1418 AACR 97th annual meeting

$TERT_{572Y}$ is an optimized cryptic peptide that induces efficient antitumoral T cell immunity but not autoreactivity *in vivo* in HLA-A*0201 transgenic mice and *in vitro* in 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. Sixty patients with advanced cancer were enrolled. The vaccination protocol consisted of two subcutaneous injections of optimized $TERT_{572Y}$ peptide followed by four injections of native $TERT_{572}$ peptide. Peptides were emulsified in Montanide ISA51. Forty one patients completed the entire vaccination program. The median follow up was 8.1 months (range 1.4-29.9). Only grade I/II toxicity was observed. $TERT_{572}$ specific cytotoxic T cells were detected in almost all patients. *Ex vivo*, $TERT_{572Y}$ tetramer positive $CD8^+$ cells varied from 0.07% to 4.5% (mean: 0.9% ± 1.3%) and ELISPot-detected $TERT_{572}$ specific $CD8^+$ cells varied from 0.001% to 1.5% (mean 0.29% ± 0.35%). $TERT_{572}$ specific CD8+ cells were still detectable one year after the sixth vaccination. They were fully functional, recognizing and killing TERT-overexpressing tumor cells. One patient experienced a partial response and twenty patients experienced stable disease for a median of 10 months (range 3.5-27). Nineteen patients died and the median overall survival was 18.7 months (range 1.4-29.9), the 1-year survival probability was 73.8%.

In conclusion, $TERT_{572Y}$ peptide vaccine is well tolerated and effective in eliciting a specific T cell immunity, allowing the initiation of more advanced and targeted clinical trials.

Safety and Immunogenicity of the Optimized Cryptic Peptide $TERT_{572Y}$ in Patients with Advanced Cancer: Latest Clinical Data.

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Introduction

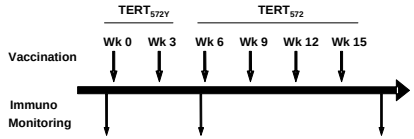
Tolerance to tumor antigens and especially to their dominant peptides is a major barrier in tumor immunotherapy. To circumvent this tolerance, we proposed vaccination with cryptic peptides. In humanized mice, we found that tolerance to cryptic peptides was weak or absent, and that cryptic peptides efficiently 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

Primary end-point : Evaluate toxicity and the risks of autoreactivity against normal tissues expressing TERT such as bone marrow, kidney, liver, intestinal epithelium *etc*…

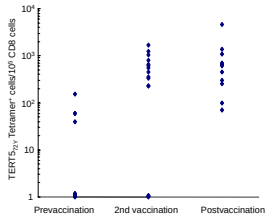
Secondary end-point : Evaluate immunogenicity

Protocol :



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

$TERT_{572Y}$ tetramer staining of PBMC



	Prevaccination	2nd vaccination	Postvaccination
	(n=14)	(n=16)	(n=11)
mean±SD	23±43	512±491	919±1267
		p<0.01	p<0.04

Frequency value for individual patient was calculated as following:
$TERT_{572Y}$ tetramer+ CD8 cells in patient – (mean $TERT_{572Y}$ treamer+ CD8 cells in healthy donors + 3SD)

Best clinical response in patients having completed vaccination

Forty one patients have completed the six cycles of vaccination

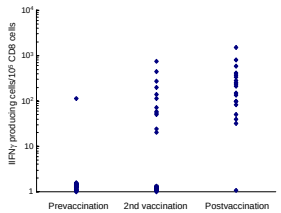
Clinical status before vaccination	Vaccination induced best response					
	PR		SD		PD	
	n	%	n	%	n	%
PR (n=1)	1	100	0	0	0	0
SD (n=7)	0	0	6	85.7	1	14.3
PD (n=33)	0	0	14	42.4	19	57.6

Demographics of patients

Demographics of patients (n=60)		n	%
Median age		60	
Cancer	NSCLC	14	23.3
	Breast	8	13.3
	Prostate	8	1.3
	Colorectal	5	8.4
	RCC	5	8.4
	Pancreas	4	6.6
	Others	17	26.7
Response to prior treatment	PD	50	83.3
	SD	9	15
	PR	1	1.7
Disease stage	III	12	20
	IV	48	80
Line of treatment	2nd	12	20
	3rd	19	31.7
	> 3	29	48.3

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

IFNγ ELISPot assay

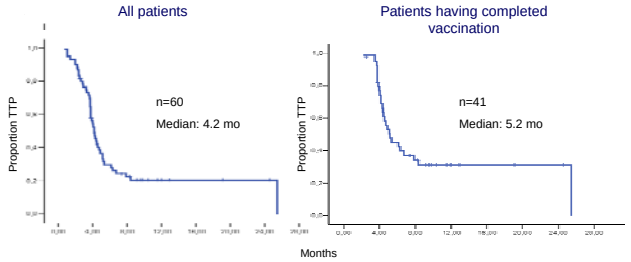


	Prevaccination	2nd vaccination	Postvaccination
	(n=31)	(n=29)	(n=19)
mean±SD	5±20	76±164	299±355
		p<0.03	p<0.002

ELISPot cultures were considered positive when there was a statistically significant difference between the $TERT_{572}$ and the irrelevant peptide stimulated groups.

In positive cultures, the number of spots in the $TERT_{572}$ stimulated group was corrected by subtracting background spots resulting from irrelevant peptide stimulated cultures.

Time to tumor progression for vaccinated patients

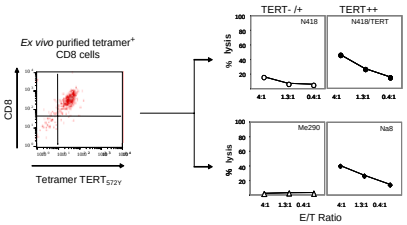


Toxicity and autoreactivity in vaccinated patients

Adverse Events	Toxicity Grade			
	Grade I	Grade II	Grade III	Grade IV
Local skin reaction	16	9	0	0
Anemia	12	14	0	0
Thrombocytopenia	2	5	0	0
Fatigue	7	6	0	0
Nausea	1	4	0	0
Anorexia	0	2	0	0
Fever	0	5	0	0
Myalgia	0	3	0	0
Diarrhoea	0	1	0	0
Constipation	1	0	0	0

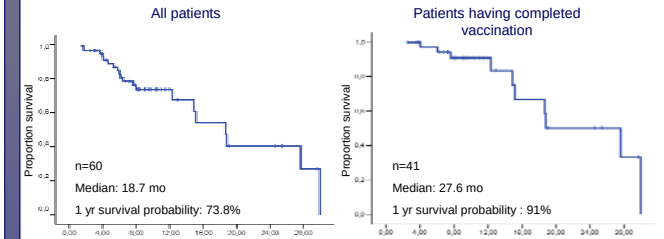
$TERT_{572}$ specific immune response in vaccinated patients (3)

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



$TERT_{572Y}$ tetramer+ cells from one vaccinated patient were purified, *in vitro* amplified with PHA and tested for cytotoxicity against $TERT^+$ and $TERT^-$ targets.

Survival for vaccinated patients



Conclusions

- $TERT_{572Y}$ vaccine was well tolerated in all patients and did not induce any autoimmunity.
- $TERT_{572Y}$ vaccine stimulated TERT specific immune response in more than 90% of vaccinated patients evaluated to date.
- 42.4% (14 out of 33) of patients with progressive desease before vaccination who have completed vaccination achieved long lasting stable desease.
- The prolonged (27.6 mo) median overall survival of patients having completed vaccination seems to indicate an anti-tumor effect of the vaccine.

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