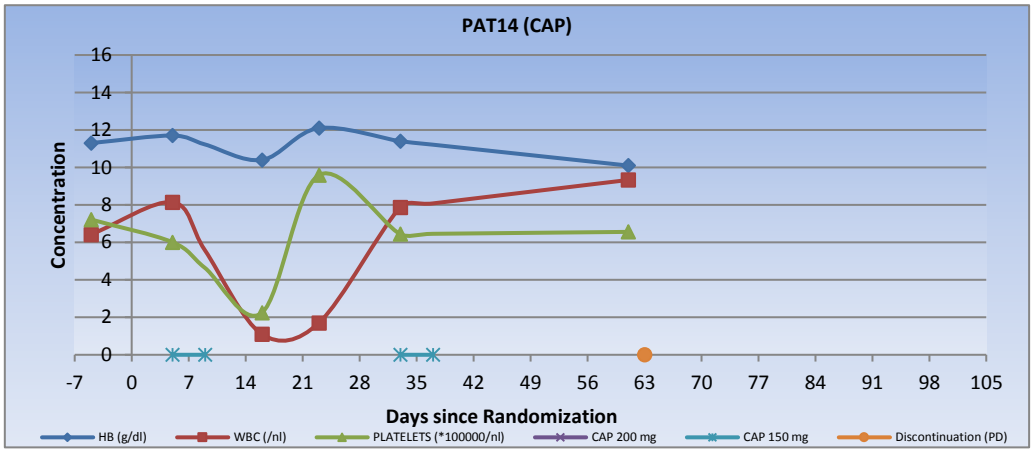
LYMPHOCYTES (/nl)
MONOCYTES (/nl)
CAP 200 mg
CAP 150 mg
Discontinuation (Death)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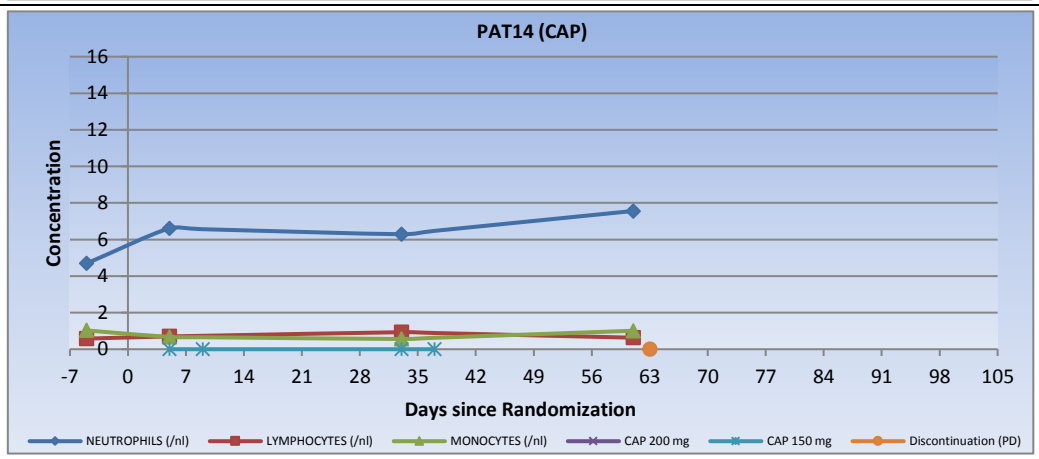
Concentration
Days since Randomization
HB (g/dl)
WBC (/nl)
PLATELETS (*100000/nl)
CAP 200 mg
CAP 150 mg
Discontinuation (PD)


PAT14 (CAP)
Concentration
Days since Randomization
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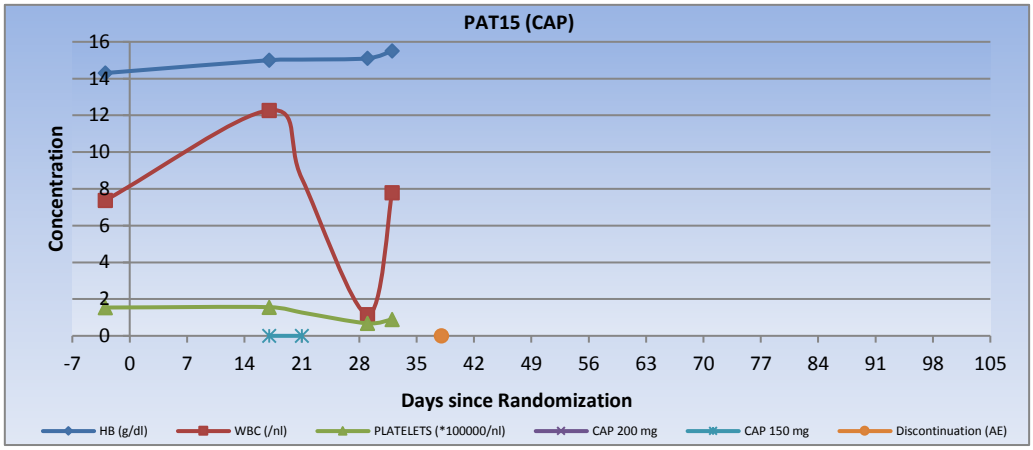
LYMPHOCYTES (/nl)
MONOCYTES (/nl)
CAP 200 mg
CAP 150 mg
Discontinuation (PD)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Concentration
Days since Randomization
HB (g/dl)
WBC (/nl)
PLATELETS (*100000/nl)
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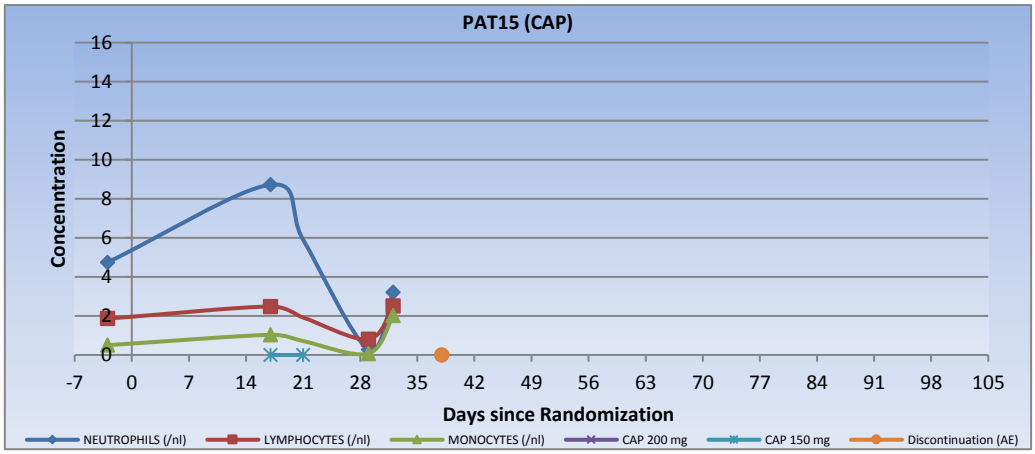
CAP 150 mg
Discontinuation (AE)


PAT15 (CAP)
Concenntration
Days since Randomization
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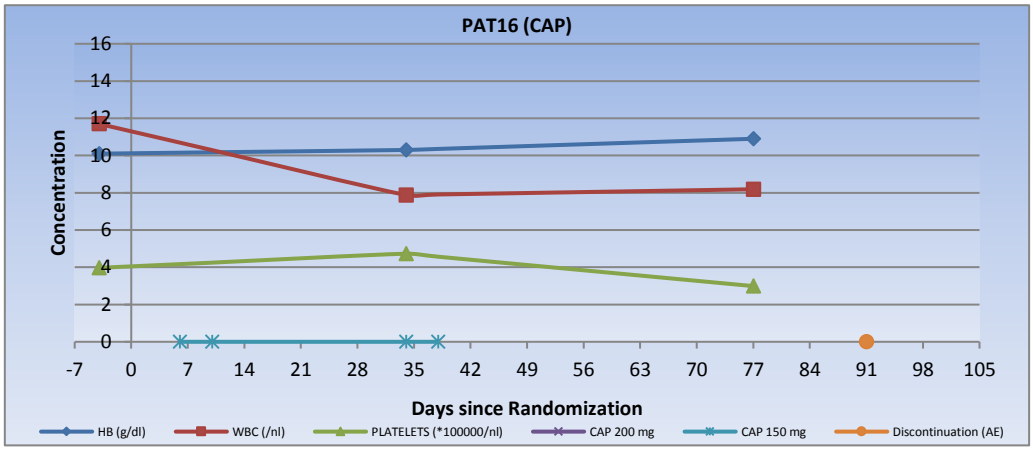
LYMPHOCYTES (/nl)
MONOCYTES (/nl)
CAP 200 mg
CAP 150 mg
Discontinuation (AE)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Concentration
Days since Randomization
HB (g/dl)
WBC (/nl)
PLATELETS (*100000/nl)
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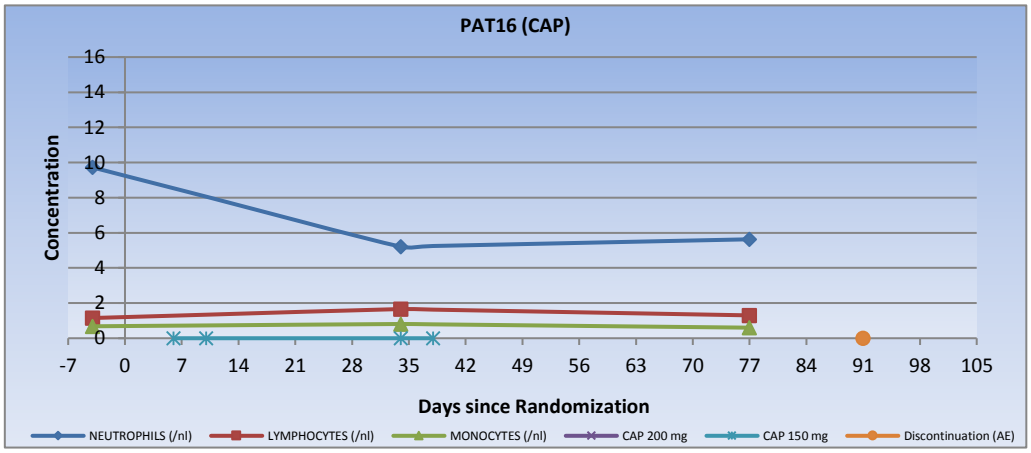
CAP 150 mg
Discontinuation (AE)


PAT16 (CAP)
Concentration
Days since Randomization
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LYMPHOCYTES (/nl)
MONOCYTES (/nl)
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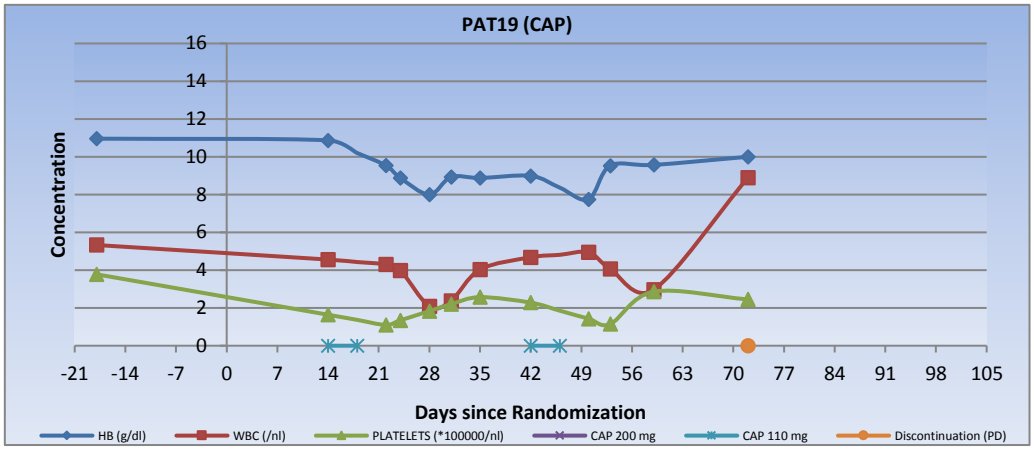
CAP 150 mg
Discontinuation (AE)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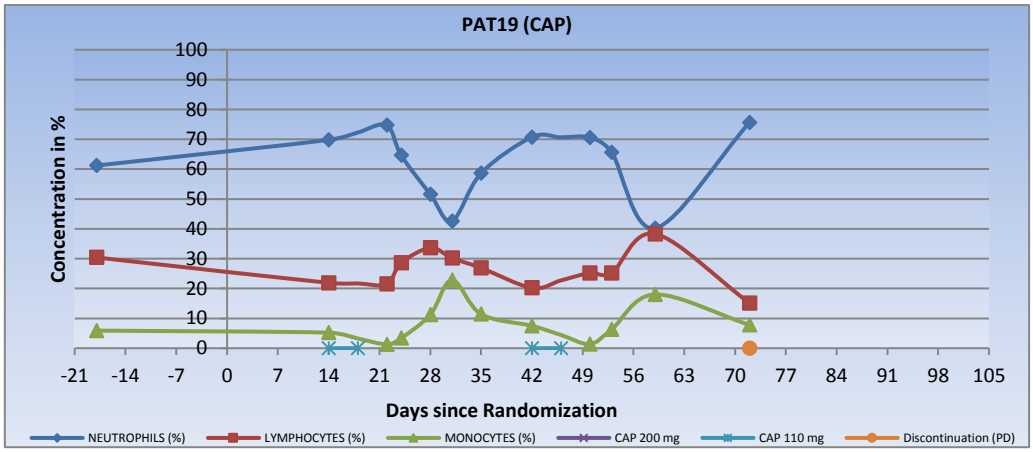
Concentration
Days since Randomization
HB (g/dl)
WBC (/nl)
PLATELETS (*100000/nl)
CAP 200 mg
CAP 110 mg
Discontinuation (PD)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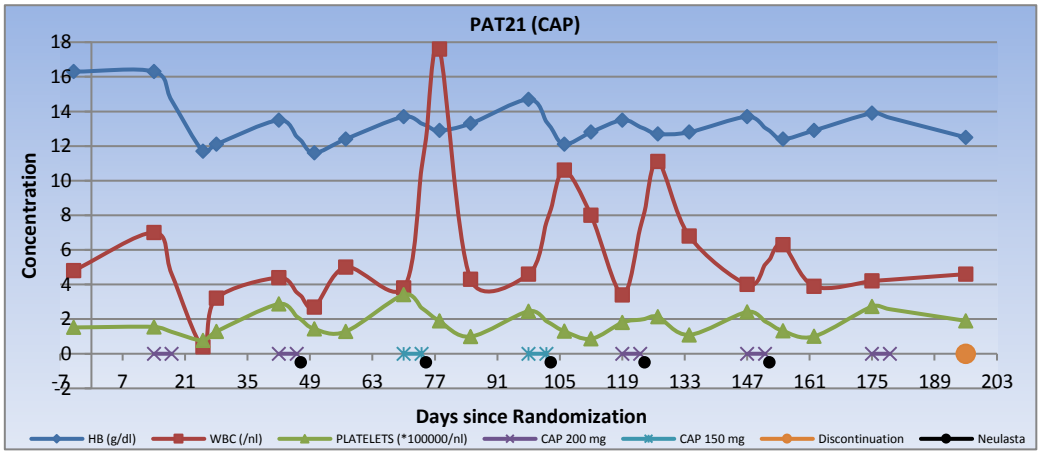
Concentration in %
Days since Randomization
NEUTROPHILS (%)
LYMPHOCYTES (%)
MONOCYTES (%)
CAP 200 mg
CAP 110 mg
Discontinuation (PD)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Concentration
Days since Randomization
HB (g/dl)
WBC (/nl)
PLATELETS (*100000/nl)
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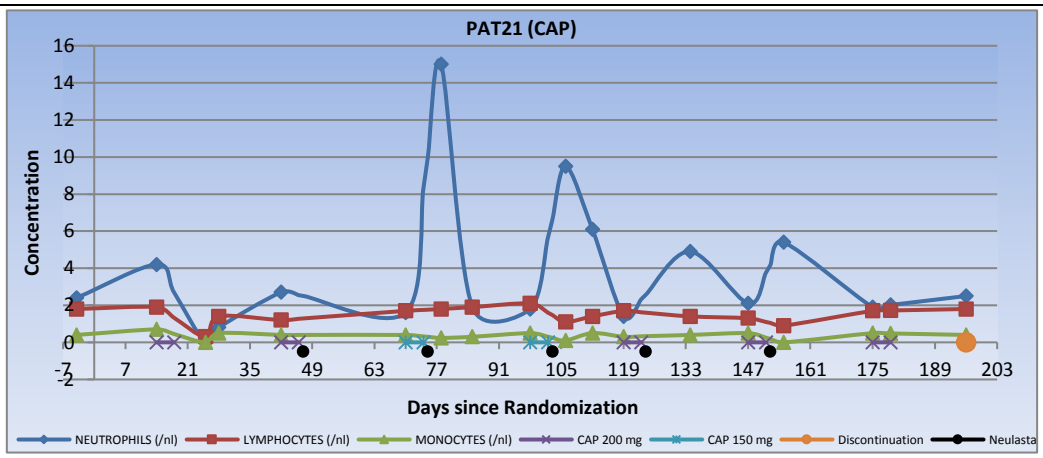
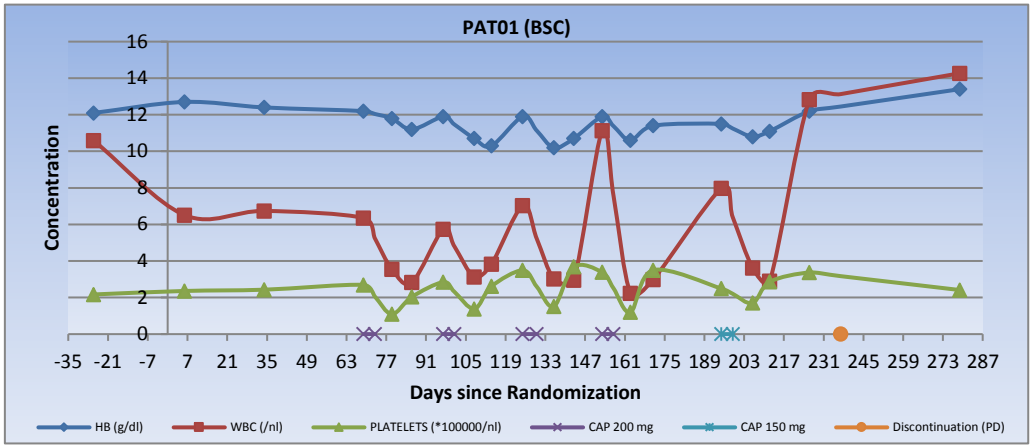
CAP 150 mg
Discontinuation
Neulasta


PAT21 (CAP)
Concentration
Days since Randomization
NEUTROPHILS (/nl)
LYMPHOCYTES (/nl)
MONOCYTES (/nl)
CAP 200 mg
CAP 150 mg
Discontinuation
Neulasta

BSC Group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Concentration
Days since Randomization
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WBC (/nl)
PLATELETS (*100000/nl)
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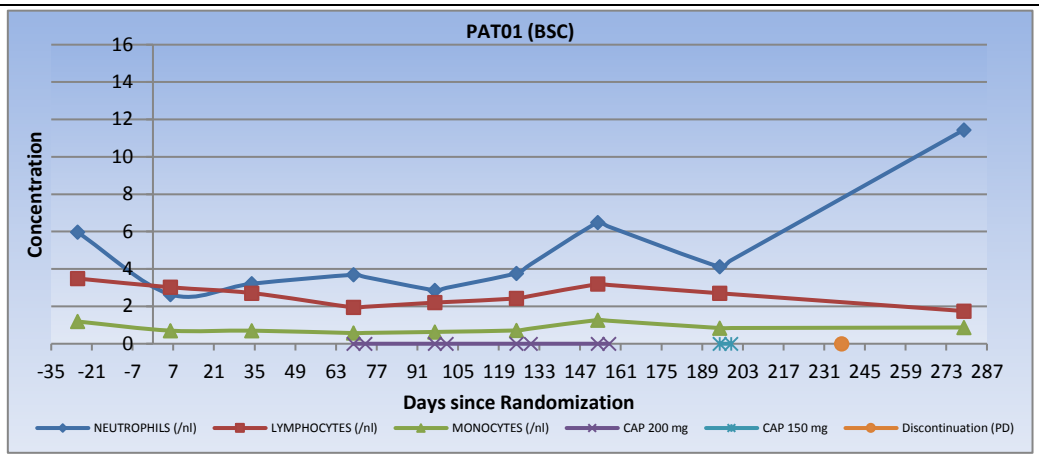
CAP 150 mg
Discontinuation (PD)


PAT01 (BSC)
Concentration
Days since Randomization
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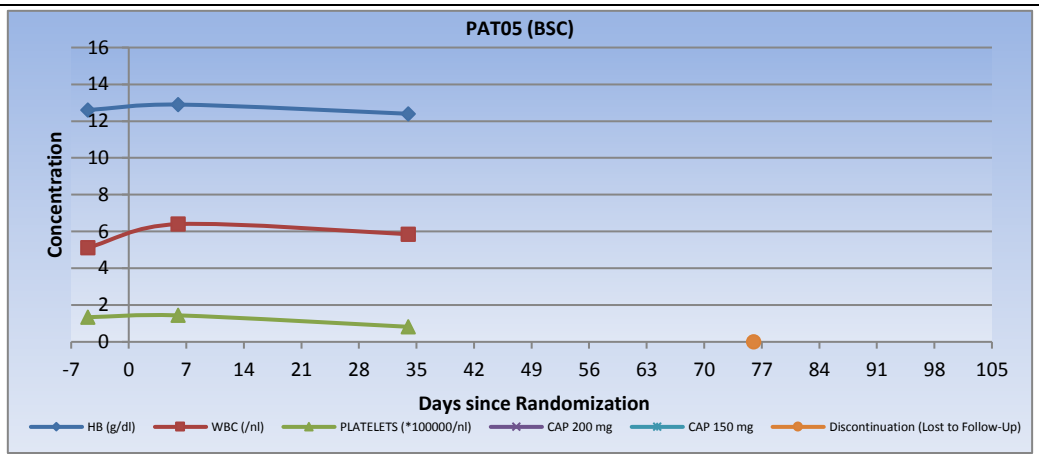
LYMPHOCYTES (/nl)
MONOCYTES (/nl)
CAP 200 mg
CAP 150 mg
Discontinuation (PD)


PAT05 (BSC)
Concentration
Days since Randomization
HB (g/dl)
WBC (/nl)
PLATELETS (*100000/nl)
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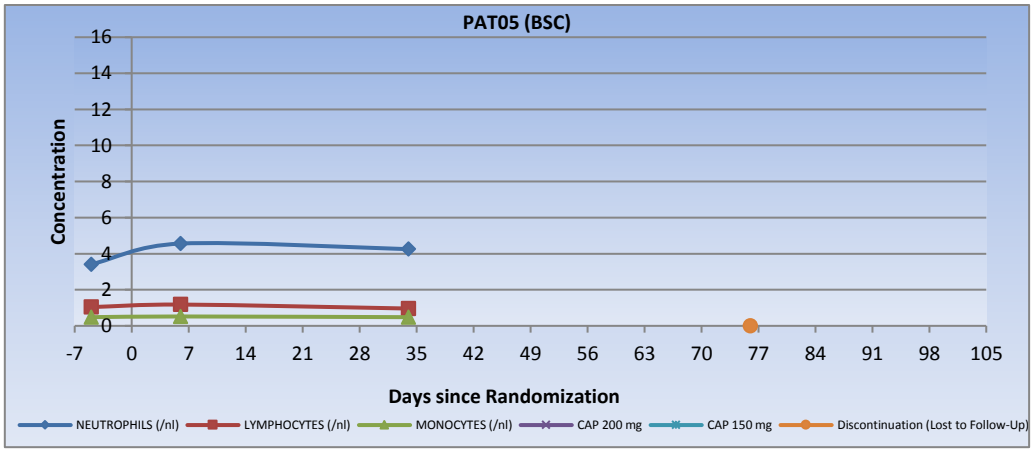
CAP 150 mg
Discontinuation (Lost to Follow-Up)


PAT05 (BSC)
Concentration
Days since Randomization
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LYMPHOCYTES (/nl)
MONOCYTES (/nl)
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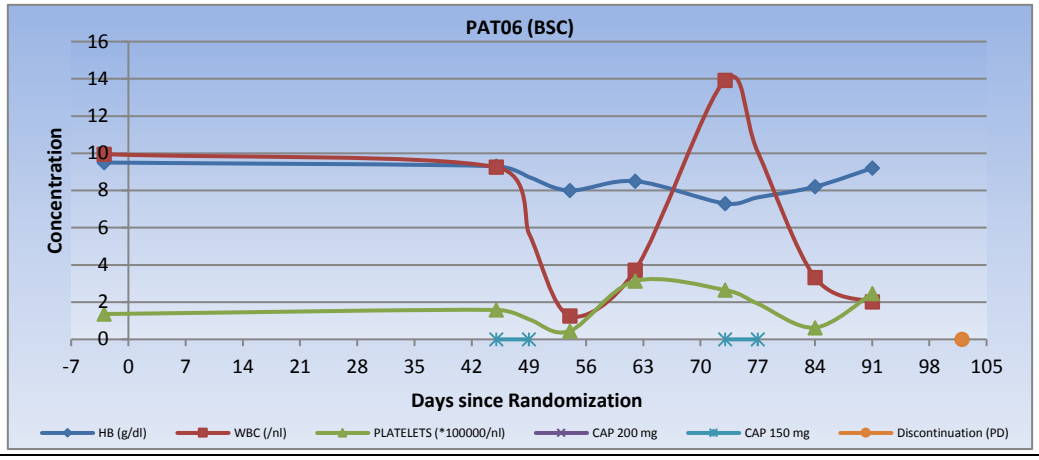
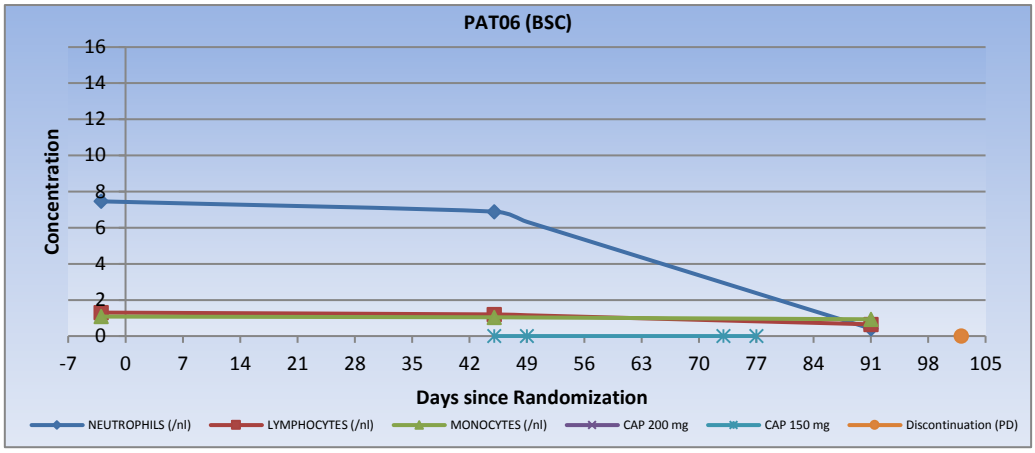
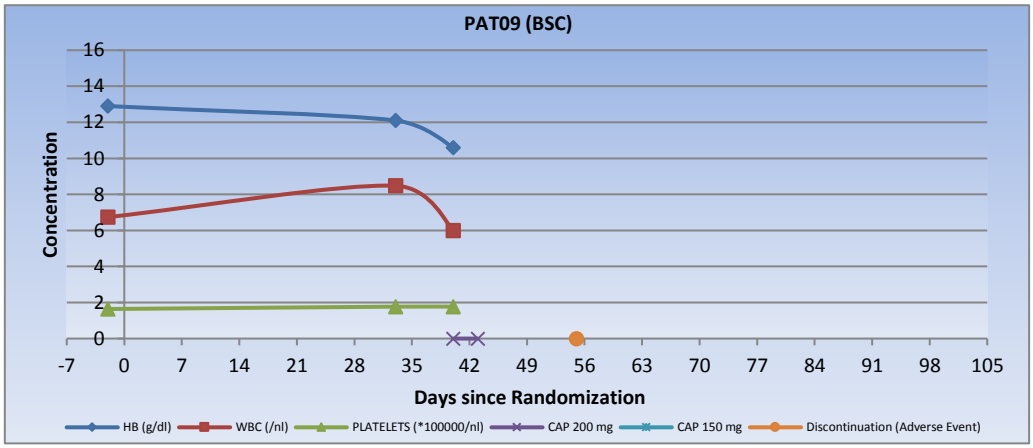
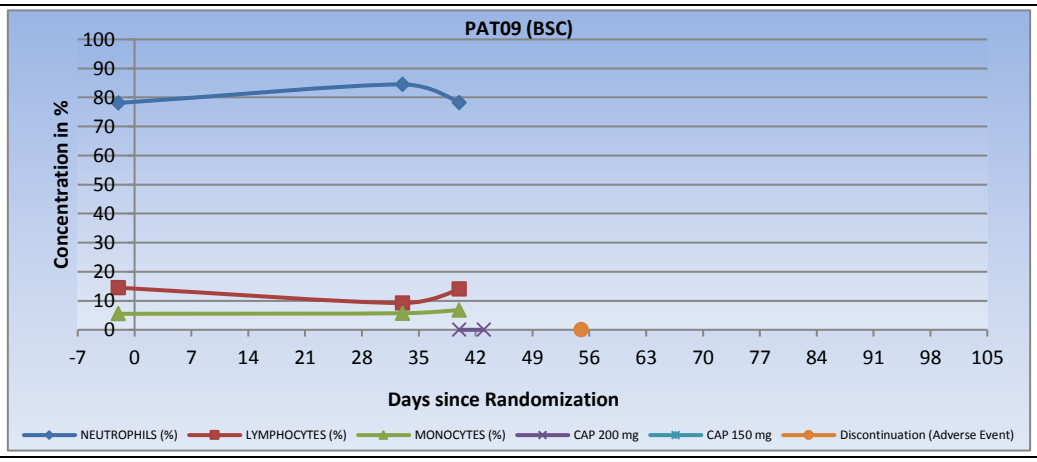
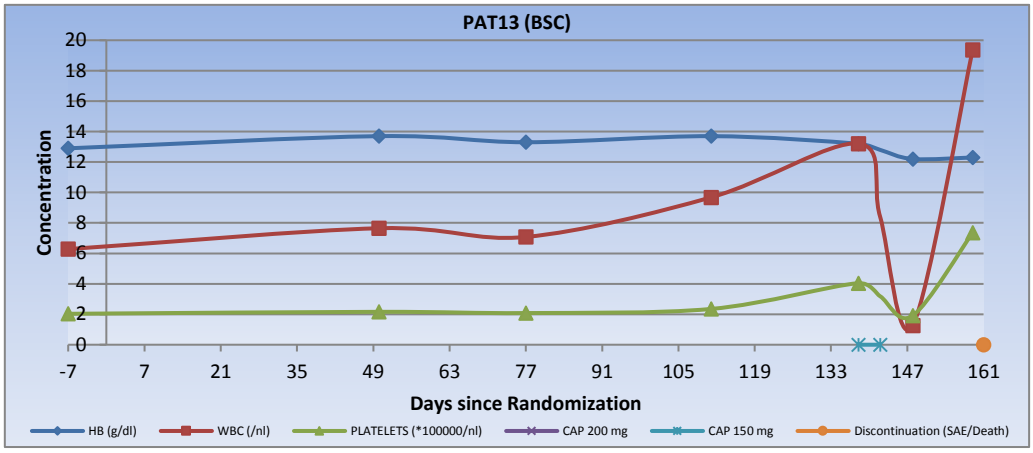
CAP 150 mg
Discontinuation (Lost to Follow-Up)

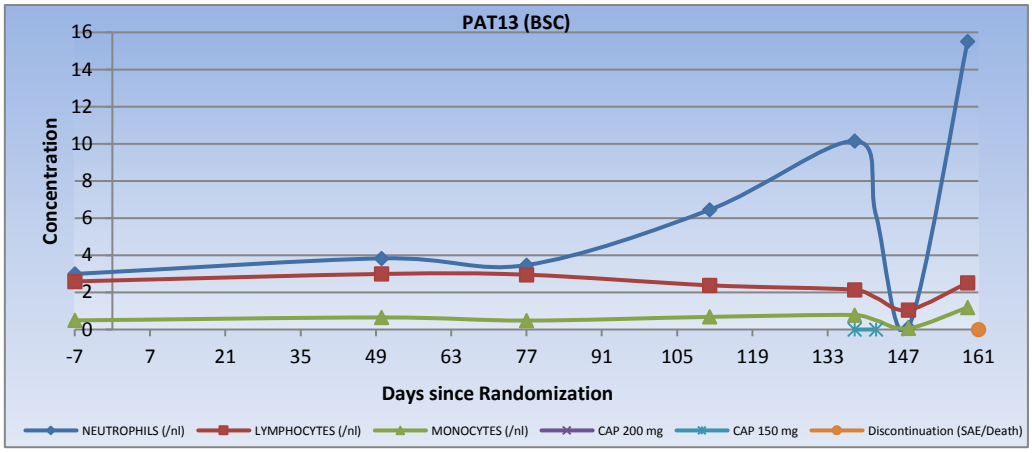

PAT06 (BSC)
Concentration
Days since Randomization
HB (g/dl)
WBC (/nl)
PLATELETS (*100000/nl)
CAP 200 mg
CAP 150 mg
Discontinuation (PD)

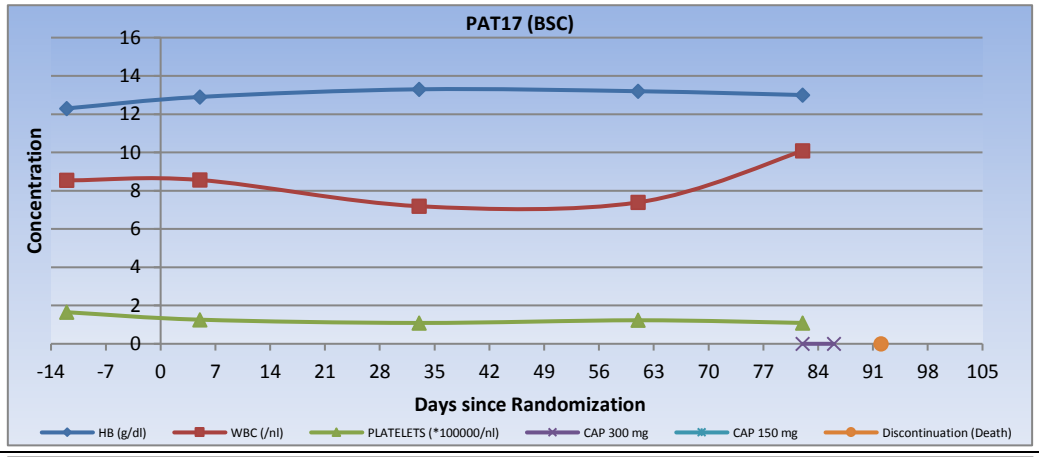

PAT06 (BSC)
Concentration
Days since Randomization
NEUTROPHILS (/nl)
LYMPHOCYTES (/nl)
MONOCYTES (/nl)
CAP 200 mg
CAP 150 mg
Discontinuation (PD)

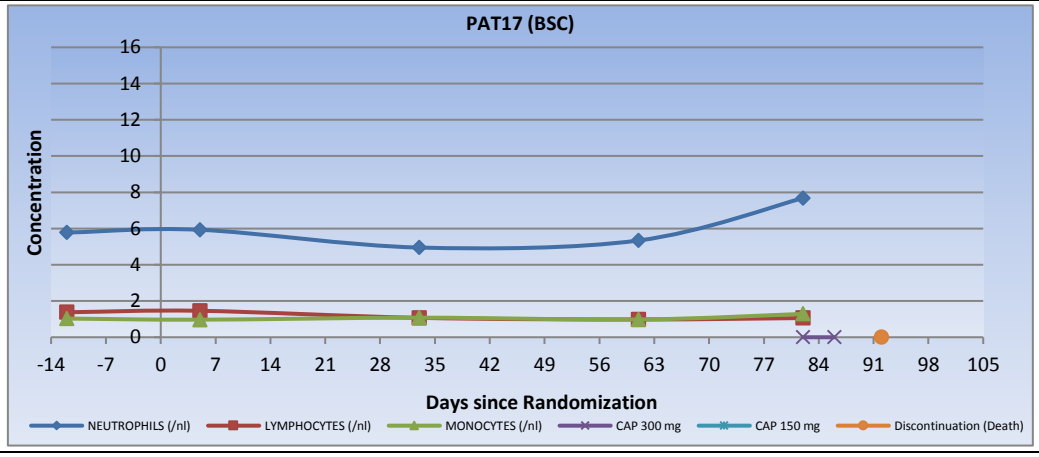

PAT09 (BSC)
Concentration
Days since Randomization
HB (g/dl)
WBC (/nl)
PLATELETS (*100000/nl)
CAP 200 mg
CAP 150 mg
Discontinuation (Adverse Event)

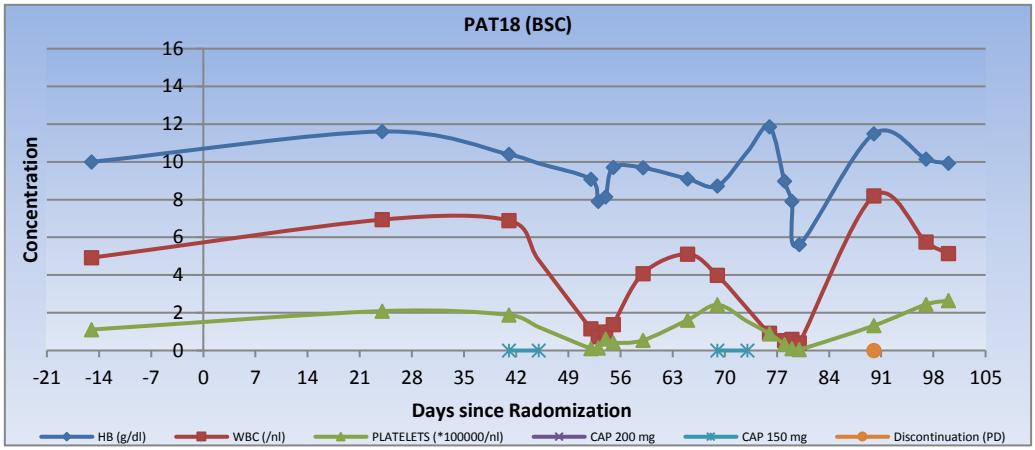

PAT09 (BSC)
Concentration in %
Days since Randomization
NEUTROPHILS (%)
LYMPHOCYTES (%)
MONOCYTES (%)
CAP 200 mg
CAP 150 mg
Discontinuation (Adverse Event)

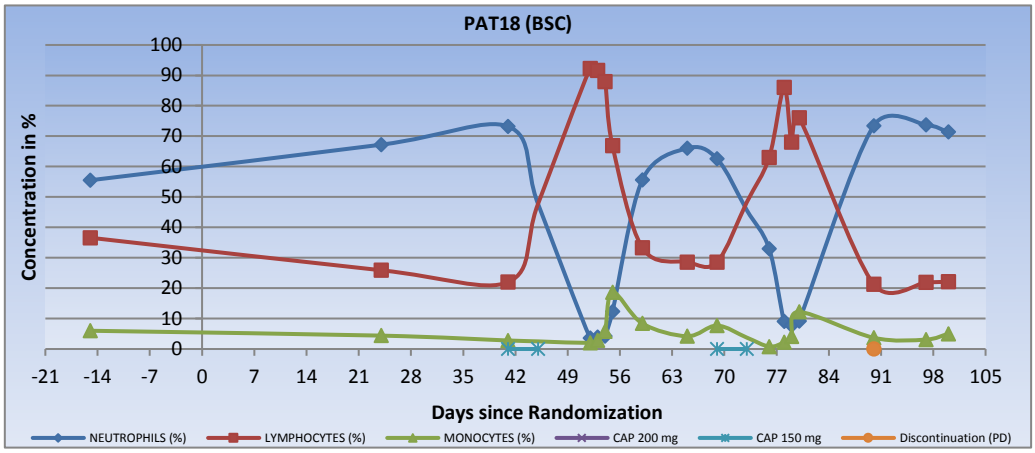

PAT13 (BSC)
Concentration
Days since Randomization
HB (g/dl)
WBC (/nl)
PLATELETS (*100000/nl)
CAP 200 mg
CAP 150 mg
Discontinuation (SAE/Death)

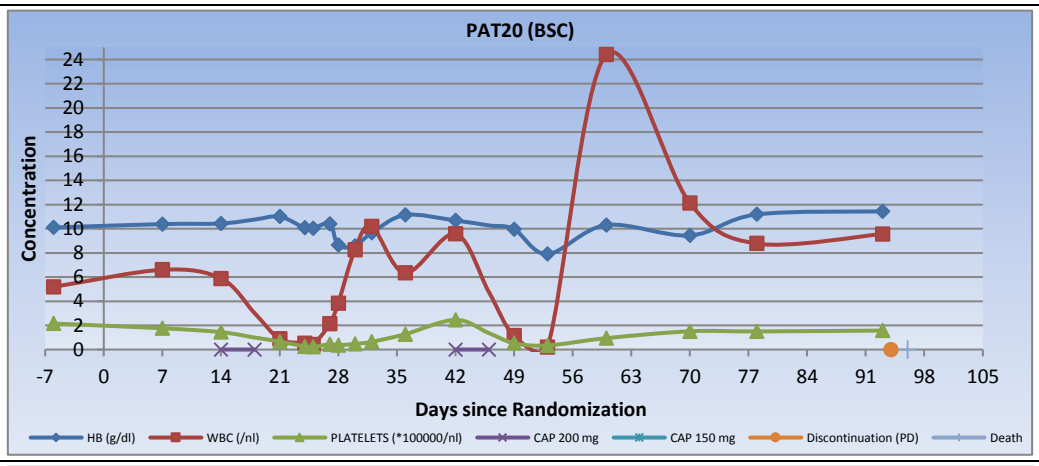

PAT13 (BSC)
Concentration
Days since Randomization
NEUTROPHILS (/nl)
LYMPHOCYTES (/nl)
MONOCYTES (/nl)
CAP 200 mg
CAP 150 mg
Discontinuation (SAE/Death)

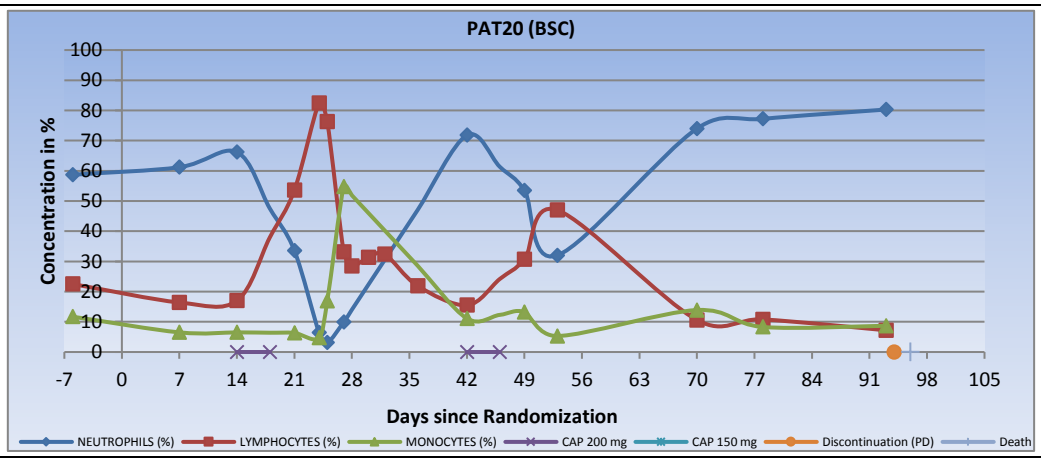

PAT17 (BSC)
Concentration
Days since Randomization
HB (g/dl)
WBC (/nl)
PLATELETS (*100000/nl)
CAP 300 mg
CAP 150 mg
Discontinuation (Death)

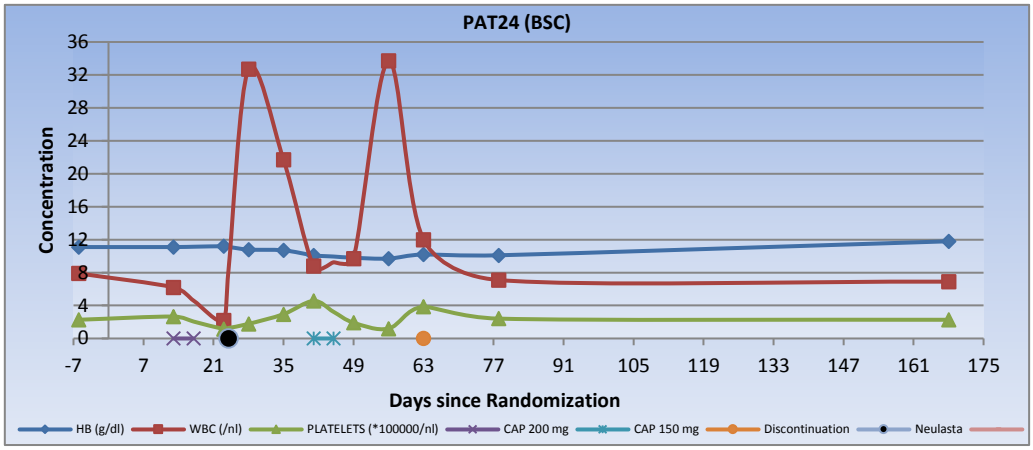

PAT17 (BSC)
Concentration
Days since Randomization
NEUTROPHILS (/nl)
LYMPHOCYTES (/nl)
MONOCYTES (/nl)
CAP 300 mg
CAP 150 mg
Discontinuation (Death)

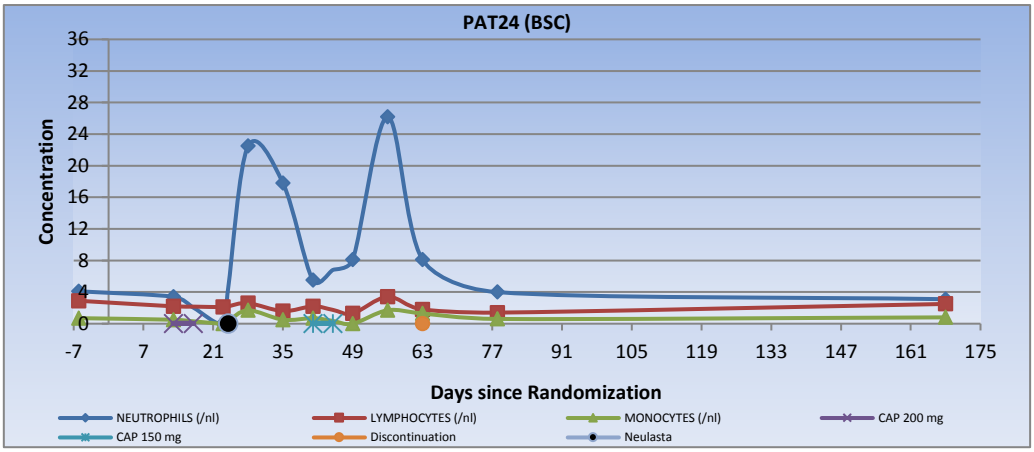

PAT18 (BSC)
Concentration
Days since Radomization
HB (g/dl)
WBC (/nl)
PLATELETS (*100000/nl)
CAP 200 mg
CAP 150 mg
Discontinuation (PD)

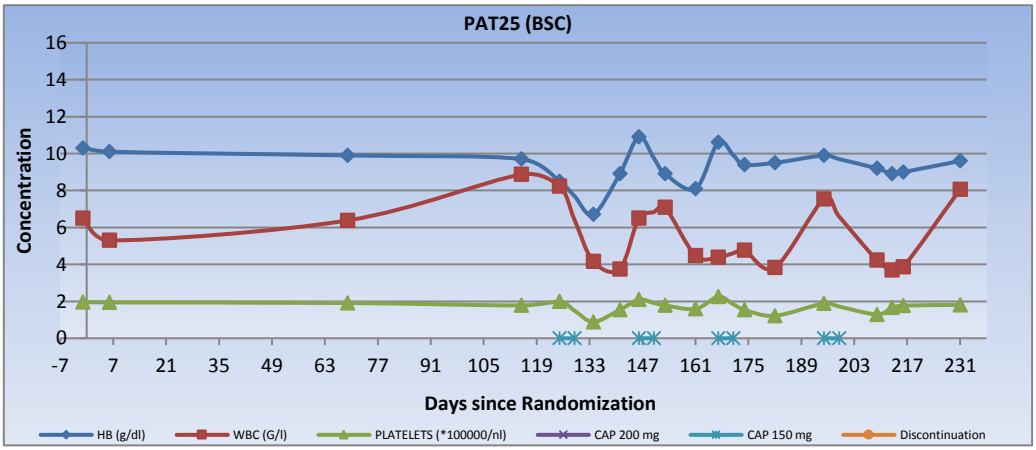

PAT18 (BSC)
Concentration in %
Days since Randomization
NEUTROPHILS (%)
LYMPHOCYTES (%)
MONOCYTES (%)
CAP 200 mg
CAP 150 mg
Discontinuation (PD)

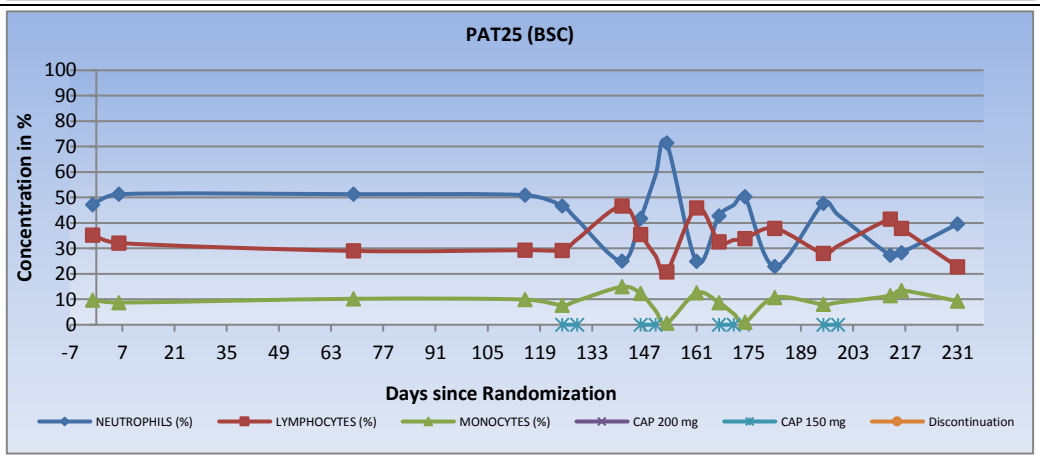

PAT20 (BSC)
Concentration
Days since Randomization
HB (g/dl)
WBC (/nl)
PLATELETS (*100000/nl)
CAP 200 mg
CAP 150 mg
Discontinuation (PD)
Death


PAT20 (BSC)
Concentration in %
Days since Randomization
NEUTROPHILS (%)
LYMPHOCYTES (%)
MONOCYTES (%)
CAP 200 mg
CAP 150 mg
Discontinuation (PD)
Death


PAT24 (BSC)
Concentration
Days since Randomization
HB (g/dl)
WBC (/nl)
PLATELETS (*100000/nl)
CAP 200 mg
CAP 150 mg
Discontinuation
Neulasta


PAT24 (BSC)
Concentration
Days since Randomization
NEUTROPHILS (/nl)
LYMPHOCYTES (/nl)
MONOCYTES (/nl)
CAP 200 mg
CAP 150 mg
Discontinuation
Neulasta


PAT25 (BSC)
Concentration
Days since Randomization
HB (g/dl)
WBC (G/l)
PLATELETS (*100000/nl)
CAP 200 mg
CAP 150 mg
Discontinuation


PAT25 (BSC)
Concentration in %
Days since Randomization
NEUTROPHILS (%)
LYMPHOCYTES (%)
MONOCYTES (%)
CAP 200 mg
CAP 150 mg
Discontinuation

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Eidesstattliche Versicherung

„Ich, Holger Jansen, versichere an Eides statt durch meine eigenhändige Unterschrift, dass ich die vorgelegte Dissertation mit dem Thema: „Clinical Evaluation of CAP7.1 in Therapy Refractory Biliary Tract Cancer in a Randomized Phase II Study“ selbstständig und ohne nicht offengelegte Hilfe Dritter verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel genutzt habe.

Alle Stellen, die wörtlich oder dem Sinne nach auf Publikationen oder Vorträgen anderer Autoren beruhen, sind als solche in korrekter Zitierung (siehe „Uniform Requirements for Manuscripts (URM)“ des ICMJE -*www.**icmje**.org*) kenntlich gemacht. Die Abschnitte zu Methodik (insbesondere praktische Arbeiten, Laborbestimmungen, statistische Aufarbeitung) und Resultaten (insbesondere Abbildungen, Graphiken und Tabellen) entsprechen den URM (s.o.) und werden von mir verantwortet.

Meine Anteile an etwaigen Publikationen zu dieser Dissertation entsprechen denen, die in der untenstehenden gemeinsamen Erklärung mit dem/der Betreuer/in, angegeben sind. Sämtliche Publikationen, die aus dieser Dissertation hervorgegangen sind und bei denen ich Autor bin, entsprechen den URM (s.o.) und werden von mir verantwortet.

Die Bedeutung dieser eidesstattlichen Versicherung und die strafrechtlichen Folgen einer unwahren eidesstattlichen Versicherung (§156,161 des Strafgesetzbuches) sind mir bekannt und bewusst.“

Datum: _____/_____/_____ Unterschrift: _____

Holger Jansen

Anteilserklärung an etwaigen erfolgten Publikationen

Herr Holger Jansen hatte folgenden Anteil an den folgenden Publikationen:

Publikation 1:

Randomized, multicenter phase II trial of CAP7.1, a novel prodrug, in patients with advanced biliary tract cancers shows promising efficacy results

Ulrich F Pape, S. Kasper, M Sinn, J Meiler, A Vogel, A Möller, A Burghardt, W Caca, Jan Kuhlmann, V. Rodriguez Lavas, A Kühl, A Ruza, U Keilholz, H Jansen, N Utku, ASCO Poster, Abstract Jan 2016

Herr Jansen hat die interim Ergebnisse der CAP7.1 phase II Studie in BTC Patienten analysiert und zu Figure-Darstellungen im Poster beigetragen.

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Unterschrift, Datum und Stempel der betreuenden Hochschullehrerin

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Unterschrift des Doktoranden

CV Holger Jansen

Mein Lebenslauf wird aus datanschutzrechtlichen Gründen in der elektronischen Version meiner Arbeit nicht veröffentlicht.