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Oblimersen combined with docetaxel, adriamycin and cyclophosphamide as neo-adjuvant systemic treatment in primary breast cancer: final results of a multicentric phase I study

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Background: Combining the Bcl-2 down-regulator oblimersen with cytotoxic treatment leads to synergistic antitumor effects in preclinical trials. This multicentric phase I study was carried out to evaluate maximum tolerated dose (MTD), safety and preliminary efficacy of oblimersen in combination with docetaxel, adriamycin and cyclophosphamide as neo-adjuvant systemic treatment (NST) in primary breast cancer (PBC).

Methods: Previously untreated patients with PBC T2–4a–c N0–3 M0 received one cycle of docetaxel 75 mg/m^2, adriamycin 50 mg/m^2 and cyclophosphamide 500 mg/m^2 administered on day 5 combined with escalating doses of oblimersen as a 24-h continuous infusion on days 1–7 followed by five cycles of combination of docetaxel, adriamycin and cyclophosphamide (TAC) without oblimersen every 3 weeks. Prophylactic antibiotic therapy and granulocyte colony-stimulating factor administration were used in all six cycles. Blood serum samples were taken throughout the treatment period for pharmacokinetic analysis.

Results: Twenty-eight patients were enrolled (median age, 50 years; ductal-invasive histology, 68%; tumorsize 2–5 cm, 61%; grade 3, 43%; hormone receptor negative, 36%; Her2 positive 18%) and received oblimersen in a dose of 3 mg/kg/day (cohort I, nine patients), 5 mg/kg/day (cohort II, nine patients) and 7 mg/kg/day (cohort III, 10 patients) respectively. No dose-limiting toxicity occurred. Following oblimersen combined with TAC, the most severe toxicity was neutropenia [National Cancer Institute—Common Toxicity Criteria (NCI-CTC) grades 1–2/3/4] which developed in 0/0/56% of patients (cohort I), 11/0/56% of patients (cohort II) and 20/20/50% of patients (cohort III). No febrile neutropenia occurred. Most common adverse events (all NCI-CTC grade ≤ 2) were fatigue, nausea, alopecia, headache and flue-like symptoms observed in 78% (cohort I), 89% (cohort II) and 90% (cohort III) of patients. With increasing dose of oblimersen, a higher incidence of grade IV leukopenia and neutropenia was noted. At the MTD of 7 mg/kg/day of oblimersen, serious adverse events occurred in 40% of the patients.

Conclusion: Oblimersen up to a dose of 7 mg/kg/day administered as a 24-h infusion on days 1–7 can be safely administered in combination with standard TAC on day 5 as NST in patients with PBC. The safety and preliminary efficacy warrants further evaluation of oblimersen in combination with every cycle of the TAC regimen in a randomized trial.

Key words: breast cancer, neo-adjuvant systemic treatment, oblimersen

introduction

Surgery is the main modality of treatment in patients with breast cancer. Surgery and/or radiotherapy can control local-regional disease in the majority of patients. However, >60% will ultimately die due to widespread disease. In the past 10 years, adjuvant hormonal or chemotherapy treatment has been increasingly used on the basis of the studies showing that adjuvant treatment can prolong time to recurrence and survival in some subsets of patients [1]. Neo-adjuvant (preoperative) systemic treatment (NST) and adjuvant chemotherapy offer patients a similar gain of survival [2]. Preoperative chemotherapy is the standard of care in advanced breast cancer.

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The combination of docetaxel, adriamycin and cyclophosphamide (TAC regimen) represents one of the standard chemotherapies applied before surgery leading to a pathologic complete response (pCR) rate of ~25% in chemosensitive primary breast cancers (PBCs) [3].

To improve the antitumor efficacy of chemotherapy, a combination with apoptosis-modulating drugs would be interesting to evaluate. Key proteins involved in the regulation of apoptosis are Bax (promoter of apoptosis) or Bcl-2 (apoptosis antagonistic) [4–6]. The antiapoptotic regulatory protein Bcl-2 demonstrates an attractive and new molecular target in the therapy of PBC [7, 8]. A significant correlation between an overexpression of Bcl-2 and a multidrug resistance to chemotherapy [9, 10] and an increased apoptosis by down-regulation of Bcl-2 through antisense oligonucleotides [9, 11–13] has been shown.

The 18-mer phosphorothiate oligonucleotide (5′-TCTCCCAGCGTGCGCCAT-3′) oblimersen (Genasense, G3139) is complementary to the first six codons of the Bcl-2 messenger RNA and depending on the dose it can down-regulate the Bcl-2 protein [14]. The antitumor effect of oblimersen was significantly improved in combination with chemotherapeutics like docetaxel [15] or anthracyclines [16]. Since it is known that the combination of oblimersen/docetaxel and oblimersen/adriamycin leads to synergistic antitumor effects in preclinical trials, a combination of TAC with oblimersen looks very promising to further increase the efficacy of TAC [17, 18]. Furthermore, oblimersen has only a moderate toxicity and could easily be combined with other chemotherapy regimens without compromising their safety [19–22]. Oblimersen can be given safely in combination with intense chemotherapy and the down-regulation of Bcl-2 may correlate with response to chemotherapy [23].

Preoperative chemotherapy in breast cancer is an ideal model to evaluate the effects (safety and activity) of new compounds in this type of tumor. The possibility to have an easy access to untreated tumor cells before treatment, to compare this with the surgical specimen after treatment and to have additional tumor material from a biopsy during treatment, enables to give an insight into biologic effects of the used treatment on the tumor cells.

The objectives of this dose-finding phase I study were to define the maximum tolerated dose (MTD), the safety profile and the preliminary clinical and pathological efficacy of one cycle oblimersen in combination with TAC followed by five cycles TAC as NST in patients with PBC.

patients and methods

study design

This phase I study was carried out to define the MTD for phase II studies, safety profile and efficacy of escalating doses of oblimersen in combination with standard dose of TAC in cycle 1 followed by five cycles of standard TAC as NST in PBC patients. This trial was stopped due to premature end of the project after three dose groups were completed. The study protocol was reviewed and approved by the ethics committee of the University of Heidelberg, Germany. The study was conducted following the Declaration of Helsinki in accordance with the Harmonized Tripartite Guideline for Good Clinical Practice and in accordance with applicable regulatory requirements. Written informed consent was obtained from each participating patient before enrollment.

patient eligibility

Patient eligibility criteria included histologically confirmed previously untreated PBC T2–4a–c N0–3 M0, age ≥ 18 years, Karnofsky performance status ≥ 80%, adequate hematologic (absolute neutrophil count $\geq 1.5 \times 10^9$/l, platelet count $>100 \times 10^9$/l), hepatic, renal and cardiac function. Patients were excluded if they had inflammatory breast cancer, any prior treatment for breast cancer or simultaneous concurrence of all of the following factors: age > 35 years, tumor size < 5 cm, estrogen receptor (ER) and progesterone receptor (PgR) positivity, no suggestion of axillary lymph node involvement by palpation or ultrasound and grading 1–2 because patients with a concomitance of these factors may not require (neo-)adjuvant chemotherapy.

immunohistochemistry and histology

Histological diagnosis was confirmed by initial core cut biopsy. Paraffin-embedded tumor tissue from this biopsy was stained using an automated immunohistochemical technique (BioTek TechMate™, BioTek Solutions, Newport Beach, CA) with strict adherence to the staining protocol. The following primary antibodies were used (clones in brackets): Her2 (A0485), ER (1D5) and PgR (PR88) (all reagents from DakoCytomation Ltd, Ely, UK). Hormone receptor positivity was assumed when the semiquantitative score was at least 3 points out of a maximum of 12 points [24]. Her2 immunoreaction was scored from 0 to 3 with respect to cell membrane staining. In the case of a Her2 score 2, FISH was carried out.

drug administration

Oblimersen in combination with TAC was given as continuous i.v. infusion on days 1–7 for one cycle in combination with a fixed dose of 75 mg/m^2 i.v. docetaxel, a fixed dose of 50 mg/m^2 i.v. adriamycin and a fixed dose of 500 mg/m^2 i.v. cyclophosphamide (TAC regimen) on day 5. Following one cycle of oblimersen combined with TAC, the TAC regimen alone was administered every 3 weeks for five cycles. The dose of oblimersen was planned to be escalated according to the scheme outlined in Table 1 until dose-limiting toxicity (DLT) would occur in more than one of three, two of six or three of nine patients. To increase the probability to have patients with adequate tumor biopsies at all biopsy time points available in all oblimersen dose levels, nine patients were included in each cohort. An intrapatient dose escalation was not allowed. DLT included febrile neutropenia (grade 4 neutropenia with a body temperature >38.5°C), grade 4 thrombocytopenia and any grade 3 or 4 non-hematologic toxicity except for grade 3 alopecia, nausea or vomiting. MTD would be defined as the dose at which not more than one of three, two of six or three of nine patients experience DLT. Dexamethason 8 mg was administered orally (p. o.) every 12 h for a total of six doses starting 12 h before TAC administration. As commonly recommended, prophylactic pegfilgrastim (6 mg s.c. fixed dose) the day

Table 1. Dose escalation scheme of oblimersen

		Dose of TAC in mg/m^2 IV	Dose of oblimersen in mg/kg/day i.v.
Dose level	Scheduled number of patients	Day 5	Days 1–7
Starting dose	9	75/50/500	3
First escalation	9	75/50/500	5
Second escalation	10	75/50/500	7

after TAC and prophylactic antibiotic therapy with ciprofloxacin 500 mg p. o. twice daily were administered for 10 days starting 5 days after TAC infusion. Adjuvant endocrine treatment in case of positive ER or PgR was applied according to current national guidelines. No trastuzumab was administered.

blood serum sample collection and analysis

Each blood sample was identified by study code, subject number, date and time of sampling and sample number. Approximately 5 ml of blood was collected at the following time points: PK1 (before oblimersen infusion on day 1), PK2 (24 h after start of oblimersen infusion on day 2), PK3 (before start of TAC infusion on day 5), PK4 (1 h after TAC infusion on day 5), PK5 (2 h after TAC infusion on day 5) and PK6 (end of oblimersen infusion on day 7). Blood samples were drawn into EDTA tubes which were placed in a wet ice bath and centrifuged for 20 min with 2200 *g* at 4°C. The resulting plasma was transferred into polypropylene tubes and frozen at −20°C until shipment. For analysis, frozen plasma samples were shipped on dry ice to the Department of Drug Metabolism Pharmacokinetics at Aventis, Bridgewater, NJ. Before sample analysis, a one-day prestudy qualification was carried out. The human plasma concentrations for oblimersen (analyte structure, 5′-TCT CCC AGC GTG CGC CAT-3′) and its N-1 (analyte structure, 5′-TCT CCC AGC GTG CGC CA-3′) and N-2 (analyte structure, 5′-TCT CCC AGC GTG CGC C-3′) metabolites were analyzed using a capillary gel electrophoresis with ultraviolet absorbance detection (CGE/UV) method [25]. Using 0.5 ml of EDTA plasma, the calibration range extended from a lower limit of quantification of 30 up to 4480 ng/ml. The quantitation range extended up to 35 000 ng/ml with 10-fold dilution for oblimersen and its N-1 and N-2 metabolite. The coefficients of determination (r^2) for the standard curves were ≥0.9739 over seven analysis runs. Accuracy, defined as the percentage bias between the nominal and the mean measured concentrations of quality controls, ranged from −1.6% to 2.7% ($n = 41$) for oblimersen, from −0.5% to −0.2% ($n = 41$) for its N-1 and from −2.5% to 11.1% ($n = 41$) for its N-2 metabolite in plasma over the analysis period. The mean accuracy of the dilution controls (1 : 10) was −3.8% for oblimersen, −6.3% for its N-1 and −6.3% for its N-2 metabolite. The precision of the assays, established by the coefficient of variation of the quality controls, was better than or equal to 9.4% in plasma.

evaluation of toxicity and clinical response

For all safety data, the observation period was divided into two segments: adverse events at cycle 1 and adverse events after cycle 1, i.e. during cycles 2–6. The terminus 'at cycle 1' encompassed the time period from start of oblimersen infusion until 21 days after the first administration of TAC. 'After cycle 1' denotes the time period from the second administration until 21 days after the sixth administration of TAC. Adverse events, i.e. any unfavorable and unintended sign, symptom, syndrome or illness that developed or worsened during the observation period, were reported according to the National Cancer Institute—Common Toxicity Criteria (NCI-CTC) version 3.0 regardless of the relationship to treatment. Clinically relevant abnormal results of diagnostic procedures including abnormal laboratory findings requiring unscheduled diagnostic procedures or treatment measures or resulting in withdrawal from the study were considered to be adverse events.

Before treatment, after two and four cycles of TAC and before surgery, the greatest tumor diameter was measured by palpation and breast ultrasound. Furthermore, before treatment and before surgery, clinical tumor response was assessed according to Revised Evaluation Criteria in Solid Tumors by mammography and, if indicated, by magnetic resonance imaging with the size of the tumor at baseline serving as reference [26]. Patients proceeded to surgery within 4 weeks after receiving the last dose of chemotherapy. If the final tumor size allowed breast-conserving surgery, the following guidelines were observed: (i) surgical margins were free of invasive or noninvasive breast cancer and, if required, repeat excision was carried out; (ii) an adequate cosmetic result was anticipated and (iii) if cosmetically acceptable, the whole previously involved area was excised. In patients without tumor in diagnostic imaging or palpation or an unfavorable ratio of tumor to breast size, a biopsy specimen of adequate size was taken from a representative area. All patients undergoing a breast-conserving procedure received standard radiotherapy to the remaining breast. Radiotherapy to the chest wall or regional lymph nodes was carried out according to the national standards. Pathological tumor response was assessed on all specimens removed at surgery according to Sinn et al. [27]. pCR was defined as no microscopic evidence of residual viable tumor cells (invasive or noninvasive) in any resected specimen of the breast and axillary nodes.

statistical analysis

Descriptive statistical methods were used for the analysis of all parameters. All results were presented by dose level. The bioanalytical data were acquired and integrated using the Millennium 32 version 4.0 software (Waters, Milford, MA). Corrected peak area results were exported from Millennium 32 software into Watson version 7.0 (PSS Inc., Wayne, PA) in an in tab-delimited text file format. Watson was used to calculate standard curve parameters and concentration data for oblimersen and its N-1 and N-2 metabolites. The normalized areas for each analyte (corrected area of analyte divided by corrected area of internal standard; where corrected area equals peak area divided by migration time) were plotted against nominal standard concentrations to construct calibration curves. The equation of best fit was obtained by fitting the data to a quadratic equation using nonlinear least-squares regression analysis with $1/x^2$ weighting and used to calculate concentrations of the unknown samples and quality controls. Concentration data were reported in three significant figures as the free acid for oblimersen and its N-1 and N-2 metabolites. Calculations of summary statistics were carried out in Watson using the rounded concentration data.

The pharmacokinetic parameters of oblimersen and its N1- and N2-metabolite were calculated using the SAS/Stat Software (SAS Institute Inc., Heidelberg, Germany). Actual sampling times were used for the pharmacokinetic analysis. For predose samples, the actual sampling time was set to zero. The pharmacokinetic parameter determined for oblimersen was area under the serum concentration versus time curve (AUC) from time point zero to 96 h after the beginning of drug administration (AUC_{0-96}). Due to large indefinable variations of the serum concentration curves, it was not possible to analyze the time point 96–168 h after begin of infusion.

results

From June 2004 to September 2005, 28 patients were enrolled into this phase I study (University of Heidelberg 20 patients, University of Munich five patients, University of Frankfurt three patients). Median age was 50 years (range 35–64), median Karnofsky performance status 90% (range 90%–100%), 61% of patients had clinically T2 tumors, 68% had a ductal-invasive histology and 86% were node positive. In all, 43% of tumors were poorly differentiated, 36% hormone receptor negative and 18% overexpressed Her2. Patient and tumor characteristics in detail are given in Table 2.

Oblimersen was given i.v. on days 1–7 in cycle 1 at three different dose levels. Nine patients received 3 mg/kg/day (cohort I), nine patients 5 mg/kg/day (cohort II) and 10 patients 7 mg/kg/day (cohort III). No deaths occurred. Five patients discontinued the study treatment prematurely. Three

Table 2. Patient and tumor characteristics

Characteristics	Dose level			
	3 mg/kg/day	5 mg/kg/day	7 mg/kg/day	Total (%)
	n = 9	*n* = 9	*n* = 10	*n* = 28
Age, years				
Median	47	49	55.5	50
Range	38–54	35–61	41–64	35–64
Karnofsky performance status, %				
Median	90	90	90	90
Range	90–100	90–100	90–100	90–100
Tumor size				
T1	0	0	0	0 (0)
T2	6	4	7	17 (61)
T3	2	5	2	9 (32)
T4	1	0	1	2 (7)
Nodal status				
N0	2	1	1	4 (14)
N1	6	7	8	21 (75)
N2	0	1	1	2 (7)
N3	1	0	0	1 (4)
Histological grading				
Well differentiated	1	0	1	2 (7)
Moderately differentiated	4	6	4	14 (50)
Poorly differentiated	4	3	5	12 (43)
Hormone receptor status				
ER and/or PgR positive	7	6	5	18 (64)
ER and PgR negative	2	3	5	10 (36)
Her2 status				
Score 3+ or 2+/FISH positive	2	2	1	5 (18)
Score 0, 1+ or 2+/FISH negative	7	7	9	23 (82)

ER, estrogen receptor; PgR, progesterone receptor; Her2, human epidermal growth factor receptor 2.

patients went off study following cycle 1. In one patient, the diagnosis was corrected to noninvasive ductal carcinoma and two patients did not wish to continue. Two patients left the study following cycle 4 due to grade 4 pancytopenia with sepsis and accidental intervertebral disc protrusion, respectively, which delayed the following treatment for >2 weeks. All five patients gave their agreement to follow further medical history. A total of 24 patients (86%) experienced an adverse event, 78%, 89% and 90% in cohort I, II and III, respectively.

At cycle 1, the most frequent adverse events were fatigue, nausea, anorexia, dysgeusia, headache and alopecia (for details see Table 3). Most adverse events were mild to moderate (NCI-CTC grade ≤ 2), one alert term event occurred. Only four serious adverse events occurred, all in cohort III, in relation to the study drug, requiring prolonged hospitalization. Two patients developed an NCI-CTC grade 3 influenza-like illness, one patient a NCI-CTC grade 3 pyrexia and one patient an NCI-CTC grade 4 life-threatening leucopoenia. There was an increasing percentage of neutropenia NCI-CTC grade 4 with increasing dose of study drug at cycle 1 (0% of patients in cohort I, 25% of patients in cohort II and 55% of patients in cohort III; Table 4).

After cycle 1, during the following treatment with five cycles of TAC, the most frequent adverse events were constipation,

Table 3. Number (%) of patients with at least one non-hematological adverse event at cycle one (OTAC)

Type of adverse event system organ class	Dose level							
	3 mg/kg/day		5 mg/kg/day		7 mg/kg/day		All	
	N = 9	%	*N* = 9	%	*N* = 10	%	*N* = 28	%
Upper abdominal pain	0	0	0	0	3	30	3	11
Constipation	0	0	3	33	0	0	3	11
Nausea	2	22	6	67	2	20	10	36
Stomatitis	1	11	0	0	3	30	4	14
Chills	0	0	1	11	2	20	3	11
Fatigue	3	33	5	56	3	30	11	39
Anorexia	1	11	3	33	1	10	5	18
Dysgeusia	1	11	3	33	0	0	4	14
Headache	1	11	1	11	2	20	4	14
Irregular menstruation	1	11	2	22	0	0	3	11
Dyspnea	1	11	2	22	0	0	3	11
Alopecia	3	33	6	67	2	20	11	39
Hyperhidrosis	1	11	1	11	1	10	3	11

nausea, stomatitis, fatigue and alopecia (for details see Table 5). Sporadic asymptomatic abnormal electrocardiograms were recorded without apparent association to study treatment which completely resolved without specific therapy. Four serious adverse events occurred. One patient in cohort I developed an NCI-CTC grade 4 hyperemesis following cycle 3. In cohort II, one patient had an NCI-CTC grade 3 pancytopenia and one patient a life-threatening sepsis following cycle 4. In cohort III, one patient developed an NCI-CTC grade 4 leucopoenia after cycle 6. There was a trend to less severe neutropenia with higher pretreatment of oblimersen (63% of patients in cohort I, 44% of patients in cohort II and 25% of patients in cohort III). Only one patient developed NCI-CTC grade 3 anemia and thrombocytopenia, no NCI-CTC grade 4 anemia or thrombocytopenia was observed.

The bioanalytical data of 153 samples from 27 patients at six different time points were assessable for analysis (PK1: 27, PK2: 26, PK3–PK6: 25). The samples of one patient could not be analyzed because of technical problems during analysis preparation. Due to a large indefinable variation of the serum concentration time curves beginning with the first blood sample following chemotherapy, only the first three blood samples (hours 0–96) were analyzed. Median serum concentration time curve profile hours 0–96 for all dose levels of oblimersen are shown in Figure 1A–C. An example of the large variation in the serum concentration time curve of oblimersen at all six different time points is shown in Figure 1D. Maximum serum concentrations were reached within 24 h after start of the infusion of oblimersen. The mean area under the serum concentration time curves from time point 0 to 96 were 113.13 μg/ml × h, 184.03 μg/ml × h and 323.87 μg/ml × h in cohort I, II and III, respectively (Table 6). Interindividual variability in all pharmacokinetic parameters of oblimersen and its N1- and N2-meabolite was relatively low.

Six patients were not assessable for response due to missing tumor lesion measurement before surgery. Among the 19 patients assessable for clinical response, seven patients (28%)

Table 4. Number of hematological adverse events during at cycle one (OTAC) and after cycle one (TAC × 5) treatment

Blood cell type	NCI—CTC grade	OTAC				TAC × 5			
		3 mg/kg/day ($N = 9$)	5 mg/kg/day ($N = 9$)	7 mg/kg/day ($N = 100$	All ($N = 28$)	3 mg/kg/day ($N = 8$)	5 mg/kg/day ($N = 9$)	7 mg/kg/day ($N = 8$)	All ($N = 25$)
Neutrophils	0	8	6	4	18	3	4	6	13
	1	–	–	–	–	–	–	–	–
	2	–	–	–	–	–	–	1	1
	3	–	–	–	–	–	–	–	–
	4	–	2	5	7	5	5	1	11
Leukocytes	0	6	3	1	10	1	2	–	3
	1	2	2	3	7	–	2	1	3
	2	–	3	–	3	2	–	2	4
	3	1	–	4	5	–	1	3	4
	4	–	1	2	3	5	4	2	11
Hemoglobin	0	6	5	7	18	3	–	2	5
	1	3	4	3	10	3	6	4	13
	2	–	–	–	–	2	2	2	6
	3	–	–	–	–	–	1	–	1
	4	–	–	–	–	–	–	–	–
Platelets	0	5	6	6	17	4	3	3	10
	1	2	2	4	8	3	4	3	10
	2	2	1	–	3	1	1	2	4
	3	–	–	–	–	–	1	–	1
	4	–	–	–	–	–	–	–	–

NTC—CTI, National Cancer Institute—Common Toxicity Criteria.

Table 5. Number (%) of patients with at least one non-hematological adverse event during TAC treatment

Type of adverse event system organ class	Dose level							
	3 mg/kg/day		5 mg/kg/day		7 mg/kg/day		All	
	$N = 8$	%	$N = 9$	%	$N = 8$	%	$N = 25$	%
Tachycardia	0	0	0	0	3	38	3	12
Conjunctivitis	1	13	3	33	1	13	5	20
Upper abdominal pain	1	13	0	0	2	25	3	12
Constipation	1	13	4	44	3	38	8	32
Diarrhea	1	13	2	22	1	13	4	16
Dyspepsia	2	25	0	0	1	13	3	12
Nausea	4	50	5	56	5	63	14	56
Stomatitis	4	50	2	22	3	38	9	36
Fatigue	3	38	6	67	5	63	14	56
Mucosal inflammation	0	0	2	22	1	13	3	12
Peripheral edema	2	25	2	22	1	13	5	20
Anorexia	1	13	3	33	2	25	6	24
Bone pain	1	13	1	11	1	13	3	12
Muscular weakness	1	13	3	33	1	13	5	20
Dysgeusia	1	13	4	44	1	13	6	24
Neuropathy	4	50	0	0	1	13	5	20
Irregular menstruation	1	13	3	33	0	0	4	16
Dyspnea	4	50	2	22	1	13	7	28
Alopecia	3	38	6	67	4	50	13	52
Nail disorder	0	0	3	33	1	13	4	16
Hot flush	1	13	2	22	1	13	4	16

achieved a complete (three patients in cohort I, two patients in cohort II and two patients in cohort III) and also seven patients (28%) a partial remission for an overall clinical response rate of 56%. Among 25 patients who received at least two cycles of chemotherapy, in 10 patients (40%) a mastectomy and in 14 patients (56%) a breast-conserving surgery was carried out; data from one patient were not available. In all patients, a conventional resection of the axilla was done with a median

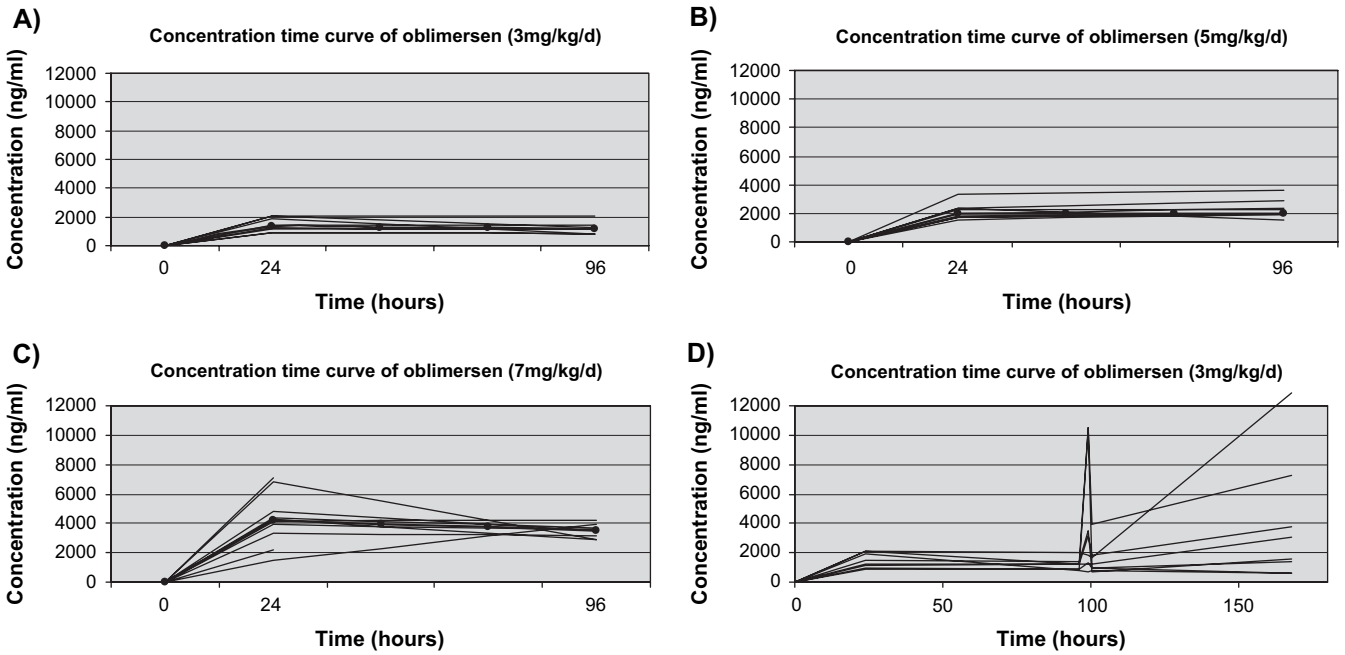

Figure 1. Concentration time curve 96 h following start of oblimersen infusion in the three different dose groups (A–C) and 168 h following start of oblimersen infusion in the 3 mg dose group (D).

Table 6. AUC_{0-96} of oblimersen, its N1 metabolite and N2 metabolite

	Dose of oblimersen (mg/kg/day)	Number of patients (*N*)	Mean µg/ml × h	Median	Minimum	Maximum	Standard deviation
Oblimersen	3	8	113.13	109.96	73.38	172.08	34.08
	5	9	184.03	169.44	142.92	286.92	44.72
	7	8	323.87	330.18	213.6	429.96	63.70
N1 metabolite	3	8	67.28	67.83	40.30	103.2	20.67
	5	9	114.26	95.04	75.53	183	36.65
	7	8	195.03	196.5	153.72	234.84	30.67
N2 metabolite	3	8	36.54	37.79	4.61	55.84	15.46
	5	9	66.05	58.51	41.92	95.66	21.65
	7	8	112.35	112.76	71.88	170.87	37.16

number of 16 axillary lymph nodes excised (range 2–29 axillary lymph nodes). None of the patients received a sentinel lymph node biopsy. It was necessary to reoperate five patients as resection margins were not free of tumor following the first surgery. Tumors showed no pathologically documented response (pathological response) in three patients. In 19 patients, regressive changes of tumor were found, five of them (20%) showed a histologic complete remission and 14 patients a partial remission (Table 7).

discussion

Several agents to control regulatory proteins that lead to apoptosis are currently undergoing clinical and preclinical evaluations. The Bcl-2-family, also overexpressed in patients with malignant tumors of the breast [8], is one of those important targets which regulate the apoptosis. Down-regulating Bcl-2 by using a Bcl-2-directed antisense oligonucleotide can increase chemotherapy-induced apoptosis and the overall effectiveness of the chemotherapy can be improved [28–30]. Combining different chemotherapeutics with oblimersen has been shown to be feasible and promising showing e.g. significantly better response rates and progression-free survival in advanced melanoma patients [19–22]. Oblimersen can also be given safely in combination with intense chemotherapy and the down-regulation of Bcl-2 may correlate with response to chemotherapy [23]. The schedule of administration for the used combination of oblimersen and TAC is on the basis of a maximal down-regulation of Bcl-2 and apoptosis by the prolonged infusion of oblimersen before and during exposure to the chemotherapy [31, 32]. The maximum reduction of bcl-2 can be noted after 72–120 h

Table 7. Tumor response in the three different dose groups after one cycle of OTAC and at least one cycle of TAC

	Dose level				
	3 mg/kg/day $N = 8$	5 mg/kg/day $N = 9$	7 mg/kg/day $N = 8$	All $N = 25$	%
Clinical response					
CR	3	2	2	7	28
PR	3	3	1	7	28
NC	1	1	3	5	20
na	1	3	2	6	24
Pathological response					
CR	3	2	0	5	20
PR	4	4	6	14	56
NC	0	2	1	3	12
na	1	1	1	3	12
Type of operation					
Mastectomy	2	4	4	10	40
BCS	6	5	3	14	56
na	0	0	1	1	4
Re-excision					
Yes	3	2	0	5	20
No	5	7	7	19	76
na	0	0	1	1	4

CR, complete response; PR, partial response; NC, no change; na, not available; BCS, breast-conserving surgery.

after start of oblimersen infusion. It is recommended to start the infusion of oblimersen at least 96 h before chemotherapy with a minimum dose above 2 mg/kg/day. Due to unclear time point of induction of apoptosis after chemotherapy, the oblimersen infusion should be continued 24–48 h after application of cytostatic treatment.

Bcl-2 which is overexpressed in patients with breast cancer is one of the most important targets that regulate apoptosis [5]. We carried out a multicentric phase I study of continuous infusion of oblimersen administered in combination with TAC as NST in patients with PBC. We found an MTD of oblimersen of 7 mg/kg/day administered on days 1–7 in combination with standard-dosed TAC administered on day 5. At that dose level, serious adverse events like leucopenia, pyrexia and influenza-like symptoms occurred in four of 10 patients. As we sought to identify a dose of oblimersen that would not compromise the ability to administer TAC at full dose and schedule, we stopped further dose escalation. The safety profile of oblimersen in combination with TAC is well consistent with the adverse events observed with TAC alone, although the addition of oblimersen might increase influenza-like symptoms, in particular pyrexia. This is in line with other studies of oblimersen and irinotecan where a higher number of pyrexia could be demonstrated [33]. This phenomenon can be explained as a reaction to the tumorlysis and the cytokine release [18, 34]. Furthermore, there was an increasing incidence of NCI-CTC grade 4 leucopoenia and neutropenia with increasing doses of oblimersen. This suggests an additional cytotoxic effect of oblimersen against the white cell line, which was shown in a study with the combination of irinotecan and oblimersen [33]. In contrast to the combination of oblimersen, fludarabine and cyclophosphamide compared with the control arm without oblimersen, the incidence of severe neutropenia was slightly higher in the control arm [18]. Anemia and thrombocytopenia were not pronounced following oblimersen as compared with TAC alone. However, numbers are rather small and interpretation should be made with caution. The mean serum concentrations in all dose groups were reached 24 h after starting the infusion. Stable plasma levels of oblimersen could also be achieved after 10–24 h in trials of oblimersen in combination with dacarbazin or docetaxel [32, 35, 36]. In this study, a large variation at the time of the chemotherapy in the serum concentrations could be seen. The recommended dose of 7 mg/kg/day of oblimersen was shown to be feasible in different phase II and III studies and could safely be combined with chemotherapy [33, 37, 38]. Following only one cycle of oblimersen in combination with TAC and five cycles of TAC alone, we observed a pCR defined as no tumor reside in breast and axilla in five of 22 patients (18%) assessable for pathological response. Only three of 22 patients did not show any pathological signs of chemotherapy response. This is in the range of pCR rates reported from NST trials with TAC alone [3]. However, oblimersen was only administered for 7 days concurrent with cycle 1. Furthermore, the primary objectives of this phase I trial were to determine MTD and safety rather than clinical and pathological efficacy. The numbers of patients are too small to draw firm conclusions regarding efficacy of oblimersen in breast cancer and further studies are needed. Additional parameters are currently evaluated (e.g. Bax, Bcl-2) to identify any pretreatment tumor or patient characteristics or posttreatment changes induced by oblimersen which are predictive of response.

In conclusion, oblimersen up to a dose of 7 mg/kg/day given as a 24-h infusion on days 1–7 is feasible and can be safely administered in combination with standard TAC regimen on day 5 as NST in patients with PBC. The preliminary efficacy warrants further evaluation of oblimersen in combination with every cycle of the TAC regimen in a randomized trial.

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