Clinical and Immunologic Responses to a B-Cell Epitope Vaccine in Patients with HER2/neu-Overexpressing Advanced Gastric Cancer—Results from Phase Ib Trial IMU.ACS.001

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ABSTRACT

Purpose: HER2/neu is overexpressed in up to 30% of gastroesophageal adenocarcinomas (GEA) and linked to poor prognosis. Recombinant mAbs to treat HER2/neu-overexpressing cancers are effective with limitations, including resistance and toxicity. Therefore, we developed a therapeutic B-cell epitope vaccine (IMU-131/HER-Vaxx) consisting of three fused B-cell epitopes from the HER2/neu extracellular domain coupled to CRM197 and adjuvanted with Montanide. This phase Ib study aimed to evaluate the optimal/safe dose leading to immunogenicity and clinical responses (https://clinicaltrials.gov/ct2/show/NCT02795988).

Patients and Methods: A total of 14 patients with HER2/neu-overexpressing GEA were enrolled, and dose escalation (10, 30, 50 μg) was performed in three cohorts (C). Immunogenicity was evaluated by HER2-specific Abs and cellular responses, clinical responses by CT scans according to RECIST version 1.1.

Results: IMU-131 was safe without vaccine-related significant local/systemic reactions or serious adverse events. A total of 11 of 14 patients were evaluable for changes in tumor size and vaccine-specific immune responses. One patient showed complete, 5 partial responses, and 4 stable diseases as their best response. HER2-specific IgG levels were dose dependent. In contrast to patients in C1 and C2, all patients in C3 mounted substantial HER2-specific Ab levels. In addition, cellular vaccine responses, such as Th1-biased cytokine ratios and reduced regulatory T cell numbers, were generated. Progression-free survival was prolonged in C3, correlating with the vaccine-specific humoral and cellular responses.

Conclusions: IMU-131 was well tolerated and safe. The induced HER2-specific Abs and cellular responses were dose dependent and correlated with clinical responses. The highest dose (50 μg) was recommended for further evaluation in a phase II trial, with chemotherapy + IMU-131 or chemotherapy alone, which is currently ongoing.

Introduction

EGFR HER2 is overexpressed in many epithelial tumors, for example, subsets of breast, gastroesophageal, ovarian, colorectal, and lung cancers, and this is associated with an aggressive phenotype and poor patient outcome (1). HER2 receptor dimerizes with other HER family members, which activates several downstream signaling pathways that are crucial for cell proliferation, survival, and differentiation (2). Thus, HER2 is a key therapeutic target for cancer treatment. Use of trastuzumab, a mAb inhibiting HER2 activation, has dramatically improved the prognosis for HER2-amplified advanced breast cancers with prolonged survival of both early-stage and metastatic patients (3). Gastroesophageal adenocarcinoma (GEA) is the fifth most frequent tumor worldwide with highest rates in Asia and HER2 overexpression ranging from 7% to 34% (4). A study in patients with GEA investigating treatment with 5-fluorouracil (or capecitabine) plus fluorouracil (or capecitabine) plus...
cisplatin chemotherapy in combination with trastuzumab showed improved overall survival and objective response rates in comparison with chemotherapy alone (5). Therefore, combination of trastuzumab with chemotherapy has become the standard therapeutic option for HER2-amplified patients with GEA in several but not all countries.

Even though cancer treatment with mAbs shows convincing therapeutic effects, passive Ab administration has several drawbacks, such as primary and secondary resistance (6), the need for frequent application in rather short time intervals, long periods of drug delivery, no preventative use in patients with comorbidities and high treatment costs. Several of these issues could be overcome by vaccine-induced active immunization against the respective tumor antigens. Active cancer vaccine approaches have mostly targeted tumor-specific T-cell responses, but have not rendered clinically relevant results partly due to MHC-restricted antigen presentation (7). In contrast to T-cell approaches, B-cell peptide vaccines are not MHC dependent.

In our previous work, we showed that vaccine-induced Abs against three selected B-cell epitope peptides from the HER2/neu extracellular domain have antitumor capacity in vitro and in vivo. Vaccination with the three selected peptides in FVB/N transgenic mice developing c-neu-overexpressing breast cancers led to delayed tumor onset and reduced tumor progression in vivo (8). The potential antitumor mechanisms were in vitro shown to be due to direct inhibition of growth of HER2/neu-overexpressing tumor cells, complement-dependent cytotoxicity (CDC), and Ab-dependent cellular cytotoxicity (ADCC; ref. 9). For the evaluation in a human phase I study with patients with metastatic breast cancer, the single peptides were coupled to influenza virosomes and this formulation was well tolerated and led to specific immune responses in 8 of 10 patients. Importantly, because trastuzumab is standard of care for patients with HER2-overexpressing breast cancer, only patients with no or low HER2 amplification could be enrolled (10). For further validation of the vaccine responsiveness, a trial including HER2-overexpressing patients was planned in countries where trastuzumab is not standard of care. As the virosomal vaccine showed poor stability, it was reformulated with respect to peptide synthesis and conjugation. CRM197, the nontoxic form of diphtheria toxoid, is a frequently used conjugate in licensed vaccines (11, 12) that rapidly induces specific memory T cells and mixed Th1/Th2 cytokines (13). Moreover, CRM197 has biologic antitumor effects by binding to its receptor heparin-binding EGF, which is frequently overexpressed on cancer cells (14). Preclinical testing of IMU-131/HER-Vaxx vaccine in mice showed polyclonal Ab responses with antiproliferative effects on HER2/neu and administration with the Th1-driving adjuvant Montanide induced high and long-lasting Ab levels and strong Th1-biased cellular responses (15).

This article provides the results from a phase I/II trial of the newly formulated B-cell vaccine, IMU-131, in patients with HER2/neu-overexpressing GEA. Patients from Eastern Europe and South-East Asia were enrolled in this study because the availability of trastuzumab as the primary treatment option for HER2-amplified GEA cancers limited potential recruitment in most Western countries. The objectives of the study were to test safety and tolerability of IMU-131, to determine the optimal dose for phase II, and to document potential clinical effects of this active immunization approach. Emphasis was given to vaccine-specific immune responses and their potential correlation with clinical responsiveness.

Patients and Methods

HER2/neu vaccine and chemotherapy

The investigated B-cell peptide vaccine IMU-131 was provided as the HER2/neu fusion peptide P467 linked to the nontoxic diphtheria protein CRM197 as sterile solution in PBS buffer (picHEM). For administration in patients, IMU-131 vaccine was prepared by study site personal on the day of injection in a homogenous water-in-oil emulsion of IMU-131 and the adjuvant Montanide ISA 51VG (SEPPIC) in a 50/50 ratio. IMU-131 was formulated into either a low (10 μg), medium (30 μg) or high dose (50 μg) of peptide antigen P467 equivalent and was administered into the deltoid region of the upper arm with a 0.60 × 25 mm 23G needle. IMU-131 in aqueous phase was stored at −15°C and Montanide at +2°C to +8°C until use.

Chemotherapy was prepared and dispensed according to Pharmacy standard practice; it consisted of cisplatin i.v. (80 mg/m²) and either 5-fluourouracil (4,000 mg/m² by continuous infusion) or capecitabine (2,000 mg/m²/day, orally). Dose and duration were varied by the respective investigators as clinically indicated for their patients.

Patients

Patients with HER2/neu-overexpressing advanced and metastatic GEA were eligible for this open-label multicenter phase I/II trial. Patients ≥ 20 years with no previous treatment with trastuzumab or other HER2/neu targeting mAbs were enrolled in South East Asia (Hong Kong, Taiwan, Thailand) and Eastern Europe (Georgia, Republic of Moldova). (Inclusion and exclusion criteria are provided Supplementary Table S1, baseline disease characteristics in Supplementary Table S2).

Confirmation of HER2/neu overexpression was required for inclusion and previous pathology results were accepted; if analysis of a tumor biopsy was required, fresh or archived tissue samples were used. HER2/neu expression was assessed by IHC and if result were equivocal (i.e., HER2+++) FISH or chromogenic in situ hybridization (CISH) were used. Patients with IHC 2+ expression without confirmation of overexpression by FISH/CISH could be included with agreement of the sponsor. Eastern Cooperative Oncology Group performance grade 0–1 was required for study inclusion and after providing written informed consent patients were allocated to treatment in a nonrandomized, sequential order.

Study design

Primary endpoints of the study were safety and tolerability of IMU-131 as well as definition of the recommended phase 2 dose (RP2D) by use of immunogenicity data, that is, HER2-specific IgG levels until day 56. Exploratory endpoints were comprehensive
humoral and cellular evaluation of vaccine immunogenicity (i.e., P467- and HER2-specific Ab levels in serum, vaccine-specific cytokine levels, and lymphocyte distributions) and clinical response rates according to radiographic data.

Patients were administered three injections of IMU-131 on days 0, 14, and 35, at a single dose level, accompanied by chemotherapy cycles every 21 days starting from day 14. For dose escalation, between 3 and 6 patients were included in each cohort (C) and the Cohort Review Committee (CRC) consisting of site investigators and a clinical immunologist reviewed dose-limiting toxicity (DLT) data from one complete cycle of chemotherapy, including three doses of IMU-131, for at least 3 patients in each dose cohort to authorize dose escalation. Patients continued receiving chemotherapy in 21-day cycles with booster doses of IMU-131 starting from day 98 and were discontinued if clinically indicated or ceased if clinically indicated (study flow diagram, Supplementary Table S3A).

RP2D was defined as the dose resulting in the most favorable safety/tolerability and immunogenicity data. RP2D was determined after the third dose cohort had completed day 56 and upon detailed analysis of safety and immunogenicity data by the CRC. Furthermore, radiographic data were analyzed descriptively to explore clinical response rates and provide information for sample size calculation for the phase II study.

The trial was conducted in accordance with the International Ethical Guidelines for Biomedical Research Involving Human Subjects (Council for International Organizations of Medical Sciences 2002), the Guidelines for GCP (ICH 1996), and the Declaration of Helsinki (World Medical Association 1996, 2008) and registered at ClinicalTrials.gov (NCT02795988).

Safety assessment

Safety and tolerability of IMU-131 were evaluated by adverse events (AE) and laboratory measurements. AEs and laboratory abnormalities were graded by Common Terminology Criteria for Adverse Events (CTCAE) v4.03.

Physical examination was performed at the screening visit, on days 0, 14, 35, 56, 77, and 98 and at long-term maintenance (LTM) visits and weight and vital signs (blood pressure, pulse, and temperature) were measured; body height was assessed only at screening. Laboratory assessments included safety laboratory testing for infections with human immunodeficiency virus, hepatitis B, and C and evaluation of hematologic and serum chemistry parameters at screening, days 0, 14, 35, 56, 77, and 98 and at LTM visits. Cardiac assessment was performed on day 0 (suitability for study inclusion) and on day 56 and was based on medical history, physical examination, 12-lead ECG and evaluation of left ventricular ejection fraction (LVEF) by echocardiography or MUGA scan. Follow-up cardiac assessment was performed when LVEF decreased by > 10% between day 0 and day 56. The vaccination site was evaluated on days 2, 16, and 37 (2 days after vaccination) for local effects of the vaccine injection and data on SAEs were collected throughout the study, including 28 calendar days after the last administration of assigned study treatment or in case of death likely related to the investigational product at any time.

Response rates

Radiographic assessment was performed for all patients at screening (for inclusion criteria assessment) and at days 56 and 98 and at all LTM visits until disease progression. CT scans of the chest, abdomen, and pelvis with contrast were used to visualize and measure tumor lesions; alternatively, MRI of the abdomen and pelvis with contrast and noncontrast CT of chest were done in patients with iodine contrast dye allergy or lesions not visible on CT scan. Sum of diameters (SOD) of the respective lesions was calculated and tumors were assessed according to the RECIST v1.1.

Vaccine-specific immunologic evaluation

Humoral immunity was assessed in serum obtained from whole blood samples taken on days 0, 14, 35, 56, 77, and 98 and at LTM visits and cellular parameters were analyzed in peripheral blood mononuclear cell (PBMC) isolated from heparinized blood samples collected on days 0 and 56 and at each LTM visit (sampling schedule, Supplementary Table S3B).

ELISA for detection of vaccine-specific IgG Abs

In brief, 96-well microtiter plates (Nunc-Maxisorp Plate, Nalge Nunc International) were coated with 2.5 µg/mL human HER2/ErbB2 Protein (Sino Biological Inc.) or 5 μg/mL P467 protein (pChem, Austria) in 100 mmol/L carbonate buffer, pH 9.6 overnight at 4°C. After washing and blocking, serial dilutions of patient sera were incubated overnight at 4°C. Bound IgG Abs were detected with horseradish peroxidases (HRP)-labeled anti-human IgG (RRID: AB_1075966; Pierce/Thermo Fisher Scientific) and tetramethylbenzidine (TMB) color development measured at 450/630 nm. HER2-specific IgG Abs were quantified with a trastuzumab standard titration curve and P467-specific IgG were calculated by use of a positive pool serum obtained from 3 patients from the previous phase I breast cancer study (10). Assay control and assay-to-assay comparability was performed with a positive and negative pool serum of defined patients (see detailed protocol in Supplementary Data S1).

Antigen-specific restimulation of PBMC

PBMC samples stored in liquid nitrogen were reestablished in culture medium RPMI1640 supplemented with 10% human AB serum (Biochrom) and 2 mmol/L l-glutamine, 50 µmol/L 2-mercaptoethanol, and 0.1 mg/mL gentamycin (all Sigma Aldrich). Cells were plated in 96-well round-bottom plates at 8 × 10⁵/well and cultured with CRM197 (Merck-Millipore) at 4 µg/well, with human HER2/ErbB2 Protein (His Tag: Sino Biological Inc.) at 4 µg/well, and with superantigen Staphylococcus Enterotoxin B (0.2 µg/well) as positive control and in culture medium only for assessment of cytokine baselines. Cultures (total volume 200 µL) were maintained for 48 hours (37°C, 5% CO₂, 95% humidity) and then supernatants were harvested, pooled, and stored at −20°C until analyses.

Quantification of cytokine production in culture supernatants

Cytokines IL2, IFNγ, TNFα, IL17A, IL10, IL5, and IL13 were quantified in culture supernatants of restimulated PBMC obtained at days 0, 56, 98, 182, 266, and 350 by use of a Luminex 200 platform and Procartaplex Multiplex Immunoassays (eBioscience, now Thermo Fisher Scientific) as described previously (16).

Cell lines and culture conditions

For evaluation of Ab-binding capacity and phosphorylation assays the HER2/neu-expressing gastric tumor cell line NCI-N87 (RRID: CVCL_1603), as well as two HER2-negative control cell lines, human 518.A2 melanoma cells (RRID: CVCL_UM77; ref. 9) and murine D2F2 breast cancer cells (17) were used. All cell lines were grown in a humidified chamber with the atmosphere containing 5% CO₂ at 37°C. The human N87 gastric cancer cells (ATCC CRL-5822) were maintained in high-glucose RPMI1640 medium (Gibco/Life Technologies),...
and the human 518.A2 melanoma cells were maintained in high-glucose DMEM (Gibco/Life Technologies). Both media were supplemented with 10% (v/v) heat-inactivated FBS (Gibco/Life Technologies), 1% (w/v) l-glutamine (PAN Biotech), 1% (w/v) penicillin/streptomycin (PAN Biotech). The human HER2/neu negative murine D2F2 breast cancer cells were kindly provided by Wei-Zen Wei (Karmanos Cancer Institute, Wayne State University School of Medicine, Detroit, MI). The D2F2 cells were maintained in high-glucose DMEM (Gibco/Life Technologies), supplemented with 10% (v/v) heat-inactivated FBS (Gibco/Life Technologies), 1% (w/v) l-glutamine (PAN Biotech), 1% (v/v) penicillin/streptomycin (PAN Biotech), 10% NCTC 109 (Gibco), 7.5% Sodium Bicarbonate (Gibco), and 1% Sodium Pyruvate (Gibco). The humidified chamber used for the incubation of the cells was routinely examined for detection of Mycoplasma.

Flow-cytometric lymphocyte analyses and IgG binding assays to HER2-overexpressing gastric cancer cells

PBMC were surface stained with fluorochrome-conjugated mAbs and stained intracellular with a mAb against transcription factor FOXP3 for characterization of regulatory T cells (Treg; see Supplemental Data S2). Fixable viability dyes eFluor-780 and eFluor-506 (eBioscience, now Thermo Fisher Scientific) were used for exclusion of dead cells. Data were acquired on a FACS Canto II flow cytometer by gating on cells with forward/side light scatter properties of lymphocytes and analyzed with FACS Diva 8.0 software.

Immunofluorescence staining of cells was evaluated to measure binding of human IgG Abs to HER2-overexpressing gastric cancer cells (NCI-N87). For this IgG from patient sera were purified: 250 μL of crude sera were used for elution of total IgG using NAB Spin Kits, 0.2 mL for Antibody Purification (Thermo Fisher Scientific) according to manufacturer’s protocol. The obtained eluate was then exchanged with PBS, and IgG concentrations were determined with a Nano ND-1000 spectrometer (PEQLAB Biotechnology GmbH).

Human N87 gastric cancer cells (passage 27) as well as HER2/neu negative human 518.A2 melanoma cells (passage 53) and murine D2F2 breast cancer cells (passage 9) were used to show specific binding of purified patient IgG to native HER2. Cells were maintained as described above. For the binding assay, 5 × 10^5 cells in 50 μL FACS buffer were incubated with 15 μg pre- and post-vaccination patients IgG, 15 μg healthy donor control IgG, or 1.25 μg therapeutic mAb trastuzumab for 2 hours at 4°C. Unbound IgG were removed with PBS, and thereafter cells were incubated with phycoerythrin-conjugated anti-human IgG Fc Ab (RRID: AB_465926; eBioscience, now Thermo Fisher Scientific) and fixable viability dye for 30 minutes at 4°C. Cells were washed in PBS and analyzed on a FACS Canto II flow cytometer. A total of 20,000 cells were gated by light-scatter assessment and single-parameter histograms were drawn.

Detection of intracellular HER2/neu and AKT phosphorylation

For detection of intracellular HER2/neu phosphorylation, human NCI-N87 gastric cancer cells were used. The cells (passage 27) were seeded into a 12-well plate and incubated in a humidified chamber (95% air, 5% CO₂) at 37°C overnight. Cell culture medium was removed, the cell monolayer was washed, and the culture medium containing the examined patients’ sera was added into the respective wells. The plate was incubated overnight at 37°C; thereafter cells were lysed using the Cell Lysis Buffer according to the manufacturer’s instructions (Cell Signaling Technology). The level of intracellular HER2 phosphorylation in the lysates of the treated cells was evaluated by ELISA, using a Phospho-HER2/ErbB2 (Tyrs 1222)/HER2 (1222) rabbit mAb (RRID: AB_490899; Cell Signaling Technology) and a HRP-conjugated goat anti-rabbit IgG (whole molecule; Sigma). For the measurement of phospho-HER2-specific Abs was calculated as follows: % inhibition = [100 – (OD(d6)/OD(d0) × 100)].

Results

Recruitment

In total, 14 patients with either gastric or gastroesophageal junction adenoma were enrolled in this phase I study. Three patients were included in the first (10 μg), 6 patients in the second (30 μg) and 5 patients in the third dose cohort (50 μg). All 14 patients were evaluated for safety and 11 patients completed the treatment period per protocol and were evaluated for response rates and immunogenicity. Patient TH03007 (C2) and patient GE01009 (C3) deceased after day 14 and patient GE01010 (C3) was excluded by physician’s decision.

Clinical responses

The study population consisted of 5 females and 9 males with mean age 57.3 years (SD = 16.7) Nine patients belonged to Asian and 5 to Caucasian race. Most patients had HER2 expression of IHC 3+ or 2+ with confirmation by FISH. Two IHC 2+ patients without FISH confirmation were included in C1 and one such patient in C3 (Table 1). Baseline findings in relation to medical history, concurrent conditions, and concomitant medications were similar across the groups.

Statistical evaluation

No formal statistical analysis was performed. Descriptive statistics including number of patients (N), means, SDs, medians, and minimum and maximum values are presented for continuous variables. Counts and percentages are presented for categorical variables. Safety and efficacy data are summarized and presented by subject, by treatment group, and by timepoint in summary tables.

Immunologic data are presented with descriptive statistics (minimum, maximum, median, geometric mean, arithmetic mean, SD) computed for each immunologic measure and each timepoint. Where appropriate, the ratio of immunologic measures assessed with descriptive statistics for these ratios (minimum, maximum, median, geometric mean, SD) calculated for each immunologic measure and each timepoint.

Radioisologic parameters was performed to have some initial signal/sign of possible immunologic effects on tumor growth.
Evaluation of safety

DLT criteria were defined and monitored by the CRC as:

- A drug-related grade 3 toxicity that cannot be resolved to grade 1 with appropriate therapy within 2 weeks, or a grade 4 or greater toxicity graded by CTCAE v4.03, related to IMU-131
- One or more patients experience a serious adverse reaction assessed as related to IMU-131 by the investigator
- One or more patients experienced anaphylaxis
- Two or more patients in a single dose cohort experienced a serious AE (SAE) explained by a diagnosis related to IMU-131 (severity grading by NCI-CTCAE v4.03.)

No DLTs were observed and clinical laboratory findings along with vital signs, electrocardiograms, and physical examinations were as expected for chemotherapy with no vaccine-related toxicity reported for the combination.

The 14 patients evaluated for safety received at least one dose of IMU-131. The 207 treatment-emergent AEs (TEAE), mainly assessed for the combination. No DLTs were observed and clinical laboratory findings along with vital signs, electrocardiograms, and physical examinations were as expected for chemotherapy with no vaccine-related toxicity reported for the combination.

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Table 1. Patient characteristics and clinical response rates.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age</th>
<th>Dose (µg)</th>
<th>HER2 Status</th>
<th>SOD (mm) Day 0</th>
<th>SOD (mm) Day 98</th>
<th>SOD (mm) Day 182</th>
<th>SOD (mm) Day 266</th>
<th>SOD (mm) Day 350</th>
<th>SAE (not IMU-131 related)</th>
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<tbody>
<tr>
<td>TH02 001</td>
<td>m</td>
<td>41</td>
<td>10</td>
<td>2+/FISH−</td>
<td>97</td>
<td>80 (SD)</td>
<td>114 (PD)</td>
<td>WD</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>TH03 001</td>
<td>f</td>
<td>33</td>
<td>10</td>
<td>2+/FISH−</td>
<td>268</td>
<td>520 (SD)</td>
<td>330 (PD)</td>
<td>Deceased</td>
<td>Convulsion</td>
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<tr>
<td>TW02 002</td>
<td>f</td>
<td>79</td>
<td>10</td>
<td>3+</td>
<td>38</td>
<td>9 (CR)</td>
<td>10 (PR)</td>
<td>14 (PD)</td>
<td>WD</td>
<td>Neutropenia</td>
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<td>53</td>
<td>30</td>
<td>2+/FISH−</td>
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<td>Inapp. ADH secretion</td>
<td></td>
<td></td>
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<tr>
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<td>30</td>
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<td>39 (SD)</td>
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<td>36 (PR)</td>
<td>35 (PR)</td>
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<td>30</td>
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<td>75 (PR)</td>
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<td>30</td>
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<td>NTL</td>
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Note: Best overall response indicated in red; continuation until study withdrawal indicated in green.

Abbreviations: ADH, antidiuretic hormone; CR, complete response; NTL, nontarget lesions; PD, progressing disease; PR, partial response; SAE, severe adverse event; SD, stable disease; SOD, sum of diameters; WD, withdrawn.

Due to two new lesions.

Due to one new lesion.

Because of the potential interaction of IMU-131 with ERG proteins at the cellular level, LVEF was assessed on day 0 and day 56 and 3 patients (2 patients in the 10µg and 1 patient in the 30µg dose group) showed a decrease between 10% and 20% from baseline. Patients with decreased LVEF at day 56 were followed-up with LVEF values.
returning to baseline levels in all retested patients. No clinical correlation was observed for these transient LVEF changes (Supplementary Table S5). Overall, IMU-131 in combination with chemotherapy was well tolerated with no significant local or systemic reactions, and no need for pretreatment or modification to the dose/treatment schedule due to safety. The selected treatment dose will be further characterized for TEAEs and overall safety in the phase II randomized and controlled study comparing IMU-131 plus chemotherapy versus chemotherapy only.

RP2D
All available data were reviewed by the CRC comprising of site investigators and clinical immunologist per cohort and after patients in C3 had completed day 56. On the basis of safety results (no DLTs, no significant injection site reactions and no IMU-131 related SAEs) and consistent moderate to high levels of HER2-specific IgG Abs, the 50 μg dose of IMU-131 was recommended and selected as RP2D.

Response rates
Eleven of 14 patients were evaluable for assessment of tumor response rates as an exploratory endpoint of this study. The overall response was calculated from responses of target and nontarget lesions and based on the RECIST v1.1 criteria for complete response (CR), partial response (PR), stable disease (SD), progressive disease (PD) and "not evaluable". Tumor size (reported as SOD) was evaluated at screening/inclusion (d0), End of Trial visit (d56), start of LTM (d98) and at each LTM visit, which were 84 days apart, until withdrawal from study. Median change in tumor size from baseline to progression (with range): C1 -18% (range, 23 to -76), C2 -18% (range, -15 to -55), C3 -41% (range, 8 to -79). The best response of 11 patients was one CR, five PRs, and four SDs; 1 patient showed PD (Table 1). C3 showed a prolongation of progression-free survival up to day 434 (Fig. 1).

Immunologic evaluation
Vaccine-specific immune responses were evaluated in 11 of 14 patients. Patients TH03007 (C2), GE01009, and GE01010 (C3) were not evaluable because they received only one or two IMU-131 doses and did not complete day 56 RECIST 1.1 tumor assessment. Patient GE01010 was terminated from the study as it was discovered that the patient was enrolled with advanced liver cirrhosis due to alcohol abuse, which was not revealed when he was included in the study. Patients TH03007 and GE01009 are included in the grade 5 AE list.

Vaccine-specific IgG antibodies
HER2- and P467 peptide-specific IgG Abs were quantified by ELISA to determine humoral responses to IMU-131. Overall, HER2-specific IgG levels in C2 (30 μg/dose) were higher than in C1 (10 μg/dose), mainly due to 2 of the 5 evaluable C2 patients showing high Ab titer increase whereas the remaining 3 showed only moderate or marginal Ab titer increases. In C3 (50 μg/dose), all evaluable patients showed moderate to high HER2-specific IgG increases upon vaccination (Fig. 2A, C, and E; Supplementary Table S6). In most patients Abs titers clearly increased 21 days after the third dose, i.e., at day 56, and
Figure 2.
HER2-specific IgG and clinical response rates in C1, C2, and C3. HER2-specific IgG Abs in C1 (A), C2 (C), and C3 (E) measured in sera that were obtained at days 0, 14, 35, 56, 77, 98, 182, 266, and 350. Concentrations in ng/mL on logarithmic scale calculated from a Herceptin Standard Curve Dilution. Change in tumor size indicated as SODs in mm calculated as positive or negative difference between SOD at day 0 and days 56, 98, 182, 266, and 350, respectively, for C1 (B), C2 (D), and C3 (F). mm, millimeters.
Ab concentrations remained at those levels or increased even further until the start of booster vaccination at day 98 (i.e., long-term maintenance). Thereafter, the majority of patients displayed a further Ab titer increase, and levels were maintained by subsequent booster vaccinations in 3-month intervals (Fig. 2C and E). The kinetic of the HER2-specific IgG Abs was strongly in parallel to the P467-specific Ab levels (Supplementary Fig. S1A–S1C).

Quantification of cytokine production in restimulated PBMC supernatants

Concentrations of cytokines IL2, IFNγ, TNFα, IL17A, IL10, IL5, and IL13 were assessed in response to CRM197 and HER2 stimulation in PBMC culture supernatants to characterize cellular immune responses to IMU-131. IL2 concentrations were very similar to IFNγ levels and thus indicate robust T-cell proliferation upon vaccination. IMU-131 also induced moderate levels of Th2 cytokines IL5 and IL13 (Supplementary Fig. S2D, S2G and S2H). IFNγ/IL10 and TNFα/IL10, as well as INFγ/IL5 and IFNγ/IL13 ratios were > 1, indicating a bias toward proinflammatory cytokines induced by active immunization with IMU-131 (Supplementary Fig. S2A and S2B; Supplementary Table S6).

Flow-cytometric lymphocyte analyses and IgG binding assays to HER2-overexpressing gastric cancer cells

Quantification of various lymphocyte subsets in PBMC by flow cytometry completed the assessment of cellular immune responsiveness. We observed that CD19⁻ B cells and CD3⁰ T cells as percentages of total lymphocytes were mostly within normal ranges. Expression of CD27 and IgD on B cells allows distinction of naïve B cells (IgD⁺/CD27⁻) and CD27⁺ memory B cells, which are either IgD⁺ (unswitched B memory) or IgD⁻ after class switch (switched B memory). The small double-negative (IgD⁻/CD27⁻) population includes class-switched, resting memory B cells lacking CD27 expression (18). In comparison with age-matched healthy donors from another trial, the study patients had lower percentages of unswitched B cells and in turn a rather expanded double-negative B-cell population. Numbers of plasma blasts (CD19⁺/CD27⁺⁺⁺/CD38high) were reduced in patients with cancer compared with healthy donors from other trials (refs. 19, 20; Supplementary Data S3).

Distributions of T-cell subsets, that is, CD4⁰ Th cells, CD8⁻ cytotoxic T cells, and natural killer (NK) T cells, in patients were similar to healthy donors. CD4⁺ and CD8⁺ T cells were divided into four subsets via CD45RA and chemokine receptor 7 expression: naïve T cells, lymph node homing central memory T cells, peripheral tissue homing effector memory T cells, and CD45RA reexpressing effector memory cells (TEmRA), which accumulate with age (21, 22). The subset distributions in patients were comparable with healthy donors with the respective age-associated changes (Supplementary Data S4). Tregs (CD4⁺/CD25⁺/FoxP3⁺) as percentages of CD4⁺ T cells were present in similar frequencies in patients versus healthy donors (Supplementary Data S4). However, the observed reductions of Tregs in a given patient at respective timepoints were often in accordance with tumor regression (Supplementary Table S6; Supplementary Fig. S3).

Direct binding of purified patient IgG to native HER2 receptor on N87 gastric cancer cells was evaluated by immunofluorescence staining and flow cytometry. As shown in Fig. 3A, IgG from a vaccinated patient with high concentrations of HER2-specific IgG showed a significant fluorescent shift in binding compared with pre-vaccination IgG and human healthy donor control IgG. This result demonstrated that vaccine-induced Abs specifically bind to HER2 overexpressed on gastric tumor cells. On both HER2-negative cell lines, that is, human 518.A2 melanoma cells (Fig. 3A) and murine D2F2 breast cancer cells (Supplementary Fig. S4), post-vaccination IgG showed no difference in binding compared with pre-vaccination IgG and human healthy donor control IgG.

Intracellular HER2 and AKT phosphorylation

In 4 of 11 patients with HER2-specific IgG serum levels inhibition of intracellular HER2 phosphorylation was observed (Supplementary Table S6, indicated in red; Fig. 3B). As phosphorylation of HER2 leads to activation (phosphorylation) of downstream signaling pathways, including the PI3kinase (PI3K)/AKT and the RAS/MAPK cascades (23), we also evaluated the level of AKT phosphorylation. Using the cell lysate of N87 cells treated with the serum of the patient with high HER2 binding antibodies (Fig. 3A), we observed in addition to a 37% reduction of HER2 phosphorylation a 13.4% inhibition of AKT phosphorylation (Fig. 3B). These results indicate the capacity of the anti-HER2 antibodies to inhibit HER2 and consequently AKT phosphorylation.

Correlation of clinical responses and immunologic parameters

Clinical responses were correlated with various immunologic parameters as exemplified in 2 patients, TW02004 and GE01004. Figure 4A shows that the tumor reduction in patient TW02004 seems to be associated with high HER2-specific Ab levels, their capacity to inhibit HER2 phosphorylation and increased ratios of CRM197-specific Th1 cytokines. In contrast, in patient GE01004 with no correlation between humoral responses and tumor reduction, high Th1 cytokine ratios supported the clinical response (Fig. 4B).

Loss of HER2 expression in a C3 patient

Patient TW02004 (21 years) in C3, diagnosed with stage IV gastric adenocarcinoma with four target lesions and five nontarget lesions, received both chemotherapy and IMU-131 vaccination for 12 months. Evaluation of the pretreatment HER2 status with FISH showed a HER2/CEP17 ratio of 4.98, that is, HER2+++ (Supplementary Fig. S5A). By day 182, the patient presented with PR for the target lesions with a SOD reduction from 177 to 52 mm (71%) and a CR for four of five nontarget lesions. By day 266, SOD of target lesions had reduced to 37 mm (79%). After day 350, the patient progressed according to RECIST 1.1 with a newly identified lesion and the reevaluation for HER2 status by FISH showed loss of HER2 amplification (HER2/CEP17 ratio of 1.63; Supplementary Fig. S5B). The FISH results for HER2 expression were confirmed by IHC staining (Supplementary Fig. S6A and S6B).

Discussion

In this phase Ib study, the safety, immunogenicity, and potential clinical responses after vaccination with the HER2-specific B-cell epitope vaccine, IMU-131, were evaluated in patients with HER2-overexpressing GEA. The vaccine comprises three B-cell epitopes of the HER2 extracellular domain fused to a single peptide which is coupled to CRM197 and was administered with Montanide ISA 51 as adjuvant.

Dose finding and determination of the RP2D were primary endpoints of the trial. In three dose cohorts (10, 30, 50 μg), three vaccinations were given in 2-week intervals in combination with chemotherapy. Because preclinical data indicated that doses <50 μg would not result in higher Ab levels (15), higher doses were not tested in the clinical trial. On the basis of the safety evaluation—which
showed no DLTs, no significant injection site reactions and no IMU-131 suspected SAEs—and the humoral vaccine responsiveness (HER2-specific IgG Abs), the 50 mg dose was chosen for use in phase II. Safety evaluation also regarded the use of the novel adjuvant Montanide ISA 51 VG, a water-in-oil emulsion consisting of a mineral oil and a mannide monooleate surfactant. Montanide’s immune-stimulatory effects are mediated by depot formation, inflammation, and immune cell recruitment (24) and when applied with cancer vaccines increased production of tumor-specific Abs and IFNγ-secreting CD4 Th cells are induced (25, 26). Montanide has been widely used as adjuvant in cancer trials and is licensed for a therapeutic vaccine against advanced non–small cell lung cancer (27). In our study, we observed two grade 1 vaccination site reactions in 2 patients with pruritus and erythema that were possibly vaccine related but represented no limitation to IMU-131 vaccine application, subsequent vaccinations were well tolerated.

Active immunization of patients with cancer aiming to generate humoral and cellular antitumor responses against cancer targets must consider the effects of concomitant chemotherapy on immune responsiveness. Lymphocyte populations are depleted by/after chemotherapy treatment (28), and especially B-cell populations are effected early during treatment (29); therefore, we started vaccination before onset of chemotherapy to effectively induce immunologic priming by the vaccine. This approach is also taken in routine vaccination of patients with cancer, where vaccines (such as against influenza) are given prior to chemotherapeutic treatment (30). We observed unaltered T-cell populations compared with healthy controls (Supplementary Data S4; ref. 29). However, while total B cells were not reduced in our study patients, lower percentages of unswitched B cells and in turn expanded class-switched terminally differentiated CD27+/CD0 memory B cells were observed (Supplementary Data S3). Accumulation of this subset could be due to the depletion of naive, unswitched, and switched populations as was described by Waidhauser and colleagues, which potentially could have an effect on vaccine responses (29).

However, neither changes in B-cell subsets nor chemotherapy, which was initiated after the first vaccine dose, limited the immunogenicity of IMU-131 in our patients, because humoral responses, that is, HER2- and peptide-specific IgG Abs, were well induced and increased at higher dose levels. After three 50 µg doses applied in C3, high Ab concentrations were observed at day 56 in 2 of 3 evaluable patients, while the 30 µg doses led to strong Ab increases only in 2 of 5 patients; both doses however were superior to the 10 µg dose. The kinetics of Ab levels indicated that HER2-specific IgG were boosted by vaccination on day 98 and thereafter were maintained by additional doses in 3-month intervals during the LTM phase.

Figure 3.
Native HER2 receptor binding assay (by FACS) and inhibition of phosphorylation (by ELISA) on N87 gastric cancer cells with patient sera. A, Flow cytometric binding analysis of patient Abs to N87 gastric cancer cells (15 µg of polyclonal patient IgG from d0 and after third vaccination). Binding of IgG from a healthy human control donor was used to determine negative cutoff. Pre-vaccination patient IgG shows background binding on 48.9% of N87 cells, while post-vaccination IgG binds on 80.9% of cells. No difference in IgG binding was observed on human HER2-negative 518A2 melanoma cells. Binding of mAb trastuzumab (Herceptin) to N87 cells is 10-fold higher than to 518A2 melanoma cells. B, Measurement of HER2 phosphorylation and phospho-AKT protein levels by ELISA. An in-house ELISA protocol was used to determine HER2 phosphorylation and the PathScan Phospho-Akt (Thr 308) sandwich ELISA kit for phospho-AKT protein levels. Inhibition of phosphorylation of HER2 and AKT by vaccine-induced Abs was calculated with the formula: % inhibition = (100 – (OD(d56)/OD(d0) x 100)). Herceptin-mediated phosphorylation inhibition was calculated versus untreated cells.
The historical target concentration for trastuzumab efficacy is 20 μg/mL, and pharmacokinetic studies of intravenous trastuzumab application show that various loading and maintenance schedules lead to steady state trough concentrations up to 50 μg/mL and more (31, 32). The highest vaccine-induced HER2-specific Ab concentrations measured in this trial were approximately 4–6 μg/mL, that is, lower than those achieved with passive Ab administration. We assume, that these concentrations might be comparable in efficacy because the half-life of endogenous IgG is 21 days (33), while a mean half-life of 5.8 days is reported for trastuzumab (34).

The effectiveness of the cancer vaccine, evaluated by progression-free survival, and tumor response, belonged to the exploratory endpoints of this phase Ib trial. Median change in tumor size from baseline to progression (with range): C1/C0 18% (range, 23 to 76), C2/C0 18% (range, −15 to −55), C3/C0 41% (range, 8 to −79). Ten of 11 patients, who were evaluable for tumor response assessment, presented with either CR, PR, or SD during the dose period (Table 1). As phase Ib, this trial was not designed with a placebo control, and thus it is difficult to assess the extent to which the clinical outcomes were due to chemotherapy, vaccination or a combination of both. The ongoing randomized phase II study may confirm the treatment benefit.

The observation that the PFS increase and tumor reduction are correlated with the highest vaccine dose may support that IMU-131 induces relevant vaccine-specific effects related to either antibodies, cellular immune responses or both, in particular as the chemo backbone remained unchanged to SOC practice. Patients who developed high Ab concentrations that could inhibit HER2 phosphorylation showed robust tumor control, for example, TW02004 in C3 (Fig. 4A). A well-known effect of trastuzumab is the inhibition of the MAPK and PI3K/Akt signaling pathways, leading to enhanced cell-cycle arrest and suppression of cell growth and proliferation, which is initiated by lack of phosphorylation of the intracellular domain of HER2 (35). We found that Abs in sera of several patients could inhibit intracellular HER2 phosphorylation in vitro (Fig. 3B; Supplementary Table S6), suggesting that IMU-131-induced Abs might have similar functionality as trastuzumab. It has been shown that AKT phosphorylates the proapoptotic Bcl-2 protein BAD, which results in sequestering BAD away from antiapoptotic Bcl-2 proteins and consequently suppresses BAD-induced cell death (36). Moreover, protease activity of caspase-9 is reduced upon phosphorylation by AKT, also leading to suppression of apoptosis (37). Our results, showing also inhibition of AKT (308) phosphorylation by vaccine-induced antibodies (Fig. 3B), are therefore indicative for apoptosis as a consequential antitumor effect. In patients with high Ab levels but without capacity to inhibit phosphorylation (TH05013, C2 and TW02003, C3) the clinical responses might be caused by other Ab functionality such as ADCC or CDC as we described previously (9).

Figure 4.
Correlation of tumor reduction and HER2-specific IgG, inhibition of HER2 phosphorylation, and CRM197-specific cytokine ratios in 2 selected patients. Changes in tumor size (SODs in mm vs. d0) correlated with HER2-specific IgG Abs levels, with percent inhibition of HER2 phosphorylation and with CRM197-specific IFNγ/IL10 ratios for (A) patient TW02004 (Cohort 3) and (B) patient GEOI004 (Cohort 2); coefficient of determination r² (+ or −) shown in graph.
Next to Abs, IMU-131 also induced cellular immune responses, as indicated by production of predominantly Th1-biased CRM197- and HER2-specific cytokines. The carrier-specific cellular responses were more robust with very high levels of IFNγ, while the HER2-specific responses were less pronounced with predominance of TNFα (Supplementary Fig. S2A–S2C and S2E). In particular, IFNγ producing Th1 cells have been shown to play a key role in controlling tumor growth via upregulation of MHC I on tumor cells and direct inhibition of tumor cell proliferation (38, 39). The measurement of low Th2 cytokine levels (IL5, IL13) supported the Th1 polarization by the vaccine (Supplementary Fig. S2G and S2H). Along these lines, we saw strong Th1 cell proliferation (38, 39). The measurement of low Th2 cytokine levels (IL5, IL13) supported the Th1 polarization by the vaccine.

Furthermore, the numbers of FOXP3+ Tregs in our patients were reduced in accordance with tumor regression (Supplementary Fig. S3; Supplementary Table S6). Tregs infiltrate into tumor tissues and suppress effective tumor immunity, which is associated with poor patient prognosis (40). Depletion of Tregs is a useful approach to enable antitumor immunity as has been shown in patients with melanoma, where administration of anti-CTLA4 mAbs not only activated T cells, but also depleted Tregs in tumor tissues (41). Previously, vaccination in healthy subjects and also in patients in our previous cancer trial led to relative reduction of Tregs in peripheral blood due to expansion of T effector populations (10, 16, 19, 42); as such, this effect might be a further benefit of the active immunization approach in cancer treatment.

It is a well-recognized phenomenon that long-term treatment with trastuzumab can quite frequently lead to a loss of HER2 expression in patients with breast cancer (43, 44). Similar observations were also reported for patients with HER2-positive GEA, even though at lower rates (45). We also observed loss of HER2 expression in 1 patient who received the highest dose, approximately 1 year after vaccination, when at day 350 the occurrence of a new lesion without HER2 expression was detected (Supplementary Fig. S5B; Supplementary Fig. S6B). As described for trastuzumab, selective pressure on HER2 might also be exerted by vaccine-induced HER2-specific Abs, giving HER2-negative cancer cell clones a survival advantage which eventually leads to tumor progression (45). This indicates that efforts to overcome treatment limitations due to loss of HER2 expression may have to extend to immunizations with cancer vaccines (46), possibly in the form of combining the IMU-131 vaccine with checkpoint inhibitors as we recently demonstrated in a preclinical setting (17).

We conclude that our novel B-cell epitope vaccine, IMU-131, was safe and immunogenic in patients with HER2-overexpressing GEA. It induced HER2-specific humoral and cellular immune responses, which were dose dependent and associated with tumor reduction. Our results suggest additional immunomodulatory effects of HER2-specific Abs and cellular responses to chemotherapy to mediate tumor control, which is an obvious advantage of an active vaccine against cancer targets over mAb treatment. On the basis of our results, a controlled phase II study (50 μg per dose) with two treatment arms, CT only and CT with IMU-131 vaccine, is currently ongoing with promising preliminary results in the vaccinated group (data on file). Further work will determine whether this HER2-targeted active B cell cancer vaccine will lead to an important alternative treatment option for HER2-overexpressing malignancies.

Authors' Disclosures

U. Wiedermann reports grants from Imugene during the conduct of the study; in addition, U. Wiedermann has a patent for US2017/0119867A1 issued. E. Garner-Spitzer reports grants from Imugene Ltd during the conduct of the study. C. Charoentum reports grants from Roche, Astrazeneca, and Novartis outside the submitted work. L.-Y. Bai reports other from Imugene Limited during the conduct of the study. M. Drinic reports grants from Imugene Ltd during the conduct of the study. C.C. Zielinski reports grants, personal fees, and nonfinancial support from Imugene and personal fees from Roche during the conduct of the study; in addition, C.C. Zielinski has a patent issued. N.J. Ede reports personal fees from Imugene Limited during the conduct of the study, as well as personal fees from Imugene Limited outside the submitted work. No disclosures were reported by the other authors.

Authors' Contributions


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References


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