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Phase I dose escalation safety and feasibility study of autologous WT1-sensitized T cells for the treatment of patients with recurrent ovarian cancer

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ABSTRACT

Background This phase I dose escalation trial evaluated the feasibility of production, safety, maximum tolerated dose, and preliminary efficacy of autologous T cells sensitized with peptides encoding Wilms' tumor protein 1 (WT1) administered alone or following lymphodepleting chemotherapy, in the treatment of patients with recurrent WT1$^+$ ovarian, primary peritoneal, or fallopian tube carcinomas.

Methods A 3+3 dose escalation design was used to determine dose-limiting toxicity (DLT). In cohort I, patients received WT1-sensitized T cells dosed at $5\times10^6/m^2$ (level I) without cyclophosphamide lymphodepletion. In cohorts II–IV, patients received lymphodepleting chemotherapy (a single intravenous dose of cyclophosphamide 750 mg/m^2), 2 days prior to the first intravenous infusion of WT1-sensitized T cells administered at escalating doses ($2\times10^7/m^2$ (level II), $5\times10^7/m^2$ (level III), and $1\times10^8/m^2$ (level IV)).

Results Twelve patients aged 23–72 years, with a median of 7 prior therapies (range 4–14), were treated on the study. No DLT was observed, even at the highest dose level of $1\times10^8/m^2$ WT1-sensitized T cells tested. Common adverse events reported were grade 1–2 fatigue, fever, nausea, and headache. Median progression-free survival (PFS) was 1.8 months (95% CI, 0.8 to 2.6); 1 year PFS rate 8.3% (95% CI, 0.5 to 31.1). Median overall survival (OS) was 11.0 months (95% CI, 1.1 to 22.6); OS at 1 year was 41.7% (95% CI, 15.2% to 66.5%). Best response was stable disease in one patient (n=1) and progressive disease in the others (n=11). We observed a transient increase in the frequencies of WT1-specific cytotoxic T lymphocyte precursors (CTLp) in the peripheral blood of 9 of the 12 patients following WT1-sensitized T-cell infusion.

Conclusion We demonstrated the safety of administration of WT1-sensitized T cells and the short-term increase in the WT1 CTLp. However, at the low doses evaluated we did not observe therapeutic activity in recurrent ovarian cancer. In this heavily pretreated population, we encountered challenges in generating sufficient numbers of WT1-reactive cytotoxic T cells. Future studies employing WT1-specific T cells generated from lymphocytes are warranted but should be done earlier in the disease course and prior to intensive myelosuppressive therapy.

Trial registration number NCT00562640.

One-sentence summary The authors describe the first human application of autologous WT1-sensitized T cells in the treatment of patients with recurrent ovarian, primary peritoneal, and fallopian tube carcinomas.

BACKGROUND

Epithelial ovarian cancer (EOC) is a leading cause of death from gynecologic malignancies. More than 21,750 cases occur annually in the USA, and 13,940 women can be expected to succumb to the disease each year.[1] Despite 70% of patients achieving clinical remission with initial chemotherapy, most patients ultimately relapse and eventually develop chemotherapy-refractory disease.[2 3] New treatment modalities and paradigms are needed.

Over the last decade, large-scale clinical trials have rekindled interest in immunotherapies, harnessing the immune system to kill cancer cells, in the treatment of ovarian cancer.[4] One promising strategy is T cell-based therapies, using adoptive transfer of tumor-reactive autologous T lymphocytes generated ex vivo to attack tumor cells.[5] Early analyses of T lymphocytes derived from ascites or tumor nodules (tumor-infiltrating lymphocytes (TILs)) of patients with ovarian cancer have documented the presence of cytotoxic T-cell clones reactive against autologous tumor cells in a proportion of patients.[6] Furthermore, the presence of T cells in ovarian tumors has been associated with significantly improved disease-free survival.[6] Early clinical trials exploring the clinical potential of autologous TILs expanded in vitro and adoptively transferred to patients with advanced disease also demonstrated clinical responses. Such responses, however, were observed in only a small subset of these patients.[7] Consistent with this finding, characterization of the

expanded TILs from these patients suggested that the cells generated were predominantly $CD4^+$ T cells and that $CD8^+$ T cells capable of lysing autologous tumor cells could be generated only from a minority of patients.

In pursuit of better strategies to stimulate and sustain effective cytotoxic T-cell responses against ovarian cancer, subsequent investigations of cell-mediated responses to ovarian cancer have focused on three areas: (1) identification of proteins differentially expressed by ovarian cancers in comparison with normal tissues; (2) definition of immunogenic peptide epitopes derived from these proteins that could be used to elicit effective T-cell responses; (3) exploration of alternative sensitization strategies designed to preferentially stimulate the generation of tumoricidal T cells in vitro or in vivo.

Wilms' tumor protein (WT1) is a human tumor-associated antigen (TAA) that is highly expressed in up to 64% of serous ovarian cancers and is a sensitive and specific biologic marker of high-grade serous ovarian cancer.[8 9] High expression of WT1 in acute myeloid leukemia (AML), myelodysplastic syndrome, and certain solid tumors is associated with poor prognosis.[10–13] Our group and others from Japan, England, and the USA demonstrated that peptides derived from the WT1 protein are immunogenic in preclinical models and human patients.[14–21] Ohminami *et al*[14] and Oka *et al*[15] first identified peptides of WT1 which, when presented by HLA-A2402 and HLA-A0201 alleles, could elicit WT1 peptide-specific T-cell clones with in vitro leukemocidal activity. Scheibenbogen *et al*[22] demonstrated evidence for spontaneous T-cell reactivity against defined WT1 antigen in patients with $WT1^+$ AML. Doubrovina *et al*[23] also identified series of novel WT1-derived immunogenic epitopes presented through different HLA alleles that are capable of inducing T-cell responses selectively cytotoxic against $WT1^+$ tumor cells in vitro in approximately 75% of normal donors.

A WT1-derived epitope, RMFPNAPYL (RMF), presented through the HLA-A0201 allele, is a well-recognized target for T cell–based immunotherapy. This RMF peptide presented by HLA-A0201 has been included in a multivalent vaccine (galinpepimut-S (GPS)) together with native long peptides of WT1. The vaccine elicited WT1-specific T-cell responses in first-in-human trials for the treatment of mesothelioma and AML.[22 24 25] A phase I study of the GPS vaccine used in combination with the anti-PD1 antibody, nivolumab, in the treatment of patients with $WT1^+$ ovarian cancers, who were in second or third remission, resulted in a 64% progression-free survival (PFS) rate at 1 year in the intention-to-treat analysis (7 of 11 patients) and 70% in those who received at least two doses of GPS and nivolumab (7 of 10 patients). Antigen-specific T-cell responses to individual WT1 peptides were observed between 6 and 15 weeks.[25]

An alternative approach is to adoptively transfer antigen-specific T cells sensitized and expanded in vitro, under conditions promoting the generation of a preponderance of cytotoxic $CD8^+$ T cells and helper $CD4^+$ T cells. Cellular immunotherapy has demonstrated efficacy in the treatment of hematologic malignancies, such as chronic myeloid leukemia and virus-associated lymphomas.[19 26] In phase II clinical trials involving the adoptive transfer of autologous antigen-specific $CD8^+$ T-cell clones against gp100 and MART-1 in patients with metastatic melanoma, even with successful clonal repopulation and evidence of in vivo antigen targeting, only transient minor tumor regressions were observed.[27] In the treatment of ovarian cancer, phase I studies of adoptive T-cell therapies have not demonstrated significant clinical benefit to date.[28 29] The study by Kershaw *et al*[28] on alpha-folate receptor-specific T cells was the first description of adoptive transfer of gene-modified tumor-reactive T cells in patients with ovarian cancer and provides insight into the safety and feasibility of adoptive therapy in metastatic ovarian cancer.

In this clinical trial, we conducted a phase I safety and feasibility trial using patient-derived polyclonal WT1-sensitized T cells. This dose escalating trial was conducted to determine the feasibility of generating autologous polyclonal WT1-specific T cells from patients with heavily pretreated ovarian cancer and to test the safety of this approach in the treatment of recurrent ovarian, primary peritoneal or fallopian tube carcinoma.

METHODS

Clinical protocol and patient population

All patients who enrolled in the trial provided written informed consent prior to undergoing leukapheresis for the subsequent generation of the WT1-sensitized T cells.

Eligible patients had recurrent or persistent, pathologically confirmed $WT1^+$ ovarian, primary peritoneal, or fallopian tube carcinomas. Tumors were tested for WT1 positivity by immunohistochemistry as previously described,[21] with positive expression graded according to an adaption of the German Immunoreactive Score (IRS, range 4–12 was considered positive).[30] Patients were required to have Karnofsky Performance Status (KPS)≥70 and normal hematologic and biochemical parameters. Prior chemotherapy must have been completed at least 3 weeks prior to leukapheresis and prior to initiation of study therapy. Patient's disease was required to be evaluable radiologically by RECIST V.1.1.

Generation of WT1-reactive T lymphocytes for adoptive therapy

Patients with confirmed $WT1^+$ tumors underwent leukapheresis. Peripheral blood mononuclear cells (PBMCs) were isolated by Ficoll/Hypaque density gradient centrifugation. Autologous B cells transformed with the B95.8 strain of Epstein-Barr virus (EBV) were used as immortalized antigen presenting cells (APCs) able to provide efficient antigen presentation and co-stimulatory signals for activation and proliferation of WT1 cytotoxic T lymphocyte (CTL).[31] The B lymphoblastoid cell lines (BLCLs) were generated as previously described.[23]

PBMCs were sensitized in vitro with irradiated autologous BLCL pre-loaded with a total pool of pentadecapeptides spanning the sequence of the WT1 protein, each 15-mer overlapping the next by 11 amino acids.[23] Autologous EBV

BLCLs were used as APCs based on prior studies indicating the potential of WT1 peptide-loaded autologous EBV BLCLs to consistently stimulate generation of higher numbers of distinct populations of $CD8^+$ and $CD4^+$ T cells exhibiting WT1-specific cytotoxic activity against EBV-negative, $WT1^+$ tumor cell targets but not against the autologous dendritic cells (DCs).[23 31] T cells were restimulated weekly in the presence of interleukin-2 (5–120 units/mL). These dual WT1/EBV-specific CTLs were expanded in vitro for 35–74 days until the dose to be administered was achieved.

After expansion in vitro, each patient's T cells were tested to ascertain their specific cytotoxic activity (tested against autologous DCs or PHA blasts used as APCs loaded with the pool of WT1 peptides) and lack of non-specific activity (tested against autologous APC and allogenic HLA mismatched APCs in the absence of the WT1 peptides). They were also tested and shown to contain at least 70% $CD3^+$ T cells and to be microbiologically sterile, mycoplasma-free and to contain ≤5 EU/mL of endotoxin. WT1 CTLs, meeting these release criteria with sufficient yield to provide the treatment dose levels, were cryopreserved in aliquots for subsequent infusion.

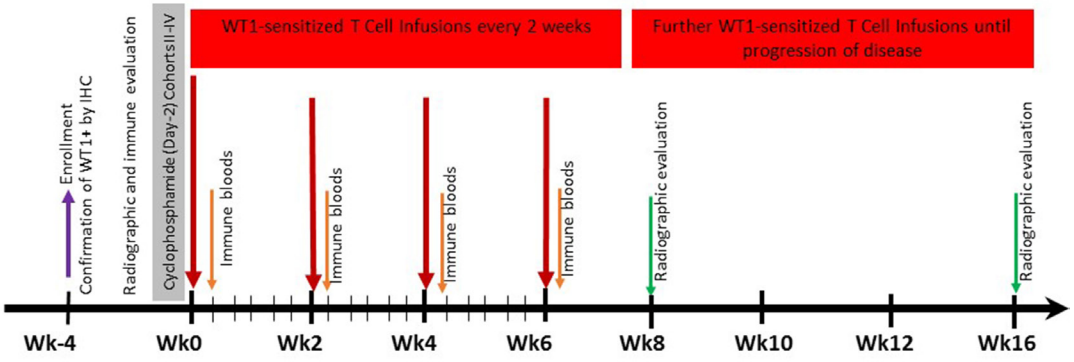
Further characterization of the WT1-specific CTLs

Aliquots of each patient's WT1 CTLs were characterized as to their content of $CD3^+CD4^+$ and $CD3^+CD8^+$, T cells and any residual B cells or NK cells. Samples were co-stained with fluorescently labeled monoclonal antibodies specific for these surface markers and tested by flow cytometry by gating on live single CD45 positive cells. $CD8^+$ and $CD4^+$ T cells generating IFN-γ in response to the total pool or to single WT1 peptides were also quantitated by FACS analysis as previously described.[25]

WT1-specific and EBV-specific CTL precursors (CTLp) were also quantitated using limiting dilution analysis as we have previously described.[32] The epitope specificities of the WT1 CTLs were identified using a matrix of WT1 peptide subpools to map peptides eliciting IFN-γ-positive T-cell responses as previously described.[23] The HLA restrictions of the WT1 CTL were then identified using a standard ^{51}Cr release cytotoxicity assay to detect T-cell responses against a panel of targets consisting of WT1 peptide-loaded and peptide-unloaded DCs or PHA blasts generated from allogeneic donors, each expressing a single HLA allele matching an HLA allele shared by the patient's WT1 CTLs.[23]

Study design and treatments

A 3+3 dose escalation design was used to determine dose-limiting toxicity (DLT) (figure 1). In cohort 1, patients received WT1-sensitized T cells (intravenous, IV) dosed at $5\times10^6/m^2$ (level I) without cyclophosphamide lymphodepletion. Patients in dose levels II, III, and IV received a standard lymphodepletion regimen consisting of a single dose of cyclophosphamide 750 mg/m^2, administered intravenously, 2 days prior to the first WT1-sensitized T-cell infusion. Patients were premedicated with diphenhydramine 25 mg and acetaminophen 650 mg 30 min prior to WT1-sensitized T-cell infusion. Patients in cohorts II–IV then received autologous WT1-sensitized T cells by intravenous infusion at escalating doses



Key:
Wk = week
Radiologic Evaluation (baseline, week 8 and then every 8 weeks)

Dose Levels of T cells and Conditioning Therapy:

Level	Patients	Cyclophosphamide	WT1-sensitized T cells
I	3-6	No	$5 \times 10^6/m^2$
II	3-6	Yes	$2 \times 10^7/m^2$
III	3-6	Yes	$5 \times 10^7/m^2$
IV	3-6	Yes	$1 \times 10^8/m^2$

Figure 1 Study design. A 3+3 dose escalation design was used to determine dose-limiting toxicity. In cohort 1, patients received WT1-sensitized T cells (intravenous) dosed at $5\times10^6/m^2$ (level I) without cyclophosphamide lymphodepletion. Patients in dose levels II, III, and IV received a standard lymphodepletion regimen consisting of a single dose of cyclophosphamide 750 mg/m^2, administered intravenously, 2 days prior to the first T-cell infusion. Patients in cohorts II–IV then received autologous WT1 peptide-sensitized T cells by intravenous infusion at escalated doses of total viable nucleated cells in the final product ($2\times10^7/m^2$ (level II), $5\times10^7/m^2$ (level III), and $1\times10^8/m^2$ (level IV))). Sequential groups of three to six patients were planned for each treatment group. IHC, immunohistochemistry; WT1, Wilms' tumor protein 1.

of total viable nucleated cells in the final product ($2\times10^7/m^2$ (level II), $5\times10^7/m^2$ (level III), and $1\times10^8/m^2$ (level IV)). Sequential groups of three to six patients were planned for each treatment group. The T cell and preconditioning chemotherapy evaluated in each group are summarized in table 1.

The first two patients in cohort I only received a single administration of WT1-sensitized T cells, whereas all subsequent patients treated on the study received additional T-cell infusions once every 2 weeks for four doses. Each cycle comprised two doses of WT1-sensitized T cells given every 2 weeks (28-day cycle). If at 8 weeks (ie, 2 weeks after four infusions (two cycles)), a patient had clinical and radiologic benefit (complete response and partial response or stable disease (SD)), additional infusions of WT1-sensitized T cells were allowed. WT1-sensitized T cells could continue to be administered once every 2 weeks until the generated stock of WT1 CTLs had been exhausted, toxicity, withdrawal of consent, or disease progression occurred.

Clinical response and toxicity evaluation

Tumor response was measured using RECIST guidelines (V.1.1) and GCIG criteria for CA125.[33 34] Safety evaluation included standard monitoring using the Common Terminology Criteria for Adverse Events (CTCAE V.4.0). Adverse events were assessed as not related, possibly related, probably related, or related to WT1-sensitized T cells.

Evaluation of WT1-specific and EBV-specific T cells in patients postinfusion

WT1-specific T cells in the blood were measured at weekly intervals post T-cell infusion by two methods: T cells generating IFN-γ specifically in response to WT1 peptide pool-loaded autologous DCs were quantitated by Fluorescence-Activated Cell Sorting (FACS) analysis. $CD8^+IFN\text{-}\gamma^+$ and $CD4^+IFN\text{-}\gamma^+$ cell populations were quantified by gating on $CD3^+$ cells (online supplemental file 1). WT1 CTLp and EBV CTLp frequencies in peripheral blood of the patients were quantitated by limiting dilution, as previously described.[32]

Statistical analyses

The sample size of the dose escalation cohort was determined by the tolerability of the study treatment according to a classical 3+3 design. Descriptive statistics were used to describe the primary endpoints of safety and feasibility. The secondary aims were addressed using descriptive statistical analyses, descriptions of time patterns for continuous variables measured over time, both on an individual level and aggregated by dose level. Wilcoxon rank-sum test was applied when comparing T-cell lysis percentages between groups. PFS was defined as the time from treatment initiation until disease progression as assessed clinically or using RECIST criteria. Overall survival (OS) was defined as duration of patient survival or time from treatment initiation until patient death. Medians of PFS and OS and PFS/OS at 1 year were estimated with the Kaplan-Meier method. Time-dependent Cox proportional hazards model was used to test the relationship between OS and the cumulated doses WT1-sensitized T cell administered.

RESULTS

A primary objective of this phase I dose escalation trial was to evaluate the feasibility of generating autologous WT1-sensitized T cells from heavily pretreated patients with recurrent ovarian, primary peritoneal, or fallopian tube cancer, and the safety and tolerability of these in vitro expanded autologous WT1-sensitized T cells as treatment, when administered alone or following lymphodepleting chemotherapy. Secondary objectives were to measure alterations in the frequencies of WT1-specific T cells in the circulation induced by infusion of different doses of WT1-sensitized T cells generated from ovarian cancer patients and to assess the effects of the adoptively transferred T cells on clinical outcomes, particularly the growth and progression of each patient's malignancy and OS.

Generation and characterization of WT1 CTLs produced from patients with recurrent ovarian cancer

Between 2007 and 2012, 25 patients in total were screened and consented, of whom 21 underwent leukapheresis for generation of WT1 CTLs. Of these 21 patients, 12 were treated on the clinical trial. Those who were not treated included: three patients who became ineligible because of declining performance status related to progression of disease (POD) during the time required for WT1 CTL generation and two who failed to meet eligibility criteria prior to treatment (renal and hepatic parameters). In addition, four patients decided to pursue another clinical trial or chemotherapy.

We were able to generate WT1-specific T cells that were cytotoxic and specific for WT1 from 19/21 patients who provided a leukapheresis. Data characterizing the WT1 CTLs for the 12 patients treated are presented in table 2 and figure 2A,B. The WT1-specific CTLs were primarily $CD8^+$ T cells (figure 2A) (14/14 products tested were used for infusions). None of the products contained residual $CD19^+$ B cells above 1%. These T cells lysed autologous WT1 total pool-loaded APC (figure 2B) but not the autologous APC alone ($p<0.001$). As expected, these T-cell lines also contained EBV-specific T cells that were cytotoxic against autologous EBV^+ BLCL (data not shown) but not against EBV-negative/WT1-negative autologous or allogeneic HLA mismatched APC (figure 2B).

Generation of a sufficient number of T cells for planned doses was problematic, potentially due to multiple prior lines of chemotherapy in patients with refractory disease who were already highly immunosuppressed. The median number of WT1-sensitized CTLs generated from a starting number of 10^8 PBMC was 5.5×10^8 cells (range 1×10^7–9.5×10^9). As a result of these low yields, generation of additional WT1 CTL lots was required for some

Table 1 Summary of patient characteristics, prior treatments, clinical outcomes, and toxicities

Cohort	Patient study ID	Age (years)	KPS	Pattern of disease	# Prior treatment lines	Positive WT1 tumor (IRS score)	Treatment course	Clinical outcome			Treatment-related toxicities
								Best overall response	Progression-free interval (months)	Overall survival (months)	
I	001	23	70	Peritoneal carcinomatosis, anterior pelvic implant	4	12+	Cohort I: 1 WT1+ T-cell infusion	PD	2.4	11.7	Gr 3 cellulitis, Gr 1 fever
	002	69	80	Peritoneal carcinomatosis, pleural, liver metastases	4	4+	Cohort I: 1 WT1+ T-cell infusion	PD	1	1.5	Gr 1 fatigue; Gr 2 fever, Gr 2 lung infection
	003	61	80	Peritoneal carcinomatosis, pleura/lung metastases	10	6+	Cohort I: 2 WT1+ T-cell infusions pt withdrew consent	Not evaluable		21.9	Gr 1 pain (headache)
II	004	61	90	Peritoneal carcinomatosis, pleural effusions	6	12+	Cohort II: Cyclophosphamide, followed by 3 WT1+ T-cell infusions	PD	1.2	4	Gr 1 fatigue and constipation
	005	47	90	Liver metastases, thoracic/ axillary lymphadenopathy	10	12+	Cohort II: Cyclophosphamide, followed by 4 WT1+ T-cell infusions	PD	3.3	12	Gr 1 bleeding Gr 2 fatigue, neuropathy, and constipation
	006	52	90	Abdominal and pelvic metastases, thoracic lymphadenopathy, pleural effusion, liver	7	9+	Cohort II: Cyclophosphamide, followed by 2 WT1+ T-cell infusions	PD	0.5	1	Gr 1 nausea and fatigue. Rapid disease progression and only received 2 WT1+ T-cell infusions
III	007	54	90	Liver metastases, retroperitoneal lymphadenopathy, paravertebral mass	8	12+	Cohort III: Cyclophosphamide, followed by 2 WT1+ T-cell infusions	PD	0.7	1	Gr 1 fatigue, nausea, and diarrhea. Gr 2 hypertension. Rapid disease progression and only received 2 WT1+ T-cell infusions
	008	69	90	Gastric implant, vaginal cuff recurrence	10	9+	Cohort III: Cyclophosphamide, followed by 7 WT1+ T-cell infusions	SD	3.7	30	Gr 1 diarrhea, nausea, headache and fatigue; Gr 2 hypertension
	009	58	90	Chest wall, axillary and supraclavicular lymphadenopathy	14	12+	Cohort III: Cyclophosphamide, followed by 4 WT1+ T-cell infusions	PD	2.5	32	Gr 1 hypokalemia, bilirubin and AST increase, fatigue, nausea, vomiting and chills
	010	69	80	Peritoneal/abdominal disease, lung nodules	4	9+	Cohort III: Cyclophosphamide, followed by 4 WT1 +T-cell infusions	PD	1.7	26	Gr 1 hyponatremia, creatinine increase, fever, chest pain and platelet decrease; Gr 3 white blood cell, neutrophil, and lymphocyte decrease

Continued

Table 1 Continued

Cohort	Patient study ID	Age (years)	KPS	Pattern of disease	# Prior treatment lines	Positive WT1 tumor (IRS score)	Treatment course	Clinical outcome			Treatment-related toxicities
								Best overall response	Progression-free interval (months)	Overall survival (months)	
IV	011	72	90	Thoracic/axillary lymphadenopathy	6	9+	Cohort IV: Cyclophosphamide, followed by 4 WT1 + T-cell infusions	PD	1.6	22.8	Gr 1 hyponatremia
	012	55	90	Liver metastases	7	12+	Cohort IV: Cyclophosphamide, followed by 4 WT1 + T-cell infusions	PD	1.9	20	Gr 1 hyponatremia

AST, aspartate transaminase; IRS, Immunoreactive Score; KPS, Karnofsky Performance Score ; SD, stable disease; WT1, Wilms' tumor protein 1.

of these patients to meet the assigned treatment dose. In comparison, the median number of WT1-sensitized T cells generated from healthy donors was 9.1×10^8 (range 3×10^7–13×10^9).

Although the WT1-sensitized T cells generated from each of the 12 treated patients exhibited WT1 peptide-specific cytotoxic activity, the frequencies of clonogenic WT1-specific CTLp in each T-cell culture varied considerably (table 2) presumably due to the individual variability of the WT1-specific T-cell response in each patient or overall immunosuppression reflected by simultaneously low EBV CTLp. Consequently, while the dose of viable T cells/m^2 administered was escalated as specified in the trial, the doses of WT1-specific CTLp administered to each patient were variable both within and among the dose cohorts. The total doses of clonogenic WT1-specific CTLp administered to each patient are specified in table 2 and are calculated based on the total number of viable cells infused per m^2 and absolute number of clonogenic WT1 CTLp per 1×10^6 of viable T cells.

Patient characteristics and treatment

A total of 12 patients were enrolled and treated on this study. Table 1 outlines patient demographics and characteristics of patients treated on the trial. These patients ranged in age from 23 to 72 years and had received a median of 7 prior lines of systemic therapy (range 4–14). The level of WT1 IRS detected in their tumor biopsies ranged from 4 to 12, with a median score of 10.

The number of WT1 CTL infusions administered ranged from 1 to 7 (table 1). The mean number of WT1 CTL infusions was 3. Cohorts I and II enrolled three patients each, for treatment and safety evaluation. In cohort III, patient 007 had early disease progression and was taken off study less than 3 weeks after study initiation; therefore, an additional patient was enrolled in cohort III (four patients in total) for safety evaluation. The study was successfully dose-escalated to cohort IV but closed prematurely in 2012 due to the lack of clinical activity observed.

Safety

Four dose levels were explored. Patients in cohort I were treated without lymphodepletion (dosed at 5×10^6/m^2), cohorts II–IV received a lymphodepleting regimen, consisting of a single dose of cyclophosphamide 750 mg/m^2, 2 days prior to the first T-cell infusion. WT1 CTLs were given at escalating doses (2×10^7/m^2 (cohort II), 5×10^7/m^2 (cohort III), and 1×10^8/m^2 (cohort IV)) (figure 1).

Infusions of WT1-sensitized T cells were well tolerated overall, even at the highest dose level tested (1×10^8 WT1-sensitized T cells/m^2). No DLTs or infusion reactions were observed in the 12 patients treated with T-cell infusions. None of the 12 treated patients experienced any life-threatening toxicities attributable to the WT1 T cells infused.

For all patients, the most common treatment-related adverse events (TRAEs) (≥20% of subjects) observed were fatigue (n=6, 50%), fever (n=3, 25%), nausea (n=3, 25%),

Table 2 Characterization of autologous WT1 CTLs used for treatment of patients with ovarian cancer

		HLA alleles of autologous WT1 CTLs infused to patients with ovarian cancer										Characterization of WT1 CTLs						
Dose cohort group	Patient study ID	A	A	B	B	C	C	DRB 1	DRB 1	DBQ 1	DBQ 1	HLA restricting alleles of WT1 CTLs	Immunodominant peptides	Absolute number of EBV CTLp cells /10^6 CTLs	Absolute number of WT1 CTLp cells /10^6 CTLs	Absolute number of WT1 reactive IFN-γ *CD3* cells /10^6 CTLs	Dominant population of cells	Total dose of clonogenic WT1 CTLp infused/m^2
I	001	2301	3101	3502	4403	0401	0401	0403	0701	0305	0303	A2301	$_{238\text{-}246}$WNQNMLGAT	1.53	1.33	30 600	CD8	6.65
I	002	0201	0201	5001	5701	0602	0602	0701	1305	0202	0301	B5001	$_{238\text{-}246}$WNQNMLGAT	1.28	1.28	29 000	CD8	6.4
I	003	0201	2601	2705	3801	0102	1203	0103	0402	0401	0302	B3801	$_{(\text{-}51)\text{-}(\text{-}42)}$LRSSGPGCLQQ $_{46\text{-}54}$SAYGSLGGP $_{94\text{-}102}$VHFSGQFTG	90 909	58 883	28 100	CD8	5 888 330
II	004	0201	6801	2705	2707	01 XX	02 XX	0701	1301	0202	0603	NA	$_{238\text{-}246}$WNQNMLGAT	27 027	2.48	24 800	CD8	148.8
II	005	0101	0201	0801	5101	0701	1402	0101	0301	0501	0201	A0201	$_{(\text{-}75)\text{-}(\text{-}67)}$AILDFLLLQ	1.28	1074	82 800	CD8	85 920
II	006	0201	2301	1801	4403	0501	401	0301	0701	0201	0202	C0501 A2301*	$_{187\text{-}201}$SLGEQQYSVPPPVYG $_{235\text{-}249}$CMTWNQMNLGATLKG $_{203\text{-}217}$HTPTDSCTGSQALLL $_{251\text{-}265}$AAGSSSSVKWTEGQS $_{331\text{-}345}$NKRYFKLSHLQMHSR $_{347\text{-}361}$HTGEKPYQCDFKDCE	1001	6289	63 600	CD8	251 560
III	007	1101	3001	4402	3501	0602	0401	0401	0101	0301	0501	NA	NA	3571	1.54	NA	CD8	154
III	008	0301	3101	4001	3701	0602	0304	0404	1001	0302	0501	B4001	NA	199 756	405	0.1	CD8	141 750
III	009	0101	2501	1801	0702	0702	1203	1501	1501	0602	0603	NA	NA	NA	NA	NA	CD4	NA
III	010	0101	0301	3503	5201	0401	1202	1201	1501	0301	0601	A0101	NA	23.7	1.28	0.1	CD4/CD8	256
IV	011	0101	3201	2703	4001	0202	0304	0401	1201	0301	0302	A3201	NA	1268	178	1	CD8/CD4	53 400
IV	012	0201	2902	3502	4403	0401	1601	0701	1104	0202	0301	A0201	$_{(\text{-}75)\text{-}(\text{-}67)}$AILDFLLLQ	2.48	18.3	23 900	CD8	7320

*HLA restricting alleles determined for patient #006: WT1 CTLs recognized WT1 primarily via HLA-C0501 allele with subdominant recognition of HLA-A2301 target loaded with WT1 total pool. Restricting HLA allele for each of the identified epitopes could not be tested individually due to lack of suitable targets at the time of testing.
CTLp, CTL precursors; CTLs, cytotoxic T lymphocytes; EBV, Epstein-Barr virus; WT1, Wilms' tumor protein 1.

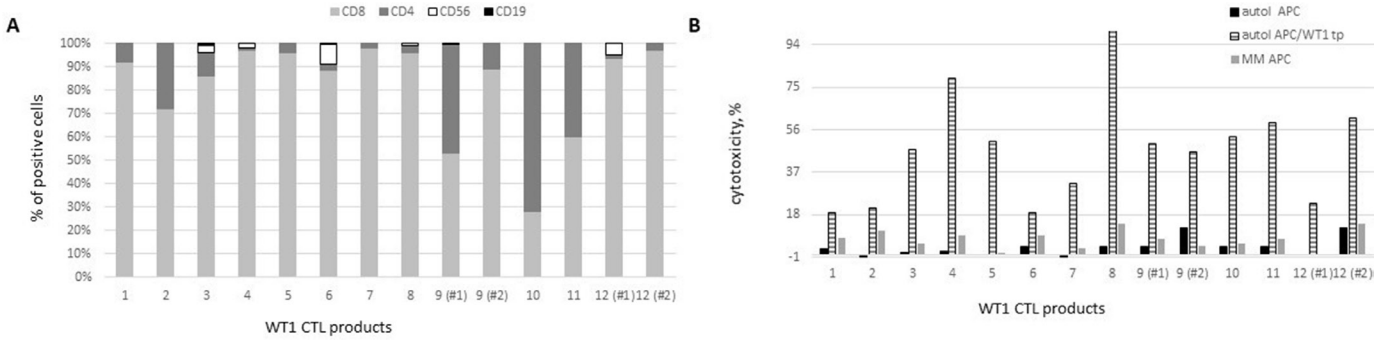


Figure 2 Characterization of the immunophenotype (A) and cytotoxicity (B) of the WT1 CTLs generated from peripheral blood mononuclear cells of the patients with ovarian cancer. (A) WT1 CTLs were tested by flow cytometry for percentage of $CD3^+CD8^+$, $CD3^+CD4^+$, $CD3^-CD56^+$, and $CD3^-CD19^+$ cells. (B) Cytolytic activity of the WT1 CTLs against autologous antigen-presenting cells (APC) loaded with total pool of WT1 pentadecapeptides (autologous APC/WT1tp) was significantly higher than their cytolytic activity against the same autologous APC alone (auto APC) ($p<0.001$) or against the non-specific allogeneic HLA-mismatched antigen-presenting cells (MM APC) ($p<0.001$) not expressing WT1. CTLs, cytotoxic T lymphocytes; WT1, Wilms' tumor protein 1.

and headache (n=3, 25%). Grade 1 hyponatremia was observed in three patients (n=3, 25%), but this laboratory abnormality was considered related to cyclophosphamide and unlikely due to the WT1-specific T cells. Other grade 1 treatment-related toxicities included an increase in bilirubin and transaminases. Table 3 provides a summary of TRAEs for all patients, as well as the full range of toxicities for each dose level.

Of the patients treated at dose level I ($5\times10^6/m^2$), two patients experienced infection: one patient developed a grade 3 cellulitis (around her pre-existing gastrostomy tube and not at the T-cell infusion site) (n=1, 8.3%) and another patient experienced a grade 2 lung infection (n=1, 8.3%), temporally related to an episode of vomiting resulting in an aspiration pneumonia that was not considered related to T-cell treatment. Neither patient had positive bacterial cultures.

Transient grade 3 myelosuppression was observed in one patient treated at dose level III ($5\times10^7/m^2$) 3 weeks after receipt of the lymphodepleting dose of cyclophosphamide. This patient had by then received two doses of WT1 CTLs.

Clinical outcome and disease response

Of the 12 patients who received study treatment, 8 completed protocol requirements as planned, 3 withdrew prior to the initial planned disease assessment for progressive disease, and 1 withdrew consent after two T-cell infusions and was lost to medical follow-up. The median PFS was 1.8 months (95% CI, 0.8 to 2.6) and 1-year PFS rate was 8.3% (95% CI, 0.5 to 31.1) (figure 3A). Median OS was 11 months (1.1–22.6). OS at 1 year was 41.7% (15.2%–66.5%) (figure 3B). Best response observed was SD (n=1); all other patients had POD (n=11). While not significant, there was a trend toward longer OS in those who received higher doses of WT1 CTLp (figure 3C).

The patient who achieved SD, patient 008, was treated at dose level III ($5\times10^7/m^2$). This patient was a 69-year-old woman who had progressed following 10 prior lines of therapy. She was treated with lymphodepleting cyclophosphamide, followed by four WT1-sensitized T-cell infusions every 2 weeks. At 8 weeks post T-cell infusion, imaging showed SD by RECIST, and she was treated with three additional infusions of WT1-sensitized T cells. Her PFS was 3.7 months, with OS of 30 months. Seven other patients (patients 001, 002, 005, 009, 010, 011, 012) completed treatment per protocol. Unfortunately, each had POD by CT scan at the 8-week time of planned evaluation and was discontinued from further study treatment. These patients survived 11.7, 1.5, 12, 32, 26, 22.8, and 20 months, respectively. The three patients (patients 004, 006, 007) who had rapid POD shortly after initiation of WT1 CTL treatment were withdrawn from the study after two to three WT1-sensitized T-cell infusions and before initial planned assessment of response. These patients died shortly thereafter due to ovarian cancer.

The patient (003) who withdrew consent 1 week after two infusions of WT1-sensitized T cells was evaluable for safety and OS; no radiologic assessment could be obtained.

Monitoring of the WT1-specific T-cell responses in patients after infusions of WT1 CTLs

We sequentially quantitated WT1-specific CTLp in the blood of 10 of the 12 patients. Increments in WT1 CTLp frequencies were detected 7 days after infusion in 6/10 patients, of whom 3 still had detectable increases over preinfusion levels at 14 days postinfusion. These 6 patients had received WT1-sensitized T cells with median of 20,865 WT1 CTLp/dose/m^2 (range 1830–294,415). None of the 4 patients who received first doses of WT1 CTLs containing lower WT1 CTLp (range 6.4–77 WT1 CTLp) had detectable CTLp at day 7 or at day 14. Following secondary doses of the WT1-sensitized T cells, increments in CTLp frequencies were detected 7–14 days postinfusion in 3 of 5 patients tested. Uniquely, patient 008 who had documented SD continued to have increases in WT1 CTLp frequencies documented through five

Table 3 Summary of treatment-related adverse events (TRAEs)

Toxicity	Grade1–2, N (%)	Grade3–4, N (%)
A. TRAEs for all patients (N=12)		
Constitutional		
Fatigue	6 (50)	0 (0)
Fever	3 (25)	0 (0)
Chills	1 (8.3)	0 (0)
Non-cardiac chest pain	1 (8.3)	0 (0)
Gastrointestinal		
Nausea	3 (25)	0 (0)
Vomiting	1 (8.3)	0 (0)
Diarrhea	1 (8.3)	0 (0)
Constipation	2 (16.7)	0 (0)
Aspartate Aminotransferase elevation	1 (8.3)	0 (0)
Bilirubin elevation	1 (8.3)	0 (0)
Neurologic		
Headache	3 (25)	0 (0)
Sensory neuropathy	1 (8.3)	0 (0)
Cardiac		
Hypertension	1 (8.3)	0 (0)
Musculoskeletal		
Arthralgias	1 (8.3)	0 (0)
Renal		
Hyponatremia	3 (25)	0 (0)
Hypokalemia	1 (8.3)	0 (0)
Creatinine increased	1 (8.3)	0 (0)
Hematologic		
White blood cell decreased	0 (0)	1 (8.3)
Lymphocyte count decreased	0 (0)	1 (8.3)
Neutrophil count decreased	0 (0)	1 (8.3)
Platelet count decreased	1 (8.3)	0 (0)
Hemorrhage	1 (8.3)	0 (0)
Infectious		
Skin infection	0 (0)	1 (8.3)
Lung infection	1 (8.3)	0 (0)
B. TRAEs for Dose Level I Patients (N=3)		
Constitutional		
Fatigue	1 (33.3)	0 (0)
Fever	2 (66.7)	0 (0)
Neurologic		
Headache	1 (33.3)	0 (0)
Infectious		
Skin infection	0 (0)	1 (33.3)
Lung infection	1 (33.3)	0 (0)
C. TRAEs for Dose Level II Patients (N=3)		
Constitutional		
Fatigue	2 (66.7)	0 (0)
Gastrointestinal		
Constipation	2 (66.7)	0 (0)
Neurologic		
Sensory neuropathy	1 (33.3)	0 (0)
Hematologic		
Hemorrhage	1 (33.3)	0 (0)
D. TRAEs for Dose Level III Patients (N=4)		
Constitutional		
Fatigue	3(75%)	0(0%)
Fever	1(25%)	0(0%)
Chills	1(25%)	0(0%)
Non-cardiac chest pain	1(25%)	0(0%)
Gastrointestinal		
Nausea	3(75%)	0(0%)
Vomiting	1(25%)	0(0%)
Diarrhea	1(25%)	0(0%)
AST elevation	1(25%)	0(0%)
Bilirubin elevation	1(25%)	0(0%)
Neurologic		
Headache	2(50%)	0(0%)
Cardiac		
Hypertension	1(25%)	0(0%)
Musculoskeletal		
Arthralgias	1(25%)	0(0%)
Renal		
Hyponatremia	1(25%)	0(0%)
Hypokalemia	1(25%)	0(0%)
Creatinine increased	1(25%)	0(0%)
Hematologic		
White blood cell decreased	0(0%)	1(25%)
Lymphocyte count decreased	0(0%)	1(25%)
Neutrophil count decreased	0(0%)	1(25%)
Platelet count decreased	1(25%)	0(0%)
E. TRAEs for Dose Level IV Patients (N=2)		
Renal		
Hyponatremia	2(100%)	0(0%)

doses over 56 days of treatment, with levels maintained through three additional treatments until day 84. This and examples of the sequential alterations in WT1 CTLp are shown in figure 4A–D.

As expected, due to significant variation in the frequencies of WT1 CTLp detected in the T-cell lines used for adoptive therapy (table 2), there was no correlation between the cumulative doses ofWT1-sensitized $CD3^+$ T cells/m^2 administered and the doses of WT1 CTLp. However, while not significant, there was a trend toward longer OS in patients receiving higher doses of WT1 CTLp (p=0.095) (figure 3C).

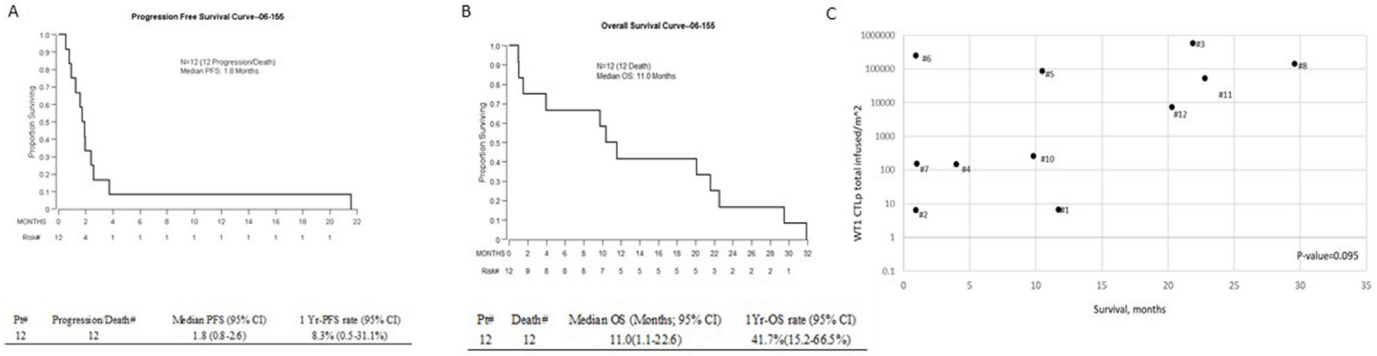


Figure 3 Progression-free survival (PFS) and overall survival (OS) of patients with ovarian cancer (n=12) after treatment with different doses of WT1-specific clonogenic T cells. (A) PFS (n=12); (B) OS (n=12); (C) correlation between overall survival (X axis) of patients with ovarian cancer and cumulative dose of WT1 CTLp (Y axis – absolute number of WT1-specific CTL precursors (CTLp)) infused per m^2 with the autologous WT1-stimulated T cells over the entire course of treatment (n=11). Each dot represents each of the 11/12 patients treated. WT1 CTLp were not tested for 1/12 WT1 CTLs due to low cell yield of the final product. WT1, Wilms' tumor protein 1. CTL, cytotoxic T lymphocyte.

Of note, patient 008 (figure 4C), who had documented SD at week 8 postinitiation of WT1-sensitized T-cell infusions and who, by 12 weeks, had received the second highest cumulative dose (141,750 WT1 CTLp/m^2) survived 30 months, the longest in this series. Patient 003, who received only two infusions of WT1 CTLs had the highest cumulative dose, 588,830 WT1 CTLp/m^2, withdrew from the study shortly after her second dose and did not follow-up per protocol; however, OS was 21.9 months.

DISCUSSION AND CONCLUSIONS

Herein, we present the results of a first human application of autologous polyclonal WT1-sensitized T cells in the treatment of patients with advanced ovarian, primary peritoneal, and fallopian tube carcinomas. Treatment with WT1-sensitized T-cell infusions was well tolerated overall. Most common TRAEs were constitutional symptoms: fatigue, fever, nausea, headache. No DLTs were observed at any dose levels (5×10^6/m^2 (level I), 2×10^7/m^2 (level II), 5×10^7/m^2 (level III)), including the highest dose level (IV) of 1×10^8 WT1-sensitized T cells/m^2. Cytokine release syndrome or infusion reactions were not observed in any patients. At the study prespecified dose levels, the WT1-sensitized T cells were both safe and tolerable in patients with recurrent ovarian, primary peritoneal, and fallopian tube cancer. Although there were no DLTs observed, this phase I trial was limited by the doses of WT1-sensitized T cells that could be generated from

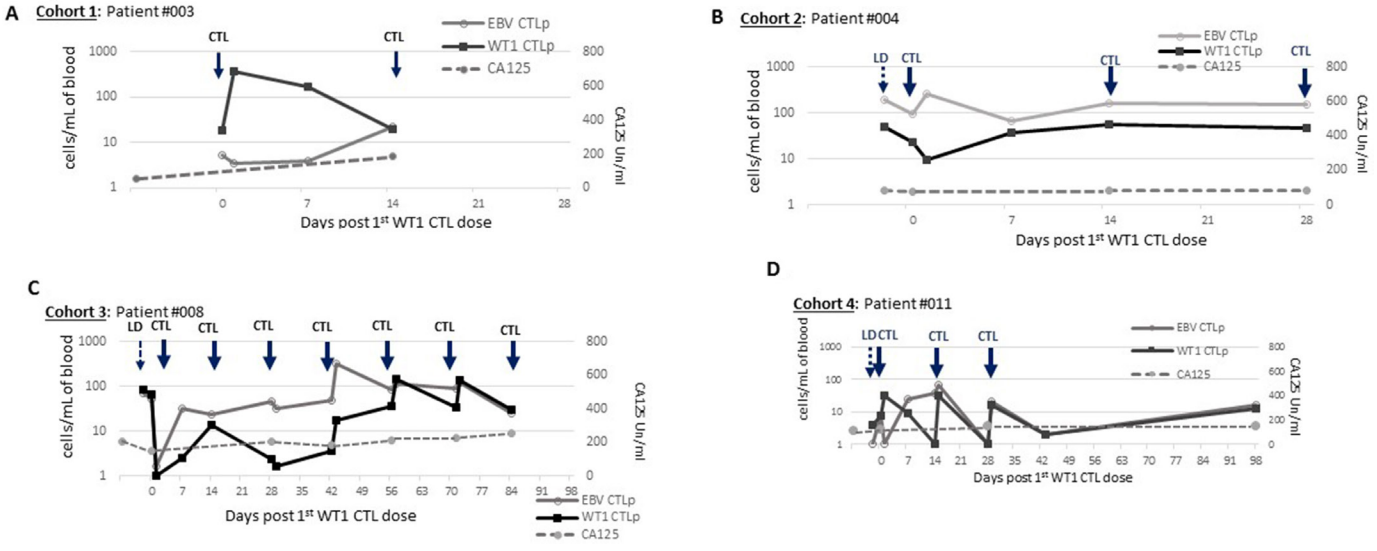


Figure 4 Monitoring of frequencies of WT1 CTLp (black lines), EBV CTLp (gray lines), and CA125 (dotted lines) in peripheral blood of representative ovarian cancer patients. (A) Patient #3 of cohort I (dose level I); (B) patient #4 of cohort II (dose level II); (C) patient #8 of cohort III (dose level III); (D) patient #11 of cohort IV (dose level IV) after treatment with different doses of autologous T cells stimulated with WT1 pentadecapeptide-loaded autologous EBV-transformed B cells. infusions of these dual WT1/EBV-specific T cells resulted in increments of both WT1 CTLp and EBV CTLp. CA125 levels were not altered by treatment with WT1-specific CTLs. CTL, cytotoxic T lymphocyte; CTLp, CTL precursors; EBV, Epstein-Barr virus.

the participating patients. This may be ascribed, in part, to the low yields of clonogenic WT1-specific T cells that we were able to generate from these heavily pretreated patients. The yields of WT1-specific CTLp/100×10^6 starting mononuclear cells were 1–2 $\log_{10}$ lower than yields obtainable from identically treated T cells from normal donors. In addition, the frequencies of clonogenic WT1 CTLp generated over 35–74 days of culture varied markedly. Thus, although each of the T-cell products exhibited comparable WT1-specific cytotoxic activity, and were administered at the escalating doses prescribed, the total doses of clonogenic WT1 CTLp infused did not correlate with the doses of T cells infused. However, those patients who received the higher doses of CTLp exhibited increments in WT1-specific T cells in the blood for periods of 7–14 days postinfusion.

At the doses evaluated in this phase I trial, we did not observe therapeutic activity in recurrent ovarian cancer. Median PFS was 1.8 (95% CI, 0.8 to 2.6) and median OS was 11.0 months (95% CI, 1.1 to 22.6). However, within this small cohort of patients, there was a trend although not significant, towards a positive correlation, between the total doses of WT1 CTLp administered per m^2 and OS (p=0.095) (figure 3C). This observation might merely be due to differences in subsequent treatment. This was comparable to historical controls of survival outcomes of patients with platinum-resistant and refractory ovarian cancer who have received multiple lines of prior treatment.[35 36] Nevertheless, the possibility that higher doses of WT1 CTLp with significant proliferative potential (as tested by LDA) could also be contributing and warrants consideration and further evaluation in future studies.

Evidence supporting the hypothesis that higher doses of WT1-specific T cells exhibiting significant proliferative potential can exert a clinically significant therapeutic effort has been reported by Chapuis *et al*[37] in patients transplanted for $WT1^+$ leukemias. In that study T-cell clones specific for the $_{126\text{-}134}$RMFPNAPYL peptide of WT1 presented by HLA A0201 allele and generated from healthy allogeneic hematopoietic cell transplant donors were used to treat or to prevent relapse in patients with AML post-transplant. These WT1-specific CTLs, cloned in the presence of IL-21, were administered in three to four doses increasing from 3.3×10^9 to $10^{10}/m^2$. Of six patients with residual disease detected at time of initial infusion, one achieved a transient clearance of blasts and one treated for minimal residual disease achieved a durable CR. Of five patients in CR at time of first infusions, four remained in remission 18–56 months post CTLs. There are also ongoing studies of WT1 T-cell receptor (TCR) therapy in patients with AML and mesothelioma (NCT02550535, NCT02408016), and we await efficacy results from these important clinical trials.

The capacity to generate high numbers of WT1-specific T-cell clones could circumvent the T-cell dose limitations encountered in our study. However, given the difficulties in generating numbers of polyclonal clonogenic WT1-specific T cells from our patients, generation of high-affinity WT1-specific T-cell clones may only be possible if the T cells are generated from blood cells obtained early before the patients have received intensive chemotherapy. An alternate approach, also being explored in patients with leukemia,[37] is the use of virus-specific T-cell clones transduced to express a WT1-specific TCR. This may have significant advantages because T cells specific for latent viruses such as EBV and Cytomegalovirus are in high frequency in the circulation and maintain significant proportions of central memory T cells that have greater potential for proliferation and persistence.

While T-cell therapies have had some success in the management of hematologic malignancies and those solid tumors from which TILs can be generated, significant responses in other solid tumors have been limited. This likely reflects current limitations in the identification of neoantigens or TAAs suitable as targets for T-cell therapy and challenges in the generation of T cells effective in trafficking to the tumor and sustaining antitumor activity in vivo.[27 29 38 39] Several antigens have been explored as targets for cellular therapy in ovarian cancer including NKG2D receptor,[40] MUC-16-CD,[41] mesothelin,[42]and folate receptor alpha[28]; but these studies have thus far yielded disappointing results.

Another promising strategy for targeting a patient's effector T cells to tumor cells is the development of bispecific T cell-engaging mAb (BiTEs) and TCR mimic mAb (TCRm). A potential advantage of these T cell-based therapies is that they are immediately available for administration and unlike chimeric antigen receptor T cells or TCR-engineered T cells, they do not require patient-specific cell engineering, in vitro cell manipulation, or cell transplantation.[43 44] Early phase trials are in progress investigating this alternative T cell-based approach, with development of BiTEs targeting cell membrane antigens differentially expressed by ovarian carcinoma cells, such as MUC16 or mesothelin.[41] The development of a TCRm mAb specifically reactive with the WT1 peptide RMF/HLA-A02 complex has also exhibited tumor-specific antibody-dependent cell-mediated cytotoxicity and potent therapeutic effects in animal models of several cancers in preclinical models.[45 46]

Although limited by a study population of patients with disease refractory to multiple prior lines of therapy and who were already highly immunosuppressed, with a significant tumor burden, the results of our study should still be informative in the design of future combination studies of WT1-sensitized T cells and other T-cell therapies. Our hope is that lessons learned from this clinical trial will provide a proof of concept for other T cell-based treatments, expanding the scope of WT1-targeted therapy as a treatment for recurrent ovarian cancer.

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Patient consent for publication Not required.

Ethics approval This clinical trial and all associated evaluations were conducted under protocol #06-155 approved by the Institutional Review Board of Memorial Sloan Kettering Cancer Center and the US Food and Drug Administration. The trial was registered at clinicaltrials.org as NCT00562640.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement All data relevant to the study are included in the article or uploaded as supplementary information.

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