Aus dem Institut für Medizinische Immunologie
der Medizinischen Fakultät Charité – Universitätsmedizin Berlin

DISSERTATION

Safety Profile of CAP7.1 obtained during Phase I Trial in adult patients with refractory malignancies

zur Erlangung des akademischen Grades
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ABSTRACT (GERMAN)

Ziel:

Durchgeführt wurde eine nicht randomisierte Phase I Studie mit CAP7.1, einem Prodrug von Etoposid, in einem Dosisfindungsdesign zur Evaluierung der Sicherheit und Verträglichkeit am Patienten, zur Definierung der dosislimitierenden Toxizität, der maximal verträglichen Dosis sowie zur Feststellung der für eine Phase II Studie geeigneten Dosis. Auch sollte das pharmakokinetische Profil ermittelt werden.

Ergebnisse:

19 Patienten (4 weiblich, 15 männlich, medianes Alter 63 Jahre) mit therapieresistenten soliden Tumoren wurden in vier sukkzessiven Dosisleveln (45mg/m²/Tag bis 200mg/m²/Tag) intravenös über eine Stunde an fünf aufeinanderfolgenden Tagen alle drei Wochen mit CAP7.1 behandelt. Insgesamt wurden 62 Zyklen verabreicht. Häufige nicht hämatologische unerwünschte Ereignisse waren Fatigue, Nausea, Alopezie und Fieber. Über 90% der nicht hämatologischen unerwünschten Ereignisse waren Grad I und Grad II (NCI-CTCAE). Hämatologische unerwünschte Ereignisse waren dosisabhängig, Leukozytopenie und Neutrozytopenie, gefolgt von Thrombozytopenie waren am häufigsten. Neutropenisches Fieber war die häufigste dosislimitiernde Toxizität. Der Leukozyten- und Neutrophilennadir wurde zwischen Tag 10 und 17 erreicht, der Nadir der Thrombozyten zwischen Tag 12 und 15.

Fazit:

Das Sicherheitsprofil von CAP7.1 ist vergleichbar mit dem anderer Topoisomerase Inhibitoren. Die hauptsächliche dosislimitierende Toxizität ist Myelosuppression, die maximal verträgliche Dosis liegt zwischen 150 und 200 mg/m²/Tag, die empfohlende Dosis für eine Phase II Studie ist 200mg/m²/Tag.

ABSTRACT (ENGLISH)

Purpose:

A non-randomized dose escalating phase I trial with CAP7.1, a prodrug of etoposide, was performed, to determine the safety profile, the dose limiting toxicities, and the recommended phase II dose. Furthermore, the pharmacokinetic profile was evaluated.

Results:

19 patients (4 female, 19 male, median age 63) with refractory solid malignancies were treated with four successive dose levels (45mg/m²/day to 200mg/m²/day) intravenously over one hour on five consecutive days every three weeks. Overall, 62 cycles were administered. Most frequent non-hematological adverse events were: fatigue, nausea, alopecia, and fever. More than 90% were either grade 1 or grade 2 (NCI-CTCAE). Hematological adverse events were dose-dependent. Leukocytopenia, neutrocytopenia, followed by thrombocytopenia were most common, the main dose limiting toxicity was neutropenic fever. The nadir of leukocytes and neutrophils was between days 10 and 17, the nadir of thrombocytes was reached between days 12 and 15.

Conclusion:

The safety profile of CAP7.1 is similar to those of other topoisomerase inhibitors, the main dose limiting toxicity is myelosuppression, the maximum tolerated dose is between 150 and 200 mg/m²/day and the recommended phase II dose is 200 mg/m²/day.

LIST OF ABBREVIATIONS

5-FU	5-fluoruracil
ABC	adenosine triphosphate-binding cassette
AE	adverse events
ALL	acute lymphoblastic leukemia
ALT	alanine aminotransferase
ANC	absolute neutrophil count
ara-C	cytosine arabinoside
AST	aspartate aminotransferase
ATP	adenosine triphosphate
AUC	area under the curve
CES	carboxylesterase
CML	chronic myelogenous leukemia
CNS	central nervous system
CR	complete response
CrCl	creatinine clearance
CSF	colony stimulating factor
CT	computed tomography
CTCAE	common terminology criteria for adverse events
DLT	dose limiting toxicity
DNA	deoxyribonucleic acid
ECOG	Eastern Cooperative Oncology Group
e.g.	exempli gratia
EOI	end of infusion
GCP	good clinical practice
Hb	hemoglobin
HIV	human immunodeficiency virus
ICH	International Conference on Harmonization
INR	international ratio
MDR	multi drug resistance

MTD	maximum tolerated dose
MTX	methotrexate
N^{o}	number
NCI	National Cancer Institute
n.d.	not documented
PD	progressive disease
PK	pharmacokinetic
PLT	platelet count
PR	partial response
PT	prothrombin time
PTT	partial thromboplastin time
RECIST	response evaluation criteria in solid tumors
RP2D	recommended phase II dose
RNA	ribonucleic acid
SAE	serious adverse event
SD	stable disease
ULN	upper limit of normal
WBC	white blood cell count
Wt	weight

1. INTRODUCTION

1.1 CONVENTIONAL CHEMOTHERAPEUTICS

Chemotherapy plays an important role in cancer therapy. Anticancer chemotherapy can either precede other anticancer therapy (neoadjuvant chemotherapy), succeed other anticancer therapy (adjuvant chemotherapy), or it can be administered on its own (Wu, 2011). Conventional chemotherapeutics damage normal cells as well as tumor cells unselectively, leading to significant and almost inevitable side effects. Typical events involve myelosuppression, stomatitis, mucositis, enterocolitis, nausea and vomiting. Myelosuppression is the dose limiting toxicity of many conventional chemotherapeutics (Barrett, 2007). Besides toxicity, drug resistance is another major problem in anticancer chemotherapy. Resistance is either intrinsic to the cancer or acquired during anticancer treatment. Tumor cells can express mechanisms of coetaneous resistance to structurally and functionally different anticancer agents (Gottesman, 2002, Liu, 2009). This so-called multidrug resistance can result from decreased uptake of the drug by the cell or higher drug efflux out of the cell. MDR often leads to cancer relapse and subsequently to death. A major efflux mechanism is through the expression of an energy-dependent pump named P-glycoprotein or multidrug transporter, a member of a group of ATP-dependent efflux pumps, known as the adenosine triphosphate-binding cassette family (Gottesman, 2002, Liu, 2009). Many commonly used drugs are substrates of multidrug transporters. The overexpression of drug transporters is important, but is only one of many mechanisms of drug resistance in anticancer chemotherapy (Wu, 2011).

One important question for future drug development in anticancer therapy will be how to circumvent drug resistance. One possible way to move forward is to design agents, which are not substrates of the mentioned drug transporters.

The most important groups of anticancer agents will be briefly reviewed in the following paragraphs. The investigated agent CAP7.1 is a topoisomerase II inhibitor activated by carboxylesterases. Topoisomerase II inhibitors and the enzymes topoisomerase and carboxylesterase will be described in greater detail.

1.1.1 Antimetabolites

Widely used and important drugs in cancer therapy include pyrimidine analogues (e.g. 5-fluoruracil, capecitabine, and cytosine arabinoside) and antifolates (e.g. methotrexate). 5-FU is an important drug in the treatment of colorectal cancer (Barrett, 2007). Its mechanism of action is through inhibition of thymidine synthase, and through incorporation into nucleic acids (Barrett, 2007). Common side effects involve myelosuppression and mucositis. Capecitabine is an oral prodrug of 5-FU, which is, after different enzymatic steps, converted into 5-FU in tumor cells (Reigner, 2001). Capecitabine is used in the treatment of metastatic colorectal and breast cancer. The toxicity and efficacy is comparable with 5-FU (Barrett, 2007). Ara-C is phosphorylated to its active form by the deoxycytidine kinase, then the active phosphate form incorporates into DNA and inhibits DNA synthesis. Ara-C is used in the treatment of hematological malignancies (Barrett, 2007).

The most common and widely used antifolate is MTX for the treatment of hematological and solid malignancies as well as for inflammatory diseases. MTX inhibits the dehydrofolate reductase, an enzyme that maintains the levels of reduced folate, which is needed for DNA synthesis. Common toxicities include mucositis and myelosuppression (Barrett, 2007).

1.1.2 Alkylating Agents

Cyclophosphamide and ifosfamide are widely used in the treatment of different solid and hematological malignancies. They are important drugs for the treatment of pediatric malignancies as well. The use is limited by tumor resistance which is due to multiple mechanisms (Zhang, 2005). Both agents are prodrugs and activated by cytochrome P450 (Chen, 2004). Mechanism of action is through alkylation of the nucleic acids DNA and RNA. Important toxicities include myelosuppression, which is dose limiting, and hemorrhagic cystitis can develop due to the toxic metabolite acreolin. There is an increased long-term risk of secondary malignancies with all alkylating agents (Barrett, 2007). Temozolomide is another important alkylating agent, which is used in the treatment of malignant melanoma and brain tumors (Stupp, 2005, Barrett, 2007).

1.1.3 Platinum Agents

Cisplatin and carboplatin form inter- and intra-strand DNA cross-links (Eastman, 1987). Inhibition of DNA replication leads to triggering of the apoptotic pathway. Both drugs are widely used in the treatment of different malignancies including testicular, ovarian, bladder, and lung cancer (Barrett, 2007). Toxicities include neurotoxicity and nephrotoxicity. Efficacy problems are likely to occur because of intrinsic and acquired resistance and different mechanisms including changes in drug transport, increased drug detoxification, and changes in DNA repair (Köberle, 2010). Oxaliplatin is used in the treatment of colorectal cancer. Dose limiting toxicity is a cumulative mixed neuropathy. Other important toxicities include myelosuppression and nausea (Barrett, 2007).

1.1.4 Antimicrotubule Agents

Taxanes are represented by paclitaxel and docetaxel, both of which bind to tubulin and interfere with the assembly microtubules. Mutations in the b-tubulin gene lead to higher levels of resistance (Monzo, 1999, Hasegawa, 2003). Both drugs are important in the treatment of breast, ovarian, prostate, small lung cell cancer and others. Relevant toxicities include myelosuppression, peripheral neuropathy, and hypersensitivity reactions (Barrett, 2007). Another group of antimicrotubule agents are the vinca alkaloids (vincristine, vinblastine, vinorelbine, and vindesine) they are active against different hematological malignancies, breast cancer, and small lung cancer. Their mechanism of action is through prevention of polymerization of tubulin. Toxicities include myelosuppression, neuropathy, and constipation.

1.2 Topoisomerase Enzymes

Topoisomerases I and II are highly conserved and ubiquitous enzymes which alter the topological state of the DNA. They are essential enzymes in DNA metabolism and cell division. The enzymes solve topological problems of the DNA by creating transient strand breaks (Osherhoff, 1989, Watt, 1994, Wang, 2002, Christensen, 2002, Nitiss, 2009). Topoisomerase I binds DNA, then it creates a

transient single-stranded break in the backbone of a DNA. Afterwards topoisomerase I passes the unbroken strand through the nick and subsequently rejoins the scission. (Osherhoff, 1989, Wang, 2002). Topoisomerase II binds DNA, afterwards it creates a transient double strand break in the DNA backbone this action requires the presence of a divalent cation (Mg^{++}). Then the enzyme passes an intact double-stranded DNA helix through the break. Topoisomerase II relegates the break. This step requires ATP (Liu, 1980, Osherhoff, 1989, Watt, 1994, Burden, 1998, Wang, 2002, Nitiss, 2009). Topoisomerase I and II can both modulate DNA over- and under-winding, in addition, topoisomerase II can remove knots or tangles from a double-strand DNA (Deweese, 2009, Nitiss, 2009). Both topoisomerase I and II are used as targets for anticancer drugs.

1.3 Topoisomerase I Inhibitors

Irinotecan and topotecan are important members of this group. Both drugs stabilize the complex formed by topoisomerase I and DNA, relegation of DNA is inhibited (Hsiang, 1989). Irinotecan is activated by carboxylesterases. It is used in the treatment of colorectal cancer. Common toxicities include myelosuppression, mucositis, and diarrhea. Topotecan is active in ovarian and non-small lung cancer (Kudelka, 1996). Its DLT is myelosuppression.

1.4 Topoisomerase II Inhibitors

Because of its essential functions for the survival of cells, topoisomerase II is target for some widely used anticancer drugs. (Burden, 1998, Hande, 2008). Most clinical active topoisomerase II inhibitors involving doxorubicin, mitoxantrone, and etoposide increase the level of topoisomerase II DNA complex, termed cleavable complex. They disrupt the normal catalytic cycle of topoisomerase II. Drugs in this group are called topoisomerase poisons as well (Watt, 1994, Bender, 2008, Nitiss, 2009, Bailly, 2012). Another group of compounds inhibits the enzyme's catalytic activity. They are therefore termed catalytic topoisomerase II inhibitors (Andoh, 1998, Larsen, 2003, Nitiss, 2009).

1.4.1 Catalytic Topoisomerase II Inhibitors

Catalytic inhibitors are a heterogeneous group able to interfere with at least one step of topoisomerase's II catalytic cycle. In contrast to topoisomerase poisons, they do not stabilize the cleavable complex. For instance, the anticancer drugs aclarubicin and suramin prevent the binding of topoisomerase II and DNA. Novobiocin blocks the ATP-binding site of topoisomerase II. Bisdioxopiperazines (ICRF-193 and others) inhibits topoisomerase II by forming a complex, which leads to inhibition of ATPase activity. In contrast to topoisomerase II poisons, catalytic inhibitors are not solely used as anticancer drugs. For example bisdioxopiperazines are used as cardioprotectors (Andoh, 1998, Larsen, 2003).

1.4.2 Topoisomerase II Poisons

Anthracycline antibiotics such as doxorubicin, daunorubicin, adriamycin and idarubicin are commonly used anticancer drugs. They are active against breast cancer, leukemias, lymphomas, sarcomas and others. They lead to apoptotic cell death through different mechanisms. Inter alia they intercalate with the DNA and inhibit topoisomerase II. Myelosuppression is dose limiting and frequent, other common toxicities include nausea, vomiting, alopecia, and mucositis. Anthracyclines are cardiotoxic, the occurrence of congestive heart failure correlates with the cumulative dose (Hande, 2008, Bailly, 2012). Mitoxantrone is the only anthraquinone used: it binds to topoisomerase II and induces DNA strand breaks. It is used for the treatment of prostate cancer, breast cancer, leukemias, and lymphomas. DLT is myelosuppression, other frequent toxicities are nausea, vomiting, alopecia, and cardiotoxicity (Hande, 2008, Bailly, 2012). Epipodophyllotoxins include Etoposide and Teniposide. Etoposide is one of the longest utilized anticancer drugs. It shows antineoplastic activity against a wide broad of solid and hematological malignancies including small lung cancers, AML, CML, Hodgkin's and Non-Hodgkin's lymphoma, testicular cancer, ovarian and gastric cancer (Hande, 1998, Bender, 2008, Bailly, 2012). Etoposide phosphate is a water-soluble ester of etoposide. It is the only prodrug of etoposide in clinical use so far, it is used in combination in the treatment of refractory testicular cancer and small lung cancer. It is rapidly and completely converted into etoposide in the plasma. Most prominent side effects of etoposide phosphate are

myelotoxicity and gastrointestinal toxicity. It is used in the treatment of testicular and small lung cell cancer (Bristol-Myers Squibb, 2011).

The toxicities of etoposide include myelosuppression, which manifests itself as leukopenia and thrombocytopenia. It is also neurotoxic. Alopecia, nausea, vomiting and diarrhea are known side effects. Besides myelosuppression, mucositis is a dose limiting toxicity (Hande, 2008). Teniposide is an analogue of etoposide. It is used in pediatric patients with poor prognosis ALL. Etoposide poses a long-term risk for the development of therapy-related secondary leukemias (Bailly, 2012).

1.5 CARBOXYLESTERASES

Carboxylesterases play an important role in the biotransformation of a large amount of various chemicals including drugs (Satoh, 1998, Satoh, 2006, Hosokawa 2008). According to the homology in the amino acid sequence, carboxylesterases can be divided into five major groups (CES1-CES5). The majority of carboxylesterases belongs to CES1 and CES2 (Hosokawa, 2008). Carboxylesterases play an important role in the pharmacokinetic behavior of a large amount of drugs, including prodrugs. The effect can be inactivation and activation of ester- or amide type prodrugs (Rooseboom, 2004, Satoh, 2006, Hosokawa, 2008). Carboxylesterases are ubiquitously expressed enzymes. The isoenzyme carboxylesterase 1 is highly expressed in the liver and very low in the gastrointestinal tract, whereas the majority of carboxylesterase 2 is present in the small intestine, colon, kidney, and liver (Imai, 2006).

1.6 CAP7.1

A novel chemotherapeutic agent CAP7.1 is a pro-drug of etoposide. A water-soluble ester group is attached to etoposide, so that the pharmacophore group is blocked. CAP7.1 is activated through carboxylesterases. CAP7.1 shows cytotoxic activity through inhibition of topoisomerase II that causes DNA damage and cell cycle arrest (Utku, 2011a).

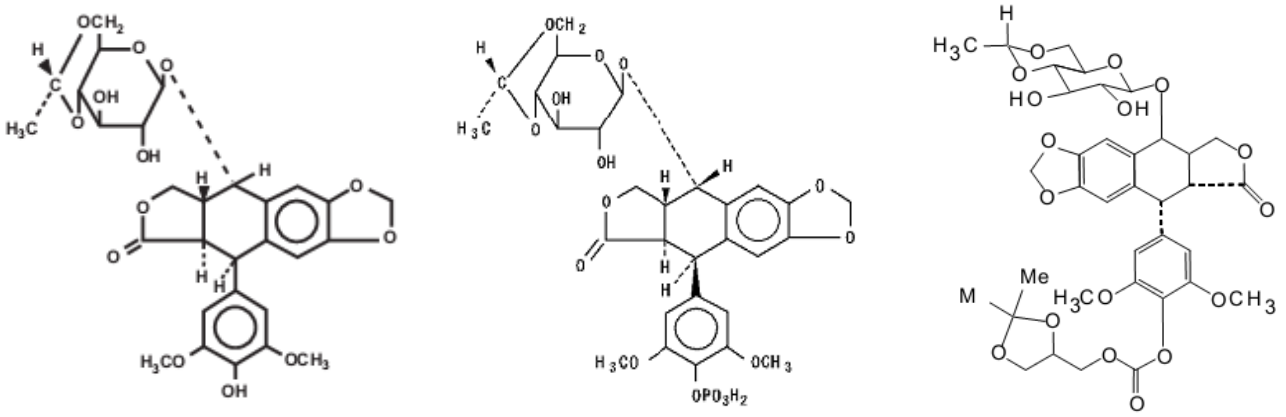


Figure 1: *chemical structure of etoposide (left, from http://www.pfizer.com/files/prodructs/upsi_toposar.pdf), etoposide phosphate (middle, from http://packageinserts.bms.com/pi/pi_etopophos.pdf) and CAP7.1 (right, Utku, 2011a)*

1.6.1 Preclinical Data

Antitumor activity, pharmacokinetics, and safety profile of CAP7.1 were preclinically tested using various in vitro and in vivo assays. Cytotoxic activity was shown in various human neoplastic cell lines, including neuroblastoma, leukemia, and solid tumors. Compared to etoposide the cytotoxic potency ranged from 0.1 to 1000 times more active in vitro. CAP7.1 also showed cytotoxic activity in an in vivo tumor xenograft model (neuroblastoma in mice, unpublished data). Furthermore CAP7.1 showed significant activity in cell lines that were resistant to etoposide. CAP7.1 is not a substrate for p-glycoprotein, a MDR gene product responsible for the transportation of toxic substances out of cells (Utku, 2011a). It is one of the important mechanisms that leads to cellular resistance to a variety of cytotoxic agents in a range of malignant diseases.

Pharmacokinetic investigations were performed in rodents and primates. In rat serum, where the activity of carboxylesterase is known to be relatively high compared to humans or primates, CAP7.1 was very rapidly converted to etoposide. The conversion to etoposide was slower in primates, with moderate levels of CAP7.1 at the end of infusion (Utku, 2011a).

Safety profile investigation included safety pharmacology, genetic toxicity, and single and repeated dose toxicity. In cynomolgus monkeys, heart rate, blood pressure, and duration of PQ, QRS, and QT

intervals were analyzed. CAP7.1 did not have any effect on the cardio-vascular system. As expected, CAP7.1 is genotoxic, it produced chromosome aberrations in human lymphocytes. The single and repeated dose toxicity was similar to etoposide, however the MTD was several fold higher than observed for etoposide. The most common effects involved weight loss, diarrhea, hair loss, leukopenia, and anemia. Usually recovery from the side effects was seen in the majority of the animals. The toxicity profile of CAP7.1 was consistent with etoposide and topoisomerase inhibition in general (Utku, 2011a).

1.6.2 Clinical Data

In an initial compassionate trial CAP7.1 was administered in combination with carboplatin to three pediatric patients with advanced stage neuroblastoma. All patients had previously received etoposide and carboplatin and were all heavily pretreated with intense systemic therapy and autologous stem cell rescue. The drug was very well tolerated at high dose levels (400-800mg/m²/day). Although the dose exceeded the therapeutic dose of etoposide in adults with solid tumors, only reduced neutrophils were observed with no further dose limiting toxicity, especially no organ toxicity was observed. The toxicity profile was similar to etoposide. Although the CAP7.1 dose exceeded the etoposide dose by several fold it was better tolerated in children. PK data was tested in one patient and showed a rapid conversion to etoposide (t/2=12-20 min) (Utku, 2011a).

2. METHODS

2.1 ETHICS

The study was authorized by the Bundesinstitut für Arzneimittel und Medizinprodukte and the ethic committee Lageso Berlin. The Studynumber is CPN70101.

2.2 STUDY DESIGN

CAP7.1 was administered in an open label, non-randomized, dose escalating trial. The dose was escalated in cohorts of 3-6 patients up to the maximum tolerated dose.

The MTD would be the dose at which dose limiting toxicities occurred in 33% or more of a cohort (1 of 3, or 2 of 6 patients). A new (higher dose) cohort may start, when at least three patients have completed at least 26 days of the study at the previous dose, without a DLT (Utku, 2011b, Keilholz, 2012).

Dose limiting toxicities were defined as grade 3 or higher non-hematological AEs, grade 4 neutropenia lasting for more than 7 days or complicated by fever, grade 4 thrombocytopenia or thrombocytopenia of any grade complicated by bleeding, which were considered to be drug-related by the investigator, using the NCI-CTCAE. DLTs that were observed during the first cycle of therapy led to expansion of cohort 3 to 6 patients (Utku, 2011b, Keilholz, 2012).

The recommended dose for phase II testing should be one dose-level below the MTD following general practice.

Since CAP7.1 is a cytotoxic agent, the study was conducted with patients with locally advanced or metastatic malignancies that were refractory to standard therapy or for which no standard therapy was available (Utku, 2011b, Keilholz, 2012).

All patients were followed until disease progression, death, initiation of alternative anticancer treatment or end of study. The end of study was defined as 26 days after the last patient has completed treatment (Utku, 2011b, Keilholz, 2012).

2.3 Study Objectives

The primary objectives of this study were to determine the MTD of CAP7.1, to determine the toxicity profile including the dose limiting toxicities of CAP7.1, and the dose suitable for phase II testing. Secondary objectives of this trial were to investigate the pharmacokinetics in adults, and to make a preliminary assessment of the anti-tumor activity of CAP7.1 with special attention to malignancies expected to be responsive to etoposide (Utku, 2011b, Keilholz, 2012).

2.4 Inclusion Criteria

- Histologically or cytologically confirmed, locally advanced or metastatic malignant disease which was refractory to standard treatment or for which no standard therapy was available.
- Measurable or non-measurable disease according to RECIST (Response Evaluation Criteria in Solid Tumors)
- Age > 18 years
- ECOG Performance status 0-2
- Life expectancy of at least 8 weeks
- Adequate bone marrow and organ function including
    - Hemoglobin > 9 g/dl
    - Absolute neutrophil count (ANC) > 1.5/mm3
    - Platelet count (PLT) > 100/mm3
    - Total Bilirubin < 1.5 times the upper limit of normal (ULN)
    - (ALT) and (AST) < 2.5 x ULN (< 5 x ULN for patients with liver involvement
    - PT-INR and PTT < 1.5 ULN

- Creatinine clearance (CrCl) > 50 mL/min, according to modified Cockcroft-Gault criteria: CrCL = Wt (kg) x (140 – age) / 72 x creatinine level x 1 (male), x 0.85 (female)

- Must have recovered from acute reversible effects of previous anti-cancer chemotherapy. Immunotherapy, radiotherapy or endocrine therapy. This means generally that at least 4 weeks should have elapsed since major surgery, radical radiotherapy or myelosuppressive chemotherapy (6 weeks for nitrosoureas or mitomycin C). At least 4 weeks must have elapsed since treatment with an investigational drug.
- Medically controlled, negative pregnancy test in all women except those who were surgically sterile or at least one year postmenopausal.
- Highly effective method of contraception which result in a low failure rate, i.e. less than 1% pregnancies per year: Female patients of child bearing potential were eligible, if they agreed to use a highly effective method of birth control throughout the study and at least 4 weeks after stopping treatment. Male patients with partners of child bearing potential were eligible, if they agreed to use contraception during the trial and for 6 months after stopping study drug, unless surgically sterile.
- Written informed consent to ICH-GCP and national/local regulations
- High probability of good compliance and orderly completion of the study

(Utku, 2011b)

2.5 Exclusion Criteria

- Known central nervous system involvement unless this has been definitively treated with radiotherapy or surgery and the patient's CNS is clinically stable at the time of study entry
- Karnofsky-Index < 70%
- Serious concurrent medical condition, which could affect compliance with protocol or interpretation of results. Patients with uncontrolled infection and patients known to be infected with the human immunodeficiency virus or with chronic hepatitis B or C virus infection were not eligible for the study.
- History of another malignancy that could affect compliance with the protocol or interpretation of results. Patients who have been treated with curative intent and remained

disease-free for at least 5 years were generally eligible, as were patients with in situ disease treated with curative intent.

- Other psychological or social conditions which in the investigator's opinion would not have made the patient a good candidate for the clinical trial.
- Pregnancy or breast-feeding
- Other anticancer therapies except endocrine therapy for prostate cancer. Bisphosphonates, where introduced prior treatment, were allowed.
- Known or suspected etoposide refractory tumors
- Known or suspected peripheral neuropathies
- Patients who had been committed to an institution by a court order or an official directive.

(Utku, 2011b).

2.6 Treatment Administration

CAP7.1 was supplied in sealed 240 ml infusion sets ready for administration. It was administered as an intravenous infusion over 60 minutes daily on five successive days. The cycles were scheduled every 21 days. In patients, who required more than three weeks for recovery from hematologic or non-hematologic toxicity, the treatment was generally stopped. The patients entered cohorts sequentially, the cohort with the next higher dose level was started when at least three patients had completed at least 26 days without the appearance of a DLT. After every two cycles patients were assessed for disease progression. In case of disease progression the treatment was stopped. Otherwise up to six cycles were administered. Furthermore, the study was stopped in case of unmanageable toxicity or at patient's request. The starting dose in this trial was 45 mg/m^2/day, the dose was planned to be escalated up to 300 mg/m^2/day, or up to the MTD, defined as the dose where DLT occurred in 1/3 or more of the patients within one cohort. Dose increments were of 45-60 mg/m^2/day when only negligible toxicity only was observed. Three patients per dose level were treated, and the cohorts were expanded to six patients when DLT was observed. Treatment was delayed by one-week increments until recovery from treatment-related toxicity. Patients may also require a dose reduction until all drug-related toxicities resolved to grade 1 or baseline, whichever was the higher within 14 days of the intended treatment day, and then treatment was resumed. If no

DLT occurred, treatment was continued at the next lowest dose level. Patients were removed from the study whenever unacceptable toxicity occurred (e.g. non-hematological NCI-CTCAE grade 4 toxicity, hematological values ≥ 3 within seven days preceding the next dosage administration) (Utku, 2011b).

2.7 Toxicity Assessment

Abnormal hematological values and signs of non-hematological toxicities were addressed by using the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE) for Cancer Clinical Trials (Version 3.0, 2006). AEs were categorized according to a 5-point scale, where grade 1=mild, grade 2=moderate, grade 3=severe, grade 4=life threatening or disabling, and grade 5=fatal. AEs and SAEs were recorded from the time the patient was registered up to and including 26 days after the last protocol treatment. Laboratory tests, including hematology (hemoglobin, WBC with differential, and PLT) and biochemistry (sodium, potassium, calcium, phosphorus, urea, creatinine, total protein, albumin, bilirubin, alkaline phosphatase, ALT, AST, glucose, and uric acid) were performed weekly. Physical examination was performed prior to each new cycle of therapy (Utku, 2011b, Keilholz, 2012).

2.8 Concomitant Medication

All patients received full supportive care, including antiemetics, antibiotics, transfusions, and analgetics, as clinically indicated. Colony stimulating factors were used in the treatment of prolonged cytopenias and its complications. Prophylactic administration of CSF was not allowed in this trial. Women of childbearing potential had to take appropriate measures to prevent pregnancy during and for at least four weeks after the study. Male patients with partners of childbearing potential had to agree to use contraception during the study and for six months after stopping the study drug (Utku, 2011b, Keilholz, 2012).

2.9 Pharmacokinetic Assessment

All patients were included in the pharmacokinetic analysis part of the study. Blood and urine samples were collected and the pharmacokinetics of CAP7.1 and its product etoposide were investigated. Blood samples were taken before the administration of CAP7.1 on all days of infusion. On day 1 blood was collected 30 min before EOI and 15 min, 45 min, 90 min, 3 h, 6 h, and 10 h after EOI. On the following days blood samples were taken before the infusion and 15 min after EOI. 24 h urine collection was started at the beginning of infusion on day 1 and additional urine samples were taken on the following days prior to the start of infusion (Utku, 2011b, Keilholz, 2012).

2.10 Efficacy Assessment

Response was assessed according to RECIST (response evaluation criteria in solid tumors), and patients were categorized as follows: complete response (CR), partial response (PR), stable disease (SD), or progressive disease (PD). Duration of response was assessed in patients receiving CR or PR. After every two cycles of therapy, all sites of disease documented at baseline were evaluated (Utku, 2011b, Keilholz, 2012).

2.11 Pretreatment and Follow-up Information

As a minimum requirement, the following was completed according the schedule of assessments prior to treatment: medical history, physical examination, and ECOG performance status, 12-lead ECG and laboratory tests (hemoglobin, WBC with differential, and PLT, sodium, potassium, calcium, phosphorus, urea, creatinine, total protein, albumin, bilirubin, alkaline phosphatase, ALT, AST, glucose, uric acid, prothrombin time and partial thromboplastin time, pregnancy test in women of childbearing potential, and urinalysis per dipstick). For tumor assessment, images of all suspected sites of disease, a baseline CT scan of the chest and abdomen and available tumor markers were also collected (Utku, 2011b).

A post-treatment safety visit was performed approximately 26 days after treatment, including physical examination, ECOG performance status, and laboratory for hematology and biochemistry (Utku, 2011b).

2.12 Literature Search and Statistics

The database of the U. S. National Library of Medicine (pubmed.org) was used in the research. Comparative clinical trials were identified as follows: Phase I clinical trials using an anticancer agent in monotherapy in the treatment of various malignancies. Trials had to be performed either with a drug with topoisomerase inhibiting properties or prodrugs activated through carboxylesterases. Furthermore other anticancer agents, that were in phase I clinical trials during the past five years were identified to get an overview of the toxicity profile of other novel anticancer drugs.

All tables were generated with Microsoft Word 2013, all calculations were performed and all charts generated with Microsoft Excel 2013.

3. RESULTS

3.1 PATIENT CHARACTERISTICS

19 patients were enrolled into the study between April 2009 and September 2011. The patient age ranged from 32 to 72 years (median 63 years). 79% were male and 21% female (one patient had genotype 46 XY, testicular feminization). The majority of patients (79%) had an ECOG performance score of one, 16% showed an ECOG of 0 and only one patient had an ECOG score of 2. All patients had previously received at least two regimes (range 1-9) of systemic chemotherapy, six patients (32%) had received five or more regimes of chemotherapy. In addition, 13 patients (68%) had received radiotherapy. Surgery had been performed in 15 patients (79%) due to malignant disease. There was a broad range of advanced solid malignancies, including cancer of testis, ovaries, esophagus, esophagogastric junction, colon, gallbladder, lung, head & neck, thymus and Merkel cell. Two patients had the same histological type (adeno-squamous) of rectal carcinoma and two patients the same type (squamous cell carcinoma) of oropharyngeal carcinoma.

Two patients did not fulfill all inclusion criteria. In both patients the last systemic chemotherapy was less than four weeks old. In patient 7, a 65 year old female with tonsil cancer and metastases in the liver and the lung, the last systemic chemotherapy, a combination of fluorouracil, folinic acid and oxaliplatin had been performed 26 days rather than four weeks previously. This patient who suffered from liver metastases also showed a more than five times higher ALT at the beginning of the study. Patient number 8, a 32-year old male with testicular cancer and multiple metastases (brain, lung, and kidney) finished his last systemic chemotherapy with both oxaliplatin and docetaxel 27 days before the first application of the study product. Patient 7 experienced dose limiting neutropenic fever during the first cycle of therapy. This patient was treated for tonsil cancer and the trial was discontinued after the second cycle of therapy due to progressing disease. Patient number 8 was treated for testicular cancer and experienced dose limiting headache during the fourth cycle of the trial, he discontinued the study due to SAE after the first infusion day of cycle 4. Patient number 11 did not experience grade 3 or 4 toxicity during the study, he discontinued the trial after cycle 1 because of noncompliance.

patient	gender	age	malignancy	ECOG	prior therapy regimes	radio-therapy	surgery
1	M	55	hypopharyngeal carcinoma	1	2	X	
2	M	48	oropharyngeal carcinoma	1	2	X	
3	M	60	thymic carcinoma	1	9		X
4	M	64	urothelial carcinoma bladder	1	1		X
5	M	64	gallbladder carcinoma	0	1		X
6	M	49	CUP-syndrome	0	1	X	X
7	F	65	tonsil carcinoma	1	5	X	X
8	M	32	testicular cancer	1	7	X	X
9	F	67	ovarian carcinoma	1	5		X
10	F	70	Merkel cell tumor	1	2	X	X
11	M	72	oropharyngeal carcinoma	2	2	X	X
12	M	52	esophagogastric junction carcinoma	0	2	X	X
13	M	50	penis carcinoma	1	1	X	X
14	M	67	lung carcinoma	1	3	X	X
15	F	63	Sertoly-Leydig cell tumor	1	2		X
16	M	48	cholangiocarcinoma liver	1	2		
17	M	66	esophageal carcinoma	1	4	X	
18	M	66	rectal carcinoma	1	9	X	X
19	M	63	rectal carcinoma	1	7	X	X

Table 1: *Patient Characteristics*

3.2 Study Drug Administration

The median duration of treatment for CAP 7.1 across all cohorts was 63 days (range 5-151 days). The median number of completed cycles administered was three (range 1-6 cycles). In total, 62 cycles were administered. Four patients (21%) completed treatment, receiving all 6 cycles of CAP7.1 treatment. All of these patients were in cohort 4.

The patients in the phase I study were treated with four successively increasing dose levels in the respective cohorts. The planned infusion time was one hour. However, it was longer than one hour in 34 cycles (55%) with a maximum length 160 minutes (patient 14, cycle 3, day 5) and shorter in 2

cycles (3%) with a minimum length of 45 minutes (patient 17, cycle 6, day 4). Infusion was interrupted for 5 minutes in patient 1 (cycle 2, day 1), in patient 3 infusion was interrupted for 14 minutes (cycle 1, day 3), in patient 11 infusion was interrupted for five minutes (cycle 1, day 1), and in patient 16 infusion was interrupted for 43 minutes (cycle 2, day 1) due to infusion reaction including flush, prickling and dyspnea. Treatment was delayed in a total of 23 cycles (approximately one third of total cycles given) due to adverse events. 19 times (in eight patients) treatment was delayed for seven days, two times (in two patients) it was delayed for 14 days, and in one patient one cycle was delayed by nine days followed by the next cycle which was delayed by another five days. All treatment delays occurred in the cohorts 3 and 4. In three cases doses were reduced due to adverse events and deteriorating physical conditions. They were all performed in the first cycle of cohort 3.

In cohort 1 three patients were treated with a daily dose administered at 45 mg/m²/day. Two patients discontinued the study because no study drug could be provided on time. Patient 1 received four cycles and patient 3 received two cycles. Patient 2 discontinued the trial after two cycles because of progressing disease. No dose reduction was necessary and no treatment was delayed in this cohort. In total, eight cycles of CAP7.1 were administered.

In cohort 2 three patients received CAP7.1 with a daily dose of 90 mg/m²/day. Two patients were withdrawn from the study after the second cycle due to progressing disease. One patient's removal was medically warranted after four cycles. In this cohort, ten cycles of the drug were administered in total, there were no dose reductions, and no cycles were delayed.

Six patients were treated with CAP7.1 in cohort 3 with a daily dose of 150 mg/m². Two of these patients completed two cycles and the other two patients four cycles of CAP7.1 treatment. One patient was withdrawn from the trial due to a serious adverse event one day after the first infusion during the fourth cycle. One patient was noncompliant and was therefore withdrawn after the first cycle. In three patients, the dose was reduced after the first cycle due to adverse events. In total, 16 cycles were completed in this cohort, and six cycles were delayed in four patients.

In cohort 4, a total of seven patients were treated with CAP7.1 at a daily dose of 200 mg/m². This cohort was expanded to initially to six patients, as one patient dropped out due to disease progress at the beginning of the study, the safety committee decided to recruit one additional patient into the cohort to expose sufficient number of patients to the study drug. In total, 28 cycles were completed. Four patients completed the full study protocol, one patient was withdrawn after the second cycle because of progressive disease and two patients were withdrawn due to SAEs during the first cycle. Study drug administration was postponed in four patients and 17 cycles. No dose reductions had to be made.

Patient	Cohort	Cycles	Treatment Time (days)	Dose Reduction	Delay of Treatment	End of study
1	1	4	63			no study drug provided
2	1	2	25			progressive disease
3	1	2	25			no study drug provided
4	2	2	25			progressive disease
5	2	4	67			progressive disease
6	2	4	67			medically warranted
7	3	2	32	neutropenic fever (cycle1)	neutropenic fever (cycle1 (7 days))	progressive disease
8	3	3	63	candidiasis (cycle 1)		reduced physical status
9	3	2	32	n. d. (cycle 1)	leukocytopenia (cycle 1 (7 days))	progressive disease
10	3	4	73		neutrocytopenia (cycle 1 (7 days))	progressive disease
11	3	1	5			noncompliance
12	3	4	88		leukocytopenia (cycle 1 (7 days))	progressive disease
					leukocytopenia (cycle 2 (7 days))	
					leukocytopenia (cycle 3 (7 days))	
13	4	6	151		anemia, leukocytopenia, thrombocytopenia (cycle 1 (7 days))	sudden death
					anemia, leukocytopenia (cycle 2 (7 days))	
					cystitis, fever (cycle 3 (9 days))	
					leukocytopenia, thrombocytopenia (cycle 4 (5 days))	
					anemia, urinary tract infection (cycle 5 (14 days))	

Patient	Cohort	Cycles	Treatment Time (days)	Dose Reduction	Delay of Treatment	End of study
14	4	6	130		leukocytopenia, neutrocytopenia, thrombocytopenia (cycle 1 (14 days))	full study protocol
					leukocytopenia, thrombocytopenia (cycle 3 (7 days))	
15	4	1	5			pulmonary embolism
16	4	6	144		leukocytopenia, neutrocytopenia, thrombocytopenia (cycle 1 (7 days))	full study protocol
					leukocytopenia, neutrocytopenia, thrombocytopenia (cycle 2 (7 days))	
					leukocytopenia, neutrocytopenia, thrombocytopenia (cycle 3 (7 days))	
					leukocytopenia, neutrocytopenia, thrombocytopenia (cycle 4 (7 days))	
					leukocytopenia, neutrocytopenia, thrombocytopenia (cycle 5 (7 days))	
17	4	6	144		leukocytopenia, neutrocytopenia, thrombocytopenia (cycle 1 (7 days))	full study protocol
					leukocytopenia, neutrocytopenia (cycle 2 (7 days))	
					leukocytopenia, neutrocytopenia (cycle 3 (7 days))	
					leukocytopenia, neutrocytopenia (cycle 4 (7 days))	
					leukocytopenia, neutrocytopenia (cycle 5 (7 days))	
18	4	1	5			sepsis
19	4	2	25			progressive disease

Table 2: *Study Drug Administration*

3.3 Adverse Events and Toxicity

3.3.1 Non-hematological Adverse Events

In the 62 cycles administered, 221 non-hematological adverse events occurred. 191 (86%) of them were observed in the cohorts 3 and 4. In 205 adverse events (93%), the severity was mild or moderate. 13 events (6%) were severe in intensity, two events (< 1%) were life threatening, and one event (< 0.5%) was fatal.

5% (eleven AEs in nine patients) of the adverse events were judged to be certainly related to the study drug. The AEs judged to be certainly related were eight cases of alopecia (n=7, grade 2; n=1, grade 1), two cases of nausea (n=1, grade 1; n=1, not documented), and one case of reduced physical status (grade 2). 7% (16 AEs in nine patients) of the non-hematological AEs were judged to be probably related to the study drug. These were six cases of fatigue (n=4, grade 1; n=1, grade 2; n=1, not documented), four cases of nausea (n=3, grade 1; n=1, grade 2), and one case each of the following: reduced physical status (grade 1), loss of appetite (grade 1), fever (grade 1), infusion reaction (grade 2), mucositis (grade 3) and headache (grade 3). Mucositis was treated with antimycotics, antibiotics and pain medication, the patient recovered after five days of therapy, and no action regarding the study drug was taken, therefore this event was not judged to be dose limiting. Since episodes of headache were in the patient's medical history and brain metastases known, the event headache (grade 3) was not judged to be dose limiting. 34% (75 AEs in 18 patients) of AEs were judged to be possibly related and 53% (117 AEs in 16 patients) of the non-hematological events were either unlikely or not related to the study drug.

Overall, the most common non-hematological AE was fatigue that occurred in 13 patients (68%) (in 15 cycles; grade 1, n=8; grade 2 n=4; not documented n=3). Nausea (in 15 cycles; grade 1, n=9; grade 2, n=5; n. d.), alopecia (in ten cycles; grade 1, n= 2; grade 2 n=8), and fever (in ten cycles; grade 1, n=9; grade 2, n=1) occurred in ten patients (53%) each. Abdominal pain occurred in seven patients (37%) (in seven cycles; grade 1, n=5, grade 2, n=2). Six patients each (32%) experienced diarrhea (in ten cycles; grade 1, n=8; grade 2, n=1; n. d., n=1) and obstipation (in six cycles; grade 1,

n=3; grade 2, n=3), and five patients (26%) suffered from vomiting (in eight cycles; grade 1, n=2; grade 2, n=5; n. d., n=1).

adverse event	number of patients	%	number of events
fatigue	13	68	15
nausea	10	53	15
fever	10	53	10
alopecia	10	53	10
abdominal pain	7	37	7
diarrhea	6	32	10
obstipation	6	32	6
vomiting	5	26	8
reduced physical status	4	21	8
back pain	4	21	8
dizziness	4	21	6
dyspnea	4	21	6
loss of appetite	4	21	5
pruritus	4	21	5
cough	4	21	5

%: calculated from total number of patients

Table 3: *common non-hematological toxicity*

3.3.2 Hematological Adverse Events

No hematological adverse events were seen in patients that received lower doses (45-90mg/m^2) of CAP 7.1 (cohorts 1 and 2).

In the 16 cycles administered in cohort 3, 13 hematological events were recognized. The most frequent one was leukopenia, which occurred six times (in three patients, cycle 1: day 7, 10, 14; cycle 2: day 14; cycle 3: day 14; cycle 4: day 14). Five cases were grade 3 (in two patients), with a median time to recovery of 12 days (range 4-13), one case was grade 4, in which the recovery time was not documented. Anemia was observed in four cycles (in four patients, cycle 1: day 5, 9; cycle 3: day 4, 7). It was grade 2 in three cases (in three patients), with a median time to recovery of 1 day

(range 0-8 days). One case was grade 3, it recovered after 14 days. One case of thrombocytopenia (cycle 1: day 7, recovery time was not documented) and one case of neutropenia (cycle 1: day 14, recovery time was 10 days) appeared in one patient each and were both grade 3. Furthermore one dose limiting case of neutropenic fever (grade 4) occurred on day 17 of cycle 1, it stabilized after six days.

In cohort 4, 87 hematological AEs (in seven patients) were counted in a total of 28 administered cycles.

The most common hematological AE was leukopenia; it was observed in 27 cycles (in seven patients). 14 cases were grade 4 (in six patients, cycle 1: day 14, 14, 14, 14, 14; cycle 2: day 13, 14; cycle 3: day 11, 15; cycle 4: day 14; cycle 5: day 14; cycle 6: day 14, 14, 15), with median time to recovery of 6 days (range 2-17). Ten grade 3 cases (in six patients, cycle 1: day 14, 14; cycle 2: day 3, 14, 14; cycle 3: day 14; cycle 4: day 4, 12, 14; cycle 5: day 14). The median recovery time was 6 days (range 3-13). Three grade 2 cases (one patient cycle 3: day 4; cycle 5: day 3; cycle 6: day 3) with a median recovery time of 11 days (range 4-12) were counted.

Neutropenia occurred 19 times (in seven patients). 14 episodes in five patients (cycle 1: day 14, 14, 14; cycle 2: day 3, 14, 14; cycle 3: day 15; cycle 4: day 14, 14; cycle 5: day 14, 14; cycle 6: day 14, 14, 15) were grade 4, the median recovery time was 7 days (range 2-17). In patient 16, grade 4 neutropenia, which lasted for more than seven days, occurred in cycles 1, 2, 5, and 6. All events recovered without the usage of CSF, no dose reduction was needed, due to this events. Treatment was delayed by seven days. Furthermore, patient 17 experienced two episodes of grade 4 neutropenia lasting for more than seven days, which recovered without medication, the next cycle of therapy was delayed by seven days. Four grade 3 episodes (in three patients, cycle 1: day 14, 22; cycle 2: day 14; cycle 3: day 14), with a median recovery time of 7.5 days (range 5-13 days) and one grade 2 episode (in one patient, cycle 4: day 4), which recovered after three days, were counted.

There were three cases of neutropenic fever (in three patients), the severity was grade 3 in two cases (in two patients, cycle 1: day 16; cycle 2 day 13), with a median recovery time of eight days (range

5-13), and one grade 4 case, that occurred on day 11 of cycle 1, it was complicated by sepsis. All three patients received CSF.

Thrombocytopenia occurred 20 times (in six patients), six cases (in two patients; cycle 1: day 14, 15; cycle 2: 14; cycle 3: day 14, cycle 4: day; cycle 5 day 14) were grade 4, with a median time to recovery of 6.5 days (range 2-9), one case (grade 2 in severity) was complicated by epistaxis. Seven cases were found to be grade 3 (in four patients, cycle 1: day 14, 14, 14, 14; cycle 2: day 13; cycle 3: day 15; cycle 6: day 14), with a median recovery time of 6 days (range 2-8). Four grade 2 cases (in two patients, cycle 3: day 15; cycle 5: day 14, 15; cycle 6: day 15) recovered after four days in median (range 1-7 days), and three cases (in two patients, cycle 3: day 11; cycle 4: day 12; cycle 6: day 14) were grade 1, the median time to recovery was seven days (range 3-9). One case of thrombocytopenia, which was grade 2 in severity and occurred on day 3 of cycle 2, was complicated by epistaxis since the epistaxis was mild and resolved after two days, with no further action taking, the event was not considered to be of dose limiting nature. Three patients received platelet concentrates.

Anemia was seen in 17 cycles (in seven patients), eight times the severity was grade 3 (in three patients; cycle 1: day 14; cycle 2: day 2, 14; cycle 3: day 8; cycle 4: day 4, 14; cycle 5: day 14; cycle 6: day 14) the median time to recovery was 5.5 days (range 1-25 days). Nine times anemia was grade 2 (in six patients; cycle 1: day 14, 15; cycle 2: day 1; cycle 3: day 7; cycle 4: day 14; cycle 5: day 14, 14, 20; cycle 6: day 2) with a median time to recovery of days 8 days (range 2-21 days). Six patients received red blood cell transfusion.

	Nº of patients	Neutropenia				Neutropenic fever				Leukopenia				Thrombocytopenia				Anemia			
Grade		1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Dose (150mg/m^2)	6			1					1			2				1			1	1	
Dose (200mg/m^2)	7			2				1	1			2	5			4	2		2	1	
Total	13			3				1	2			4	5			5	2		3	2	

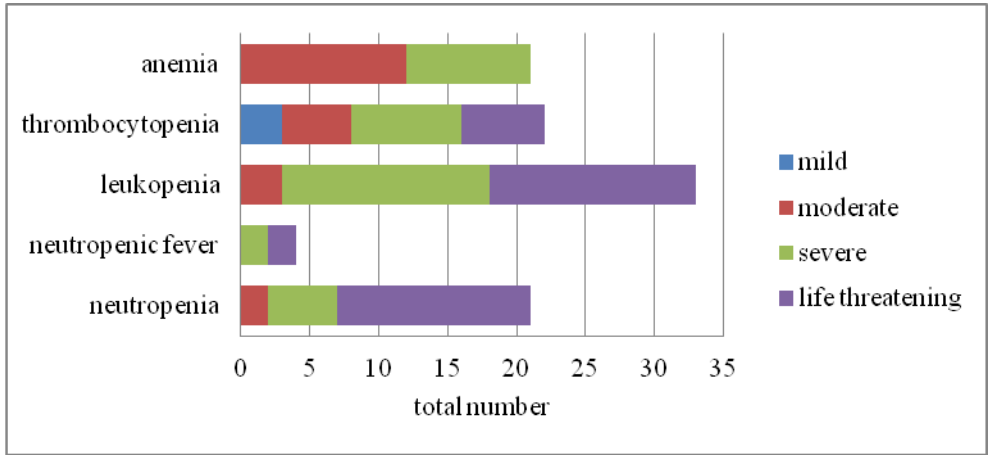
Table 4: *hematological toxicity first cycle by NCICTCAE grade (version 3) in cohorts 3 and 4*

	Nº of patients	Neutropenia				Neutropenic fever				Leukopenia				Thrombocytopenia				Anemia			
Grade		1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Dose (150mg/m²)	6			1				1				5	1			1			3	1	
Dose (200mg/m²)	7		1	4	14			2	1		3	10	14	3	5*	7	6		9	8	
Total	13		1	5	14			2	2		3	15	15	3	5	8	6		12	9	

*one case was complicated by epistaxis

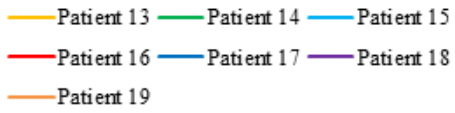
Table 5: *hematological toxicity all cycles*

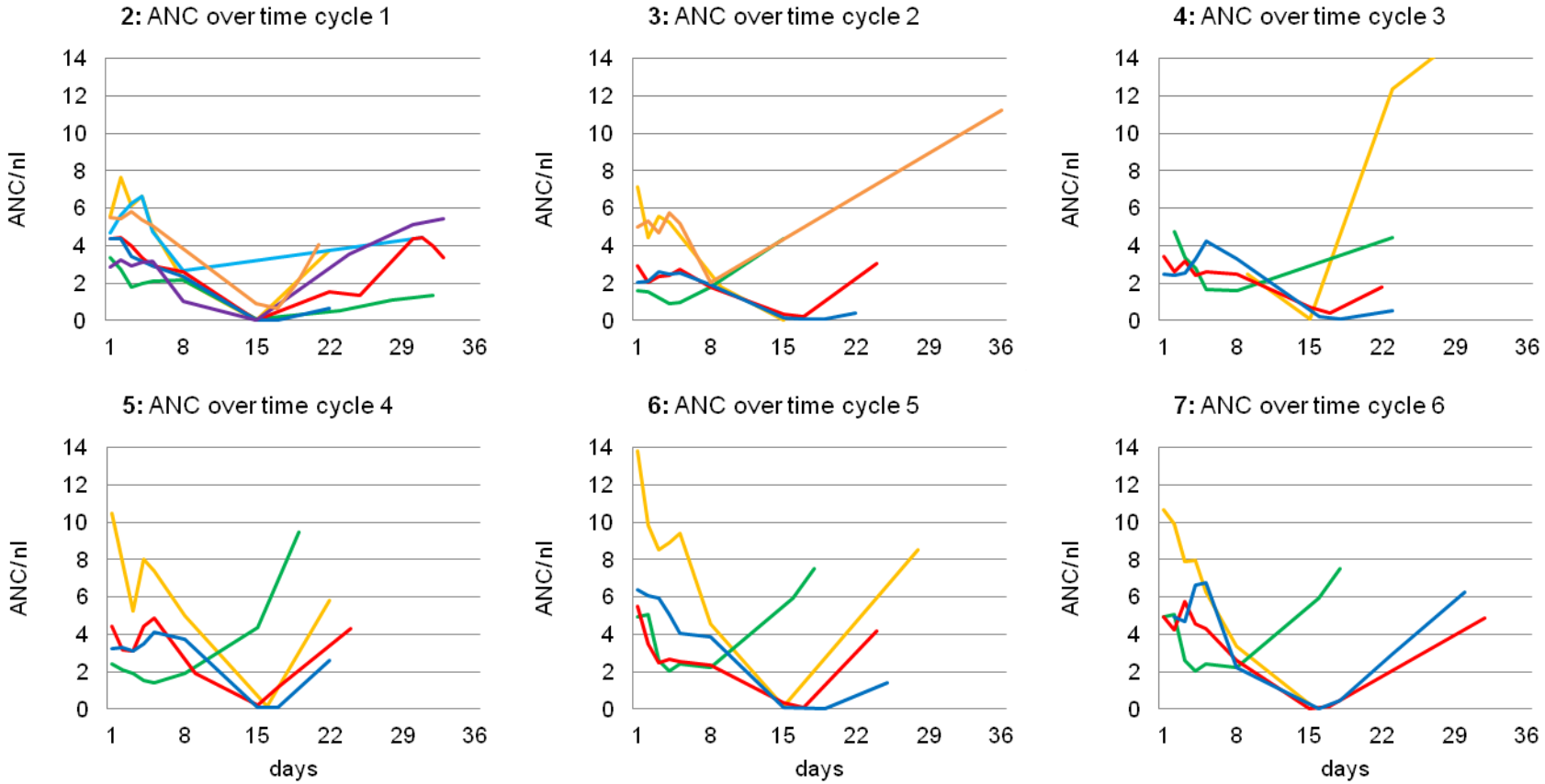
Chart 1: *hematological toxicities highest grade per cycle (all cycles)*



Charts 2-7 *represent the change of absolute neutrophil count over time in cohort 4 (dose level 200 mg/m^2) in all cycles. Filgrastim or Pegfilgrastim were administered to patient 14 cycle 2 / day 7, cycle 3 / day 7, cycle 4 / day 7, cycle 5 / day 7, and cycle 6 / day 4), to patient 18 (cycle 1 / day 16), and to patient 19 (cycle 1 / day 11).*

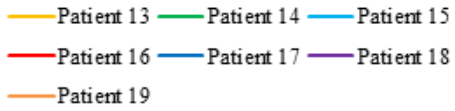
Legend:

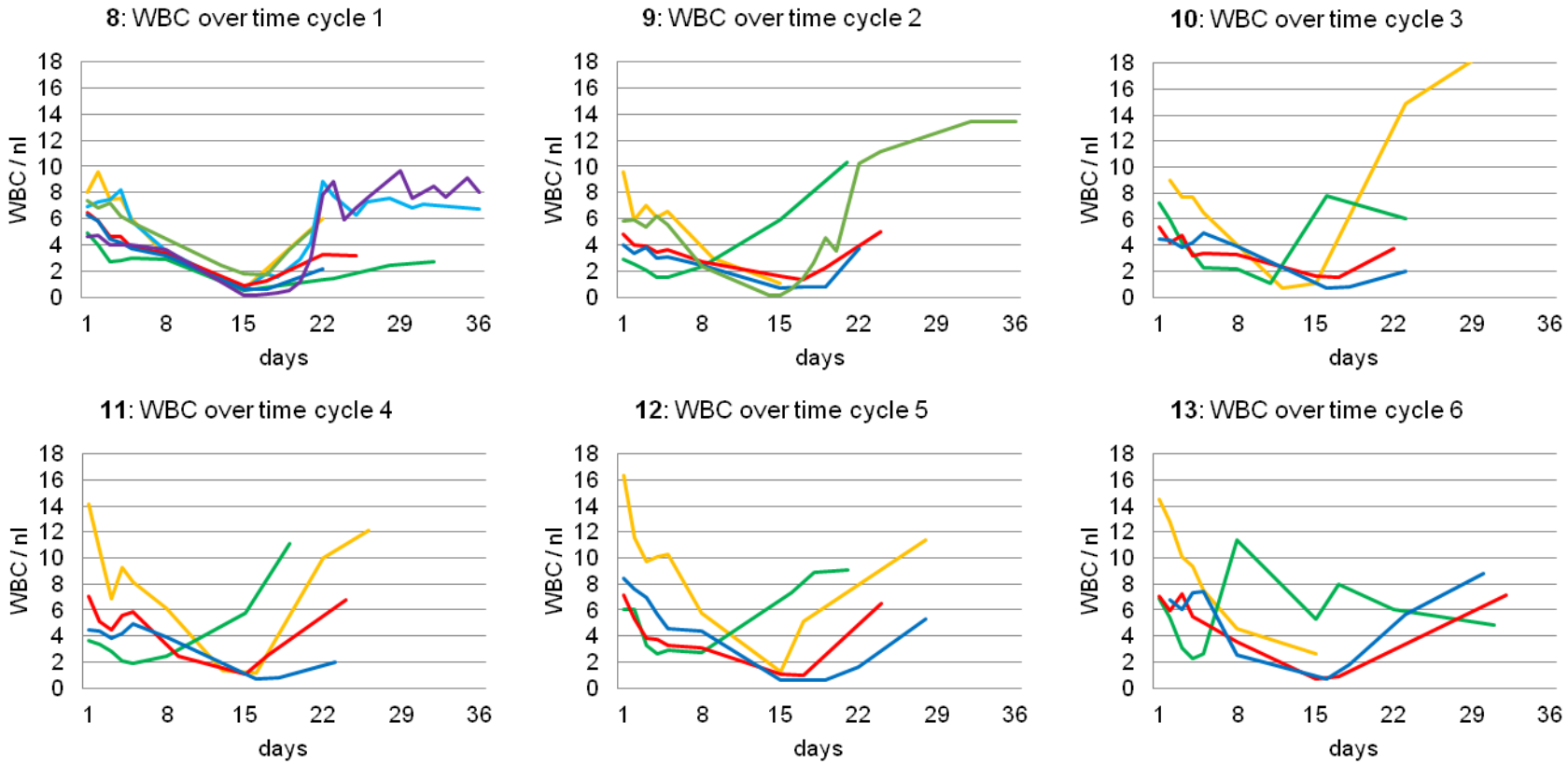




Charts 8-13 *represent the change of white blood cell count over time in cohort 4 (dose level 200 mg/m^2) in all cycles. Filgrastim or Pegfilgrastim were administered to patient 14 cycle 2 / day 7, cycle 3 / day 7, cycle 4 / day 7, cycle 5 / day 7, and cycle 6 / day 4), to patient 18 (cycle 1 / day 16), and to patient 19 (cycle 1 / day 11).*

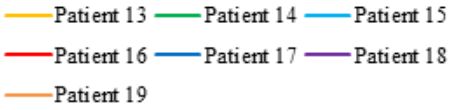
Legend:

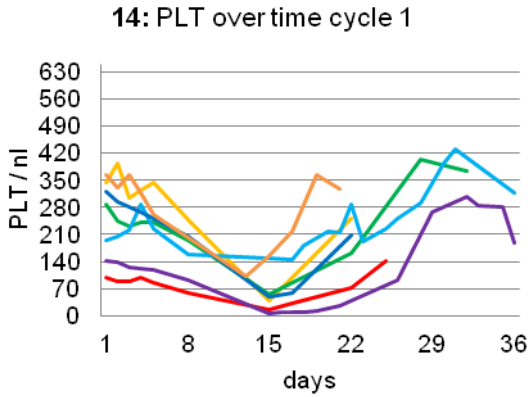


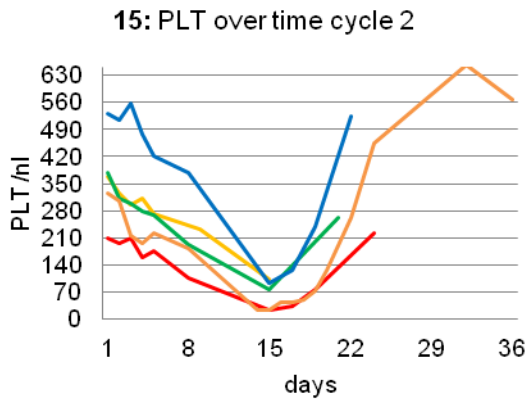


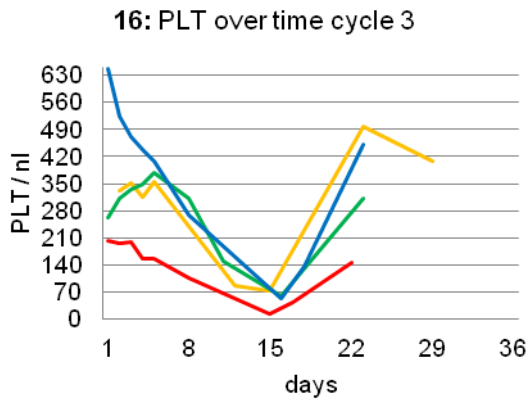
Charts 14-20 *represent the change of platelet count over time in cohort 4 (dose level 200 mg/m^2). Platelet concentrates were administered to patient 16 (cycle 1 /day 14, cycle 3 / day 14, and cycle 6 / day 14), to patient 18 (cycle 1 / day 15 of cycle one and to patient 19 on day 14 of cycle 2).*

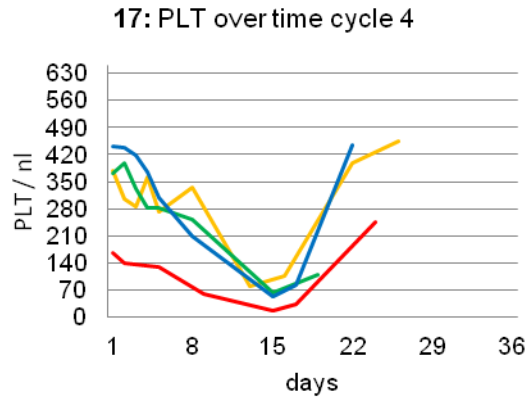
Legend:

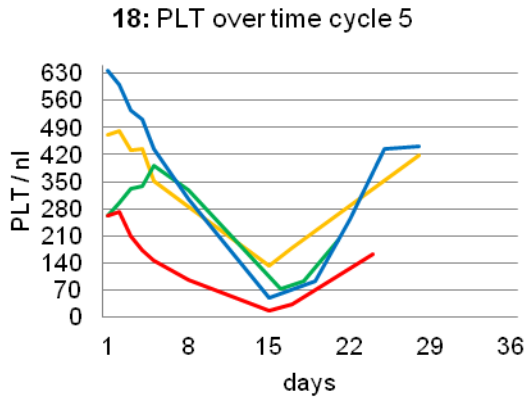


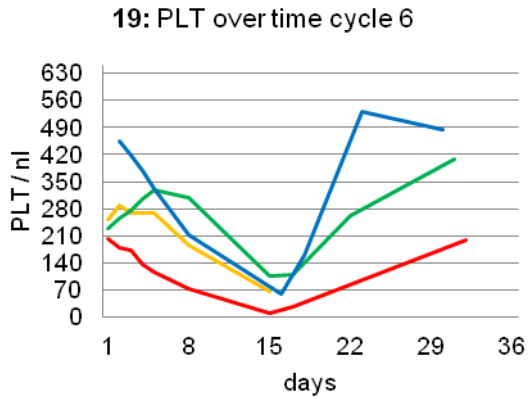






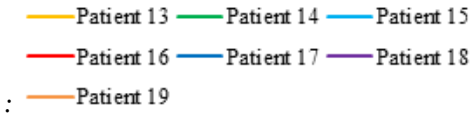






Charts 20-26 *represent the change of hemoglobin over time in cohort 4 (dose level 200 mf/m²) in all cycles. Erythrocyte concentrates were administered to patient 13 (cycle 1 / day 14, cycle 4 / day 12, cycle 5 / days 2 and 16, and cycle 6 / day 4), to patient 14 (cycle 3 / day 8), to patient 16 (cycle 5 / day 16), to patient 17 (cycle 2 / days 18, cycle 4 / day 14, cycle 5 / day 14), to patient 18 (cycle 1 / day 32) and to patient 19 (cycle 2 / days 14 and 17).*

Legend:



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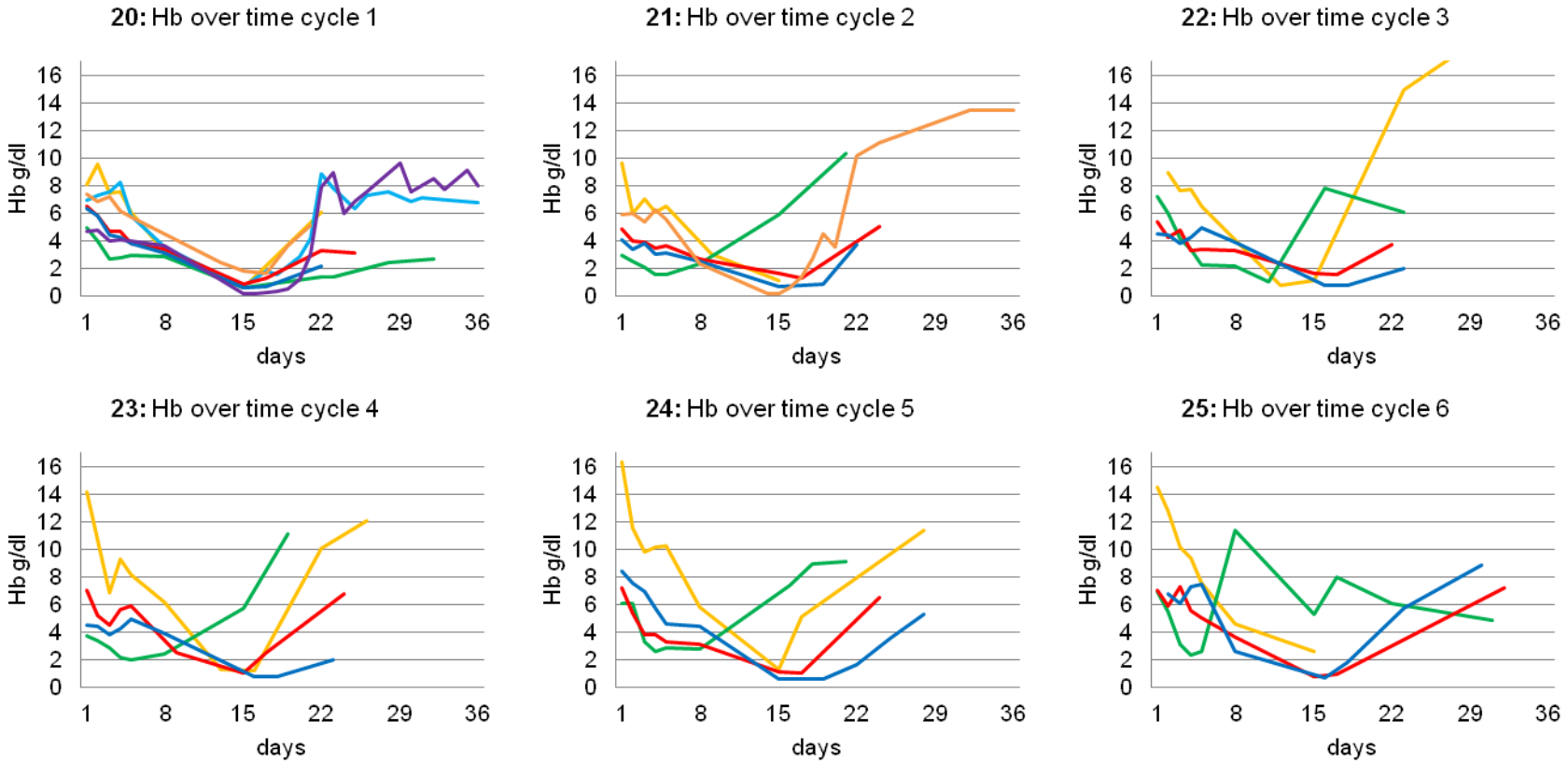

3.3.3 DOSE LIMITING TOXICITIES

Dose limiting toxicities did not occur in cohort 1 and in cohort 2.

Cohort 3: Patient 7 who suffered from tonsil cancer experienced a hematological dose limiting toxicity which was neutropenic fever, it appeared twelve days after the most recent administration of CAP 7.1 (cycle 1, day 5) and was assessed as SAE as well. The patient was hospitalized and treated with antibiotics and antimycotics. The event stabilized after seven days. The event was life threatening and judged to be certainly related to the study drug. A dose reduction of the drug was performed and the treatment was postponed by seven days. The patient also experienced grade 3 mucositis on the same day, it was judged as probable related to the study drug. The patient received medication and recovered after 5 days.

Cohort 4: Patient number 13 died twelve days after he completed the study protocol after receiving full 6 cycles of the study drug suddenly at home. No autopsy was performed and the cause of death remained unclear. The event was judged to be possibly related to the study drug. The lab results from two days before the fatal event showed severe anemia and thrombocytopenia and life threatening leukopenia and neutropenia. The patient was at the local maintenance laboratory for blood tests and talked to the study site for a visit, but died at home before further medical action could be taken.

Patient 14 was treated with CAP7.1 because of lung cancer with metastases. The patient experienced dose limiting neutropenic fever 13 days after the most recent study drug application (cycle 1, day 5). He was hospitalized and recovered after five days as a result of treatment with antibiotics and granulocyte colony stimulating factor. The event was severe and probably related to CAP7.1.The following cycle was delayed by 14 days. The event was judged to be a serious adverse event too. The patient also suffered from moderate thrombocytopenia complicated by epistaxis on third day of infusion of cycle 2. The event was classified probable related to study compound it recovered without further action after four days.

Patient 16 experienced hematological dose limiting toxicities in all 6 cycles of the study. 10 days after the last day of infusion in the first cycle grade 4 neutropenia, which recovered after eight days, occurred. In the same laboratory test grade 4 thrombocytopenia was seen it recovered after two days. The next cycle was delayed by one week due to this event. In the second cycle the patient experienced grade 4 neutropenia which lasted for nine days and grade 4 thrombocytopenia lasting for two days the next cycle was postponed by seven days. Ten days after the most recent infusion of cycle 3 grade 4 thrombocytopenia occurred it recovered after seven days and led to the delay of the next cycle. Grade 4 thrombocytopenia also appeared in the cycle four it lasted for two days and the next infusion was postponed. In the cycle 5 grade 4 neutropenia and thrombocytopenia happened 13 days after the last day of infusion both event lasted for nine days. The next cycle was delayed by one week. Ten days after the last infusion day in cycle 6 the patient experienced grade 4 neutropenia it recovered after 17 days. Thrombocyte concentrates were administered in the cycles 1, 3 and 6.

Patient 17 experienced grade 4 neutropenia 14 days after the last infusion of cycle 2 it lasted for 14 days, another case of grade 4 neutropenia occurred 14 days after the most recent infusion of cycle 5 it recovered after ten days. Due to both events the next cycles had to be delayed by one week.

Patient 18 who received CAP7.1 because of rectal carcinoma experienced a febrile neutropenia seven days after the most recent infusion of CAP7.1 (cycle 1, day 5). The event was life threatening and possibly related to study drug. Two days after the onset of the neutropenic fever grade 4 thrombocytopenia occurred and after another three days the patient experienced a dose limiting sepsis which was assessed as a SAE and prolonged the hospitalization, the patient was treated with antibiotics and received granulocyte colony stimulating factor as well as thrombocyte concentrates. The lab results showed a urinary tract infection. The event was life threatening and possibly related to study drug. The patient was withdrawn from the study due to this event and recovered after 26 days. One week after the onset of the sepsis the patient suffered from a phlegmon of the left hand he was undergoing surgery and recovered after 6 days. The event was grade 3 in severity and judged possibly related to study compound.

Patient 19 that was treated for rectal carcinoma underwent a dose limiting febrile neutropenia nine days after the most recent application of CAP 7.1 (cycle 2, day 5). He was hospitalized on the same

day and treated with an intravenous combination of antibiotics and received granulocyte colony stimulating factor. The event was assessed as severe, probably related to the study drug it was assessed also as SAE. The patient recovered after eleven days. No action regarding study drug was taken.

Since DLTs occurred during the first cycle of therapy in cohorts 3 and 4, the cohorts were expanded to 6 and 7 patients, respectively.

Dose	Patients, n	Patient Nº	Type	Grade	Cycle
150 mg/m²	6	7	neutropenic fever	4	1
			mucositis	3	1
200 mg/m²	7	13	death	5	6
		14	neutropenic fever	3	1
			thrombocytopenia complicated by bleeding	2	2
		16	neutrocytopenia	4	1
			thrombocytopenia	4	1
			neutrocytopenia	4	2
			thrombocytopenia	4	2
			thrombocytopenia	4	3
			thrombocytopenia	4	4
			neutrocytopenia	4	5
			thrombocytopenia	4	5
			neutrocytopenia	4	6
		17	neutrocytopenia	4	2
			neutrocytopenia	4	5
		18	neutropenic fever	4	1
			thrombocytopenia	4	1
			neutropenic sepsis	4	1
			phlegmon	4	1
		19	neutropenic fever	3	2

Table 6: *Dose Limiting Toxicities (all cycles)*

3.3.4 Severe Adverse Events

SAEs were observed in eight patients, they only appeared in cohorts 3 and cohort 4.

Cohort 3: Patient number 7 was treated with CAP7.1 because of tonsillar cancer with metastases in the liver and the lung. She experienced three SAEs during the treatment with CAP7.1. Febrile neutropenia in cycle 1 was discussed in the section above. In the second cycle three days after most recent study product administration (cycle 2, day 5), patient number 7 suffered from reduced physical status due to emesis which was of moderate intensity and possibly related to the study drug. The gastroscopy showed a high grade stenosis of the upper duodenum and a progressing retroperitoneal manifestation of lymph nodes was confirmed. The duodenum was dilated with a balloon and a stent placed in the area of the stenosis. The event stabilized after 16 days. The third SAE was subacute abdomen due to perforation of the duodenal stent. It started 21 days after the most recent infusion of CAP7.1 (cycle 2, day 5). The patient was hospitalized on the same day and treated with clyster and pethidine. She also received a stomach tube. The event stabilized after 17 days. It was reported as not related to the study drug and severe in intensity.

Patient 8 was treated with CAP 7.1 because of testicular cancer. He suffered from two SAEs. Oral candidiasis occurred ten days after the first application of the study product. The patient was hospitalized and treated with antibiotics, antimycotics and pain medication. The event resolved five days after onset. The event was judged to be unlikely related to the study product and severe in intensity. The dose was reduced due to this event. On day 2 of cycle 4 the study was discontinued permanently due to the SAE reduced physical status. The event was assessed as severe and unlikely to be related to the study drug. The patient suffered further from severe headache (see 3.3.3) and pain in both legs. The lab results showed a urinary tract infection that was stabilized after seven days with antibiotics.

Patient number 9 received the study drug because of ovarian cancer. She sustained hematemesis 49 days after the first study drug administration. The event was serious, of severe intensity and unlikely to be related to the study drug. The patient was hospitalized on the same day and received intravenous pantoprazole until the event stabilized after three days. In the

esophagogastroduodenoscopy no cause of hemorrhage was visible. The patient had intra-abdominal and peritoneal metastases. Multiple episodes of vomiting occurred during the study and even prior to the first study drug application.

Cohort 4: Patient number 13 received CAP 7.1 because of penis cancer with liver and lung metastases. He confracted a urinary tract infection on day 5 of the fifth cycle. The patient was hospitalized and treated with antibiotics and pain medication. The infection stabilized 13 days after onset. The event was severe in intensity and unlikely to be related to the study drug. Treatment was delayed by 14 days. The patient died after receiving all cycles of therapy suddenly at home (event discussed in 3.3.3)

Patient number 14 experienced dose limiting neutropenic fever the event is discussed in the section above.

Patient number 15 received the study medication because of a Sertoly-Leydig-cell tumor. Her general condition deteriorated 16 days after the first study drug application. The event was judged to be severe and possibly related to the study drug. The patient was hospitalized on the same day. She received subcutaneous anti-coagulation medication for suspected deep venous thrombosis. Five days after the onset of the first SAE she suffered from pulmonary embolism and was transferred to an intensive care unit and treated with heparin. The event was stabilized 18 days after onset. The event was reported as life threatening and not related to the study drug. The patient was withdrawn from the study due to the SAE.

Patient 18 received CAP7.1 because of rectal carcinoma with metastases in the liver and lung. He experienced two SAEs. Leukocytopenia occurred 14 days after first infusion of the study product. He was hospitalized on the same day. The event stabilized after five days. A dose limiting case of sepsis occurred two days after the onset of the first events and is described in the section above.
Patient 19 was included in the study because of metastasized rectal cancer. The SAE renal congestion took place seven days after the first study drug application. The patient was hospitalized on the same day. Due to this event the double J catheter (it was implanted prior the beginning of the study due to renal congestion) was changed, and the patient recovered five days after. The event was

not related to study drug and severe in intensity. In cycle 2 dose limiting neutropenic fever assessed as SAE appeared (see 3.3.1).

4. DISCUSSION

4.1 KEY FINDINGS

In this phase I dose-escalation trial in adult patients with solid refractory malignancies, CAP7.1 was administered daily for five days every 21 days as a 60-minute intravenous infusion.

Frequent non-hematological toxicity involved fatigue (n=13), nausea (n=10), alopecia (n=10), fever (n=10), abdominal pain (n=7), diarrhea (n=6), obstipation (n=6), and vomiting (n=5). Non-hematological AEs were mainly mild or moderate. Hematological toxicity was frequent at higher doses and clearly dose related. It manifested itself by anemia (n=11), leukopenia (n=10), thrombocytopenia (n=8), neutropenia (n=6), and neutropenic fever (n=4). Hematological adverse event usually appeared during the first cycle for the first time and were repeatedly observed in subsequent cycles. Cumulative hematological toxicity was not seen. The nadirs of WBC and ANC were between day 10 and day 17 of each cycle. The median recovery time was 12 days (range 4-13 days).

Myelotoxicity was the main DLT and manifested itself by neutropenic fever (including one patient with a neutropenic sepsis), neutropenia lasting for more than seven days without aggravation and thrombocytopenia (complicated by bleeding in one case).

Two DLTs occurred in one patient in cohort 3 (dose 150 mg/m2/day). The cohort was expanded and since no DLTs were observed in the other five patients, the dose was further escalated. At the next dose level (dose 200 mg/m2/day) six out of seven patients experienced AEs meeting the criteria for a DLT. Four patients in this cohort could receive all six cycles of therapy anyhow. Patient 14 received colony stimulating factor due to neutropenic fever in the first cycle of therapy and patient 16 received thrombocyte concentrates due to decreased platelet count, in all other cycles in this two patients and in patient 17 solely treatment had to be delayed for bone marrow recovery. Patient 13 died after he received all cycles of therapy.

The MTD was considered between 150 and 200 mg/m2/day. Since good bone marrow recovery was seen and four patients in cohort 4 received all 6 cycles of therapy the Data Review Committee defined RP2T 200 mg/m2/day with an option for delay of treatment cycle as well as dose reduction to 150 mg/m2/day when bone marrow toxicity occurs.

4.2 Study Design

4.2.1. Study Drug Administration

Infusion time was not exactly one hour in all patients and in most cases a reason for this could not be identified retrospectively. One infusion reaction occurred and in one case a defect infusion pump is documented in the CRF.

4.2.2 Inclusion And Exclusion Criteria

Patient 7 who did not fulfill all inclusion criteria, the last chemotherapy was not at least 28 days old and ALT was elevated more than 5 times upper normal limit (>34 U/l). She was probable included into the study anyway since merely two days lacked (last chemotherapy ended 26 days before first study drug application) and she had recovered from bone marrow toxicity and showed good bone marrow function and the ALT was with 171 U/l only 1 U/l higher than allowed for a patient with liver involvement. Anyway the patient suffered from neutropenic fever and mucositis (both dose limiting) in the first cycle of therapy and had to be hospitalized due to those events.

In patient 8 the last prior chemotherapy ended 27 days before first study drug application, since he showed good bone marrow function he was probable included into the trial. Patient 8 experienced two SAEs one meeting the criteria for a DLT as well. And the study was discontinued due to the second SAE (reduced physical status due to urinary tract infection).

Since both patients experienced DLTs it is more than questionable to have led those patients participate in this trial. The reason for inclusion is not definitely clear.

4.3 Dose Limiting Toxicities and Severe Adverse Events

Patient 7, who was treated with CAP7.1 due to tonsil cancer with metastases in the liver and lung suffered from several SAEs and two DLTs. First she experienced neutropenic fever which was probably due to study drug. Mucositis which occurred during the first cycle of therapy was probable due to study drug too, since it appeared in other patients too, and was not in the patient`s medical record furthermore it is well known side effect of etoposide. In the second cycle the patient experienced reduced physical status due to emesis (SAE). A stent was placed because of duodenal stenosis, which was due to progressive retroperitoneal manifestation of lymph nodes, the stent perforated later and led to abdominal pain (SAE). These events were not related to the study product but to malignant disease.

Patient 8 experienced SAE oral candidiasis in cycle 1. It was judged as unlikely related to study product and does therefore not meet the criteria of a dose limiting toxicity, but this event could also been seen in context with the study drug, since candidiasis is common side effect of CAP7.1. mother`s compound and occurred in other patients participated in this trial.

Patient 13 suffered from urinary tract infection (SAE). Before the onset of the urinary tract infection the ANC was 0.14/nl and the WBC was 1.23/nl. The patient had a record of urinary retention. He was treated for penis cancer, radiotherapy of the small pelvis was performed in the past. After the patient completed the full study protocol, he died suddenly at home (SAE). The lab results from two days before the fatal event showed severe myelosuppression. All lines, including hemoglobin (7.9 g/dl) platelet count (67/nl), white blood cell count (2.6/nl) and absolute neutrophil count (0.25/nl) were decreased. The patient was at the local maintenance laboratory for blood tests and contacted the study site for an appointment, but died at home before that. Since no autopsy was performed, the cause of death remaines unclear. The patient had repeated tumor hemorrhage and hematuria in his history, considering the last PLT, it seemed to assume that bleeding was the cause of death. The

patient also suffered from coronary heart disease with myocardial infarction in his record. Coronary heart disease aggravated by anemia should be considered as a possible reason for the sudden death as well.

Patient 14 suffered from dose limiting neutropenic fever in the first cycle of therapy, the DLT was probable in the context of CAP7.1, the patient received granulocyte colony-stimulating factor for recovery. Moderate thrombocytopenia in cycle 2 was probable due to the study product too it was complicated by epistaxis and therefore meets the criteria of a DLT. Since heavier cases of thrombocytopenia did not lead to bleeding complications in other patients (except maybe in patient 13) there might be other reason for the epistaxis. But epistaxis was not in the patient´s medical record and no predisposal condition was known.

The general condition of patient 15 deteriorated with dyspnea (SAE) and five days later pulmonary embolism (SAE) was diagnosed. A deep venous thrombosis was suspected. The event was probably tumor related. No thrombosis, embolism or coagulopathy was in the patient's medical history.

Patient 16 experienced hematological dose limiting toxicities in every cycle (neutrocytopenia and thrombocytopenia) of therapy. Complications like fever or bleeding did not occur and the patient could receive all six cycles of therapy but cycles 2 to 6 had to be delayed by one week, for bone marrow recovery and the patient received thrombocyte concentrates in the first cycle of therapy. Those event are clearly in context of the study drug.

Grade 4 neutropenia lasting for more than seven days occurred in the cycles 2 and 5 in patient 17. They are due to CAP7.1 the following cycles had to be delayed by one week due to recovery. The other cycles also had to be delayed due to hematological AE, which did not meet the criteria of a dose limiting toxicity. The patient received all 6 cycles of therapy.

Patient 18 developed sepsis (DLT and SAE) in the first cycle of therapy. Two days before onset the white blood cell and absolute neutrophil count had decreased massively (WBC 0.18/nl and ANC 0.01/nl), this neutropenic sepsis was probably due to study drug. Seven days after the onset of sepsis the patient developed a phlegmon on the left hand, that was rated possibly related to study drug.

Since the white blood cell count was normal at this time after receiving granulocyte colony-stimulating factor had to be administered.

Patient 19 developed a dose limiting case of febrile neutropenia nine days after the most recent application of CAP 7.1 (cycle 2, day 5). The event was due to study product and the patient had to be treated in hospital, with antibiotics and colony stimulating factor had to be administered.

4.4 Comparison of the Toxicity of CAP7.1 with other Inhibitors of Topoisomerase II

4.4.1 Etoposide (VP-16)

Etoposide is a semisynthetic derivative of epiphylotoxin, that inhibits topoisomerase II and hence DNA synthesis. Etoposide is used in the treatment of a wide range of solid tumors such as lung cancer, bladder cancer, brain tumors, head and neck cancers and others. It is also used for the treatment of lymphomas and leukemias. It is usually administered in daily doses of 50-120 mg/m^2/day (with a maximum dose per cycle of 1000 mg/m^2. Common side effects of etoposide are fatigue, alopecia (8-66%), anorexia (10-13%), diarrhea (1-13%), nausea and vomiting (31-43%), stomatitis (1-6%), and hypersensitivity during intravenous infusion (1-3%) (BC Cancer Agency, Cancer Drug Manual, 2006). Mixed lineage leukemia is described as a secondary malignancy. Myelotoxicity as a result of treatment with etoposide appears at standard doses. It is mainly represented by neutropenia and thrombocytopenia. Higher doses ($\geq$ 2400 mg/m^2/day) result in mucositis which is dose limiting as well.

4.4.2 Etoposide phosphate

Etoposide phosphate is a water-soluble prodrug of etoposide, which is rapidly converted into etoposide. It is used in the treatment of small cell lung cancer and refractory testicular cancer. The principal and dose limiting toxicities in phase I trials was myelotoxicity. Neutropenia and

leukopenia were both dose related. Etoposide phosphate was tested in different phase I trials at doses ranging from 10 to 200 mg/m².

In a phase I dose escalating trial 36 patients were treated. Etoposide phosphate was administered as a 5-minute infusion on days 1, 3, and 5 every 21 days. The drug was administered in etoposide equivalent doses which ranged from 50 to 200 mg/m^2. All patients experienced significant alopecia, fatigue (WHO grade 2 or higher) was seen in approximately 20% of the patients, anorexia occurred in 4%, and constipation in 3%. Nausea and vomiting were observed in the trial but could be controlled easily. Myelosuppression was the major toxicity and dose dependent. Grade 3 or 4 granulocytopenia was present in 23 courses (overall 110 courses were administered) and leukopenia in 16 courses (Budman, 1994).

28 patients were enrolled in another phase I trial using etoposide equivalent doses between 50 and 125 mg/m^2 and the drug was administered as a daily 30-minute infusion for five days. Cycles were repeated every 21 days, and the dose was escalated. Frequent non-hematological AEs were nausea/vomiting (n=18), fatigue/weakness (n=16), anorexia (n=9), diarrhea (n=7); and mucositis (n=7), they were mostly of grade 1 or 2 (WHO grade), one case of mucositis was grade 4. Myelosuppression was clearly dose dependent and dose limiting. In the four dose levels grade 3 or higher myelotoxicity appeared in 11%, 20%, 37%, and 50% of the courses. Six patients developed neutropenic fever (Thompson, 1995).

In a similar phase I trial etoposide phosphate was administered in different dose levels (50 to 150 mg/m^2 equivalent to etoposide) to 39 patients as a 30-minute infusion on days 1, 3, and 5 of a 21-day cycle. Non-hematological toxicity was generally mild. Vomiting/nausea occurred in 24 patients, alopecia in 15, and mucositis in 6 patients. Myelotoxicity was the major dose limiting toxicity. Grade 3 or 4 (WHO) toxicity were leukocytopenia (n=11), neutrocytopenia (n=11), anemia (n=10), and thrombocytopenia (n=3) (Fields, 1995).

Another phase I trial was performed with 23 patients. The study drug was administered in different dose levels, ranging from 10 to 30 mg/m^2 as a continuous infusion using ambulatory pumps for six weeks, followed by a two weeks rest. Most common non-hematological AEs were alopecia (n=10),

fatigue (n=9), mucositis (n=7, two cases were dose limiting), and nausea/vomiting (n=4). Myelosuppression was the major toxicity and it was dose dependent and dose limiting. Anemia and leukopenia occurred in 13 patients each, neutropenia in seven and thrombocytopenia in five. Grade 4 (NCI-CTC) toxicity was observed for neutropenia (n=3) and leukopenia (n=3) (Soni, 1996).

The toxicity profile of etoposide phosphate is comparable to CAP7.1. The main DLT is dose dependent myelotoxicity in both. The non-hematological AEs were quite similar and generally mild or moderate. Unlike the trials with etoposide phosphate, mucositis was not a common AE in the trial with CAP7.1. The main DLT (bone marrow toxicity) of etoposide occurs at doses higher than 500 mg/m^2/cycle, side effects of etoposide phosphate in phase I trials occurred at various doses of 840 mg/m^2/cycle (administered as a continuous infusion over six weeks) (Soni, 1996) 375 mg/m^2/day in etoposide equivalent doses (Budman, 1994), 500 mg/m^2/cycle in etoposide equivalent doses (Thompson, 1995), and at 450 mg/m^2/cycle (Fields, 1995), depending on the administration scheme. Comparable AEs related to bone marrow toxicity such as neutropenia after CAP7.1 treatment occurred at doses 750-1000mg/m^2/cycle.

Substance	CAP7.1	Etoposide Phosphate	Etoposide Phosphate	Etoposide Phosphate	Etoposide Phosphate
Study	Keilholz, U.	Budman, R	Thompson, DS.	Fields, SZ.	Soni, N.
Nº of patients	19	36	28	39	23
Frequent Toxicities	fatigue (n=13), nausea (n=10), alopecia (n=10), fever (n=10), abdominal pain (n=7), diarrhea (n=6), obstipation (n=6), vomiting (n=5)	alopecia (n=36), fatigue (n=7),	nausea/vomiting (n=18), fatigue/weakness (n=16), anorexia (n=9), diarrhea (n=7), mucositis (n=7)	nausea/vomiting (n=24), alopecia (n=15), mucositis (n=6)	alopecia (n=10), fatigue (n=9), mucositis (n=7)
Leukopenia (grade ≥ 3)	n=10	**m*=16	*[3]	n=12	n=7
Neutropenia (grade ≥ 3)	n=6	**m*=*[2]23	*[3]	n=11	n=5
Neutropenic fever	n=4	n. d.	n=6	n=3	n=2
Thrombocytopenia (grade ≥ 3)	n=6	n. d	n. d.	n=3	n=1
Anemia (grade ≥ 3)	n=4	n. d.	n. d.	n=10	n=1
Major DLTs	neutropenia (n=3), neutropenic fever (n=3), thrombocytopenia (n=3)	leukopenia and neutropenia (not further specified)	leukopenia and neutropenia (not further specified)	leukopenia and neutropenia (not further specified in the trial)	leukopenia (n=2, 1 with neutropenia), leukopenia / neutropenia /anemia (n=1) leukopenia / neutropenic fever (n=3), mucositis (n=3, 1 with fatigue)

**m* represents the number of cycles, in total 103 cycles were administered

*[2]all granulocytes were counted

*[3]toxicities were not available in a form suitable for comparison

Table 7: *Comparison of toxicity of CAP7.1 and etoposide phosphate in phase I clinical trials*

4.4.3 NK611

NK611 is a water-soluble podophyllotoxin-derivative. Its mechanism of action is through inhibition of topoisomerase II. In a phase I clinical trial it was administered either orally (four daily doses) or intravenously (30-minute infusion) to 21 patients at different doses. Treatment was repeated every 4 weeks. Neutropenia was the main manifestation of toxicity. Grade 4 (CTC) neutropenia developed in 19% of the cycles and appeared to be dose related. Anemia occurred in 32% of the cycles and did not seem to be dose related. Thrombocytopenia of grade 2 or higher occurred in 5 patients. Non-hematologic toxicity was moderate and included alopecia in all patients and nausea (n=3). One case of grade 3 stomatitis occurred as well. DLTs were manifested by neutropenia and thrombocytopenia. The study was focused mainly on the bioavailability and pharmacokinetic of the drug (Pagani, 1996).

4.4.4 VORELOXIN

Voreloxin is a novel naphthyridine analogue structurally related to quinolone class of compounds. It intercalates with DNA and inhibits topoisomerase II. Voreloxin was tested in two dose escalating schedules in which 41 patients were treated intravenously on day 1 of a 21 day cycle. 27% of the patients completed all cycles. The most frequent adverse events were nausea, which occurred in 61% of the patients, followed by vomiting (42%), neutropenia (37%), fatigue (32%), and constipation (29%). DLTs were grade 4 neutropenia (n=3, one with fever), grade 4 neutropenia and thrombocytopenia (n=1), grade 3 neutropenia with pneumonia (n=1), and one case of grade 2 oral thrush, which lasted for more than 29 days. All DLTs occurred, when high doses were administered. In the same trial 21 patients were treated on days 1, 8, and 15 of a 28 day cycle. Two patients completed all cycles. Most common adverse events were nausea and constipation (n=8) abdominal pain (n=6), diarrhea (n=5), vomiting (n=5), pyrexia (n=5), and pain in extremity (n=5). They were generally mild or moderate in intensity. Two patients experienced dose limiting grade 3 neutropenia that lasted for more than 14 days (one was with pleural effusion) (Advani et al, 2010).

4.4.5 AQ4N

AQ4N is a prodrug, which is reduced in hypoxide tumor to AQ4, an inhibitor of topoisomerase II. In a phase I clinical trial it was administered to 16 patients as a 30-minute infusion on days 1, 8, and 15 of a 28 day cycle, in different dose levels. Most frequent non-hematological adverse events were chromaturia (100%), skin discoloration (81%), fatigue (38%), diarrhea (31%), nausea (25%), vomiting (25%), and anorexia (13%). Myelosuppression was dose dependent and generally mild, except for one patient, who developed persistent pancytopenia. Dose limiting toxicities were manifested by one case each of fatigue and respiratory failure (Papadopoulos, 2008).

4.4.6 C-1311

C-1311 is a member of the imidazoacridinone family. Its mechanism of action is through DNA intercalate and inhibition of topoisomerase II. A phase I trial was performed, in which the drug was administered to 22 patients weekly during three consecutive weeks followed by one week´s rest. The dose was escalated. The most common non-hematologic AE were nausea (n=11), vomiting (n=6), asthenia (n=5), and diarrhea (n=4). They were mostly of grade (CTCAE) 1 or 2. Hypo-albuminemia was seen in 86%, hyponatremia in 55% and abnormal liver function tests in 36-50% of the patients. Most frequent hematological AEs were neutropenia (n=11) and anemia (n=5). Neutropenia was grade 4 in 5 cases and dose limiting (Isambert, et al. 2010).

4.4.7 NSC 655649

NSC 655649 is an antibiotic with antitumor activity, and a water-soluble analog to rebeccamycin, that inhibits topoisomerase II and I. In a dose escalating phase I clinical trial it was administered to 45 patients intravenously over 30 to 60 minutes once every three weeks. Most common non-hematologic AEs were nausea and vomiting (n=23), diarrhea (n=10), and stomatitis (n=10). Myelotoxicity was the main toxicity, it was dose dependent and dose limiting. It was mainly

manifested by neutropenia (n=28), with three cases of grade 4 (NCI-CTC), one aggravated by fever and thrombocytopenia (n=18, including 3 cases of grade 4 cases) (Tolcher, 2001).

In another phase I study with NSC 655649 30 patients were treated intravenously over 1 or 2 hours. Cycles were repeated every three weeks and the dose was escalated. All patients experienced superficial phlebitis (central venous access was used at higher doses). Other frequent non-hematological AEs in the first cycle were nausea (n=10), mucositis (n=8), elevated ALT/AST (n=8), vomiting (n=7). All events mentioned were only of grade (NCI-CTC) 1 or 2. Hematologic toxicity was dose dependent. During the first cycle, neutropenia (n=16) was most common, followed by thrombocytopenia (n=9), and anemia (n=5). Neutropenia was dose limiting and occurred at the highest dose level (Dowlati, 2001).

In a third phase I trial NSC 655649 was given in both single- (as a 30-60-minute infusion) and multiple- (dose divided into three consecutive daily doses) dose formats. In total 69 patients were treated. The most frequent non-hematological toxicities were nausea (n=38), vomiting (n=34), fatigue (n=20), and phlebitis (n=20). They were mainly of grade 1 or 2 (NCI-CTC). Grade 3 or 4 hematologic toxicity was represented by leukopenia (n=12) and neutropenia (n=10). Neutropenia was the dose limiting toxicity in this trial (Merchant, 2002).

Substance	CAP7.1	NK611	Voreloxin	AQ4N	C-1311	NSC 655649	NSC 655649	NSC 655649
Study	Keilholz, U.	Pagani, O.	Advani, RH.	Papadopoulos, KP.	Isambert N.	Tolcher, AW.	Dowlati, A.	Merchant, J.
Nº of patients	19	21	62	16	22	45	31	69
Frequent Toxicities	fatigue (n=13), nausea (n=10), alopecia (n=10), fever (n=10), abdominal pain (n=7), diarrhea (n=6), obstipation (n=6), vomiting (n=5)	alopecia (n=21)	nausea (n=33), vomiting (n=17), fatigue (n=13), constipation (n=20), abdominal pain (n=6)	chromaturia (n=16), skin discoloration (n=13), fatigue (n=6), diarrhea (n=5), vomiting (n=4)	hypoalbuninemia (n=19), hyponatremia (n=12), nausea (n=11), vomiting (n=6), asthenia (n=5), diarrhea (n=4)	nausea (n=23), vomiting (n=23), stomatitis (n=10), diarrhea (n=10)	*[7]phlebitis (n=31), nausea (n=10), ALT/ AST elevation (n=9), vomiting (n=7), mucositis (n=8)	nausea (n=38), vomiting (n=34), fatigue (n=20), phlebitis (n=20)
Leukopenia (grade ≥ 3)	n=10	n. d.	n. d.	n=0	n. d.	n. d.	n. d.	n=12
Neutropenia (grade ≥ 3)	n=6	**m*=28	n=17	n=0	n=11	*[2]*m*=21	*[8]n=9	n=10
Neutropenic fever	n=4	n=1	n=1	n=0	n=2	*[3]*m*=2	n=1	n=0
Thrombocytopenia (grade ≥ 3)	n=6	n=2	n=2	n=0	n=1	*[4]*m*=3	*[9]n=5	n=1
Anemia (grade ≥ 3)	n=4	n. d.	n=4	n=0	n=1	n=5	*[10]n=2	n=2
Major DLTs	neutropenia (n=3), neutropenic fever (n=3), thrombocytopenia (n=3)	*neutropenia (*m*=8)	neutropenia (n=7), neutropenic fever (n=2), thrombocytopenia (n=2)	fatigue (n=1), respiratory failure (n=1)	neutropenia (n=5)	*[5]neutropenia (*m*=4), *[6]neutropenic fever (*m*=2), thrombocytopenia (n=2)	neutropenia (n=5)	leukopenia (n=12), neutropenia (n=10)

**m* represents number of cycles, in total 54 cycles were administered *[2- 6]*m* represents number of cycles, in total 130 cycles were administered

*[7-10] toxicity is listed for the first cycle of therapy only

Table 8: *Comparison of toxicity of CAP7.1 and topoisomerase II inhibitors in phase I clinical trials*

4.5 Comparison of the Toxicity of CAP7.1 with Topoisomerase I Inhibitors

4.5.1 Irinotecan (CPT-11)

Irinotecan is a camptothecin derivative, whose mechanism of action is through inhibition of topoisomerase I. Like CAP7.1 irinotecan is activated through carboxylesterase II. The drug has undergone various phase I trials.

In 1993, 32 patients were treated with CPT-11, administered as 90-minute infusion every week for four consecutive weeks in six different dose levels. The most frequent hematologic adverse event was neutropenia, which occurred in 18 patients during cycle 1. Grade 3 or 4 neutropenia (NCI Toxicity grade) occurred, when high doses were administered. The most common non-hematologic AEs with NCI Grade > 3 were diarrhea (n=6), dehydration (n=4), nausea and vomiting (n=3), and asthenia (n=3). Grade 4 diarrhea was the dose limiting toxicity in this trial. It occurred in 4 out of 6 patients and was dose dependent (Rothenberg, 1993).

In another trial 59 patients were treated with CPT-11 intravenously at different dose levels using a weekly schedule. Hematologic toxicity was represented by neutropenia (n=40) including three grade 4 (WHO grade) cases, and leukopenia (n=40), two patients experienced a grade 4 leukopenia. The intensity of hematologic toxicity was dose dependent. Mild or moderate anemia was seen in 64% of the patients. Most common non-hematological AE were diarrhea (n=43), nausea (n=43) and vomiting (n=43), asthenia (n=33), alopecia (n=23), and abdominal pain (n=13). Diarrhea was the dose limiting toxicity in this study, two cases of grade 4 diarrhea occurred in the highest dose level (de Forni, 1994).

In one trial CPT-11 was administered to 46 patients as a 30 minute infusion over three consecutive days every three weeks and the dose was escalated. Leukopenia (n=25) and anemia (n=36) were the principal hematological AEs, the frequency and intensity were both dose related. Common non-hematological AEs were diarrhea (n=46) nausea (n=40), vomiting (n=40), alopecia (n=27), fatigue (n=24), and abdominal pain (n=17). Diarrhea and leukopenia were dose limiting events and in this trial the severity was clearly dose-related (Catimel, 1995).

Irinotecan was administered to 64 patients as a 30-minute infusion every three weeks. The dose was escalated. Grade 2 or higher granulocytopenia occurred in 27 patients. At the highest dose level all seven patients experienced a grade 4 granulocytopenia. Anemia (grade 2 or higher) was seen in 28 patients, the severity was clearly dose related. The main non-hematological AE was diarrhea (n=48), this AE was dose dependent. Other frequent toxicities (grade 3 or higher) included alopecia (53%) asthenia (14%), nausea and vomiting (9%). DLTs were granulocytopenia and diarrhea (diarrhea could be controlled through high dose loperamide administration) (Abigerges, 1995).

A feasibility study with high doses of irinotecan (MTD or one dose level below) was performed as a 30-minute infusion once every three weeks in 35 patients. At the MTD, 78% of the patients evolved a grade 3 or 4 neutropenia regularly aggravated by fever. One toxic death occurred. At the dose level below grade 3 or 4 neutropenia occurred in 41% of the patients and no episodes of neutropenic fever were observed. Other toxicities (grade 3 or higher) included diarrhea (37%), nausea and vomiting (29%), and alopecia (51%). 63% of the patients experienced cholinergic symptoms, one patient suffered from acute cholinergic syndrome (Merouche, 1997).

Administered once every three weeks as a 90-minute infusion, irinotecan was given to 34 patients in another dose-escalating phase I study. Most common hematological toxicities were neutropenia (n=25) and leukopenia (n=24) the severity was clearly dose dependent. Frequent non-hematological AEs were diarrhea (n=27), nausea (n=27), anorexia (n=21), and vomiting (n=18). DLTs were gastrointestinal (diarrhea, vomiting and nausea) and hematological (neutropenia, febrile neutropenia) (Pitot, 2000).

26 patients were treated with irinotecan in a phase I trial using a 96-hour infusion weekly. The dose was escalated during treatment. Non-hematological toxicities included diarrhea (n=21), nausea and vomiting (n=26), 29% of the patients experienced a mild anorexia and in 36% constipation was observed. DLTs were diarrhea (n=3) and thrombocytopenia (n=2). No grade 3 or 4 neutrocytopenia was observed and only one patient experienced a grade 3 leukocytopenia. DLTs were diarrhea and thrombocytopenia (Takimoto, 2000).

Pharmacokinetics and pharmacodynamics of irinotecan were examined by Chabot, et al. in 1995. It was shown, that CPT-11 AUC significantly correlates with decrease of leucocytes and granulocytes.

Substance	CAP7.1	Irinotecan	Irinotecan	Irinotecan	Irinotecan	Irinotecan	Irinotecan	Irinotecan
Study	Keilholz, U.	Rothenberg, ML.	de Forni, M.	Catimel, G.	Abigerges, G.	Merrouche, Y.	Pitot, HC.	Takimoto, CH.
Nº of patients	19	32	59	46	64	35	34	26
Frequent Toxicities	fatigue (n=13), nausea (n=10), alopecia (n=10), fever (n=10), abdominal pain (n=7), diarrhea (n=6), obstipation (n=6), vomiting (n=5)	*[1] diarrhea (n=4), dehydration (n=4), nausea (n=4), asthenia (n=3)	diarrhea (n=43), nausea (n=43), vomiting (n=43), asthenia (n=33), alopecia (n=23)	diarrhea (n=46), nausea (n=40), vomiting (n=40), alopecia (n=27), fatigue (n=24)	*[5]diarrhea (n=12), asthenia (n=9) nausea (n=6), vomiting (n=6),	nausea/ vomiting (n=29), diarrhea (n=28), asthenia (n=26), alopecia (n=23), cholinergic symptoms (n=22),	diarrhea (n=27), nausea (n=27), anorexia (n=21), vomiting (n=18)	nausea (n=26), vomiting (n=26), diarrhea (n=21), constipation (n=8), anorexia (n=6)
Leukopenia (grade ≥ 3)	n=10	n. d.	n=15	n=10	n=18	n=16	n=11	n=1
Neutropenia (grade ≥ 3)	n=6	*[2] n=4	n=11	*[4]n=9	*[6]n=21	*[8]n=10	n=13	n=0
Neutropenic fever	n=4	n=0	n=1	n=8	*[7]n=8	*[8]n=12	n=1	n=0
Thrombo cytopenia (grade ≥ 3)	n=6	n=0	n=2	n=7	n=8	n=4	n=1	n=2
Anemia (grade ≥ 3)	n=4	n=1	n=1	n. d. (all grades: n=36)	n=6	n=4	n. d.	n=1
Major DLTs	neutropenia (n=3), neutropenic fever (n=3), thrombocytopenia (n=3)	diarrhea (n=4)	*[3]diarrhea (m=5), leukoneutro penia (m=5)	diarrhea (n=16)	granulocyto penia,(n=12)	diarrhea (n=13), neutropenia (n=10, febrile granulocyto penia(n=12)	diarrhea (n=5), vomiting (n=4), neutropenia (n=4), neutropenic sepsis (n=1)	diarrhea (n=4), thrombocyte penia (n=2)

*[1]toxicities listed occurred during first cycle of treatment and were grade ≥3 *[2]toxicities listed occurred during first cycle of treatment

*[3]*m* represents the number of cycles, in total 304 cycles were administered *[4, 6, 7 and 8]all granulocytes were counted *[5]toxicities listed are ≥ grade 3

Table 9: *Comparison of toxicity of CAP7.1 and irinotecan in phase I clinical trials*

4.5.2 TOPOTECAN

Topotecan is a semisynthetic camptothecin analog and a specific inhibitor of topoisomerase I which was tested in a phase I trial in 2010. It was administered to 16 patients intravenously over 30 minutes weekly for three weeks repeated every 28 days. Anemia (n=11), thrombocytopenia (n=10), leukopenia (n=8), and fatigue (n=8) were the most frequent adverse events followed by neutropenia (n=5). Non hematological toxicity was represented by fatigue (n=8), alopecia (n=5), nausea (n=5), and vomiting (n=4). One patient experienced dose limiting grade 4 hematological toxicity (leukopenia, thrombocytopenia, and anemia) (Curtis, 2010).

4.5.3 NK-012

NK-012 is a SN 38 loaded polymeric micelle. SN-38 is an analogue of camptohecin and is released from NK-012 in a nonenzymatic manner. A phase I clinical trial was performed in 2010. 24 patients were treated with a 30-minute infusion every three weeks. The dose was escalated. Common non-hematological AEs were nausea (n=20), anorexia (n=19), vomiting (n=9), alopecia (n=8), and fatigue (n=6). These events were generally mild or moderate in intensity. Leukopenia (n=20) and neutropenia (n=20) were the main hematological AEs. The severity was dose dependent. Thrombocytopenia occurred 13 times, but only one was grade 3 and none grade 4. Seven DLTs occurred during the trial, five were neutropenia or related. One patient had atrial flutter and one an increased GGT. DLTs only happened in the high dose cohorts (Hamagushi, 2010).

4.5.4 DIFLOMOTECAN (N80915)

Diflomotecan is a homocamptothecin derivate: it targets DNA topoisomerase I. In a phase I trial diflomotecan was administered to 24 patients once every three weeks as a 20-minute infusion and the dose was escalated. Hematological toxicity was dose dependent. 13 patients experienced grade 4 (NCI-CTC) neutropenia and three patients grade 4 thrombocytopenia. Nausea, vomiting, and diarrhea were common non-hematological adverse events. 16 patients experienced a dose limiting

toxicity at some point in the study, which was either infection of hematological toxicity or fatigue. One patient with febrile neutropenia died. DLTs were dose related (Trocòniz, 2005).

In another phase I study diflomotecan was administered to 31 patients intravenously on days 1-5 every three weeks in different dose levels. Grade 3 or 4 (NCI-CTC) neutropenia and leukopenia occurred in eleven patients each. Thrombocytopenia was seen in six patients. Most frequent non-hematological AE was fatigue, followed by alopecia (n=14), mucositis (n=12), nausea (n=8), vomiting (n=5). DLT were neutropenia (grade for lasting for more than 7 days, n=5), neutropenic fever (n=1), neutropenic infection (n=1), diarrhea (n=1), rash (n=1), stomatitis (n=1), increased bilirubin (n=1) (Scott, 2007).

In a phase I trial diflomotecan was administered to 13 patients at two starting doses as a 20 minute infusion every 21 days. Grade 3 or 4 (NCI-CTC) hematologic toxicity were neutropenia (n=7), thrombocytopenia (n=2), anemia (n=1), non-hematological AEs of grade 3 or 4 were one case each of diarrhea, peripheral neuropathy, oral candidiasis, fatigue, cholangitis, pleural effusion, and anaphylaxis. Grade 4 neutropenia (n=4) was the DLT in this trial (Graham, 2009)

4.5.5 DELIMOTECAN

Delimotecan is a polysaccharide prodrug of camptothecin that selectively inhibits topoisomerase I. In a phase I trial 22 patients were treated in eight different dose levels. Delimotecan was administered as a 3-hour infusion once every six weeks. Most common non-hematological AEs were fatigue (n=14), diarrhea (n=11), rash (n=9), nausea (n=8), vomiting (n=7), and anorexia (n=5). Anemia (n=12), leukocytopenia (n=11), neutropenia (n=8), and thrombocytopenia were dose dependent. Grade 3 or 4 (NCI-CTC) myelotoxicity occurred only at the three highest dose levels. Dose limiting toxicities included one case of multiorgan failure, stomatitis (n=2), and thrombocytopenia (n=1) (Veltkamp, 2008).

4.5.6 S-CKD-602

S-CKD-602 is a pegylated liposomal formulation of CKD602, a camptothecin analogue. It was administered intravenously every three weeks in a dose escalating phase I trial. 45 patients were treated. Most frequent non-hematologic toxicities were nausea (n=25), fatigue (n=23), diarrhea (n=12), vomiting (n=9), and anorexia (n=8). They were mainly mild or moderate in intensity. Hematologic toxicity was represented by neutropenia (n=13), anemia (n=13), and thrombocytopenia (n=4). DLTs were anemia (n=3), neutropenia (n=2), thrombocytopenia (n=2), febrile neutropenia (n=1), and mucositis (n=1) (Zamboni, 2009).

4.5.7 7-t-Butyldimethylsilyl-10-Hydroxycamptothecin (AR-67)

AR-67 is a novel camptothecin analogue that was tested in phase I trial with 26 patients in 2010. It was infused five times over one hour daily every 21 days. The dose was escalated. In total 61 courses were administered. The major toxicities were hematologic and represented by leukopenia (*m*=41), thrombocytopenia (*m*=37), anemia (*m*=26), and neutropenia (*m*=19). Frequent non-hematological AEs were fatigue (*m=26*), followed by nausea (*m*=14), constipation/dehydration (*m*=14) anorexia (*m*=12). (*m* represents AEs in number of cycles). DLTs were thrombocytopenia (n=3), febrile neutropenia (n=1), and fatigue (n=1) (Arnold, 2010).

Substance	CAP7.1	Topotecan	NK-012	Diflomotecan	Diflomotecan	Diflomotecan	Delimotecan	S-CKD-602	AR 67
Study	Keilholz, U.	Curtis, KK.	Hamagushi, T.	Tróconiz, IF.	Scott, L.	Graham, JS.	Veltkamp, SA.	Zamboni, WC.	Arnold, SM.
Nº of patients	19	16	24	24	31	13	22	45	26
Frequent Toxicities	fatigue (n=13), nausea (n=10), alopecia (n=10), fever (n=10), abdominal pain (n=7), diarrhea (n=6), obstipation (n=6), vomiting (n=5)	fatigue (n=8), alopecia (n=5), nausea (n=5), vomiting (n=4)	nausea (n=20), anorexia (n=19), vomiting (n=9), alopecia (n=8), fatigue (n=6)	*fatigue (n=21), vomiting (n=10), diarrhea (n=4), nausea (n=4)	*alopecia (n=14), mucositis (n=12), nausea (n=8), vomiting (n=5)	*[2] diarrhea (n=1), peripheral neuropathy (n=1), oral candidiasis (n=1), fatigue (n=1), anaphylaxis (n=1)	fatigue (n=14),diarrhea (n=11), rash (n=9), nausea (n=8), vomiting (n=7)	nausea (n=25), fatigue (n=23), diarrhea (n=12), vomiting (n=9), anorexia (n=8)	*[3]fatigue (*m*=26), followed by nausea (*m*=14), constipation/ dehydration (*m*=14) anorexia (*m*=12)
Leukopenia (grade ≥ 3)	n=10	n=3	n=12	n. d.	n=11	n. d.	n=7	n. d.	*m*=8
Neutropenia (grade ≥ 3)	n=6	n=4	n=16	n=16	n=11	n=7	n=4	n=8	*m*=6
Neutropenic fever	n=4	n=0	n=1	n=6	n=1	n=0	n=0	n=1	n=1
Thrombocytopenia (grade ≥ 3)	n=6	n=1	n=1	n=10	n=6	n=2	n=3	n=4	*m*=16
Anemia (grade ≥ 3)	n=4	n=1	n=1	n=6	n=6	n=1	n=2	n=4	*m*=6
Major DLTs	neutropenia (n=3), neutropenic fever (n=3), thrrombocytopenia (n=3)	panzytopenia (n=1)	neutropenia (or related event) (n=5)	neutropenia (n=16, including 6 with infection), thrombocytopenia (n=10), fatigue (n=10)	neutropenia (n=5), neutropenic fever (n=1), neutropenic infection (n=1)	neutropenia (n=3)	multiorgan failure (n=1), stomatitis (n=2), thrombocytopenia (n=1)	anemia (n=3), neutropenia (n=2), thrombocytopenia (n=2), febrile neutropenia (n=1), mucositis (n=1)	thrombocytopenia (n=3), febrile neutropenia (n=1), fatigue (n=1)

*toxicities listed are of grade 2 and 3 only *[2]toxicities listed are of grade 3 and 4 only *[3]*m* represents the number of cycles, in total 61 cycles were administered

Table 10: *Comparison of toxicity of CAP7.1 and topoisomerase I inhibitors in phase I clinical trials*

4.6 Comparison of the Toxicity of CAP7.1 with New Cytotoxic Drugs in Phase I Trials

4.6.1 Eribulin Mesylate (E7389)

Eribulin mesylate is a synthetic analogue of halichondrin B. It is a non-taxane microtubule dynamics inhibitor and causes an irreversible mitotic block that leads to cell cycle arrest in the G2-M phase and then to apoptosis. In a phase I trial eribulin was administered to 32 patients on days 1, 8, and 15 of a 28 day cycle as a 1-hour infusion with different doses. Most common non-hematological AEs were fatigue (n=17), nausea (n=13), anorexia (n=12), diarrhea (n=7), and alopecia (n=5). Myelotoxicity was dose related. Neutropenia developed in 14 and anemia in ten patients. Neutropenia (n=2) and fatigue (n=2) were dose limiting (Goel, 2009).

In another phase I trial, eribulin was administered to 21 patients as a 1-hour infusion every 21 days with escalating doses. Fatigue (33%) and alopecia (33%) were the main non-hematological AEs. Hematological AEs included neutropenia (n=8), leukopenia (n=7), and anemia (n=5). In this trial febrile neutropenia (n=3) was the principal DLT and dose dependent, one case was aggravated by mucositis. No other DLTs were seen (Tan, A.R., 2009).

In a phase I study eribulin mesylate was administered in escalating doses on days 1 and 8 of a 21 day cycle to 15 Japanese patients. Most frequent non hematological AEs were hyperglycemia (n=6), fatigue (n=5), and alopecia (n=3). Hematological toxicity included leukopenia (n=12), neutropenia (n=11), lymphocytopenia (n=7), febrile neutropenia (33%), anemia (n=4), and thrombocytopenia (n=2). Five patients developed DLTs during the first cycle of therapy (neutropenia (n=3) and febrile neutropenia (n=2)) (Mukohara, 2011).

4.6.2 Cabazitaxel

Cabazitaxel is a taxane that showed broad antitumor activity in human xenograft models. In a dose escalating phase I clinical trial it was administered to 21 patients every 3 weeks. Most common non-hematologic AEs included gastrointestinal toxicities (67%), which were mostly represented by

diarrhea followed by constitutional symptoms (24%) such as fatigue and fever, neurological disorders (14%). Hematologic toxicity included neutropenia (n=16), anemia (n=20), and thrombocytopenia (n=3). DLTs that occurred during the first cycle were diarrhea (n=2), neutropenia (n=4), febrile neutropenia (n=1), and infection (n=1) (Diéras, 2012).

4.6.3 UTD1

UTD1, an epitholone analog which inhibits microtubule dynamics, was tested in a phase I clinical trial with 21 patients in different dose levels. UTD1 was administered as a 3-hour intravenous infusion every three weeks. Gastrointestinal discomfort (n=16) was the most common non-hematologic AE, followed by paresthesia/neurotoxicity (n=15), myalgia/arthralgia (n=14), fatigue (n=12), and alopecia (n=8). The only hematological AE was neutropenia (n=1). DLTs were ataxia (n=2) and nausea/vomiting (n=1) (Zhang, 2010).

4.6.4 BMS-31705

BMS-31705 is a water-soluble semisynthetic epitholone B analog, which is cytotoxic through microtubule stabilization. It was given to 59 patients as a 15-minute infusion in a phase I trial on two different schedules. (Days 1, 8, and 15, followed by one week´s rest, and day 1 and 8 followed by one week rest). Most common non-hematological toxicities during the first cycle were diarrhea (n=27), asthenia/fatigue (n=26), vomiting (n=14), myalgia (n=13), nausea (n=10), and paresthesia (n=6). Main hematological toxicity was neutropenia (n=22). DLTs during the first cycle were diarrhea (n=5), neutropenia (n=2), vomiting (n=1), and thrombocytopenia (n=1). A cumulative late onset of paresthesia was observed and peripheral neuropathy (n=13) was the main reason for treatment discontinuation due to toxicity (Sessa, 2006).

Substance	CAP7.1	Eribulin Mesylate	Eribulin Mesylate	Eribulin Mesylate	Cabazitaxel	UTD 1	BMS-31705
Study	Keilholz, U.	Goel, S.	Tan. AR.	Mukohara T.	Diéras, V.	Zhang, P.	Sessa, C.
Nº of patients	19	32	21	15	21	21	30
Frequent Toxicities	fatigue (n=13), nausea (n=10), alopecia (n=10), fever (n=10), abdominal pain (n=7), diarrhea (n=6), obstipation (n=6), vomiting (n=5)	fatigue (n=17), nausea (n=13), anorexia (n=12), diarrhea (n=7), and alopecia (n=5)	fatigue (n=7), alopecia (n=7), nausea (n=4)	hyperglycemia (n=6), fatigue (n=5), and alopecia (n=3)	gastrointestinal (n=14, including diarrhea n=10), constitutional symptoms (n=5)	gastrointestinal discomfort (n=16), paresthesia / neurotoxicity (n=15), myalgia / arthralgia (n=14), fatigue (n=12)	*diarrhea (n=27), asthenia/fatigue (n=26), vomiting (n=14), myalgia (n=13), and nausea (n=10)
Leukopenia (grade ≥ 3)	n=10	n. d.	n=5	n=10	n. d.	n=0	n. d.
Neutropenia (grade ≥ 3)	n=6	n=12	n=7	n=10	n=10	n=0	*n=4
Neutropenic fever	n=4	n=1	n=3	n=5	n=2 (including one infection)	n=0	n. d.
Thrombocytopenia (grade ≥ 3)	n=6	n=0	n=0	n=0	n=1	n=0	n=0
Anemia (grade ≥ 3)	n=4	n=2	n=0	n=0	n=2	n=0	n. d.
Major DLTs	neutropenia (n=3), neutropenic fever (n=3), thrrombocytopenia (n=3)	neutropenia (n=1), neutropenic fever (n=1), fatigue (n=2)	neutropenic fever (n=3)	*neutropenia (n=3), neutropenic fever (n=2)	*diarrhea (n=2), neutropenia (n=4), neutropenic fever (n=1), neutropenic infection (n=1)	ataxia (n=2), nausea / vomiting (n=1)	*diarrhea (n=5), neutropenia (n=2), vomiting (n=1), and thrombocyte penia (n=1)

*toxicities are listed for the first cycle of therapy only

Table 11: *Comparison of toxicity of CAP7.1 and new cytotoxic drugs in phase I clinical trials*

4.7 CONCLUSION

CAP7.1 was administered intravenously as a 60 minute infusion on five consecutive days in four different dose levels. Non-hematologic toxicity was generally moderate. The most common non-hematological adverse events were fatigue, nausea, alopecia and gastrointestinal and constitutional symptoms.

Myelotoxicity was the most frequent toxicity in this study and it was dose dependent. DLTs were represented by neutropenia and thrombocytopenia. Myelotoxicity usually appeared during the first cycle of therapy and then recurred in the following cycles. Cumulative myelotoxicity was not seen. In general, bone marrow related toxicities recovered within 12 days. In further studies it will be necessary to pay attention especially to WBC, ANC and PLT changes. Since the nadir of the white blood cells appeared between days 10 and 17, scheduling laboratory between these days of each cycle should be considered and if decrement in white blood cells is seen, action could be taken to prevent complications such as infection or sepsis. PLT nadir was seen between days 12 and 15 and should be monitored carefully as well to prevent complication.

Compared to other topoisomerase inhibitors, the toxicity profile in terms of hematotoxicological effects was similar. In contrast to its mother compound etoposide, no specific organ toxicities were associated with CAP7.1 in this trial. Since CAP7.1 could be safely administered in this trial further studies with this drug should be performed, to evaluate the anticancer activity. In this trial eleven patients achieved a SD and one patient a PR as best overall response (Rhode, 2012, Keilholz, 2012). Especially etoposide-resistant malignancies could be treated. Since CAP7.1 is not a product of the MDR, resistance might be overcome.

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EIDESSTATTLICHE VERSICHERUNG

Ich, Philipp-Mathias Mehlitz, versichere an Eides statt durch meine eigenhändige Unterschrift, dass ich die vorgelegte Dissertation mit dem Thema "Safety Profile of CAP7.1 obtained during Phase I Trial in adult patients with refractory malignancies" selbstständig und ohne nicht offengelegte Hilfe Dritter verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel genutzt habe. Mir lagen zur Auswertung dieser Phase I Studie sämtliche CRFs, die SAE reports, die Clinical Investigators Brochure und das Study Protocol vor.

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 Strafgesetzbuch) sind mir bekannt und bewusst.

Philipp-Mathias Mehlitz

CURRICULUM VITAE

Mein Lebenslauf wird aus datenschutzrechtlichen Gründen in der elektronischen Version meines Lebenslaufes nicht veröffentlicht.

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Auswertung der Daten bezüglich der Wirksamkeit des Medikamentes CAP7.1 aus Phase I, Präsentation der Studienergebnisse auf dem "24th EORTC-NCI-AARC Symposium on Molecular Targets and Cancer Therapeutics" im November 2012

Philipp-Mathias Mehlitz Priv. Doz. Dr. med. N. Utku