Comprehensive results of a Phase Ib study with a HER2/neu B-cell peptide vaccine administered with cisplatin and 5-fluorouracil or capecitabine chemotherapy show safety, immunogenicity and clinical response in patients with HER2/neu overexpressing advanced gastric cancer

MEDICAL UNIVERSITY OF VIENNA #

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IMUGENE Developing Cancer Immunotherapies ##

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Background:

HER2/neu is overexpressed in 15-25% of gastric cancer patients and associated with poor prognosis. Recombinant monoclonal antibodies (mAbs) against HER2/neu have been shown to be effective in clinical application but alternative treatments are needed due to cost and global availability issues of mAbs.

Thus a **B-cell peptide vaccine (IMU-131)** was developed at the Medical University of Vienna # (1), consisting of three fused B-cell epitopes (p467) from the HER2/neu extracellular domain coupled to CRM197 and administered with the adjuvant Montanide.

The goal of the study was to evaluate the optimal and safe vaccine dose which leads to immunogenicity and clinical responses.

Material & Methods:

An **open label multicenter Phase 1b trial** was performed in SE-Asia (Taiwan, Thailand) and Eastern Europe (Georgia, Moldova).

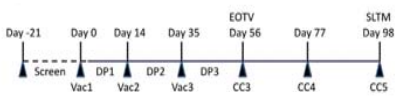
Fourteen patients with HER2/neu overexpressing gastric or gastro-esophageal junction adenocarcinoma were enrolled.

Dose escalation with 10, 30, and 50 µg of IMU-131 was performed in 3 cohorts, each including 3 to 5 patients.

Immune responsiveness to **IMU-131** was evaluated according to antibody levels against p467 peptide and HER2 and by cellular responses.

HER2- and p467-specific IgG levels were determined by ELISA, cytokine levels were measured by Luminex Technology in PBMC cultures after 48h of HER2-stimulation and lymphocyte subsets were analyzed by FACS.

Timeline of interventions:



DP Dose Period
CC Chemotherapy Cycle
Vac IMU-131 administration
EOTV End of Treatment Visit
SLTM Start of long-term maintenance

Results:

Our Ph1b study data indicate that IMU-131 is safe and tolerated with no significant local or systemic reactions, and no need for pre-treatment or for modification to the dose or treatment schedule due to safety. No SAEs related to administration of IMU-131 were reported.

Eleven of fourteen patients were evaluable for vaccine-specific immune responses and tumor response assessment (Table 1, Fig. 2).

Higher HER2-specific IgG levels were observed in cohort 2 (30 µg/dose) compared to cohort 1 (10 µg/dose). Three of five patients in cohort 2 displayed moderate or little increase in antibody titers. In contrast, all patients in cohort 3 (50µg/dose) showed a moderate or high increase of HER2-specific Ab levels upon vaccination (Fig. 1).

Response rate was an exploratory endpoint of this clinical trial and of 11 evaluable patients one showed complete response, 5 partial response and 4 stable disease (RECIST response, Tables 1 & 2).

In cohort 3, antibody levels correlated strongly with clinical responses /changes in tumor size (Fig. 1, 2 & 3). In 5 of 11 patients tumor reduction was associated with high HER2-specific IgG levels, which could inhibit HER2-phosphorylation (Table 3, Fig. 4). Several patients showed marked tumor reduction in association with high HER2-specific Ab levels w/o phosphorylation inhibition (function to be determined) or low Ab levels. In these patients, a Th1 biased cytokine profile, i. e. increased IFNg/IL10 and/or TNFa/IL10 ratios, as well as reduction of suppressive T-regulatory cells was observed (Table 3).

	Patient	gender	age	Dose (µg)	HER2 Status	SOD d0 (mm)	SOD d56 (mm)	SOD d98 (mm)	SOD d182 (mm)	SOD d266 (mm)	SOD d350 (mm)	SAE (not IMU-131 related)
C1	TH02 001	m	41	10	2+/Fish-	97	80 (SD)	114 (PD)	Withdrawn			none
	TH03 001	f	33	10	2+/Fish-	268	320 (SD)	330 (PD)	Deceased			Convulsion
	TW02 002	f	79	10	3+	38	9 (CR)	10 (PR)	14 (PD)	Withdrawn		Neutropenia
C2	TH05 013	f	54	30	2+/Fish+	49	39 (SD)	39 (SD)	Deceased			Hyponatraemia
	TH05 015	m	55	30	3+	78	66 (SD)	36 (PR)	35 (PR)	Deceased		none
	GE01 004	m	58	30	3+	122	103 (SD)	75 (PR)	Deceased			Pneumonia
	TW01 001	f	80	30	3+	56	40 (PD)*	Withdrawn	Withdrawn			Kidney failure
	MD01 003	m	64	30	3+	NTL**	NTL (SD)	NTL (PD)	Withdrawn			none
C3	TW02 003	m	75	50	2+/Fish+	66	36 (PR)	39 (PR)	42 (PR)	65 (PD)	Withdrawn	Gastritis
	MD01 007	m	66	50	2+/Fish -	26	28 (SD)	18 (PR)	18 (PR)	18 (SD)	20 (SD)	none
	TW02 004	f	21	50	3+	177	105 (PR)	99 (PR)	52 (PR)	37 (PR)	(PD)	Leucocytosis

Table 1: Patient characteristics; SOD - sum of diameters

* due to 2 new lesions; ** NTL non-target lesions

Best Overall Response	IMU-131 10 µg (n=3)	IMU-131 30 µg (n=6)	IMU-131 50 µg (n=5)
Complete Response (CR)	1 (33.3%)	0 (0.0%)	0 (0.0%)
Partial Response (PR)	0 (0.0%)	2 (33.3%)	3 (60.0%)
Stable Disease (SD)	2 (66.7%)	2 (33.3%)	0 (0.0%)
Progressive Disease (PD)	0 (0.0%)	1 (16.7%)	0 (0.0%)
Not Evaluable (NE)	0 (0.0%)	0 (0.0%)	1 (20.0%)
Not Applicable (NA)	0 (0.0%)	1 (16.7%)	1 (20.0%)
Objective Response (CR+PR)	1 (33.3%)	2 (33.3%)	3 (60.0%) *
Disease Control Rate (CR+PR+SD)	3 (100.0%)	4 (66.7%)	3 (60.0%)

Table 2: Best overall response

* In cohort 3, the 3 patients evaluated at Day 98 after completed IMU-131 vaccinations showed 100% objective response (CR+PR), while 2 patients dropped out early due to incorrect enrolment or SAE (non-vaccine related).

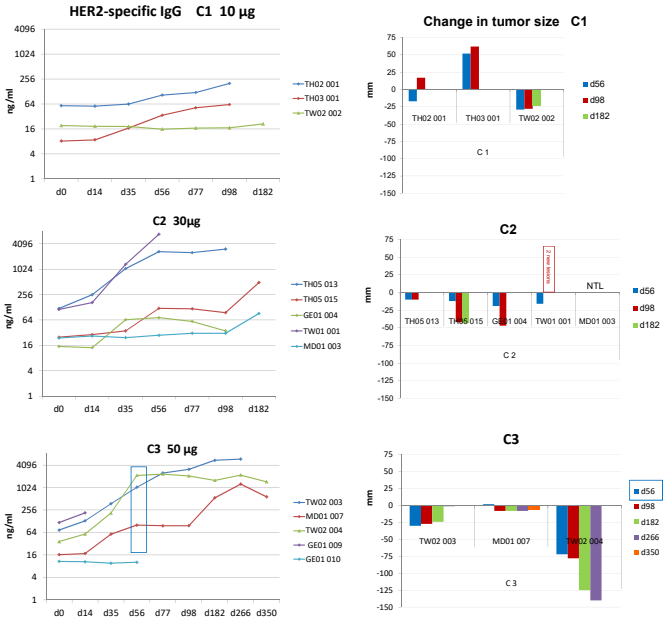


Fig. 1: HER2-specific IgG Abs in Cohort 1, 2, and 3 measured in sera obtained at Day 0, 56 and 98. Concentrations in ng/ml calculated from a Herceptin Standard Curve Dilution.

Fig. 2: Change in tumor size (in mm) from Day 0 to Day 56, 98, 182, 266 and Day 350 in C1, C2 and C3.

	C1						C2										C3					
	TH02 001		TH03 001		TW02 002		TH05 013		TH05 015		GE01 004		TW01 001		MD01 003		TW02 003		MD01 007		TW02 004	
	d56	d98	d56	d98	d56	d98	d56	d98	d56	d98	d56	d98	d56	d98	d56	d98	d56	d98	d56	d98	d56	d98
Her 2-specific IgG	✓	0	0	0	0	0	✓	✓	✓	✓	✓	0	✓	X	0	0	✓	✓	✓	✓	✓	✓
Inhibition of phosphorylation	✓	0	0	0	✓	0	0	0	✓	0	0	0	✓	X	✓	✓	0	0	✓	✓	✓	✓
↑ IFNg/IL10 ratio TNFa/IL10 ratio	0	X	X	0	✓	0	✓	X	✓	✓	✓	✓	✓	X	✓	0	X	✓	0	0	0	X
T-reg reduction	X	X	X	X	✓	✓	X	✓	0	✓	✓	✓	0	X	X	0	✓	0	0	✓	X	X

Table 3: Parameters correlated with tumor size reduction, 0= no correlation, X= not determined

✓ = correlation tumor reduction and HER2-specific Abs

✓ = correlation tumor reduction and Abs with HER2 phosphorylation inhibition

✓ = correlation tumor reduction and cellular parameters

Inhibition of phosphorylation: HER2-expressing gastric cancer cells (N87) were incubated with patient sera, Herceptin or left untreated and cell lysates were tested with capture ELISA for phosphorylated HER2.

IFNg/IL10 & TNFa/IL10 ratios: Cytokine concentrations of IFN-g, TNF-a and IL-10 were measured in supernatants of HER2 re-stimulated PBMC cultures. Ratios of IFN-g/IL-10 and TNFa/IL-10 were calculated.

T-regulatory cells: Tregs (CD4+/CD25+/Foxp3+) were quantified as % of CD4+ T-helper cells by Flow cytometry. Difference/reduction of Tregs was calculated vs. Tregs as % of CD4 at Day 0.

Results:

- In 5 of 11 patients, tumor reduction was associated with high HER2-specific IgG levels, which showed capacity to inhibit HER2-phosphorylation.
- In patients showing tumor reduction in association with low Her2-specific Abs (TW02 002) or Abs without capacity to inhibit phosphorylation, increased IFNg/IL10 and TNFa/IL10 ratios and/or reduction of T-regulatory cells were observed.

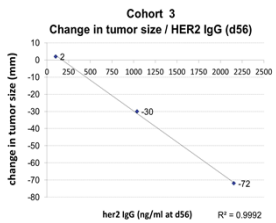


Fig. 3: Correlation of change in tumor size from Day 0 to Day 56 and HER2-specific IgG levels at Day 56 in Cohort 3.

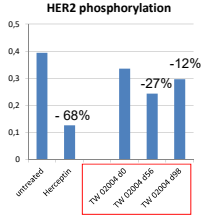


Fig. 4: Example of inhibition of HER2-phosphorylation by Herceptin and patient sera. Percent reduction by Herceptin vs. untreated cells and by patient sera (C3, TW02 004) from Day 56 and Day 98 vs. Day 0.

Conclusions:

The vaccine was well tolerated and safe, with antibody responses at the highest dose (50 µg) showing a strong correlation with clinical responses. Thus, a dose of 50 µg was recommended for further evaluation in a Phase II trial, featuring two arms of either IMU-131 plus chemotherapy (CT) or CT alone, which has been initiated (first patient in).

Proposed mechanisms of action:

Direct effect of Abs on phosphorylation status of HER2 and/or HER2-specific Th1-biased cytokine profile and/or decrease of T-regulatory cells.

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[1] *Tobias J, Jasinska J, Baier K, Kundi M, Ede N, Zielinski C, Wiedermann U.* Enhanced and long term immunogenicity of a Her-2/neu multi-epitope vaccine conjugated to the carrier CRM19 7in conjunction with the adjuvant Montanide. BMC Cancer. 2017

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Correlation with tumor reduction:

	TH02 001		TH03 001		TW02 002			TH05 013		TH05 015			GE01 004		TW01 001		MD01 003			TW02 003				MD01 007					TW02 004				
	d56	d98	d56	d98	d56	d98	d182	d56	d98	d56	d98	d182	d56	d98	d56	d98	d56	d98	d182	d56	d98	d182	d266	d56	d98	d182	d266	d350	d56	d98	d182	d266	d350
Her 2 Abs	✔							✔	✔	✔	✔	✔	✔		✔					✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔
Inhibition of phosphory-lation	✔				✔		tbd			✔		tbd			✔	nd	✔	✔	tbd					✔	✔			tbd	✔	✔	✔	✔	tbd
IFNg/IL10 TNFa/IL10					✔			✔		✔	✔	tbd	✔	✔	✔	nd			tbd	nd	✔	tbd	tbd			tbd	tbd	tbd		nd	tbd	tbd	tbd
Reduced Tregs	nd	nd	nd	nd	✔	✔	tbd	nd	✔		✔	tbd	✔	✔		nd		nd	tbd	✔		tbd	tbd		✔	tbd	tbd	tbd	nd	nd	tbd	tbd	tbd

Correlation with tumor reduction:

	TH02 001		TH03 001		TW02 002		TH05 013		TH05 015		GE01 004		TW01 001		MD01 003		TW02 003		MD01 007		TW02 004	
	d56	d98	d56	d98	d56	d98	d56	d98	d56	d98	d56	d98	d56	d98	d56	d98	d56	d98	d56	d98	d56	d98
Her 2-specific IgG	✔	0	0	0	0	0	✔	✔	✔	✔	✔	0	✔	nd	0	0	✔	✔	✔	✔	✔	✔
Inhibition of phosphorylation	✔	0	0	0	✔	0	0	0	✔	0	0	0	✔	nd	✔	✔	0	0	✔	✔	✔	✔
↑ IFNg/IL10 ratio TNFa/IL10 ratio	0	nd	nd	0	✔	0	✔	nd	✔	✔	✔	✔	✔	nd	✔	0	nd	✔	0	0	0	nd
T-reg reduction	nd	nd	nd	nd	✔	✔	nd	✔	0	✔	✔	✔	0	nd	nd	0	✔	0	0	✔	nd	nd