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 5×10^6/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 (2×10^7/m^2 (level II), 5×10^6/m^2 (level III), and 1×10^6/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 1×10^6/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 (CTLPs) 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 CTLPs. 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.

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expanding 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.6–9 High expression of WT1 in acute myeloid leukemia (AML), myelodysplastic syndrome, and certain solid tumors is associated with poor prognosis.10–15 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 al4 and Oka et al5 first identified peptides of WT1 which, when presented by HLA-A0201 alleles, could elicit WT1 peptide-specific T-cell clones with in vitro leukemiodial activity. Scheibenbogen et al22 demonstrated evidence for spontaneous T-cell reactivity against defined WT1 antigen in patients with WT1+ AML. Doubrovina et al23 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 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 al30 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 (BLCs) were generated as previously described.23

PBMCs were sensitized in vitro with irradiated autologous BLCLs 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.25 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). 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.31 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 51Cr 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 5×10^6/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

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 5×10^6/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-sensitized T cells by intravenous infusion at escalating doses

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of total viable nucleated cells in the final product (2×10⁷/m² (level II), 5×10⁷/m² (level III), and 1×10⁸/m² (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. 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. CD8¹IFN-γ⁺ and CD4¹IFN-γ⁺ 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 10⁶ PBMC was 5.5×10⁶ cells (range 1×10⁶–9.5×10⁶). 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 | Progression-free interval (months) | Overall survival (months) | Treatment-related toxicities |
|--------|-----------------|-------------|-----|-------------------|------------------------|----------------------------|-----------------|-----------------|-----------------------------------|--------------------------|----------------------------|
| 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, pleural/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              | 3.3                              | 12                       | Gr 1 bleeding Gr 2 fatigue, neuropathy, and constipation |
|        | 005             | 47          | 90  | Liver metastases, thoracic/axillary lymphadenopathy | 10                     | 12+                       | Cohort II: Cyclophosphamide, followed by 4 WT1+ T-cell infusions | PD              |                                  |                          |                                                                                      |
|        | 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
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/m² 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 m² and absolute number of clonogenic WT1 CTLp per 1×10^9 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/m²), cohorts II–IV received a lymphodepleting regimen, consisting of a single dose of cyclophosphamide 750 mg/m², 2 days prior to the first T-cell infusion. WT1 CTLs were given at escalating doses (2×10^7/m² (cohort II), 5×10^7/m² (cohort III), and 1×10^8/m² (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/m²). 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%),
| Dose | Patient study ID | HLA alleles of autologous WT1 CTLs infused to patients with ovarian cancer | Characterization of WT1 CTLs | 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 | WNQNMLGAT | 1.53 | 1.33 | 30600 | CD8 | 6.65 |
| I    | 002             | 0201 0201 5001 5701 0602 0602 0701 1305 0202 0301 | B5001 | WNQNMLGAT | 1.28 | 1.28 | 29000 | CD8 | 6.4 |
| I    | 003             | 0201 2601 2705 3801 0102 1203 0103 0402 0401 0302 | B3801 | LRSSGPGLQQ SAYGSLGPL VHFSGQLFTG | 90909 | 58883 | 28100 | CD8 | 58883.3 |
| II   | 004             | 0201 6801 2705 2707 01 XX 02 XX 0701 1301 0202 0603 | NA | WNQNMLGAT | 27027 | 2.48 | 24800 | CD8 | 148.8 |
| II   | 005             | 0101 0201 0801 5101 0701 1402 0101 0301 0501 0201 | A0201 | AILDFLLQQ | 1.28 | 1.074 | 82800 | CD8 | 85920 |
| II   | 006             | 0201 2301 1801 4403 0501 401 0301 0701 0201 0202 | C0501 | A2301 | 1001 | 6289 | 63600 | CD8 | 251560 |
| 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 | 199756 | 405 | 0.1 | CD8 | 141750 |
| III  | 009             | 0101 2501 1801 0702 0702 1203 1501 1501 0602 0603 | NA | NA | 199756 | 405 | 0.1 | CD8 | 141750 |
| III  | 010             | 0101 0301 3503 5201 0401 1202 1201 1501 0301 0601 | A0101 | NA | 23.7 | 1.28 | 0.1 | CD4/CD8 | 2.56 |
| IV   | 011             | 0101 3201 2703 4001 0202 0304 0401 1201 0301 0302 | A2301 | NA | 1268 | 178 | 1 | CD4/CD8 | 53400 |
| IV   | 012             | 0201 2902 3502 4403 0401 1601 0701 1104 0202 0301 | A2001 | AILDFLLQQ | 2.48 | 18.3 | 23900 | 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.
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 (5×10⁶/m²), two patients experienced infection: one patient developed a grade 2 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 (5×10⁷/m²) 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 (5×10⁷/m²). 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/m² (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
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 of WT1-sensitized CD3⁺ T cells/m² 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).

### Table 3

#### Table 3 Continued

| 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          |                 |                 |

### 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          |                 |                 |
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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/m²) 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/m², 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 (5×10⁶/m² (level I), 2×10⁷/m² (level II), 5×10⁷/m² (level III)), including the highest dose level (IV) of 1×10⁸ WT1-sensitized T cells/m². 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 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 m² 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.

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 presence of IL-21, were administered in three to four courses of WT1-specific T cells and other T

Evidence supporting the hypothesis that higher doses of WT1-specific T cells exhibiting significant proliferative potential can exert a clinically significant therapeutic activity in recurrent ovarian cancer who have received multiple lines of prior treatment. 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.

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. 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. The development of a TCRm mAb specifically reactive with the WT1 peptide RMF/HLA-A0201 complex has also exhibited tumor-specific antibody-dependent cell-mediated cytotoxicity and potent therapeutic effects in animal models of several cancers in preclinical models.

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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