Safety and immunogenicity of the optimized cryptic peptide$TERT_{572Y}$ in patients with advanced malignancies: a Phase I clinical study.

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Abstract: Background: $TERT_{572Y}$, an optimized cryptic peptide homologous to TERT induces efficient antitumoral T cell cytotoxic immunity but not autoreactivity *in vivo* in HLA-A*0201 transgenic mice and healthy blood donors and prostate cancer patients (J. Clin. Invest., 2004, 113,425). A phase I trial evaluating the safety and immunogenicity of the cryptic $TERT_{572Y}$ peptide was conducted in HLA-A*0201 cancer patients. **Methods**: Nineteen patients with chemotherapy refractory and progressing malignant tumors were enrolled in the study. The vaccination protocol consisted of two injections of optimized $TERT_{572Y}$ peptide followed by four injections of native $TERT_{572}$ peptide. Peptides were injected emulsified in Montanide ISA51. Patients were vaccinated with escalated doses of peptide ranging from 2 to 6 mg. Toxicity and peptide-specific immune responses were evaluated. **Results**: Fourteen patients completed the entire vaccination program. Only grade I toxicity was observed, affecting 13 of the 19 patients. $TERT_{572Y}$-specific cytotoxic T cells were detected in the peripheral blood of 13 out of 14 evaluate patients, as early as 3 weeks after the 2nd vaccine injection. CTLs were fully functional and killed TERT-overexpressing tumor cells. Four (29%) of 14 evaluable patients experienced stable disease for a median of 10 months. **Conclusions**: $TERT_{572Y}$ peptide vaccine is well tolerated and effective in eliciting a specific T cell immunity. This is the first clinical trial demonstrating that cryptic peptides are promising candidates for cancer immunotherapy.

Introduction

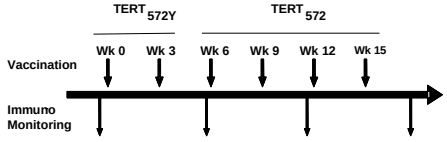
Tolerance to tumor antigens and especially to their dominant peptides is a major barrier in tumor immunotherapy. To circumvent this tolerance, we proposed vaccination with cryptic peptides. In humanized mice, we found that tolerance to cryptic peptides was weak or absent, and that cryptic peptides efficiently induced antitumor immunity *in vivo*, providing their immunogenicity had been optimized (J. Clin. Invest., 2004, 113, 425). We have previously described a peptide sequence modification that optimizes immunogenicity of HLA-A*0201-restricted cryptic peptides (Eur. J. Immunol., 2000, 30, 3411).
$TERT_{572Y}$ is an HLA-A*0201-associated optimized cryptic peptide derived from TERT, an antigen overexpressed by 85% of tumors. $TERT_{572Y}$ differs from native $TERT_{572}$ at the first amino acid position, where arginine (R) is replaced by tyrosine (Y). This substitution enhances affinity for the HLA-A*0201 molecule and optimizes immunogenicity of $TERT_{572}$ peptide (J. Immunol., 2002, 168, 5900). $TERT_{572Y}$ is present in both human and murine TERT and is able to induce antitumor immunity in HLA-A*0201 transgenic mice; no autoimmunity against normal TERT-expressing tissues is observed even one year after vaccination (J. Clin. Invest., 2004, 113, 425). *In vitro*, $TERT_{572Y}$ stimulates antitumor CTL from both healthy donors and prostate cancer patients. CTL kill TERT-overexpressing tumor cells but not TERT-expressing normal cells (J. Immunol., 2002, 168, 5900; PNAS 2002, 99, 12275).

Clinical study protocol

Patients enrolled :3 NSCLC, 4 colon, 2 ovarian, 2 breast, 1 prostate, 1 cervical, 1 esophagus, 1 head and neck cancers, 1 melanoma, 2 RCC, 1 cancer of unknown origin

Primary end-point : evaluate toxicity and the risks of autoireactivity against normal tissues expressing TERT such as bone marrow, kidney, liver, intestinal epithelium etc…
Secondary end-point : evaluate immunogenicity

Protocol



Evaluation of toxicity and autoreactivity

Toxicity was evaluated every week during vaccination and every month thereafter for one year or until the death of the patient. Toxicity measurements were particularly focused on hematopoiesis, and renal and hepatic functions. Bone marrow, kidney and liver express TERT and might, therefore, be targets of a TERT specific CTL response generated in vaccinated patients.

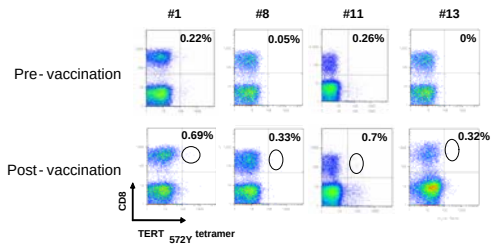
Toxicity and autoreactivity in vaccinated patients

Adverse Events	Toxicity Grade			
	Grade I	Grade II	Grade III	Grade IV
Local skin reaction	10	0	0	0
Anemia	3	0	0	0
Thrombocytopenia	2	0	0	0
Fatigue	1	3	0	0
Nausea	0	2	0	0
Anorexia	0	2	0	0

Evaluation of $TERT_{572Y}$ immunogenicity

The immunogenicity of $TERT_{572Y}$ was evaluated by measuring the frequency of vaccine-specific CD8 cells in the patients' peripheral blood assessed by staining CD8 T cells with $TERT_{572Y}$ tetramer. In a preliminary study, $TERT_{572Y}$ tetramer labeled less than 0.11% of CD8 cells in seven HLA-A*0201 healthy donors (mean 0.035±0.035, range 0.0-0.11%). The positivity cutoff for specific immunity was therefore set at 0.14% (mean+3SD).

$TERT_{572Y}$ specific immune response in vaccinated patients (1)



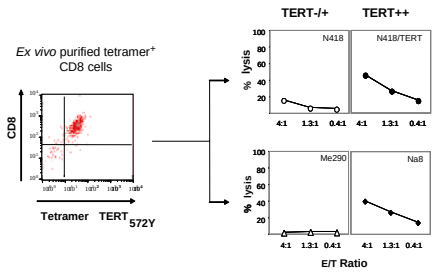
$TERT_{572Y}$ specific immune response in vaccinated patients (2)

% of $TERT_{572Y}$ specific CD8 cells in the peripheral blood

Patient	Pre-vaccination	During vaccination	Post-vaccination
#1	0,29	1,25	NT
#2	0	0,11	NT
#3	0	1,14	NT
#4	NT	4,00	0,48
#5	0	0,36	NT
#6	0,01	4,20	NT
#7	0,14	0,42	0,36
#8	0,33	0,98	NT
#11	0,30	1,30	0,52
#12	0,11	0,98	NT
#13	0	0,88	0,48
#15	0,04	0,45	NT
#18	0	0,62	NT
#19	0	0,73	NT

$TERT_{572Y}$ specific immune response in vaccinated patients (3)

Killing of TERT expressing tumor cells by $TERT_{572Y}$ specific T cells



Evaluation of clinical response

Evaluation of clinical responses was based on CT-scan, performed before the first vaccine injection and after the sixth injections in patients who completed the vaccination protocol. Disease progression was considered when existing lesions grew by more than 25% or new lesions appeared. Surrogate tumor markers were also measured. When there was discrepancy between CT-scan and surrogate tumor markers the diagnosis of disease progression was based exclusively on CT-scan.

Clinical response in fully vaccinated patients

Patient	Cancer	Dose of vaccine	Clinical response
#2	breast	2mg	Progression
#3	melanoma	2mg	Progression
#4	lung	3mg	Progression
#6	cervix	3mg	Progression
#7	Head and neck	4mg	Progression
#8	colorectal	4mg	Progression
#9	renal	4mg	Stabilization 17+ months
#10	colorectal	5mg	Progression
#11	lung	5mg	Stabilization 9 months
#12	breast	5mg	Stabilization 17+ months
#13	renal	6mg	Stabilization 14+ months
#15	colorectal	6mg	Progression
#16	prostate	6mg	Progression
#17	ovarian	6mg	Progression

Conclusion

This is the first human clinical trial testing optimized cryptic peptides:

- $TERT_{572Y}$ was well tolerated in all patients and did not induce any autoimmunity
- $TERT_{572Y}$ stimulated fully functional immune response in most patients evaluated to date
- Almost 30% patients having completed vaccination protocol displayed stability of their disease

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