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CYTOTOXIC T-LYMPHOCYTE IMMUNOTHERAPY FOR OVARIAN CANCER: A PILOT STUDY

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Abstract

The objective was to evaluate the toxicity and feasibility of intraperitoneal (IP) infusion of tumor-specific cytotoxic T-lymphocytes (CTL) as therapy for recurrent ovarian cancer, and to determine if repetitive cycles of CTL generation and infusion measurably increases the host's ovarian cancer immune response.

In this study, seven subjects with recurrent ovarian cancer confined to the peritoneal cavity underwent up to 4 cycles, each cycle beginning with a leukapheresis for collection of precursor lymphocytes, which were stimulated in vitro with MUC1, a tumor-specific antigen found commonly in ovarian cancer cells. The resulting new CTL for each cycle were re-introduced into the host via IP infusion. Immunological parameters (killer cells, cytokine production, memory T-lymphocytes and natural killer (NK) cells) were studied. Toxicity, CA-125, and survival data were also evaluated.

The tumor marker CA-125 was non statistically significantly reduced after the first month of immunotherapy. However, after that, it rose. Killer cells, cytokine production and memory T-lymphocytes increased after the first cycle of stimulation, but plateaued or reduced thereafter. The percent of NK cells inversely correlated with other immune parameters.

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Median survival was 11.5 months. One subject is free of disease since December, 2000. Multiple cycles, beyond one cycle, of T-cell stimulation followed by adoptive T cell infusion, may not enhance the in vivo immune response.

Keywords

Adoptive immunotherapy; Stimulation; CTL; human; MUC1

Introduction

Subjects with recurrent ovarian cancer respond poorly to second line chemotherapy and virtually all will succumb to the disease, usually as a result of inanition due to extensive involvement of the peritoneal cavity and intestinal tract with tumor [1]. The evaluation of new therapeutic strategies is, therefore, needed. The peritoneal route of administration would seem desirable since the tumor is localized to this cavity. Intraperitoneal infusion of in vitro expanded tumor infiltrating lymphocytes (TIL) was shown to produce some clinical activity in subjects with ovarian cancer [2]. Another trial examined intraperitoneal infusion of autologous lymphocytes retargeted by a bispecific monoclonal antibody and IL-2. Clinical response was limited and toxicity, presumably due to the IL-2, was considered moderate [3]. An alternative approach is to stimulate T-lymphocytes with a specific tumor antigen in vitro.

The immune system recognizes tumors; however, the tumor microenvironment generates immunosuppressive cells leading to immune evasion of cancer [4]. An approach to overcome the immunosuppressive tumor microenvironment is to generate cells in vitro that will kill tumor cells. Cellular immunotherapy, in the form of cytotoxic T-lymphocytes (CTL), has been successful in treating viral-associated malignancies [5], some hematologic malignancies [6] and even certain solid tumors [7,8]. Antigen-specific CD8 T cell clones have also been shown to be effective for treatment of malignant disease [9], implying specificity to antigens on the tumor cells. Since CTL from ovarian cancer subjects recognize MUC1-expressing cancer cells [10,11], we used ex vivo stimulation of CTL with MUC1 to generate killer cells for adoptive immunotherapy. These CTL were used to attempt to augment the host immunologic response to tumor cells in subjects with recurrent ovarian cancer.

Materials and methods

Human Subjects Protection. The protocol was approved by the TTUHSC Investigational Review Board (IRB) and conducted under an IND of the Food and Drug Administration (FDA), which required subjects with relapsed cancer. Subjects were entered onto the protocol after obtaining consent. The goal was for 20 subjects, but was terminated because the FDA added a requirement for an endotoxin assay of cells in the infusion bag, which would have taken over two hours and reduced the cell number by half.

Trial Design

This was a study of subjects with recurrent epithelial ovarian cancer confined to the peritoneal cavity. Chemotherapy was completed within 4–6 weeks of protocol entry. Subjects underwent leukapheresis for collection of precursor lymphocytes on day 0, which were stimulated in vitro with MUC1. The resulting CTL were re-introduced into the host via intraperitoneal (IP) infusion, through an IP Infus-A-Port (Infusaid, Wysox, PA 18854), on days 9 – 11, for the first infusion, and on days 16 -- 18, for the second infusion, of each month. The cycle was repeated monthly up to 4 times. Subjects received no other

intervention for the recurrent adenocarcinoma and no other therapy for the remainder of the cycle. Toxicity, CA-125, an ovarian cancer tumor antigen, and survival were compiled. Carcinoma cell cytotoxicity and cytokines, including G-IFN, GM-CSF and TNF-alpha, and immunophenotyping were also evaluated.

Apheresis and generation of CTL

Procedures were as detailed [12,13]. PBMC were collected from each subject by apheresis, without any stimulant to raise the PBMC count. The goal of each collection was a minimum of 1 x 10^9 mononuclear cells. PBMC contain dendritic cells and the precursor-CTL that were later stimulated and expanded using the MUC 1 antigen. PBMC were cultured until tumor cell cytotoxicity or type 1 cytokines were statistically significantly elevated over day 0 unstimulated levels or for a maximum of one month. The infused cell number of 1–4 x 10^8 CTL/m^2 was based on an optimized study with non-specific stimulated PBMC [14], and was infused IP after one week (first infusion) or two weeks (second infusion) in culture. The process of apheresis for precursor-CTL collection, ex vivo stimulation and infusion was repeated every four weeks to complete four cycles or until recurrent disease was detected (whichever occurred first).

MUC1 Peptides

A single repeat of optimized context of MUC1-VNTR1 peptide GSTAPPAHGVTSAPDTRPAP [15] was synthesized by Peninsula Laboratories, Inc., Palo Alto, CA, USA.

Cell Culture Conditions

Procedures were as detailed [12,13]. MC were obtained from humans with ovarian adenocarcinomas by apheresis. Cells were cultured at 2 x 10^6 cells/ml in AIM-V (Registered) serum free lymphocyte medium (Life Technologies GIBCO-BRL, Grand Island, NY, 14072, USA) and maintained in a 37°C humidified 5% CO_2 atmosphere. IL-2 (Cetus) was added twice per week at 100 IU/ml. Cells were stimulated with MUC1-VNTR1 peptide at 45 x 10^{-8} M (1ug/ml) on days 0, after freezing aliquots for assays with later aliquots, and 7. Cells were harvested on day eight, or on the indicated day, if either percent specific lysis of a mucin containing adenocarcinoma cell line, MCF-7, or G-IFN production was statistically significantly elevated over prestimulation, day 0, values. Cells were frozen for other assays.

Immunophenotyping

Procedures were as detailed [10,17]. Briefly, cells (5 x 10^5 per aliquot) were obtained from ex vivo cultures on the indicated days, stained with fluorochrome-conjugated mAbs against the indicated CD markers (BD Pharmingen Inc., San Diego, CA, 14072, USA) and analyzed by flow cytometry using the Becton Dickinson FACScan/Lysis II (Registered) or the BD Biosciences FACSCalibur (Registered) system, per manufacturer's instructions.

Cytotoxicity Assays

MCF-7, a HLA-A2 breast cancer cell line, was obtained from, and cultured as recommended, by the American Type Culture Collection (ATCC). MCF-7 expresses hypoglycosylated mucin [18]. This cell line was used as the CTL target cell line in a XTT assay (Registered) (Roche Diagnostics Corp., Indianapolis, IN, 46250-0414, USA) [19], which was performed per manufacturer's instructions. K562, a natural killer (NK)/ lymphokine-activated killer (LAK) sensitive target [20] cell line, and RAJI, a NK-relatively resistant/(LAK) -sensitive target [21] cell line were used as target cell lines in an alamarBlue® assay (Biosource International Inc., Camarillo, CA, USA). K562 and RAJI

were cultured in RPMI-1640 (Gibco-BRL, Life Technologies, Inc. Grand Island, NY, USA) supplemented with 10% FBS and 1% L-glutamine. All the cells were maintained in a 37°C humidified and 5% CO_2 atmosphere. The effector cells were tested at the effector to target (E:T) cell ratio of 10:1.

Cytokine Assays

Levels of cytokines [G-IFN, GM-CSF, IL-10, and TNF-Alpha (BD Pharmingen Inc., San Diego, CA, 92121, USA)], were performed per manufacturer's instructions from supernatants of MUC1-stimulated mononuclear cells (M1SMC).

Toxicity

Toxicity was evaluated by NCI Common Toxicity Criteria [22].

Statistical Analysis

The goal of this analysis was to determine if the distribution of each variable was changing over time (or month); preferably, we would like to see a monotonically increasing relationship. In order to test this assumption, the method of summary-statistics was used to analyze all data. Data from each subject was reduced to a single summary measure, in this case the Spearman's rank correlation coefficient, r_s, between the number of the month (t = 0, 1, 2, 3, 4 for pre-treatment, month 1, 2, 3 and 4, respectively.) and the measured variable. Spearman's correlation coefficient, a non-parametric correlation, was utilized as the small sample sizes and the boxplots (shown below) indicate that it cannot be assumed the variables were normally distributed. Due to the missing data from patient drop out, each rank correlation coefficient was weighted according to the square root of the number of measurements taken. The nonparametric sign test and Wilcoxon Signed Rank Test was then used to test the hypothesis that $r_s=0$ versus $r_s>0$. Student's t test was used in paired comparisons. Mean (M) +/− standard error of the mean (SEM) are the values given in the text.

Results

Clinical

Seven subjects were enrolled in the protocol. Patient characteristics and clinical course summary are described in Table 1. All subjects had recurrent ovarian cancer, following resection and chemotherapy consisting of cisplatin or carboplatin and paclitaxel containing regiments. After recurrence of ovarian cancer, Patient 1 received topotecan, oral etoposide and intravenous liposomal doxorubicin. Subjects 2 and 3 had partial and complete, respectively, resection following recurrence. Chemotherapy was completed within 4–6 weeks of protocol entry. Their pre-study CA-125s varied from normal for Patient 2 to over a thousand. The number of 4 planned paired infusions was reduced due to disease progression in Subjects 3, 6 and 7. In addition, because of a port rupture, possibly due to a fall by Patient 3, or port occlusion in Subjects 4 and 5, the subjects refused another port. The latter two subjects received intraperitoneal chemotherapy prior to the cellular therapy trial. A fibrous sheath was found around each port. There was no toxicity except for grade 1 abdominal pain in Patient 2, at the time of infusion. Survival varied from two months to one patient (No. 2) being alive with no evidence of disease (NED) since December, 2000. This latter patient (No. 2), who had a rising CA-125 and increasing size of tumor, had resection of gross disease and concurrent cisplatin chemotherapy and radiation therapy after the completion of immunotherapy. Those with the highest pre-study CA-125s, Subjects 1 and 6, had the shortest survival of three and two months, respectively. The others survived from six months to one with NED (No. 2). There was no correlation between survival and the number of

infusions of M1SMC. One of the shortest survivors of three months (No. 1) and the only long-term survivor (No. 2) both received the maximum planned number of four paired infusions.

The principal objective of this study was to determine if adoptive immunotherapy using MUC1 VNTR1-stimulated CTL was effective in reducing the tumor marker of subjects with recurrent ovarian cancer. The therapy was well tolerated. The only clinical side effect was abdominal pain in one patient. Tumor marker CA- 125 dropped after the first month in four subjects (1, 3, 5, 6), stabilized in two subjects (2 (which was normal),4) and progressively increased in one subject (7) and another after the second infusion (1) (Figure 1) (mean values were: prestudy, 413; post month 1, 246; post month 2, 1395; post study, 3132). None of the differences between the time points studied were statistically significantly different.

Human leukocyte antigen (HLA) typing

MC were HLA typed, although we [10,16] and others [23] have found cytotoxicity by M1SMC may be non-MHC restricted. Subjects 4, 5 and 7 were HLA I matched with MCF-7 (Table 2). Subjects 5 and 7 were HLA I A2, the HLA I known to present a MUC1 peptide [24]. A region of MUC1, PG STAPPAHGV T, has been found to be class II MHC restricted.[25]. All subjects tested (1, 2, 3–5) were found to be HLA II matched with MCF-7, but not at DRB3, the HLA II known to present PG STAPPAHGV T [25] (Table 2). Whether other HLA I or II alleles present MUC1 peptides is unknown. There was no correlation between HLA restriction and survival.

Cytotoxicity

To determine if killer cells increased with each monthly cycle of stimulation of PBMC with MUC1, lysis of MUC1-expressing MCF-7 and nonspecific targets of NK (K562) and LAK (RAJI) activities, were analyzed after two simulations with MUC1 each month. The measurements for the lysis of the target cells initially (prestudy) at apheresis and after stimulation each month were taken in triplicate. The average of the 3 measurements was used for boxplot comparison and calculation of the mean and median value across subjects initially (prestudy) and after stimulation each month. At each apheresis, lysis remained stable or decreased, except for one or two subjects, for one or two apheresis (figure 2). The subjects and month of apheresis differed for lysis of MCF-7, K562 and RAJI (figure 2). Regarding lysis of MCF-7 (Figure 2, top panel), there were no statistically significant differences between prestudy (19 +/− 3%) to post stimulation in month 1 (29 +/− 4%), 2 (19 +/− 5%) or 3 (29 +/− 8%). However, in month 4 the lysis of MCF-7 was statistically significantly greater (39 +/− 5%, $p = 0.001$) than prestudy (due to subjects 1, 2 and 7). There were no statistical differences from one month to the next. Lysis of K562 also did not show a statistically significant increase from prestudy (16 +/− 2%) to month 1 (24 +/− 4%), from prestudy to month 2 (35 +/− 6%, $p = 0.004$), or from one month to the next (Figure 2, middle panel). However, in months 3 (42 +/− 6%, $p = 10^{-6}$) (due to subjects 2, 3 and 7) and 4 (42 +/− 7%, $p = 10^{-5}$) (due to subjects 1, 2 and 7) the lysis of K562 was statistically significantly greater than prestudy (Figure 2, middle panel). Similarly to lysis of MCF-7 and K562, lysis of RAJI did not show a statistically significant increase from prestudy (12 +/− 1%) to month 1 (22 +/− 4%) ($p = 0.016$), or month 2 (28 +/− 6%, $p = 0.005$) ; however, a statistically significant difference was noted between prestudy and month 3 (43 +/−11%, $p = 0.0006$) (due to subject 7). No such difference was found from month 1 to 2 or month 2 to 3 (Figure 2, lower panel). Also, as opposed to lysis of MCF-7 and K562, lysis of RAJI showed a nonstatistically significant decrease from prestudy (12 +/− 1%) to month 4 (8 +/− 1%) and from month 3 (43 +/− 11%) to 4 (8 +/− 1%) (Figure 2, lower panel). However, as opposed to others, subject 2 increased at month 4 infusion. There were no statistical differences

between the lysis of MCF-7, K562 or RAJI at months 1, 2 or 3. Lysis of MCF-7 and K562 were statistically significantly greater than RAJI at month 4 ($p = 10^{-6}$).

Cytokines

To determine if cytokine production was increased with each monthly cycle of stimulation of PBMC with MUC1, levels of cytokines (G-IFN, GM-CSF, IL-10, and TNF-Alpha) were analyzed from supernatants of M1SMC after two stimulations with MUC1 each month. The measurements for the cytokine production of M1SMC initially (prestudy) and after stimulation each month were taken in triplicate. The average of the 3 measurements was used for boxplot comparison and calculation of the mean and median value across subjects initially (prestudy) and after stimulation each month. Cytokines were undetectable at each apheresis. Regarding production of G-IFN, all measurements and the time (t = 0, 1, 2, 3, 4) in which they were taken were used in the calculation of the Spearman's correlation coefficient. The boxplot comparison, mean and median measurements all show a detectable increase in the measured variable, G-IFN (Figure 3, top panel). The exact sign test and Wilcoxon signed rank test both indicate a difference over time in the distribution using Spearman's correlation, although not statistically significant when multiple comparisons are considered using Bonferroni correction (combined α=0.05). The sign and Wilcoxon p-values for a 2-tailed test are p=0.036 and p=0.031, respectively. Evaluating the change between prestudy and month 1 and between each following month showed less increases with time. There was a statistically significant increase between prestudy (1.8 +/− 0.6 pg/ml) to post stimulation in month 1 (29 +/− 4 pg/ml, $p = 10^{-7}$) (due to all subjects except 1 and 3). The difference was not significant, accounting for multiple comparisons between month 1 and 2 (50 +/− 9 pg/ml, p = 0.04), and not between 2 and 3 (57 +/−16 pg/ml) or 3 and 4 (96 +/− 14 pg/ml). There were no increases with subject 3 in any of the months, whereas that of subject 2 showed the greatest increase in months 2, 3 and 4. Production of GM-CSF also showed smaller increases in the increment with time (Figure 3, middle panel). There was a statistically significant increase between prestudy (0.5 +/− 0.2 pg/ml) to post stimulation in month 1 (60 +/− 15 pg/ml, $p = 4x10^{-4}$) (due to subjects 4, 5, 6 and 7). The difference was not statistically significant between month 1 and 2 (167 +/− 35 pg/ml, p = 0.007) (due to subjects 1, 2, 4 and 7), and not between month 2 and 3 (218 +/− 51 pg/ml) or 3 and 4 (169 +/− 40 pg/ml), which decreased. There were no increases with subject 3 except slightly in month 3, whereas that of subjects 2 and 7 showed the greatest increase in months 2, 3 and 4. As opposed to production of G-IFN and GM-CSF, IL-10 only increased from prestudy (0.0 +/− 0.0 pg/ml) to month 1 (140 +/− 39 pg/ml, $p = 8x10^{-4}$) (due to all subjects except 1 and 3) and then decreased in months 2 (27 +/− 6 pg/ml), 3 (27 +/− 7 pg/ml) and 4 (16 +/− 5 pg/ml) (Figure 3, lower panel). There were no increases with subject 3 except slightly in month 3, whereas that of subject 2 showed the greatest increase in months 2, 3 and 4. TNF-Alpha was not detected except in month 4 in patient 2, at 17 pg/ml.

Immunophenotyping

To determine if the % of memory T-lymphocytes or NK cells were changed with each monthly cycle of stimulation with MUC1, immunophenotypes for memory (CD45RO+) CD4+ (CD4+CD45RO+) (Figure 4, top panel) and CD8+ (CD8+CD45RO+) (Figure 4, middle panel) T-lymphocytes were analyzed after two simulations with MUC1 each month. At each apheresis, the percent of both remained stable or decreased except for one subject (number 2) at apheresis four. Although the mean showed a tendency for the CD4+CD45RO + and CD8+ CD8+CD45RO+ to increase slightly from prestudy to month 1, the exact sign test and Wilcoxon signed rank test indicate no significant difference of r_s from the prestudy values. CD4+CD45RO+ T-lymphocytes increased, non-significantly, from prestudy (35 +/− 12 %) to month 1 (45 +/− 10 %) and then decreased in months 2 (35 +/− 10 %), 3 (27 +/− 10 %) and 4 (21 +/− 10 %) (Figure 4, top panel). Subject 2 was the only one to show a

gradual increase from apheresis 2 to infusion 4. CD8+CD45RO+ T-lymphocytes lymphocytes increased, non-significantly, from prestudy (10 +/− 3 %) to month 1 (18 +/− 8 %) but then remained stable in months 2 (20 +/− 9 %), 3 (19 +/− 8 %) and 4 (21 +/− 8 %) (Figure 4, middle panel). Subject 2 showed an increase at infusions 3 and 4, whereas subject 1 showed a gradual decrease until infusion 4 where they increased. The percent of natural killer (NK) cells (CD 56+ %) decreased from prestudy (14 +/− 2 %) to month one (3 +/− 1 %), rose at apheresis two (8 +/− 2 %), then remained stable or decreased except in a six-month survivor, subject 3, where it rose at apheresis 3 and remained elevated through infusion 4 (figure 4, lower panel). The NED survivor, subject 2, had one of the lowest percent of CD 56+ (NK) cells throughout the study, after apheresis one (prestudy).

Discussion

This study shows that M1SMC can be given safely intraperitoneally to subjects with recurrent ovarian cancer, after resection and chemotherapy. There were, however, complications with the intraperitoneal port in three of seven subjects which prevented further therapy. This route of delivery may thus be limited. Subjects who have received previous intraperitoneal chemotherapy may be at increased risk for a reactive process which causes occlusion of the port. Others have experienced similar problems and now recommend against the use of fenestrated type ports, but rather recommend use of single lumen ports designed for intravenous access [26]. There was no correlation between survival and the number of infusions of M1SMC. One patient was a long-term survivor, with no evidence of disease (NED). This patient had resection of gross disease with concurrent chemotherapy and radiation therapy after the completion of immunotherapy. Subjects with recurrent ovarian cancer are not cured with the other therapies she received [1]. A case-control group of 42 similar treated subjects with relapsed ovarian cancer at the Harrington Cancer Center showed no five year survivors. There is precedent for immunotherapy eliminating microscopic disease with Her2 – specific CTL, where clearance of bone marrow disease was observed in a breast cancer subject [27]. There was no correlation between HLA restriction and survival. This corroborates other studies which showed that MUC1 tumor cell killing may be non-MHC restricted [10,16,23]. The tumor marker CA 125 was reduced after the first month of immunotherapy. However, after that, the tumor marker rose.

M1SMC were evaluated for cytotoxicity, cytokine production and immunophenotype. Killer cells were increased in the first month. However, in the second month, killer cell activities, either decreased (lysis of MUC1-expressing MCF-7) or plateaued (NK and LAK). The percent of NK cells (CD 56+) inversely correlated with other immune parameters. Cytokine production was also increased in the first month. However, after the second month, the levels of the type I cytokines (G-IFN, GM-CSF) plateaued while the type II cytokine interleukin (IL)-10 decreased. Thus cytokine production followed the trend of the killer cell activities. The % of memory T-lymphocytes increased in the first month. However, in the second month, the % of memory T-lymphocytes, either decreased (CD4+CD45RO+) or plateaued (CD8+ CD8+CD45RO+). This implies that induction of immune activated T-cells with memory phenotype was produced in the initial month of stimulation, but was not increased with other cycles of immune stimulation and infusion of adoptive T cells. If this data is validated in a larger trial, it would imply that multiple cycles of immunotherapy may not be necessary.

Others [28] have found a lower in vivo survival of tumor-specific CTL at the third treatment. However, the same group reported a direct correlation with the number of treatment cycles and tumor response [29]. This implies that the function or survival of the CTL may not correlate with clinical response. Others have found the reverse, where the survival of the CTL is directly correlated with clinical response [30]. Procedures to develop

more effective CTL from PBMC are under investigation. Dendritic cells may prolong the survival of T-cells, and have been shown to enhance the efficacy of T-cells therapy in preclinical studies (Wang, unpublished), as well as function alone in humans [31]. Other approaches include enhancers for immune stimulation, such as, co-stimulatory molecules [32,33,34], as well as modifying the host immune environment, depleting or inhibiting suppressor T cellsand myeloid-derived suppressor cells; in addition to disrupting inhibitors of activated immune cells [34]. In this regard, we found in a subset of four subjects that completed three cycles of immunotherapy that the systemic Fox P3/CTLA – 4 memory T cell ratio inversely correlated with disease-free survival [35]. Some of these elements may be added as chimeric antigen receptors in T-cell therapy, which has shown enhanced regression of cancer in preclinical models [36].

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Abbreviations

ATCC	American Type Culture Collection
CTL	cytotoxic T-lymphocytes
E:T	effector to target
FDA	Food and Drug Administration
HLA	Human leukocyte antigen
IP	intraperitoneal
IL	interleukin
IRB	Investigational Review Board
LAK	lymphokine-activated killer
M1SMC	MUC1-stimulated mononuclear cells
MUC 1	mucin 1
NK	natural killer
NED	no evidence of disease
PBMC	peripheral blood mononuclear cells
TIL	tumor infiltrating lymphocytes
VNTR	variable number of tandem repeats

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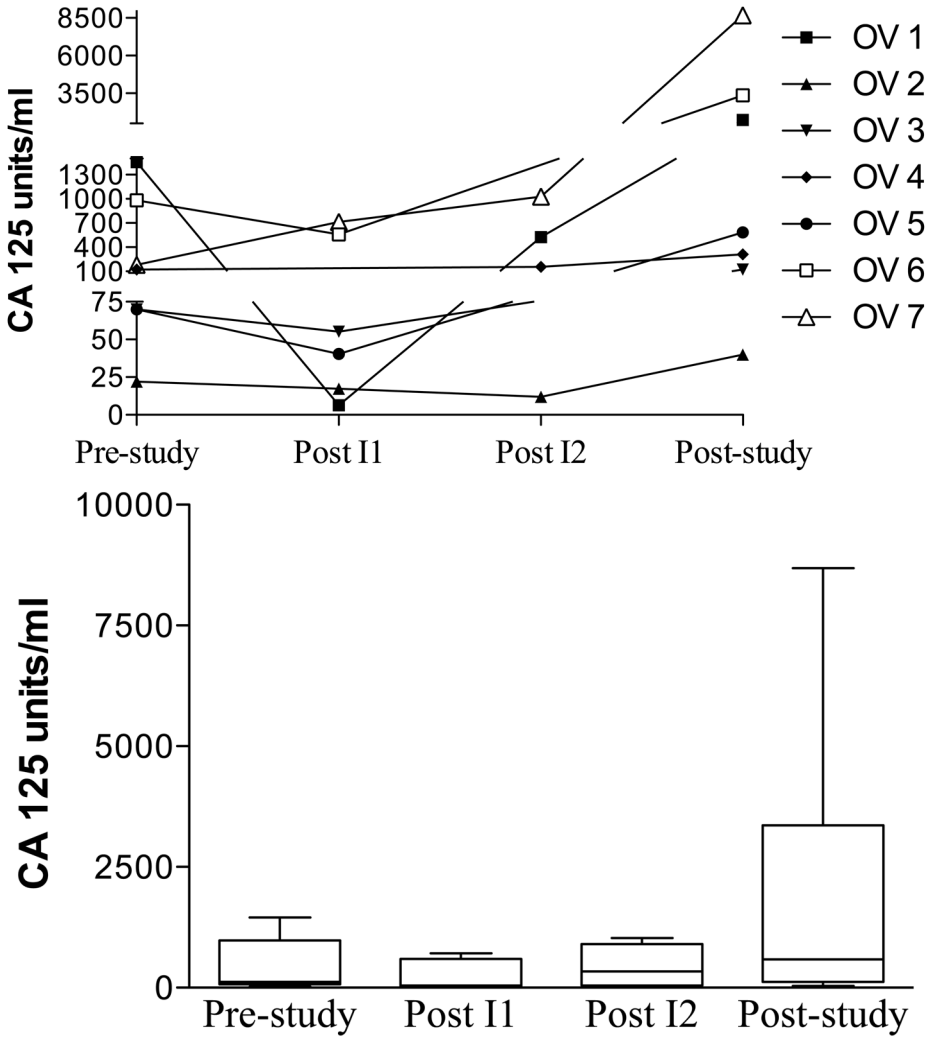
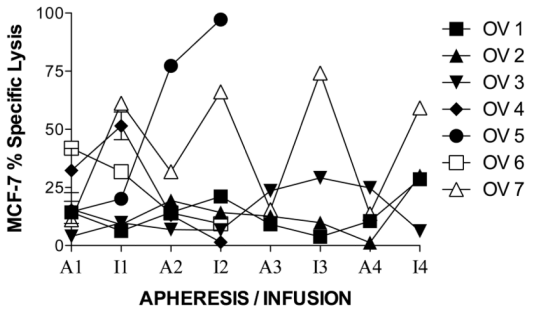
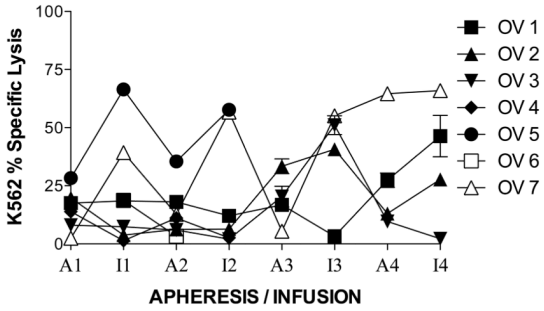
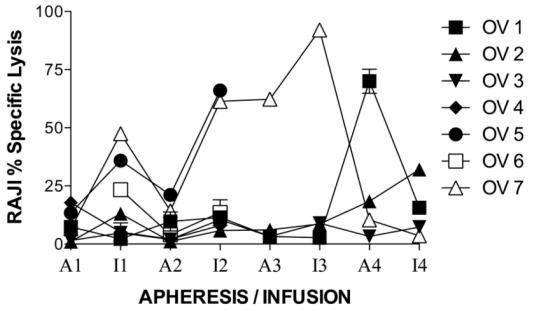


Figure 1.

Tumor marker CA125 levels before, during and after treatment. Aphereses, followed by 9 – 11 days in culture, were separated by 28 days (month). Pre-study (0) was within 5 weeks of the first apheresis. Post month 1 and 2 infusions were at the second and third aphereses, respectively. Post study was after all 4 monthly treatment cycles. A. Each patient is represented by a line. B. Boxes are 10th–90th percentiles and bars are 5th – 95th percentiles of the mean of a single determination for each patient. Median is a line in the box.







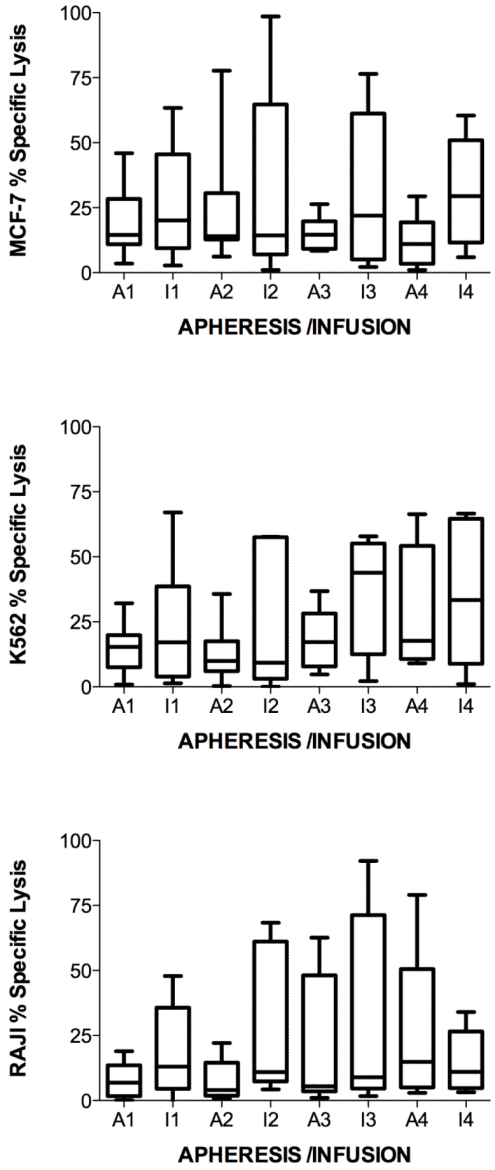
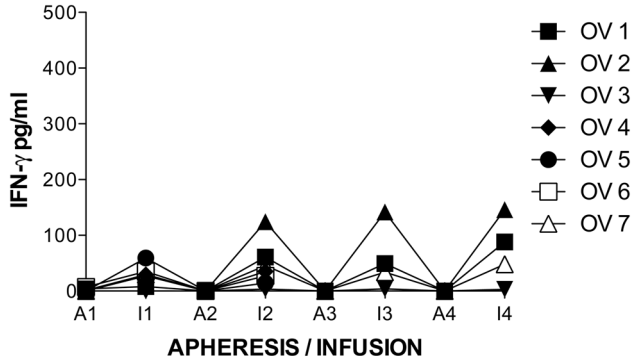
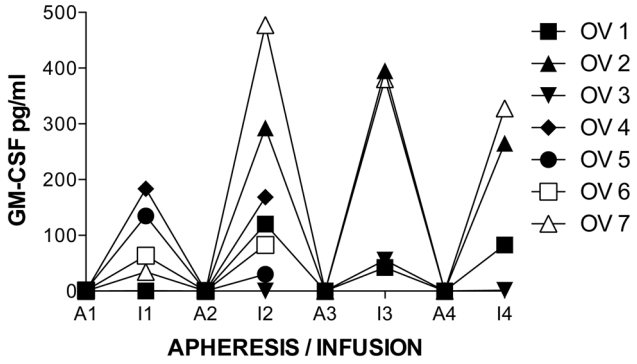
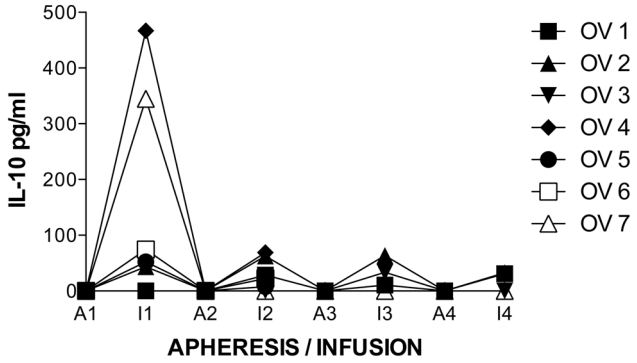


Figure 2.

MUC1-stimulated PBMC specific lysis of targets at effector to target cell ratio = 10:1. Aphereses were monthly. Infusions were at days 9 – 11 in culture. Apheresis (A) or infusion (I) and month (number) is indicated on the horizontal axis. A. Each patient is represented by a line. B. Boxes are 10th–90th percentiles and bars are 5th – 95th percentiles of the mean of 3 or 6 repetitions from each patient. Median is a line in the box.







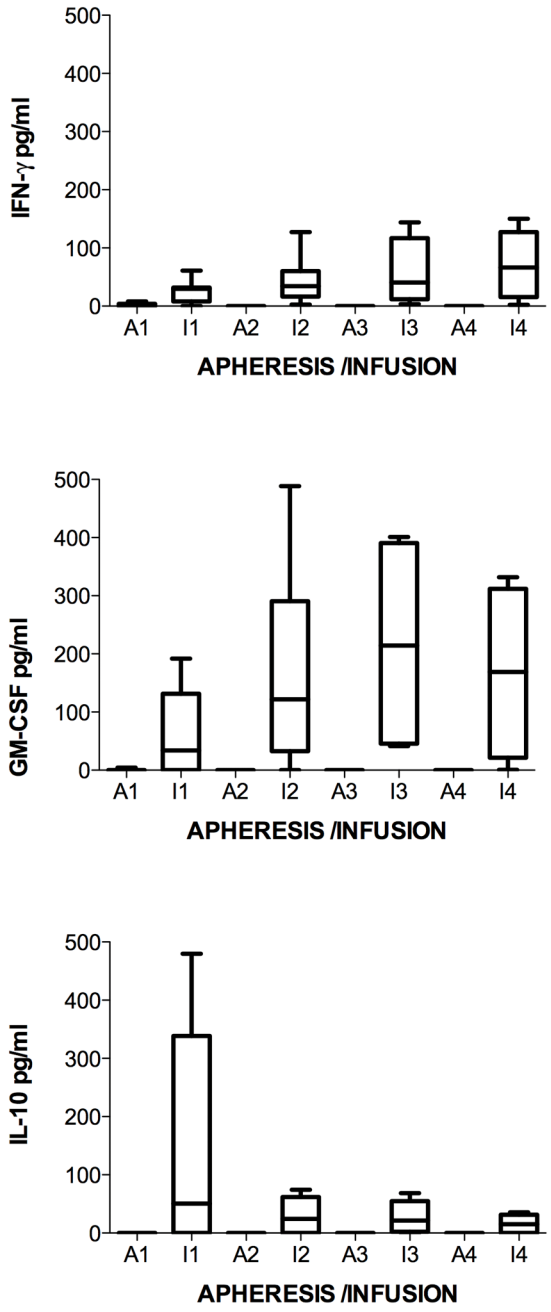
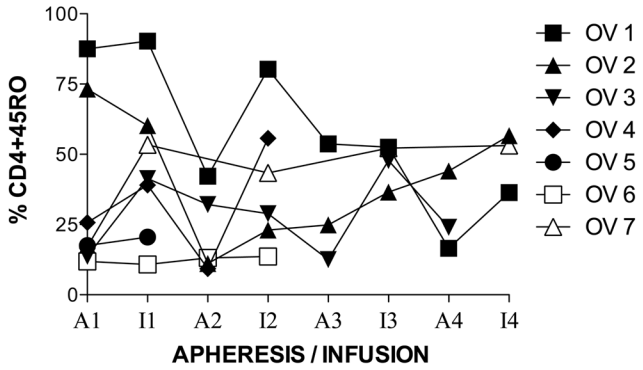
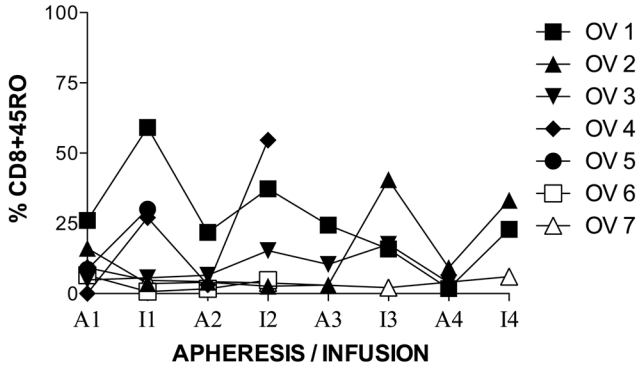
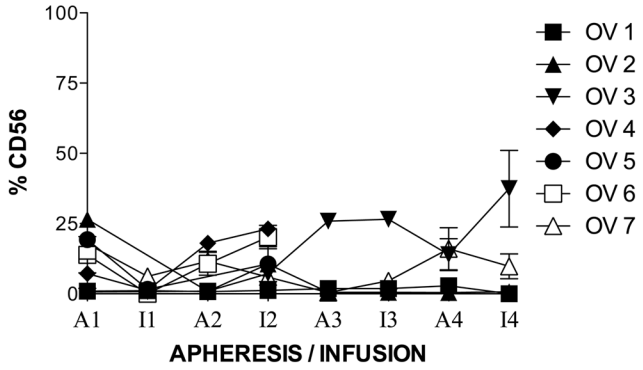


Figure 3.

Cytokine levels from supernatants of MUC1-stimulated PBMC. Aphereses were monthly. Infusions were at days 9 – 11 in culture. Apheresis (A) or infusion (I) and month (number) is indicated on the horizontal axis. A. Each patient is represented by a line. B. Boxes are 10th–90th percentiles and bars are 5th – 95th percentiles of the mean of 3 or 6 repetitions from each patient. Median is a line in the box.







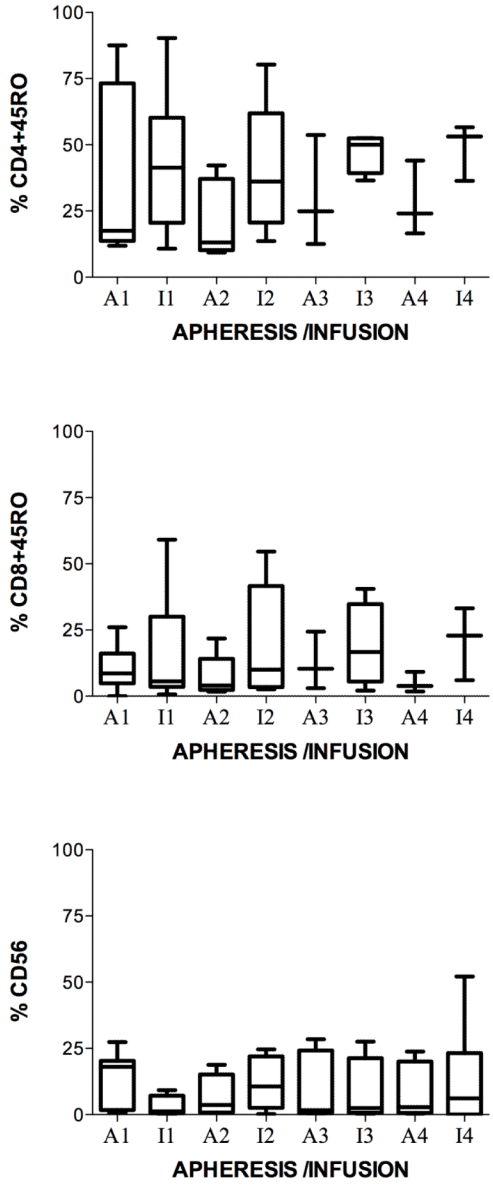


Figure 4.

Immunophenotyping of MUC1-stimulated PBMC for memory T-lymphocytes and NK cells. Aphereses were monthly. Infusions were at days 9 – 11 in culture. Apheresis (A) or infusion (I) and month (number) is indicated on the horizontal axis. A. Each patient is represented by a line. B. Boxes are 10th–90th percentiles and bars are 5th – 95th percentiles of the mean of a single determination for each patient. Median is a line in the box.

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Table 1

Patient characteristics and clinical course summary

Patient No.	Histology	Pre-study Therapy	Disease Status	Pre-study CA-125	Number of Paired* infusions/month/4 maximum	Complications with intraperitoneal port	Toxicity NCI Common Toxicity Criteria [22]	Survival (months)**
1	Poorly differentiated/ Epithelial papillary serous adenocarcinoma	Resection Chemotherapy***	Recurrent	1455	4	None	None	3
2	Moderately differentiated/ Epithelial not otherwise specified adenocarcinoma	Resection Chemotherapy Partial Resection	Recurrent	22	4	None	Grade 1 abdominal pain	NED**** since Dec, 2000
3	Poorly differentiated/ Epithelial papillary adenocarcinoma	Resection Chemotherapy Resection	Recurrent	70	3 Progressive Disease	Ruptured	None	6
4	Poorly differentiated/ Epithelial papillary serous adenocarcinoma	Resection Chemotherapy	Recurrent	122	2.5	Occluded	None	18
5	Poorly differentiated/ Epithelial papillary serous adenocarcinoma	Resection Chemotherapy	Recurrent	56	1	Occluded	None	6
6	Well differentiated/ Epithelial papillary serous adenocarcinoma	Resection Chemotherapy	Recurrent	983	2 Progressive Disease	None	None	2
7	Poorly differentiated/ Epithelial papillary serous adenocarcinoma	Resection Chemotherapy	Recurrent	183	4	None	None	15

*After one week (first infusion) or two weeks (second infusion) in culture

** After completion of MUC1-stimulated mononuclear cells (M1SMC) therapy

*** Chemotherapy was cisplatin or carboplatin and paclitaxel containing regiments were completed within 4–6 weeks of protocol entry.

**** NED = no evidence of disease (after resection of gross disease and concurrent cisplatin chemotherapy and radiation therapy)

Table 2

HLA TYPING

HLA/Subject	MCF-7	#1	#2	#3	#4	#5	#6	#7
A	2/10	3/11	1/3	3/31	1/29	1/2	26/74	2/−
B	18/44	7/35	8/51	14/40	7/44	14/57	15/53	27/40
C		W4/W	W7/W	W3/W	W7/W	W4/W	W6/W1	W01/W
		7	-	8	-	6	4	03
DRB1	3/15	13/14		13/16	1/15	15		
DRB3	2	52A/52B		52C				
DRB5	1			51	51	51		
DQB1	2/6	5/6		6/7	5/6	6		
MCF-7 HLA I match		no	no	no	yes	yes	no	yes
MCF-7 HLA II match		yes	NA*	yes	yes	yes	NA	NA

*NA = not available