SUPPLEMENTARY MATERIAL

**Supplementary Methods**

**Procedures for Immune Assays**

Blood was drawn and assays run for all time points. Peripheral blood leukocytes (PBLs) were isolated from 60 ml of venous blood by using Ficoll density gradient centrifugation (Pharmacia Biotech, Inc., Piscataway, NJ). The isolated leukocytes were washed in calcium- and magnesium-free PBS and counted on a Coulter counter (Coulter Corp., Miami, FL). Aliquots of 6 x 106 isolated PBLs were suspended again in 0.6 ml of RPMI 1640 supplemented with 10% fetal bovine serum (HyClone, Logan, UT), 2 mercaptoethanol (BME; Sigma, St. Louis, MO), and 100X antibiotic-anti-mycotic stock, HEPES, sodium bicarbonate, and L-glutamine (all from Life Technologies, Inc., Grand Island, NY).

Numeration of Total T-lymphocyte Counts, T-Cell Subsets, and NK Cells. PBLs were labeled with florescent-conjugated monoclonal antibodies specific for the following cell surface markers: total T lymphocytes (CD3, FITC), T4 subsets (CD4, rhodamine), T8 subset (CD8, FITC), and NK cells (CD56, rhodamine). Monoclonal antibodies were purchased from Beckman Coulter Corp. Briefly, an aliquot of PBLs (2.5 x 106 cells) was treated with Erythrocyte Lysis Buffer (154 mM NH4Cl, 10 mM KHCO3, 0.082 mM EDTA-Na), resuspended in Dulbecco’s phosphate-buffered saline, and centrifuged for 5 min at 3300 rpm. Cells (0.5 x 106) were incubated with the appropriate monoclonal antibodies for 15 min in the dark on ice. After the incubation, the labeled cells were washed with Dulbecco’s phosphate-buffered saline and were fixed with 2% formaldehyde (made using 10% ultrapure formaldehyde). Dual-labeled IgG was used to determine nonspecific immunofluorescence binding. Samples were analyzed with a Coulter EPICS XL- MCL flow cytometer.

NK Cell Cytotoxicity**.** Briefly, PBLs were resuspended in complete medium at a density of 2.5 X 106 cells/ml and were seeded into 96-well V-bottomed microtiter plates in a volume sufficient to provide an effector to target (E:T) cell ratio of 100:1, 50:1, and 25:1 (triplicate wells). Complete medium was added to each well to give a total volume of 200 µl. The NK-sensitive human myeloid K562 cell line was used as the target in a 51Cr assay (12, 13). K562 cells were harvested from culture, labeled with 51Cr, and washed. Then, 5 x 103 K562 target cells were added to each well in a volume of 50 µl. Plates were centrifuged at 300 X *g* for 5 min and were incubated for 5 hrs in an atmosphere of 5% CO2 at 37°C. After this incubation, the plates were again centrifuged at 300 x*g* for 5 min, and 100µl of supernatant were harvested and counted using a Beckman 5500 gamma counter. Minimum and maximum 51Cr release was determined using target cells that had been incubated in complete medium or 5% SDS detergent solution, respectively. Cytotoxicity was calculated using the following equation: Experimental 51Cr release – (Minimum release/Maximum release) - Minimum Release

Blastogenic Response to PHA and ConA. The serial dilutins used for each were 2.5, 5.0, and 10.0 µg/ml. For the assays, isolated PBLs, resuspended in supplemental RPMI without phenol red, were seeded in triplicate at 0.5 x 105 cells/well and incubated for 68 hrs at 37°C, in an atmosphere of 5% CO2 in sterile 96-well flat-bottomed plates. Wells were pulsed for the final 4 hrs with MTS, *i.e.,* 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt (Promega Corp., Madison, WI) and phenazine methosulfate (PMS), an electron coupling reagent, to measure proliferative response. The amount of proliferation was determined via optical density readings in the suspension well compared with cells and media alone, using an HTS7000 Bioassay microplate reader (Perkin-Elmer) at a determination wavelength of 492 nm and a reference wavelength of 690 nm

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Supplementary Table 1.**  Means of outcome measures at each time point | | | | | | | | | | | | |
|  | **Initial (n=113)** | **4 (n=90)** | **8 (n=90)** | **12 (n=86)** | **18**  **(n=81)** | **24 (n=79)** | **30 (n=77)** | **36 (n=70)** | **42 (n=67)** | **48 (n=59)** | **54 (n=62)** | **60 (n=59)** |
| IES | 26.28 (14.46) | 18.39 (15.95) | 17.16 (14.11) | 15.42 (13.20) | 13.32 (12.83) | 13.67 (13.58) | 12.58 (13.91) | 9.24 (10.44) | - | 10.36 | - | 8.63 (10.45) |
| CES-D | 5.98 (3.46) | 4.90 (3.95) | 4.03 (3.86) | 4.09 (3.387) | 3.88 (3.44) | 4.22 (3.96) | - | 4.04 (4.09) | - | 4.03 (4.27) | - | 4.04 (4.00) |
| NKCC | 34.92 (15.56) | 37.54 (18.67) | 40.54 (16.23) | 42.79 (15.94) | 48.66 (17.91) | 45.56 (20.93) | 46.61 (18.95) | 49.44 (16.32) | 48.71 (16.80) | 47.99 (19.78) | 50.22 (18.49) | 48.64 (18.83) |
| Con A | 0.18 (0.12) | 0.16 (0.12) | 0.18 (0.10) | 0.14 (0.11) | 0.21 (0.16) | 0.14 (0.11) | 0.13 (0.10) | 0.19 (0.16) | 0.18 (0.12) | 0.22 (0.17) | 0.18 (0.16) | 0.19 (0.16) |
| PHA | 0.28 (0.15) | 0.22 (0.14) | 0.25 (0.14) | 0.20 (0.13) | 0.30 (0.19) | 0.25 (0.16) | 0.26 (0.17) | 0.27 (0.15) | 0.25 (0.17) | 0.35 (0.15) | 0.27 (0.18) | 0.25 (0.19) |

Online only. IES, Impact of Events Scale; CES-D, Center for Epidemiological Studies- Depression Scale; NKCC, natural killer cell cytotoxicity (lytic units at 25:1 ratio); Con A, concanavalin A (optical density at 5 ug/ml); and PHA, phytohemaggluttinin (optical density at 5 ug/ml

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Supplementary Table 2.** Attrition Data for Participants by Time Point (N=113) | | | | | | |
| **Category** | **Initial** | **12-months** | **24-months** | **36-months** | **48-months** | **60-months** |
| Completed | 113 | 86 | 79 | 70 | 59 | 59 |
| Not Completeda | 0 | 9 | 3 | 8 | 12 | 6 |
| Study Drop | 0 | (13b) 13 | (7) 20 | (1) 21 | (2) 23 | (5) 28 |
| Recurred/Deceased | 0 | (5) 5 | (6) 11 | (3) 14 | (5) 19 | (1) 20 |
| Total | 113 | 113 | 113 | 113 | 113 | 113 |

Online only

aPatients still enrolled in trial but assessment not completed.

bValue in parentheses reflects number of new cases added to the cumulative total in this period.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Supplementary Table 3.** Adjuvant Treatment Use by Agent or Combination in Reviewed Immunologic Studies | | | | | | |
| **Treatment Regimen** | **Standish et al., 2008**  **N=14** | **Thornton et al., 2007**  **N=113** | **Mozaffari et al., 2009**  **N=41** | **Kang et al., 2009**  **N=80** | **Wiltschke et al.,**  **N=90** | **Andersen et al., 2004; Carson et al., 2004**  **N=227** |
| Adriamyacin/Doxorubicin | 100% | 73% | -- | -- | -- | 74% |
| Cyclophosphamide/Cytoxan | 100% | 86% | 51% | -- | \*\* | 82% |
| Docetaxel/Taxotere | 21% | -- | -- | -- | -- | -- |
| Epirubicin | -- | -- | 51% | -- | -- | -- |
| Fluorouracil | -- | 19% | 51% | -- | \*\* | 18% |
| Gemcitabine | 7% | -- | -- | -- | -- | -- |
| Methotrexate | -- | 14% | -- | -- | \*\* | 12% |
| Taxol/Paclitaxel | 71% | 20% | -- | -- | -- | 21% |
| Trastuzumab | 14% | -- | -- | -- | -- | -- |
| Adriamycin + Cytoxan Combinationa | -- | -- | -- | 40% | -- | -- |
| Adriamycin + Cytoxan +Taxol or Taxotere Combinationa | -- | -- | -- | 53% | -- | -- |
| Other combination (e.g., Cyclophosphamide + Methotrexate + Fluorouracil)a | -- | -- | -- | 8% | -- | -- |
| Radiation Therapy | 100% | 51% | 100% | 32% | 100% | 54% |
| Hormone Therapy (e.g., tamoxifen) | 71% | 80% | 63% | 75% | \*\* | 75% |

Online only

aAgent combination values provided for studies in which percentage use of each individual agent could not be calculated.

(--) = not administered

(\*\*) = administered but no specific n provided