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Original Research

A phase I study of PankoMab-GEX, a humanised glyco-optimised monoclonal antibody to a novel tumour-specific MUC1 glycopeptide epitope in patients with advanced carcinomas

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Abstract ***Background:*** A phase I open-label dose-escalation study was conducted to define the safety, tolerability, and pharmacokinetics (PK) of PankoMab-GEX, a glyco-optimised humanised IgG1, with high affinity to a novel tumour-specific glycopeptide epitope of MUC1 (TA-MUC1) with excellent preclinical anti-tumour activity.
Patients and methods: Seventy-four patients with advanced TA-MUC1-positive carcinomas received PankoMab-GEX intravenously every 3 (Q3W), 2 (Q2W), or 1 (QW) week in doses of 1–2200 mg in a three-plus-three dose-escalation design until disease progression (NCT01222624).
Results: No maximum tolerated dose was reached. Adverse events were mainly mild-to-moderate infusion-related reactions (IRRs) by the first infusion in 45% of patients. Only one dose-limiting toxicity, a grade III IRR, was observed. PankoMab-GEX exhibited linear PK over all doses. Mean terminal half-life was 189 ± 66 h (Q3W), without dose dependency. A target trough level ≥50 μg/mL was reached after one infusion with doses ≥1700 mg Q3W in

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80% of patients. Clinical benefit in 60 evaluable patients included one complete response in a patient with ovarian cancer treated 483 d and confirmed disease stabilisation in 19 patients lasting a median (range) of 23 (10–109) weeks. All but two of the patients with clinical benefit had received a compounded total dose ≥700 mg over a 3-week period, including 8 of 12 (67%) patients with ovarian cancer.

Conclusion: PankoMab-GEX is safe, well tolerated, and showed promising anti-tumour activity in advanced disease. A phase IIb study is ongoing evaluating the efficacy of PankoMab-GEX as a maintenance therapy in advanced ovarian cancer.

1. Introduction

MUC1 is a transmembrane mucin expressed on the ductal cell surface of normal glandular epithelia and on some haematopoietic cells, which is overexpressed and aberrantly glycosylated in carcinomas, including cancer stem cells [1–3]. Tumour-associated alterations of MUC1 not only favour tumour progression and metastasis but also turn it into a tumour antigen that can be targeted specifically for cancer immunotherapy [4,5]. The molecule's large extracellular domain consists mainly of a variable number of normally highly glycosylated peptide tandem repeats, which are underglycosylated in tumour-associated MUC1 [6]. Exposure of immunogenic core protein epitopes and carbohydrate antigens, such as Tn and Thomsen Friedenreich (TF), through aberrant glycosylation generate (glyco)peptide epitopes that provide tumour-specific targets for immunotherapy [7–11].

PankoMab-GEX is a glyco-optimised humanised IgG1 monoclonal antibody (MAb) that binds with high affinity to a novel carbohydrate-induced conformational epitope (TA-MUC1) on MUC1 that is highly expressed in a broad variety of carcinomas and is virtually absent on normal tissues or blood cells [5,8,9,12,13]. Tn or TF on the highly immunogenic PDTRP sequence of the tandem repeat are a crucial part of the epitope of PankoMab-GEX. Its dependence on glycosylation, its tumour specificity, and its affinity differentiate PankoMab-GEX from other anti-MUC1 antibodies [12]. PankoMab-GEX also binds effectively to cancer stem cells (article in preparation).

Glycosylation of an antibody influences its anti-tumour efficacy and effector mechanisms [14,15]. The glycosylation of PankoMab-GEX was optimised with the GlycoExpress™ system using human glyco-engineered production cell lines to give it a human glycosylation pattern, leading to improved antibody-dependent cell cytotoxicity (ADCC) and phagocytosis (ADCP), as well as apoptosis of TA-MUC1-expressing tumour cells (data on file; Glycotope GmbH, Berlin, Germany). Tumour cell killing through natural killer (NK) cell and macrophage-mediated ADCC and ADCP relies on the constant (Fc) domain of the antibody, but its efficacy is strongly influenced by Fc gamma receptor IIIa (FcγRIIIa) polymorphism [16]. Glyco-optimisation can lead to increased ADCC and ADCP activity [17], whereby the minimisation of core fucose and the maximisation of galactose on the Fc N-glycans play the crucial roles for enhanced activity, with increase in bisecting GlcNAc also involved in enhancing ADCC. PankoMab-GEX has been improved by approximately fivefold to eightfold in its NK cell-mediated cytotoxicity and expresses also a particularly strong ADCP against TA-MUC1-positive tumour cells. Extent of ADCC of tumour cell lines expressing TA-MUC1 mediated by PankoMab-GEX depended on TA-MUC1 expression levels by the individual cell lines and donor peripheral blood mononuclear cells (PBMC). Maximum specific lysis of target cells was achieved at PankoMab-GEX™ concentrations between 3 and 20 μg/mL depending on the different donors. The ability of PankoMab-GEX to induce phagocytosis was shown in a conjugate formation assay using differently fluorescent-labelled TA-MUC1-positive target cells and monocyte-derived macrophages (investigators brochure, data on file; Glycotope GmbH). Co-localisation of macrophages and tumour cells in the presence of PankoMab-GEX was observed by flow cytometry and ingestion of tumour cells by macrophages by confocal microscopy. PankoMab-GEX™ induced apoptosis of target cell lines expressing TA-MUC1 after cross-linking by protein G. It is expected that *in vivo*, cross-linking of the antibody is induced by Fcγ-receptor-bearing cells. As reported for MUC1 antibody induced by specific vaccination [18], no CDC activity of PankoMab-GEX was observed using human serum complement and the TA-MUC1-positive cell line ZR-75-1. The *in vivo* anti-tumour activity of PankoMab-GEX was studied in nude mice xenografted with TA-MUC1-positive human tumour cell lines. The models showed strong anti-tumour activities in dose levels ranging from 0.02 to 12.5 mg/kg (investigators brochure, data on file; Glycotope GmbH).

The present study in patients with advanced metastatic carcinomas was undertaken to investigate the safety and tolerability of PankoMab-GEX, to establish

the dose for phase II trials, and to evaluate its pharmacokinetics (PK), immunogenicity, and preliminary clinical efficacy.

2. Patients and methods

2.1. Study population

This multicentre phase I study was conducted in three institutions in Italy, Switzerland, and Germany between November 2009 and May 2013. The study population consisted of patients with advanced TA-MUC1-positive solid tumours measurable according to RECIST 1.1 guidelines [19] that had failed and exhausted available standard therapy and had progressive disease at study entry. TA-MUC1 positivity was assessed by PankoMab-GEX staining of tumour sections (Supplement A). Inclusion/exclusion criteria are summarised in Supplement A.

Local ethics committee approval and patient's written informed consent were obtained. The study was conducted in accordance with Good Clinical Practice guidelines and the Declaration of Helsinki.

2.2. Study design and dosing

PankoMab-GEX (PankoMab-GEX™; Glycotope GmbH) was administered intravenously in 250–500 mL saline, in 2 h for doses up to 500 mg and 3 h for higher doses, in three dosing schedules: every 3 (Q3W), 1 (QW), and 2 weeks (Q2W). Patients were sequentially enrolled in a three-plus-three dose-escalation design, starting with Q3W (1, 10, 50, 150, 300, 500, 700, 900, 1100, 1300, 1500, 1700, and 2200 mg flat dose), followed by QW (300, 400, 500, 600, and 700 mg) once the Q3W cohort of 900 mg was completed. Based on PK evaluation, Q2W (a 900-mg loading dose followed 1 week later by 1200 mg every 2 weeks) was tested. Premedication was introduced in the course of the study (H1 and H2 antagonists, paracetamol and corticosteroids) to minimise infusion-related reactions (IRR). Treatment was continued until disease progression, occurrence of intolerable toxicity, or withdrawal of consent.

2.3. Dose-limiting toxicity

Toxicities were graded according to NCI CTCAE, version 3.0. Dose-limiting toxicity (DLT) was defined as any haematological or non-haematological toxicity of grade III or more, a grade II allergic reaction at first infusion, or a grade II autoimmune reaction during or after first infusion of PankoMab-GEX. Three evaluable patients were entered at each dose level. If a DLT occurred, the cohort was expanded to six patients before escalating to the next dose level; an maximum tolerated dose (MTD) was reached if more than two patients experienced a DLT at any given dose in the first cycle.

2.4. Pharmacokinetic analysis

Blood for PK analysis was collected at specified time points, and PankoMab-GEX serum levels were measured (Glycotope GmbH; see Supplement A).

PK parameters were derived from the individual patient serum concentration–time profiles using non-compartmental methods (FUNCALC 3; Prolytic GmbH, Frankfurt, Germany). The maximum (C_{max}) and minimum (C_{min}) serum concentration after administration were directly taken from analytical data. Dose linearity and proportionality of the PK parameters C_{max}, C_{min}, $AUC_{0-\infty}$ and $AUC_{0-tlast}$ were investigated over the dose range, based on the individual values by linear regression analysis. A trough level of 50 μg/mL of the drug was set as target for the study based on *in vitro* tests in which maximum ADCC is achieved at PankoMab-GEX concentrations of 3–20 μg/mL depending on the FcγRIIIa polymorphism of the donor PBMCs and reported trough levels of established antibodies, such as cetuximab and trastuzumab, which also mediate ADCC [20,21]. Accumulation of PankoMab-GEX was assessed by dividing the trough concentrations after the second and subsequent doses by the trough concentration after the first dose.

2.5. Immunogenicity

Samples were screened for anti-drug antibody (ADA) and ADA titration was performed on samples confirmed as positive (Glycotope GmbH; see Supplement A). Cytokines IL-1β, IL-8, IFN-γ and TNF-α serum levels were analysed during the first infusion at specified time points. Additionally, factor C3a (19 patients) and eosinophilic cationic protein (ECP, 27 patients) were measured within 24 h after the first PankoMab-GEX infusion.

2.6. MUC1 serum levels

MUC1 serum levels were measured for Q3W and QW with a CA15-3 commercial ELISA (MP Biomedicals, Orangeburg, NY, USA).

2.7. Tumour assessment

Tumour response in patients with measurable disease was evaluated according to RECIST 1.1 guidelines [16]. Baseline imaging was assessed within 4 weeks before the first PankoMab-GEX dose and then every 8 weeks until withdrawal from study. Imaging included computed tomography and/or magnetic resonance imaging of target lesions. Clinical activity was assessed by measuring the response (complete response [CR], partial response [PR], stable disease [SD]). SD and PR needed imaging confirmation after 8 and 4 weeks, respectively.

2.8. Statistical analysis

Descriptive statistics were used on the intent-to-treat population to summarise patient demographics and baseline characteristics, treatment administration, safety parameters, PK variables, and efficacy end-points (SAS 9.1). The distribution of anti-tumour responses was analysed in contingency tables according to dose levels.

3. Results

3.1. Patient characteristics

Demographics and disease characteristics of the 74 patients enrolled in the study are contained in Table 1. Upon entering the study, all patients had progressive advanced metastatic disease and had exhausted available standard treatment procedures.

3.2. Drug exposure, safety and tolerability

The three dosing schedules had similar adverse event profiles. Reason for study termination was disease progression (65 cases), death (1), adverse event (4), withdrawal of informed consent (1), lost to follow-up (1), and investigator's decision (2).

Number of infusions administered, drug exposure and incidence of IRRs are listed in Table 2. The majority of IRRs was mild-to-moderate and resolved quickly after a pause in the infusion and symptomatic medication. Infusion duration was extended from 2 to 3 h after an IRR grade II (Q3W, 500 mg), initially erroneously classified, as an allergic reaction occurred. The drug was withdrawn and three additional patients were recruited at the same dose level without further incidents. A grade III IRR, classified as a DLT, was observed in a patient (Q3W, 900 mg; premedication, anti-histamine), consisting of facial rash, abdominal

Table 1
Demographic and baseline disease characteristics of the study population.

	Q3W (N = 52)	QW (N = 18)	Q2W (N = 4)	Total (N = 74)
Age in years (median, range)	58 (25–81)	54.5 (41–74)	58 (50–70)	57 (25–81)
Gender (N, %)				
- Male	15 (28.8)	5 (27.8)	1 (25)	21 (28.4)
- Female	37 (71.2)	13 (72.2)	3 (75)	53 (71.6)
Ethnic origin (N, %)				
- Caucasian/white	51 (98.1)	18 (100)	4 (100)	73 (98.6)
- Hispanic	1 (1.9)			1 (1.4)
ECOG performance status (N, %)				
- 0	30 (57.7)	7 (38.9)	3 (75)	40 (54.1)
- 1	22 (42.3)	11 (61.1)	1 (25)	34 (45.9)
Time from diagnosis in months (median, range)[a]	37.6 (6–290)	27.3 (12–103)	41 (7–104)	35 (6–290)
Primary tumour (N, %)				
- Colorectal cancer	21 (40.4)	3 (16.7)	1 (25)	25 (33.8)
- Ovarian cancer	16 (30.8)	3 (16.7)	1 (25)	20 (27)
- Breast cancer	6 (11.5)	1 (5.6)	–	7 (9.5)
- Non-small cell lung cancer	–	7 (38.9)	–	7 (9.5)
- Pancreatic cancer	3 (5.8)	2 (11.1)	–	5 (6.8)
- Gastro-oesophageal cancer	1 (1.9)	1 (5.6)	–	2 (2.7)
- Bladder cancer	1 (1.9)	–	–	1 (1.4)
- Prostate cancer	1 (1.9)	–	–	1 (1.4)
- Pseudomyxoma peritonei	1 (1.9)	–	–	1 (1.4)
- Cholangiocarcinoma	–	1 (5.6)	–	1 (1.4)
- Adenoid cystic carcinoma (salivary gland)	1 (1.9)	–	–	1 (1.4)
- Cervix carcinoma	–	–	1 (25)	1 (1.4)
- Oropharyngeal carcinoma	1 (1.9)	–	–	1 (1.4)
- Carcinoma of unknown primary	–	–	1 (25)	1 (1.4)
Prior antibody therapy (N, %)				
Any prior antibody therapy[b]	22 (42.3)	5 (27.8)	1 (25)	28 (37.8)
- Bevacizumab	15 (28.8)	4 (22.2)	1 (25)	20 (27)
- Cetuximab	9 (17.3)	2 (11.1)	1 (25)	12 (16.2)
- Panitumumab	1 (1.9)	0	0	1 (1.4)
- Ramucirumab	1 (1.9)	0	0	1 (1.4)
- Trastuzumab	2 (3.8)	1 (5.6)	0	3 (4.1)
CA15-3 serum levels in U/mL, (median, range)[c]	30 (7–1633)	33 (14–857)	25 (19–49)	31 (7–1633)

ECOG = Eastern Cooperative Oncology Group.
[a] Date of first dose of study drug − date of initial diagnosis of the disease + 1.
[b] Nine patients received two antibodies.
[c] CA15-3 was measured before start of the first (all patients) and the second infusion (27 and 17 patients for Q3W and QW, respectively).

Table 2
Extent of exposure to PankoMab-GEX, number of infusions administered, and number (%) of patients with IRR following the first infusion of PankoMab.

Schedule	Every 3 weeks (Q3W)								Every 2 weeks (Q2W)	Every week (QW)						Total population
Extent of exposure in days, median (range)									121 (1–210)	36 (15–288)						
	23 (1–760)															
Number of infusions administered, median (range)									10 (1–15)	6 (3–36)						
	2 (1–33)															
Dose (mg)	1–300	500	700	900	1100	1300	1500	1700	2200	1200	300	400	500	600	700	
No. of patients	16	6	3	6	3	3	3	6	6	4	3	3	3	3	6	74
IRR, no. of patients (%)[a,b]																
- Grade I	0	1 (17)	2 (67)	4 (67)	0	0	0	1 (17)	3 (50)	0	0	0	0	0	1 (17)	12 (16)
- Grade II	0	3 (50)	1 (33)	0	2 (67)	1 (33)	2 (67)	3 (50)	1 (17)	1 (25)	2 (67)	1 (33)	0	3 (100)	0	20 (27)
- Grade III	0	0	0	1 (17)	0	0	0	0	0	0	0	0	0	0	0	1 (1)
Total	0	4 (67)	3 (100)	5 (84)	2 (67)	1 (33)	2 (67	4 (67)	4 (67)	1 (25)	2 (67)	1 (33)	0	3 (100)	1 (17)	33 (45)

IRR = infusion-related reaction.
[a] Percentage is calculated using the number of patients in the column heading as denominator.
[b] Symptoms of IRR included dyspnoea (12 patients, 36%), face flushing (5 patients, 15%), feeling hot (8 patients, 24%), skin rash (8 patients, 24%), pruritus (2 patients, 6%), chills (2 patients, 6%), tachycardia (3 patients, 9%), hypertension (2 patients, 6%), abdominal pain (5 patients, 15%), back (subscapular) pain (4 patients, 12%), cough (4 patients, 12%), nausea (2 patients, 6%), and choking sensation (2 patients, 6%).

pain, choking sensation, and hypotension that resolved quickly after withdrawal of the drug and symptomatic medication. Three additional patients were recruited at the same dose level; no further DLTs were observed in the course of the study. An MTD was not reached. The drug was withdrawn in one additional patient who experienced a grade II IRR during the second infusion (total of drug withdrawals due to IRRs 3 of 74, 4%). Only 4 of 63 (6.3%) patients experienced mild-to-moderate IRRs with the second infusion; the patients had received premedication. Only six IRRs (1.9%) were observed in all 322 subsequent infusions, of which three were of grade II, and the patients had received no premedication.

Initially, no premedication was administered. It was introduced for the first and all subsequent infusions from Q3W, 900 to 1700 mg dose, but was later restricted to the first infusion or the one following an IRR in the previous infusion for Q3W 2200 mg, Q2W and QW to limit the negative effect of steroids on ADCC [22].

Treatment emergent adverse events (TEAEs) other than IRR possibly related to the drug were mild-to-moderate and few in number. Seven patients experienced a possibly drug-related grade III TEAE: three cases of asthenia and one case each of decreased white blood cell count, increased transaminases, nausea, and pneumonia were observed. No grade IV or V drug-related TEAE occurred.

No increase of cytokines, C3a, or ECP serum levels was observed. Seven patients developed low titres of ADA between the second and tenth infusion and 28 d after the last infusion. The highest log 2 titre was 6.91.

3.3. Pharmacokinetics

Mean serum concentrations of PankoMab-GEX per dose cohort measured in the Q3W schedule during the first infusion are illustrated in Fig. S1 and PK parameters for the three schedules are contained in Table S1. PK could be evaluated in 49 of 52 patients in the Q3W schedule. PankoMab-GEX exhibited linear PK with respect to dose across the whole 1- to 2200-mg dose range, as demonstrated by the dose-linear increase in C_{max}, C_{min} and $AUC_{0-tlast}$ (Fig. S2). No dose dependency was observed for $t^1/_2$. For Q3W, $t^1/_2$ (mean ± standard deviation) was 189 ± 66 h; comparable values were obtained for Q2W. Lower $t^1/_2$ values (108 ± 28 h) were observed for QW, reflecting the shorter dosing interval. CL and VZ showed comparable values over the dose range.

A trough level (C_{min}) of 50 μg/mL was reached after one infusion with doses ≥1700 mg Q3W and ≥500 mg QW and in Q2W in 8 of 10 (80%), 11 of 11 (100%) and 1 of 3 (33%) evaluable patients, respectively. The accumulation ratios of C_{min} in Q3W after three infusions (seven patients) ranged from 1.22 to 2.36; a steady state was achieved after three infusions in three

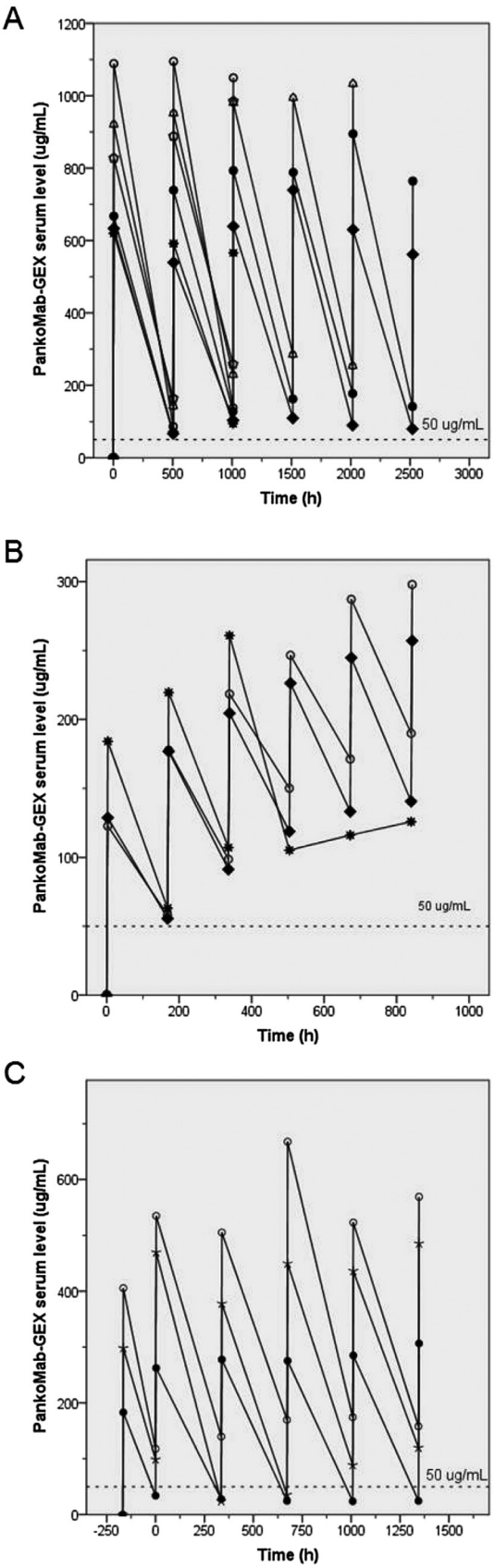

Fig. 1. Concentration–time profiles of repeated infusions of PankoMab-GEX measured in individual patients before and 3 h after start of infusion: (A) three-weekly schedule (Q3W), dose cohort 2200 mg, *N* 6; (B) weekly dosing schedule (QW), dose cohort 500 mg, *N* 3; (C) two-weekly dosing schedule (Q2W), 1200 mg (time point 0), preceded a week before by a 900-mg loading dose, *N* 3. The dotted line indicates the targeted trough level of 50 μg/mL of PankoMab-GEX.

of seven patients, with very small variations observed in the other four patients. The accumulation ratios of C_{min} in QW after five infusions ranged from 1.91 to 3.49 without dose dependency; a steady state had not been attained in most of the patients. No reliable statement can be given for Q2W due to the low number of patients. An example of individual serum concentration–time profiles for each dose schedule is illustrated in Fig. 1.

3.4. MUC1 serum levels

MUC1 (CA15-3) serum levels before first infusion of PankoMab-GEX are contained in Table 1. The percentage of CA15-3 (mean ± standard deviation) in relation to its corresponding baseline values was 89.8 ± 36.8% and 80.8 ± 21.9% before the second infusion, for Q3W and QW, respectively.

3.5. Clinical anti-tumour activity

All patients had progressive disease at study entry. Tumour response was evaluated in 60 patients. Fourteen patients were not evaluated because of either premature withdrawal (13 patients) or lost to follow-up (1 patient). A clinical benefit was observed in 28 of 60 (47%) patients: 1 CR and 27 SD, with 19 of the SD confirmed. Anti-tumour activity (1 CR and 17 confirmed SD) was observed in 18 of 42 (43%) of patients treated with a compounded total dose of PankoMab-GEX ≥700 mg over a 3-week period, but only in 2 of 18 (11%) patients who received <700 mg PankoMab-GEX in the same period ($p = 0.019$). No correlation was found between MUC1 expression levels on the primary tumour and clinical response.

One patient with serous ovarian cancer (Q3W, 1100 mg, 23 infusions) progressive after debulking surgery and chemotherapy with carboplatin/paclitaxel and carboplatin/doxorubicin achieved CR before progressing after 483 d on therapy (Fig. 2). Twenty-three of 42 (53%) patients treated with a compounded total dose ≥700 mg PankoMab-GEX over a 3-week period had a best overall response of SD (median 19 weeks, range 9–109 weeks) that was confirmed in 17 (40%) patients (median 23 weeks, range 10–109 weeks), 10 patients in Q3W, 3 in Q2W and 4 in QW. A patient with non-small cell lung cancer (NSCLC) (QW, 600 mg, 36 infusions) progressive after three chemotherapy regimens and radiotherapy achieved a (unconfirmed) PR after 164 d of treatment; the response was classified as an SD, which lasted 295 d. The longest confirmed SD (759 d) with long-lasting

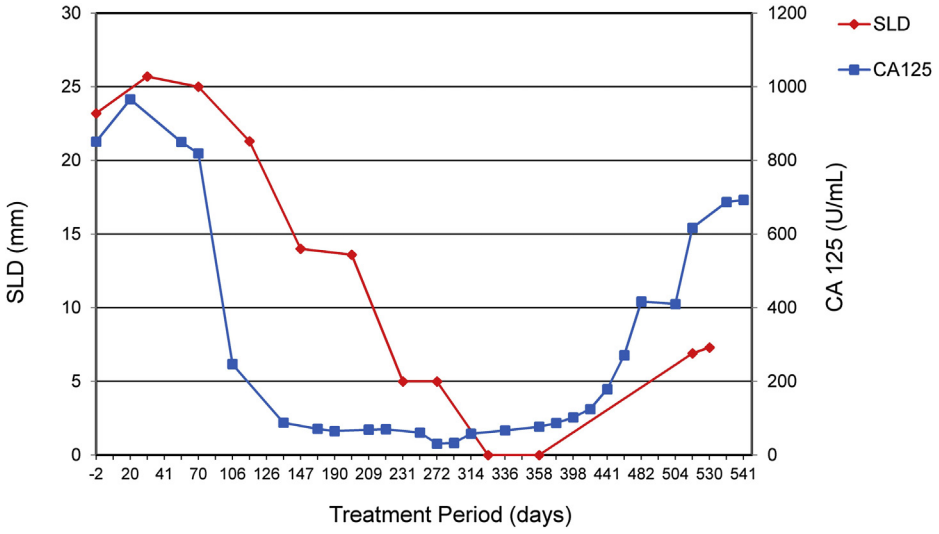

Fig. 2. Sum of longest diameter (SLD, in mm) of target lesions (two peritoneal nodules) and CA 125 serum levels (U/mL) during treatment with 1100 mg PankoMab-GEX every 3 weeks (Q3W) in a patient with a serous papillary carcinoma of the ovary. An initial increase of target lesions and CA 125 levels was followed by a slow and sustained decrease until normalisation of CA 125 levels by day 272 and disappearance of target lesions by day 315 of treatment. Non-target lesions (axillary, para-aortal and aorto-caval lymph nodes, all smaller than 15 mm at baseline) had all disappeared by day 191 of treatment. The patient received a total of 23 infusions until progression after 483 d of treatment.

stabilisation of all target lesions (21% reduction) and reduction of CA 125 to normal levels was observed in a patient (Q3W, 900 mg, 33 infusions) diagnosed with pseudomyxoma peritonei stage IV 2 years previously and progressive after neoadjuvant chemotherapy, debulking surgery and chemotherapy. Clinical benefit was observed in a broad variety of primary tumours, but more frequently in ovarian and NSCLC. Primary tumours of patients with clinical benefit included 8 of 15 (53%) ovarian cancer and 3 of 7 (43%) NSCLC. Seven patients experienced a confirmed SD for at least 210 d, three of them (43%) had ovarian cancer. Waterfall plots of changes from baseline of target lesions are shown in Fig. 3.

4. Discussion

This is the first clinical study of a humanised monoclonal antibody directed to a MUC1 conformational glycopeptide epitope highly expressed in adenocarcinomas and glycoengineered for enhanced Fc-mediated anti-tumour activity. PankoMab-GEX was safe and very well tolerated after repeated administration in three different schedules. The MTD was not reached after a maximum dose of 2200 mg. Similar to other therapeutic MAbs, IRRs were mostly mild-to-moderate and confined to the first infusion [23]. IRRs were not associated with cytokine release, activation of complement, or an allergic reaction. Only seven patients developed a low ADA response that was not related to dose and did not interfere with prolonged treatment. Interestingly, five in seven of them had SD, ranging from 133 to 760 d.

Consistent with its high specificity, PankoMab-GEX exhibits a long half-life that allows one to three weekly schedules and shows linear PK over all doses. The lack of an antigen sink agrees with the virtual absence of expression of its epitope on normal tissues. Circulating MUC1 did not affect the linearity of distribution of the drug, reflecting a lack of significant binding of PankoMab-GEX to it. The target trough level of 50 μg/mL was amply reached at the higher doses with all three administration schedules. Based on the PK and clinical results, a loading dose of 500 mg PankoMab-GEX followed a week later by 1700 mg administered every 3 weeks was chosen for the first phase II study as a maintenance therapy in ovarian carcinoma.

As PankoMab-GEX binds to a tumour-specific conformational glycopeptide epitope on MUC1 that is extensively expressed in many tumour types, and its potential for immunotherapy is very wide. TA-MUC1 is expressed in >80% of lung, breast and ovarian adenocarcinomas [24–26] and in 92–100% of clear cell, endometroid and serous adenocarcinomas of the ovary [26]. A clinical benefit that was more frequent at higher doses was observed in 47% of evaluable patients, all with advanced progressive disease at study entry and a broad variety of adenocarcinomas. The best responses and the highest frequency of confirmed SD were observed in patients with ovarian and lung cancer. In the subgroup of ovarian cancer patients, the clinical benefit rate at total dose levels of ≥700 mg was 75% (9 of 12), including one complete responder and SD in four patients resistant/refractory to platinum therapy. As is characteristic of immunotherapy [27], responses take time to establish and can be preceded by an initial

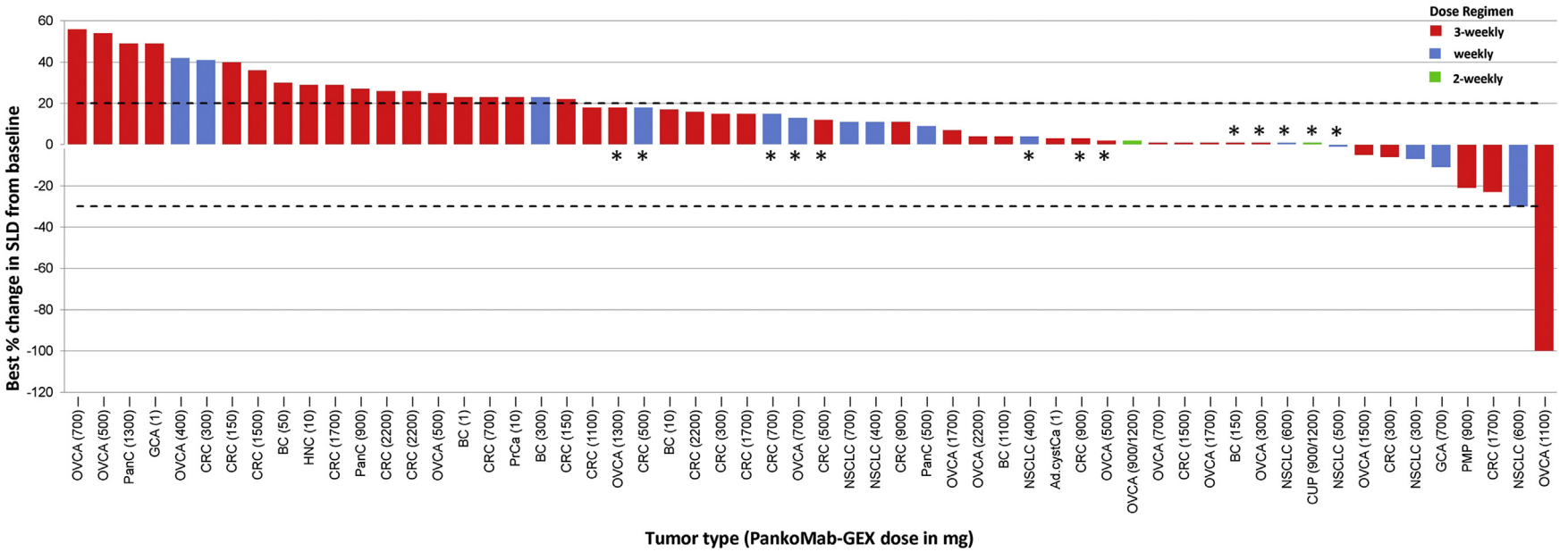

Fig. 3. Waterfall plots of the best percent change from baseline in sum of longest diameters (SLD) for target lesions. Baseline is defined as the last non-missing value before the first dose of PankoMab-GEX. Only 58 patients in the total population (*N* 74) had valid baseline and post-baseline values. Tumour assessment was not performed in 16 patients because of early withdrawal due to clinical deterioration (*N* 10) or adverse event (*N* 3) or no target lesions (*N* 3). The dotted lines indicate the cutoff for PR (−30%) and progressive disease (+20%). Bars marked with an asterisk denote 13 patients with stable target lesions but progression because of new lesions. Abbreviations: Ad.cystCa, adenoid cystic carcinoma; BC, breast cancer; CRC, colorectal cancer; CUP, carcinoma of unknown origin; GCA, gastric cancer; HNC, head and neck cancer; NSCLC, non-small cell lung cancer; OVCA, ovarian cancer; PanC, pancreatic cancer; PMP, pseudomyxoma peritonei; PrCa, prostate cancer.

increase in lesion size and markers, as was observed in the patient who developed a CR (Fig. 2).

In conclusion, PankoMab-GEX was very well tolerated with mild-to-moderate adverse events, mainly IRRs at first infusion. Following the promising preliminary efficacy in patients with ovarian cancer, a double-blind, placebo-controlled, randomised phase IIb study in patients with advanced ovarian carcinoma has been started.

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Conflict of interest statement

Marc Salzberg, Bruno Dietrich, Hans Baumeister, and Steffen Goletz are employees of Glycotope GmbH. All remaining authors have declared no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejca.2016.05.003.

References

[1] Zotter S, Hageman PC, Lossnitzer A, Mooi WJ, Hilgers J. Tissue and tumor distribution of human polymorphic epithelial mucin. Cancer Rev 1988;11–12:55–101.
[2] Dent GA, Civalier CJ, Brecher ME, Bentley SA. MUC1 expression in hematopoietic tissues. Am J Clin Pathol 1999;111:741–7.
[3] Karsten U, Goletz S. What makes cancer stem cell markers different? SpringerPlus 2013;2:301.
[4] Kufe DW. Mucins in cancer: function, prognosis and therapy. Nat Rev Cancer 2009;9:874–85.
[5] Karsten U, von Mensdorff-Pouilly S, Goletz S. What makes MUC1 a tumor antigen? Tumor Biol 2005;26:217–20.
[6] Hattrup CL, Gendler SJ. Structure and function of the cell surface (tethered) mucins. Annu Rev Physiol 2008;70:431–57.
[7] Burchell J, Taylor-Papadimitriou J. Effect of modification of carbohydrate side chains on the reactivity of antibodies with core protein epitopes of the *MUC1* gene product. Epithelial Cell Biol 1993;2:155–62.
[8] Karsten U, Diotel C, Klich G, Paulsen H, Goletz S, Müller S, et al. Enhanced binding of antibodies to the DTR motif of MUC1 tandem repeat peptide is mediated by site-specific glycosylation. Cancer Res 1998;58(12):2541–9.
[9] Karsten U, Serttas N, Paulsen H, Danielczyk A, Goletz S. Binding patterns of DTR-specific antibodies reveal a glycosylation-conditioned tumor-specific epitope of the epithelial mucin (MUC1). Glycobiology 2004;14(8):681–92.
[10] Tang CK, Katsara M, Apostolopoulos V. Strategies used for MUC1 immunotherapy: human clinical studies. Expert Rev Vaccines 2008;7(7):963–75.
[11] von Mensdorff-Pouilly S, Petrakou E, Kenemans P, et al. Reactivity of natural and induced human antibodies to MUC1 mucin with MUC1 peptides and n-acetylgalactosamine (GalNAc) peptides. Int J Cancer 2000;86(5):702–12.
[12] Danielczyk A, Stahn R, Faulstich D, Löffler A, Märten A, Karsten U, et al. PankoMab: a potent new generation anti-tumour MUC1 antibody. Cancer Immunol Immunother 2006; 55(11):1337–47.
[13] Fan XN, Karsten U, Goletz S, Cao Y. Reactivity of a humanized antibody (hPankoMab) towards a tumor-related MUC1 epitope (TA-MUC1) with various human carcinomas. Pathol Res Pract 2010;206(8):585–9.
[14] Natsume A, Niwa R, Satoh M. Improving effector functions of antibodies for cancer treatment: enhancing ADCC and CDC. Drug Des Devel Ther 2009;3:7–16.
[15] Goede V, Klein C, Stilgenbauer S. Obinutuzumab (GA101) for the treatment of chronic lymphocytic leukemia and other B-cell non-Hodgkin's lymphomas: a glycoengineered Type II CD20 antibody. Oncol Res Treat 2015;38(4):185–92.
[16] Dall'Ozzo S, Tartas S, Paintaud G, Cartron G, Colombat P, Bardos P, et al. Rituximab dependent cytotoxicity by natural killer cells: influence of FCGR3A polymorphism on the concentration-effect relationship. Cancer Res 2004;64:4664–9.
[17] Herter S, Birk MC, Klein C, Gerdes C, Umana P, Bacac M. Glycoengineering of therapeutic antibodies enhances monocyte/macrophage-mediated phagocytosis and cytotoxicity. J Immunol 2014;192(5):2252–60.
[18] Ragupathi G, Liu NX, Musselli C, Powell S, Lloyd K, Livingston PO. Antibodies against tumor cell glycolipids and proteins, but not mucins, mediate complement-dependent cytotoxicity. J Immunol 2005;174(9):5706–12.
[19] Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). Eur J Cancer 2009;45: 228–47.
[20] Erbitux – Annex I – Summary of product characteristics. http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/000558/WC500029119.pdf.
[21] Bruno R, Washington CB, Lu JF, Lieberman G, Banken L, Klein P. Population pharmacokinetics of trastuzumab in patients with HER2+ metastatic breast cancer. Cancer Chemother Pharmacol 2005;56(4):361–9.
[22] Kumai T, Oikawa K, Aoki N, Kimura S, Harabuchi Y, Kobayashi H. Assessment of the change in cetuximab induced ADCC activity of NK cells by steroid. Head Neck 2015. http://dx.doi.org/10.1002/hed.23906 [Epub ahead of print].
[23] Maggi E, Vultaggio A, Matucci A. Acute infusion reactions induced by monoclonal antibody therapy. Expert Rev Clin Immunol 2011;7(1):55–63.
[24] Dian D, Janni W, Kuhn C, et al. Evaluation of a novel anti-mucin 1 (MUC1) antibody (PankoMab) as a potential diagnostic tool in human ductal breast cancer; comparison with two established antibodies. Onkologie 2009;32(5):238–44.
[25] Kuemmel A, Single K, Bittinger F, Faldum A, Schmidt LH, Sebastian M, et al. TA-MUC1 epitope in non-small cell lung cancer. Lung Cancer 2009;63(1):98–105.
[26] Dian D, Lenhard M, Mayr D, Heublein S, Karsten U, Goletz S, et al. Staining of MUC1 in ovarian cancer tissues with PankoMab-GEX detecting the tumour-associated epitope, TA-MUC1, as compared to antibodies HMFG-1 and 115D8. Histol Histopathol 2013;28(2):239–44.
[27] Wolchok JD, Hoos A, O'Day S, Weber JS, Hamid O, Lebbé C, et al. Guidelines for the evaluation of immune therapy activity in solid tumors: immune-related response criteria. Clin Cancer Res 2009;15(23):7412–20.