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A phase I study with adecatumumab, a human antibody directed against epithelial cell adhesion molecule, in hormone refractory prostate cancer patients

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ABSTRACT

Aim of the study: Adecatumumab (also known as MT201) is a human recombinant IgG1 monoclonal antibody binding with low affinity to epithelial cell adhesion molecule (EpCAM). To explore safety, pharmacokinetics and pharmacodynamics of adecatumumab, a phase I trial in patients with hormone refractory prostate cancer (HRPC) was performed.
Methods: Twenty patients were treated with two adecatumumab infusions on days 0 and 14 in cohorts with doses of ten up to 262 mg/m^2.
Results: Adecatumumab was well tolerated at all doses tested, and no maximum tolerated dose reached. Most adverse events were mild or moderate with pyrexia and nausea being most frequent. The highest dose of adecatumumab induced shortly after infusion robust and transient increases of TNF-alpha serum levels. At all doses, significant transient declines of peripheral natural killer cells were observed shortly after antibody infusions. Adecatumumab had a serum half-life of 15 days, and immune responses to the antibody were not detected.
Conclusions: A benign safety profile, long serum half-life and low immunogenicity do warrant further exploration of adecatumumab for treatment of EpCAM-expressing neoplasia.

1. Introduction

EpCAM is a surface glycoprotein expressed on many carcinomas of different origin, including prostate, breast and gastrointestinal cancers.[1] Its presumed biological function is to serve as a homotypic, calcium-independent cell adhesion molecule.[2] More recent studies have shown that overexpression of EpCAM confers an oncogenic phenotype to quiescent cells, and is required for the invasive, migratory and proliferative potential of certain tumour cells.[3,4] EpCAM may also contribute to immune evasion of tumour cells. When ectopically expressed or transferred to dendritic cells by tumour cell

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debris, EpCAM strongly impairs MHC class II-dependent antigen presentation.[5] Using tissue microarray technology, 3900 samples from 134 histologically different tumour types and subtypes were analysed for EpCAM expression.[6] Particularly high and frequent expression was found in patients with prostate cancer, consistent with three other immunohistochemical studies.[7–9]

Because of its overexpression on a wide variety of epithelial tumour cells, many immunotherapeutic approaches have employed EpCAM as target antigen. These include murine[10] and humanised monoclonal antibodies,[11,12] immunotoxins,[13] and vaccine-based approaches.[14] Most clinical experience to date has been gathered with the low-affinity murine anti-EpCAM antibody edrecolomab (Panorex®).[10] Major limitations of this murine antibody are its short serum half-life in man, its high immunogenicity and its low effectiveness with immune effector cells of human origin. The latter results in considerably reduced antibody-dependent cellular cytotoxicity (ADCC) with human immune effector cells when compared to humanised or human anti-EpCAM antibodies.[15] Nevertheless, clinical activity was reported for edrecolomab in Dukes C colorectal cancer patients in two out of three phase III trials.[16,17] Moreover, edrecolomab was well tolerated. This is in contrast to two humanised anti-EpCAM antibodies, ING-1 and 3622W94, both of which caused acute pancreatitis as a dose limiting toxicity in phase I studies.[11,12] It is not known whether this acute organ toxicity was due to their higher binding affinity for EpCAM, their human IgG1 format, recognition of a different epitope on EpCAM, or combinations thereof.

Adecatumumab (also known as MT201) is a human, recombinant monoclonal IgG1 antibody binding to EpCAM with rather low affinity.[15,18] Being fully human, adecatumumab is expected to be less immunogenic than murine, chimeric or humanised antibodies, potentially translating into a lower frequency of neutralising antibody responses in patients. The human nature of adecatumumab is also expected to result in a half-life as observed with other human IgG1, and in optimal compatibility with human immune effector mechanisms. Adecatumumab has demonstrated cytotoxic activity against tumour cell lines of multiple origins including breast, prostate, ovarian, gastric and colon cancers *in vitro*,[15,19,20] in an animal model,[15] and *ex vivo* with human ovarian cancer samples.[19] The prime mechanisms of action of adecatumumab are antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC).

This is the first phase I study performed with adecatumumab for assessment of safety and tolerability in patients with HRPC and to determine pharmacokinetic (PK) and pharmacodynamic (PD) properties of the antibody.

2. Patients and methods

2.1. Description of study drug

Adecatumumab is a human monoclonal antibody of the IgG1 isotype binding with low affinity to EpCAM.[15] The variable domains of the antibody were derived from human B cell repertoires ensuring closeness to human germline and lowest possible immunogenicity.[18] For clinical use, adecatumumab was recombinantly produced by a contract manufacturer in CHO cells. The antibody is formulated in phosphate-buffered saline at 10 mg/mL antibody and can be stored at 4–8 °C for more than 1 year. Preclinical data have shown that adecatumumab eliminated prostate cancer cell lines 22RV1 and LNCAP by ADCC, while PC3 and DU145 had to low an EpCAM level to show susceptibility to ADCC by adecatumumab. The antibody was well tolerated in cynomolgus monkeys treated for 3 months with multiple doses up to 180 mg/kg.

2.2. Trial design and selection of starting dose

The starting dose of 10 mg/m^2 was selected based on toxicology data from two other humanised anti-EpCAM IgG1 antibodies called ING-1 and 3622W94. Both of these high-affinity humanised antibodies had an MTD of 30 mg total dose, i.e. approximately 17 mg/m^2.[11,12] Given that adecatumumab is of the same immunoglobulin isotype as ING-1 and 3622W94, we considered it reasonable to start dosing of adecatumumab below the MTD of the two clinically tested antibodies. A standard 3+3 design was used to escalate by increasing the adecatumumab dose with each cohort by 100%, and in the case of the occurrence of CTC grade 1 or 2 toxicities, by 60%. Cohort 1 had only two patients. Because of the limited amount of available antibody, adecatumumab doses had to be restricted to two biweekly administrations and a maximum of 262 mg/m^2 (6–7 mg/kg). Continuation of the study with newly produced material was not a possibility because the production process for adecatumumab for phase I material had been terminated in favour of development of a new cell line and fermentation process. Nevertheless, maintenance of trough levels for 4 weeks and reaching an antibody dose as used by other IgG1 cancer therapies was considered sufficient for an initial safety assessment of adecatumumab in man.

2.3. Study design and patient selection

This was an open-label, non-randomised, multi-center, dose-escalation phase I study in which adecatumumab was administered intravenously to patients with advanced HRPC. The study objectives were to assess safety, pharmacokinetics, pharmacodynamics and the maximum tolerated dose (MTD) of adecatumumab in patients with HRPC given as two single intravenous (i.v.) infusions at a 2-week interval.

Patients aged over 18 with histologically confirmed relapsed HRPC were enrolled in two centers (Augsburg and Munich; Germany). Relapse was confirmed by a continuous increase of PSA during two consecutive measurements at least 2 weeks apart. Patients with previous monoclonal antibody treatment, a high tumour burden, Eastern Cooperative Oncology Group (ECOG) status >2, chemo- or radiotherapy within 3 months, CNS metastases, poor life expectancy, other severe diseases, or liver, renal or bone marrow dysfunction were excluded. Hormonal therapy at entry had to be left unchanged during the study.

Each patient received two i.v. infusions of adecatumumab over 30 min separated by 2 weeks provided that there was no dose limiting toxicity during or after the first infusion. A total

of 20 patients were treated in seven dose cohorts. Two patients were treated with the lowest dose of 10 mg/m^2 and three patients each at doses of 20, 40, 64, 102, 164 and 262 mg/m^2.

The study was conducted in accordance with the Declaration of Helsinki, and Good Clinical Practice. All patients gave their voluntary informed consent and signed a consent document that had been approved by the institutional review boards of both participating centres.

2.4. *Safety measurements*

Adverse events (AEs) and vital signs were recorded every other day until study day 23, and weekly thereafter. During infusion, vital signs and oxygen saturation were recorded every 5 min, and at longer intervals thereafter. Physical examination, clinical laboratory values, ECG, oxygen saturation and vital signs were recorded before the start of the study and weekly during the treatment and follow up periods. Adverse drug reactions (ADRs) that occurred after the start of the study treatment were graded by the investigator according to NCI Common Toxicity Criteria (CTC, version 2.0). The frequency of grade 3 or 4 toxicities (i.e. ADRs or AEs considered at least possibly related to the study drug by the investigator) was evaluated for each cohort with the aim to determine a maximum tolerated dose (MTD). MTD was defined as one dose level below the dose level that caused, in at least two out of three patients, a grade 3 or 4 toxicity. This toxicity was defined as dose limiting.

2.5. *Cell culture*

Chronic myeloid leukaemia cell line K562 was used for determination of natural killer (NK) cell activity and derived from the American Type Culture Collections (Manassas, VA, USA). Cells were cultured at 37 °C in a 5% CO_2 incubator in Iscove's modified Dulbecco's medium with 4 mM L-glutamine adjusted to contain 1.5 g/L sodium bicarbonate supplemented with 10% fetal bovine serum (Invitrogen GmbH, Karlsruhe, Germany).

2.6. *Pharmacodynamic measurements*

Cytokine levels for gamma-interferon (IFN-γ), tumour necrosis factor alpha (TNF-α), and interleukin 1 beta (IL-1β) were measured at baseline and frequently throughout the study using the Immulite system, an automated solid phase ELISA (enzyme linked immunoabsorbent assay) assay (DPC Biermann GmbH, Bad Nauheim, Germany). Interleukin 2 (IL-2) concentrations were measured using a standard ELISA (Beckman-Coulter GmbH, Krefeld, Germany). Prostaglandin E_2 (PGE_2) concentrations were determined using a validated commercial ELISA assay (Assay Designs Inc., Ann Arbor, MD, USA).

2.7. *Pharmacokinetic measurements*

Adecatumumab serum concentration was measured at predose and frequently during the treatment and observation period in i.v. collected blood samples. Time points of blood sample collection are depicted in Fig. 1. To determine adecatumumab concentrations, an ELISA-based assay has been developed and validated at Micromet. Monoclonal rat anti-adecatumumab antibody HD4A4 7B1 (Micromet AG, Germany) was coated at a final concentration of 1 mg/mL to a 96-well microtitre plate overnight at 2–8 °C. A blocking step was performed using PBS buffer with 1% BSA. Quality control samples, calibration standards and patient samples were diluted 1:20 and 1:50 in phosphate buffered saline (PBS) containing 5% and 2% human serum, respectively. Samples were incubated for 1–2 h at 25 ± 2 °C following washing using an ELISA plate washer (Tecan GmbH, Crailsheim, Germany). Next, a final concentration of 5 μg/mL biotinylated chicken polyclonal anti-adecatumumab solution (Davids Biotechnologie, Regensburg, Germany) containing 1% bovine serum albumin (BSA) was incubated for 1 h at 25 °C. After washing, 0.5 μg/mL streptavidin alkaline phosphatase (AP) solution with 1% BSA was added and incubated for 30 min at 25 °C. pNPP was then used as substrate for AP. Colour intensity of the antibody staining reaction was measured in a Power Wave plate reader with a 405 nm filter (Bio-Tek Instruments GmbH, Bad Friedrichshall, Germany). For evaluation of unknown adecatumumab concentrations in patient samples, KC4 software (Bio-Tek Instruments GmbH, Bad Friedrichshall, Germany) was used. The lower limit of quantification of the assay was 200 ng/ml serum.

2.8. *Immunogenicity measurements*

Human antibody responses against adecatumumab were determined using an ELISA-based assay. Adecatumumab (Micromet AG, Germany) was coated at a final concentration of 0.2 μg/mL to a 96-well microtitre plate overnight at 2–8 °C. Blocking was performed using PBS buffer with 1% BSA for 1 h at 25 °C. After washing, negative control serum from healthy donors, patient near pre- and post-dose serum as well as serum spiked as positive control with serial dilutions of rat monoclonal anti-adecatumumab antibody HD4A4 7B1 (Micromet AG, Germany), were transferred to the microtitre plates and incubated for 1.5 h at 25 °C. Plates were washed using

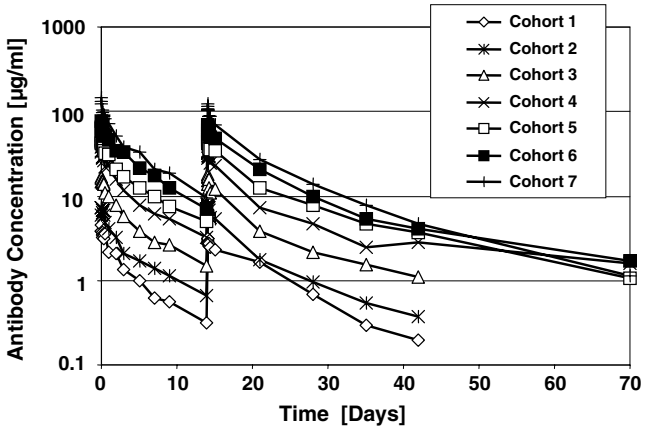

Fig. 1 – Serum concentration/time profiles of adecatumumab for all seven cohorts. Depicted is the arithmetic mean of each cohort.

an ELISA plate washer (Tecan GmbH, Crailsheim, Germany) followed by an incubation of biotinylated adecatumumab (Micromet AG, Germany) at a final concentration of 2 μg/mL in PBS containing 1% BSA for 1 h at 25 °C. Detection, staining and evaluation was carried out as described for the adecatumumab pharmacokinetic assay (see above). The assay sensitivity was adjusted to an upper limit of 95% negative and 5% false positive results to exclude false negative results, using samples from healthy donors as well as from a patient population ($n = 50$). Samples for immunogenicity evaluations were taken before and after treatment. The lower limit of quantitation of the assay was 100 ng/mL.

2.9. *Determination of natural killer cell number and activity*

Natural killer (NK) cell numbers were determined at baseline and at various time points after treatment using the Simultest IMK Lymphocyte Kit (Becton Dickinson GmbH, Heidelberg, Germany) following the manufacturer's recommendations. Antibody-labeled cells were analysed using a FACSCalibur flow cytometer (Becton Dickinson GmbH, Heidelberg, Germany). CD16/CD56 double-positive peripheral blood lymphocytes, i.e. NK cells, were determined as absolute numbers and as a percentage of total blood lymphocytes.

For determination of NK cell cytotoxic activity, peripheral blood mononuclear cells (PBMC) were prepared before and at various time points after treatment following conventional procedures. PBMC were washed with PBS buffer and resuspended in RPMI medium supplemented with 10% foetal calf serum (Invitrogen, Karlsruhe, Germany). K562 target cells lacking MHC class I and II molecules were labelled with 3,3′-dioctadecyloxacarbo-cyanine perchlorate (Sigma, Aldrich, Seelze, Germany), a fluorescent cell membrane dye allowing for flow cytometric separation of effector PBMC from target cells. Next, 5×10^6 PBMC were incubated with 1×10^5 labelled K562 cells at 37 °C over 2 h in RPMI medium in the absence or presence of 200 U/mL recombinant cytokine IL-2 (Sigma Aldrich, Seelze, Germany). Basal and IL-2 induced NK cell activity was monitored by uptake of propidium iodide (PI) dye (1 μg/mL) into nuclei of lysed K562 cells. Aliquots of 20,000 cells were analysed by flow cytometry using a FACSCalibur instrument. Cytotoxicity was quantitated as a percentage of K562 cell lysis.

2.10. *Clinical and biochemical response measurements*

Tumour lesions were assessed using standard imaging techniques such as computerised tomography (CT), magnetic resonance tomography (MRT), and standard X-ray before the start of study treatment and at day 42. Responses were re-evaluated by a reference radiologist. Tumour response was defined according to the Response Evaluation Criteria in Solid Tumours (RECIST).

PSA serum levels were determined immediately before the start of treatment (day 0) and in weekly intervals thereafter until week 6 (day 7, 14, 21, 28, 35, and 42) and at week 10 (day 70) using an Elecsys instrument and respective reagents (Roche Diagnostics, Penzberg, Germany) in a central laboratory. PSA measurements, before the start of the study, were performed in local laboratories using various assay procedures, and were employed to verify PSA rise. PSA levels at baseline were compared to day 42 and, where available, day 70 values. A 50–79% decline below starting level qualified as 'partial PSA response', a decline of 80% or greater as complete PSA response. PSA doubling time and weekly PSA net production were also assessed.

2.11. *Statistical analyses*

A two-sided sign test was performed based on per protocol data to assess whether there were statistically significant differences in the number of peripheral NK cells before and 12 h after the first dose, or, independently, after the second dose of adecatumumab. Statistical analysis of TNF serum levels was performed on all patients' data by a two-sided Wilcoxon rank sum test to analyse whether there was a significant difference of relative changes of TNF levels between low (1–5) and high dose levels (6 and 7).

3. Results

3.1. *Study population*

Twenty patients with HRPC were enrolled between September 2001 and July 2002 (Table 1). All cohorts with the exception of cohort 5 contained patients from both centres. All patients had advanced disease and were extensively pretreated. The mean time from first diagnosis was 3.6 years (range: 0.7–11.1 years). Twelve patients had tumour stages T3 or T4, and in 11 patients (55%) the Gleason score was $\geqslant 7$. Sixteen patients had a radical prostatectomy and all but one patient received hormonal treatment before study inclusion. All but one patient with a T4N1 tumour stage had documented metastatic disease. Total PSA levels before adecatumumab treatment varied between 2.0 and 1400 ng/mL with a mean PSA serum concentration (±SD) of 156.82 (± 324.43) ng/mL. Four of the six patients in cohorts 6 and 7 had the highest PSA baseline levels in the study. While all 20 patients were evaluable for safety assessment, three patients had to be excluded

Table 1 – Patient characteristics

Baseline characteristics	Value
Patient number (N):	20
Age (years): mean ± SD (range)	64.1 ± 5.4 (56–74)
Prior therapy N (%)	
Chemotherapy	6 (30%)
Hormone therapy	19 (95%)
Orchiectomy	6 (30%)
Radiotherapy	15 (75%)
Prostatectomy	16 (80%)
PSA levels (ng/ml)	
<4	2 (10%)
4–9.9	4 (20%)
10–19.9	2 (10%)
20–99.9	7 (35%)
100–999.9	4 (20%)
>1000	1 (5%)

from the per protocol response analysis for concomitant hormonal treatment or chemotherapy.

3.2. Safety assessment

Adecatumumab was generally well tolerated at all doses administered. No patient discontinued the study medication due to an adverse event. The majority of adverse events (AEs) were of mild or moderate severity. Only six out of a total of 120 AEs were considered severe, of which none were classified as related to the study drug by the investigators. Among a total of 77 clinical AEs considered to be drug-related by the investigators (Table 2), pyrexia was the most frequent (45%) followed by nausea (25%), feeling cold, diarrhea and vomiting (15% each). Laboratory abnormalities considered as drug-related were lymphopenia (25%), elevation in lactate dehydrogenase and decrease in activated PTT (20% each), increase in transaminases and disorders of white blood cells (15%). There was a significant increase in the incidence of adverse events per patient in cohorts 6 and 7 versus cohorts 1 to 5 ($p = 0.0003$, and $p = 0.0001$ for the drug-related AE; two-sided Wilcoxon rank sum test), suggesting a relationship between the dose of adecatumumab and frequency of adverse events.

Only two patients experienced grade 3 toxicities with a possible relationship to the study medication (Table 2). One was an elevation of glutamate transaminase (γ-GT) in cohort 3 and one a transient lymphocytopenia in cohort 7. No grade 4 toxicities according to CTC criteria were observed. In contrast to what has been observed with high-affinity humanised anti-EpCAM IgG1 antibodies[11,12] adecatumumab did at no dose, and at no time point during treatment and follow-up period, cause acute pancreatitis. Neither a significant increase of serum amylase or lipase above baseline levels was observed. A total of four serious adverse events were reported in four patients. Only one SAE, i.e. prolonged hospitalisation due to fever in cohort 3, was considered possibly related to the study drug.

Table 2 – Safety assessment of adecatumumab and summary of patients with possible drug-related CTC toxicities

Toxicities	Grade 1/2	Grade 3	Total **N = 20**
Clinical abnormalities	(Number of patients with toxicities)		
Fever	9	0	9 (45%)
Nausea	6	0	6 (30%)
Diarrhea	3	0	3 (15%)
Rigors, chills	3	0	3 (15%)
Abdominal pain or cramping	2	0	2 (10%)
Fatigue	2	0	2 (10%)
Hot flashes / flushes	2	0	2 (10%)
Sweating	2	0	2 (10%)
Vomiting	2	0	2 (10%)
Dyspnea	1	0	1 (5%)
Palpitation	1	0	1 (5%)
Salivary gland changes	1	0	1 (5%)
Laboratory abnormalities			
Hepatic other: LDH	4	0	4 (20%)
PTT	4	0	4 (20%)
γ-GT	2	1	3 (15%)
SGOT	3	0	3 (15%)
SGPT	3	0	3 (15%)
Alkaline phosphatase	1	0	1 (5%)
Hematology			
Lymphopenia	4	1	5 (25%)
Neutrophils/granulocytes	3	0	3 (15%)
Platelets	1	0	1 (5%)
Urine analysis			
Hematuria	1	0	1 (5%)
Proteinuria	1	0	1 (5%)

3.3. Immunogenicity

Sera of all 20 patients were analysed at baseline and at days 28, 35, and 42 for the presence of human antibodies against adecatumumab. Antibodies against adecatumumab were not detected at any of the time points. Likewise, there was no evidence for a neutralising response to adecatumumab from the pharmacokinetic analysis (see Fig. 1), as the serum half-life of adecatumumab was not significantly reduced in any of the 20 patients after the second infusion of the antibody.

3.4. Pharmacokinetics

The mean serum concentration and clearance profiles of adecatumumab from all seven cohorts are shown in Fig. 1. The main pharmacokinetic parameters of adecatumumab following the second intravenous administration of adecatumumab are summarised in Table 3. Dose-linearity for the parameters C_{max}, AUC_{τ}, AUC_{last} and AUC_{inf} was evident. Clearance and volume of distribution showed no dose dependency and no major differences after the first and the second dose were apparent. Compartmental analysis was consistent with a three compartment model with half-lives of 0.565 days ($t_{1/2}\alpha$), 3.78 ($t_{1/2}\beta$) and 13.3 days ($t_{1/2}\lambda z$)). The half-life of the terminal phase is in accordance with the non-compartmental analysis, which showed an apparent terminal half-life of 14.74 ± 4.23 days. The apparent terminal half-life was determined to be approximately 7 days for single dose (calculated from day 7 to 14) and 15 days for multiple dose administration (calculated from days 28 to 42 or 35 to 70). The difference in half-life values between first and second dose was a consequence of the longer evaluation period after the second dose. The mean apparent terminal half-life for cohorts 4 to 7, as determined between days 35 and 70, was on average 17 days (see Table 3). After 70 days, the adecatumumab serum concentrations of all patients from cohort 4 to 7 had not yet reached the lower limit of assay quantification of 200 ng/mL. The $T_{1/2}$ values derived from such limited time courses may underrepresent the true value.

3.5. Pharmacodynamic effects

Serum levels of the inflammatory cytokines IFN-γ, TNF-α, IL-1β, IL-2 as well as for PGE_2 and C-reactive protein (CRP) were determined at various time points after administration of adecatumumab. Natural killer (NK) cells were also analysed

Table 3 – Multiple-dose pharmacokinetic parameters for adecatumumab

Multi dose	Dose (mg/m^2)	$t_1/2$ (Day)	V_{ss} (L)	Cl_{ss} (L/day)	C_{max} (μg/mL)	$AUC_{T(14–28)}$ (Day*μg/mL)	AUC_{inf} (Day*μg/mL)
Cohort 1	10	10.01	11.42	1.063	3.4	22.46	30.60
Cohort 2	20	10.78	10.62	1.168	8.9	36.6	50.94
Cohort 3	40	14.20	11.88	1.046	18.3	81.7	131.7
Cohort 4	64	16.95	11.92	0.941	34.7	158.7	288.3
Cohort 5	102	16.11	12.11	0.878	51.1	246.0	411.0
Cohort 6	164	22.37	12.78	0.956	74.6	365.8	601.2
Cohort 7	262	12.77	9.98	1.034	127.1	563.4	788.4
Mean		14.74	11.53	1.01			
SD		4.23	0.95	0.10			
CV		28.7	8.2	9.4			
Min		10.01	9.98	0.88			
Max		22.37	12.78	1.17			
Geo Mean		14.25	11.50	1.01			
Median		14.20	11.88	1.03			

Abbreviations used are: $t_{1/2}$, terminal half life; V_{ss}, steady-state volume of distribution; Cl_{ss}, steady-state clearance; C_{max}, maximum serum concentration; AUC_T, area under the curve up to the last measured time point; AUC_{inf}, area under the curve to infinity; SD, standard deviation; CV, coefficient of variation; Min, minimum; Max, maximum; GeoMean, geometric mean.

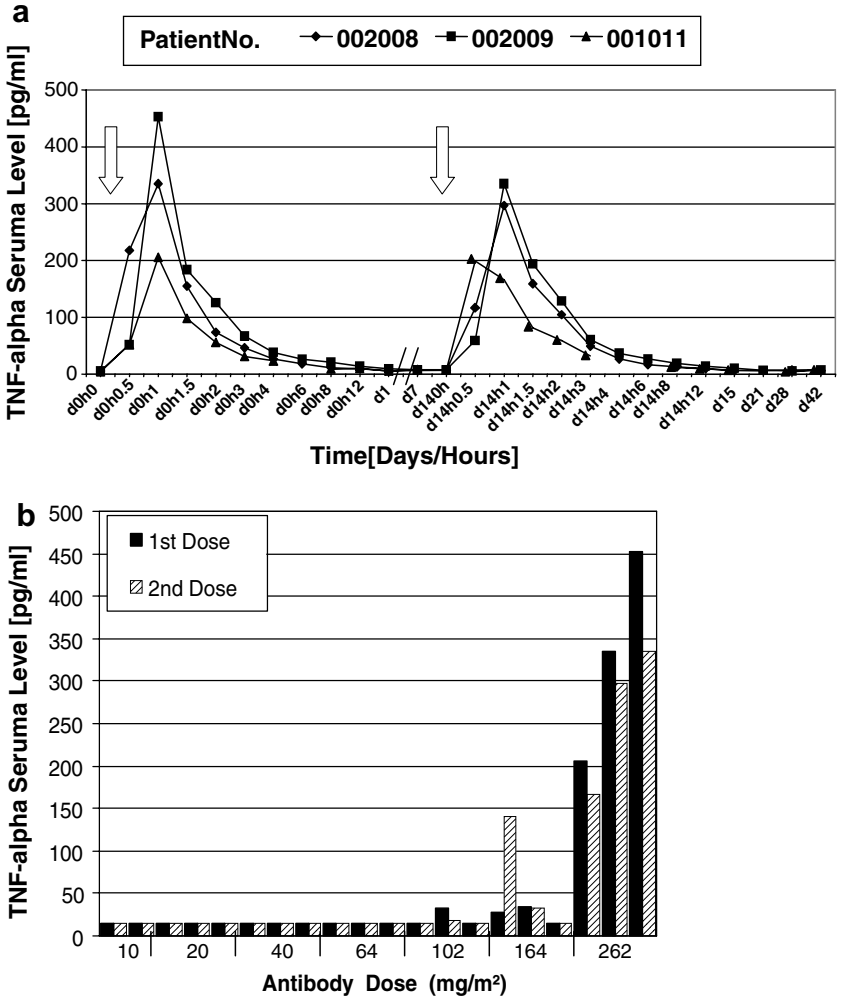

Fig. 2 – Response of TNF-alpha serum levels to adecatumumab infusion and dose. (a) TNF-alpha concentration/time profiles for all three patients of cohort 7 are shown. Patients were treated with two 1-h adecatumumab infusions at 262 mg/m^2, the beginning of which are indicated by arrows. Time points after the first antibody dose are shown on the X-axis in days (d) and hours (h). (b) TNF-alpha peak levels as reached by all 20 patients following first and second adecatumumab infusions.

in detail because they were shown to be key effector cells for ADCC as induced by adecatumumab and other human IgG1 antibody therapies.[21] Here, the total and relative number, cytotoxic activity and activation state of peripheral NK cells were investigated in response to adecatumumab.

Transient increases in TNF-α serum concentrations above baseline were observed in cohorts 5, 6 and 7. All three patients in cohort 7 reached TNF-α peak levels between 206 and 453 pg/mL 1 h after the first adecatumumab infusion, which returned to baseline values or below after 8 h (Fig. 2a). All other patients in the study did not exceed serum levels of 35 pg/mL TNF-α after the first infusion of adecatumumab (Fig. 2b). When compared to the TNF-α response in cohorts 1–5, the transient TNF-α increase in cohorts 6 and 7 was significant ($p = 0.0002$ at +1 h; two-sided Wilcoxon rank sum test on all patient data). The TNF-α increase in response to adecatumumab was thus fast, transient, and dose dependent.

Transient increases after administration of adecatumumab were also observed for IFN-γ. Five patients from different cohorts had peaks of IFN-γ reaching 3–6.8 IU/mL (baseline <1 IU/mL) between 2 h and 1 day following the first antibody dose. No significant change in serum concentration was seen for IL-1β. Serum levels of PGE_2 and IL-2 showed random peaks, and strong fluctuations were observed for CRP serum levels.

A time- but not dose-dependent correlation was seen between adecatumumab administrations and the absolute and relative number of peripheral NK cells. Fourteen of 17 patients (82%) in the per protocol analysis set showed a statistically significant decline in total NK cell number within 12 h post start of each adecatumumab infusion. Declines were statistically significant for the first ($p = 0.0127$) and second infusion ($p = 0.049$; two-sided sign tests). In the vast majority of cases, NK cell numbers had returned to baseline when measured 2 weeks after adecatumumab infusion (data not shown).

The basal and IL-2-stimulated cytotoxic activity of peripheral NK cells against MHC class I-negative K562 cells and the percentage and total number of peripheral NK cells expressing the early activation marker CD69 showed a wide variation during adecatumumab treatments (data not shown), but was not observed to correlate with dose or administration of adecatumumab.

3.6. *Response assessment*

A variety of PSA measurements were performed in this phase I study, including biochemical response, PSA-doubling time and determination of weekly PSA production. No biochemical response was observed. Although inhibitory effects on PSA doubling time and weekly PSA production were noted in retrospective analyses during adecatumumab treatment (data not shown), the low number of patients per cohort, the highly variable PSA starting values and the short duration of treatment altogether reduced the significance of such observations.

All 20 patients had baseline assessments and 19 had follow-up assessments of tumour lesions by CT scans. Of these, nine patients had measurable target lesions at baseline. Tumour response assessment on day 42 showed one partial response (PR), six stable and two progressive diseases. The partial response could not be confirmed 4 weeks later because the patient did not attend the scheduled visit.

4. Discussion

This is the first time a human anti-EpCAM monoclonal antibody of low binding affinity has been tested in man. Adecatumumab was generally well tolerated at all doses tested. The majority of reported AEs were mild or moderate, and reversible. Four adverse events were reported in four patients, of which only one was rated as possibly related to the study drug by the investigator; and no grade 4 adverse events were reported. The significant increase in the incidence of adverse events in cohorts 6 and 7 may have been related to the release of TNF-α at the highest dose levels. Patients with inflammatory liver disease might experience *de novo* expression of EpCAM on hepatocytes.[21] In some patients with pre-existing liver enzyme elevations from inflammatory or other liver impairments, a further transient and mild increase in these laboratory parameters was observed upon infusion of adecatumumab, which could have been related to EpCAM neoexpression on hepatocytes.

The tolerability of adecatumumab at the highest antibody exposure in the present trial is favourable in comparison to two high-affinity antibodies called ING-1 and 3622W94. The latter have equilibrium dissociation constants in the low nano-molar range and have been reported to cause pancreatitis as a dose-limiting toxicity in patients with solid tumours.[11,12] Both ING-1 and 3622W94 had an MTD of 1 mg/kg, which may not support sustained serum trough levels as reached by other currently approved IgG1 cancer therapies. Based on the experience with high-affinity anti-EpCAM IgG1 antibodies, a very careful dose escalation was therefore performed in the present study, and serum concentrations of pancreatic lipase and amylase were closely monitored. No significant changes of these enzymes occurred during this study with serum antibody levels expected to be sufficient to saturate accessible EpCAM on target tissues.[15] Of note, the two high-affinity mAbs ING-1 and 3622W94 induced acute pancreatitis during antibody infusion, while adecatumumab did not even affect basal serum levels of pancreatic enzymes. No maximum tolerated dose was reached yet with adecatumumab but this is not uncommon for monoclonal antibody therapies. Antibody side effects are mostly target antigen-dependent and will be maximal once all accessible target is bound by the antibody. From the current study, it would appear that with respect to safety adecatumumab is more related to the murine IgG2a antibody edrecolomab than to humanised antibodies ING-1 or 3622W94, suggesting that high-affinity EpCAM binding or recognition of a different epitope rather than presence of a human IgG1 Fcγ portion may have been responsible for the low tolerability of ING-1 and 3622W94. The safety profile of adecatumumab over longer periods of time remains to be established.

Adecatumumab had a serum half-life after repeated injection of approximately 15 days, which permits infrequent dosing of the antibody and facilitates long-term dosing, as is possible with other currently approved antibody therapies. No antibody responses directed against the human monoclonal antibody were found during the course of the study. This may be explained by the fact that the variable heavy and light chain domains of adecatumumab are 95% identical to human

germ line sequences due to their origin from human B cell repertoires.[18] Yet, larger studies with prolonged dosing are required to precisely determine the frequency of a HAHA response to adecatumumab.

With two doses of 262 mg/m^2 given every other week, serum levels were achieved for adecatumumab as appear necessary for several other therapeutic IgG1 antibodies used in oncology, including anti-CD20 mAb rituximab (375 mg/m^2, weekly), anti-EGFR mAb cetuximab (400/250 mg/m^2, weekly) and anti-HER-2 mAb trastuzumab (140/70 mg/m^2, weekly). The need for high antibody doses in man is in contrast to the relatively high *in vitro* cytotoxic efficacy of most therapeutic IgG1 antibodies. This may be due to a number of factors. One is that part of the efficacy of IgG1 can be mediated by ADCC, which employs effector cells, such as NK cells, bearing the low-affinity Fcγ receptor (FcR) type III (CD16).[21–23] It is thus the affinity of IgG1 for CD16 and not for the target antigen, which is rate-limiting for ADCC. The low affinity FcγR/IgG1 interaction is further reduced by excess serum IgG, which can very effectively compete for binding of therapeutic IgG1 to CD16.[15,24] High antibody concentrations may also be needed for CDC, which in *in vitro* assays requires antibody concentrations at or in excess of 10 μg/mL.[15] High antibody titres may furthermore facilitate target tissue penetration by creating a steeper concentration gradient, compensate for antibody loss by internalisation or binding to soluble antigen, and compensate for a reduced affinity of antibodies to certain Fcγ receptors with frequent polymorphisms.[22,23] For ongoing phase II studies in prostate and breast cancer, the present phase I study helped to define 2 mg/kg and 6 mg/kg as dose levels for efficacy testing, which approximately correspond to dose levels of cohorts 6 and 7.

Because there is no established PD marker in blood for cytotoxic IgG1 activity, a number of pharmacodynamic (PD) parameters were explored in this phase I study. Pre-clinical experiments have shown a key role of NK cell-mediated ADCC of adecatumumab against human cancer cell lines,[24] which is why we have focused on determining the number, activity and activation state of peripheral NK cells and inflammatory cytokines produced by NK and other immune cells. While there was an obvious redistribution of peripheral NK cells in response to adecatumumab infusions this was seen with all antibody doses and does therefore not provide a dose response parameter. Most obvious was a dose-dependent increase of TNF-α serum levels in response to adecatumumab, while other cytokines and mediators tested did not show a significant antibody dose-dependent response. Presently, it is unclear which immune cell types produced TNF-α in all three patients of cohort 7 and much less pronounced in patients of cohort 6. By selecting doses of 2 and 6 mg/kg for phase II studies, two doses will be tested for efficacy, which are different in their capacity to induce TNF-α in patients. This may allow further exploration as to whether a TNF-α serum response at these dose levels has potential as a PD marker for adecatumumab.

Conflict of interest statement

None declared.

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