European Journal of Cancer 100 (2018) 17–26

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.ejcancer.com

Original Research

A phase I dose-escalation study of IMAB362 (Zolbetuximab) in patients with advanced gastric and gastro-oesophageal junction cancer☆

Ugur Sahin [a], Martin Schuler [b,c], Heike Richly [c], Stefan Bauer [d], Anna Krilova [e], Tobias Dechow [f,k], Markus Jerling [g], Magdalena Utsch [h], Christoph Rohde [h], Karl Dhaene [i], Christoph Huber [a], Özlem Türeci [j,*]

[a] *TRON – Translational Oncology, Medical Center, Johannes Gutenberg University, Mainz, Germany*
[b] *West German Cancer Center, University Hospital Essen, Essen, Germany*
[c] *German Cancer Consortium (DKTK), Partner Site University Hospital Essen, Essen, Germany*
[d] *Gemeinschaftspraxis Hematology and Oncology, Lebach, Germany*
[e] *Piejuras Hospital, Oncology Clinic, Liepaja, Latvia*
[f] *Formerly of Klinikum Rechts der Isar, Munich, Germany*
[g] *Markus Jerling Consulting AB, Bromma, Sweden*
[h] *Formerly of Ganymed GmbH, Mainz, Germany*
[i] *MD Dhaene Pathology Lab Bvba, Destelbergen, Belgium*
[j] *CI3 – Cluster of Individualized Immune Intervention, Mainz, Germany*
[k] *Onkologie Ravensburg, Ravensburg, Germany*

Received 14 March 2018; accepted 9 May 2018
Available online 21 June 2018

KEYWORDS
Immunotherapy;
Gastric cancer;
Gastro-oesophageal junction;
Phase I clinical trials;
Biomarkers

Abstract ***Introduction:*** IMAB362 (Zolbetuximab) is a chimeric monoclonal antibody that binds to Claudin-18.2, a target antigen specific to cancer cells. *In vitro*, IMAB362 mediates cell death through antibody-dependent cellular cytotoxicity and complement-dependent cytotoxicity; thus, IMAB362 may serve as a potent, targeted immunotherapeutic agent.
Methods: This first-in-human phase I study enroled adult patients (N = 15) with advanced gastric or gastro-oesophageal junction cancer into five sequential single dose–escalation cohorts (33, 100, 300, 600, and 1000 mg/m^2) following a 3 + 3 design. Safety/tolerability, including determination of maximum tolerated dose and recommended phase II dose, were the primary objectives; secondary objectives included assessment of the IMAB362 pharmacokinetic profile, immunogenicity, and antitumour activity (assessed by Response Evaluation Criteria in Solid Tumors v1.0).

☆ This study was supported by Ganymed GmbH (formerly GanymedAG), a wholly owned subsidiary of Astellas Pharma, Inc.
* *Corresponding author*: CI3 – Cluster of Individualized Immune Intervention, Hölderlinstraße 8, Mainz, Germany. Fax: +49 (0) 6131 50 193 23.
E-mail address: tureci@uni-mainz.de (Ö. Türeci).

https://doi.org/10.1016/j.ejca.2018.05.007

Results: IMAB362 was generally well tolerated at all doses, with gastrointestinal toxicities being the most commonly observed treatment-related adverse events. As dose-limiting toxicity was not observed within 4 weeks of treatment, a maximum tolerated dose was not established. The pharmacokinetic profile of IMAB362 appeared to be proportional across the dose range; and mean half-life ranged from 13 to 24 d. While most patients showed progressive disease at weeks 4–5 after a single intravenous IMAB362 infusion, one patient in the 600 mg/m^2 dose group achieved and maintained stable disease for approximately 2 months postinfusion.
Conclusions: Findings from this study demonstrate that IMAB362 is generally well tolerated and support further evaluation in patients with gastric/gastro-oesophageal junction cancer.
Clinical trial registry: ClinicalTrials.gov, Identifier NCT00909025.

1. Introduction

Gastric cancer (GC) is among the leading causes of cancer-related deaths worldwide, with a survival rate of ≤5% at 5 years for patients with metastatic disease [1]. Because symptoms of early disease are mostly nonspecific, the majority of patients with GC or GEJ cancer in countries where routine screening is not available have advanced disease at initial diagnosis [2]. Chemotherapy with platinum and fluoropyrimidine derivatives is currently recommended as first-line treatment of unresectable or metastatic GC/GEJ cancer [3]; however, patients eventually experience progressive disease on first-line therapy, with a median progression-free survival of 5–7 months and a median overall survival of 9–11 months [4,5]. Targeted therapies for GC/GEJ cancer are currently limited. [6–13].

Claudin 18 (CLDN18) is a member of the claudin family of tetraspanin membrane proteins expressed at epithelial tight junctions; claudin 18.2 (CLDN18.2) is a splice variant of CLDN18 [14]. There is evidence to suggest that expression of CLDN18.2, in normal tissue, is strictly confined to tight junctions of the gastric mucosa, buried within a supramolecular complex [14]. CLDN18.2 is largely inaccessible to intravenous (IV) antibodies; however, upon malignant transformation, perturbations in cell polarity lead to epitopes of CLDN18.2 being exposed on the cancer cell surface, targetable by antibodies [14].

CLDN18.2 expression is maintained in up to 80% of GC tumours and aberrantly activated in a number of other tumour types, such as oesophageal, pancreatic, ovarian, biliary and lung adenocarcinomas [14,15]. IMAB362 (Zolbetuximab) is a first-in-development chimeric IgG1 monoclonal antibody that specifically binds to CLDN18.2 on the cell surface and mediates cell death through antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC) [16]. The primary objectives of this phase I study were to assess the safety and tolerability of single escalating doses of IMAB362, including determining the maximum tolerated dose (MTD) in pretreated patients with advanced GC and GEJ cancer, as well as establishing a recommended phase II dose (RP2D). Secondary objectives were assessment of the IMAB362 pharmacokinetic (PK) profile, immunogenicity, and antitumour activity.

2. Methods

2.1. Patients

Patients aged ≥18 years with metastatic, refractory, or recurrent advanced GC or GEJ cancer who had positive CLDN18.2 tumour expression (Supplemental Information), one or more measurable sites of the disease (according to Response Evaluation Criteria in Solid Tumors [RECIST], version 1.0), estimated life expectancy ≥3 months, adequate organ function, and an Eastern Cooperative Oncology Group performance status <2 or Karnofsky 70–100% were eligible for enrolment. Patients with ongoing anticancer therapies, an allergy to mAbs, or those who had had <3 weeks since a prior antitumour treatment were excluded.

2.2. Study design and conduct

This multicenter, first-in-human, phase I, single dose–escalation study was conducted at six centres in Germany and Latvia from July 2009 to May 2010. All patients received a single dose of IMAB362 as a 2-h IV infusion and remained hospitalised at the study center for 24 h postinfusion. A 4-week (±28 d) observation period followed treatment.

The study had five dose-escalation cohorts (33, 100, 300, 600 and 1000 mg/m^2) with three patients enroled at each dose level. Initially, one patient in each dose cohort received IMAB362. If no dose-limiting toxicities (DLTs; as defined in Supplemental Information) were observed within the first 5 d following treatment, two additional patients within each cohort were treated. In the absence of DLTs, and with approval from the Data and Safety

Monitoring Board, doses were escalated to the next increment until the 1000 mg/m^2 dose was reached or at least one patient experienced a DLT (Supplemental Fig. 1).

The initial starting dose of 33 mg/m^2 was based on the lowest dose level with no observable adverse effect in nonclinical toxicity studies in mice (100 mg/kg), including a safety factor of 10 (data on file; Astellas Pharma Inc.). Therefore, a dose of 33 mg/m^2 was considered to be a safe starting dose, with sequential planned dose escalations to 100, 300, 600, and 1000 mg/m^2.

2.3. Study end-points and assessments

2.3.1. Safety and tolerability

Safety and tolerability were based on monitoring of DLTs and adverse events (AEs), as well as on physical examinations, clinical laboratory evaluations, and electrocardiogram (ECG) monitoring.

2.3.2. Pharmacokinetic profile

Blood samples were drawn prior to infusion and upon completion of infusion (0 h) and at 3, 8, 12 and 24 h and 2, 4, 7, 14 and 28 d after the end of infusion. IMAB362 serum concentrations were determined at a central laboratory (Vivo Science GmbH, Gronau, Germany) using an enzyme-linked immunosorbent assay (ELISA) specific for the detection and quantification of IMAB362. The concentration limit of quantification was from 2 ng/mL to 20 ng/mL (at 80–120% recovery). Standard PK parameters (eg, maximum observed serum concentration [C_{max}], time to C_{max} [T_{max}], area under the serum concentration–time curve [AUC] and terminal half-life [$t_{1/2}$]) were calculated for IMAB362 using non-compartment analysis. For the calculation of compartmental parameters (eg, volume of distribution at steady state [Vss], systemic clearance [CL]), a 2-compartment model was used.

2.3.3. Immunogenicity

Immunogenicity of IMAB362 was assessed by collecting serum samples immediately prior to infusion and post-infusion on days 14 and 28 (final visit) and analysed for antidrug antibodies by ELISA. The ELISA system was validated for the detection of free ADA present in human serum samples based on a sandwich of captured IMAB362 and biotinylated IMAB362.

2.3.4. Antitumour activity

Tumour lesions were assessed according to RECIST, v1.0 by computerised tomography within 6 weeks prior to and 2–5 weeks after IMAB362 treatment. Cancer antigens in patient serum, including CA125, CA15-3, CA19-9, and carcinoembryonic antigen (CEA), were collected on infusion day (day 1) and on days 14 and 28 after IMAB362 infusion, and measured at a central laboratory (Interlab GmbH, München, Germany).

2.3.5. ADCC and CDC assays

Peripheral blood mononuclear cells (PBMCs) taken from untreated healthy donors or enroled patients with GC/GEJ 14 d posttreatment were used as effector cells in the presence of IMAB362 on CLDN18.2-positive NUGC-4 human GC target cells (effector-to-target [E:T] ratio of 20:1) to determine the patients' capability to induce ADCC. For determination of the kinetics of IMAB362-mediated ADCC response, healthy donor PBMCs were co-cultured with CLDN18.2-expressing luciferase-transfected NUGC-4 human GC target cells (E:T 40:1) in the presence of postinfusion serum samples (days 0, 14 and 28–32); a healthy donor serum pool, spiked with a saturating IMAB362 concentration, was used as control (ADCC max). To calculate fold-change lysis, patient-matched pre-infusion serum spiked with IMAB362 was used as pre-infusion serum control.

The CDC capability of IMAB362 was tested by spiking pre-infusion serum samples from patients with GC/GEJ and healthy donor with IMAB362 and by incubating CLDN18.2-expressing Chinese hamster ovary (CHO-K1) target cells with the serum. For determination of the kinetics of IMAB362-mediated CDC response, luciferase-transfected CLDN18.2-positive CHO-K1 target cells were incubated in serum samples from patients postinfusion; a healthy donor serum pool, spiked with a saturating IMAB362 concentration, was used as a control (CDC max).

For measuring IMAB362-mediated CDC kinetics, luciferase-transfected CLDN18.2-positive CHO-K1 target cells were incubated with post IMAB362 infusion serum (20% v/v) for 80 min at 37 °C. A healthy donor serum pool, spiked with a saturating IMAB362 concentration (10 μg/mL), was used as a control (CDC max). To calculate fold lysis, patient-matched pre-infusion serum samples spiked with 10 μg/mL IMAB362 were used as a control.

2.4. Statistical analysis

All adverse events (AEs) and laboratory data were summarised using descriptive statistics. Pharmacokinetic parameters were evaluated by dose cohort; calculations were performed using Phoenix WinNonlin Professional, version 6.3 software (Certara L.P., Princeton, US) and reported using descriptive statistics computed with Statistica 12.0 software (StatSoft Inc., Tulsa, US). Data on ADCC and CDC were analysed using Excel and GraphPad software.

2.5. Study oversight

This study was conducted in accordance with the Declaration of Helsinki ethical principles, Good Clinical Practices, principles of informed consent, and requirements of public registration of clinical trials (ClinicalTrials.gov Identifier, NCT00909025). The

Table 1
Patient demographics and baseline disease characteristics.

	Cohort 1 33 mg/m^2 (n = 3)	Cohort 2 100 mg/m^2 (n = 3)	Cohort 3 300 mg/m^2 (n = 3)	Cohort 4 600 mg/m^2 (n = 3)	Cohort 5 1000 mg/m^2 (n = 3)	Total (N = 15)
Median age, years (range)	54.3 (49–61)	58.3 (49–76)	67.0 (63–70)	64.7 (57–72)	62.3 (46–71)	61.3 (46–76)
Gender, n (%)						
Male	3 (100)	1 (33)	2 (67)	3 (100)	2 (67)	11 (73)
Female	0	2 (67)	1 (33)	0	1 (33)	4 (27)
Median time since first diagnosis, years (range)	1.4 (0.9–1.5)	2.1 (1.0–3.3)	3.6 (1.3–4.0)	0.6 (0.6–2.3)	1.7 (1.0–2.4)	1.5 (0.6–4.0)
Primary tumour location, n (%)						
Stomach	0	3 (100)	2 (67)	2 (67)	1 (33)	8 (53)
Gastro-oesophageal junction	3 (100)	0	1 (33)	1 (33)	2 (67)	7 (47)

protocol was approved by site-specific institutional review boards. Written informed consent was obtained from each subject at enrolment.

3. Results

3.1. Disposition, patient demographics, and baseline disease characteristics

Of the 29 patients who were screened, 15 met the inclusion criteria, were sequentially enroled into five dose groups (33, 100, 300, 600 and 1000 mg/m^2) and received a single infusion of IMAB362. As detailed in Table 1, the median age of the total population was 61.3 years (range: 46–76), and the median time since first diagnosis was 1.5 years (range 0.6–4.0). All patients had received ≥1 prior chemotherapy, seven patients had also undergone previous radiotherapy and one patient from the 33 mg/m^2 and 1000 mg/m^2 dose levels and two from each of the 100, 300 and 600 mg/m^2 dose levels had also undergone gastrectomy. Except for one patient in the 300 mg/m^2 group, patients showed CLDN18.2 expression.

3.2. Safety and tolerability

Across the entire dose range (33–1000 mg/m^2) studied, IMAB362 was generally well tolerated; no adverse event lead to study discontinuation. No DLTs were observed within 4 weeks postinfusion in any of the dose groups, and thus the MTD was not identified. Overall, 13 of 15 patients experienced ≥1 AEs, with gastrointestinal disorders being most commonly reported. Mild-to-moderate gastrointestinal toxicities (eg, nausea, vomiting), starting shortly after initiation of infusion, were the most common treatment-related AEs (Table 2). Of the grade 3 AEs observed in three patients, one event (vomiting in a patient treated with 1000 mg/m^2) was classified as related to the study medication. While gastrointestinal AEs were potentially related to on-target effects of IMAB362, no clear dose dependency was observed. Furthermore, no unexpected serious AEs were reported, and neither of the two reported serious AEs (grade 2 odynophagia [300 mg/m^2]; grade 3 urinary retention [600 mg/m^2]) were considered by investigators as treatment related. No significant safety observations were made regarding vital signs, ECG, haematology, clinical chemistry and coagulation parameters.

3.3. Immunogenicity

In this study, no patient was confirmed positive for antidrug antibodies after receiving a single dose of IMAB362 over the dose range of 33–1000 mg/m^2.

3.4. Pharmacokinetic profile

Single-dose IMAB362 serum concentrations over time are presented in Fig. 1a. IMAB362 demonstrated a linear PK profile as both C_{max} and AUC_{inf} increased proportionally across the dose range with no indication for target-mediated drug disposition (Fig. 1b and Table 3). The overall mean elimination phase half-life of IMAB362 was 17 d, ranging from a mean of 13–24 d for the dose groups.

3.5. Preliminary antitumour activity

Clinical response was assessed during weeks 4–5 after treatment. Most patients (n = 12/15; 80%) showed progressive disease after a single IMAB362 IV infusion. However, one patient (patient 4) in the 600 mg/m^2 dose group had stable disease in conjunction with a steady decline of previously elevated serum levels of tumour markers CA125, CA15-3, CA19-9, and CEA during the 4-week follow-up post IMAB362 infusion (Supplemental Fig. 2 and Supplemental Table). It should be noted that this phase I study was neither designed nor powered to evaluate the antitumour activity of IMAB362.

3.6. Immune effector–activating capacity of a single dose of IMAB362

Based on potent ADCC- as well as CDC-mediating antitumour activity in nonclinical studies [16], a series of experiments were conducted to confirm ADCC and

CDC activity of IMAB362 using samples from enroled patients, thereby generating *ex vivo* pharmacodynamic data to help inform the RP2D determination. The patients' PBMCs, collected 14 d postinfusion and used as a source for FcR^+ immune effectors, efficiently executed IMAB362 dose-dependent lysis of CLDN18.2-positive NUGC-4 human GC cells, with the maximal lysis similar to that obtained with healthy donor PBMCs (Fig. 2a and b). Similarly, pre-infusion sera of patients, as a source of complement factors supplemented with IMAB362, lysed CLDN18.2-transfected CHO-K1 cells as efficiently as the healthy donor serum pool (Fig. 2c).

In this small set of patients, the kinetics of the immune effector–activating capacity of a single dose of IMAB362 was evaluated by *ex vivo* testing of circulating IMAB362 in the patients' postinfusion sera collected on days 1, 14, and 28. The magnitude of the fold change in ADCC- and CDC-mediated lysis was dose-dependent and gradually declined over time in accordance with the serum levels of IMAB362 (Fig. 3, left panels). In patients who had received a dose of at least 300 mg/m^2, the amplitude of both responses at day 28 postinfusion was still above 50% of the respective maximum amplitude, indicating a long lasting bioactivity. This effect was more apparent when ADCC and CDC responses of these patients were plotted against the received IMAB362 dose. Independent of the received dose, ADCC and CDC responses were close to maximum on day 1 (Fig. 3, right panels). Doses above 300 mg/m^2 resulted in robust and durable kinetics of both modes of action over more than 4 weeks, further supporting dose levels of ≥300 mg/m^2 for additional clinical development.

3.6.1. Determination of the recommended phase II dose

As no DLT was observed in this study, RP2D determination was based on the IMAB362 PK profile as well as on the estimate of biologically active doses. The objective was to maintain IMAB362 serum levels, by repeated administration, in the range of EC_{95} values for IMAB362-induced ADCC (0.3–28 μg/mL) and CDC (30–183 μg/mL) [16]. In this study, patients who had received a single dose of ≥300 mg/m^2 were exposed to IMAB362 serum levels in the EC_{95} range for both ADCC and CDC up to 14 d after infusion of IMAB362. Furthermore, modelling and simulation of the single-dose PK parameters predicted marked accumulation of IMAB362 at weekly dosing intervals, which is in line with the long $t_{1/2}$; a 2-week dosing interval predicted reduced accumulation. Importantly, IMAB362 doses of ≥300 mg/m^2 were supported by *ex vivo* measurement of ADCC and CDC activity of circulating IMAB362 in conjunction with the patient's respective immune effectors. Based on these PK and PD data, a dose range of 300–600 mg/m^2 every 2 weeks was selected as the IMAB362 dose for further evaluation.

Table 2
Overview of treatment-related adverse events.

Dose group	Patient number	MedDRA preferred term	Intensity	Outcome
Cohort 1	None reported			
33 mg/m^2 (n = 3)				
Cohort 2	1	Nausea	Moderate	Recovered
100 mg/m^2 (n = 3)		Vomiting	Mild	Recovered
	2	Vomiting	Moderate	Recovered
		Malaise	Mild	Recovered
		Myocardial ischaemia	Moderate	Recovered
		Decreased appetite	Moderate	Recovered
		Salivary hypersecretion	Moderate	Recovered
	3	Decreased appetite	Mild	Recovered
		Saliva altered	Mild	Recovered
		Dysgeusia	Mild	Unknown
Cohort 3	1	Nausea	Moderate	Recovered
300 mg/m^2 (n = 3)				
Cohort 4	1	Nausea	Moderate	Recovered
600 mg/m^2 (n = 3)		Hypertension	Moderate	Recovered
		Haematemesis	Moderate	Recovered
		Haemorrhagic gastritis	Moderate	Recovered
Cohort 5	1	Vomiting	Severe	Recovered
1000 mg/m^2 (n = 3)	2	Diarrhoea	Mild	Recovered
	3	Vomiting	Moderate	Recovered
		Nausea	Moderate	Recovered
		Increased GGT	Moderate	Not resolved
		Vertigo	Mild	Recovered
		Oesophageal disorder	Mild	Recovered

GGT, gamma-glutamyl transferase.

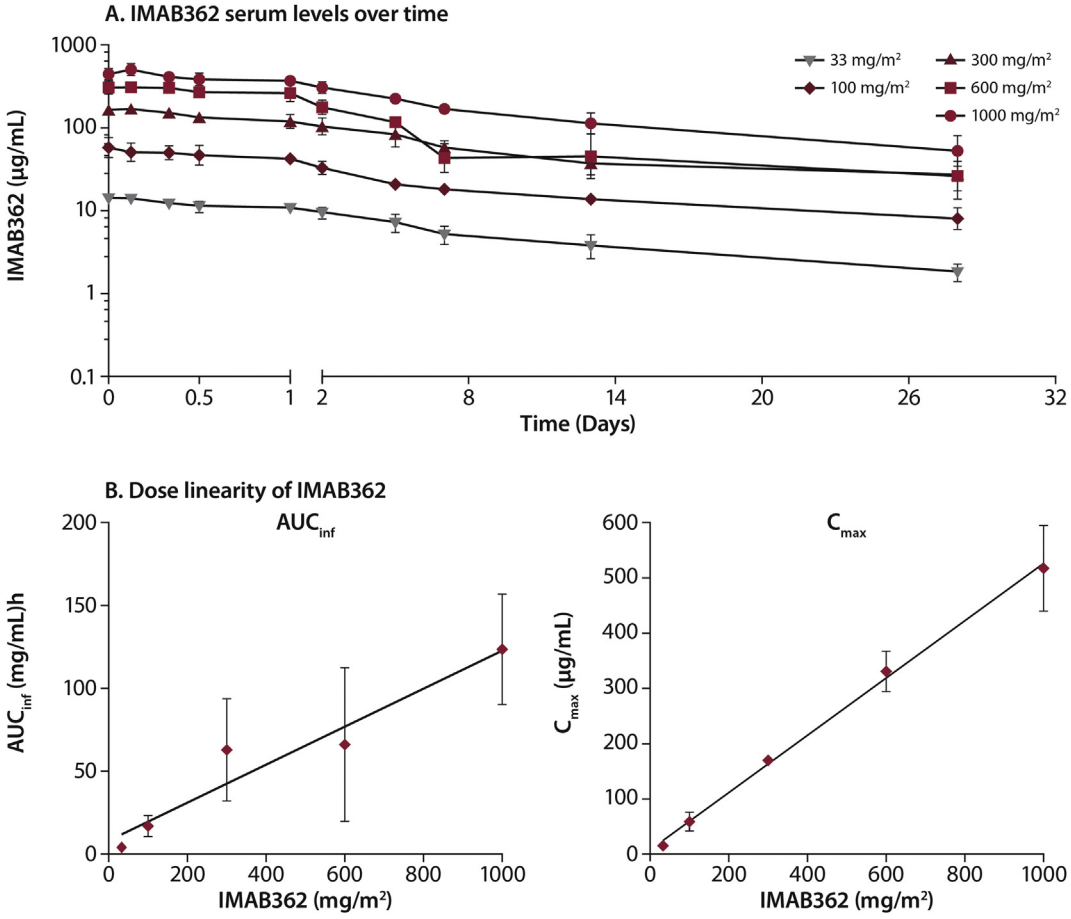

Fig. 1. **Pharmacokinetics of IMAB362 in patients with advanced GC/GEJ cancer. (A)** IMAB362 serum levels over time in patients following a single intravenous administration of different IMAB362 doses (infusion time range: 105–167 min). **(B)** AUC_{inf} (**left panel**) and C_{max} (**right panel**) of IMAB362 in patient serum were estimated with a non-compartmental model. Data are mean ± SD of n = 3 per IMAB362 dose with corresponding linear regression. AUC_{inf}, area under the concentration–time curve from time 0 to infinity; C_{max}, maximum observed concentration.

Table 3
PK parameters of IMAB362 at different doses in patients with advanced GC/GEJ cancers.

PK parameter, mean (range)	33 mg/m^2 (n = 3[a])	100 mg/m^2 (n = 3)	300 mg/m^2 (n = 3[a])	600 mg/m^2 (n = 3)	1000 mg/m^2 (n = 3)
C_{max}[b] (µg/mL)	15.1 (14.9–15.4)	58.7 (41.1–75.6)	170 (164–176)	331 (290–362)	517 (466–606)
AUC_{0-672}[b] (mg/mL × h)	3.1 (2.4–3.8)	11.0 (9.7–13.2)	34.5 (23.7–41.9)	46.9 (26.1–73.8)	96.2 (87.9–111.0)
AUC_{inf}[b] (mg/mL × h)	4.1 (3.1–5.2)	16.9 (12.9–24.2)	62.9 (28.2–87.6)	66.1 (26.5–117.3)	123.5 (101.2–161.9)
$t_{1/2}$[b] (days)	14.9 (14.3–15.4)	19.3 (14.4–28.2)	24.1 (11.3–33.8)	14.6 (6.0–20.8)	13.1 (9.8–17.4)
V_{ss} (L)[c]	6.91 (5.34–8.49)	7.34 (6.69–7.96)	6.79 (6.55–7.04)	8.95 (5.84–14.0)	6.36 (5.99–7.05)
CL (mL/h)[c]	14.3 (11.8–16.8)	11.8 (7.6–14.1)	14.5 (8.1–21.0)	24.7 (7.3–49.4)	15.1 (11.3–18.3)

AUC_{0-672}, area under the concentration–time curve between time 0 and 672 h (28 d) postinfusion; AUC_{inf}, area under the concentration–time curve from time 0 to infinity; CL, clearance; C_{max}, maximum observed concentration; PK, pharmacokinetic; $t^1/_2$, terminal elimination phase half-life; V_{ss}, volume of distribution at steady state.

[a] In the 33 mg/m^2 and 300 mg/m^2 dose cohort, one patient was excluded from the estimation of the terminal elimination phase as it was poorly estimated due to a large variability in concentrations.

[b] Obtained from non-compartmental analysis.

[c] Obtained from a two-compartmental pharmacokinetic model.

4. Discussion

Single doses of IMAB362, up to 1000 mg/m^2, showed a favourable safety profile and were well tolerated in a patient population with advanced/metastatic GC/GEJ cancer. No life-threatening AEs or hypersensitivity reactions, a typical class-specific AE occurring with mAbs, were reported; there were no discontinuations due to an AE. This is consistent with the lack of IMAB362 binding site expression in all explored normal tissues, except

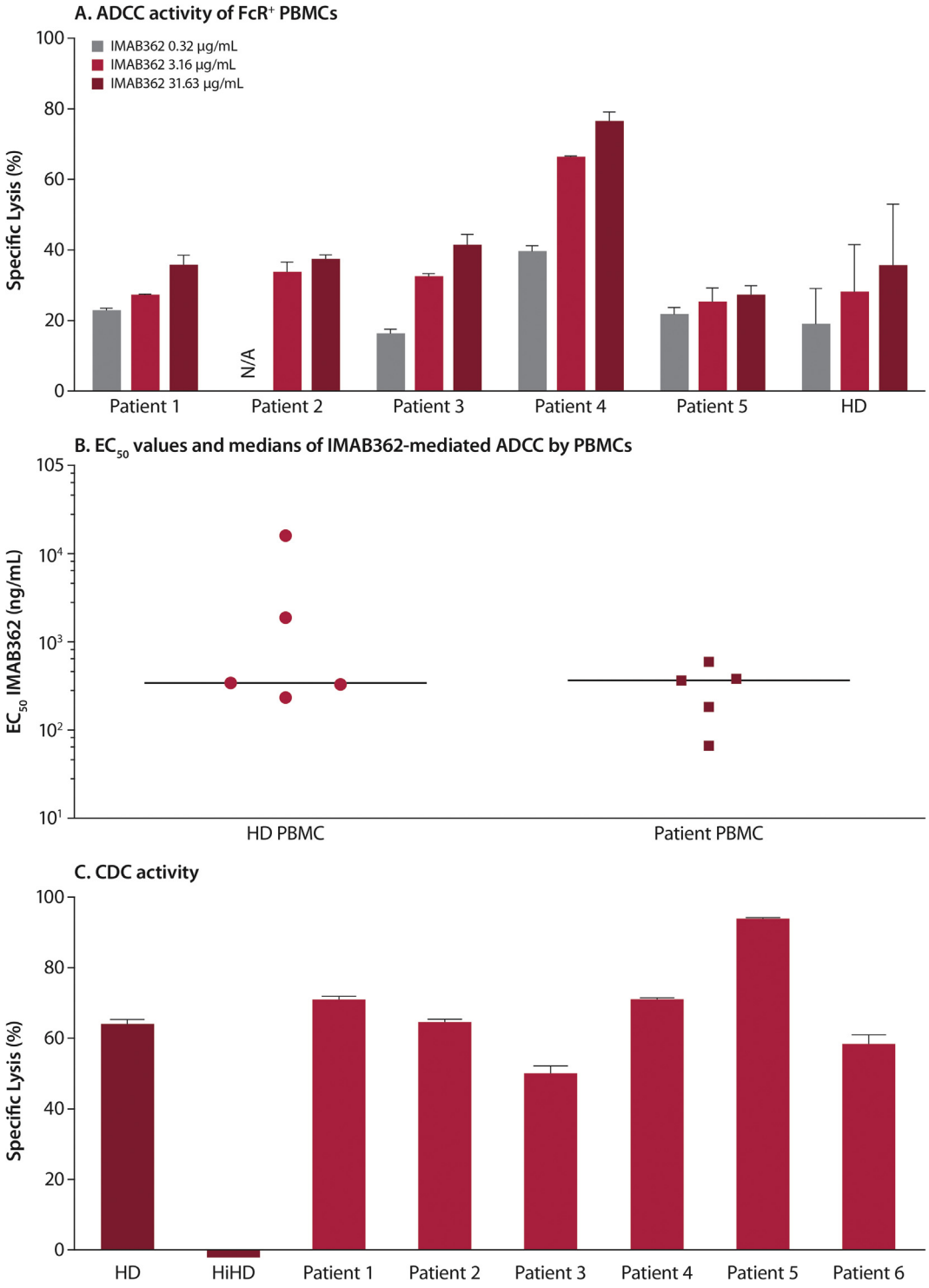

Fig. 2. **Baseline capability of patients to induce ADCC and CDC. (A)** ADCC activity of FcR^+ PBMC of five patients at baseline was tested *ex vivo* against the CLDN18.2-positive human gastric cancer cell line NUGC-4 at an effector-to-target ratio of 20:1 and compared with PBMC from six healthy donors in the presence of ascending concentrations of IMAB362. Data are mean ± SD of triplicate measurements per patient or mean ± SD of data from all healthy donors. **(B)** EC_{50} values and medians of IMAB362-mediated ADCC by PBMC from patients versus healthy donors against NUGC-4 cells. **(C)** CDC activity against CLDN18.2-transduced CHO-K1 cells was tested *ex vivo* in pre-infusion sera of six patients and compared with pooled healthy donor sera (both 20% v/v serum) as a source of complement factors in the presence of 0.5 µg/mL (patient and healthy donor serum) or 10 µg/mL (heat-inactivated, healthy donor serum pool) IMAB362. A heat-inactivated healthy donor serum pool served as negative control. Data are mean ± SD of triplicate measurements per patient or healthy donor serum pool. ADCC, antibody-dependent cellular cytotoxicity; CDC, complement-dependent cytotoxicity; EC_{50}, half-maximal effective concentration; FcR^+, Fc receptor positive; HD, healthy donor; HiHD, heat-inactivated, healthy donor; PBMC, peripheral blood mononuclear cell.

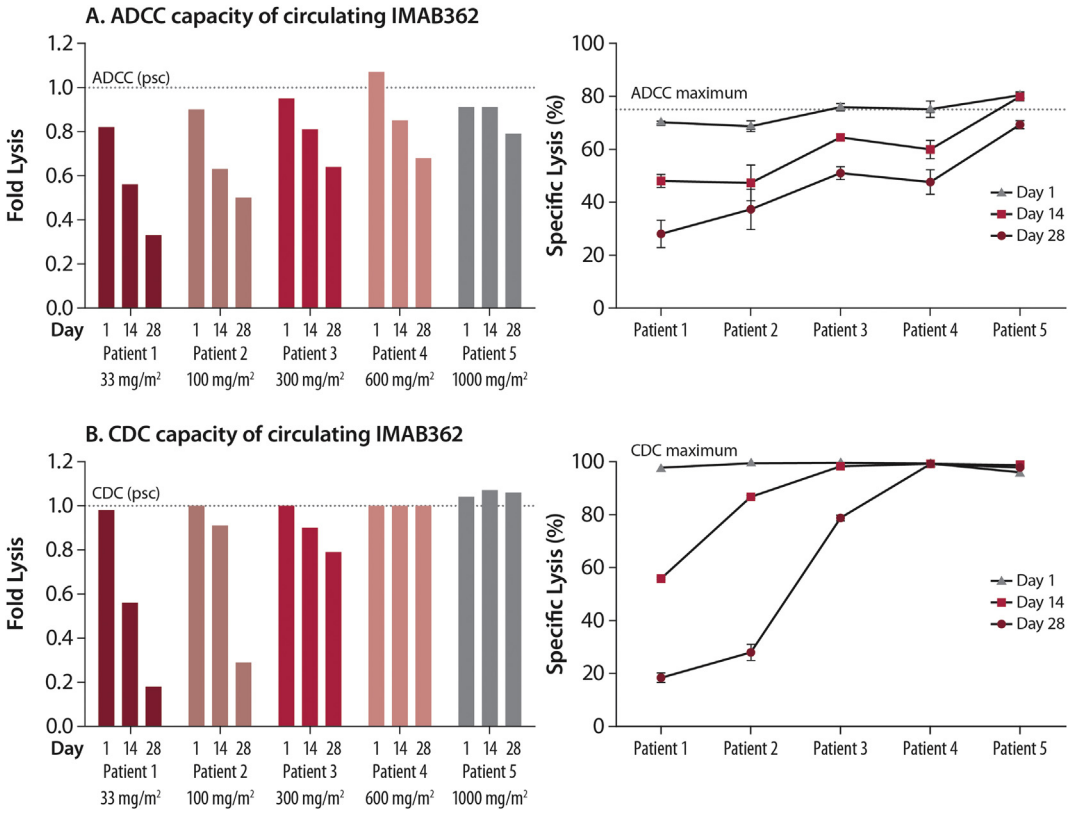

Fig. 3. **Kinetics and Dose–Response of Circulating IMAB362 to Mediate ADCC and CDC. (A)** ADCC capacity of circulating IMAB362 in patient serum against CLDN18.2-positive target cells was determined with HD PBMC. Serum samples obtained from patients treated with IMAB362 on days 1, 14 and 28 at the indicated doses served as the IMAB362 source. To block parallel CDC activity, patient serum samples were heat inactivated. **Left panel**: Fold lysis was calculated at the indicated time points based on patients' maximum ADCC capacities, determined with pre-infusion serum spiked with saturating 200 μg/mL IMAB362 antibody (ADCC psc) and set to 1 (dotted line). **Right panel**: ADCC responses per patient were plotted against the received IMAB362 dose. Maximum ADCC (ADCC max) was determined with an HD serum pool spiked with saturating 200 μg/mL IMAB362 (dotted line). Data are mean ± SD of quadruplicate measurements per patient. **(B)** CDC capacity of circulating IMAB362 was determined in serum samples collected on days 1, 14 and 28 as the IMAB362 source from patients receiving the indicated IMAB362 doses. **Left panel**: Fold lysis was calculated at the indicated time points based on patients' maximum CDC capacities, determined with pre-infusion serum spiked with saturating 10 μg/mL IMAB362 (CDC psc) and set to 1 (dotted line). **Right panel**: CDC responses per patient plotted against the received IMAB362 dose. Maximum CDC (CDC max) was determined with HD serum pool spiked with 10 μg/mL IMAB362 (dotted line). Data are mean ± SD of triplicate measurements per patient. ADCC, antibody-dependent cellular cytotoxicity; CDC, complement-dependent cytotoxicity; HD, healthy donor; max, maximum; psc, pre-infusion serum control.

gastric epithelia [14]. As CLDN18.2 is a highly selective gastric epithelial lineage marker, the only toxicity-relevant organ is the stomach; however, no major manifestations of mucosal injury, such as ulceration, gastritis or perforation, were reported. The most commonly reported AEs were grade 1/2 nausea and vomiting. The ability to fully assess dose-related safety/tolerability trends was limited due to the small number of patients in each dose cohort and due to the nature and advanced phase of this disease, patients enroled in this study already displayed gastrointestinal symptoms at baseline.

Pharmacodynamic data from patients with GC/GEJ cancer in this study demonstrate that circulating IMAB362 retains the ability to stimulate ADCC and CDC and that patient serum and effector cells can be stimulated to elicit ADCC and CDC. Taken together, these data support the results of nonclinical studies [16] that suggest the mechanisms of action of IMAB362 are ADCC and CDC via immune effector stimulation following mAb–target binding.

This first-in-human trial was not designed to assess antitumour activity of IMAB362. Because patients with varying levels of CLDN18.2 expression were enroled, no conclusions can be made regarding potential association of CLDN18.2 expression level and IMAB362 activity. Nevertheless, antitumour activity was observed in one patient treated with a single 600 mg/m^2 dose of IMAB362. Interestingly, this patient had the highest CLDN18.2 expression level and presented the highest capacity to induce ADCC and CDC in conjunction with IMAB362.

A key objective of this study was to determine an appropriate dosing for future clinical studies. Because DLTs were not observed in dose levels up to 1000 mg/m^2, dose-related safety concerns could not be factored into the dose-finding estimates. Pharmacokinetic parameters obtained for IMAB362 correspond to those of other IgG1 antibodies. In this study, IMAB362 demonstrated a dose-proportional PK profile with a mean $t_{1/2}$ of 17.2 d without any signs of target-mediated drug disposition. By evaluating PK data and estimates of biologically active dose levels based on preclinical pharmacology, a dose range of 300–600 mg/m^2 was determined as suitable for further evaluation. This range was further supported by *ex vivo* measurements of the ADCC and CDC activity of circulating IMAB362 in conjunction with individual patients' respective immune effectors.

5. Conclusions

The results from this study help to establish the single-dose safety profile of IMAB362 across a wide dose range as well as a dosing regimen for consideration in further studies. These data prompted the initiation of clinical trials addressing the antitumour activity of IMAB362 as a single agent and in combination with standard therapeutics.

Role of the funding source

This study was supported by Ganymed GmbH (formerly Ganymed AG), a wholly owned subsidiary of Astellas Pharma, Inc. Ganymed GmbH (formerly Ganymed AG) collected and analysed the data and contributed to the interpretation of the study. Financial support for the development of this manuscript, including medical writing and editorial assistance under the authors' guidance, provided by Regina Switzer, PhD of SuccinctChoice Medical Communications (Chicago, IL, USA), was funded by Astellas Pharma, Inc. (Northbrook, IL, USA).

Authors' contributions

U.S. and Ö.T. were involved in the conception and design of the study and provided guidance in the analysis and interpretation of the data, and contributions to the writing and revision of the manuscript. M.S. and C.H. advised on the study design. M.S., S.B., H.R., A.K., T.D., C.R. and K.D. were integral in data collection and contributed to development and review of the manuscript. M.J. and M.U. provided data analysis and interpretation and critically reviewed the manuscript. All authors are accountable for all aspects of the work and approved the manuscript for submission.

Conflict of interest statement

For the work under consideration, S.B., T.D., K.D., A.K., H.R. and M.S. have nothing to declare. C.H. received grant funding from the Federal Ministry of Education and Research (Germany); M.J. received consultancy fees from (Ganymed GmbH); at time of the work, C.R. and M.U. were employees at Ganymed GmbH. U.S. and Ö.T. were co-founders, stock owners and shareholders of Ganymed GmbH; U.S., M.U. and Ö.T. held patents broadly related to the work. Ö.T. was the CEO of Ganymed GmbH until acquired by Astellas Pharma, Inc.

Acknowledgements

The authors would like to thank Vilmos Posevitz for preparing figures and to acknowledge all investigators, coordinators, and study site personnel, as well as patients and their families, for their participation in this study.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.ejca.2018.05.007.

References

[1] Lasithiotakis K, Antoniou SA, Antoniou GA, Kaklamanos I, Zoras O. Gastrectomy for stage IV gastric cancer. A systematic review and meta-analysis. Anticancer Res 2014;34:2079–85.
[2] Maconi G, Manes G, Porro GB. Role of symptoms in diagnosis and outcome of gastric cancer. World J Gastroenterol 2008;14:1149–55.
[3] A National Comprehensive Cancer Network. Clinical practice guidelines in oncology gastric cancer, Version 5. 2017. https://www.nccn.org/professionals/physician_gls/pdf/gastric.pdf. [Accessed 23 February 2018].
[4] Iacovelli R, Pietrantonio F, Farcomeni A, et al. Chemotherapy or targeted therapy as second-line treatment of advanced gastric cancer. A systematic review and meta-analysis of published studies. PLoS One 2014;9:e108940.
[5] Kim HS, Kim HJ, Kim SY, et al. Second-line chemotherapy versus supportive cancer treatment in advanced gastric cancer: a meta-analysis. Ann Oncol 2013;24:2850–4.
[6] De Vita F, Di Martino N, Fabozzi A, et al. Clinical management of advanced gastric cancer: the role of new molecular drugs. World J Gastroenterol 2014;20:14537–58.
[7] Bang YJ, Van Cutsem E, Feyereislova A, et al. Trastuzumab in combination with chemotherapy versus chemotherapy alone for treatment of HER2-positive advanced gastric or gastro-oesophageal junction cancer (ToGA): a phase 3, open-label, randomised controlled trial. Lancet 2010;376:687–97.
[8] Wilke H, Muro K, Van Cutsem E, et al. Ramucirumab plus paclitaxel versus placebo plus paclitaxel in patients with previously treated advanced gastric or gastro-oesophageal junction adenocarcinoma (RAINBOW): a double-blind, randomised phase 3 trial. Lancet Oncol 2014;15:1224–35.

[9] Jomrich G, Schoppmann SF. Targeted therapy in gastric cancer. Eur Surg 2016;48:278–84.
[10] Pasini F, Fraccon AP, Modena Y, et al. Targeted therapies for advanced and metastatic adenocarcinoma of the gastroesophageal junction: is there something new? Gastric Cancer 2017;20:31–42.
[11] Sibertin-Blanc C, Ciccolini J, Norguet E, Lacarelle B, Dahan L, Seitz JF. Monoclonal antibodies for treating gastric cancer: promises and pitfalls. Expert Opin Biol Ther 2016;16:759–69.
[12] Updated data in KEYNOTE-061: Pembrolizumab in previously treated gastric or gastroesophageal junction adenocarcinoma. [Accessed 26 January 2018]. http://www.ascopost.com/News/58377.
[13] Kang YK, Boku N, Satoh T, et al. Nivolumab in patients with advanced gastric or gastro-oesophageal junction cancer refractory to, or intolerant of, at least two previous chemotherapy regimens (ONO-4538-12, ATTRACTION-2): a randomised, double-blind, placebo-controlled, phase 3 trial. Lancet 2017;390:2461–71.
[14] Sahin U, Koslowski M, Dhaene K, et al. Claudin-18 splice variant 2 is a pan-cancer target suitable for therapeutic antibody development. Clin Cancer Res 2008;14:7624–34.
[15] Heinz C, Mitnacht-Kraus R, Kreuzberg M, Wöll S, Sahin U, Türeci Ö. Preclinical evaluation of the anti-CLDN18.2 antibody, IMAB362, in pancreatic carcinoma. In: European Society of Medical Oncology Annual Congress; 2017. Poster 376P.
[16] Minacht-Kraus R, Kreuzberg M, Utsch M, Sahin U, Yamada T, Tureci O. Preclinical characterization of IMAB362 for the treatment of gastric carcinoma. Ann Oncol 2017:28. European Society of Medical Oncology Annual Congress 2017. Poster 378P.