Copy 9-29-00

AD______________

Award Number: DAMD17-97-C-7056

TITLE: Phase I Trials with TALL-104 Cells for Treatment of Metastatic Breast Cancer

AD-A382839

PRINCIPAL INVESTIGATOR: Daniela Santoli, Ph.D.

CONTRACTING ORGANIZATION: The Wistar Institute
Philadelphia, Pennsylvania 19104

REPORT DATE: October 1999

TYPE OF REPORT: ~~Annual~~ Final

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public release;
distribution unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

20001017 060

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503.

1. AGENCY USE ONLY *(Leave blank)*

2. REPORT DATE
October 1999

3. REPORT TYPE AND DATES COVERED
~~Annual~~ Final (1 Oct 98 - 30 Sep 99)

4. TITLE AND SUBTITLE
Phase I Trials with TALL-104 Cells for Treatment of Metastatic Breast Cancer

5. FUNDING NUMBERS
DAMD17-97-C-7056

6. AUTHOR(S)
Daniela Santoli, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
The Wistar Institute
Philadelphia, Pennsylvania 19104

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for public release; distribution unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 words)*

The human cytotoxic T cell line TALL-104 has displayed anti-tumor effects in several animal species. A Phase I trial was conducted to determine toxicity of TALL-104 cells in women with refractory breast cancer. Fifteen patients with metastatic infiltrating ductal (N=12), lobular (N=2) or medullary (N=1) carcinoma received escalating doses of lethally irradiated TALL-104 cells (10^6, 3 X 10^6, 10^7, 3 X 10^7 and 10^8 cells/kg) for 5 consecutive days. Patients without progressive disease received monthly maintenance 2-day infusions at the same dose level. Mild grade I/II toxicity developed in 11 patients regardless of cell dose. One grade IV toxicity consequent to hepatic tumor necrosis occurred in a patient given 10^8 cells/kg, 3 weeks after the induction course. Nine patients progressed within one month from induction and 5 patients had stable disease for 2-6 months. One patient (at 3 X 10^7/kg) had improvement of liver metastases and ascites, and a second patient (at 10^6/kg) experienced a dramatic relief in bone pain. Increases in blood NK cell activity and levels of IFN-γ, interleukin (IL)-10 and activation markers (sIL-2R and sICAM-1) were often seen. Only 1 patient developed anti-HLA class I antibody responses against TALL-104 cells; specific CTL activity developed in 3 patients during induction and in 4 patients during the maintenance boosts. In conclusion, TALL-104 cells were well tolerated by patients with metastatic breast cancer at the doses and regimen tested.

14. SUBJECT TERMS
Breast Cancer

15. NUMBER OF PAGES
61

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

FOREWORD

Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the U.S. Army.

___ Where copyrighted material is quoted, permission has been obtained to use such material.

___ Where material from documents designated for limited distribution is quoted, permission has been obtained to use the material.

___ Citations of commercial organizations and trade names in this report do not constitute an official Department of Army endorsement or approval of the products or services of these organizations.

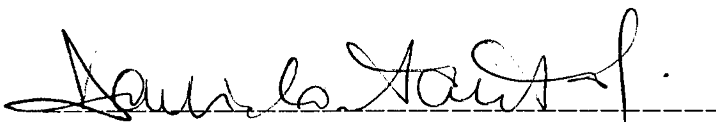
X_ In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and use of Laboratory Animals of the Institute of Laboratory Resources, national Research Council (NIH Publication No. 86-23, Revised 1985).

X_ For the protection of human subjects, the investigator(s) adhered to policies of applicable Federal Law 45 CFR 46.

N/A In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institutes of Health.

N/A In the conduct of research utilizing recombinant DNA, the investigator(s) adhered to the NIH Guidelines for Research Involving Recombinant DNA Molecules.

X_ In the conduct of research involving hazardous organisms, the investigator(s) adhered to the CDC-NIH Guide for Biosafety in Microbiological and Biomedical Laboratories.



PI - Signature

Date 11/8/99

Table of Contents

Page

Foreword 3

Table of Contents 4

Introduction 5

Body 5

Key Research Accomplishments 6

Reportable Outcomes 6

Conclusions 7

Appendix 7

Bibliography of Publications and Meeting Abstracts 7

Personnel receiving pay from the research effort 7

Introduction

Despite great improvements in the treatment of early stage breast cancer, metastatic disease is, to date, still incurable. A variety of new therapeutic approaches have recently been suggested, such as a more rationale use of existing drugs (*i.e.*, via dose intensification and drug combinations), and testing of new drugs (*i.e.*, taxanes, gemcitabine, vinorelbine), anti-angiogenic agents (*i.e.*, endostatin and angiostatin), and gene therapy or immunotherapy (*i.e.*, cancer vaccines, dendritic cell therapy). Although each of the above strategies has merits, immunological approaches are particularly appealing because their toxicity is usually low and non cross-reactive with the toxicity of chemotherapy; in addition, they might induce a tumor-specific and permanent resistance, thus protecting from recurrence. Among immunological approaches, therapy with major histocompatibility complex (MHC) non-restricted effectors, such as patient-derived lymphokine-activated killer (LAK) cells, has the distinct advantage that tumoricidal activity does not require prior sensitization to a specific tumor antigen.

The human MHC non-restricted killer cell line TALL-104 ($CD3^+$, $CD8^+$, $CD56^+$ $CD16^-$) developed in this laboratory, might represent a powerful new immunotherapeutic approach to cancer, as suggested by accumulated evidence in animal models. Specifically, adoptive transfer of lethally-irradiated (40 Gy) TALL-104 cells into severe combined immunodeficiency (SCID) mice has induced regression of transplantable human hematopoietic and nonhematopoietic malignancies. In addition, remarkable anti-tumor effects were seen in immunocompetent mice bearing syngeneic leukemia and in pet dogs with spontaneous tumors. Interestingly, multiple systemic delivery of TALL-104 cells in some of these animals appeared to be followed by the development of protective anti-tumor immunity, which is crucial for induction and maintenance of tumor regression. Recent biodistribution studies in dogs and mice have shown that TALL-104 cells, injected i.v, localize primarily in the lungs for the first 2 h and then redistribute to liver, spleen and kidneys in the following 24 h. Importantly, in tumor bearing animals, irradiated TALL-104 cells accumulated at the site of both the primary tumor and distant metastases.

Our previous pre-clinical studies in SCID mice engrafted with human breast carcinomas and in dogs and cats with spontaneous metastatic mammary tumors have shown that breast cancer is highly responsive to TALL-104 cells. These encouraging results prompted us to initiate this Phase I clinical trial to explore the safety and potential efficacy of escalating doses of lethally irradiated TALL-104 cells administered systemically to women with refractory metastatic breast cancer. The maximal dose tested (10^8/kg/day) was the one proven effective in preclinical veterinary studies.

Body

This investigation was designed as a single center, dose-escalating study, with 5 dose levels to be tested: 10^6, 3×10^6, 10^7, 3×10^7, and 10^8 cells/kg of body weight. Each dose level was to be tested in three patients. No dose escalation was proposed within the same patient. Each patient received a first cycle consisting of 5 consecutive days of intravenous (i.v.) injections of TALL-104 cells (at the dose level correspondent to the entry number) followed by 2-day monthly boosts (at the same dose level) until disease progression. The 15 subjects planned to be enrolled in the study were enrolled in the first year of the grant and follow-up analysis was done in the second year. The highest dose level of TALL-104 cells to be tested (10^8/kg) was reached without any significant clinical toxicity and/or result. The study has been completed and a manuscript has been recently submitted to Clinical Cancer Research (please see Appendix).

Key Research Accomplishments

- TALL-104 cells were well tolerated by patients with advanced breast cancer at the doses and regimen tested.
- Despite their advanced disease, several patients showed responsiveness to TALL-104 therapy, including disease stabilization and clinical improvements.
- The patients who received the highest cell dose (10^8/kg) are still alive at 13-16 months from the end of cell therapy.
- Breast tumor biopsies were sensitive to in vitro killing by TALL-104 cells.
- Only one of five tumor specimens grew in SCID mice and metastasized to lymph nodes.
- Increases in NK activity and serum levels of cytokines and immune activation markers were often seen.
- Only 1 out of 15 patients developed antibodies against TALL-104 cells and 3 developed TALL-104 specific CTL activity (see Appendix for details).

Reportable Outcomes

Manuscripts:

S. Visonneau, A. Cesano, D.L. Porter, S.L. Luger, L. Schuchter, M. Kamoun, M.H. Torosian, K. Duffy, E.A. Stadtmauer, and D. Santoli. 1999. Phase I Trial of TALL-104 Cells in Patients with Refractory Metastatic Breast Cancer. Clinical Cancer Research, submitted.

Santoli, D., Visonneau, S. 1999. Breast Cancer: Immunotherapy and gene therapy. In: Breast Cancer- Multidisciplinary Management. (M.H. Torosian, ed.) Humana Press, Totowa, NJ, in press.

Abstracts:

E.A. Stadtmauer, S. Visonneau, A. Cesano, D.L. Porter, K.M. Duffy, S.L. Luger, and D. Santoli. 1999. Phase I trial of adoptive immunotherapy with TALL-104 cells in women with advanced metastatic breast cancer. Eunice and Irving Leopold Annual Scientific Symposium and Academic Retreat, University of Pennsylvania Cancer Center, Philadelphia, PA

S. Visonneau, A. Cesano, D. Porter, E. Stadtmauer, and D. Santoli. 1999. A Phase I study of TALL-104 cells in patients with metastatic breast cancer. AACR Annual Meeting, April 10-14, 1999, Philadelphia, PA.

D.L. Porter, E.A. Stadtmauer, S. Visonneau, A. Cesano, K.M. Duffy, S.M. Luger, L.M. Schuchter, and D. Santoli. Phase I trial of adoptive immunotherapy (AI) with TALL-104 cells for women with advanced metastatic breast cancer. 1999 ASCO Meeting Abstract.

Conclusions

Results from this phase I trial indicated that TALL-104 cells are well tolerated by terminally ill breast cancer patients up to the maximal intended dose. In addition, encouraging clinical responses were observed, including a minor response and stabilization of disease in some patients; further studies are warranted to evaluate the optimal regimen of TALL-104 cell administration resulting in significant anti-tumor effects.

Appendix

Manuscript submitted:

S. Visonneau, A. Cesano, D.L. Porter, S.L. Luger, L. Schuchter, M. Kamoun, M.H. Torosian, K. Duffy, E.A. Stadtmauer, and D. Santoli. 1999. Phase I Trial of TALL-104 Cells in Patients with Refractory Metastatic Breast Cancer.

Bibliography of Publications and Meeting Abstracts

Manuscripts:

S. Visonneau, A. Cesano, D.L. Porter, S.L. Luger, L. Schuchter, M. Kamoun, M.H. Torosian, K. Duffy, E.A. Stadtmauer, and D. Santoli. 1999. Phase I Trial of TALL-104 Cells in Patients with Refractory Metastatic Breast Cancer. Clinical Cancer Research, submitted.

Reviews:

Santoli, D., Visonneau, S. 1999. Breast Cancer: Immunotherapy and gene therapy. In: Breast Cancer- Multidisciplinary Management. (M.H. Torosian, ed.) Humana Press, Totowa, NJ, in press.

Abstracts:

E.A. Stadtmauer, S. Visonneau, A. Cesano, D.L. Porter, K.M. Duffy, S.L. Luger, and D. Santoli. 1999. Phase I trial of adoptive immunotherapy with TALL-104 cells in women with advanced metastatic breast cancer. Eunice and Irving Leopold Annual Scientific Symposium and Academic Retreat, University of Pennsylvania Cancer Center.

S. Visonneau, A. Cesano, D. Porter, E. Stadtmauer, and D. Santoli. 1999. A Phase I study of TALL-104 cells in patients with metastatic breast cancer. AACR Annual Meeting, April 10-14, 1999, Philadelphia, PA.

D.L. Porter, E.A. Stadtmauer, S. Visonneau, A. Cesano, K.M. Duffy, S.M. Luger, L.M. Schuchter, and D. Santoli. Phase I trial of adoptive immunotherapy (AI) with TALL-104 cells for women with advanced metastatic breast cancer. 1999 ASCO Meeting Abstract.

Personnel receiving pay from the research effort

D. Santoli - Principal Investigator
A. Cesano - Senior Scientist
P. Sanders - Research Technician

S. Sheriden - Research Technician
C. Brando - Research Scientist
H. Buchinsky - Research Technician
E. Stadtmauer - Lead Investigator
D. Porter - Co-Investigator
C. Sickles - Data Manager
P. Mangan - Research Nurse

Phase I Trial of TALL-104 Cells in Patients with Refractory Metastatic Breast Cancer[1]

S. Visonneau, A. Cesano, D.L. Porter, S.L. Luger, L. Schuchter, M. Kamoun, M.H. Torosian, K. Duffy, E.A. Stadtmauer, and D. Santoli[2]

Running head: TALL-104 Cell Therapy of Breast Cancer

The Wistar Institute [S. V., A. C., D. S.], The University of Pennsylvania Cancer Center [D. L. P., S. L. L., L. S., M. K., K. D., E. A. S.], and The Fox Chase Cancer Center [M. H. T.], Philadelphia, PA.

Keywords: Cell therapy, Phase I clinical trial, MHC non-restricted effectors, metastatic breast cancer

(Footnotes)

[1]Supported by USAMRMC, and partially, by the Breast Cancer Fund, the Expedition Inspiration Fund, a gift from Felicity and Peter Benoliel, and the NIH Core Grant CA10815-30.

[2]To whom requests for reprints should be addressed, at The Wistar Institute, 3601 Spruce Street, Philadelphia, PA 19104. Telephone: (215) 898-3978; Fax: (215) 573-7919; Email: santoli@wistar.upenn.edu

[3]The abbreviations used are: i.v., intravenously; PD, progressive disease; MHC, major histocompatibility complex; LAK, lymphokine-activated killer; SCID, severe combined immunodeficiency; CBC, complete blood count; rhIL, recombinant human interleukin; PBMC, peripheral blood mononuclear cells; FACS, fluorescence activated cell sorting; AHG-CDC, anti-human globulin-enhanced complement-dependent lymphocytotoxicity; ABMT, autologous bone marrow transplant; SCT, stem cell transplant; GVT, graft-versus-tumor; NK, natural killer

ABSTRACT

The human cytotoxic T cell line TALL-104 displays anti-tumor effects in animals with implanted and spontaneous malignancies. A Phase I trial was conducted to determine toxicity of TALL-104 cell therapy in women with metastatic refractory breast cancer. Fifteen patients with metastatic infiltrating ductal (N=12), lobular (N=2) or medullary (N=1) carcinoma received escalating doses of lethally irradiated TALL-104 cells (3 patients per group received 10^6, 3 x 10^6, 10^7, 3 x 10^7 and 10^8 cells/kg) for 5 consecutive days (induction course). Patients without progressive disease received monthly maintenance 2-day infusions at the same dose level. Mild grade I/II toxicity developed in 11 patients regardless of cell dose. One grade IV toxicity consequent to hepatic tumor necrosis occurred in a patient given 10^8 cells/kg, 3 weeks after the induction course. Nine patients progressed within one month from induction and 5 patients had stable disease for 2-6 months. One patient (at 3 x 10^7/kg) had improvement of liver metastases and ascites, and a second patient (at 10^6/kg) experienced a dramatic relief in bone pain. Increases in blood NK cell activity and levels of IFN-γ, interleukin (IL)-10 and activation markers (sIL-2R and sICAM-1) were often seen. Only 1 patient developed anti-HLA class I antibody responses against TALL-104 cells; specific CTL activity developed in 3 patients during induction and in 4 patients during the maintenance boosts.

In conclusion, TALL-104 cells were well tolerated by patients with metastatic breast cancer at the doses and regimen tested. The clinical responses observed in

this preliminary trial demonstrate that further investigation of TALL-104 cell therapy is warranted.

INTRODUCTION

Despite great improvements in the treatment of early stage breast cancer, metastatic disease is, to date, still incurable. A variety of new therapeutic approaches have recently been suggested, such as a more rationale use of existing drugs (*i.e.*, via dose intensification and drug combinations), and testing of new drugs (*i.e.*, taxanes, gemcitabine, vinorelbine), anti-angiogenic agents (*i.e.*, endostatin and angiostatin), and gene therapy or immunotherapy (*i.e.*, cancer vaccines, dendritic cell therapy) (1). Although each of the above strategies has merits, immunological approaches are particularly appealing because their toxicity is usually low and non cross-reactive with the toxicity of chemotherapy; in addition, they might induce a tumor-specific and permanent resistance, thus protecting from recurrence. Among immunological approaches, therapy with major histocompatibility complex (MHC)[3] non-restricted effectors, such as patient-derived lymphokine-activated killer (LAK) cells, has the distinct advantage that tumoricidal activity does not require prior sensitization to a specific tumor antigen (2, 3).

The human MHC non-restricted killer cell line TALL-104 ($CD3^+$, $CD8^+$, $CD56^+$ $CD16^-$) developed in this laboratory (4, 5), might represent a powerful new immunotherapeutic approach to cancer, as suggested by accumulated evidence in animal models. Specifically, adoptive transfer of lethally-irradiated (40 Gy) TALL-104 cells into severe combined immunodeficiency (SCID) mice has induced regression of transplantable human hematopoietic and nonhematopoietic malignancies (6-10). In addition, remarkable anti-tumor effects were seen in

immunocompetent mice bearing syngeneic leukemia (11) and in pet dogs with spontaneous tumors (12-14). Interestingly, multiple systemic delivery of TALL-104 cells in some of these animals appeared to be followed by the development of protective anti-tumor immunity, which is crucial for induction and maintenance of tumor regression (11-13). Recent biodistribution studies in dogs and mice have shown that TALL-104 cells, injected i.v, localize primarily in the lungs for the first 2 h and then redistribute to liver, spleen and kidneys in the following 24 h (15, 16). Importantly, in tumor bearing animals, irradiated TALL-104 cells accumulated at the site of both the primary tumor and distant metastases (8, 16).

Our previous pre-clinical studies in SCID mice engrafted with human breast carcinomas (8, 16) and in dogs and cats with spontaneous metastatic mammary tumors (17) have shown that breast cancer is highly responsive to TALL-104 cells. These encouraging results prompted us to initiate this Phase I clinical trial to explore the safety and potential efficacy of escalating doses of lethally irradiated TALL-104 cells administered systemically to women with refractory metastatic breast cancer. The maximal dose tested (10^8/kg/day) was the one proven effective in preclinical veterinary studies (12-14, 17).

MATERIALS AND METHODS

Patients Eligibility. The patients enrolled in this study (Table 1) were required to have histologically proven metastatic breast cancer relapsed after at least two forms of conventional therapy (including stem cell transplantation). Other eligibility criteria included: age > 18 years, performance status 0-1 (by the criteria of the Eastern Cooperative Oncology Group), life expectancy > 8 weeks, and adequate hematopoietic (ANC > $1{,}500/ml^3$ and platelet > $100{,}000/ml^3$), hepatic (total bilirubin level < 1.5 mg/dl) and renal (creatinin concentration < 1.5 mg/dl) function.

Patients with medical conditions requiring steroid administration were excluded, based on *in vitro* observations that steroids interfere with the anti-tumor activity (cytotoxicity and lymphokine production) of TALL-104 cells (not shown). Written informed consent was obtained from all patients, and the protocol was approved by the Institutional Review Boards at both the Hospital of The University of Pennsylvania and the Wistar Institute.

Trial Design. The trial was designed as a single center, open label, dose escalation study. Five dose levels of lethally irradiated (40 Gy) TALL-104 cells (10^6/kg, 3 x 10^6/kg, 10^7/kg, 3 x 10^7/kg, and 10^8/kg) were tested. The schedule of administration included a first 5 day cycle (induction course) of TALL-104 cells administered by i.v. infusion (100 ml) over 60 min, followed by 2-day monthly boosts until disease progression. No intrapatient dose escalation was allowed. Three patients per dose level were treated. All patients received pre-medication

with diphenylhydramine and acetoaminophen within 1 h before the start of cell infusion.

Pre-Treatment and Follow-Up Studies. Histories, physical examinations (including pain assessment), routine laboratory studies and complete tumor staging (using appropriate radiological techniques) were performed prior to TALL-104 treatment. When available, excess tumor tissue was cryopreserved and used later on for a) engraftment studies in SCID mice and b) *in vitro* studies measuring sensitivity to killing by TALL-104 cells and/or patient's CTL. Irradiated TALL-104 cells were infused at a slow rate over 60 min; all patients were hospitalized during the 5-day treatment in the Clinical Research Center unit at the Hospital of the University of Pennsylvania. During cell infusion, patients were closely monitored and their vital signs recorded at times 0, 5, 10, 15, 30, 60, 75, 90, 120, and 240 min, and every 24 h thereafter. Together with the clinical evaluation for toxicity, a complete blood count (CBC) including differential, and serum biochemistry tests (electrolytes, alkaline phosphatases, urea and creatinin, liver function tests) were performed daily during each cell course. The tumor marker CEA was checked before treatment and weekly thereafter. Patients were reviewed weekly for symptoms and signs of toxicity for at least 4 weeks after the last cell injection. Tumor assessment was repeated before each cell cycle. Treatment was discontinued when PD was documented.

Manufacturing of the TALL-104 Clinical Product. Manufacturing of clinical grade TALL-104 cells was performed under GLP conditions in a specially designated facility at The Wistar Institute. TALL-104 cells were grown in endotoxin-free Iscove's modified Dulbecco's medium (Life Technologies, Inc., Grand Island, NY) supplemented with 10% heat-inactivated fetal bovine serum (Atlanta Biologicals, Norcross, GA) and 100 U/ml recombinant human interleuken (rhIL)-2 (Chiron Therapeutics, Emeryville, CA) in humidified incubators at 37°C with 10% CO_2 in T-175 vented cap flasks (Falcon, Franklin Lakes, NJ). Mycoplasma contamination was monitored weekly on cell samples taken from at least two flasks from each incubators using a commercial PCR kit (ATCC, Rockville, MD). Three times a week, cells were harvested by centrifugation in 250 ml conical tubes (Corning, New York, NY), washed twice in saline (Abbott Laboratories, King of Prussia, PA), γ-irradiated (40 Gy) using a ^{137}Cs source, resuspended in freezing medium, consisting of human plasma protein fraction 5% (American Red Cross Blood Services, Philadelphia, PA) and 10% DMSO (Rimso, Tera Pharmaceuticals, Buena Park, CA), and then transferred into Fenwal® transfer pack containers (Baxter Healthcare Corporation, Deerfield, IL). The TALL-104 cell packs were stored at -70°C. Each lot of frozen TALL-104 cell packs was tested for sterility, mycoplasma contamination, endotoxin, purity, identity, phenotype, proliferation and cytotoxic function (quality control assays). Only lots that met the standard criteria summarized in Table 2 were used for patients administration. When needed for infusion, frozen TALL-104 cell packs were rapidly thawed in a 37°C water-bath, and saline was added to reach a final volume of 100 ml. Cell aliquots were removed from each pack for determination of

cytotoxic and proliferative activities and sterility (quality control assays). The TALL-104 cell packs were then handed to the clinical personnel for patient injection, as described above. A Gram stain (STAT) was performed at HUP just prior to infusion.

Immunological Monitoring. Immunological studies were performed on serum and peripheral blood mononuclear cells (PBMC) samples (pre-treatment, before each TALL-104 cell injection, and during the follow-up period) to monitor the development of TALL-104-specific humoral and cellular immune responses, respectively.

PBMC were isolated from heparinized blood samples by Accu-Prep™ (specific gravity: 1,077 g/ml; Accurate Chemical, Westbury, NY) lymphocyte gradient centrifugation, and tested as effectors in an 18 h ^{51}Cr-release assay against TALL-104 cells and the NK sensitive human leukemic cell line K562, as previously described (5, 18). When available, tumor cells harvested from patients' tumor biopsies by mechanical dissociation (8, 19), were tested in 18 h ^{51}Cr-release assays for susceptibility to the lytic activity of TALL-104 cells and autologous PBMC.

Monitoring of anti-TALL-104 cell antibodies in the patients sera was performed by immunofluorescence. Briefly, the sera were diluted 1:100 and 1:1000 in fluorescence activated cell sorting (FACS) buffer (11, 12-14, 17) and incubated for an hour with the TALL-104 cells. After three washes in FACS buffer, fluorescein isothiocyanate conjugated goat anti-human IgG (whole molecule) and anti-human IgM (μ-chain specific) (Sigma, St. Louis, MO) were added at a 1:100 dilution in FACS

buffer for another hour. After final washes in FACS buffer, the % reactivity of the sera with TALL-104 cells was calculated as previously described (11, 12-14, 17). HLA class I antibody screening in the patients' sera was performed using a cell panel from 35 volunteers and anti-human globulin-enhanced complement-dependent lymphocytotoxicity (AHG-CDC) assay. Furthermore, indirect immunofluorescence flow cytometry technique using HLA class I microbeads (One Lamba Inc., Canoga Park, CA) was performed to specifically detect anti-HLA class I antibodies, as described (20, 21).

Variations in serum levels of two markers for non-specific immune activation, namely sIL2-R and sICAM-1, were also investigated, using commercially available ELISA kits (Endogen), according to the manufacturer's instructions. The sensitivity of the assays was 24 U/ml for sIL2-R and 0.3 ng/ml for sICAM-1.

Cytokine Assays. Patients sera were collected pre-TALL-104 cell infusion (time 0) and at 2, 4, and 6 h post TALL-104 cell infusion, on day 1; before each TALL-104 infusion on days 2, 3, and 5, and weekly thereafter until disease progression. The presence of IFN-γ, TNF-α, TNF-β, GM-CSF, and IL-10 in the sera was tested using human cytokine-specific ELISA kits, according to the manufacturer's instructions. The sensitivity of the assays was 2 pg/ml for IFN-γ and GM-CSF, 5 pg/ml for TNF-α, 3 pg/ml for IL-10 (Endogen, Woburn, MA), and 7 pg/ml for TNF-β (R&D, Minneapolis, MN).

PCR Analysis. Patients PBMC samples, obtained immediately before each TALL-104 cell infusion, and at various intervals during and after cell infusions, were subjected to DNA extraction using standard techniques (8, 12-14, 17). The presence of circulating TALL-104 cells in each cell extract was evaluated by PCR analysis using two primers specific for the human minisatellite region YNZ.22. An oligonucleotide probe recognizing 24 nucleotides in the middle of the amplified sequence was used to demonstrate the specificity of the PCR products by Southern blot hybridization, as previously described (8, 12-14, 17).

Statistical Analysis. The significance of hematological and immunological changes within each patient during and after TALL-104 cell infusions was tested by paired Student's t test.

RESULTS

Patient Population. The characteristics of the 15 patients enrolled in this study are summarized in Table 1. Median age was 49 years (range 33-63); primary diagnosis was infiltrating ductal carcinoma (12 patients), infiltrating lobular carcinoma (2 patients), and medullary carcinoma (1 patient). All patients had failed a combination of at least two different treatments including chemotherapy, radiation therapy, hormonal therapy, immunotherapy with HER2/neu antibodies, and PB stem cell transplant (SCT).

Some exceptions to the eligibility criteria were made in this trial: one patient (#008) had only one chemotherapy regimen because of the development of severe cardio-pulmonary syndrome that precluded further chemotherapy; two patients (#011 and #013) had platelets $< 100{,}000/ml^3$ due to history of autologous SCT, two patients (#010 and #011) had increased alkaline phosphatase levels due to metastatic disease, and one patient (#015) had history of brain metastases.

Toxicity. Table 3 summarizes, for each enrolled patient, the TALL-104 cell dose injected, the number of monthly maintenance boosts subsequent to the 5-day induction course, and the total number of cell infusions. Twenty-eight treatment courses were administered in this trial, for a total of 101 cell injections (Table 3). The DMSO present in the cell product was responsible for taste change and alithosis, an unanimous complaint by both patients and caregivers. No anaphylactic-like reactions and/or significant changes in vital signs occurred at any of the cell doses

tested (not shown). Few adverse events were observed, listed in Table 4: 2 patients, who received the lowest cell dose (10^6/kg), had bilateral ulnar eminence erythema and urticaria on day 4 of the induction course; symptoms resolved within a week; grade I nausea with or without vomiting was observed in 6 patients regardless of the dose level; three grade I and one grade II hyperglycemia (blood glucose, 130, 131, 154, and 180 mg/dl, respectively) were observed in 4 patients and resolved within 5 to 7 days in all cases. Other toxicities included: grade I hypocalcemia in 1 patient (calcium, 8.4 mg/dl) which returned to normal levels the following day, hypoglycemia in 1 patient (blood glucose, 62 mg/dl) which resolved within a week, elevated liver enzymes (SGOT, 40 and 44 IU/L) and elevated alkaline phosphatases (131 and 139 IU/L) in 2 patients, both of which returned to normal levels within 5 days. Patient #015 had grade I leukopenia (WBC, 3.2 thousands/mm^3) which resolved in a week, patient #011 had grade II leukopenia (WBC, 2.7 thousands/mm^3) and grade I neutropenia (granulocytes, 1.65 thousands/mm^3) that resolved within 5 days (Table 4; Fig. 1). One patient (#010) developed grade I fever (up to 100°F) on day 2 of the induction course, which resolved the following day. Finally, patient #014 was diagnosed with necrotic hepatic metastases (symptoms were fever up to 102°F, profuse sweating, nausea and decreased appetite), 3 weeks after the induction course; the event was serious enough (grade IV) to require hospitalization. No other grade III and IV toxicities were seen. No toxic effects were observed in the patients who underwent maintenance therapy.

Laboratory Findings. Fig. 2 illustrates the effects of TALL-104 cell administration (induction course) on the blood cell counts of the 15 patients enrolled in the study. When the mean WBC counts pre-treatment (day 0) were compared to the day 5 counts, a decrease was observed regardless of the dose level. By contrast, the mean monocyte counts at the same time points showed a trend towards an increase (not shown); values 3.2 times higher than the basal value were seen in patient #002 (at 10^6 cells/kg). A similar trend towards an increase was observed for the mean eosinophil counts at any dose level. These changes were not statistically significant, and consistently normalized within a week from the last cell injection. Granulocytes, lymphocytes, and platelet counts remained mostly unaltered (not shown).

Serum Cytokines, Immune Activation Markers and Tumor Markers. The presence of cytokines (IL-10, IFN-γ, TNF-α, TNF-β and GM-CSF) known to be released by TALL-104 cells *in vitro* upon interaction with tumor cells (22) was measured in patients sera before and at different time points during the TALL-104 cell induction course. None of the patients had detectable serum levels of TNF-α, TNF-β (< 5 pg/ml) nor GM-CSF (< 2 pg/ml) before and during cell treatment, except for patient #001 who had increased TNF-α levels (7.6 pg/ml) starting on day 4 of the induction course. IFN-γ was present in the sera of 13 patients and increased in most cases (9/13) regardless of TALL-104 cell dose injected, either on day 1, starting at 2 h (patients #002, 003, 010, 013, 014), 4 h (patients #004, 012), or 6 h (patient #011), or on

day 5 (patient #007) (Table 5). Two more patients who had no detectable levels of IFN-γ pre-treatment, had significant levels of this cytokine during the induction course, starting on day 3 (patient #005) or on day 1, at 4 h (patient #008) (Table 5). Nine patients had baseline, and sometimes elevated, levels of IL-10, which increased in 6 patients during cell treatment, starting at 2 h (#013, 014) or 4 h (#008, 012, 015) of day 1, or on day 4 (#007) of the induction course, independent of TALL-104 cell dose (Table 5). IL-10 was also found in the serum of four patients receiving TALL-104 cells (#001, 002, 004, and 006), starting at 2 h (patient #004) and 4 h (patients #001, 002, and 006) of day 1 of the induction course although no IL-10 was detectable prior to cell therapy.

Serum levels of the sIL-2R and sICAM-1 markers, associated with non-specific immune activation, were measured at the same time points. As shown in Fig. 3, there was a trend in increase in both markers during TALL-104 cell infusions which was evident as soon as day 1 post-treatment. Interestingly, the levels of sIL-2R and sICAM-1 returned to the baseline by day 5 if they were high pre-treatment (8 and 7 patients, respectively, panels A and C), but persisted until day 5 post-infusion when they were low pre-treatment (7 and 8 patients, respectively, panels B and D).

Elevated levels of the CEA tumor marker were present pre-treatment in patients #001, 009, 011, and 014 (492, 68.6, 254, 175.5 U/L, respectively); CEA values remained stable in patients #011, 014 while progressively increased in patients #001, 009 up to 2.6 and 6.6 times their basal value, 2 months and 15 days, respectively, from start of treatment. In the other 11 patients who did not have elevated levels of

CEA before enrolling in the trial (range 1.0 - 17.0 U/L), no significant changes were observed during and post-TALL-104 infusions (not shown).

Humoral Immune Response Against TALL-104 Cells. FACS analysis on patients sera before and after TALL-104 cell therapy showed that none of the patients developed antibodies against TALL-104 cells up to 6 months follow-up, except for patient #012, whose serum became 89.1% reactive with TALL-104 cells as early as day 7 from the start of therapy and remained positive until the date off study (3 weeks from the start of TALL-104 infusions). By the AHG-CDC bioassay and indirect immunofluorescence flow cytometry analyses with HLA class I microbeads, it could be determined that the post-immune serum from patient #012 reacted with HLA class I antigens whereas the pre-immune serum was not reactive (Fig. 4). Furthermore, the post-immune serum was reactive by AHG-CDC with the cell panel. The HLA specificity of this serum was broad and included the TALL-104 HLA class I antigens (not shown). Importantly, the post-immune serum did not inhibit TALL-104 killer activity against the patient's own tumor biopsy in *in vitro* cytotoxicity assays (not shown).

NK and Specific CTL Activities. Basal (pre-treatment) levels of peripheral blood cell cytotoxicity against the NK sensitive K562 cell line were present in most patients, and increased slightly on day 5 of the induction-course (Fig. 5). The limited number of patients in the study and the large variability of NK activity among the patient population precluded an analysis of the statistical significance of this observation.

Five patients had baseline NK activity against TALL-104 cells (> 15% specific ^{51}Cr-release). Interestingly, this activity decreased slightly during cell treatment (Fig. 6*A*); however, specific killing of TALL-104 cells developed or increased in 3 patients (20%) (#002, 007, and 012) (Fig. 6*B*) regardless of cell dose.

***In Vitro* and *In Vivo* Studies on Tumor Biopsies.** Tumor biopsies, consisting of surgical lymph nodes (from 4 patients) and chest wall mass (from 1 patient), were used for both *in vitro* and *in vivo* studies. Four of these specimens were sensitive to *in vitro* lysis by TALL-104 cells and all 5 induced cytokine production in TALL-104 cells (Table 6). The levels of cytokines induced by lymph node biopsies were low (#003, 012, and 015, Fig. 7*A*) or intermediate (#009, Fig. 7*B*) while those induced by the chest walls from patient #008 were remarkably high (Fig. 7*C*).

PBMC from patients #003, 008, 009, and 015 were tested for ability to kill autologous tumor cells before and after TALL-104 treatment; none of the patients displayed killer activity against their own tumors (< 1% ^{51}Cr-release at effector:target ratio of 100:1), except for patient #008, who developed tumor specific CTL (28% ^{51}Cr-release) 1 month after TALL-104 cell infusions.

Tumor biopsies (5x5 mm fragments) were implanted in SCID mice, as previously described (8, 19). Six months later, a palpable tumor mass developed at the site of engraftment in only two of the four female mice engrafted with patient #009 specimen lymph node only. At necropsy 15 months after engraftment, macroscopic examination of these two mice revealed the presence of a small, soft, and well vascularized primary tumor mass (570 mg), small metastatic axillary

lymph nodes, and slight splenomegaly. Tumor biopsies from other patients failed to engraft during the 18 months follow-up (Table 6).

Detection of Circulating TALL-104 Cells. Short (1 week)- and long (up to 2 months)-term monitoring of circulating TALL-104 cells was performed in 5 patients by PCR analysis. TALL-104 cells were not detectable in their peripheral blood by day 7 from the last cell injection in each cycle (not shown).

Clinical Responses. Five patients receiving 10^6 (#001 and 003), 3 x 10^6 (#005 and 006), and 10^8 (#013) cells/kg had stable disease for 2-6 months after the induction course, thus qualifying for monthly maintenance boosts; among them, patient #001 had a significant reduction in narcotic requirements for bone pain. Nine patients had progressive disease in the month after the induction course; one patient (#011) had a documented marginal clinical response after receiving the TALL-104 induction course at 3 x 10^7/kg: comparison of abdomen and pelvis CT scans before and 3 weeks after TALL-104 cell therapy showed a decrease in size of liver metastases (many of the lesions having become necrotic) and in the amount of peri-hepatic and pelvis fluid. Unfortunately, this patient withdrew from the study for personal reasons before receiving the first monthly boost, precluding further evaluation.

As of October 1999 (exactly a year after the end of the study), 3 patients (#013, 014, and 015) are still alive with disease.

Immunological Monitoring During Maintenance Therapy. The 5 patients who had stable disease (see Table 3) were immunologically monitored during the 2-

day monthly maintenance infusions. None of these patients had detectable serum levels of TNF-α, TNF-β or GM-CSF before or during the boosts. The IFN-γ and IL-10 levels observed on day 5 of the induction course (see Table 5) consistently returned to baseline levels before each subsequent monthly boost and always increased on day 2 of each boost (not shown). sIL2-R and sICAM-1 serum activation markers followed the same pattern described for the cytokines with return to baseline levels pre-TALL-104 cell infusion, followed consistently by an increase on day 2 of each monthly boost (not shown). In the case of patient #001, high levels of IL-10 (61.8 pg/ml) were seen as soon as 4 h after the first TALL-104 infusion and decreased with time, but did not return to baseline levels before the subsequent boost. An increase in IL-10 was also observed on day 2 of the first boost and significant IL-10 levels were still present in the patient's serum up to 3 months after the start of therapy (Fig. 8*A*).

Peripheral blood NK activity against K562 cells was highly variable among patients and also varied within each individual patient when tested at different times. An example is shown in Fig. 8*B* for patient #005 whose peripheral blood NK activity, seen at day 5 of the induction course, remained stable until pre-TALL-104 cell infusion on boost 2; her NK activity increased at this time, stabilized until administration of the third boost, decreased until administration of boost 4, and rised again after boost #4 and during boost #5 (6 months after initiation of cell therapy), reaching the maximum value (Fig. 8*B*).

No antibody responses against TALL-104 cells developed in any of the 5 patients who received monthly boosts for 2 to 6 months (not shown). By contrast,

TALL-104 specific cellular immune responses (CTL activity) developed in all patients receiving boosts, with the exception of patient #013 who did not have CTL activity up to 3 months from initiation of therapy (not shown). In patient #005, baseline cytotoxicity against K562 and TALL-104 cells consistently increased on day 2 of each boost up to 6 months from initiation of cell therapy (Fig. 8*B*).

DISCUSSION

Currently, surgery, chemotherapy, hormonal therapy, and radiation therapy remain the main forms of treatment for breast cancer. Although these approaches reduce the risk of death and can induce complete remissions in a majority of patients, many tumors will recur as metastatic lesions. In recent years, novel therapies have been developed that work independently or in conjunction with conventional treatments to minimize side effects and enhance therapeutic efficacy (1). In particular, autologous and allogeneic effector cell-based approaches are being evaluated in the clinic that have either a direct tumoricidal function or act by stimulating the immune system against the patient's own tumor cells (2, 3). Trials using activated autologous lymphocytes have been disappointing. Specifically, two Phase II trials of high-dose IL-2/LAK therapy were performed in patients with either advanced breast carcinoma or advanced cancers arising in other sites. Of all patients, one with adenocarcinoma of the breast had a partial response of 17 weeks' duration (23). Very few breast cancer patients have been treated with TILs, and in those treated, there have been no responses (24).

Allogeneic donor T cells can mediate a potent graft-versus-tumor (GVT) reaction in patients who have relapsed after allogeneic BMT (25, 26). Transfer of allogeneic effectors may also be effective as primary therapy for patients with various malignancies (27). Breast cancer may be an appropriate target for GVT induction as a clinically significant GVT effect was suggested after HLA-matched sibling SCT or BMT in patients with metastatic breast cancer (28, 29). Or *et al.* (30)

have also investigated possible GVT effects in six patients with metastatic breast cancer cytoreduced with high-dose chemotherapy and autologous SCT, and treated by adoptive transfer of HLA-matched donor PBL activated with rhIL-2. Treatment was well tolerated. Two patients developed symptoms compatible with GVHD grade I-II, one of whom showed no evidence of disease at more than 34 months out. In the remaining patients, progression-free survival ranged between 7 and 13 months (30).

Unlike HLA-matched sibling/donor-derived effector cells, TALL-104 cells represent a universal donor system and provide an unlimited and reliable source of tumoricidal cells with stable cytotoxic activity, which is ideal for adoptive immunotherapy approaches. Although dependent on IL-2 for expression of cytotoxicity and long-term survival *in vitro,* TALL-104 cells can exert anti-tumor effects *in vivo* without the concomitant administration of IL-2, thus eliminating the toxicity observed with LAK and/or TIL therapies in conjunction with IL-2 (2, 3). The major objective of this study was to evaluate the potential toxicities associated with systemic administrations of lethally irradiated, non-dividing TALL-104 cells in women with metastatic refractory breast cancer. In addition, immunological studies were done to find surrogate markers for toxicity and/or clinical responses.

Abnormalities in liver functions were seen in 2 of the treated patients, regardless of the dose injected; their correlation with TALL-104 therapy was sometimes difficult to assess because of PD involving the liver parenchyma. However, when liver alterations could be ascribed to TALL-104 cell injections, they

were always limited to SGOT and alkaline phosphatases, moderate (grade I), transient and completely reversible upon termination of therapy. It has been suggested that IL-2-activated lymphocytes are hepatotoxic and the strong correlation between IL-2 priming and serum transaminase levels supports this contention (31). Similar to our findings, LAK/IL-2 therapy was reported to frequently alter hepatic functions (*i.e.*, serum transaminases, alkaline phosphatase, and total billirubin); the LAK cell-induced liver abnormalities usually returned to baseline values after completion of the therapy, indicating that the insult to the liver was transient and reversible (32). Severe toxicity (grade IV) was observed in one patient receiving the highest cell dose and was related to hepatic tumor necrosis developing 3 weeks after the induction course. While it is difficult to prove that this toxicity was consequent to TALL-104 cell therapy (often in patients with PD, liver metastases outgrow their blood supply), it is tempting to speculate that it actually reflected a marginal response, based on the fact that TALL-104 cells do induce necrotic tumor cell death *in vitro* (5, 18) and in animal studies (6-11, 12-14, 17). However, no liver tissue was available from this patient to conduct histological and/or *in situ* hybridization studies to definitively document an anti-tumor response.

Mild, grade I gastrointestinal toxicity (nausea, vomiting) was observed during TALL-104 treatment in 40% of the treated patients. This has also been reported to occur in 80% of cancer patients during LAK/IL-2 therapy (33), and was reported in 5% of TALL-104 treated dogs in our study (12). Taste change/alithosis was observed in 100% of the cases and was likely associated with the presence of DMSO as cryostabilizer in the TALL-104 cell packs. The range of DMSO given with each

TALL-104 cell infusion varied from 2 to 5 ml corresponding to a dose of 0.06-0.2 g/kg; this dose was much lower than that (0.2-1.3 g/kg)administered during BMT trials using cryopreserved grafts, thus explaining, at least in part, the lack of toxic signs (flushing, pulmonary and abdominal toxicity) attributed to DMSO in those trials (34, 35). Although two patients at the lowest TALL-104 cell dose complained of erythema and urticaria, our use of antihistaminic premedication may have prevented more severe toxicities resulting from DMSO-induced histamine release in the rest of the patients. Another explanation for the low incidence of side effects in our study may lie on the low level of cell lysed products infused (TALL-104 cell viability after thawing was 85-90%). Comparatively, in thawed marrow grafts, the final amount of cell lysis products injected may be very high, depending on the quantity of contaminant mature cells present, which are usually poorly preserved (34, 35).

No severe toxicities, such as hypotension, secondary to increased capillary permeability, were observed in our study. These effects have been reported to be induced by conventional LAK/IL-2 therapy (36). Although the mechanism by which IL-2 and LAK cells induce a vascular leak syndrome is unknown, LAK cells have been shown to bind and lyse normal human vascular endothelial cells *in vitro* (37, 38). Whether this applies also to TALL-104 cells is now under investigation in this laboratory. However, the absence of vascular leak syndrome in mice, dogs, cats, and monkeys treated with up to 10^8/kg TALL-104 cells in the absence of exogenous sIL-2 (6-17, 39), suggests that this effects does not reach biological significance with TALL-104 cells.

None of the hematological abnormalities induced by LAK/IL-2 therapy (anemia; lymphopenia followed by rebound lymphocytosis, and thrombocytopenia with coagulation disorders) (32) were detected in the present study. Interestingly, leukopenia with neutropenia were seen in 1-2 patients. It was of fast onset, moderate (grade I-II), and rapidly reversible once therapy was halted. Most patients developed absolute and relative monocytosis and eosinophilia; the latter abnormality has also been reported in patients treated with IL-2 and LAK cells (32). Although the cause of these changes is not clear, it may be related to the production of cytokines by TALL-104 cells in response to tumors (22). Indeed, an increase in the production of IFN-γ and IL-10 was observed in 69% and 27% of the patients treated, respectively. Whether TALL-104 cells or the patients own tumors were responsible for the observed increases in serum cytokines and immune activation markers (sIL-2R and sICAM-1) remains unclear. In fact, sICAM-1 and sIL-2R are known to be associated with human breast cancer progression (40, 41); moreover IL-10 has been detected in primary breast cancers, associated with the induction of T-cell anergy (42), as a result of T cell proliferation and function, reduced IL-2 production, and antigen presentation (43).

To our knowledge, in previous trials with IL-2 activated lymphocytes (LAK cells), no correlation was shown between clinical and laboratory immunological markers. In particular, neither the LAK cell dose infused nor the *in vitro* tumoricidal activity of the effectors were found to be predictive of clinical efficacy or toxicity (32). The mean total number of LAK cells infused per patient ranged from

5.6 x 10^9 to 5.1 x 10^{10} in different clinical trials (44). In our study, the maximum number of TALL-104 cells injected was 6.6 x 10^{10} (patient #013).

Xenogeneic antibody responses were consistently observed in TALL-104 pre-clinical studies in mice, dogs, and monkeys; such antibodies did not have neutralizing activity *in vitro* but were responsible for progressively faster kinetics of TALL-104 cell clearance with boosts (12-15, 17, 39). Surprisingly, in the present study, non-neutralizing anti-HLA class I antibodies against TALL-104 cells developed only in one of 15 patients. It is possible that the dose, route, and time of TALL-104 cell administration, combined with the host compromised immune status, resulted in a humoral tolerant response *versus* the allogeneic cells. This would be consistent with previous findings demonstrating that female first transplant candidates induced panel-reactive antibodies in only 5-10% of the recipients (45, 46). The mechanism of low anti-HLA antibody responses is not known but it has been suggested that each transfusion induces some degree of clonal expansion that is too low in magnitude to produce a persistent antibody response; subsequent transfusions continue to expand memory cells, but such expansion remains clinically insignificant as long as the number of transfusions remains below 20 (45, 46). With higher numbers of transfusions, an increasing proportion of patients develops a critical mass of memory T and B cells capable of sustaining a persistent antibody response (significant clonal expansion). In this respect, TALL-104 cells might represent a setting of allo-immunization similar to the one described for allogeneic transfusions and/or transplantations (45, 46). Antibody responses were indeed observed in only 1 of the recipients and the maximum total number of

infusions given was 15 (patient #005). A higher number of patients with a more extensive follow-up and longer periods of treatment needs to be studied to clarify this important issue. Contrary to antibody responses, specific CTL responses to TALL-104 cells occurred in 3 patients during the induction course, and in 4 patients over the monthly boosts.

Based on the advanced clinical stage and heavy pre-treatment of the 15 patients in this Phase I study, observing even a marginal clinical response in one patient and noting significant pain relief in a second patient is encouraging. These findings, together with the disease stabilization seen in 5 patients for 2-6 months, suggest biological activity of TALL-104 cells and point to their potential clinical benefit. Future trials will investigate the efficacy of TALL-104 cell therapy in the adjuvant setting and determine their kinetics of distribution at sites of metastases. Additional Phase I/II clinical trials are planned to establish the most effective TALL-104 cells dose, delivery method and schedule, and to identify highly responsive tumor targets.

ACKNOWLEDGMENTS

We thank Linda Knox R.N., Ph.D., Laura Manley, R.N., and Patricia Mangan, R.N. for patient care, Cheryl Sickles for coordinating patients entry and collecting clinical data, Thuy Tran and Shelley Sheridan for manufacturing clinical grade TALL-104 cells, Jeffrey Faust for assistance with Flow Cytometry analysis, and the Wistar Editorial Department for preparing this manuscript.

REFERENCES

1. Santoli, D., and Visonneau, S. Breast cancer: immunotherapy and gene therapy. *In*: M. H. Torosian (ed.), Breast Cancer-Multidisciplinary Management. Totowa, NJ, The Humana Press, 1999 (in press).

2. Rosenberg, S., Lotze, M., Muul, L., Chang, A. E., Avis, F. P., Leitman, S., Linehan, W. M., Robertson, C. N., Lee, R. E., Rubin, J. T., Seipp, C. A., Simpson, C. G., and White, D. E. A progress report on the treatment of 157 patients with advanced cancer using lymphokine-activated killer cells and interleukin-2 or high-dose interleukin-2 alone. N. Engl. J. Med., *316*: 889-897, 1987.

3. Law, T., Motzer, R., Mazundar, M., Sell, K. W., Walther, P. J., O'Connell, M. O., Khan, A., Vlamis, V., Vogelzang, N. J., and Bajorin, D. F. Phase III randomized trial of interleukin-2 with or without lymphokine-activated killer cells in the treatment of patients with advanced renal cell cancer. Cancer 76:824-832, 1995

4. O'Connor, R., Cesano, A., Lange, B., Finan, J., Nowell, P. C., Clark, S. C., Raimondi, S. C., Rovera, G., and Santoli, D. Growth factor requirements of childhood acute T lymphoblastic leukemia: correlation between presence of chromosomal abnormalities and ability to grow permanently *in vitro*. Blood, *77*: 1534-1545, 1991.

5. Cesano, A., and Santoli, D. Two unique human leukemic T-cell lines endowed with stable cytotoxic function and different spectrum of target reactivity. Analysis

and modulation of their lytic mechanisms. In Vitro Cell. Dev. Biol., *28A*: 648-656, 1992.

6. Cesano, A., Visonneau, S., Cioé, L., Clark, S. C., Rovera, G., and Santoli, D. Reversal of acute myelogenous leukemia in humanized SCID mice using a novel adoptive transfer approach. J. Clin. Invest., *94*: 1076-1084, 1994.

7. Cesano, A., Visonneau, S., and Santoli, D. Treatment of experimental glioblastoma with a human MHC non-restricted cytotoxic T-cell line. Cancer Res., *55*: 6-101, 1995.

8. Visonneau, S., Cesano, A., Torosian, M., and Santoli, D. Cell therapy of a highly invasive human breast carcinoma implanted in immunodeficient (SCID) mice. Clin. Cancer Res., *3*: 1491-1500, 1997.

9. Cesano, A., Visonneau, S., Rovera, G., and Santoli D. Synergistic effects of adriamycin and TALL-104 cell therapy against a human gastric carcinoma in vivo. Anticancer Res., *17*: 1887-1892, 1997.

10. Cesano, A., Visonneau, S., and Santoli, D. TALL-104 cell therapy of human solid tumors implanted in immunodeficient (SCID) mice. Anticancer Res., *18*: 2289-2295, 1998.

11. Cesano, A., Visonneau, S., Pasquini, S., Rovera, G., and Santoli, D. Anti-tumor efficacy of a human MHC non-restricted cytotoxic T cell line (TALL-104) in immunocompetent mice bearing syngeneic leukemia. Cancer Res., *56*: 4444-4452, 1996.

12. Cesano, A., Visonneau, S., Jeglum, K. A., Owen, J., Wilkinson, K., Carner, K., Reese, L., and Santoli, D. Phase I clinical trial with a human major histocompatibility complex nonrestricted cytotoxic T-cell line (TALL-104) in dogs with advanced tumors. Cancer Res., *56: 3021-3029, 1996.*

13. Visonneau, S., Cesano, A., Tran, T., Jeglum, K. A., and Santoli, D. Successful treatment of canine malignant histiocytosis with the human MHC non-restricted cytotoxic T cell line TALL-104. Clin. Cancer Res., *3*: 1789-1797, 1997.

14. Visonneau, S.*, Cesano, A.*, Jeglum, K. A., and Santoli, D. Adjuvant treatment of canine osteosarcoma with the human cytotoxic T cell line TALL-104. Clin. Cancer Res., *5*: 1868-1875, 1999 (*authors contributed equally).

15. Cesano, A.*, Wortman J. A.*, Pourdehnad, M., Visonneau, S., Mozley, D., Bhatnagar, A., Alavi, A., and Santoli, D. Effects of a multiple dose administration schedule on the biodistribution of TALL-104 cells in healthy dogs. Implications for adoptive cell therapy of cancer. Int. J. Oncol., *14*: 233-244, 1999 (*authors contributed equally).

16. Cesano, A., Visonneau, S., Tran, T., and Santoli, D. Biodistribution of human MHC non-restricted TALL-104 killer cells in healthy and tumor bearing mice. Int. J. Oncol., *14*: 245-251, 1998.

17. Visonneau, S., Cesano, A., Jeglum, K. A., and Santoli, D. Adoptive therapy of canine metastatic mammary carcinoma with the human MHC non-restricted cytotoxic T-cell line TALL-104. Oncol. Reports, *6*: 1181-1188, 1999.

18. Cesano, A., Visonneau, S., Clark, S. C., and Santoli, D. Cellular and molecular mechanisms of activation of MHC non-restricted cytotoxic cells by IL-12. J. Immunol., *151*: 2943-2957, 1993.

19. Visonneau, S., Cesano, A., Torosian, M. H., Miller, E. J., and Santoli, D. Growth characteristics and metastatic properties of human breast cancer xenografts in immunodeficient mice. Am. J. Pathol., *152*: 1299-1311, 1998.

20. Pei, R., Wang, G., Tarsitani, C., Rojo, S., Chen, T., Takemura, S., Liu, A., and Lee, J. Simultaneous HLA class I and class II antibodies screening with flow cytometry. Hum. Immunol., *59*: 313-322, 1998.

21. Zachary, A. A., Klingman, L., Thorne, N., Smerglia, A. R., and Teresi, G. A. Variations of the lymphocytotoxicity test. Transplantation, *60*: 498-503, 1995.

22. Cesano, A., and Santoli, D. Inducible expression of granulocyte-microphage colony-stimulating factor, tumor necrosis factor-α, and interferon-γ in two human cytotoxic leukemic T cell lines. In Vitro Cell. Dev. Biol., *28A*: 657-662, 1992.

23. Sparano, J. A., Fisher, R. I., Weiss, G. R., Margolin, K., Aronson, F. R., Hawkins, M. J., Atkins, M. B., Dutcher, J. P., Gaynor, E. R., and Boldt, D. H. Phase II trials of high-dose interleukin-2 and lymphokine-activated killer cells in advanced breast carcinoma and carcinoma of the lung, ovary, and pancreas, and other tumors. J. Immunother., *16*: 216-223, 1994.

24. Wei, W. Z., and Heppner, G. Breast cancer immunology. Cancer Treat. Res., *83*: 395-410, 1996.

25. Horowitz, M., Gale, R., Sondel, P., Goldman, J., Dersey, J., Kolb, H., Rimm, A., Ringden, O., Rozman, C., Speck, B., Truitt, R., Zwann, F., and Bortin, M. Graft-versus-leukemia reactions after bone marrow transplantation. Blood, *75*: 555-562, 1990.

26. Porter, D., and Antin, J. Graft-versus-leukemia effect of allogeneic bone marrow transplantation and donor mononuclear cell infusions. *In*: J. Winter (ed.), Blood Stem Cell Transplantation, pp 57-86. Norwell, MA, Kluwer Academic Publishers, 1997.

27. Porter, D. L., Connors, J. M., Van Deerlin, V., Duffy, K. M., McGarigle, C., Saidman, S., Leonard, D., and Antin, J. A pilot trial of donor leukocyte infusions as primary adoptive immunotherapy for patients with malignancies. J. Clin. Oncol., *17*: 1234-1243, 1999.

28. Ueno, N., Rondon, G., Mirza, N., Geisler, D., Anderlini, P., Giralt, S., Andersson, B., Claxton, D., Gajewki, J., Khouri, I., Korbling, M., Mehra, R., Przepiorka, D., Rahman, Z., Samuels, B., van Besien, K., Hortobagyi, G., and Champlin, R. Allogeneic peripheral blood progenitor cell transplantation for poor risk patients with metastatic breast cancer. J. Clin. Oncol., *16*: 986-993, 1998.

29. Eibl, B., Schwaighofer, H., Nachbauer, D., Marth, C., Gachter, A., Knapp, R., Bock, G., Gassner, C., Schiller, L., Petersen, F., and Niederwieser, D. Evidence for a

graft-versus-tumor effect in a patient with marrow ablative chemotherapy and allogeneic bone marrow transplantation for breast cancer. Blood, *88*: 1501-1508, 1996.

30. Or, R., Ackerstein, A., Nagler, A., Kapeluschnik, J., Naparstek, E., Samuel, S., Amar, A., Bruatbar, C., and Slavin, S. Allogeneic cell-mediated immunotherapy for breast cancer after autologous stem cell transplantation: a clinical pilot study. Cyto. Cell. Mol. Ther., *4*: 1-6, 1998.

31. Boldt, D. H., Mills, B. J., Gemlo, B. T., Holden, H., Mier, J., Pajetta, E., McMannis, J. D., Escobedo, L. V., Sniecinski, I., Rayer, A. A., Hawkins, M. J., Atkins, M. B., Ciobanu, N., and Ellis, T. M. Laboratory correlates of adoptive immunotherapy with recombinant interleukin-2 and lymphokine-activated killer cells in humans. Cancer Res., *48*: 4409-4416, 1988.

32. Huang, C. M., Elin, R. J., Ruddel, M., Silva, C., Lotze, M. T., and Rosenberg, S. A. Changes in laboratory results for cancer patients treated with interleukin 2. Clin. Chem., *36*: 431-434, 1988.

33. Margolin, K. A., Rayer, A. A., Hawkins, M. J., Atkins, M. B., Dutcher, J. P., Fisher, R. I., Weiss, G. R., Doroshow, J. H., Jaffe, H. S., Roper, M., Parkinson, D. R., Viernik, P. H., Creekmore, S. P., and Boldt, D. H. Interleukin-2 and lymphokine activated killer cells therapy of solid tumors: analysis of toxicity and management guidelines. J. Clin. Oncol. *7*: 486-498, 1989.

34. Stroncek, D. F., Fautsch, S. K., Lasky, L. C., Hurd, D. D., Ramsay, N. K. C., and McCullough, J. M. Adverse reactions in patients transfused with cryopreserved marrow. Transfusion, *31*: 521-526, 1991.

35. Davis, J. M., Rowley, S. D., Braine, H. G., Piantadosi, S., and Santos, G. W. Clinical toxicity of cryopreserved bone marrow graft infusion. Blood, *75*: 781-786, 1990.

36. Rosenstein, M., Ettinghausen, J. E., and Rosenberg, S. A. Extravasation of intravascular fluid mediated by systemic administration of interleukin 2. J. Immunol., *137*: 1735-1742, 1986.

37. Damle, N. K., Doyle, L. V., Bender, J. R., and Bradley, E. C. Interleukin-2-activated human lymphocytes exhibit enhanced adhesion to normal vascular endothelial cells and cause their lysis. J. Immunol., *138*: 1779-1785, 1987.

38. Kotasek, D., Ochoa, A. C., Vercellotti, G. M., Bach, F. H., and Jacob, H. S. LAK cell-mediated endothelial injury: a mechanism for capillary leak syndrome in patients treated with LAK cells and IL-2. Clin. Res., *35*: 660A, 1987.

39. Cesano, A., Visonneau, S., Wolfe, J. H., Jeglum, A., Fernandez, J., Gillio, A., O'Reilly, R., and Santoli, D. Toxicological and immunological evaluation of the MHC-non-restricted cytotoxic T cell line TALL-104. Cancer Immunol. Immunother., *44*: 125-136, 1997.

40. Klein, B., Levin, I., Kfir, B., Michaeli, M., Shapira, J., and Klein, T. The significance of soluble interleukin-2, soluble interleukin-2 receptors, soluble ICAM-1 and β2-microglobulin in breast cancer patients. Tumor Biol., *16*: 290-296, 1995.

41. Lissoni, P., Barni, S., Rovelli, F., Viviani, S., Maestroni, G., Losti, A., and Tancini, G. The biological significance of soluble interleukin-2 receptors in solid tumors. Eur. J. Cancer, *26*: 33-36, 1990.

42. Becker, J., Czerny, C., and Brocker, E. Maintenance of clonal anergy by endogenously produced IL-10. Int. Immunol., *6*: 1605-1612, 1994.

43. Beissert, S., Hosoi, J., Grabbe, S., Asahina, A., and Granstein, R. D. IL-10 inhibits tumor antigen presentation by epidermal antigen-presenting cells. J. Immunol., *154*: 1280-1286, 1995.

44. Rosenberg, S. A. Cell transfer therapy: clinical applications. *In*: V. T. DeVita. S. Hellman, S. A. Rosenberg (eds.), Biologic Therapy of Cancer, pp. 487-506, Chapter 19. Philadelphia, PA, JB Lippincott Co., 1995.

45. Scornik, J. C., Brunson, M. E., Howard, R. J., and Pfaff, W. W. Alloimmunization, memory, and the interpretation of crossmatch results for renal transplantation. Transplantation, *54*: 389-394, 1992.

46. Opelz, G., Graver, B., Mickey, M. R., and Teraski, P. I. Lymphocytotoxic antibody responses to transfusions in potential kidney transplant recipients. Transplantation, *32*: 177-183, 1981.

Fig 1 TALL-104 related hematological toxicities (grade II leukopenia and grade I neutropenia) occurring during the induction course in patient #011 who experienced a marginal clinical response.

Fig 2 Effects of TALL-104 cell administration (induction course) on blood cell counts (3 patients/dose level).

Fig 3 Serum levels of sIL2-R and sICAM-1 during the TALL-104 induction course. The increase of both markers noted on day 1 either persisted or resolved by day 5 when low or high levels, respectively, of both markers were present pretreatment.

Fig 4 Serum reactivity of patient #012 to TALL-104 cells and HLA class I antigen panel;---------, serum collected before TALL-104 therapy (day 0), ——, serum collected 3 weeks from the start of TALL-104 therapy. Results are shown as % serum reactivity (mean fluorescence intensity).

Fig 5 Peripheral blood NK activity against K562 cells during the TALL-104 induction course. E:T ratio = 100:1

Fig 6 (*A*) TALL-104 cell lysis by patients PBMC during the induction course. (*B*) Development or increase of TALL-104 cell lysis in three patients. E:T ratio = 100:1

Fig 7 *In vitro* cytokine production upon incubation of TALL-104 cells with cells from patients' tumor biopsies. TNF-α, TNF-β, IFN-γ, GM-CSF, and IL-10 were measured in the supernatants of 18 h cocultures of TALL-104 cells with tumor cell

suspensions from 5 patients. (*A* and *B*) tumor biopsies originated from lymph nodes, (*C*) tumor biopsy originated from chest walls.

Fig 8 (*A*) IL-10 levels in patient #001 serum during the induction course. (*B*) Peripheral blood cytotoxic activity of patient #005 against K562 cells and TALL-104 cells during the five TALL-104 monthly maintenance infusions. E:T ratio = 100:1.

Table 1 Patients characteristics

Characteristics	No. of patients
Sex (F/M)	15/0
Age, years	
Median	49
Range	33-63
Performance status	
0	8
1	7
Previous treatment	
Radiation	10
Chemotherapy	15
Chemotherapy +radiation therapy	10
Hormonotherapy	12
Immunotherapy	1
PBSCT	2
Diagnosis	
Infiltrating ductal carcinoma	12
Infiltrating lobular carcinoma	2
Medullary carcinoma	1

Table 2 Quality controls (QC) and criteria for acceptance of TALL-104 cells for clinical use

QC assay	Accepted results
Viability (Erythrosin B dye-exclusion)	≥80%
Mycoplasma (PCR)	Negative
Identity (fingerprinting)	Equal to parental TALL-104 cells
Purity (PCR)	Negative for murine cell contamination
Sterility (SOB test incubation for 15 days)	No bacterial or fungal contamination
Proliferation (^{3}H-TdR uptake)	None
Biological activity (K562 killing)	> 60% (E:T=10:1)
Endotoxin	≤ 0.05 EU/ml
Phenotype	≥90% $CD3^+$, $CD8^+$, $CD56^+$

Table 3 TALL-104 cell dose/courses administered to the patients

Patient #	Dose (cells/kg)	No. of monthly maintenance boosts[a]	Total no. of TALL-104 cell infusions
001	10^6	2	9
002	10^6	0	5
003	10^6	3	11
004	3×10^6	0	5
005	3×10^6	5	15
006	3×10^6	1	7
007	10^7	0	5
008	10^7	0	5
009	10^7	0	5
010	3×10^7	0	5
011	3×10^7	0	5
012	3×10^7	0	5
013	10^8	2	9
014	10^8	0	5
015	10^8	0	5

[a] Each boost consisted of 2 injections given over 2 consecutive days.

Table 4 TALL-104 related toxicities during or after the induction course

Toxicity	Patient #	TALL-104 cell dose/kg/day	Grade
Erythema/urticaria	001	10^6	I
	002	10^6	I
Nausea/vomiting	001	10^6	I
	005	3×10^6	I
	007	10^7	I
	011	3×10^7	I
	012	3×10^7	I
	014[a]	10^8	IV
	015	10^8	I
Neutropenia	011	3×10^7	I
Leukopenia	011	3×10^7	II
	015	10^8	I
Increased SGOT and alkaline phosphatases	003	10^6	I
	009	10^7	I
Hypocalcemia	003	10^6	I
Hypoglycemia	001	10^6	I
Hyperglycemia	003	10^6	I
	011	3×10^7	II
	013	10^8	I
	015	10^8	I
Fever	010	3×10^7	I
	014[a]	10^8	IV

[a]Fever, sweating, and nausea developed in this patient 3 weeks after the induction course and was felt related to hepatic tumor necrosis.

Table 5 Serum levels (pg/ml) of cytokines during the induction course

		IL-10		IFN-γ	
Patient #	Cell dose/kg/day	Pre-infusion	Post-infusion	Pre-infusion	Post-infusion
001	10^6	<3.0	61.8	6.1	5.9
002	10^6	<3.0	8.4	2.7	2.9
003	10^6	<3.0	<3.0	3.0	3.4
004	3×10^6	<3.0	4.9	2.7	3.0
005	3×10^6	<3.0	<3.0	<2.0	2.2
006	3×10^6	<3.0	4.2	2.5	<2.0
007	10^7	5.6	14.6	3.1	10.2
008	10^7	4.3	4.6	<2.0	3.0
009	10^7	7.6	5.6	2.8	2.8
010	3×10^7	9.0	9.0	2.4	2.6
011	3×10^7	15.0	12.7	3.5	3.8
012	3×10^7	7.1	9.3	2.5	3.2
013	10^8	5.7	6.2	3.5	3.8
014	10^8	8.8	11.5	3.0	3.8
015	10^8	6.5	7.4	3.2	3.2

Table 6 *In vitro* and *in vivo* assays with patients tumor biopsies

Patient #	Biopsy site	Tumor lysis	Cytokine production	No. of SCID mice engrafted/no. of mice with tumor take	Length of follow-up (months)
003	Lymph node	<1%	+	4/0	18
008	Chest walls	56%	+	4/0	16
009	Lymph node	28%	+	4/2	15
012	Lymph node	67%	+	4/0	12
015	Lymph node	24%	+	3/0	6

Tumor lysis is expressed as % specific ^{51}Cr-release at E:T=100:1. Human IFN-γ, TNF-α, TNF-β, GM-CSF, and IL-10 were measured in the supernatants of 18 h co-cultures of TALL-104 cells with tumor cells isolated from the patients biopsies.

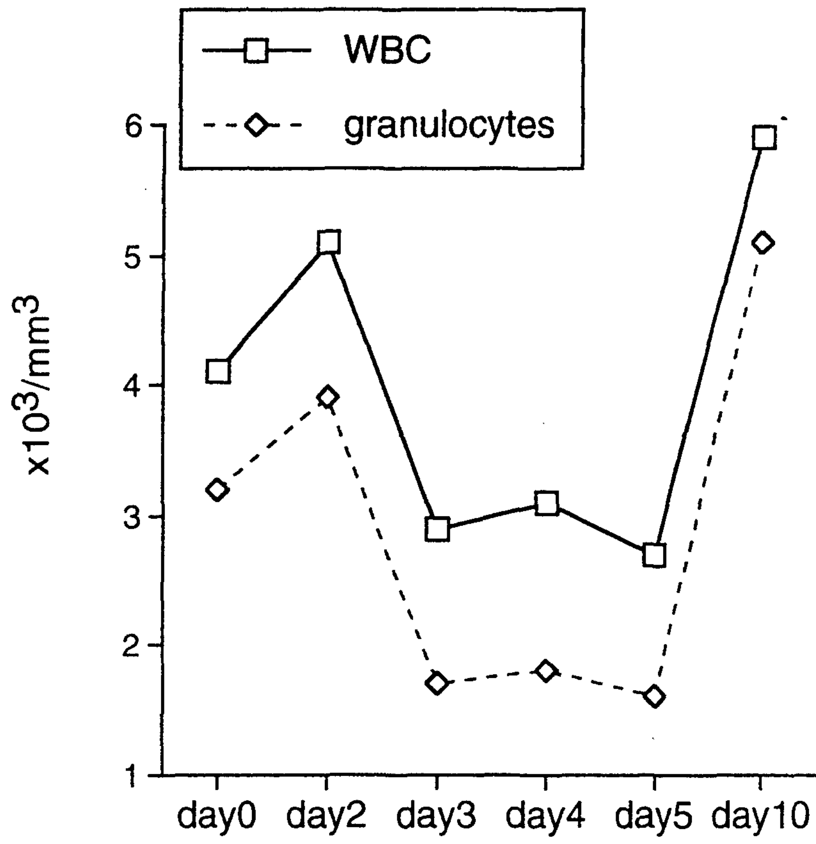
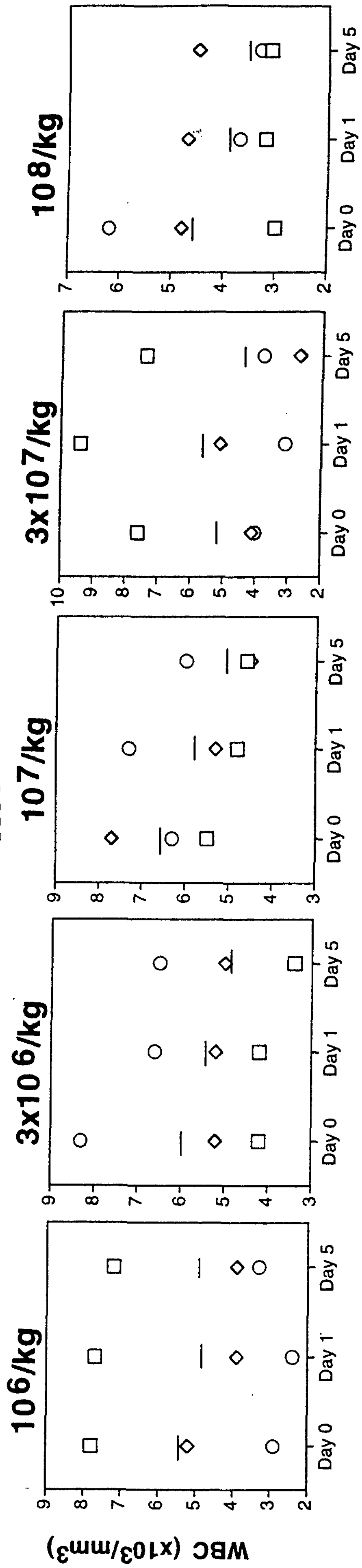


FIGURE 1

FIGURE 2



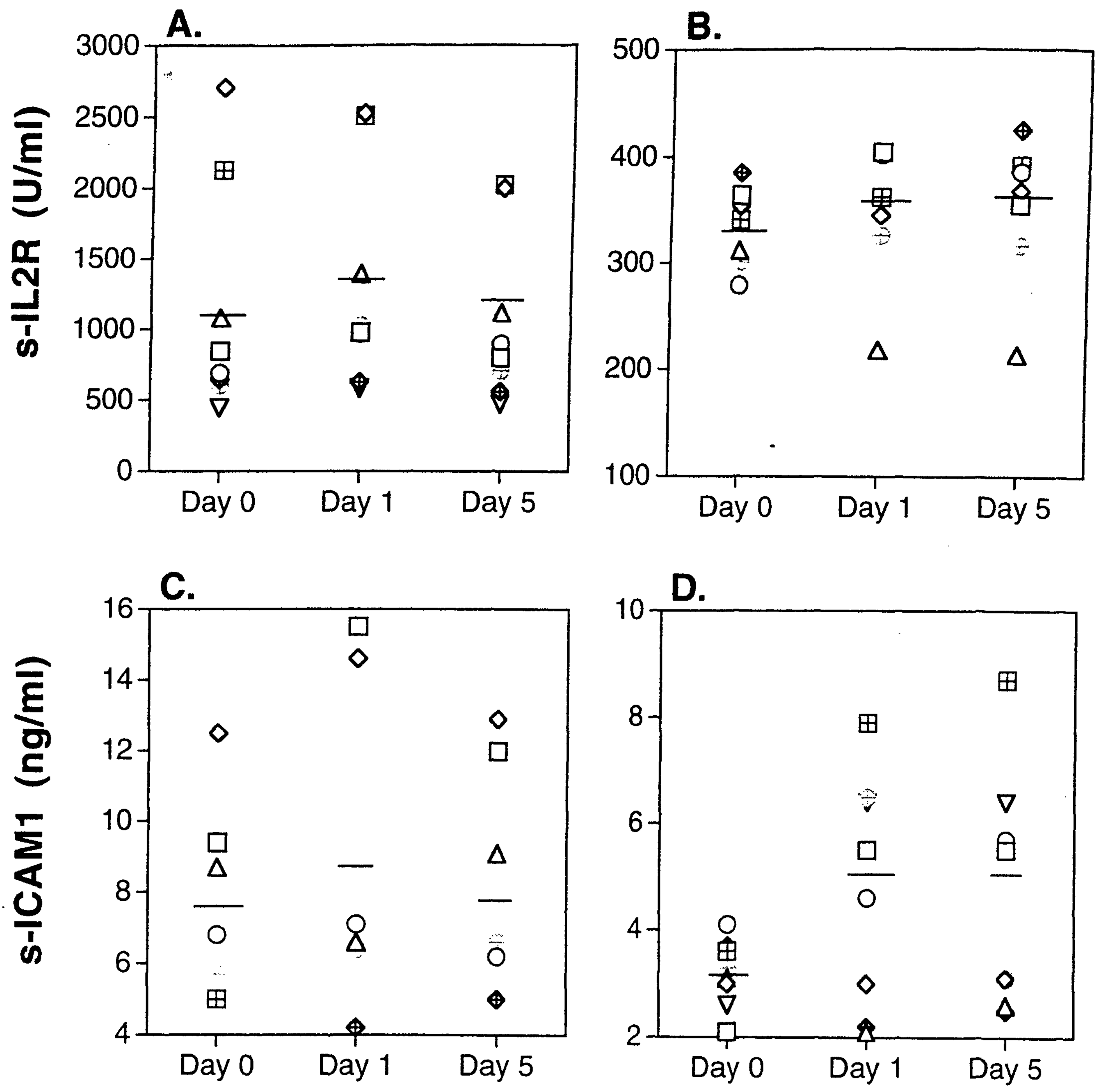
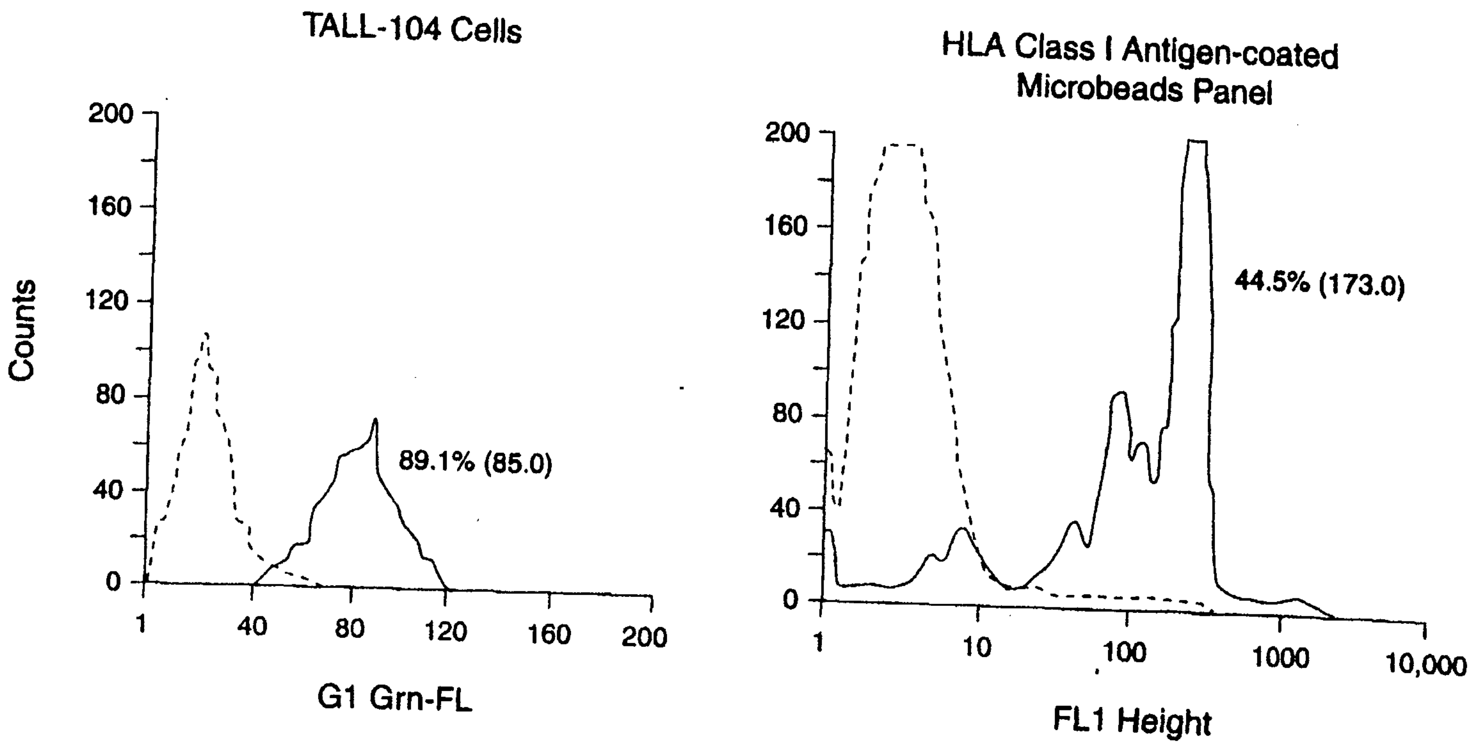


FIGURE 3

FIGURE 4



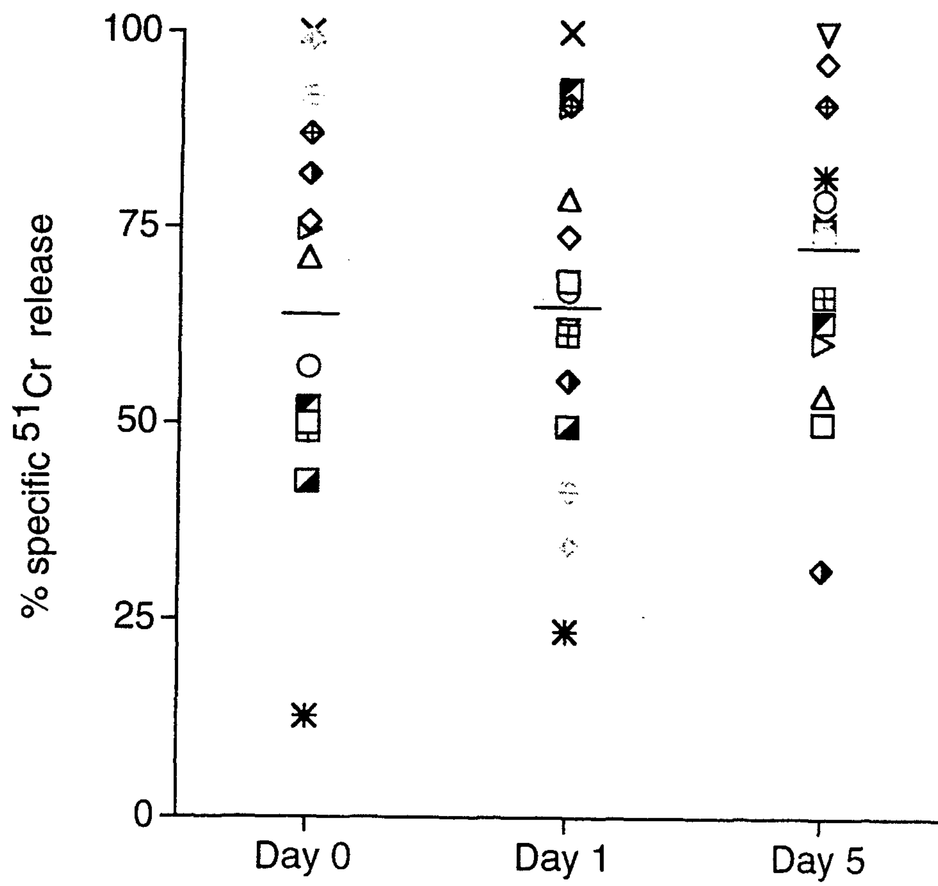
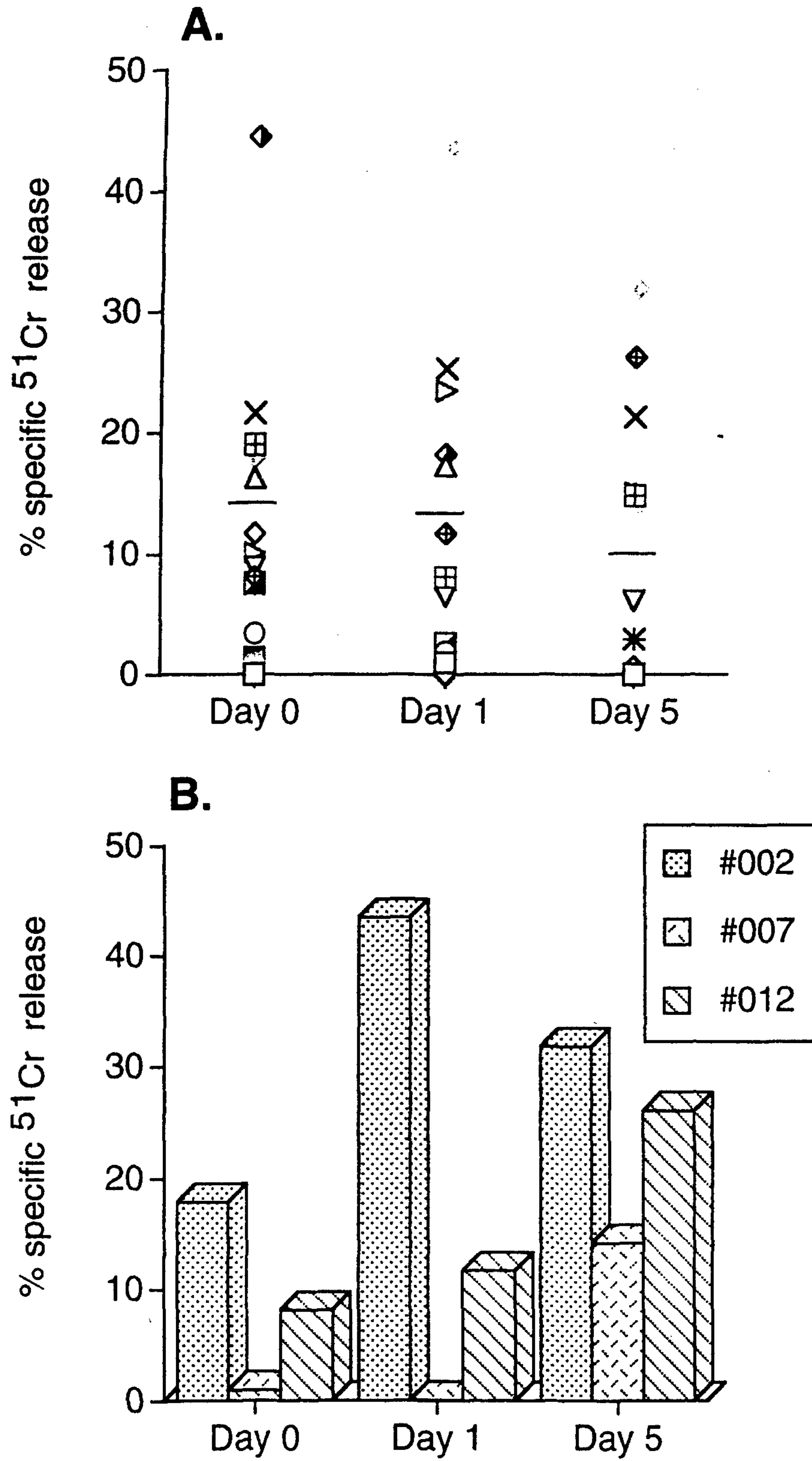


FIGURE 5

FIGURE 6

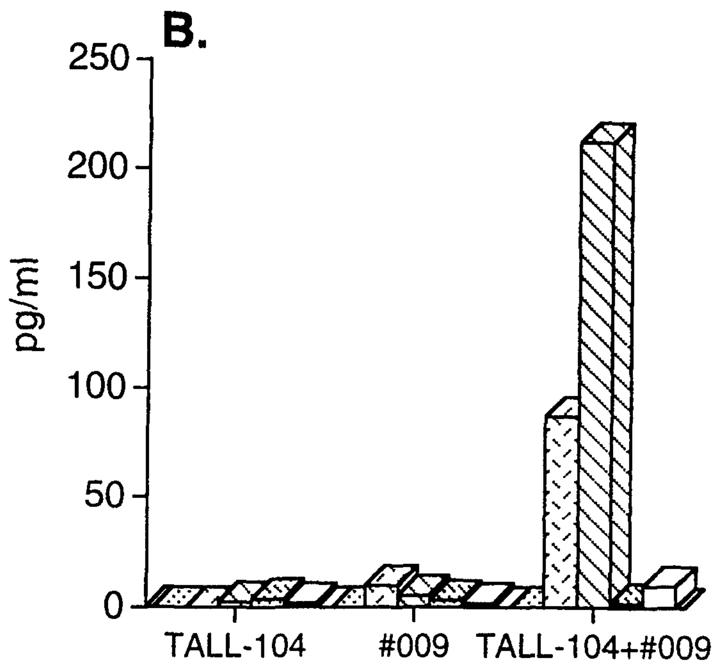


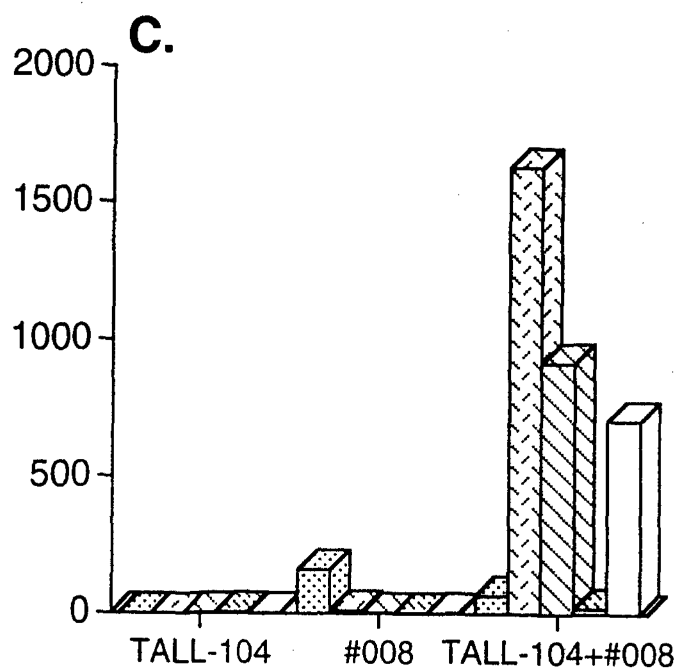
A.

TNF-α
TNF-β
GM-CSF
IFN-γ
IL-10

pg/ml
30
25
20
15
10
5
0
TALL-104
#003
#012
#015
TALL-104+#003
TALL-104+#012
TALL-104+#015

FIGURE 7





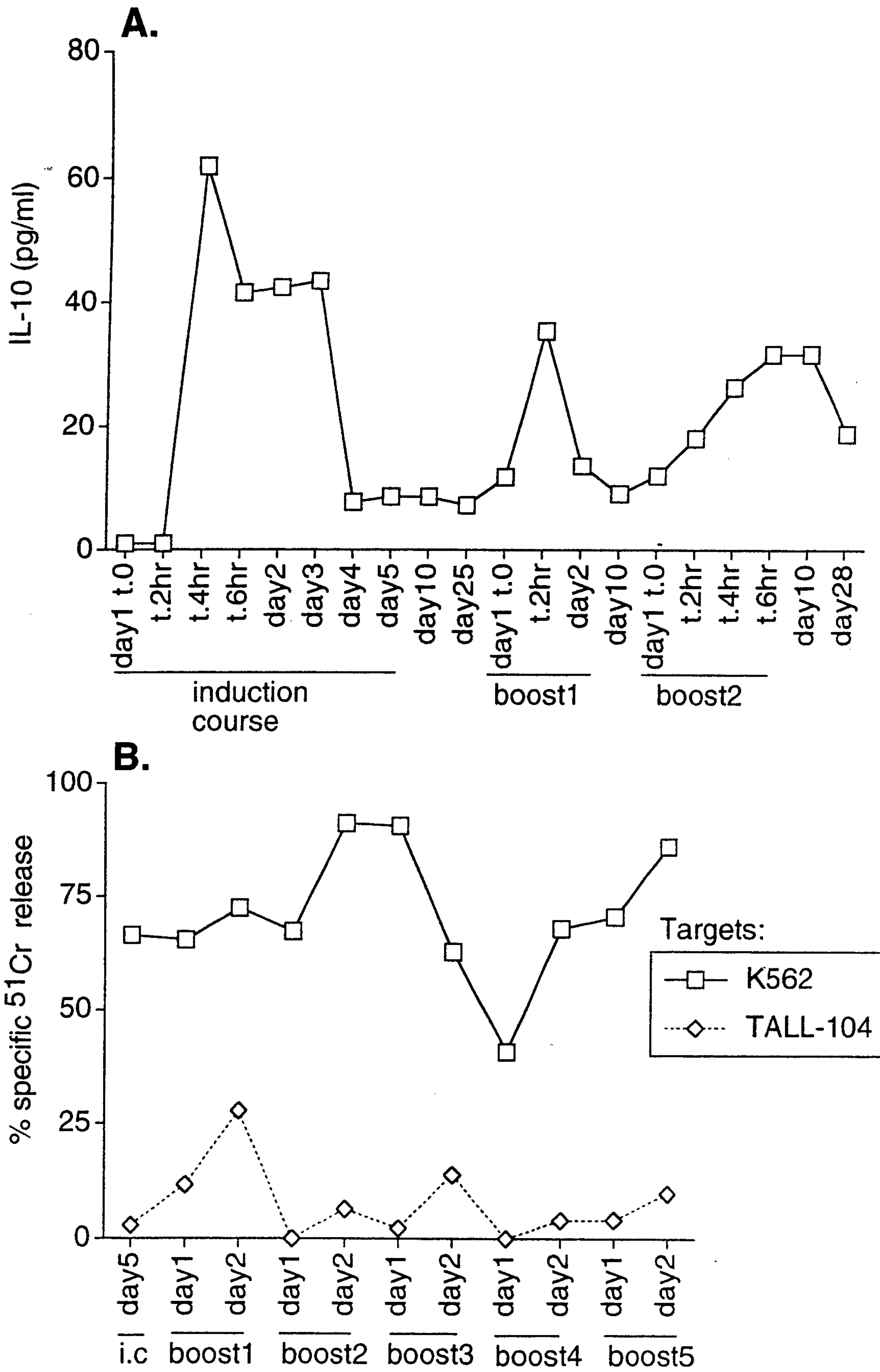


FIGURE 8