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DISSERTATION

Analysis of Initial Efficacy Results of a New Cytotoxic Prodrug, CAP7.1, in Adults with Therapy Refractory Solid Tumours in a Phase I Clinical Trial

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List of Abbreviations

5-FU	5-Fluorouracil
ADEPT	Antibody-directed enzyme prodrug therapy
ALT	Aspartate transaminase
AML	Acute myeloid leukaemia
ANC	Absolute neutrophil count
AST	Alanine transaminase
ATP	Adenosine triphosphate
BCRP	Breast cancer resistance protein
CD	Cluster of differentiation
CES 2	Carboxylesterase 2
CINV	Chemotherapy-induced nausea and vomiting
CLL	Chronic lymphocytic leukaemia
CR	Complete response
CrCl	Creatinine clearance
CT	Computed tomography
CUP	Cancer of unknown primary
DLT	Dose limiting toxicity
DNA	Deoxyribonucleotide acid
ECG	Electrocardiography
ECOG	Eastern Cooperative Oncology Group
FDA	U.S. Food and Drug Administration
G-CSF	Granulocyte colony-stimulating factor
HIV	Human immunodeficiency virus
ICH-GCP	International Conference on Harmonisation–Guidelines for Good Clinical Practice
LD10	Lethal dose in 10% of cases
LRP	Lung resistance protein
MDR	Multiple-drug resistance
MRI	Magnetic resonance imaging
MRP1	Multi-drug resistance-associated protein
MTD	Maximum tolerated dose
MTX	Methotrexate
NDEPT	Neuroblastoma directed enzyme prodrug therapy

NSCLC	Non-small-cell lung cancer
PD	Progressive disease
PR	Partial response
PT/INR	Prothrombin time / International normalized ratio
PTT	Partial thromboplastin time
RECIST	Response Evaluation Criteria in Solid Tumours
SCLC	Small-cell lung cancer
SD	Stable disease
U.S.	United States of America
ULN	Upper limit of normal
WHO	World Health Organisation

1 Abstract

This work analyzes the preliminary anti-tumour activity of the new cytotoxic compound CAP7.1 in a first in human phase I trial in adults. Cancer is one of the leading diseases and causes of death worldwide, but therapeutic options are often unsatisfactory. Etoposide is a widely prescribed and highly effective cytotoxic drug, but its therapeutic use is limited by systemic toxicity and induction of drug resistance mechanisms. CAP7.1 is a newly developed prodrug of etoposide and is enzymatically activated by carboxylesterase (CES). Preclinical data showed an improved safety profile and better efficacy (compared to etoposide) in animals and different cell lines, including etoposide resistant cell lines.

Eligible for this open-label, non-randomized, dose escalating trial were adult patients with refractory malignancies, adequate bone marrow and organ function and good performance status. CAP7.1 was administered on 5 consecutive days as a 60-minute intravenous infusion every 21 days for up to 6 cycles. Tumour assessment was scheduled every second cycle and analyzed according to RECIST 1.0 criteria.

19 patients with a wide range of tumours were included; their median age was 63 years. Patients were treated in four different escalating dose cohorts (dosage: 45, 90, 150 mg/m²/day up to the maximum tolerated dose (MTD) of 200 mg/m²/day). In total, 62 cycles of CAP7.1 were administered (range 1-6 cycles). Four patients, all in the last cohort, completed all six cycles. 17 patients were assessable for tumour response. One partial response was observed in a heavily pre-treated patient who suffered from Merkel cell carcinoma. 11 patients achieved stable diseases (SD), with a wide range of tumour entities. Six patients had an SD duration of over 3 months. 10 patients survived over six months. The longest overall survival (25 months) was seen in a patient who suffered from gallbladder carcinoma, the second longest survival (20 months) was assessed in a patient diagnosed with cancer of unknown primary (CUP).

Overall efficacy showed promising initial results in various tumour entities. Due to the small number of patients, only limited comparison of efficacy of this trial with other studies is possible, being a conceivable issue in phase I testing. A preliminary anti-tumour efficacy was demonstrated and CAP7.1 compares favourably with most of the other compounds analysed. Therefore, further investigation in clinical trials with various tumour entities is warranted.

2 Zusammenfassung in deutscher Sprache

Ziel dieser Arbeit ist die Analyse und Bewertung der initialen Wirksamkeit von CAP7.1, einer neuen zytotoxischen Substanz, welche kürzlich in einer Phase-I-Studie an Patienten mit therapierefraktären Krebserkrankungen getestet wurde. Maligne Erkrankungen sind eine der führenden Todesursachen weltweit, therapeutische Optionen sind jedoch nach wie vor unzureichend. Ein weit verbreitetes, hoch effektives zytotoxisches Medikament ist Etoposid, welches jedoch aufgrund von systemischer Toxizität und enzymatisch aktivierten Resistenzmechanismen nur begrenzt einsatzfähig ist. CAP7.1 ist ein neu entwickeltes Prodrug von Etoposid, welches durch eine Carboxylesterase (CES) aktiviert wird. Präklinische Daten aus Versuchen an Tieren und verschiedenen Ziellinien (incl. etoposidresistente Zelllinien) zeigten ein verbessertes Nebenwirkungsprofil sowie eine bessere Wirksamkeit.

Anlehnend an Resultate, die bisher in präklinischen Analysen und in Fallbeispielen in Kindern mit Neurofibromatose ist die Hypothese der Arbeit, dass CAP7.1 (in 2-3x höheren Dosen der Etoposide) eine gute Wirksamkeit und Vertäglichkeit bei therapie-resistenten Tumoren im Erwachsenen aufweist.

In diese “open-label” nicht-randomisierte Dosiseskalationsstudie wurden volljährige Personen mit refraktärem malignen Tumorleiden mit angemessener Knochenmarks- und Organfunktion sowie einem ausreichenden Allgemeinzustand eingeschlossen. CAP7.1 wurde in einem Zyklus von 21 Tagen an fünf aufeinander folgenden Tagen, mit einem Maximum von sechs Zyklen, als jeweils 60-minütige Infusion verabreicht. Die Tumorevaluation erfolgte jeden zweiten Zyklus und wurde nach den RECIST 1.0 Kriterien ausgewertet.

In die Studie wurden 19 Patienten mit verschiedensten Tumorentitäten eingeschlossen, welche in vier Kohorten eingeteilt wurden (Dosierungen von jeweils 45, 90, 150 mg/m²/Tag bis zur maximal tolerierbaren Dosis (MTD) 200 mg/m²/Tag). Das mediane Alter betrug 63 Jahre. Insgesamt wurden 62 Zyklen (Bandbreite: 1-6 Zyklen) verabreicht. Vier Patienten aus der letzten Kohorte vervollständigten dabei die sechs Zyklen. Eine Tumorevaluation konnte bei insgesamt 17 Patienten erfolgen. Eine “partial response” (PR) wurde bei einer bereits mehrfach vorbehandelten Patientin mit Merkelzellkarzinom beobachtet. Bei elf Patienten mit unterschiedlichsten Tumorentitäten konnte eine “stable disease” (SD) erreicht werden, welche bei sechs Patienten mehr als drei Monate anhielt. Zehn Patienten überlebten länger als sechs Monate. Ein Patient mit Gallenblasenkrebs zeigte die längste Überlebenszeit (25 Monate), gefolgt von einem Patienten mit einem CUP (cancer of unknown primary) (Überlebenszeit: 20 Monate).

Da eine initiale Wirksamkeit von CAP7.1 demonstriert werden konnte und die Ergebnisse der Phase-I von CAP7.1 mit anderen Phase-I-Studien bereits zugelassener Substanzen vergleichbar sind, ist eine Weiterentwicklung in klinischen Studien mit verschiedenen Krebsindikationen gerechtfertigt.

3 Introduction

This work aims to analyse preliminary anti-tumour efficacy of the new anti-cancer agent CAP7.1 during a phase I clinical trial in adult patients with advanced stage solid cancers.
This work is part of the phase I study of CAP7.1 with the following study objectives approved prior to study begin by Bundesinstitut für Arzneimittel und Medizinprodukte (BfArM):

- Determine the maximum tolerated dose (MTD) of CAP7.1
- Determine toxicity profile (including dose limiting toxicity, DLT) of CAP7.1
- Determine the dose of CAP7.1 suitable for phase II testing
- Investigate the pharmacokinetics of CAP7.1 in adults
- Make a preliminary assessment of the anti-tumour activity of CAP7.1.

Based on findings in the preclinical assessment and in clinical use of the prodrug in children, our hypothesis of the phase I study is that CAP7.1 is capable to overcome therapeutic resistance of solid tumors and exhibits an initial efficacy at several fold higher dose as conventional etoposide accompanied by a good tolerability.
Although a phase I study serves as base for safety analysis, the efficacy results are an important part of the study to find the effective dose and indications selection for phase II.
The reason for the evaluation of the efficacy of the prodrug CAP7.1 is several fold:
1. This is a new prodrug of a well known drug etoposide and tested first time in adult patients it is not possible to continue further clinical phase II studies without an initial efficacy evaluation as addressed by a number of other clinical phase I studies.
2. Similar to other trials in oncology, this Phase I study protocol is designed to continue the treatment until tumour progression. Therefore the patients have to show a benefit after the treatment with the study drug to continue in the study. Thus the efficacy of the compound has to be evaluated to continue or discontinue the treatment during the phase I study.
3. The evaluation of efficacy along with safety serves as decision base to select the recommended dose and more importantly the target indication for further phase II trials.
4. Additionally, pharmacological studies are sponsored studies and to carry out further studies an initial prove of efficacy even in a smaller patient group is necessary.
Taken together, the analysis of the safety pharmacokinetic results in context with efficacy, although limited due to small patient numbers plays a crucial role for further clinical development and testing of the drug CAP7.1.

In order to outline the importance and the development of such a new cancer therapy, epidemiological data of cancer, chemotherapeutics currently used in the clinic, and their cardinal limitations are summarized. Furthermore, several possibilities to overcome these limitations are described. Since CAP7.1 is a prodrug of etoposide, etoposide and CAP7.1 are characterized in detail.

3.1 Cancer Facts

Cancer, which accounts for around 8.2 million deaths and 14.1 million new cases in 2012 worldwide, is one of the major diseases and leading causes of death in the world.[1] The World Health Organisation (WHO) expects an increase in deaths due to cancer to 11.8 million cases in 2030.[2] The most likely reasons for this development are the aging and growth of the world population and an increase in cancer-causing behaviours, such as tobacco use, imbalanced dietary or alcohol consumption.[3]

The National Institutes of Health of the United States of America (U.S.) estimated the total costs of cancer at $263.8 billion in 2010 for the U.S., which include $102.8 billion for medical treatment and $161 billion for indirect costs (like the decrease in productivity).[4] This shows that cancer is not only a growing health problem, but also has many financial and political implications. Therefore, development of more specific treatments should be given priority in current and future research programs.

3.2 Brief Overview of Currently Used Chemotherapeutics

Apart from radiation therapy and surgery, systemic chemotherapy continues to play an important role in the treatment of cancer. In recent years, two major classes of drugs have been established for clinical use; systemic cytotoxic drugs and target-specific therapeutics.

The beginning of the modern era of cytotoxic drugs dates back to 1942 and actual results of research were first published in 1946. At that time patients with Hodgkin's disease, lymphosarcoma, and chronic leukaemia which were treated with nitrogen mustard showed a significant improvement. [5, 6]

Today, various cytotoxics are available and can be divided into five major groups in terms of their molecular mechanisms – alkylating agents, antimetabolites, cytotoxic antibiotics, microtubule-targeting agents, and topoisomerase inhibitors.

Among the most commonly used chemotherapeutics are the *alkylating agents*. The most successful representative in clinical use is cyclophosphamide, a derivative of nitrogen mustard.[7] It is widely used in cancer treatment, including solid tumours, like breast cancer, ovarian cancer, or sarcomas, and haematological malignancies, e.g. chronic lymphocytic leukaemia (CLL).[8-11] Alkylating agents induce apoptosis by forming DNA-alkyl adducts. Additionally, most alkylating agents form interstrand or intrastrand DNA cross-links leading to cellular cytotoxicity. [7,12]

Antimetabolites interfere with cell division and cell metabolism by replacing natural metabolites, resulting in non-functional macromolecules or blockage of enzymes. Main representatives of the antimetabolites are antifolates, purine, and pyrimidine analogues.

Antifolates are the oldest antimetabolites and among the first modern anticancer drugs to be used. In the 1950s, the antifolate methotrexate (MTX) was first introduced for cancer treatment.[13] MTX leads to cell death by inhibition of dihydrofolate reductase. This results in a decrease of tetrahydrofolate coenzymes. These coenzymes are needed by, for instance, the thymidylate synthase. The decrease causes inhibition of synthesis of thymidylate and purine precursors, which leads to inhibition of DNA synthesis.[13,14] In clinical oncology, MTX is used for the treatment of leukaemia, lymphoma, choriocarcinoma, head and neck cancer, and osteogenic sarcoma.[15]

An often used example for pyrimidine analogues is 5-Fluorouracil (5-FU), which is an analogue of uracil. 5-FU is a prodrug which reduces cancer cell replication by inhibition of pyrimidine synthesis through blockage of thymidylate synthase.[16] It is used in the treatment of various solid tumours such as colorectal and breast cancer.

Some antibiotics are used not only in anti-microbial treatment but also as antitumour medication. *Cytotoxic antibiotics* are a heterogeneous group, but they all have a common their origin; they are products of fermentation in microbial cultures.[17] In 1952, the first anticancer antibiotic, sarcomycin, produced by *Streptomyces*, was discovered and first published in 1953 by Umezawa et al., after observing in the proceeding years that microorganisms could produce various kinds of cytotoxic agents. [18, 19]

Anthracycline antibiotics are a widely used subgroup of cytotoxic antibiotics. The antitumour activity of daunorubicin and adriamycin was first described in the early 1960s.[20] The mechanism of action of daunorubicin and adriamycin is still not completely understood, but it seems that different mechanisms lead to its cytotoxicity depending on drug concentration. Discussed are the inhibition of DNA synthesis, interference with DNA unwinding and strand separation, free

radical formation, lipid peroxidation, direct membrane effects, and topoisomerase II inhibiting effects.[21] Anthracyclines are used in first-line therapy of acute myeloid leukemia (AML) as well as in solid tumours, for example breast cancer. [22, 23]

Microtubule-targeting agents interfere with microtubular structure and function. Microtubules are fibrillar structures and play an important role in cellular activity such as intracellular transport, cell shape, motility, and mitosis.[24]

Vinca alkaloids, like vincristine, vinblastine, and vinorelbine, are the oldest substances that interfere with microtubules.[25] Vinca alkaloids inhibit polymerization into microtubules by binding at the central position of the ß-tubulin subunit. The second group, the taxanes, have a different mechanism and lead to apoptosis by stabilizing the microtubules.[25]

Both are widely used in the treatment of various malignancies, including standard first line therapies for example in metastatic breast cancer and ovarian cancer. [26,27]

The mechanism of action of *topoisomerase inhibitors* is based on interfering in enzymes regulating the topology of the double-helix structured DNA during cellular cycle.[28] For replication and transcription, removal of supercoiled DNA is required. Topoisomerase I relaxes supercoiled DNA by forming single strand DNA breaks and subsequently closing them without requiring ATP for the process.[29] Topoisomerase I inhibitors are the camptothecin derivatives of topotecan used in the treatment of relapsed small cell lung cancer (SCLC) and gynaecological tumours[30,31] and irinotecan, used especially in the treatment of colorectal cancer.[32]

Topoisomerase II relaxes (ATP-dependent) negatively and positively supercoiled DNA by cleaving double stranded complementary DNA and rejoining the separated DNA segments afterwards.[33] Vertebrates exhibit two isozymes: topoisomerase IIα and topoisomerase IIß. They have similar catalytic activities, but differ in their time and location of expression. Topoisomerase IIα is mainly expressed during S, G2, and M phase in rapidly dividing cells, whereas topoisomerase IIß is expressed in all kind of cells and is not cycle dependent.[34]

Topoisomerase II inhibitors are divided into two groups; topoisomerase II catalytic inhibitors and topoisomerase II poisons. Topoisomerase II catalytic inhibitors, for example aclarubicin and suramin, are pure inhibitors of the enzyme and block either the ATP binding site, stabilize non-covalent DNA topoisomerase II complexes, or prevent the nucleotide binding, thus having cytotoxic impact.[35] Catalytic inhibitors are used in the treatment of, for example, haematological malignancies, such as AML and myelodysplastic syndrome[36], and solid tumours, like hormone-refractory prostate cancer or advanced platinum-resistant ovarian cancer.[37]

Topoisomerase II poisons build covalent ternary enzyme-drug-DNA complexes, hereby interrupting transcription and replication. Anticancer agents in clinical use are etoposide, teniposide, doxorubicin, daunorubicin, mitoxantrone, idarubicin, amsacrine, and ellipticine.[28,34] Etoposide as a representative of topoisomerase II poisons is further described in detail as it is the active compound released from CAP7.1 - a new cytotoxic agent analyzed in this work.

3.3 Side Effects and Limiting Aspects of Cytotoxic Drug Application

Nearly all cytotoxic drugs have basic side effects. This is due to the fact that toxicity affects all cells without selectivity, especially rapidly dividing cells. Those side effects can be divided into two groups: short and long term effects. Short term side effects include toxic effects during chemotherapy and mostly resolve within months after treatment, at the latest. Long term side effects occur several weeks or months after treatment. Both are dependent on dose and duration of treatment and on the agents used and vary across individuals.[38]

***Table 1*. Common Side Effects of Systemic Chemotherapy.**

Short Term Effects	Long Term Effects
Nausea & Vomiting	Weight gain
Diarrhea	Infertility
Malabsorption	Cardiac dysfunction
Stomatitis	Secondary malignancies
Alopecia	
Myelosuppression	
Thromboembolism	
Neuropathy	
Fatigue	

Chemotherapy-induced nausea and vomiting (CINV) is still among the most disturbing side effects of chemotherapy, with 37% of patients experiencing acute episodes during treatment, increasing over the cycle.[39] Evidence-based guidelines have been developed, but CINV still remains a significant problem.[40] The most common dose limiting side effect (dose limiting toxicity (DLT)) of chemotherapy is myelosuppression, especially neutropenia leading to infections. Granulocyte colony-stimulating factor (G-CSF) is used as prophylactic or therapeutic treatment of chemotherapy associated neutropenia.[41] It reduces severe neutropenia (absolute neutrophil count (ANC) < 500/ml), febrile neutropenia (ANC < 1000/ml and temperature > 38.2°C for more than one hour), the incidence of infections and increases the possibility to continue treatment according to the scheme.[42]

Besides the various side effects, another limiting factor for application of cytotoxics is an increase in resistance. Cancer cells often utilize multiple different pathways to obtain resistance. Various mechanisms can be broadly classified into pharmacodynamic or pharmacokinetic pathways. Pharmacodynamic pathways are, for example, the reduced sensitivity to apoptosis through overexpression of the anti-apoptotic Bcl-2/Bcl-x protein.[43] Another mechanism is the alteration of the drug's target, for instance in topoisomerase, by reducing expression and mutations that reduce the affinity of drug binding.[44] One major pharmacokinetic pathway is the expression of multi-drug efflux pumps which leads to multiple drug resistance (MDR). MDR results in an overexpression of the mdr-1 encoded p-glycoprotein which works as an ATP-dependent drug efflux pump. The result is a cross-resistance of tumour cells, including anthracyclines (doxorubicin, daunorubicin), etoposide, vinca alkaloids (vincristine, vinblastine) and taxanes (taxol, taxotere).[45] Other examples of multi-drug efflux pumps are multidrug resistance-associated protein (MRP1) and breast cancer resistance protein (BCRP).[43] A further mechanism is the metabolic biotransformation and inactivation of drugs, for instance seen in 5-FU, catabolized by dihydropyrimidine-dehydrogenase which is overexpressed in cancer cells.[44] There are multiple other additional, different mechanisms leading to resistance which are not further explained in detail here due to their complexity.

3.4 New Development and Modification of Chemotherapeutics

To overcome the current lack of selectivity and the increase of MDR tumour cells, target-specific agents and cytotoxic substances that overcome MDR have been developed.

New strategies, already in wide clinical use, are targeted therapies which use substances that interfere with a specific molecular target. This is typically a protein playing a role in tumour growth or progression, therefore leaving normal cells unaffected. This results in a better toxicity and a decrease in general side effects compared to ordinary cytotoxics.[46] In addition to a number of antibodies, many small molecules have been developed. Examples are tyrosinkinase inhibitors (erlotinib) or proteasome inhibitors (bortezomib). Current monoclonal antibodies in clinical use target different growth factors, for instance vascular endothelia growth factor (bevacizumab), epidermal growth factor receptor (cetuximab), and HER2/neu (trastuzumab), or they target CD antigens, like CD52 (alemtuzumab) and CD20 (rituximab).[47] Although remarkable improvements in response to treatment and overall survival have been made in certain tumour entities, other entities do not seem to respond to the new therapy options. Reasons are insufficient cytotoxic effects and development of multiple resistance to many targeting agents.[48]

Therefore, further development of targeting therapies, new technologies, and research into new possible mechanisms of action became absolutely necessary.

One new approach is to conjugate targeting molecules (for example antibody) and highly cytotoxic drugs, combining strong cytotoxic effects and selectivity. The complexes formed are internalized by receptor mediated endocytosis of only the targeted cells, whereas other cells remain unaffected. For a wide clinical application, more analysis and research still need to be done.[49] A different approach is to increase the tumour selectivity of a cytotoxic drug itself, by conjugating the cytotoxin with a moiety, being split off enzymatically in cancer cells. The so called prodrug itself is not toxic and develops cytotoxicity only in cells which express the accordant enzyme to release the parent drug.[50] Due to their high proliferation rate, tumour cells often have an elevated level of certain enzymes. This leads to high bioconversation of certain prodrugs, especially in tumour cells which causes a larger measure of selectivity.[51] Another new strategy combining the two mechanisms is called antibody-directed enzyme prodrug therapy (ADEPT), where a specific enzyme linked to an antibody targeting a tumour antigen is administered. Consequently the prodrug is converted into its active compound by this specific enzyme only in the targeted tumour tissue. In this manner, tumour selectivity is achieved.

Additionally to the development of new agents, it is necessary to develop and research, for example, in molecular and genetic profiles of tumours or effective biomarkers. Especially now, as treatment is getting increasingly specific for different targets or mechanisms, detailed knowledge is needed to have an optimized, individual, and effective treatment scheme.[5]

One example of a newly developed antineoplastic drug is CAP7.1 - a prodrug of etoposide. Due to its pharmacokinetics and pharmacodynamics, better efficacy and less intense side effects are expected. Preliminary efficacy of CAP7.1 is analyzed here, which was obtained during a first phase I clinical trial.

3.5 Etoposide

Etoposide or VP-16 is a semi-synthetic derivate of podophyllotoxin, a naturally occurring extract of plants in the genus *Podophyllum*. It occurs in North America as *Podophyllum peltatum* and as *Podophyllum hexandrum* in India.[52]

Podophyllin, already used by the Native Americans and natives of the Himalaya as a cathartic and anthelmintic, was removed from the U.S. Pharmacopoeia in 1942 because of its severe toxicity. Its anti-mitotic effect was already known in 1946, but due to its general toxicity, high enough dosages to give significant clinical activity in humans could not be reached.[53] During the

1960s and 1970s, Sandoz Laboratories synthesized a large number of podophyllin derivates and analyzed their biological effects, hoping for better efficacy and less toxicity. In 1966, etoposide was first synthesized. In 1971, first clinical trials began and in 1983 etoposide was launched as VePesid on the U.S. market for treatment of testicular cancer.[54]

Etoposide **1** is a 4´-demethylepipodophyllotoxin-9-(4,6-O-ethyldene-ß-D-glucopyranoside). It differs from podophyllotoxin **2** by epimerization and substitution of ethyldene-ß-D-glucopyranoside at the C-ring and demethylation of C-4´ at the E-ring (figure 1).[55]

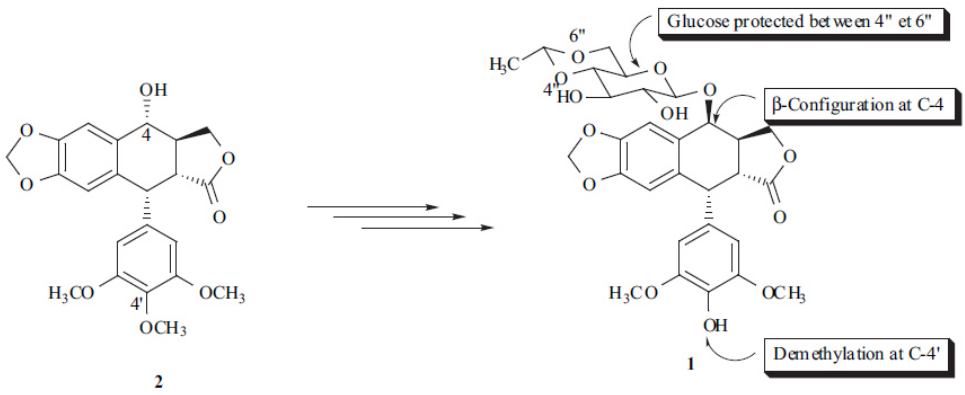

Figure 1. from Meresse, P, Dechaux, E et al.[45], **1** etoposide, **2** podophyllotoxin

Etoposide is a phase specific cytotoxic drug and prevents cells from entering mitosis. Already in 1975, it was shown that in contrast to podophyllotoxin, etoposide causes accumulation of cells in G2 phase and can only induce cell cycle arrest in metaphase of mitosis when used in high dosages.[56] It was noticed by Wozniak et al. that etoposide induces single and double stranded DNA breaks and that DNA-Protein cross-links are temperature dependent, therefore the involvement of an enzyme seemed to be likely.[57] In 1984 this led to the identification of the enzyme topoisomerase II as the target of etoposide.[58]

Etoposide inhibits topoisomerase II by stabilizing the enzyme-DNA cleavable complex during the catalytic cycle of the enzyme and forms a covalently bound ternary complex.[45]

Recently, the crystal structure of a large fragment of topoisomerase II complexed to DNA and two drug molecules of etoposide have been described, as shown in figures 2 and 3.[59]

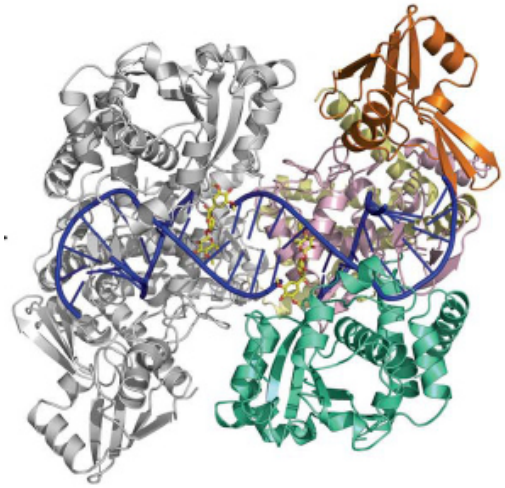

Figure 2. from Wu et al. [59]
3D Structure of Ternary Cleavage Complex.
(DNA: blue, etoposide: yellow and red)

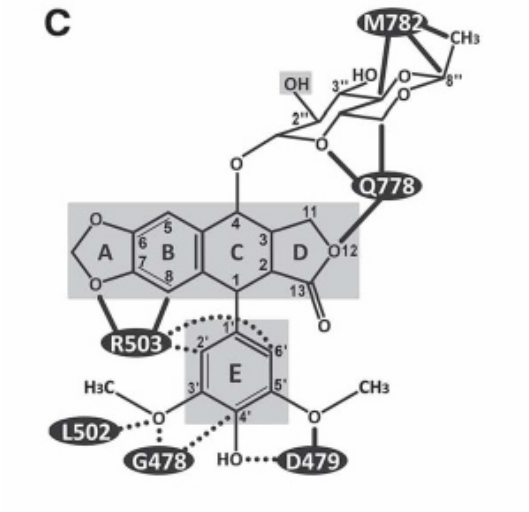

Figure 3. from Wu et al. [59]
Drug-Interacting Residues of Etoposide.
(Drug-enzyme interactions: solid and dashed lines, Drug-DNA interactions: shaded grey)

By forming the stabilized complex TOP2cc (transient formation of topoisomerase II when covalently linked to two 5´ends of DNA), ligation activity of topoisomerase is inhibited and, therefore, DNA breaks result.[60] This leads to activation of several DNA damaging molecules, such as ATM, ChK 1/2, H2AX, p53 or RPA, subsequently resulting in cell cycle arrest, non-homologue end joining, non-homologue recombination, and apoptosis of the cell.[61]

Etoposide is a widely prescribed chemotherapeutic drug. As a combination therapy, it is licensed in Germany for SCLC, advanced non small cell lung cancer (NSCLC), subtypes of lymphomas, AML in children and adults after failure of standard therapy, testicular cancer, and in choriocarcinoma in female patients (dependent on WHO-staging).[62] It is mostly combined with cisplatin, carboplatin, and cyclophosphamide, as, for example, in PEB regime (bleomycin, etoposide and cisplatin) for standard induction chemotherapy for progressed testicular cancer.[45,63] In monotherapy it is used for palliative treatment of progressed ovarian cancer, after failure of platinum analogues and relapsing refractory testicular cancer. Standard dosage is 50-120 mg/m² etoposide for 3-5 days with a free interval of 3-4 weeks with generally 3-4 cycles, but it varies on protocols for different indications.[62]

One of the dose limiting side effects of etoposide is its highly myelosuppressive effect, particularly seen as neutropenia but also thrombocytopenia.[64] Common side effects of cytotoxic drugs like digestive toxicity, nausea, and vomiting are not very frequent and can be treated easily by antiemetics. Mucositis and diarrhoea are only seen when using high dosages.[45]

It is also observed that treatment with etoposide induces secondary acute leukemia two or three

years later mostly having distinctive translocations involving the region 11q23.[64]
It has been shown that etoposide is highly schedule dependent and shows better efficacy and less systemic toxicity in prolonged low-dose application than short high-dose application.[65-67] Slevin et al. suggested that prolonged exposure to low serum levels is the main determinant of cytotoxic efficacy, which was also seen in *in vitro* experiments. [65, 68] In 1993 Thompson et al. postulated that myelosuppression is dependent on peak serum level and, therefore, highly dose dependent.[66] Today, it is suggested that antitumour activity can be achieved at plasma concentrations of ca. 0.5-1µg/mL, whereas side effects occur at ca. 10µg/mL.[69] Therefore, specific complex application schemes and drug monitoring seem to be required.
Another limiting factor is the development of resistances through two different mechanisms. One is the development of MDR by overexpression of p-glycoprotein and other proteins like MRP (multidrug resistance protein) and LRP (lung resistance protein).[45,70] Additionally, tumour cells also form specific resistance by alteration of the enzyme topoisomerase II, the target of etoposide.[71]
This shows the necessity for the development of etoposide analogues which overcome current limitations - one being CAP7.1.

3.6 CAP7.1

3.6.1 Preclinical Data

Despite internal preclinical investigations, several unpublished in vivo and in vitro characterisations of CAP7.1 have been performed prior to starting the investigations for this clinical trial.

3.6.1.1 Chemistry and Activation

To create a hydrolytically activated prodrug of etoposide, the hydroxyl group on C4 of etoposide was esterified with a propyl-carbonoxy moiety. The hydroxyl group on C4 of etoposide is one active binding site with topoisomerase II (see figure 3 above) and, therefore, blocked by the new added moiety. This creates an inactivation of the topoisomerase inhibitor, which results in reduced general unspecific initial toxicity.

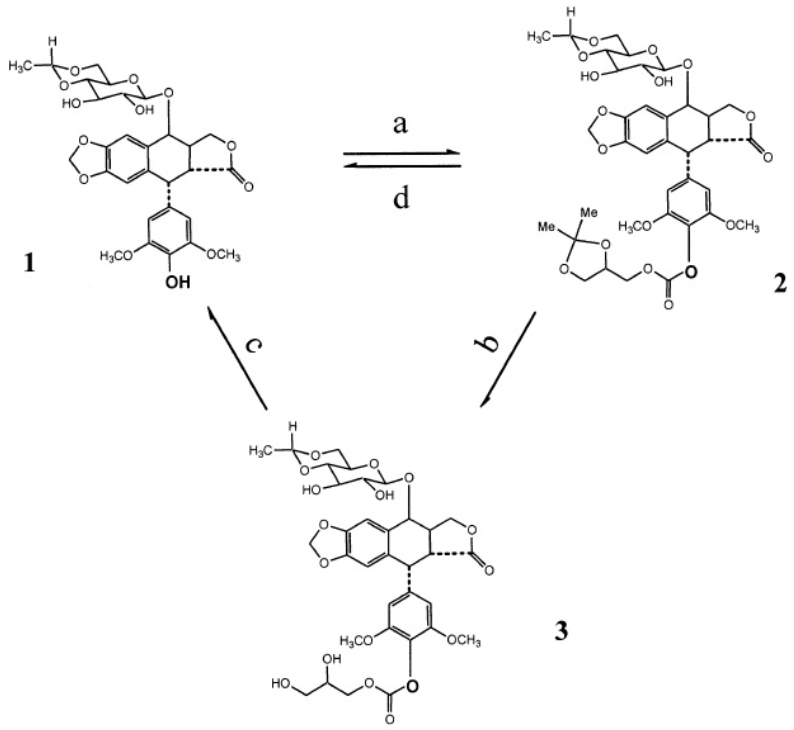

Figure 4. **1** Etoposide, **2** proVP 16 I, now CAP7.1, **3** proVP 16 II [72]

In 2001, Wrasidlo et al. first synthesized two new derivates of etoposide: proVP 16 II and proVP 16 I. Pro VP I was eventually named CAP7.1 (figure 4).[72]

However, proVP 16 II underwent several further investigations but was not further developed for clinical use.

By then, it was suspected that CAP7.1 (proVP 16 I) converts into proVP 16 II in an acidic environment, being subsequently activated into etoposide.[72,73] This could not be confirmed by internal preclinical data. Hydrolytical activation through carboxylesterase, also observed by Wrasidlo et al., was confirmed in the new preclinical investigations. In vitro and in vivo experiments in rodents and primates showed a carboxylesterase dependent conversion of CAP7.1 into etoposide. The particular classes of enzymes involved have not been determined yet, but carboxylesterase 2 (CES 2) seems to be the most likely one. CES 2 is present in various normal tissues, as it has the highest expression in liver, small intestine, kidney and adrenal cortex cells.[74] The presence of CES 2 in human tumour cells was demonstrated and shown to have a wide range of intensity of expression between different tumour types but also within one tumour entity in nearly all tumour tissues tested.[75]

3.6.1.2 *In Vitro* Experiments

Concerning multi-drug resistant cell lines (expressing MDR-1 gene), significant difference with an increase of up to three orders of magnitude of cytotoxicity compared to etoposide was observed (see figure 5). This indicates that CAP7.1 somehow circumvents drug resistance mechanisms; one possible mechanism discussed is the prodrug's direct interaction and decrease in MDR-1-mediated substrate efflux.[72,73,76]

An increase in cytotoxic activity compared to etoposide was also observed in various cancer cell lines, including cells from human neuroblastoma, leukaemia, and solid tumours (see figure 6). An observed slow release mechanism of the free drug could be one explanation.[73,76]

Furthermore, an additional G2/M-phase arrest, with complete synchronisation of cells in the cell cycle not seen with etoposide, was observed. This suggests an additional target of the prodrug itself. [73,76] Possible mechanisms have not been determined yet.

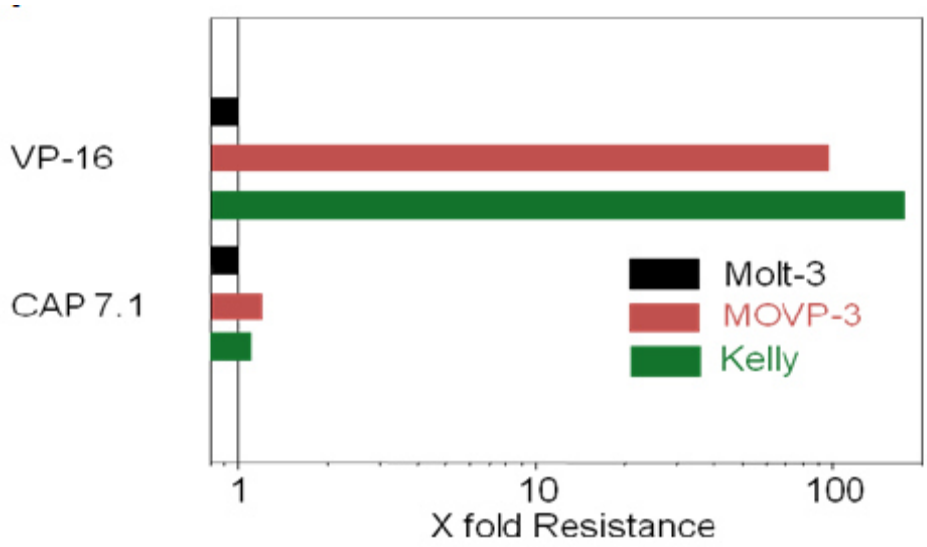

Figure 5. **Resistance of CAP7.1 Compared to Etoposide.** Significant difference between CAP7.1 and etoposide (VP-16) ($p<0.001$). Molt-3 human T-lymphoblastic cell line, MOVP-3 and Kelly are etoposide-resistant cell lines (MDR-1 mediated).[73]

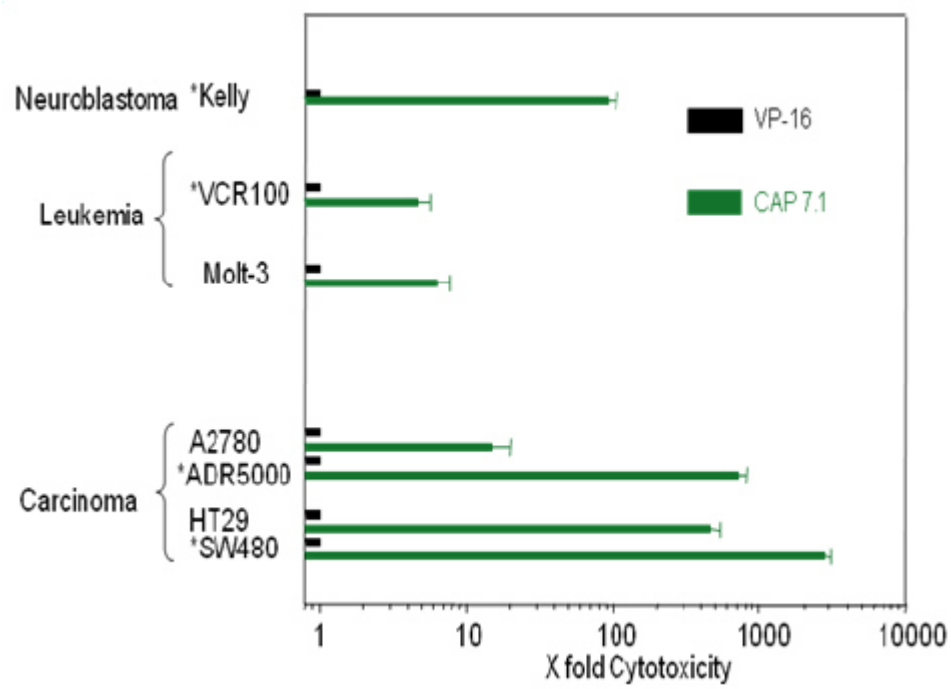

Figure 6. **Cytotoxicity of CAP7.1 Compared to Etoposide.** Significant difference between CAP7.1 and etoposide (VP-16) ($p<0.01$) in all cell lines. * amplified MDR-1 expression.[73]

3.6.1.3 *In Vivo* Experiments

In vivo experiments were performed in rodents and primates. Pharmacokinetics, toxicology, and efficacy were analyzed (internal data).

Pharmacokinetic data showed that CAP7.1 was converted extremely rapidly into etoposide in rodents, leading almost to the same toxicity profile as that of etoposide. Therefore, studies in primates followed which were more similar to human circumstances. This showed a slower conversion explained through the less active carboxylesterase in primates which results in less side effects.

Toxicological analyses showed typical effects of cytotoxic agents which are mostly similar to those of etoposide.

Efficacy was demonstrated in a model of neuroblastoma in mice (cell line NXSC injected s.c.) and showed a significant inhibition of tumour growth (85% reduction by day 19 with treatment compared to control) (unpublished data, Lode H et al.).

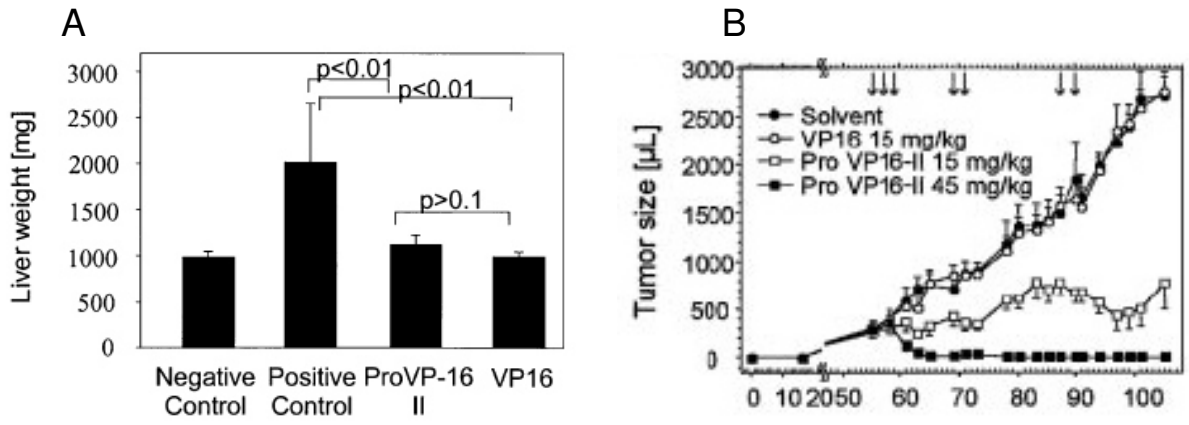

Figure 7. **Effect of ProVP-16 II in a (A) Mice Model of Non-Resistant NXS2 Cell Injection** (significant reduction of liver metastasis compared to untreated controls) and **(B) Mice Model with Subcutaneously Injected MOVP-3 Cells** (significant difference between tumour size of mice treated with ProVP16-II and control groups).[73,76]

3.6.2 Clinical Data

CAP7.1 was administrated to three pediatric patients with recurrent metastatic neuroblastoma stage 4 in 2003 and 2004 (unpublished report Gaedicke et al.). The heavily pre-treated children (failure of prior chemotherapies including etoposide) were treated with a combination of CAP7.1 and carboplatin, escalated up to a dosage of 800 mg/m²/day of CAP7.1. The observed toxicities were similar to those of etoposide (including hematological toxicity grades 3 and 4) and evaluated low especially considering the escalated doses. Pharmacokinetic data (of one patient) showed similar results as in preclinical investigations.

One 5-year-old patient developed progression of the disease and died (maximal administrated dose 200 mg/m²).

The second patient (6 years old) responded with a stable disease over a period of 9 months (maximal administrated dose 600 mg/m²).

The third patient (12 years old) achieved a partial response and was in stable remission for 2 years before tumour relapse (maximal administrated dose 800 mg/m²).

3.7 Other Examples of Prodrugs of Etoposide

Besides the two synthesised prodrugs mentioned above, several other approaches to create a prodrug of etoposide have been made.

The most successful one is etoposide phosphate, a phosphate ester of etoposide, which is rapidly

hydrolysed by alkaline phosphatase to its active compound etoposide.[77] In Germany, similar to etoposide, it is licensed in combination with other chemotherapeutic drugs for treatment of SCLC, palliative therapy of NSCLC, reinduction therapy of M. Hodgkin and AML, Non-Hodgkin lymphomas (intermediate and high aggressive entities), testicular cancer, and choriocarcinoma (in women). As monotherapy it is used in palliative treatment of ovarian cancer (refractory to platinum agents).[62]

Other approaches made by adding a moiety to the parent substance etoposide led to the substances etoposide 4´-sulfate (ADEPT by antibody-arylsulfatase conjugates) and glucuronide-etoposide (activated by ß-glucuronidase occurring in necrotic tumour areas).[78,79] A new NDEPT (neuroblastoma directed enzyme prodrug therapy) approach was to select the enzyme tyrosine hydroxylase (specific for neuroblastoma) to specifically activate a special designed prodrug - proVP-16 IV.[80] A different concept was to create a dual prodrug-enzyme complex (dpVP-16 combined with irinotecan both activated by rabbit carboxylesterase), suggested by Yoon et al..[81] Furthermore, a catalytic antibody-prodrug system was developed, creating a non-immunogenic approach for targeted therapy.[82] However, none of these substances has reached the market or even the lead of clinical studies. Examples of clinically tested substances (mostly phase I, but even phase II trials) are the podophyllotoxin derivates TOP-53, GL331, NK611, or Tafluposide, but none of these reached the market.

3.8 Hypothesis

This phase I study was performed at Charité Universitätsmedizin Berlin after the approval of the regular German authorities (Federal Institute for Drugs and Medical Devices; „Bundesinstitut für Arzneimittel und Medizinprodukte (BfArM)“) on the following study objectives:

- Determine the maximum tolerated dose (MTD) of CAP7.1
- Determine toxicity profile (including dose limiting toxicity, DLT) of CAP7.1
- Determine the dose of CAP7.1 suitable for phase II testing
- Investigate the pharmacokinetics of CAP7.1 in adults
- Make a preliminary assessment of the anti-tumour activity of CAP7.1.

The work presented here focuses solely on the analysis of drug efficacy although only preliminary but crucial for further clinical studies and indication selection.

The pharmacokinetic assessment and safety assessment were part of the study, wherefore the analysis was performed, but not subject on this thesis. The full study report is available which contains all data about the study outcome.

Our hypothesis is that CAP7.1, as a prodrug of etoposide, can overcome the current therapeutic resistance of tumour cells and exhibits efficacy in especially lung cancer patients, testicular tumours and ovarian tumours. In addition, because of suspected CES 2 driven conversion of CAP7.1 into etoposide, we expect to see efficacy in tumour tissues with a high CES 2 expression such as gastrointestinal tissues and lung.

4 Methods

This trial was an open-label, non-randomized phase I dose-escalating trial. In the study, the dose of CAP7.1 was escalated in cohorts of three to six patients until the maximum tolerated dose (MTD) was reached. All patients should be followed until disease progression, death, initiation of alternative anticancer treatment, or the end of the trial. The end of the trial was defined as 26 days after the last patient completed treatment, the date the last patient's tumour progressed, or the patient died, if this is earlier. No patients received placebo medication, and all patients received full supportive care including antiemetics, antibiotics, and analgesics as clinically indicated. Patients were assessed for disease response or progression at regular intervals (as defined in the schedule of assessments), and those who appeared to be benefiting from therapy continued to receive cycles of CAP7.1 treatment until a maximum of six cycles, documented disease progression, unacceptable toxicity, or patient request.

A large team consisting of several disciplines such as statisticians, pharmacists, study nurses and oncologists, pharmacologists and monitors. Ph. D. students e.g. Laura Rohde has been involved from the beginning till the end and beyond (study report and publication of results) in the study in the team. The author was involved during patient recruitment (selection of patients according to exclusion-includion criteria), management of data for the efficacy assessments, organizing data for dose escalation meetings (decision for continuation, dose selection and dose escalation of the trial), organization and follow up of the Recist evaluation of computer tomograms for the analysis of the drug efficacy, decision taking process for continuation of the treatment of patient during the phase I study, patient follow up and monitoring the documentation of the patient relevant data into the CRF during the entire study and supporting data cleaning after finalization of the study. Laura Rohde was also involved into the assessment and analysis of the data base after close out of the study for the analysis of all drug and treatment related data of the patients and finalization of the full study report.

4.1 Selection of Study Patients

All potential patients were screened according to following criteria. If a patient was eligible and confirmed by the principal investigator, the patient was registered for this study.

4.1.1 Inclusion Criteria

All patients were required to fulfil all of the inclusion criteria to be eligible for the study.
Inclusion criteria were:

- Histologically confirmed 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 criteria (Response Evaluation Criteria in Solid Tumours)
- Age ≥ 18 years
- Eastern Cooperative Oncology Group (ECOG) Performance Status of 0-2
- Life expectancy of at least eight weeks
- Adequate bone marrow and organ function including:
    - Haemoglobin ≥ 9.0 g/dL
    - ANC ≥ 1 500/mm³
    - Platelet count ≥ 100 000/mm³
    - Total bilirubin ≤ 1.5 times the upper limit of normal (ULN)
    - Alanine transaminase (ALT) and aspartate transaminase AST ≤ 2.5 x ULN (≤ 5 x ULN for patients with liver involvement)
    - PT-INR (prothrombin time - international normalized ratio) and PTT (partial thromboplastin time) < 1.5 x ULN
    - Creatinine clearance (CrCl) > 50 mL/min, according to modified Cockcroft-Gault criteria:

      CrCl = weight (kg) × (140-age)/72 × Creatinine level (male x 1, female × 0.85)
- Patient had to have recovered from the acute reversible effects of previous anti-cancer chemotherapy, immunotherapy, radiotherapy, or endocrine therapy. This meant at least four weeks had to have elapsed since major surgery, radical radiotherapy, or myelosuppressive chemotherapy (six weeks for nitrosoureas or mitomycin C). At least 4 weeks had to have elapsed since treatment with an investigational agent.
- 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 resulted in a low failure rate, i.e. less than 1 % pregnancies per year.

Female patients of child-bearing potential were eligible if they had agreed to use a highly effective method of birth control throughout the study and for at least 4 weeks after stopping the treatment. Male patients with partners of child-bearing potential were eligible if they had agreed to use contraception during the trial and for 6 months after stopping study drug, unless surgically sterile.

- Written informed consent according to ICH-GCP (International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceutics for Human Use – Harmonised Tripartite Guidelines – Guidelines for good Clinical Practice E6(R1),1996), and national/local regulations.
- High probability of good compliance and orderly completion of the study

4.1.2 Exclusion Criteria

All patients were required to fulfil none of the exclusion criteria to be eligible for the study.
Exclusion criteria were:

- Known central nervous system involvement unless it had been definitively treated with radiotherapy or surgery and the patient's central nervous system disease was clinically stable, at the time of study entry.
- Karnofsky-Index < 70%
- Serious concurrent medical condition which could have affected compliance with the protocol or interpretation of results. Patients with uncontrolled infection and patients who had known to be infected with the human immunodeficiency virus (HIV) or with chronic hepatitis B or C virus infections were not eligible for the study.
- History of another malignancy which could have affected compliance with the protocol or interpretation of results. Patients who had been treated with curative intent and had remained disease-free for at least five years were generally eligible, as were patients with *in situ* disease that had been treated with curative intent.
- Other psychological or social conditions which in the investigator's opinion would not make the patient a good candidate for the clinical trial.
- Pregnancy or breast-feeding
- Other anti-cancer therapies, except endocrine therapy for prostate cancer. Bisphosphonates if introduced prior to enrolment, were allowed.
- Known or suspected etoposide refractory tumours
- Known or suspected peripheral neuropathies

- Patients who had been committed to an institution according to an order of court or an official directive

4.1.3 Patient Removal

Patients could withdraw from the study at any time for any reason.
Patients could be withdrawn from study treatment or from the study itself in the event of intercurrent illness, adverse events, treatment failure, protocol violation, administrative, or for other reasons. Patients should be removed from the study at any time due to unacceptable toxicity or if such removal is medically warranted.

4.2 Treatment

4.2.1 Treatment Administration

CAP7.1 was delivered as a sterile solution in a 240 ml intravenous infusion bag. It was administered in 60 minutes as an intravenous infusion for five consecutive days. Repetitions were scheduled every 21 days. The treatment could be delayed by one week until the patient recovered from treatment-related toxicities. Generally, if a patient required more than three weeks' delay, drug administration had to be stopped completely, unless discussed and agreed otherwise (benefit – risk – evaluation).
Patients who seemed to be benefiting from treatment (tumour evaluation was scheduled every second cycle) continued treatment up to a total of six cycles, on condition that unmanageable toxicity, tumour progression or patient's request to withdraw study were absent.
Treatment started within one week after registration for the study.

4.2.2 Selection of Dose Level

The starting dose was 45 mg/m² per day for five consecutive days. Maximum dose administered was planned on 350 mg/m² per day for five consecutive days, considering preclinical and prior clinical data.
Preclinical studies on rats had showed a LD10 (lethal dose in 10%) of 714 mg/m². The starting dose of 45 mg/m² was a 15 fold decrease, the maximum dose of 350 mg/m² more than two-fold lower than observed LD10 in rats. In preclinical studies on primates, the dose of 240 mg/m² had caused minor haematological toxicities and was therefore five-fold higher than the starting dose.

Clinical data in paediatric patients had shown a tolerable dose of up to 800 mg/m², which was more than two-fold higher than the planned maximum dose for this trial.

Dose escalation from starting dose proceeded in increments of 45-60 mg/m² in a total of four major cohorts (Cohort 1-4, see table 3), which each consisted of at least three patients. A new cohort started when at least three patients completed a minimum of 26 days on treatment. If minor toxicities were observed, increments would have only been 15-35 mg/m² (including intermediate cohorts 2*-4*). If dose limiting toxicities (DLT) were observed in a cohort in one of three patients, this cohort was expanded to six patients. If DLT were observed in a cohort in two of six patients, immediate starting and/or expansion into lower intermediate cohort should have been initiated (cohort 1-4 into 1*-4*). If DLT occurred in two of six patients in an intermediate cohort, data should have been reviewed and further actions should have been discussed separately in detail.

Table 2. **Scheme for Dose Escalation.** 1-7 Major cohorts, 1*-7* Intermediate cohorts, DLT Dose Limiting Toxicity

Cohort	Dose	DLT in 1 of 3 Patients	DLT in 2 of 6 Patients
1*	30 mg/m²	Expand to 6 patients	Suspend recruitment & review data
1	**45 mg/m²**		Start and/or expand cohort 1*
2*	60 mg/m²		Suspend recruitment & review data
2	**90 mg/m²**		Start and/or expand cohort 2*
3*	125 mg/m²		Suspend recruitment & review data
3	**150 mg/m²**		Start and/or expand cohort 3*
4*	175 mg/m²		Suspend recruitment & review data
4	**200 mg/m²**		Start and/or expand cohort 4*

4.2.3 Dose Modification Within a Patient

Due to toxicity of CAP7.1, dose reductions in one patient were allowed in the following cases:

- If abnormally low blood count values were detected before and up to ten hours after the substance infusion, especially in connection with suspicion, signs, and/or symptoms of infection.
- Abnormal haematological values were detected preceding next dosage.
- Signs of non-haematological toxicities were found.

Treatment was first delayed by one week until recovery. If the patient did not recover within 14 days from treatment-related toxicity to grade 1 or baseline (when higher than grade 1), treatment was either continued on the next lowest dose level or the patient was removed from the study due to unacceptable toxicity.

4.2.4 Concomitant Therapy

All patients received full supportive care, including antiemetics, antibiotics, transfusions, and analgesics, as clinically indicated. Prophylactic colony stimulating factors were not allowed, but colony stimulating factors could be used to treat a patient experiencing prolonged cytopenia or its complications, if clinically indicated. Patients who already had started bisphosphonates for bone metastases or hormone therapy for supportive care, continued to receive these treatments. Contraceptive medication, as required in inclusion criteria, was continued, including systemic contraceptives, diaphragm with intravaginal spermicide, cervical cap with intravaginal spermicide, intrauterine device, and condoms with intravaginal spermicide.
It was not allowed to administer any other anti-cancer medication or investigational agent for the duration of the study. Initiation of another anti-cancer medication was regarded as evidence for disease progression. If a patient required palliative radiotherapy for pain, she/he had to be assessed for any evidence of disease progression. If there was no evidence of disease progression, palliative radiotherapy could be given as long as it was in a small area, was not expected to result in myelosuppression or exacerbate myelosuppression, and did not affect assessment of tumour response.

4.3 Efficacy Evaluation

4.3.1 Primary Variables

In this study, to evaluate efficacy, the tumour size, response, duration of response, and progression free survival were analysed.
Response was assessed according to RECIST 1.0 criteria. Response was categorized into remission, with either the options of complete response (CR) and partial response (PR) and into patients with stable disease (SD) or tumour progression (PD). As this was a phase I trial, minor tumour response was also documented, which was considered as a decrease in tumour size that

fulfilled criteria neither for PR nor for CR, nor showed any tumour progression as categorized in PD. Furthermore, unconfirmed and transient responses were noted.
Duration of response was assessed in patients who achieved SD and PR. Duration of PR or CR was defined as the time from the first documented response (PR or CR) until documented progression of disease or death (of any cause). Duration of SD was defined as the time from the first treatment until PD or death (of any cause). Progression free survival was defined as the time from enrolment for the study until documented progression of disease or death (of any cause).
To evaluate the patient's status, aside from tumour response, ECOG performance scale was also analyzed during the study.

4.3.1.1 Response Evaluation Criteria in Solid Tumours – RECIST Criteria

RECIST (Response Evaluation Criteria in Solid Tumours) criteria referred to in this trial were published in 2000, and they are currently widely used to evaluate tumour response in clinical trials [83] :
Method of Assessment. For objective evaluation, different methods of tumour assessment could be used. However, the same method and technique should be used in one patient for baseline evaluation and further tumour assessment. In this trial, CT scan (computed tomography), MRI (magnetic resonance imaging) images, and tumour markers were assessed. According to RECIST criteria, imaging-based techniques should be preferred. CT and MRI were currently the best reproducible methods and were widely available. Conventional CT and MRI had to be performed at a slice thickness of 10 mm or less. Spiral CT had to be used in an algorithm of 5 mm. Tumour markers could only be evaluated regarding other methods and when returned to normal. CR was considered only when all other tumour lesions had disappeared.
Measurability. In general, measurable and non-measurable lesions were categorized. Measurable lesions were defined as lesions that could be accurately measured in at least one diameter (longest diameter must be recorded) and were at least 20 mm in diameter in conventional techniques or at least 10 mm in spiral CT. Measurable lesions should be recorded in metric notation (by ruler or callipers). Non-measurable lesions were defined as lesions with a smaller diameter and truly non-measurable lesions (for instance bone lesions, pleural effusions, ascites).
Baseline Evaluation. The baseline evaluation had to be performed as close as possible to the start of treatment and had not to be closer than four weeks before treatment. Subsequent measurements were compared to baseline tumour burden. Only patients with measurable disease

at baseline were included. Measurable disease was defined as the presence of at least one measurable lesion. Solitary lesions were confirmed cytologically or histologically.

Lesions were categorized into targeted and non-targeted lesions. All measurable lesions (up to a maximum of ten total lesions and five lesions per organ) were identified as targeted lesions. Criteria in favour of selection as targeted lesion were lesions with longer diameters (including longest diameter) and lesions which could be measured accurately when measurement was repeated. A baseline sum longest diameter was calculated by taking the sum of the longest diameter of each categorized targeted lesion. This sum was the parameter referred to in further tumour response and compared to the sum of longest diameter of always the same targeted lesions in following performed CT/MRI. All other lesions were categorized as non-targeted lesion and were not further calculated with, but reported as present or absent.

Response Criteria. Targeted and non-targeted lesions were evaluated separately regarding response. Tables 4 and 5 show criteria for complete response (CR), partial response (PR), stable disease (SD), and progressive disease (PD). Tumour re-evaluation was suggested to be performed every six to eight weeks.

Table 3. **Evaluation of targeted lesions according to RECIST.**

CR	Disappearance of all targeted lesions
PR	At least 30% decrease in the sum of longest diameter of all targeted lesions, while taking the baseline sum longest diameter as reference
SD	Neither sufficient shrinkage to qualify as PR nor sufficient increase to qualify as PD
PD	At least 20% increase in the sum of longest diameter of all targeted lesions, while taking as reference the smallest sum of longest diameter documented since the start of treatment or appearance of any new lesions

Table 4. **Evaluation of non-targeted lesions according to RECIST.**

CR	Disappearance of all non-targeted lesions and normalization of tumour marker level
SD	Persistence of one or more non-targeted lesion(s) and/or the maintenance of tumour marker level above normal limits
PD	Appearance of one or more new lesions and/or unequivocal progression of existing non-targeted lesions

Overall tumour response was dependent on response of targeted lesions, non-targeted lesions and the appearance of new lesions. PR and CR had to be confirmed by repeating the assessment in no less than four weeks after the first documented response. SD had to be confirmed by repeating the assessment in an interval of no less than eight weeks. The best tumour response was defined

as the best overall response documented from the beginning of treatment until disease progression/recurrence.

Duration of SD was defined as time of first treatment until PD or death of any cause. Progression free survival was defined as the duration of enrolment for the study until documented progression or death of any cause. Overall survival was defined as the time from entering the study until death or date of last patient contact.

Table 5. **Overall Tumour Response According to RECIST.**

Targeted Lesions	Non-Targeted Lesions	New Lesions	Overall Response
CR	CR	No	CR
CR	SD	No	PR
PR	SD or CR	No	PR
SD	SD or CR	No	SD
PD	Any	Yes or No	PD
Any	PD	Yes or No	PD
Any	Any	Yes	PD

4.3.1.2 ECOG Performance Scale

The ECOG (Eastern Cooperative Oncology Group) performance score quantifies how the cancer disease affects daily living abilities and tries to evaluate the patient's general well-being. ECOG performance of 0-2 was generally described as a good performance status, whereas ECOG score of 3-4 was considered a poor performance status.

Table 6**. ECOG Performance and Related Karnofsky Index as Published by Oken et al. in 1982.[84]**

ECOG		Karnofsky Index
0	Fully active, able to carry on all pre-disease performance without restriction	100 90
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work	80 70
2	Ambulatory and capable of all self-care but unable to carry out any work activities. Up and about more than 50% of waking hours	60 50
3	Capable of only limited self-care, confined to bed or chair more than 50% of waking hours	40 30
4	Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair	20 10
5	Dead	0

4.3.2 Assessment

Prior to first treatment, following screening tests were performed:

- Medical history, demographic data, previous and current diseases, and medication were documented
- Physical examination (incl. weight, height, vital signs)
- 12- lead ECG (electrocardiography)
- ECOG performance status
- Tumour assessment:
    - All suspected sites of disease were imaged and documented (incl. bone scan for suspected bone metastases and ultrasound to evaluate e.g. eventual hepatic malignant lesions)
    - Baseline CT scan of chest and abdomen (pelvis when required)
    - Measurement of tumour markers, if established markers were available for the tumour type
- Laboratory tests (incl. haematology, biochemistry, coagulation panel, pregnancy test (if required) and urine analysis by dipstick)

The baseline sum of longest diameters of targeted lesions was determined in the screening CT. Tumour assessment followed every two cycles of treatment. Images which documented the baseline tumour burden were repeated. Here, the same method of assessment as in baseline evaluation was used. Patients who achieve CR or PR had the following CT scan at least four weeks later for eventual confirmation, even if not scheduled for regular tumour assessment. Images of sites which did not involve any targeted lesions were not repeated unless clinically indicated or to confirm any response. Available tumour markers were also collected after every second cycle. ECOG performance scale was likewise documented every two cycles.

A follow-up examination was performed 26 days after the last application of CAP7.1. Results of a physical examination, ECOG performance, and laboratory analysis (incl. haematology, biochemistry urine analysis by dipstick and tumour markers) were documented. Patients who discontinued treatment early for other reasons than progression of disease had a further tumour assessment. Tumour evaluation was not required when the last assessment was less than six weeks old and no evidence of progression was seen.

4.4 Additional Definitions

As this thesis focused solely on the evaluation of preliminary efficacy, the selected definitions explained in this work are only the definitions which are relevant to the analysis of efficacy and do not represent a complete overview about all methods used during this phase I study.
The efficacy analysis of the study is important aspect for the selection of the target indications and for the continuation of the evaluation beyond phase I trial.
For detailed methods on the safety or pharmacokinetics evaluation, I hereby refer to the thesis, which also describes a part of the phase I study, which concentrates solely on the safety aspects (Safety Profile of CAP7.1 obtained during Phase I Trial in adult patients with refractory malignancies, Philipp Mehlitz and full study report of Phase I including all aspects of the study (available on demand)) and the approved study protocol.
The preliminary maximum tolerated dose (MTD) was defined as the highest dose level below the maximum administered dose at which one of three patients experienced a dose limiting toxicity (DLT).
DLTs were defined as:

- grade 3 or higher non-hematological adverse event,
- grade 4 neutropenia lasting for more than seven days or complicated by fever,
- grade 4 thrombocytopenia or thrombocytopenia of any grade complicated by bleeding,

which were considered to be drug related.
Adverse events were categorized according to a five point scale with grade 1 (mild), grade 2 (moderate), grade 3 (severe), grade 4 (life threatening or disabling), and grade 5 (fatal). 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).
Adverse events were recorded from the time the patient was registered up to and including 26 days after the last protocol treatment.
The evaluation of the safety aspects and pharmacological aspects in this phase I trial of CAP7.1 was not subject to this work and therefore analysed separately.

4.5 Criteria for Studies Included in the Comparison

Only phase I clinical trials with documented tumour response were considered for comparative analysis. Although inclusion and exclusion criteria varied, they were based on principles similar

to those considered in the CAP7.1 trial. They were adult patients (at least 18 years old patients) with advanced, refractory and/or resistant confirmed malignancies, adequate bone marrow and organ function, adequate time since last major therapy, sufficient general condition, sufficient life expectancy and written informed consent. Only studies which conducted monotherapies were analyzed and compared in order to ensure comparability to monotherapy with CAP7.1 investigated in this study. Studies that had been initiated before 2000 often referred to WHO criteria in response evaluation, as RECIST criteria had not been published. Investigations concluded that RECIST criteria are comparable to WHO criteria, therefore, comparison of results of this study with other phase I studies also included studies using WHO criteria.[85]
Research was done on the database PubMed.*gov* (of the U.S. National Library of Medicine). Additionally, results of clinical trials published directly by the investigating company were analyzed if data was not available on Pubmed.*gov*.

4.6 Statistical Methods

All figures (e.g. bar charts) that show data were generated with Microsoft Office Excel® 2003. Calculated median was based on the following formula:

$$x_{median} = \frac{1}{2}(x_{\frac{n}{2}} + x_{\frac{n}{2}+1}) \qquad \textit{for n = even number}$$

$$x_{median} = x_{\frac{n+1}{2}} \qquad \textit{for n = uneven number}$$

All further calculations were based on ordinary standard mathematical methods, as specific statistical calculations were not practical due to the small sample size (low number of patients) and inhomogeneity of the sample group (different dosages, tumours etc.).

5 Results

5.1 Patient Characteristics

Between April 27, 2009 and September 20, 2011, 23 patients were enrolled in the study of whom 19 patients were eligible for this study.

Inclusion criteria were completed fully in 16 of the 19 patients. In two patients, the last systemic chemotherapy was less than four weeks (28 days) old, which was only 26 days in patient 7 and 27 days in patient 8 before the start of treatment on CAP7.1. Both patients had recovered from acute reversible effects of prior chemotherapy and were, nevertheless, included in the study. Additionally, patient 7 had an increase of liver enzymes, with AST 3.7 times over upper normal limit (128 U/l) and ALT 5.4 times over upper normal limit (184 U/l). This was explained by the implantation of a stent in September 2008 due to progressive cholestasis. Even that ALT was more than 5 times above the upper normal limit (Inclusion criteria for patients with liver involvement: ALT $\leq$ 5 x UNL), patient 7 was also included. Even though a Creatinine clearance < 50 ml/min was seen in patient 11, with 43.7 ml/min according to modified Cockcroft-Gault criteria, the patient was included. No exclusion criteria could be identified in any patient.

The median age was 63 years, with a range of 32 years to 72 years. Both genders were represented, with four female (one patient had genotype XY 46 with testicular feminisation, phenotype female) and a majority of 15 male patients.

The majority had a good ECOG performance status, with ECOG status of 0 in 16 % and ECOG status of 1 in 79 %. Only Patient 11 had an ECOG performance of 2. No patients with ECOG performance > 2 participated.

All patients were pre-treated. All patients received prior systemic chemotherapy, 32 % even received five or more prior regimes. Five patients received prior treatment with etoposide (table 9). Four patients with chemotherapeutic regimes including etoposide received a complete response. One patient, who had been treated twice with etoposide, achieved a partial response. Three regimes in two different patients (patient 3 received three regimes including the drug etoposide) brought about a stable disease. The longest progression-free interval of twelve years, after treatment with etoposide, cisplatin, and bleomycin, was seen in patient 15. 13 patients had prior fractionated radiotherapy, and 15 patients had to undergo prior surgery which was related to the cancer diagnosis. Patient characteristics are summarized in table 7.

A wide range of malignancies was observed (table 8). Rectal, oesophageal (one adenous and one squamous), and testicular cancer occurred twice in patients, although the histological type varied

in patients suffering from testicular cancer, with one Sertoli-Leydig cell tumour and one mixed seminiom/non-seminiom histological differentiation.

Table 7. **Patient Characteristics.**

	Cohort 1 n = 3	**Cohort 2** n = 3	**Cohort 3** n = 6	**Cohort 4** n = 7	**Total** n = 19	**Total** in %
Gender						
Male	3	3	3	6	15	78.95
Female	0	0	3	1	4	21.05
Age ***[years]***						
Range	48 - 60	49 - 64	32 - 72	48-67	32-72	
Median	55	64	65,5	63	63	
ECOG Performance						
0	0	2	1	0	3	15.79
1	3	1	4	7	15	78.95
2	0	0	1	0	1	5.26
Prior Treatment						
Systemic Chemotherapy	3	3	6	7	19	100
Radiotherapy	2	1	5	5	13	68.42
Surgery	1	3	6	6	16	84.21
Number of Prior Regimes						
1	0	3	1	1	5	26.32
2	2	0	2	2	6	31.58
3 to 4	0	0	0	2	2	10.53
≥ 5	1	0	3	2	6	31.58

Table 8. **Tumour Entities and TNM Classification.** CUP cancer of unknown primary, n.k. not known

		Malignancy Type	TNM Classification
Cohort 1	Patient 1	Hypopharynx	IVa-IVb
Dose: 45 mg/m²/day	Patient 2	Oropharynx	IVa-IVb
	Patient 3	Thymus	IVc
Cohort 2	Patient 4	Urinary bladder	n.k.
Dose: 90 mg/m²/day	Patient 5	Gallbladder	IVc
	Patient 6	CUP	n.k.
Cohort 3	Patient 7	Tonsils	IVc
Dose: 150 mg/m²/day	Patient 8	Testis	III
	Patient 9	Ovary	III
	Patient 10	Merkel cell carcinoma	II
	Patient 11	Oral cavity	II
	Patient 12	Cardia	IVa-IVb
Cohort 4	Patient 13	Penis	III
Dose: 200 mg/m³/day	Patient 14	Lung	IVa-IVb
	Patient 15	Testis	III
	Patient 16	Cholangiocarcinoma	IVa-IVb
	Patient 17	Oesophagus	IVa-IVb
	Patient 18	Rectum	III
	Patient 19	Rectum	II

Table 9. **Patients Who Received Prior Etoposide Therapy.** yr years, m month

	Regime		*Best Response and Outcome*	
Patient 3	1st	Cisplatin, *Etoposide*, Ifosfamid	SD	End of treatment, based on histological evaluation
	3rd	Carboplatin, *Etoposide*, Epirubicin, Melphalan	CR	Treatment completed, Relapse: 3yr 7m
	8th	*Etoposide*	SD	Progression of disease
Patient 6	1st	Platinum, *Etoposide*	CR	Treatment completed, Relapse: 1yr 8m
Patient 8	1st	Cisplatin, *Etoposide*, Ifosfamide	SD	Progression of disease
Patient 10	2nd	Carboplatin, *Etoposide*	CR	Treatment completed, Relapse: 6m
Patient 15	1st	Cisplatin, *Etoposide,* Bleomycin	CR	Treatment completed, Relapse: 12yr
	2nd	Cisplatin*, Etoposide*, Ifosfamide	PR	Treatment completed

5.2 Delivered Treatment

The 19 patients were grouped into four different cohorts (cohorts 1-4). Cohorts 1 and 2 consisted of three patients each, cohort 3 of six, and cohort 4 of seven patients. Dose escalation was performed from 45 mg/m² per day in cohort 1, up to the dose of 200 mg/m² per day in cohort 4.

All patients in *cohort 1* received five days of 45 mg/m² per day in each cycle according to planned starting dose of the administration scheme. Of the three patients assigned to cohort 1, two had to discontinue the study for administrative reasons (sufficient amount of study drug beyond two cycles could not be provided continuously at the beginning of the study) and received therefore only four cycles (patient 1) and two cycles (patient 3). Further participation in the study was ended in patient 2 due to progression of the tumour, seen in a CT scan after cycle two. As no major toxicities were observed and dosage was given to all patients in accordance with the scheme in a total of eight cycles, the second cohort started with a dose increment of 45 mg/m²/day.

All patients in *cohort 2* received the planned 90 mg/m² per day in a total of ten cycles. Two patients were withdrawn due to progression of disease. The third patient showed a stable disease after four cycles and the patient was, therefore, withdrawn for medically warranted reasons. No major toxicities were observed, and dosage followed the planned scheme in all ten cycles, therefore cohort 3 was started with an increment of 60 mg/m²/day to a dosage of 150 mg/m²/day.

In *cohort 3* a total of 16 completed cycles were administrated. In three patients the dose was reduced and the cohort was extended from initially three to six patients. A delay of treatment was necessary in a total of six cycles, which occurred in four different patients.
Patient 7 needed a dose reduction from 150 mg/m²/day to 90 mg/m²/day and a delay of treatment of seven days on cycle two due to febrile neutropenia, which occurred 16 days after the first cycle (with 150 mg/m²/day). Patient 8 also received a dose reduction to 90 mg/m²/day in cycles two, three, and four. The reason here was the appearance of severe oral candidiasis ten days after the first treatment. Patient 8 also failed to complete cycle four (the treatment was administered only on the first day) due to reduction of physical status, probably as a result of a urinary tract infection. The dose reduction in patient 9 (also to 90 mg/m²/day) came about as a result of leucopoenia observed seven days after the day of first treatment, which resulted in an additional delay of seven days on second cycle.

Patient 10 had a delay of six days on the second cycle due to neutropenia occurring 15 days after the first treatment. A treatment postponement of seven days (each in cycle two, three, and four) occurred in patient 12. They all were consequences of leucopoenia.
In cohort 3, three patients were withdrawn from the study because of progression of the malignant disease. Two patients had severe adverse events which led to discontinuation of the study; patient 8 showed a reduced physical status, and patient 9 suffered abdominal pain with massive haematemesis due to tumour location complications. One patient was withdrawn because of non-compliant behaviour.

Cohort 4 was started with an increment of 50 mg/m²/day to a total dose of 200 mg/m² per day. All patients in cohort four received the planned 200 mg/m²/day in all 28 administered cycles. Two patients had to be withdrawn from the study after receiving only one cycle of CAP7.1 due to serious adverse events; patient 15 was diagnosed with pulmonary embolism after having been previously hospitalized for reduced general physical status and patient 18 developed a sepsis after febrile neutropenia was observed. Patient 19 had to end the study after scheduled tumour evaluation (after second cycle) which showed a progression of the disease. The other four patients fully completed treatment (six cycles), although one patient (patient 13) could not complete the study entirely due to sudden death before the last follow up at the end of the study. All patients who achieved six cycles had numerous delays on treatment. Patients 16 and 17 both had all cycles delayed by seven days due to leucopoenia, neutropenia, or thrombocytopenia. These also occurred in the other patients of this cohort which delayed treatments, with a maximum delay of 14 days. One patient (patients 13) also suffered from urinary tract infections and anaemia, as a result of which there was a delay in treatment. No further dose escalation was performed because of adverse events and probable reaching of the maximum tolerated dose for this patient's group. Under the study protocol dose escalation was to be discontinued the in case of DLT and on reaching the MTD.
Administered treatment and reasons for discontinuation are summarized in table 10.

The administration time of 60 minutes + 5 minutes was mostly adhered to. In the total of 62 cycles, 13 excessively long (maximum of 1 hour and 50 minutes) and two exessively short administrations (minimum of 45 minutes) were observed. Four administrations were interrupted, with a maximum of 43 minutes.
The Maximum delay of treatment was 14 days on cycle six in patients 13 and 14 (maximum allowed delay under the study protocol is 21 days).

In total, 62 cycles of treatment were completed. The least possible number of completed cycles (only one cycle) occurred in patient 11, who was withdrawn from the study after the first cycle due to non-compliance and patients 15 and 18, who had to withdraw from the study due to severe adverse events (pulmonary embolism and sepsis). The maximum of six cycles, i.e., the full course of treatment, was given to a total of four patients (patients 13, 14, 16 and 17); they were in cohort 4. The total median of completed cycles was three cycles. The most cycles were achieved in cohort 4 (28 cycles), with a median of six cycles.

Median time on treatment was 63 days, with a lower range of five days (in patients 11, 15 and 18) and highest up to 151 days in patient 13.

Table 10. **Treatment Delivered to Each Patient and Reasons For Discontinuation of Study.**

	Patient Number	Cycles Completed	Time of Treatment *[days]*	Dose Applied *[mg]*	Reason for Dose Reduction / Delay of Treatment	Reason for Discontinuation
Cohort 1 Dose= 45 mg/m²/day	1	4	67	cycle 1: 70.2 cycle 2: 70 cycle 3: 70.2 cycle 4: 69.75		no further study drug provided
	2	2	25	cycle 1: 77.85 cycle 2: 77.85		progressive disease
	3	2	25	cycle 1: 85.5 cycle 2: 84.6		no further study drug provided
Cohort 2 Dose= 90 mg/m²/day	4	2	25	cycle 1: 145 cycle 2: 146		progressive disease
	5	4	67	cycle 1: 190 cycle 2: 188 cycle 3: 189 cycle 4: 189		progressive disease
	6	4	67	cycle 1: 174 cycle 2: 172 cycle 3: 173 cycle 4: 174		removal medically warranted, because of stable disease
Cohort 3 Dose= 150 mg/m²/day	7	2	32	cycle 1: 215 cycle 2×: 130*	Febrile Neutropenia	progressive disease
	8	3	63	cycle 1: 312.6 cycle 2: 186* cycle 3: 184* cycle 4°: 187*	Candidiasis	SAE
	9	2	32	cycle 1: 219 cycle 2×: 127*	Leucopoenia	SAE
	10	4	73	cycle 1: 304 cycle 2×: 304 cycle 3: 304.5 cycle 4: 304.5	Neutropenia	progressive disease

	11	1	5	cycle 1: 220		patient non-compliant
	12	4	88	cycle 1: 281 cycle 2×: 279 cycle 3×: 278 cycle 4×: 273	Leucopoenia	progressive disease
Cohort 4 **Dose= 200 mg/m²/day**	13	6	151	cycle 1: 396 cycle 2×: 288 cycle 3×: 370 cycle 4×: 370 cycle 5×: 376 cycle 6×: 376	Anaemia, Leucopoenia, Thrombocytopenia, UTI	treatment completed
	14	6	130	cycle 1: 400 cycle 2×: 396 cycle 3: 394 cycle 4×: 394 cycle 5: 392 cycle 6: 394	Neutropenia, Thrombocytopenia, Leucopoenia	treatment completed
	15	1	5	cycle 1: 400		SAE
	16	6	144	cycle 1: 424 cycle 2×: 418 cycle 3×: 424 cycle 4×: 428 cycle 5×: 416 cycle 6×: 420	Neutropenia, Thrombocytopenia, Leucopoenia	treatment completed
	17	6	144	cycle 1: 380 cycle 2×: 376 cycle 3×: 378 cycle 4×: 376 cycle 5×: 370 cycle 6×: 370	Neutropenia, Thrombocytopenia, Leucopoenia	treatment completed
	18	1	5	cycle 1: 396		SAE
	19	2	25	cycle 1: 390 cycle 2: 386		progressive disease
Median		**3**	**63**			

* Dose reduction to 90 mg/m²/day, × Delay of treatment, ° Cycle not completed

5.3 Efficacy

In this phase I trial, efficacy according to RECIST criteria and ECOG performance were analysed. Therefore, targeted lesions and non-targeted lesions are evaluated at first. Subsequently, tumour overall response is assessed and the development of ECOG performance is summarized.

5.3.1 Evaluation of Targeted Lesions

Tumour evaluation of targeted lesions was performed every second cycle and additionally in a follow-up examination 26 days after last treatment with CAP7.1. Targeted lesions were selected on screening CT according to RECIST 1.0 criteria. Overall, 17 patients were available for measurement of tumour response.

Out of the 19 patients, the *first tumour evaluation* (CT scan after the second cycle) was only performed in 16 patients. Patient 11 (cohort 3) was non-compliant. Patients 15 and 18 (cohort 4) suffered a severe adverse event (pulmonary embolism and sepsis) and therefore, received only one cycle of CAP7.1 administration, therefore no additional CT scan was scheduled.
No partial or complete response was observed in the first CT scan evaluation after the second cycle. According to RECIST criteria, the disease was stable in eleven patients and five patients showed a progression of their targeted tumour lesion of more than 20 %. In cohorts 1 and 2, in two out of three cases, the disease was stabilized and one third displayed a progression of the disease. In cohort 3, three out of six patients showed a stable disease, whereas two developed a progression of their lesions. Four stable diseases and one progressive disease were determined in cohort 4 (table 11).
As minor response is also analysed, figure 8 shows the development of targeted lesions after two cycles of CAP7.1 administration in relation to the performed baseline CT.
Overall, six out of the 16 patients showed tumour regression after the second cycle. Among all the patients achieving SD, six patients showed tumour reduction, whereas five showed tumour enlargement.
Best minor response with a decrease of targeted lesions of 14 % was seen in a 66-year-old patient suffering from oesophageal cancer (patient 17), treated with a dosage of 200 mg/m²/kg of CAP7.1 in cohort 4. The squamous oesophageal cancer was first diagnosed two years before entering the study and had been treated with three chemotherapy regimes, radiation therapy, and

surgery prior to study medication. Over the years, multiple metastases occurred, and the carcinoma was recurrent three months before entering the study. On date of entry, the tumour was classified as TNM IVa-IVb. Three additional patients (patients 8, 9, 13) had a decrease of around 10 % (+ 3 %) and suffered from recurrent testicular (mixed seminiom/non-seminiom), recurrent and metastatic adenous ovarian cancer and metastatic penis carcinoma. All were assessed as TNM stage III. These three were pre-treated, among them a 32-year-old patient who was diagnosed with testicular cancer and had a prior chemotherapy regime which included etoposide. Two of these patients were treated with dosages of cohort 3 (with dose reductions to level of cohort 2) and the patient who suffered from penis carcinoma received dosages of cohort 4.

An increase of sum diameter of targeted lesions of more than 30 % was determined in three patients (who were patients 4, 10, 19) diagnosed with bladder cancer, Merkel-cell carcinoma, and rectal carcinoma. They were treated in cohorts 2, 3, and 4. The maximum increase of 43 % occurred in a 64-year-old patient (patient 4) who suffered from an urothelial cell carcinoma with metastases in lung and liver.

Cohort	SD	PD
1	2 *(67%)*	1 *(33%)*
2	2 *(67%)*	1 *(33%)*
3	3 *(50%)*	2 *(33%)*
4	4 *(57%)*	1 *(14%)*
Total	11 *(69%)*	5 *(31%)*

Table 11. **First Tumour Evaluation After Second Cycle.**

The *second tumour evaluation* was scheduled after the fourth cycle of administration of CAP7.1. Out of the 16 patients who were available for evaluation after the second cycle, nine patients received the scheduled CT scan after the fourth cycle. Four patients were withdrawn from study after having had a progressive disease after the second cycle tumour evaluation (patients 2, 4, 7, 19). One patient (patient 3) discontinued after two cycles of administration due to administrative reasons (the study drug could not be provided) and was therefore not scheduled for a CT scan. Furthermore, two patients (patients 8 and 9) suffered from serious adverse events during the following administrations and, therefore, did not reach the scheduled time for the CT evaluation. According to RECIST criteria, the disease in six of the nine remaining patients achieved a stable status, and three patients showed progression of the disease. In cohorts 1 to 3, two cases of stable

disease and three progressive diseases were observed. All remaining patients in cohort 4 still showed stable disease (table 12).

In analyzed minor response (figure 9), highest decrease of sum of longest diameters was measured in the 66-year-old patient who suffered from oesophageal cancer as mentioned above (patient 17) and who achieved a decrease of 16 % (in relation to baseline sum of longest diameters). A decrease of 12 % was measured in a 50-year-old patient diagnosed with penis carcinoma (patient 13), who was pre-treated with one regime of chemotherapy, radiation, and surgery.

Unequivocal tumour enlargement of over 40 % was measured in two patients (patient 5 and 12), who suffered from adenosquamous gallbladder and adenous oesophageal cancer. For patient 10, no actual CT data could be supplied, but an obvious tumour progression was described by the investigator.

Cohort	SD	PD
1	1	0
2	1	1
3	0	2
4	4	0
Total	6	3

Table 12. **Second Tumour Evaluation After Fourth Cycle.**

Follow-up CT scans were only performed in five patients. Two patients still showed a stable disease, with a maximum shrinkage of 2 % in targeted lesions. One patient (patient 14) suffered from recurrent adenosquamous lung cancer with multiple spinal and occipital metastases, with TNM IVa-IVb. The other patient (patient 17) was the 66-year-old man diagnosed with oesophageal cancer. The other three patients all showed a progression of their targeted lesions with a minimum of 25 % and a maximum of 43% increase in sum of longest diameters (figure 10).

An *unscheduled CT scan* after the first cycle was performed in patient 10, who had histological confirmed Merkel-cell carcinoma. The 69-year-old woman was pre-treated with etoposide and received a dosage of 150 mg/m²/day of CAP7.1 (cohort 3). The CT scan was performed after one cycle of treatment and showed a reduction of targeted lesions of 42 %, which means partial response according to RECIST criteria. The partial response could not be confirmed in the

scheduled CT a month later (after second cycle of treatment), and targeted lesions had an increment of 34 %.

Overall (regardless of cycles or dosages applied), eight patients showed a shrinkage of targeted tumour lesions as best response, and nine patients had an enlargement of targeted lesions as best response achieved during the whole study. As visualized in figure 11, tumour shrinkage occurred predominantly in patients of cohorts 3 and 4.

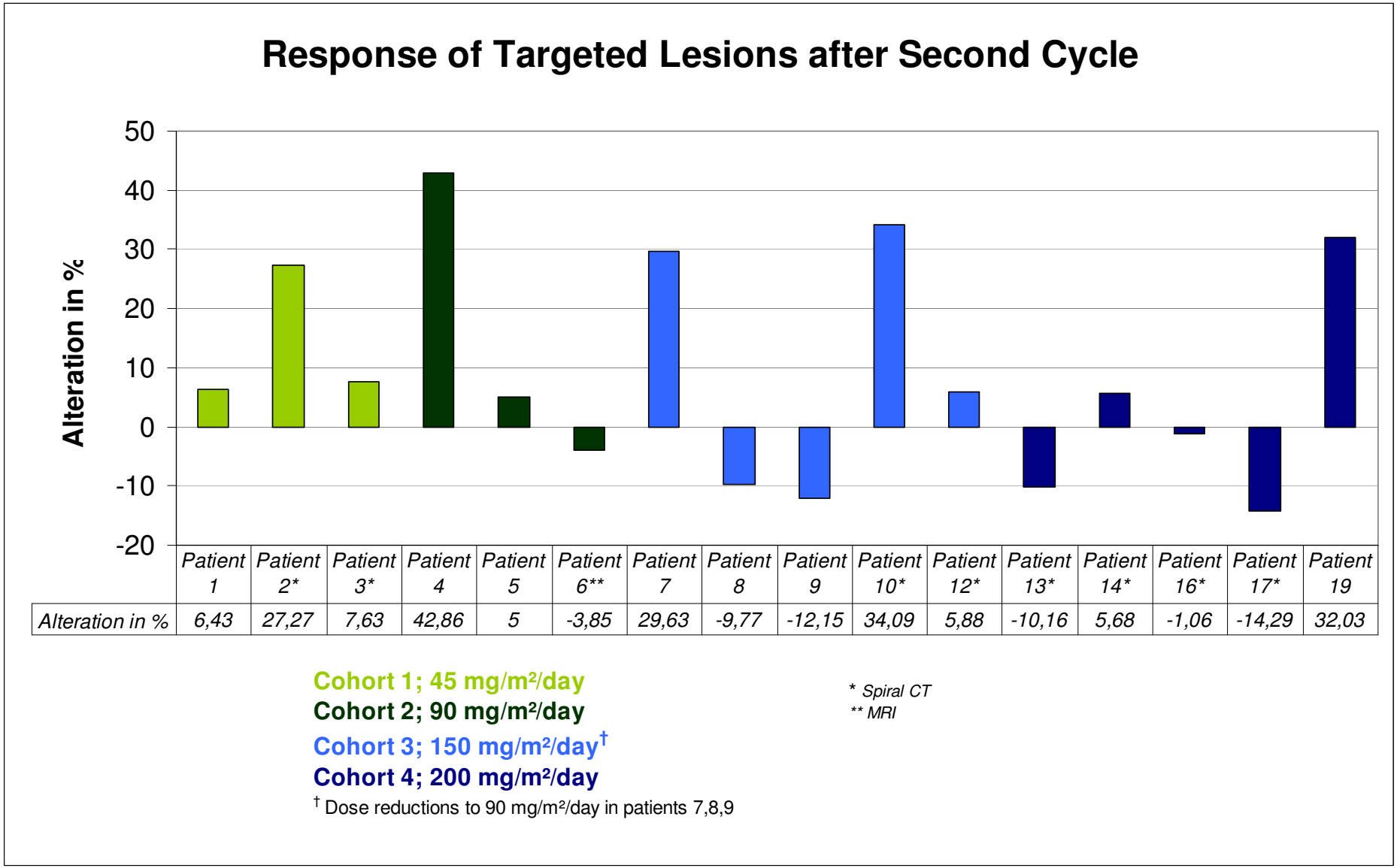

Figure 8. **Response of Targeted Lesions After Second Cycle in Relation to Baseline Sum of Longest Diameters Measured.**

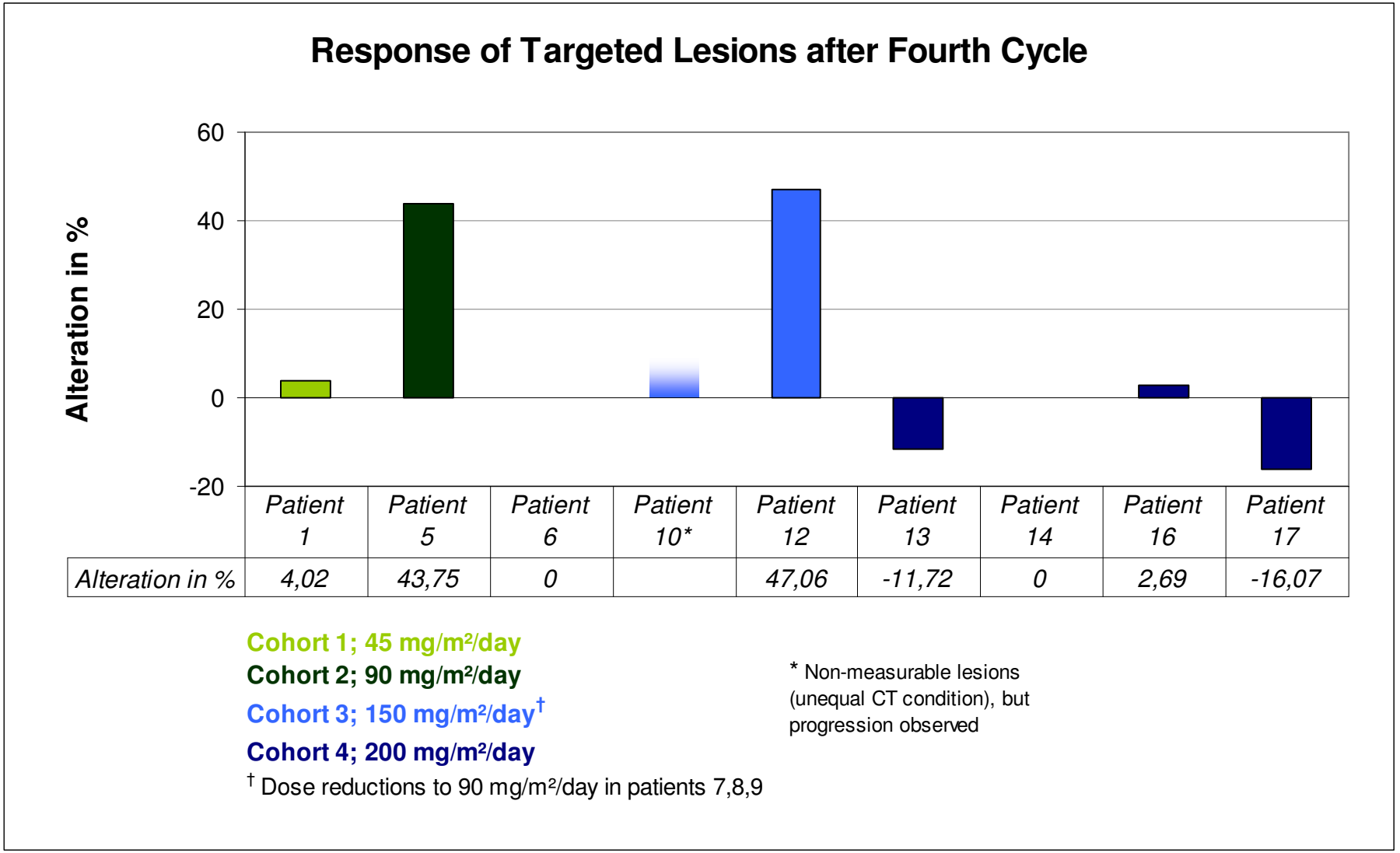

Figure 9. **Response of Targeted Lesions After Fourth Cycle in Relation to Baseline Sum or Smallest Sum of Diameter Measured.**

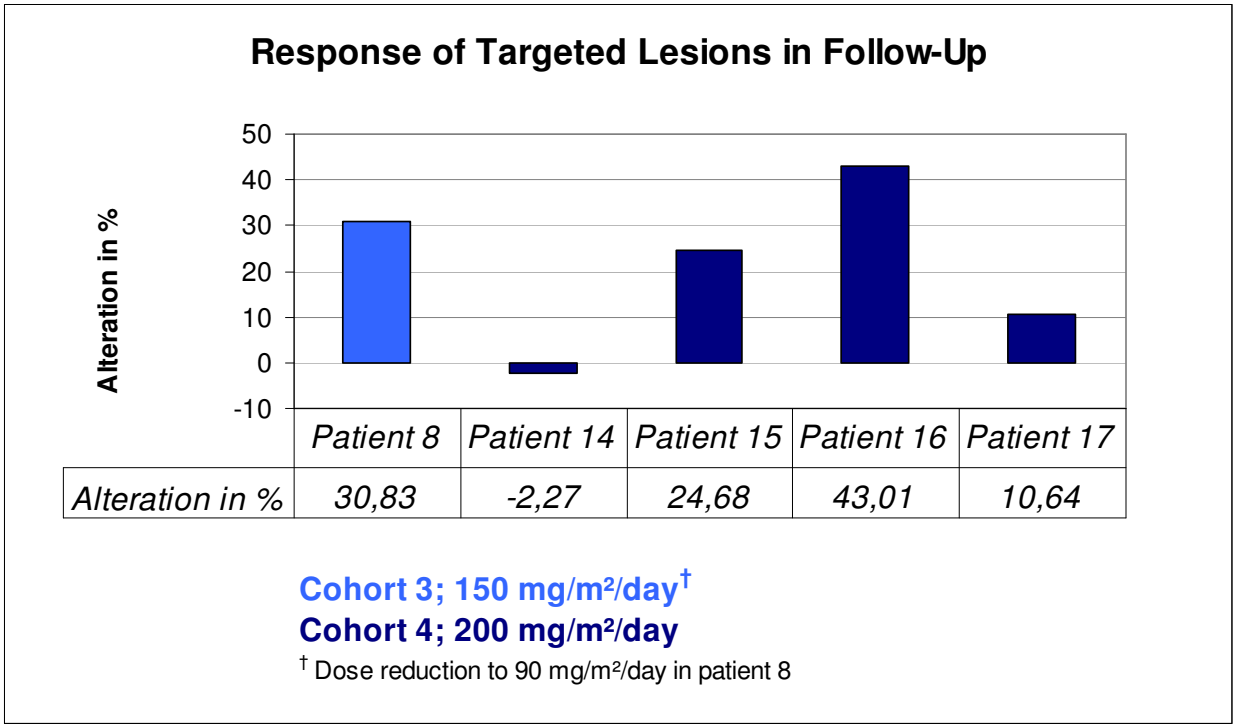

Figure 10. **Response of Targeted Lesions in Follow Up CT Scans in Relation to Baseline Sum or Smallest Sum of Diameter Measured.**

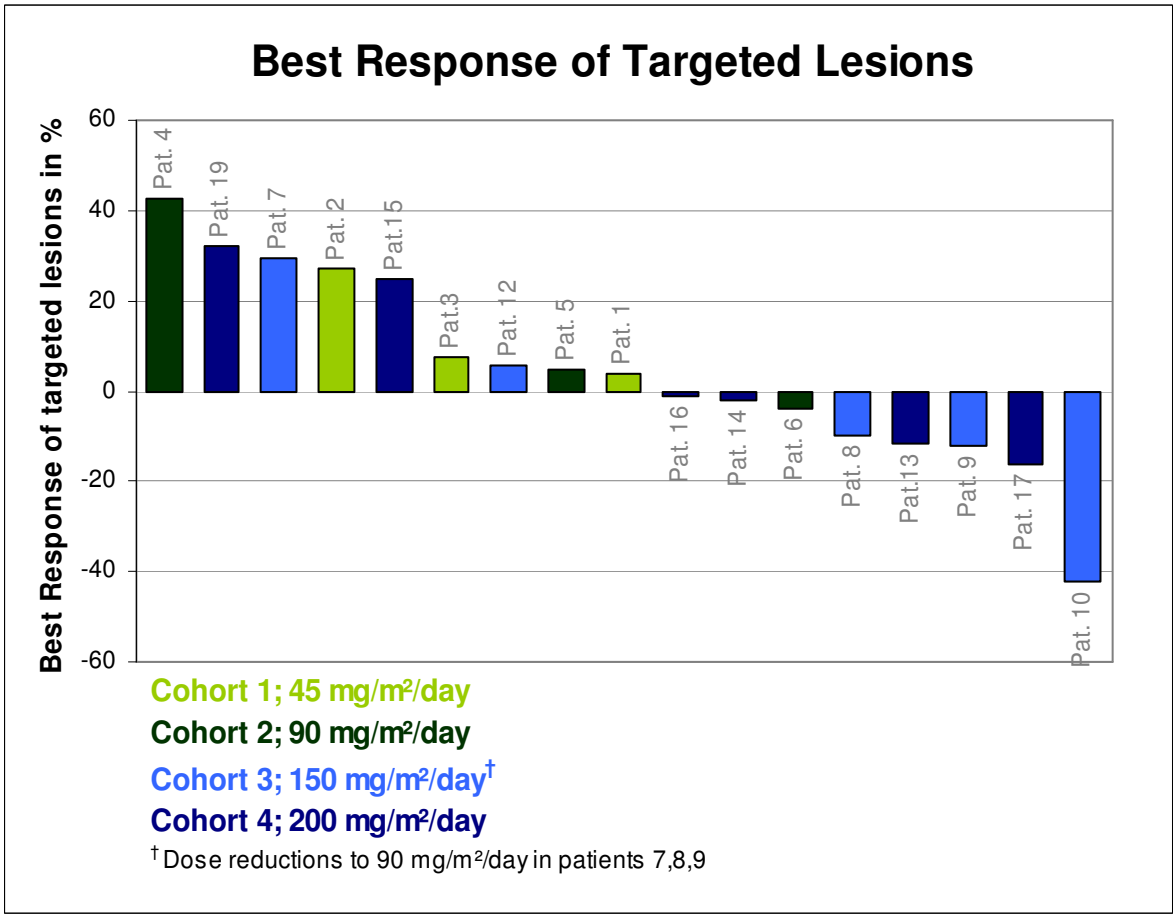

Figure 11. **Waterfall Plot of the Best Response of Targeted Lesions Achieved During Whole Study by Patient.**
Pat. patient

5.3.2 Evaluation of Non-Targeted Lesions

Non-targeted lesions were documented in eleven of nineteen patients. Non-targeted lesions were analyzed according to RECIST criteria. Most non-targets described were simple lesions (often multiple metastases). Three patients had diagnosed pleural effusions, and one patient suffered from ascites. Except for three non-targeted multiple lesions, all other lesions were stable during tumour assessment. Progression of lesions occurred in three patients who had urinary bladder carcinoma (patient 4 after second cycle, cohort 2), tonsil carcinoma (patient 7 after second cycle, cohort 3), and gallbladder cancer (patient 5 after fourth cycle, cohort 2). Follow up assessment could not be evaluated due to non-applicable documentation. Development of non-targeted lesion of every patient is further discussed in the latter part in combination with targeted lesions and new lesions, when overall tumour response is analyzed.

Table 13. **Evaluation of Non-Targeted Lesions.**

	Evaluation after 2nd Cycle		Evaluation after 4th Cycle	
	SD	PD	SD	PD
Multiple Lesions	7	2	6	1
Pleural Effusion	3	-	1	-
Ascites	1	-	-	-

5.3.3 Overall Tumour Response

The overall tumour response depends, according to RECIST criteria, on the development of targeted lesions, non-targeted lesions and the appearance of new lesions. Table 14 shows the overall tumour response of each assessment performed over the duration of the study. The overall tumour response after ending or withdrawal from the study is also assessed and identified as the final tumour response, while the actual time on study, time on treatment, or number of performed CT scans is not considered. Best overall tumour response obtained is also analyzed separately. Of the 19 patients who participated in this study, 17 were available for at least one follow-up CT scan for tumour assessment. One patient (patient 11) was non-compliant, the other patient (patient 18) had suffered a serious adverse event; therefore, no further CT scans (other than baseline scan) were performed in these two patients.

Best Overall Response

The best response observed differs from the overall tumour response, as one patient had a major response (partial response, 6 %), a majority of eleven patients had stable diseases (65 %) and five patients had progressive diseases (29 %). In each cohort, more stable diseases as the best response achieved were observed than progressive diseases. Best response rate with one partial response, three stable diseases, and only one progressive disease were determined in cohort 3. Cohorts 1 and 2 had the same response rate with a ratio of 2:1 of SD:PD. In cohort 4, two times more stable diseases than progressive diseases were observed. The best response during the whole study was the partial response observed in an unscheduled CT in patient 10.

Final Tumour Overall Response

In this phase I study, seven patients (41 %) achieved a stable disease, and ten patients (59 %) suffered from progression of their disease as final overall tumour response. No major response (partial response or complete response) could be achieved as final tumour overall response. Most progressive diseases were observed in cohort 3, with four times more progressive diseases than stable diseases and most stable diseases were observed in cohort 4, with three patients who achieved stable disease, which was 50 % of the cohort.

In the following part, the single observed partial response is discussed in detail first, followed by a more detailed view on the achieved stable diseases. Next, characteristics of patients with progression of their cancer disease are analyzed. At the end of this section, overall response time is evaluated, followed by table 25, which summarizes all analyzed information for efficacy.

Table 14. **Overall Tumour Response after Each Cycle, Final Overall Tumour Response and Best Overall Response.**

Patient	*Tumour Assessment after Cycle No.*	*Targeted Lesion Response*	*Non-Targeted Lesions Response*	*New Lesions*	*Cycle-dependent Tumour Response*	*Final Overall Tumour Response*	*Best Overall Response*
1	2nd 4th	SD SD	SD SD	No No	SD SD	Stable Disease	Stable Disease
2	2nd	PD	-	Yes	PD	Progressive Disease	Progressive Disease
3	2nd	SD	-	No	SD	Stable Disease	Stable Disease
4	2nd	PD	PD	Yes	PD	Progressive Disease	Progressive Disease
5	2nd 4th	SD PD	SD PD	No Yes	SD PD	Progressive Disease	Stable Disease
6	2nd 4th	SD SD	SD SD	No No	SD SD	Stable Disease	Stable Disease
7	2nd	PD	PD	No	PD	Progressive Disease	Progressive Disease
8	2nd follow up	SD PD	- -	No Yes	SD PD	Progressive Disease	Stable Disease
9	2nd	SD	SD	No	SD	Stable Disease	Stable Disease
10	unscheduled 2nd 4th	PR PD PD	- - -	No No Yes	PR PD PD	Progressive Disease	Partial Response
11	Patient non-compliant						
12	2nd 4th	SD PD	- -	No No	SD PD	Progressive Disease	Stable Disease
13	2nd 4th	SD SD	SD SD	No No	SD SD	Stable Disease*	Stable Disease*
14	2nd 4th follow up	SD SD SD	SD SD SD	No No No	SD SD SD	Stable Disease*	Stable Disease*
15	follow up	PD	-	Yes	PD	Progressive Disease	Progressive Disease

16	2nd 4th follow up	SD SD PD	SD SD N/A	No No No	SD SD* PD	Progressive Disease	Stable Disease*
17	2nd 4th follow up	SD SD SD	SD SD N/A	No No No	SD SD SD	Stable Disease*	Stable Disease*
18	Discontinuation due to SAE						
19	2nd	PD	N/A	No	PD	Progressive Disease	Progressive Disease
** confirmed stable disease (at least 8 weeks interval in between CTs showing SD), N/A not applicable, SAE serious adverse event*							

Table 15. **Summary of Overall Response and Best Overall Response.**

Cohort	Final Overall Response		Best Overall Response	
1	SD PD	2 1	SD PD	2 1
2	SD PD	1 2	SD PD	2 1
3	SD PD	1 4	PR SD PD	1 3 1
4	SD* PD	3 3	SD* PD	4 2
Total *(n=17)*	**SD** **PD**	**7** **10**	**PR** **SD** **PD**	**1** **11** **5**
** confirmed stable disease*				

The one *partial response* achieved in patient 10 was measured in an unscheduled CT scan after the first cycle of 150 mg/m²/day CAP7.1 administration. The 69-year-old woman suffered from Merkel-cell carcinoma on her left arm, with a TNM II classification. The tumour had distant metastases and was recurrent. The cancer disease was diagnosed a year before the patient entered the study, and the patient received two prior chemotherapy regimes, which included a treatment with etoposide. Radiation therapy and various surgeries had been performed, but the carcinoma was recurrent. The patient had a positive cancer history with a mamma carcinoma diagnosed and successfully treated five years before the current cancer diagnosis. The unscheduled spiral CT showed a regression of targeted lesions of 42 %. No non-targeted lesions or new lesions were reported. The next scheduled CT (after second cycle), was performed 32 days later and showed an increase of targeted lesions of 34 %. A further CT scan about one month later showed even new appeared lesions, which led to a in a final overall response of a progressive disease.

Stable diseases were observed in eleven patients (64.7 %) as best response. All patients were exhaustively pre-treated: Seven patients had a history of prior chemotherapy, radiation therapy, and surgery performed for tumour treatment. Two patients received chemotherapy and surgical intervention, and two had just undergone chemotherapy (two and nine regimes). Tumour types had a wide range which included abdominal cancer, genital carcinomas, head and neck cancers and pulmonary cancer (table 16). Of these four, SD could be confirmed, which means, that the interval between the CT scan when SD was first documented and the second CT showing SD, was at least eight weeks. All patients with confirmed stable diseases were treated with dosages of cohort 4, whilst the tumour entities varied (table 17).

Table 16. **Characteristics of Patients with SD.**

Cohort	Total Number of SD	Percentage of Cohort	Malignancy Type
1	2	67 %	Hypopharynx Thymus
2	2	67 %	Gallbladder CUP
3	3	60 %	Testicular Ovary Oesophagus
4	4	67 %	Penis Lung Cholangiocarcinoma Oesophagus
Total	11	65 %	

Table 17. **Characteristics of Patients with Confirmed Stable Disease.** CT Chemotherapy, RT Radiation therapy

Patient	*Tumour Location*	*TNM*	*Histological Analyses*	*Prior Treatment*	*Cohort*
13	Penis	III	not known	CT (1 regime) Surgery RT	4
14	Lung	IVa-IVb	Adenosquamous	CT (3 regimes) Surgery RT (2)	4
16	Cholangiocarcinoma	IVa-IVb	Adenosquamous	CT (2 regimes)	4
17	Oesophagus	IVa-IVb	Squamous	CT (3 regimes) Surgery RT	4

Duration of SD (time of first treatment until PD or death of any cause) varied from a minimum of one month and five days to a maximum of six month and seven days. The achieved SDs lasted a median time of three month and nine days. It has to be considered that various durations are only the minimum durations of SD in each patient due to the fact that in most cases CT scans were not forwarded to the investigator after the end of the study. In these cases, the last CT scan which showed an SD was considered as minimum duration of the SD. An SD over three months was observed in six patients, of which four patients were in cohort 4. Five patients had an SD for less than three months, of which two patients had an SD that lasted less than two month. None were in cohort 4. Tumour types varied. Only oesophageal cancer occurred twice within an SD duration over three months.

Table 18. **Duration of SD > 3 Month.** min. minimum

Patient	*Malignancy Type*	*Month*	*Days*	*Cohort*
1	Hypopharynx	6	7	1
12	Oesophagus	3	6	3
13	Penis	5	12	4
14	Lung	min. 4	20	
16	Cholangiocarcinoma	5	1	
17	Oesophagus	min. 5	9	

Table 19. **Duration of SD < 3 Month.** min. minimum

Patient	*Malignancy Type*	*Month*	*Days*	*Cohort*
3	Thymus	min. 1	5	1
5	Gallbladder	2	16	2
6	CUP	min. 2	9	
8	Testis	2	29	3
9	Ovary	min. 1	14	

The total number of *progressive diseases* (in final response) was ten patients, which accounts for 59 % of all patients available for tumour assessment. Progression of disease as evidenced by the appearance of new lesions was observed in 60 % (six patients) of these ten patients. No new lesions but an increase of tumour burden, to fulfil RECIST criteria for progressive disease, were seen in 40 % of these ten patients. No relation to either tumour type, TNM classification, histological classification or dosage of CAP7.1 could be seen. Only five patients had progressive disease as best response achieved and showed no better response in prior assessments. Nor could an obvious cluster regarding tumour type, TNM classification, histological classification or dosage of CAP7.1 be found in this patient-group (details see table 20).

Table 20. **Characteristics of Patients with PD as Best Response.**

Patient	*Tumour Location*	*TNM*	*Histological Analyses*	*Cohort*
2	Oesophagus	IV	Squamous	1
4	Urinary Bladder	not known	Urothelial cells	2
7	Tonsils	IV	Squamous	3
15	Testis	III	Sertoli-Leydig cells	4
19	Rectum	II	adenosquamous	4

Progression free survival is defined as the duration of enrolment in the study until documented progression or death of any cause. Eleven patients had a documented progressive disease. One additional patient with stable disease died suddenly before the scheduled end of the study. Five patients still had ongoing SD; therefore, exact dates of possible PD after the study could not be provided. One patient was non-compliant, and a second one suffered from SAE, which led to exclusion from follow up assessment. Maximum progression free survival was observed in Patient 16, who suffered from recurrent cholangiocarcinoma of the liver and had a PD over six months after entering the study. The 48-year-old patient was treated with the highest dosage of CAP7.1 in cohort 4 and completed the treatment with six cycles. Three additional patients had progression free survivals of about five to six months. They suffered from the following cancer types: metastasised hypopharynx, metastasised penis and metastasised recurrent oesophageal cancer.

Table 21. **Progression Free Survival and Patient Details.** *SD still ongoing when end of study.

Patient	*Tumour Location*	*Best Response*	*Month*	*Days*	*Cohort*
1	Hypopharynx	SD	6	9	1
2	Oropharynx	PD	1	14	
4	Urinary bladder	PD	1	8	2
5	Gallbladder	SD	2	22	
7	Tonsils	PD	1	7	3
8	Testis	SD	3	9	
10	Merkel cell carcinoma	PR	1	15	
12	Oesophagus	SD	3	11	
13	Penis	SD	5	25	4
15	Testis	PD	0	27	
16	Cholangiocarcinoma	SD	6	11	
19	Rectum	PD	2	1	
3*	Thymus	SD	min. 1	5	1
6*	CUP	SD	min. 2	9	2
9*	Ovary	SD	min. 1	14	3
14*	Lung	SD	min. 4	20	4
17*	Oesophagus	SD	min. 5	9	

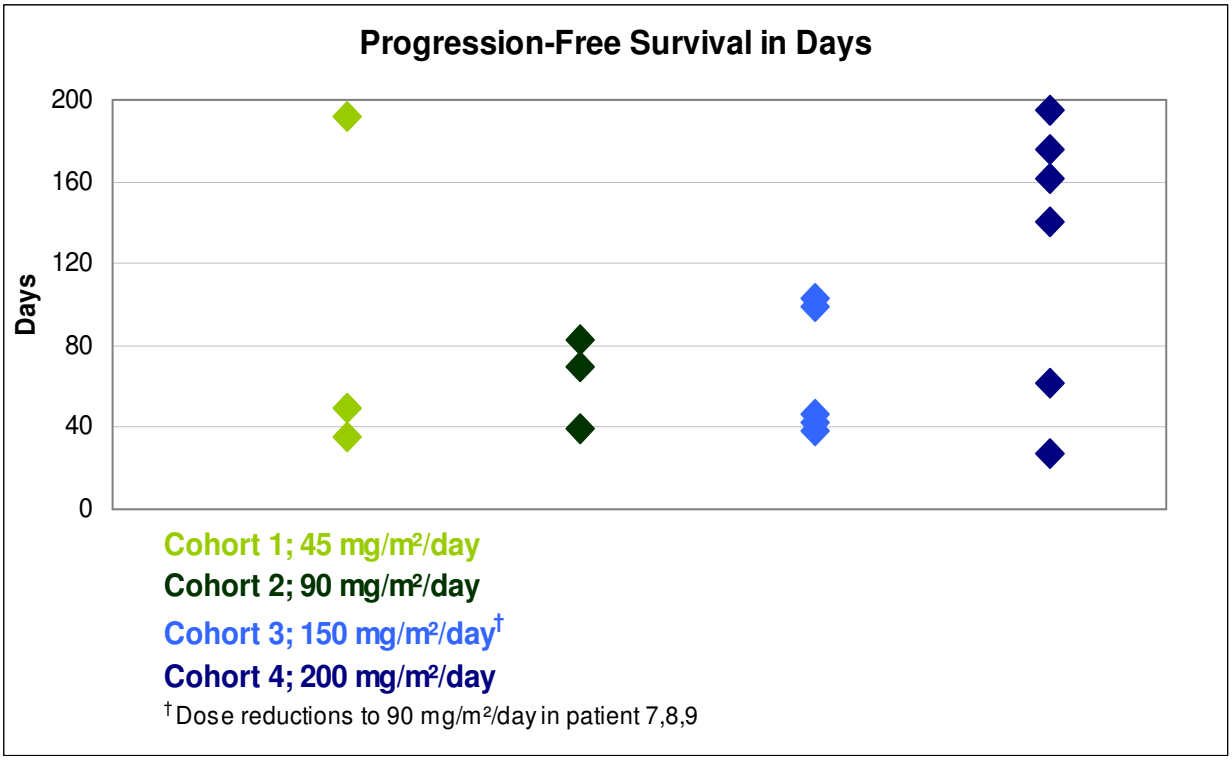

Figure 12. **Progression Free Survival in Days in the Different Treatment Cohorts.**

Overall survival is defined as the time from entering the study until death or date of last patient contact. Out of 19 patients, the actual date of death is known in six patients. Of three patients the last known status was admission to a hospice. The dates of last patient contact in all other patients are based on documented additional therapies (chemotherapy or surgery) or hospital admissions for other reasons. One patient was lost to follow up. Figure 13 visualises overall survival of the study population.

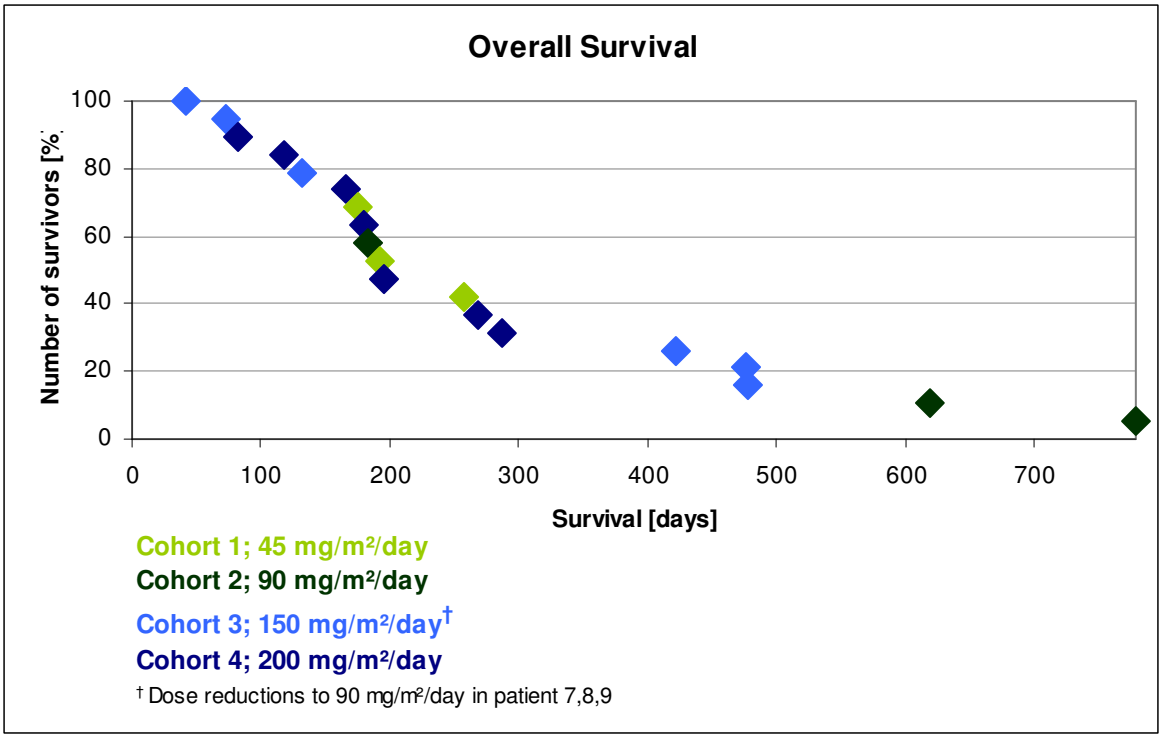

Figure 13. **Overall Survival.**

Longest overall survival (25 months and 19 days) was seen in patient 5, who suffered from gallbladder carcinoma. The 64-year-old man was diagnosed with adenosquamous carcinoma of the gallbladder a year before he entered the study. At the time of inclusion in this CAP7.1 trial, the cancer had distant metastases and was recurrent after prior chemotherapy (gemcitabine) and prior surgery. He was treated in cohort 2 and received four cycles of 90 mg/m²/day CAP7.1. Best response was SD, which had the duration of two months and 16 days. The patients had to discontinue the study after they were assessed as having PD on scheduled CT scan after the fourth cycle. An additional chemotherapy was administered one year and three months after discontinuance of the CAP7.1 trial. The last patients contact was dated 25 months and 19 days after the entry into the study. Further information could not be assessed.

A second patient survived over 20 months and suffered from CUP, with the histology subtype of squamous cell carcinoma of the lung. The 49-year-old patient was also treated in cohort 2 and received four complete cycles of CAP7.1. Overall response was SD with a duration of at least

two months and nine days. Prior chemotherapy regime included a treatment with etoposide and platinum (besides radiation therapy and surgery). Last contact was due to subsequent radiation therapy and surgical extirpation of lymph nodes a year and five months after the discontinuation of the CAP7.1-trial.

In general, 53 % (10 patients) achieved an overall survival of more than six months (table 22). Six patients (32%) could be monitored for three to six months (table 23). Only three patients reached less than three months. This included one patient who was lost for any follow up due to non-compliance. He was, therefore, only monitored for about one month (table 24). Median overall survival was 6 months and 14 days.

Table 22. **Characteristics of Patients With Overall Survival > 6 Months.** † known date of death

Patient	*Malignancy Type*	*Best Response*	*Month*	*Days*	*Cohort*
1 †	Hypopharynx	SD	6	9	1
2	Oropharynx	PD	8	12	
5	Gallbladder	SD	25	19	2
6	CUP	SD	20	12	
8 †	Testis	SD	15	22	3
10 †	Merkel cell carcinoma	PR	13	26	
12	Oesophagus	SD	15	21	
14 †	Lung	SD	9	14	4
15	Testis	PD	8	23	
16	Cholangiocarcinoma	SD	6	11	

Table 23. **Characteristics of Patients With Overall Survival 3-6 Months.** † known date of death

Patient	*Malignancy Type*	*Best Response*	*Month*	*Days*	*Cohort*
3	Thymus	SD	5	13	1
4	Urinary bladder	PD	5	30	2
9 †	Ovary	SD	4	12	3
13 †	Penis	SD	5	25	4
17	Oesophagus	SD	5	27	
18	Rectum	-	3	26	

Table 24. **Characteristics of Patients With Overall Survival < 3 Months**. * patient non-compliant

Patient	*Malignancy Type*	*Best Response*	*Month*	*Days*	*Cohort*
7	Tonsils	PD	2	14	1
11*	Oral cavity	-	1	12	3
19	Rectum	PD	2	21	4

Table 25. **Summary of Efficacy Data.** m month(s), d day(s),* dose reduction in patient 7, 8, 9 to 90 mg/m²/d

Patient	*Malignancy Type*	*Cohort*	*Completed Cycles*	*Best Overall Response*	*Duration of Response*	*Overall Survival*
1	Hypopharynx	1 45 mg/m²/d	4	SD	6 m 7 d	6 m 9 d
2	Oropharynx		2	PD	-	8 m 12 d
3	Thymus		2	SD	1 m 5 d	5 m 13 d
4	Urinary bladder	2 90 mg/m²/d	2	PD	-	5 m 30 d
5	Gallbladder		4	SD	2 m 16 d	25 m 19 d
6	CUP		4	SD	2 m 9 d	20 m 12 d
7	Tonsils	3* 150 mg/m²/d	2	PD	-	2 m 14 d
8	Testis		3	SD	2 m 29 d	15 m 22 d
9	Ovary		2	SD	1 m 14 d	4 m 12 d
10	Merkel cell carcinoma		4	PR	0 m 32 d	13 m 26 d
11	Oral cavity		1	-	-	1 m 12 d
12	Oesophagus		4	SD	3 m 6 d	15 m 21 d
13	Penis	4 200 mg/m²/d	6	SD	5 m 12 d	5 m 25 d
14	Lung		6	SD	4 m 20 d	9 m 14 d
15	Testis		1	PD	-	8 m 23 d
16	Cholangiocarcinoma		6	SD	5 m 1 d	6 m 11 d
17	Oesophagus		6	SD	5 m 9 d	5 m 27 d
18	Rectum		1	-	-	3 m 26 d
19	Rectum		2	PD	-	2 m 21 d

5.4 Evaluation of ECOG Performance

To evaluate not only tumour response but also general well being, ECOG performance scale is also analyzed by comparison of scores before and after treatment (follow up assessment). Before treatment, all patients had good ECOG performance scores (ECOG 0-2) with the majority (79 %) achieving the ECOG performance score of 1. At follow up assessments only 15 patients were available. Most patients had ECOG scores of 1 (42 % of all 19 patients). 21 % had ECOG performance scores of 2, i.e., good ECOG performance scores (ECOG 0-2) were still seen in the majority of patients. Deterioration of scores on follow up was always only one level below ECOG performance score before treatment. Exceptions were patients with poor ECOG performance scores of 4 and 5, which were determined in two patients. One patient suffered from tonsil carcinoma. His ECOG score dropped from 1 to 4 and he suffered adverse events and PD. One patient died suddenly, which was possibly related to the study drug. In general, ECOG performance appeared to deteriorate over the period of treatment.

6 Discussion

This phase I trial was the first clinical trial which was conducted with the new etoposide prodrug CAP7.1 in adults. Preclinical in vitro and in vivo experiments showed a significant increase in cytotoxicity in various cancer cell lines (compared to its mother compound etoposide) and anti-tumour efficacy in tumour xenograft mouse models. CAP7.1 was administered in a companionate trial in three children who were suffering from neuroblastoma and who showed astonishingly good results in two patients (PR and SD, unpublished report Gaedicke et al.). This promted further clinical investigations.

In this phase I trial, CAP7.1 was administered to adult patients with refractory malignancies on five consecutive days as a 60-minute intravenous infusion, which was repeated every 21 days up to a maximum of six cycles. This trial was an open-label, non-randomised, and dose escalating study.

Primary objectives were to determine the maximum tolerated dose, toxicity profile, and the dosing for future phase II trials. Secondary objectives include pharmacokinetic analyses and preliminary assessment of anti-tumour activity.

Although a phase I study serves mainly (primary objectives) as base for safety analysis, the efficacy results are a very important part of phase I studies nowadays.

As this is a new compound, and was tested in this phase I trial first within adult human patients, it was not possible to continue further clinical phase II studies without any positive efficacy outcome in phase I.

This phase I study conducts a prodrug of a well known drug; etoposide. Safety aspects of etoposide are well known and were analyzed in different studies and observed during years of clinical application. CAP7.1 was testes in children in very high dosages and the side effects were limited to the bone marrow only as one would expect with etoposide at low doses. Therefore side effects of CAP7.1 were suspected to be alike the ones of etoposide, but in different dosage-equivalents. Because the drug has a suspected CES II related efficacy it is therefore very important to have a close view on efficacy data and not only safety aspects. As side effects were expected to be like the ones of etoposide, efficacy data was expected to show different results from etoposide due to its chemical structure which acts as a prodrug of etoposide and is therefore suspected to show results in different tumour entities. The analysis of efficacy therefore plays, in this study, a very important rule, as the active drug itself is no new compound and therefore needs to show its advantages to etoposide to justify any further testing.

As pharmaceutical studies (as this phase I trial) are sponsored studies they can only be carried out with prove of efficacy in phase I. As one can see in the part of this thesis where CAP7.1 is compared to other phase I studies efficacy outcome is a very often analyzed aspect of a phase I study and is even published in a more detailed way in the more recent studies compared to older phase I studies.

The efficacy outcome in phase I was for this particular study one of the decisive factors for further clinical testing in phase II and crucial for the further development and further investment into the drug in the pharmaceutical company.

In this paper, results of efficacy of this phase I CAP7.1 trial are discussed. CAP7.1 is compared to other recently developed chemotherapeutics of different classes for an initial impression of its clinical performance. Parts of this paper were presented at the 24th EORTC-NCI-AARC Symposium on Molecular Targets and Cancer Therapeutics in November 2012.[86]

6.1 Key Findings and Consideration of Possible Explanations

In this phase I trial, 19 patients with refractory malignancies were treated in four different cohorts with CAP7.1. All patients had been pre-treated with at least one chemotherapy regime, and a majority had received prior radiation therapy and/or surgery. In some cases, patients had received multiple prior regimes of chemotherapy, which had consisted of up to nine combinations of chemotherapeutics. Eligible patients showed a wide range of solid tumour entities. All patients included had progressed or relapsed tumours. Of the 19 participating patients, 17 were assessable for tumour response. As best overall tumour response, one PR, eleven (65 %) SD, and five (29 %) PD could be observed.

Considering the advanced nature of tumours treated in this study, the overall response is promising.

Key aspects of the individual responses and their valuation for the study are further discussed.

One major response (PR) was observed. It suggests anti-cancer activity, even if the short duration and unconfirmed status are considered. The patient who suffered from Merkel cell carcinoma showed a decrease of targeted lesions of 43 % after the first cycle of CAP7.1 on an unscheduled CT scan.

The carcinoma, however, showed an increase of 34 % on the next scheduled CT scan after the second cycle. One might argue that this measurement might be an individual outlier, imprecise measurement, eventual bias, or other reasons could be the cause of the calculated PR.

To strengthen the tendency of tumour regression, it has to be emphasized that a shrinkage of 22 % of the targeted lesions would have shown on the scheduled CT (after second cycle), if the unscheduled CT (after the first cycle) had been totally ignored. This would have resulted in SD. However aside from that, the patient would still have been the best responder in evaluation after the second cycle (next best shrinkage was 14 %). These results show a definite tumour response as initial evidence of anti-tumour efficacy of CAP7.1.

Keeping all the patients' pre-study history in mind (as mentioned above), even SD is a substantial tumour response as the prior therapy failed. All eleven patients achieving SD had been heavily pre-treated: The majority even had had radiation therapy and surgery additionally to multiple chemotherapy regimes. Of these eleven patients, eight patients, which is 42 % of all participants, showed shrinkage in targeted tumour lesions during or after treatment with CAP7.1 (see figure 11).

This was predominantly in cohorts 3 and 4, which suggests a positive influence of CAP7.1 on tumour growth and indicates an effect of dosage on the activity of the drug. Confirmed SD, which all had a duration over three month, were only seen in the patient group treated with the highest dosage of 200 mg/m²/day in cohort 4. Furthermore, two thirds of all observed SD which lasted more than three months were achieved in this cohort 4. It also suggests that there is an unequivocally tendency of tumour response which is dependent on a higher dosage; therefore, tumour response seems drug related and a dosage-efficacy relation is likely.

No cluster concerning tumour type, TNM classification, or histological type could be seen. While taking into account the diversity, with unfortunately nearly no tumour entity occurring twice, it was conceivable that the identification of a tendency concerning tumour entities was unlikely in this study population. More patients and a different study protocol would have been required to make any reliable predication on efficacy on certain tumour entities (as included for example in phase II studies). Aside from that, it is not the primary objective of a phase I trial due to the study design to assess definite, extensive and detailed information on tumour response in specific different tumour types, but only to give a preliminary impression.

PD as best response was assessed in five patients. Non-responding tumours or lack of efficacy of CAP7.1 in these certain tumour types is suggested. Two of the non-responders were treated in cohorts 1 and 2 with very low dose levels (only 23 % and 45 % of MTD assessed at 200 mg/m²/day in this trial). Thus, there is reason to presume that these dose levels are too low to result in high enough CAP7.1 blood concentrations to have any possible effect on tumour cell growth of these tumours. This also indicates a dosage-efficacy relation of CAP7.1 as already mentioned above. In a detailed review, it was found that one patient treated in

cohort 3 did not fulfil eligibility criteria at screening assessment for the study but was nevertheless included by the investigator. The patient had insufficient liver function and the interval since last chemotherapy was not in the required time frame. That might have influenced the decision to reduce the patient's dose and to delay treatment after the first cycle. This has to be considered while discussing and evaluating the response, because the patient received only one cycle of the determined dose in cohort 3 and the second cycle on the 60 mg/m²/day lower dose level of cohort 2. Therefore, an insufficient dosage and excessively low CAP7.1 plasma-concentrations to harm the tumour tissue could be a possible reason for no tumour response in a total of three patients. It is important to take this into consideration. Nevertheless, two PD cases were observed in cohort 4 which was the highest dose level administered. Of these, one patient received only one cycle of CAP7.1 due to the occurrence of a serious adverse event and a PD was seen on a follow-up CT scan. It is likely that one cycle is not sufficient to significantly inhibit cell growth, due to the fact that CAP7.1 is a cell cycle specific agent. Unfortunately, tumour response in two other patients who also received only one cycle could not be assessed; therefore possible, similar relation could not be ascertained. However, lack of efficacy was also observed in a patient who suffered from adenosquamous rectum carcinoma and was treated in cohort 4. This might suggests a non-responding tumour entity, despite of adequate dosage.

Encouraging results were further detected in overall survival times. This is especially due to the fact that the assessed durations represent mostly only the minimum of overall survival, for the actual date of death was known only in six patients. As this phase I trial did not have overall response as primary objective, it is suggested that detailed data should be assessed in further trials as data show promising results. Data analyzed here give only an impression of overall response and shows a tendency of development due to the phase I stud design. Median overall survival was 6 months and 14 days. Consequently, the majority survived for more than six month. Five patients even lived longer than a year. Furthermore, median overall survival data are very promising, since 9 of 19 patients had stage IV TNM classification.

Two patients in particular had surprisingly good results and further aspects of these patients are now discussed in more detail. One patient who suffered from gallbladder carcinoma stage IV had an overall survival of 25 months and 16 days. Median survival of stage IV gallbladder carcinoma is very poor with no treatment. Standard first line chemotherapy for advanced non-resectable gallbladder carcinoma is currently a combination of gemcitabine and cisplatin. Median overall survival in a phase 3 trial that administered this combination therapy was 11.7 months.[87] The patient's best response on CAP7.1 was SD with a progression of the disease

after the fourth cycle of 90 mg/m²/day of CAP7.1. The patient received an additional regime of 5-FU and cisplatin one year and three months later. Unfortunately, further information could not be obtained, but the doubled survival time (in comparison to median survival on gemcitabine and cisplatin) was a surprising result. Especially since CAP7.1 was given as monotherapy and for the fact that spoken generally chemotherapeutic response of gallbladder carcinoma is limited due to insufficient knowledge of the cancer's biology. As this is one individual case, further investigation is absolutely necessary, and no generalisation can be made. Furthermore, it is not possible to determine an absolute causal relationship between treatment with CAP7.1 and the actual prolonged survival observed in the patient. CAP7.1 might have prolonged survival, but it is not clear which impact it finally had on the tumour development as further therapy was applied after this study. Therefore, it seems absolutely recommendable for the subsequent phase II trial to include patients who suffer from advanced, refractory, non-resectable gallbladder carcinoma in order to evaluate efficacy in more detail.

A second patient had an overall survival of at least 20 months and 12 days. The patient was diagnosed with CUP that occured in the region of the neck and was treated with four cycles of CAP7.1. An SD was achieved an in all CT scan evaluations performed. Median survival of CUP-patients is poor (only three to eleven months).[88] Therefore, a survival of 20 months seems an astonishing result. It is known that different subtypes of CUP have very different response rates to chemotherapy and a wide range of prognosis. The patient included in this study had the histological classification of squamous cell carcinoma, possibly of the lung. In different studies, the histological subtype of squamous cell carcinoma in CUP appears to have the best survival rates, like for example 31 months in a recently performed retrospective analysis.[89] It has to be mentioned that the patient in this study had been diagnosed three years before he entered the study and had, therefore, an unexpectedly good survival time prior to the treatment in the study. Nevertheless, a patient who had been pre-treated with radiation therapy, chemotherapy and surgery an who had suffered recurrent cancer over a period of three years, showed a SD and a survival of over 20 months. This represents a good and encouraging result and supports further phase II testing of the drug.

As CAP7.1 is converted by CES 2 to its cytotoxic compound etoposide, the assumption is suggested that CES 2 is highly expressed by the tumour entities showing response or prolonged survival. Unfortunately, no data is available that analyzes CES 2 expression in Merkel cell carcinoma as the only assessed PR. Recently CES 2 expression in various tumour cells was analysed. Regarding the patients who had a prolonged survival, however, no

expression of CES 2 was observed in tissue of adenocarcinomas of the gallbladder, and the expression in tissue of squamous lung carcinoma was only weak. An intense expression was seen in colon (adenocarcinoma), hepatocellular, adenous kidney and thyroid papillary cancer.[75] Unfortunately, none of these tumour types with intense expression was involved in the study population as it was not suitable to include only specific tumour entities for a first phase I study design. No correlation of general CES expression and efficacy of CAP7.1 could be seen, which is unexpected. A possible explanation might be an earlier conversion of CAP7.1 into etoposide outside the tumour tissue. The wide occurrence of CES 2 in common tissues, as it is highly expressed in the liver and kidney and has a moderate expression even in blood vessels and capillaries, could be one possible way of prodrug activation.[74] Further pharmacokinetic and pharmacodynamic analyses might provide explanatory insight and is needed.

Evaluation of ECOG performance showed deterioration during CAP7.1 treatment. Nevertheless, it should be emphasized that ECOG performance after treatment was still good in the majority of patients. The patient with an ECOG score of 4 on follow up assessment is the same one who did not entirely fulfil inclusion criteria, therefore, it was more likely that possible side effects would occur. The patient suffered various adverse events and severe adverse events, which could explain the heavy deterioration of ECOG performance. One patient died during the study (ECOG 5). Here a relation to the study drug was considered possible. Of course it has to be proved that treatment was not the immediate cause of death. The evaluation of safety of this phase I trial with CAP7.1, which was analyzed parallel to this evaluation of efficacy serves as a reference (“Safety Profile of CAP7.1 obtained during Phase I Trial in adult patients with refractory malignancies”, Philipp Mehlitz). In total, development of general wellbeing shows an acceptable result.

With one PR and almost two-thirds who had SD as best response, preliminary anti-tumour efficacy could be demonstrated, and preclinical data of anti-tumour impact could be confirmed as an important aim of this phase I trial. Prolonged survival could be assessed, and general well being had an acceptable development. The data that a dosage-efficacy relation is likely even as the data only represent a tendency due to the phase I study design. The results also underpin the promising effect of CAP7.1 administered to three children who suffered from neuroblastoma, which included one SD and one PR. Therefore, in terms of CAP7.1 efficacy, further testing in clinical trails is sensible.

6.2 Trial Limitations

It is very important to identify and discuss limitations and potential error sources in order to evaluate results and draw appropriate conclusions for further investigations. Especially as this is a phase I trial, and results build the foundation for decisions to be made with regard to future development, being the planning for phase II or a necessary additional phase I trial. Weaknesses concerning the evaluation of anti-tumour efficacy were found in various different aspects in this CAP7.1 trial. It has to be kept in mind that it was not the primary objective of this phase I study design to evaluate efficacy, and all limitations and errors discussed must be seen in this context. To have a better overview, the aspects discussed are categorized into aspects of study design, study performance, and study evaluation, even if a distinct placement is not always possible.

In general, all results obtained for efficacy evaluation are only preliminary nature and explorative which is the main limitation of the analysis of efficacy in this study. However, this evaluation is mandatory for the selection of the target patient population on the phase II study and for continuation of the therapy during phase I beyond two cycles (until progression) which was nessecary in more than 50% of patients during the phase I trial. However, the plan is to confirm data obtained during the phase I study in a randomized phase II study in respective indication selected based on findings during the phase I study.

The study protocol of this CAP7.1 trial is very similar to other phase I clinical trial study designs. One major aspect that limits the validity of results is the small number of participating patients. Even if phase I trials are usually relatively small studies (including 20-60 patients), the 19 patients participating in this phase I trial still constituted a very small group.[90] In the study protocol the number of patients per cohort was set at three to six per dose level. As only four dose levels were administered, due to occurring DLT, the participating patients were limited to this very small number. In the proceeding administration of CAP7.1, children were treated up to a dose level of 800 mg/m² without severe toxicities. Dosing scheme of this trial was, therefore, set to levels in which previous human data and toxicology studies were taken into consideration.

The MTD in this trial was reached at the relatively low point of 200 mg/m². A possible explanation is the advanced nature of diseases treated in this study, with which patients were more vulnerable to possible side effects due to a generally reduced status of health. Additionally, the previously treated patients were children, whereas in this study only adults

were included. Children generally tolerate chemotherapy better than adults and possible effects of the drug (positive or negative) cannot be assumed to apply fully to adults.
As a consequence, recruitment ended earlier than expected and the number of patients was kept limited to 19.
Extended statistical analyses were, therefore, not practicable (the number was too small and the characteristics too inhomogeneous for reliable statistical analyses), and all results represent individual cases and can only show a tendency of possible tumour development. To include more patients by extending the cohorts or by starting a new intermediate cohort (which was mentioned in the study protocol) could have been a possible solution to enlarge the patient group. This, however, was not done. Dosing schemes in future trials should be adjusted according to these lower dose levels now known in order to ensure a sufficient number of patients.
The study design also produces a wide range of observed tumour entities due to the relatively small number of patients and the study design which includes all tumour entities as this is a first in-human phase I trial. This is another limiting aspect. It is necessary to include various tumour types in a phase I study to evaluate possible efficacy even in unexpected tumour entities. In a larger number of patients it might have been possible to asses at least certain tumour groups with similar characteristics and response to CAP7.1. Unfortunately, almost no tumour entity occurred twice in this population. Therefore no correlation of efficacy to tumour location, histological subtype, or TNM classification could be observed. One might assume that it could help to select specific tumour entities for a study, but the approach to restrict a study to a certain group of tumours is not recommendable, as this is the first in-human study of CAP7.1.
As this is an open label trial, with all participants getting study medication, eventual bias is not ruled out, and direct comparability of efficacy is not given. Results might be more enlightening with a double-blind, placebo-controlled study. However it is ethically not acceptable to refuse someone treatment since patients included in this study had advanced refractory cancers with no alternative standard therapy, and it is out of the question to do so in this first phase I trial.
Inclusion and exclusion criteria were typical for phase I trials and also have a influence on efficacy results. As heavily pre-treated patients with recurrent and advanced stages of disease with no other therapeutic option were included, it was conceivable that results would not show extraordinary response rates. A healthier population can be included in phase II, therefore, it can be assumed that efficacy will improve.

In regard of the tumour assessment according to protocol, follow up CT scan for tumour evaluation was not necessary if the last CT scan was less than six weeks old and no evidence of tumour progression was seen. Thus some SDs could not be confirmed or duration could not be assessed. If the evaluation of efficacy is the priority in future trials, additionally scheduled CT scans will be necessary. For longterm results, it is absolutely essential to schedule another follow up status assessment (months or years later), as information about the patient's whereabouts in this trial was only sporadic. Not to be informed of the patient's status after the end of treatment with the study drug distorts results, particularly in the evaluation of the overall survival and the duration of response.

Reasons for eventual limitations are also within the realization of the study protocol. They are mostly a disregard of specifications set in the protocol. In three patients, inclusion and exclusion criteria were not fulfilled completely. Nevertheless, they were included and distorted results were the consequence. Two of these patients suffered adverse events and could not receive CAP7.1 medication according to scheme in the protocol, and they did not only influence efficacy results (details mentioned above), but also had an impact on safety and pharmacokinetic data. Additionally, the dose reductions were not suggested in the study protocol in these particular medical conditions. Eventually only three of the original six patients in cohort 3 received the actual planned dose, and, therefore, confounded the effect of dosage on efficacy.

Two patients who participated in the study had to withdraw due to delays in the production of CAP7.1. This might have had a detrimental impact on efficacy due to less time on study medication. However, all administrative problems in manufacturing were solved after the first cohort and will not occur again in further trials.

A potential source of imprecise data is also the measurement and evaluation of the targeted lesions on the CT scans. In quite a few cases, RECIST 1.0 criteria for tumour assessment were not adhered to, and data needed to be corrected afterwards. This is an additional potential error source. As not all measurements were done by the same person, individual differences in measuring techniques, even if measuring instruction is given, cannot be excluded but probably influenced data only slightly. Even considering that tumour response was not the primary objective, tumour assessment was unsatisfactory in some cases. In particular, it was often not possible to confirm an SD, which might have had a positive effect on efficacy data. Failure to perform scheduled CT scans, as mentioned in protocol for administrative or unknown reasons, interfered with the confirmation of SD, and prolonged SD could not be assessed.

In conclusion, better communication between investigators and performing clinicians and providing clinicians with a better theoretical background could be a possible solution to avoid the same problems in further trials.

An aspect in every clinical trial after discussing results and limitations is the validity of the results. External validity (or generalisability) analyzes if the results can be generalized for other circumstances, like patients with different age, sex, or stage of disease. As this is only a small phase I trial and, as mentioned above, results can only show individual cases, generalizations cannot be made and were not meant to be made. In this trial, we saw a PR in a patient who suffered from Merkel cell carcinoma and prolonged survival in patients who suffered from gallbladder carcinoma and squamous cell CUP. These results need to be confirmed in further investigations in order to make any concrete predictions on CAP7.1 efficacy for individual patients and different tumour entities.

Internal validity is mainly the accurate elimination of possible bias, which optimally requires a randomized, double-blind, placebo-controlled study, which is, as mentioned before, ethically not acceptable in this type of study (first in-human phase I study of a chemotherapeutic agent). It also includes the need for heterogeneous characteristics of patients (age, sex etc.), which is definitely given in this trial. Validity criteria are designed for randomized controlled trials. Thus, even though validity criteria are not met, standardized methods and criteria (e.g. RECIST) ensure, that the results of this study are comparable and reliable.

However, it has to be emphasized once more that the purpose of a phase I trial is to assess safety and determine the dosage for phase II. Preliminary tumour efficacy is only a secondary objective. Therefore, restrictions regarding tumour evaluation have to be made.

In general the main limiting aspecst are due to the study design of a phase I trial. Therefore these results have to be considered rather as initial and explorative at this stage.

In brief, the limitations of evaluating efficacy were mainly the small number of patients. All aspects mentioned above might influence the results of this study, but the fundamental findings remain valid. As some of these problems could have been prevented and as they also concern other evaluations (e.g. safety profile and pharmacokinetics), special attention has to be paid, and further actions have to be taken regarding these aspects to improve future clinical trials.

6.3 Comparison of CAP7.1 with Other Chemotherapeutics

In this chapter the results of tumour evaluation will be evaluated in comparison to other early clinical studies, divided into different groups of chemotherapeutics.
First, etoposide phosphates as the only other prodrug of etoposide that passed phase I clinical trial will be compared to CAP7.1. Followed by other prodrugs activated through the same enzyme as CAP7.1, which is carboxylesterase. Furthermore, results of CAP7.1 are put into context with substances that have a similar mechanism of action. Here CAP7.1 is compared to several topoisomerase inhibitors. In the last section, various new chemotherapeutics which reached the market recently are compared with CAP7.1.
To maintain comparability, only phase I studies which did not focus on a certain tumour entity (but included a range of different tumours (refractory advanced tumour stages)) and studies which applied only monotherapy of the certain chemotherapeutic were included in this comparison.

6.3.1 Efficacy of CAP7.1 Compared with Etoposide Phosphate

Etoposide phosphate is a water-soluble prodrug of etoposide and is rapidly converted to etoposide after administration. Etoposide phosphate, like etoposide, is a highly schedule dependent drug with which several phase I studies have been performed. In four phase I studies (applying monotherapy, encompassing 121 patients) tumour response was analyzed.[77,91-93]
In trials which administered etoposide phosphate, two CRs in 121 patients were observed in patients who suffered either from ovarian or cervical cancer. One patient with ovarian cancer in the CAP7.1-trial achieved an SD with a minimum duration of one month (discontinuance due to serious adverse event). The three patients who achieved PR in etoposide phosphate trials were diagnosed with ovarian, hepatocellular, and NSCLC. As no CR was observed after the administration of CAP7.1, etoposide phosphate achieved better efficacy with a total of 1.7% with CR. However in terms of observed PR, CAP7.1 percentage is more than two-fold higher. A correlation between tumour types could not be observed. Adding CR and PR percentages and define it as actual responsive tumour tissue, CAP7.1 (5.9%) and etoposide phosphate (4.2%) reached a quite similar percentage range in terms of tumour response. However, the difference in kinetics of CAP7.1 (prolonged serum levels up to 6 hrs) in comparison to etoposide phosphate (detectable 30 min in serum) might make it possible to

achieve better efficacy and safety in a larger and homogeneous patient population. In addition, unlike to CAP7.1, efficacy is not demonstrated in therapy-refractory upper gastrointestinal and head and neck tumours using etoposide phosphate as a monotherapy. Thus, CAP7.1 might give grounds for hope for further successful development in cancer entities not addressed by the current etoposide associated compounds.

Table 26. **CAP7.1 and Phase I Trials of Etoposide Phosphate.**

Study	Fields et al.	Thompson et al.	Millward et al.	Soni et al.	Total	CAP7.1
Administration Scheme	Day 1,3,5 à 30 min	5 days à 30 min	5 days à 30 min	6 weeks continuous		5 days à 60 min
Number of Patients	39	28	31	23	121	19 §
Median Age	61,6	55	52	66		63
Response*						
CR		2 *(7,1)*			2 *(1,7)*	
PR			1 *(3,2)*	2 *(8,7)*	3 *(2,5)*	1 *(5,9)*
MR		3 *(10,7)*		1 *(4,3)*	4 *(3,3)*	*not analysed*

n *(in %)*, * WHO criteria, MR Minor Response, PR Partial Response, CR Complete Response, min minutes, § 17 Patients assessable for tumour response

6.3.2 Efficacy of CAP7.1 Compared with Other Prodrugs Activated by Carboxylesterase

CAP7.1 is converted to its active compound, etoposide, by the enzyme carboxylesterase. There are a couple of anticancer prodrugs, which are also converted by carboxylesterase. Two substances, irinotecan and capecitabine, already passed through phase I clinical trial and are comparable to CAP7.1 in matters of efficacy. Other substances, however, have so far only been investigated in preclinical experiments; they include Pentyl PABC-Doxaz (Pentyl 4-(*N*-doxazolidinylcarbonyloxymethyl)phenylcarbamate) a carbamate of doxazolidine (a derivate of doxorubicin) or Paclitaxel-2-ethylcarbonate (a prodrug of paclitaxel applied through gene-directed enzyme prodrug therapy (GDEPT)).[94,95]

Irinotecan is a semi-synthetic derivate of camptothecin and exhibits anticancer activity by inhibiting topoisomerase I. It is enzymatically converted into its active metabolite SN-38 by carboxylesterase. Various application schemes that applied monotherapy were explored in phase I in clinical trials (total number of patients: 311, eight trials).[96-103] As far as they are comparable, CR and PR percentages of CAP7.1 are below the average of the eight compared studies of irinotecan (PR irinotecan: 8% vs. CAP7.1: 5.9%, CR irinotecan: 1.3% vs. CAP7.1: 0%). Results of patients with an SD were only published in one irinotecan study and are,

therefore, not truly representative. Nevertheless CAP7.1 shows a 1.88 fold higher SD-rate.[101] Tumour entities observed in patients with CR, while on treatment with irinotecan, were non-Hodgkin lymphoma, cervix, head/neck, and colon carcinomas.[97,100,103] Most PR were seen in patients who suffered from colon carcinoma (20/25). Keeping in mind the intense CES 2 expression observed in some colon carcinoma cell lines, there might be a correlation between CES 2 expression and efficacy of the CES activated prodrug in these tumour cells.[75] Unfortunately, however, no patient with colon carcinoma was included in this trial to confirm similar results.

Capecitabine is a carbamate of fluoropyrimidine, which is converted into 5-FU in three steps, with carboxylesterase as one necessary enzyme. Three phase I oral capecitabine-monotherapy studies that evaluate tumour response are compared here.[104-106] Breast cancer (with one CR and four PR), oesophageal (one PR), colon (one PR) and rectal cancer (one PR) showed objective tumour response, with no obvious correlation with tumour entities analysed in this trial. CAP7.1 efficacy is lower compared with the average of capecitabine, however a head-to-head comparison is difficult as the tumour entities differ in both trials.

In total, the single CAP7.1 phase I study (19 patients) compared to eleven phase I trials of CES-activated prodrugs (in nearly 400 patients) seems to have less favourable efficacy results than other carboxylesterase activated drugs. However irinotecan and capecitabine were tested in more than one clinical trial, including a significantly higher number of patients (386 patients, i.e. more than 20 times more patients than in this CAP7.1 trial). Direct comparison of efficacy can only give a very limited impression. As a result, further testing of CAP7.1 with significantly more patients seems to be advisable and is absolutely needed to obtain more reliable and comparable results.

Table 27. **CAP7.1 and Phase I Trials of Irinotecan.**

Study	De Forni et al.	Abigerges et al.	Catimel et al.	Takimoto et al.	Pitot et al.	Rothenberg et al.	Rowinski et al.	Merrouche et al.	Total	CAP7.1
Administration Scheme	Weekly for 3 weeks	Once every 3 weeks	3 days every 3 weeks	96h for 1,5 weeks every 3 weeks	Once every 3 weeks	Weekly for 4 weeks	Once every 3 weeks	Once every 3 weeks		5 days every 3 weeks
Number of Patients	59	64	46	26	34	32	32	18	311	19 §
Median Age	54	51	56	56	61	55	49	55		63
Response*										
CR		2 *(3,1)*			1 *(2,9)*			1 *(5,6)*	4 *(1,3)*	
PR	1 *(1,7)*	6 *(9,4)*	2 *(4,3)*	1 *(3,8)*	4 *(11,8)*	2 *(6,25)*	3 *(9,4)*	6 *(33,3)*	25 *(8,0)*	1 *(5,9)*
MR	4 *(6,8)*		2 *(4,3)*	1 *(3,8)*			3 *(9,4)*		10 *(3,2)*	*not analysed*
SD	*n. m.*	*n. m.*	*n. m.*	*n. m.*	*n. m.*	11 *(34,4)*	*n. m.*	*n. m.*		11 *(64,7)*

n *(in %)*, * WHO Criteria, MR Minor response, CR complete response, PR partial response, h hour, § 17 Patients assessable for tumour response, *n.m.* not mentioned

Table 28**. CAP7.1 and Phase I Trials of Capecitabine.**

Study	Mackean et al.*	Saeki et al.*	Peutheroudakis et al.	Total	CAP7.1
Administration	2x/day for 2 weeks	2x/day for 6 weeks	2x/day on weekdays, weekend off		5 days every 3 weeks
Number of Patients	34	16	25 (24)	74	19 §
Median Age	57	64	67		63
Response					
CR	1 *(2,9)*			1 *(1,4)*	
PR	3 *(8,8)*	1 *(6,3)*	3 *(12,5)*	7 *(9,5)*	1 *(5,9)*
MR	7 *(20,6)*	1 *(6,3)*		8 *(10,8)*	*not analysed*

n *(in %),* *WHO criteria, CR complete response, PR partial response, MR Minor Response, § 17 Patients assessable for tumour response

6.3.3 Efficacy of CAP7.1 Compared with New Topoisomerase Inhibitors

Many chemical substances that act as topoisomerase inhibitors have been developed, and several of these have been tested clinically. The substances mentioned are mostly still undergoing clinical development, or investigations have stopped due to various reasons.

Table 29. **New Topoisomerase Inhibitors Which Completed Phase I Clinical Trial.**

Topoisomerase II Inhibitors	NK 611 Vosaroxin C-1311 R(+)NK469 AEZS-112 Banoxantrone XL 119 Elsamitrucin
Topoisomerase I Inhibitors	Elsamitrucin TLC 388 XMT-1001

NK 611 is a semi-synthetic podophyllotoxin derivate and inhibits topoisomerase II by stabilizing the covalently bound DNA-enzyme complex. It was tested in various phase I trials during the 1990s with the application of various dose schemes due to its suspected schedule-dependency, which is similar to etoposide.[107-112] However, further development was discontinued for unpublished reasons.

The new topoisomerase II inhibitor ***vosaroxin (SNS-595, AG-7352, AT-3639, voleroxin)*** is the first member of quinolone derivates used for the treatment of cancer. It is currently evaluated in a phase III clinical trial in patients with relapsed or refractory AML by Sunesis pharmaceuticals. Two application schemes have been tested in phase I clinical trials. [113]

C-1311 (Symadex ™) is a new anticancer agent of the class of imidazoanidiones, which inhibits topoisomerase II and a certain tyrosine-kinase receptor. Symadex™ finished phase I, but its clinical development was put on hold by the investigating company for administrative reasons (Antisoma Plc). [114-116]

R(+)NK469 is a quinoxaline anti-cancer medication, with pre-clinical data that showed a possible topoisomerase II inhibitory aspect. However, a clear insight into the drug's physiological effects has not yet been provided. Even so, R(+)NK469 was not developed further after phase I clinical trial due to insufficient clinical activity .[117,118]

AEZS-112 is a new substance which inhibits topoisomerase II and has a tubulin inhibition as an additional effect. In April 2009, phase I results were published and, according to the

investigating company (Æeterna Zentaris Inc.), further promotion and development plans are still being discussed at the moment.[119]

Banoxantrone (AQ4N) is a substance which converts into its cytotoxic compound under hypoxic conditions and inhibits topoisomerase II. Due to administrative problems of the investigating company, further development was not conducted. [120]

XL 119 (Becatecarin) is a rebeccamycin analogue (antitumour antibiotic) and inhibits only topoisomerase II. Phase II results in patients with SCLC were published in April 2012, but they were not better than those achieved with existing chemotherapeutic regimes.[121-123]

Elsamitrucin is an antitumour antibiotic and inhibits both topoisomerase I and II. The first phase I trials were published in 1992, but up-to-date information on status of development is not available.[124]

TLC 388 (Lipotecan®) is a topoisomerase I inhibitor, and phase I clinical trial results were presented at the AACR-NCI-EORT meeting in 2011. It is undergoing further clinical trials at Taiwan Liposome Company.[125]

XMT-1001 is a prodrug of camptothecin; therefore, it inhibits only topoisomerase I. Phase II trials were planned to start in 2012 by Mersana Therapeutics.[126]

Figure 14 shows summarized data of newly developed topoisomerase inhibitors in comparison with CAP7.1. CAP7.1 shows better efficacy results than all other tested substances. It has to be mentioned that SD was not mentioned separately in some studies. Hence, the data might give a skewed impression, but in these cases the PR-rate of CAP7.1 at least shows a higher percentage than other tested substance. Especially interesting is the comparison with substances that passed phase I successfully and are currently undergoing further trials. Compared to vosaroxin (being currently in phase III), CAP7.1 shows more than double the percentage in PR rates (vosaroxin: 2%, CAP7.1: 5.9%) and a 14.7% higher rate in SD percentages (vosaroxin: 50%, CAP7.1: 64.7%). A phase II trial was also initiated on XL 119. It showed a total PR rate of 2% and had a SD rate of 20-38%, which are clearly lower than those of CAP7.1. It is indeed noteworthy that vosaroxin underwent two, and XL 119 underwent three different phase I trials. Consequently, statistical data is more reliable. Even so, better results in SD rates and PR percentages could be seen in comparison to TLC 388 and XMT-1001 (TLC 388: PR 3%, SD 58%, XMT-1001: PR 0%, SD 24%). Phase II trials are planned or in progress for these substances and only one phase-I trial was performed and published.

Tumour responses were assessed in a wide range of tumours and showed no explicit similarities to tumour response in this trial. Interestingly, a response (tumour shrinkage of 47%) and

prolonged SD were observed in three patients who suffered from gallbladder carcinoma in a study with XL 119. A prolonged survival of a patient with gallbladder carcinoma was also seen in this CAP7.1 trial. Both substances act as a topoisomerase II inhibitor. This aspect may point to responsive tumour tissue in gallbladder carcinoma. Therefore, patients with gallbladder carcinoma should definitely be included in further clinical testing.[122]

Overall, CAP7.1 shows superior results compared to other topoisomerase inhibitors, and since it was only recently developed, this effectively encourages the further investigation in CAP7.1 as a potentially new cytotoxic agent.

Table 30. **Analyzed Phase I Studies of New Topoisomerase Inhibitors.**

Substance	**Study**	**Number of Patients**	**Median Age**	**Response**			
				CR	**PR**	**MR**	**SD**
NK 611	Schilling et al.*	26	56				*n.m.*
	Fukuoka et al.*	26	63			2	*n.m.*
	Raßmann et al.* (1995)	18	60				*n.m.*
	Raßmann et al.* (1996)	18	64				*n.m.*
	Pagani et al.*	21	60,5				4
	Raßmann et al.* (1998)	45	54		4		5
Vosaroxin	Advani et al. I	41	59,5		1		21
	Advani et al. II	21	61				10
C-1311	Isambert et al. (2006)	16	*n.m.*				3
	Thomas et al.	36	*n.m.*		1		17
	Isambert et al. (2010)	22 (21)	56,5				6
R (+)NK469	Alousi et al.	81	60		1		*n.m.*
	Undevia et al.	22	55,6				*n.m.*
AESZ-112	Æeterna Zentaris	44	*n.m.*				20
Banoxantrone	Papadopoulos et al.	16	57				2
XL 119	Dowlati et al.*	30	66		2	2	6
	Tolcher et al.*	45	55			2	*n.m.*
	Merchant et al.*	69	56		1	1	26
Elsamitrucin	Raber et al.	49	56				*n.m.*
TLC 388	Ghamande et al.	54(36)	*n.m.*			1	21
XMT-1001	Sausville et al.	49	62				12

n.m. not mentioned,* Response criteria differ from RECIST criteria, (n) number of patients assessable for tumour response

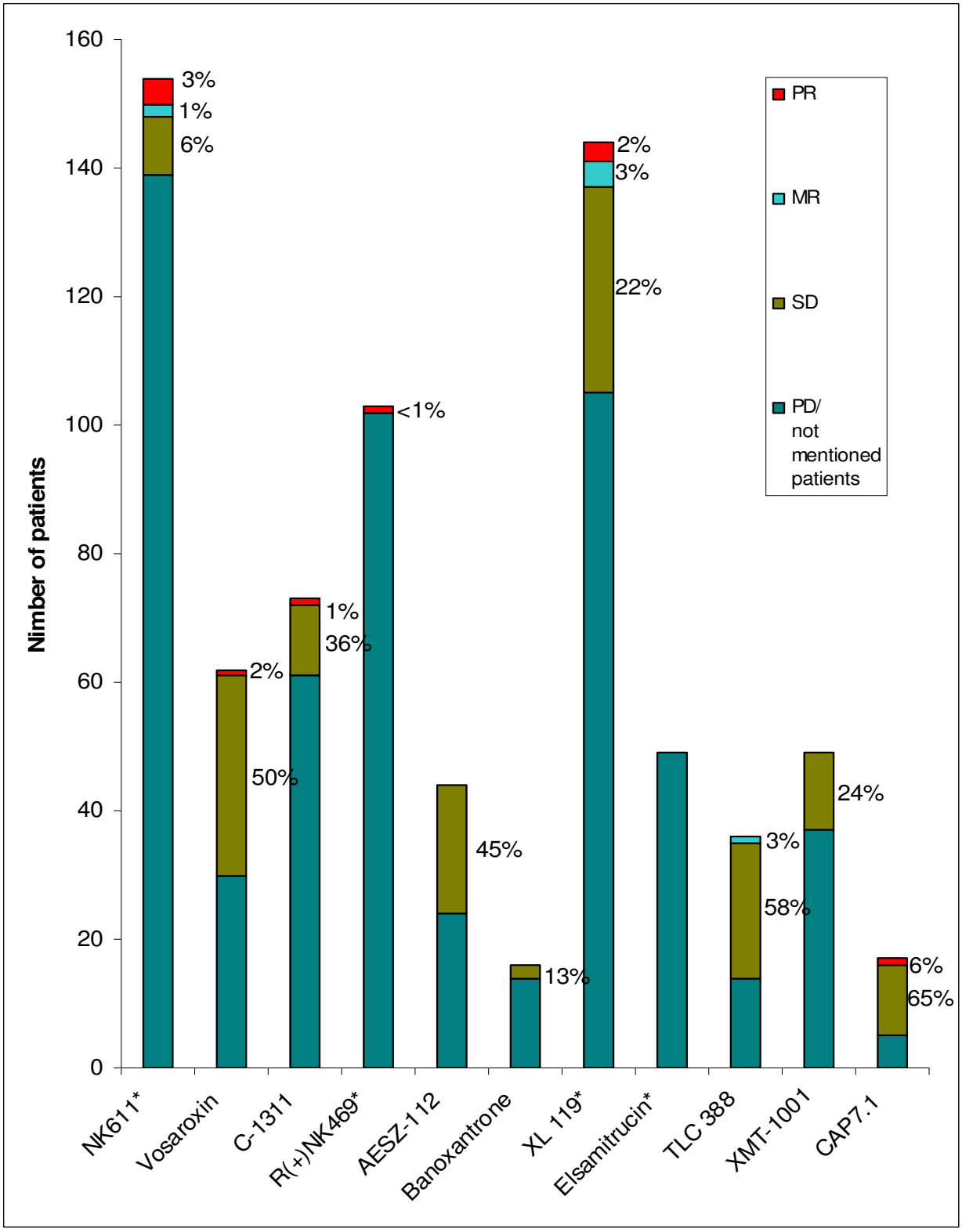

Figure 14. **Summary of Phase I Studies of Other Newly Developed Topoisomerase Inhibitors.** *SD not mentioned in all included studies

6.3.4 Efficacy of CAP7.1 Compared with Recently Approved Cytotoxic Drugs

Javlor® (Vinflunine) is a semi-synthetic vinca-alkaloid and has been on the European market since November 2009. Since 2010 Javlor® is listed in the guidelines of the European Association of Urology as a monotherapy for the treatment of advanced or metastasised urothelial carcinoma after failure of cisplatin treatment. Javlor® is currently undergoing several clinical trials with various new indications, like metastatic breast cancer and new combination therapies, e.g. capecitabine. [127-130]

Yondelis® is the first anticancer drug isolated from a marine organism (*Ecteinascidia turbinata*) that has been approved in Europe. Since 2007, it is licensed for the treatment of soft tissue sarcoma (after failure of prior treatment). Since 2009, Yondelis® has its second marketing authorization for the treatment of relapsed platinum sensitive ovarian cancer in combination with pegylated liposomal doxorubicin. Various phase I/II trial are currently being carried out. [131-136]

Ixempra® (Ixabepilone) is a semi-synthetic epothilone B analog and acts as a cytotoxic agent via the interference with the microtubular structure. Ixempra® is approved by the U.S. Food and Drug Administration (FDA) for the treatment of aggressive and metastatic or locally advanced breast cancer (resistant to other chemotherapeutic regimes), but is not authorized for the European market. [137-143]

Halaven® (eribulin mesylate) is a microtubule inhibiting agent, and it is a synthetic analogue of a marine naturally occurring sponge species. In the U.S. (since 2010) and in Europe (since 2011) it is licensed for the treatment of local advanced or metastatic breast cancer (after failure of at least two prior chemotherapy regimes, including taxanes and anthracyclines).[144-148]

Jevtana® (Cabazitaxel) is a new taxane and was approved for treatment of hormone-refractory prostate cancer (second-line treatment) in 2010 by the FDA. Only one phase one trial has been published.[149]

Gimatecan is a derivate of camptothecin that acts as a topoisomerase I inhibitor. It is approved in the U.S. by the FDA as an orphan drug for the treatment of malignant glioma.[150-152]

In order to gain a notion on possible future development, it is important to examine the preliminary efficacy of CAP7.1 in relation to other phase I studies of substances that have finished all clinical trials successfully. At first glance at figure 15, CAP7.1 seems to have a good efficacy compared to the other substances. On closer examination of figure 15, PR rates in all compared studies vary from 0 % to 21 %, with a median percentage of 5. As CAP7.1 had achieved a PR rate of 5.9 %; it is the same as, or even slightly higher than, the average of all studies compared. In regard to SD data, the range varied from 8 to 68 %, in studies which

mentioned the number of patients with identified SD. Median percentage is 29 %, and CAP7.1 is with 64.7 % of patients with an assessed SD more than two-fold higher than the median SD percentage. In all 23 studies analyzed, less than one percent of participating patients had CR. This is an insignificant number, and it shows the same tendency as in this CAP7.1 trial with no observed CR.

Overall, CAP7.1 efficacy is in the same percentage range as regards efficacy. It even shows above-average results compared to phase I studies from recently approved drugs, as is shown in figure 15. These results justify the prediction of an encouraging future for CAP7.1 as a substance that actually reaches the market. Eventually, it is not possible to predict results of the clinical development in the future. Nonetheless, compared to other phase I results of cytotoxic substances, CAP7.1 phase I results decidedly warrant further investigations.

Table 31. **Analysed Phase I Studies of Recently Approved Cytotoxic Drugs.**

Substance	**Study**	**Number of Patients**	**Median Age**	**Response**			
				CR	**PR**	**MR**	**SD**
Javlor®	Delort et al.*	14	55			1	*n.m.*
	Bennounan et al.*	31 (25)	53		3		4
	Johnson et al.*	16 (14)	51				6
	Calvo et al.	36 (34)	60		1		23
Yondelis®	Taamma et al.*	52	58		3		4
	Ryan et al.*	21	59			2	*n.m.*
	Villano-Calero et al.*	42	53			3	*n.m.*
	Twelves et al.*	72 (49)	57	1	1		14
	Forouzesch et al.*	63	46		1		18
	Pardo et al.*	33	54		1		3
Ixempra®	Abraham et al.	27	*n.m.*		5		*n.m.*
	Mani et al.	26	59		2	2	*n.m.*
	Aghajanian et al.	61	58	2	6		*n.m.*
	Gadgeel et al.	18	54,5				4
	Shimizu et al.	14	55		1		6
	Awada et al.	87	55		5		*n.m.*
	Kunz et al.	23 (18)	59				5
Halaven®	Synold et al.	40 (38)	61		2	3	12
	Minami et al.	15	*n.m.*		3		3
	Goel et al.	32	57		1		10
	Tan et al.	21	62		1		12
	Mukohara et al.	15 (14)	58		3		4
Jevtana®	Mita et al.*	25	60		3	2	12
Gimatecan	Sessa et al.	108 (97)	57		6		*n.m.*
	Zhu et al.	33 (26)	61				4

n.m. not mentioned,* Response criteria differ from RECIST criteria, (n) number of patients assessable for tumour response

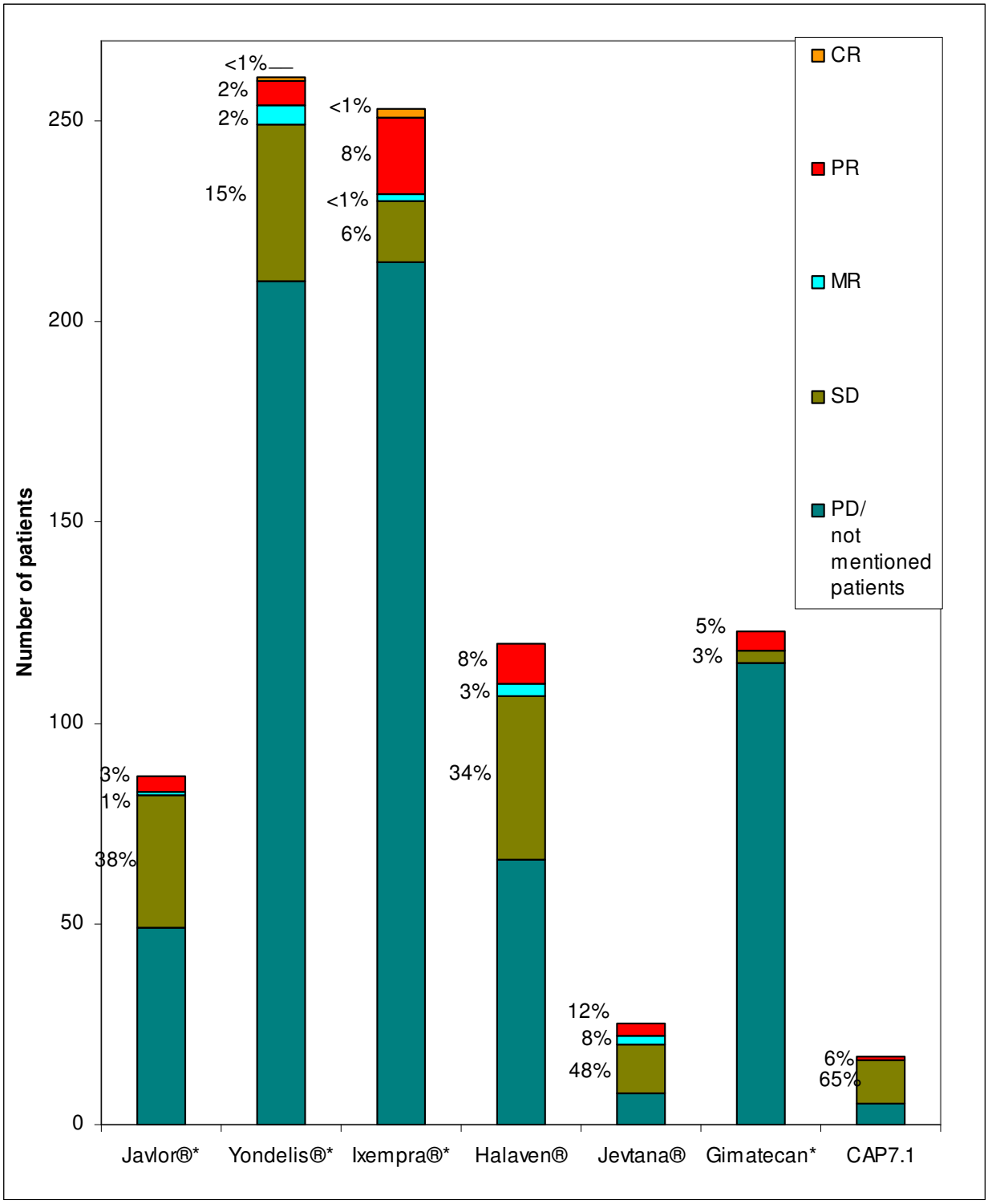

Figure 15. **Summary of Phase I Studies of Recently Approved Drugs.** *SD not mentioned in all included studies

6.4 Conclusion

In this phase I study of CAP7.1 preliminary anti-tumour efficacy as secondary objective could be demonstrated; further clinical trials in general are therefore justified. Nevertheless, owing to the very small number of participants the efficacy results can only give a limited impression and represent only tendencies.

At the end of the analysis and discussion of all data, the next step is to decide how to continue CAP7.1 development. In particular, there are two different possibilities. They are either to conduct a phase II or to initiate another additional phase I (Phase Ia) trial.

A reason to proceed directly with a phase II is the fact that the objective of this study, to show preliminary anti-tumour efficacy, was demonstrated successfully. Moreover CAP7.1 results could be compared effectively to other cytotoxic agents, of which some also moved directly into phase II. Additionally, a phase II would probably have efficacy as a primary observed parameter, whereby efficacy data will be a lot more detailed and conclusive. As the population of patients will be homogeneous, larger, healthier and randomized against control (not only patients with highly advanced and recurrent malignancies) in phase II, efficacy results might give more realistic information, and data will have greater validity to predict tumour response in general. The aspect of the small number of patients and therefore rather limited predication on future efficacy outcome can be seen as an inevitable consequence of a phase I study design, which makes an additional phase I with more patients redundant. However, findings in orphan indications such as oesophageal, stomach or gallbladder cancer might account for a larger effect of the drug due to lower incidence of the disease in a limited patient group. In the currently ongoing, multicentre, randomized phase II trial of CAP7.1, the treatment of patients with therapy refractory biliary tract, gallbladder cancer and lung cancers demonstrate a high rate of response according to Recist criteria in comparison to the control group (best supportive care) supporting the predictions in the phase I study. Thus, these unpublished results confirm findings of the initial efficacy analysis of the phase I trial.

Another important aspect is that proceeding directly to phase II would accelerate development and therefore could help patients in the corresponding patient group earlier. It has to be taken into account that proceeding directly to phase II would save financial resources, but this should not be the decisive factor.

A counter-argument for proceeding directly to phase II is that the assessed results are not definite and only preliminary.

In view of the number of patients and the diversity tumour entities included, the value of analysis is limited due to few patients and partially incomplete analysis and realisation of efficacy assessment. Even though some of these aspects were predictable due to the phase I study design. Moreover, there were certain restrictions in the realisation of the study, which could be avoided in an additional phase I to make it more accurate and detailed (as discussed above). Another argument which favours an additional phase I is the chemical structure of CAP7.1. CAP7.1 is a prodrug of etoposide which is a highly schedule dependent drug. Therefore it can be assumed that CAP7.1 has similar characteristics. To ensure the best schedule for a phase II testing, in the past, in different substances which are also schedule depended, additional phase I trials were initiated. These additional phase I trials applied various application schemes. As this is likely to apply to CAP7.1, an additional phase I with a different application schedule seems very sensible. Other arguments in favor of an extra phase I originate from the newly assessed information about CAP7.1 in humans during the trial. As the MTD is now known, which is lower than the initially expected dose, a new application scheme could be adjusted accordingly. Therefore enough patients will be enrolled to ensure a sufficient number of patients for every cohort and in total. But even this would not change the objectives of a phase I study, where efficacy will also be a secondary objective and therefore the results preliminary. From an academic point of view, more data revealing possible relations of tumour response, for instance to tumour entities or dosing, would be very interesting and could be assessed in an extra phase I with an adequate study protocol. Imaginable are also protocols including a combination with other chemotherapeutic drugs. Furthermore, correlations, for example with pharmacokinetic data, could be assessed. This was not possible in this trial due to unexpected pharmacokinetic behaviour of the drug. Consequently, changes in methods are needed as new aspects in terms of pharmacokinetics of the drug in humans are now known.

Only all analyzed results of this CAP7.1 trial taken together can build the basis for any further decision. This includes especially the safety profile. From an academic point of view and taking only the results of efficacy of the drug into consideration, it appears to be preferable to initiate an additional phase I trial, in which a different application scheme is applied, which includes more patients and uses a different schedule. Keeping in mind the objectives of this phase I trial, regarding all analyzed aspects of this study, a different conclusion has to be drawn.

Data on safety analysis are currently only preliminary, but they show a better toxicity profile than etoposide. It was astonishing to discover that haematological toxicities were reversible in a short time and that no organ-toxicities could be observed. As safety was the primary objective,

these results are highly promising and distinguish CAP7.1 from etoposide, which is not tolerable at the doses that were given in the phase I study.

Therefore, considering the entire study with its primary objectives and all results, it appears more reasonable to proceed directly into phase II instead of an additional phase I.

If a phase II is initiated, the question as to which tumour entities should be included is highly related to efficacy analysis in phase I. Since the data is not statistically confirmed, only a limited statement can be made, which is more a suggestion. Etoposide as the mother-compound of CAP7.1 is licensed for the treatment of lung cancer (NSCLC, SCLC). Data showed the prolonged survival of a patient who suffered from CUP, with cells probably originating from the lung. Additionally one of the four confirmed SDs was observed in a patient diagnosed with lung cancer. All this speaks in favor of including of patients who suffer from lung carcinoma in phase II. Due to the prolonged survival data observed in a patient with gallbladder carcinoma, this entity should also be included. Therefore, the one PR observed in a patient with Merkel cell carcinoma supports the inclusion of this tumour type in future studies. Schedule and administration scheme should be adjusted, paying attention also to pharmacokinetic and safety evaluations, all the while keeping in mind the assessed MTD at 200 mg/m²/day.

An issue for further discussion can be the question as to whether future testing should also include children, since CAP7.1 showed good results in children with neuroblastoma in a previous administration. It is always a difficult decision at what age to start the testing in underage patients without endangering them. As this trial shows a good safety profile, results of the subsequent trial (phase II) should be taken into account in making this decision. If they show an acceptable profile, a trial in children with neuroblastoma should be seriously considered as a new option for a clinical trial.

In summary, it can be stated that from an academic point of view an additional phase I trial would be preferable, if only efficacy data would be the decisive factor. However, with regard to the study, goals and its results in their entirety, a phase II testing is clearly more sensible.

Regarding the hypothesis of this work a preliminary winti-tumour efficacy as study objective could be demonstrated and CAP7.1 schowed efficacy in tumour entities pre-treated with etoposide. A sout statement concerning the efficacy in certain tumours (which concern CES II expression or tumour entity) could not be assessed due to a wide diversity of tumour entities. But as mentioned above the results suggest the inclusion of lung cancer, gallbladder carcinoma and Merkel cell carcinoma in phase II testing as the results suggest a slightly favourable outcome in these tumour types.

In conclusion, the CAP7.1 trial finished successfully, and the substance showed preliminary anti-tumour efficacy as a crucial factor for further development and secondary objective of this study. CAP7.1 did quite well in the comparison with other cytotoxic compounds, which definitely supports its further testing in clinical trials.

7 References

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8 Tabellarischer Lebenslauf

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

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9 Eidesstattliche Erklärung

„Ich, Laura Rohde, versichere an Eides statt durch meine eigenhändige Unterschrift, dass ich die vorgelegte Dissertation mit dem Thema: "Analysis of Initial Efficacy Results of a New Cytotoxic Prodrug, CAP7.1, in Adults with Therapy Refractory Solid Tumours in a Phase I Clinical Trial" selbstständig und ohne nicht offengelegte Hilfe Dritter verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel genutzt habe.
Alle Stellen, die wörtlich oder dem Sinne nach auf Publikationen oder Vorträgen anderer Autoren beruhen, sind als solche in korrekter Zitierung (siehe „Uniform Requirements for Manuscripts (URM)" des ICMJE -*www.**icmje**.org*) kenntlich gemacht. Die Abschnitte zu Methodik (insbesondere praktische Arbeiten, Laborbestimmungen, statistische Aufarbeitung) und Resultaten (insbesondere Abbildungen, Graphiken und Tabellen) entsprechen den URM (s.o) und werden von mir verantwortet.
Meine Anteile an etwaigen Publikationen zu dieser Dissertation entsprechen denen, die in der untenstehenden gemeinsamen Erklärung mit dem/der Betreuer/in, angegeben sind. Sämtliche Publikationen, die aus dieser Dissertation hervorgegangen sind und bei denen ich Autor bin, entsprechen den URM (s.o) und werden von mir verantwortet.

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

Datum Unterschrift

10 Anteilserklärung an erfolgten Publikationen

Laura Rohde hatte folgenden Anteil an den folgenden Publikationen:

Keilholz U, Knoedler M, Schmittel A, Kümmerlen V, Klinghammer K, Rohde L, Mehlitz P, Gehringer C, Joel S, Utku, First-in-man Dose Escalating and Pharmacokinetic Study of CAP7.1, a Novel Etoposide Prodrug in Adults with Heavily Pretreated Solid Tumors, Eur J Cancer 2012;48(S6):189

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

Priv. Doz. Dr. med. N. Utku

Laura Rohde

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