

Significant Impairment in Immune Recovery following Cancer Treatment

(Running head: Immune recovery post-cancer therapy)

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1 **Abstract:**

2 Background. Although immunosuppression from cancer adjuvant therapy has been documented,
3 how these suppressed immune responses recover to baseline values after completion of cancer
4 adjuvant therapy has not been studied systematically.

5 Objectives: The objective of this study was to examine the probability of immune recovery post-
6 adjuvant therapy and the potential impact of adjuvant therapy type and cancer stage on immune
7 recovery in newly diagnosed breast cancer patients.

8 Method. Immune responses were measured 4 times over a 1-year period in 80 early stage breast
9 cancer patients: prior to, and at 2, 6, and 12 months from the beginning of adjuvant therapy. Natural
10 killer cell activity (NKCA), lymphokine-activated killer cell activity, lymphocyte proliferation, CD
11 subsets (CD4, CD8, and CD56) and cytokines (IFN- γ , IL-2, IL-4, IL-6, and IL-1 α) were selected for
12 their relevance to breast cancer.

13 Results. Delayed immune recovery to pretreatment baseline levels continued to the 12-month
14 timepoint in all parameters: The probability of immune recovery ranged from only 17.6% to 77.3% of
15 the patients, varying among immune parameters. Overall, immune recovery was poorer for IFN- γ ,
16 IL-2, IL-4, lymphocyte proliferation and NKCA (18.5 – 33.0%) than CD subsets and IL-6 (61.5 –
17 77.3%). The type of adjuvant therapy, not cancer stage, showed selective influence on immune
18 recovery: Chemotherapy or chemo- and radiotherapy combination significantly suppressed IL-2
19 recovery, whereas radiotherapy significantly suppressed IL-4 recovery.

20 Discussion. Immune recovery in breast cancer patients is significantly delayed for an extended time
21 period in numerous immune parameters. The type of cancer adjuvant therapy has only selective
22 influence on immune recovery. Clinical significance of and factors influencing poor immune recovery
23 warrant further investigation in relation to patients' health outcomes and quality of life.

24

25 **Keywords:** immune recovery, breast cancer, cancer treatment

1 INTRODUCTION

2 With the exception of skin cancer, breast cancer (BC) is the most common type of cancer and
3 second leading cause of cancer death in women in the United States (American Cancer Society,
4 2007). Treatments for BC include surgery, chemotherapy, radiotherapy, and hormonal and targeted
5 therapy, often combining two or more modalities. With advances in early diagnosis and treatment,
6 the overall survival rates are 88% at 5 years and 80% at 10 years across all stages of BC to
7 consider BC as a chronic disease (American Cancer Society, 2007). Accordingly, increasing
8 attention has been paid to the long-term effects of BC diagnosis and treatment, but primarily to
9 psychosocial outcomes, not to physiological responses.

10 The immune system is a major regulatory defense mechanism. Although cancer diagnosis
11 and adjuvant chemotherapy are known to significantly alter immune responses (Gardner, 1999; van
12 der Most, Currie, Robinson, & Lake, 2006), surprisingly little has been investigated as to how altered
13 immune responses recover and how the type of cancer therapy and cancer stage interact with
14 immune recovery over time. The significance of the topic might be even greater given that BC
15 patients are found to have lower baseline immune responses than healthy counterparts (Hakim,
16 1988; Konjevic & Spuzic, 1993). Poor immune recovery may further increase an individual's
17 susceptibility to adverse health outcomes. There is evidence that poor recovery of lymphocyte
18 proliferation (LP) and lymphocyte count predicted more disease recurrence in patients with early
19 stage BC (Wiltschke et al., 1995) and poorer disease-free and overall survivals in metastatic BC
20 patients (Nieto et al., 2004; Porrata, Ingle, Litzow, Geyer, & Markovic, 2001).

21 Immune parameters selected for the relevance to BC in the study include: Natural killer (NK)
22 cell activity, lymphokine-activated killer (LAK) cell activity, CD subsets, LP, and cytokines. NK cells
23 participate in the resistance to and the control of malignancies (Brittenden, Heys, Ross, & Eremin,
24 1996; Pross & Lotzova, 1993; Whiteside & Herberman, 1995). NK cell activity was incrementally
25 reduced with advances in BC stage (Konjevic & Spuzic, 1993), and lower levels of NK cell activity
26 were associated with greater numbers of positive axillary lymph nodes (Levy, Herberman, Maluish,

1 Schlien, & Lippman, 1985). LAK cell activity was a sensitive predictor for emotional outcomes in BC
2 patients (Sachs et al., 1995). For cytokines, interleukin (IL)-2 and interferons (IFNs) can enhance NK
3 cell activity (Sinkovics & Horvath, 2005), but IL-2 was found to be significantly lower in BC patients
4 than in healthy controls prior to cancer treatment (Elsasser-Beile, von Kleist, & Gallati, 1991;
5 Elsasser-Beile, von Kleist, Sauther, Gallati, & Monting, 1993). Lower levels of IL-2 were a predictor
6 for a shorter survival time (Lissoni, Barni, Rovelli, & Tancini, 1991) and increased risk for relapse in
7 BC (Arduino et al., 1996). Similarly, decreased IFN- γ production was significantly correlated with
8 tumor burden (Elsasser-Beile et al., 1993). In contrast, elevated serum IL-6 and TNF-alpha levels
9 were significant predictors of shorter disease-free survival and overall survival in metastatic BC
10 patients (Bachelot et al., 2003; Bozcuk et al., 2004). Furthermore, LP was impaired in 58% of BC
11 patients leading to a significant inverse association with positive nodal status (Head, Elliott, & McCoy,
12 1993).

13 Although these findings clearly indicate the relevance of cellular immune parameters to
14 clinical outcomes, recent studies have been largely focused on specific molecular or genetic
15 markers, leaving little attention to fundamental cellular immune parameters. As a first step to a
16 renewed understanding of the role of cellular immune responses in long-term clinical outcomes, the
17 purposes of this study were to: (1) examine how immune responses recover post-cancer adjuvant
18 therapy over the first year of cancer diagnosis and treatment and (2) determine what effects the type
19 of adjuvant therapy and cancer stage have on immune recovery in early stage (I-III) BC patients. We
20 hypothesized that immune responses would return to pretreatment baseline values by the 12-month
21 time point; and immune recovery would differ based on the type of adjuvant therapy and cancer
22 state. Data were collected as part of a parent study designed to examine the effects of an 8-week
23 intervention integrating cognitive behavioral modification and exercise training on psychosocial,
24 immune and symptom outcomes in newly diagnosed BC patients, controlling for intervention effects.

25

26 **PATIENTS AND METHODS**

1 **Participants**

One hundred women newly diagnosed with breast cancer who were to undergo cancer adjuvant therapy were recruited from the interdisciplinary and other breast clinics in the university health system. Inclusion criteria were: (1) women 30 years or older with stage I-III BC and receiving chemotherapy and/or radiotherapy; (2) absence of a defined psychosis; (3) no uncontrolled cardiopulmonary or other serious medical conditions that would prohibit moderate intensity exercise; (4) not participating in other structured support or exercise program; (5) ability to comprehend and follow instructions, speak and respond in English; and (6) medical clearance from a primary medical oncologist to participate in the study. Exclusion criteria were: (1) pregnancy; (2) distance preventing weekly participation in intervention; (3) diffuse bony metastasis with high risk of pathologic fractures; and (4) lack of access to a telephone. After obtaining written informed consent, patients were first stratified by cancer stage (stage I-II vs. III) and randomized into either intervention or wait-list control group using a computer-generated randomization table. The 8-week integrated intervention was implemented at the start of adjuvant therapy, and patients were encouraged to continue their learned activities at home. The control group received usual standard cancer care. Eighty women completed the study after 10 from each group withdrew from the study for reasons, such as chemotherapy side effects (4), time commitment (2), and other family and personal issues (14). The protocol was approved by the Institutional Review Board.

Data Collection Procedure and Immune Measurements

Blood samples for immune measurements were collected prior to the start of adjuvant therapy (chemotherapy and/or radiotherapy) as baseline, and at 2, 6, and 12 months after the start of adjuvant therapy. Baseline samples were collected at least 2-3 weeks after initial surgery to control for any surgery-related impact on immune responses. All blood samples (20 ml) were collected between 0800 and 1200 into heparin-containing vacutainers. All assays were performed following well-established protocols.

Cell Separation. Mononuclear cells (MNCs) were separated using the Ficoll-Hypaque (1.077 sg; Sigma) density-gradient method. MNCs were washed twice with sterile phosphate-buffered saline (PBS without Ca^{2+} and Mg^{2+} , pH 7.4; Gibco), centrifuged at 450 g for 10 min, and resuspended in complete RPMI 1640 (supplemented with HEPES 25 mM, L-glutamine 2 mM, 50 U Penicillin and 50 μg Streptomycin/ml) at 2×10^6 cells/ml. This method has yielded > 98% cell viability in previous work (Kang, Coe, & McCarthy, 1996; Kang, Coe, McCarthy, & Ershler, 1997).

Natural Killer Cell and Lymphokine-activated Killer Cell Activity. The standard chromium-51 (Cr-51) release cytotoxicity assay was used using K562 target cells (Kang et al., 1997). Target cells were labeled with 125 μCi Cr-51 for 1 hr at 37° C, washed and centrifuged twice, and resuspended at 4×10^4 cells/ml. MNCs from patients were incubated in triplicate with labeled K562 target cells and 60% heat-inactivated pooled human serum in three effector-to-target cell (E:T) ratios--100:1, 50:1 and 25:1. Spontaneous and maximal lysis was determined by incubating target cells with medium alone and 10% sodium dodecyl sulfate solution. Following a 4-hr incubation in 5% CO_2 at 37° C, cytotoxicity was determined from supernatant using a gamma counter. Cytotoxicity was calculated as follows: NK cell activity (%) = [(sample release - spontaneous release)/(maximum - spontaneous release)] x 100. The assay sensitivity was $\geq 0.1\%$, with 2-6% intra-assay coefficient variation in our previous work.

1 For LAK cell activity, a protocol modified from studies by Brenner et al. (Brenner, Gryllis,
2 Gornitsky, Cupples, & Wainberg, 1991) and Nagao et al. (Nagao, Yabe, Xu, & Okumura, 1995) was
3 used: MNCs (1×10^6 cells/ml) were incubated in complete RPMI medium containing 10% fetal calf
4 serum and 100 U/ml recombinant IL-2 for 3 days in a humidified air of 5% CO_2 at 37°C. Next day,
5 LAK cells were washed twice in complete RPMI medium, cells were counted, and the same Cr-51
6 NK assay protocol was used (Brenner et al., 1991). The assay sensitivity and variation remains the
7 same.

8 Lymphocyte Proliferation. MNCs (1×10^5) were cultured in triplicate with 60% heat-
9 inactivated pooled human serum and phytohemagglutinin (PHA) at 5 and 10 $\mu\text{g}/\text{ml}$. Following 54 hr-

1 incubation in humidified air with 5% CO₂ at 37°C, cells were pulsed with 1 μCi of tritiated thymidine,
2 incubated for additional 18 hr, harvested onto glass-fiber filters (MASH harvester, Otto Hiller) and
3 were counted by a liquid scintillation counter (Packard Tricarb 300 CD). The results indicate net
4 counts per minute controlling for unstimulated cell counts.

5 Cytokine Production. Whole blood cell culture assay was used, which is thought to be a
6 better method to reflect the in vivo condition than the isolated MNC method. Blood was diluted
7 1:10 with the complete RPMI medium and was incubated for 4 days at 37°C with 5% CO₂ with
8 PHA 10 μg/ml and lipopolysaccharide 2.5 μg/ml. Culture supernatant was collected and stored in
9 aliquots at –80° C until assayed. Cytokine levels were determined by a standard two-step
10 sandwich enzyme-linked immunosorbant assay (ELISA) using commercial kits. The assay
11 sensitivity for cytokines has been reported to be 0.04 - 5 pg/ml with intra- and inter-assay %
12 coefficients of variation being 4 - 9.6% for all (Biosource, CA; R & D Systems, MN).

13 CD Cell Subsets. Direct immunofluorescence method was used. Cells suspended in PBS
14 supplemented with 0.1% sodium azide were stained using fluorescein (FITC)- and phycoerythrin
15 (PE)-conjugated monoclonal antibodies against CD4, CD8, CD56 and CD3+ (pan T cells) surface
16 markers. Cells were incubated on ice for 30 min, washed with PBS, and were analyzed using a flow
17 cytometer (FACScan, Becton-Dickinson) at the FACS Shared Core Facility on campus.

18

19 **Statistical Analysis**

20 The response variable, Immune Recovery to Baseline or above, was binary in nature, and
21 evaluated at 2, 6, and 12 months post baseline. Immune Recovery was coded as 1 if immune
22 response was equal or greater than the baseline value, and as 0, if immune response was less than
23 the baseline value. A Generalized Linear Mixed Models (GLMM) approach, as implemented in SAS
24 PROC GLIMMIX (SAS Institute Inc., 2005), was used to analyze the data. This approach
25 accommodates the binomial distribution of the response variables through use of a logit link function,
26 model dependencies produced by the repeated measures, and incorporate time-varying covariates

1 and factors (Little, Schnabel, & Baumert, 2000; McCullagh & Nelder, 1997; McCulloch & Searle,
 2 2001). Multivariate models were examined for NK, LAK, and LP immune factors to accommodate
 3 the multiple target/effector ratios or stimulations used for those factors. Each model was composed
 4 of first-order type of cancer adjuvant therapy (chemotherapy, radiotherapy, or combination), cancer
 5 stage (Stage 2 or less, greater than 2), hormone replacement (no, yes), menopause (pre-, peri, post-
 6 menopause), and time (2, 6, and 12 Months) effects, as well as all significant second-order effects
 7 involving the time factor. Statistically significant interactions were followed up with simple main
 8 effects analyses. Residuals were examined for conformity to assumptions. Probability of immune
 9 recovery was estimated using $\hat{P}\{\text{Recovery}\} = \left(1 + e^{-\hat{\beta}}\right)^{-1}$. Significance level was set at 0.05, with
 10 adjustment for multiple comparisons using the SIMULATE adjustment option implemented within
 11 PROC GLIMMIX (SAS Institute Inc., 2005).

12

13 **RESULTS**

14 **Participant Characteristics**

15 Demographic characteristics and medical information of the participants are shown in Tables
 16 1 and 2. Participants had a mean age of 49.5 years and tended to be Caucasian (75%),
 17 postmenopausal (57.5%) and working full time or part-time (81.3%). Participants were overweight on
 18 average (mean body mass index 28.8), had stage I-III BC, and most frequently received both
 19 chemo- and radiotherapy. Typical regimens of chemotherapy included about 40% of the patients
 20 receiving Adriamycin 60mg/m² + Cytosin 600 mg/m² together every 2-3 weeks for 4 doses and
 21 about 50% of the patients receiving either Adriamycin 60mg/m², Taxol 145mg/m², and Cytosin 600
 22 mg/m² sequentially every 2-3 weeks for 3 doses each or Adriamycin 50mg/m² + Cytosin 500
 23 mg/m² together every 2-3 weeks for 4 doses followed by Taxotere 75mg/m² every 2-3 weeks for 4
 24 doses. Typical radiotherapy included a total dose of 45-65 Gy over six weeks with 15-20 Gy boost
 25 dose toward the end.

26 **Immune Recovery**

1 The probability of immune recovery to baseline or above ranged from 20.5% to 65.1% at 2
2 months, 22.2% to 71.4% at 6 months, and 17.6% to 77.3% at 12 months (Table 3). Statistically
3 significant interactions for cancer adjuvant therapy with time were observed for CD4%, IFN- γ , and
4 LAK cell activity. Significant differences in probability of immune recovery among three different time
5 points were noted in CD4%, IFN- γ , IL-2, IL-6, LAK cell activity, and LP responses. IL-2 and
6 lymphocyte responses, for example, showed further decrement in the probability of immune
7 recovery at 12 months than earlier time points. At 12 months, the overall probability of immune
8 recovery differed among immune parameters: CD subset % (with the exception of CD4% in
9 chemotherapy patients) and IL-6 showed a higher probability of recovery to baseline or above, 61.5
10 – 77.3%, whereas IFN- γ , IL-2, IL-4, and NK cell activity showed a lower probability of recovery to
11 baseline and above, 18.5 – 33.0%.

12 With regard to the impact of types of cancer adjuvant therapy (chemotherapy only,
13 radiotherapy only, or combination) and cancer stage on immune recovery (Tables 4 and 5), only the
14 type of cancer adjuvant therapy had significant effects on the probability of IL-2 and IL-4 immune
15 recovery across all three time points as well as of CD4% and LAK cell activity at 12 months. The
16 recovery of IL-2 responses was significantly poorer in women who received chemotherapy or
17 combination therapy than radiotherapy, whereas the recovery of IL-4 responses was significantly
18 poorer in women received radiotherapy than chemotherapy or combination therapy. In addition, the
19 recovery of CD4% and LAK response was significantly lower in women received chemotherapy than
20 radiotherapy or combination at 12 months.

21

22 **DISCUSSION**

23 The findings of this study indicate that immune recovery to pretreatment levels within the first
24 year of cancer adjuvant therapy is significantly delayed in large proportions of BC patients.
25 Furthermore, there is considerable variability in the extent of immune recovery between different
26 immune parameters and patients. Not surprisingly, the type of adjuvant therapy showed significant

1 effects on immune recovery, but those effects differed among the various immune parameters.
2 Cancer stage did not show any significant effects on immune recovery in this study, perhaps
3 because of only a few participants with more advanced disease.

4 The probability of immune recovery to baseline or above ranged from 17.6% to 77.3% across
5 all three time points in our study. Significant delays in immune recovery and large variability
6 persisted at 12 months, which was about 6-10 months after completion of any adjuvant therapy. It is
7 interesting to note that CD subsets (with the exception of CD4% in chemotherapy patients) and the
8 proinflammatory cytokine, IL-6, seem to recover faster than other more immune enhancing IFN- γ , IL-
9 2 and NK cell activity. The differential speed of recovery in different CD subsets in our study is
10 consistent with previous findings that CD8+ and CD16+/CD56+ cells recovered faster than CD4+
11 cells (Belka et al., 1999; Santin et al., 2000), and that CD4+ cell recovery remained below baseline
12 at 4 months after a low dose radiotherapy (Belka et al., 1999). In advanced BC patients, high-dose
13 chemotherapy led to an inversion of CD4/CD8 ratio and significant reductions in T LP that persisted
14 for 6-12 months (Avigan et al., 2000). Prolonged reductions in LP were also reported in early stage
15 BC patients (Wiltschke et al., 1995). Furthermore, immune recovery was noted to not always
16 improve with time. The probability of recovering IL-2 responses to baseline or above was only about
17 18% at 12 months, lower than that of earlier timepoints. A similar decreasing trend was noted in T
18 LP responses. These findings suggest that adjuvant therapy may have a delayed impact on
19 recovery of certain immune responses beyond the immediate period after completion of therapy.

20 The type of cancer adjuvant therapy had a differential influence on immune recovery in
21 selective immune measures. Chemotherapy had significant selective immunosuppressive impact on
22 the recovery of IL-2 responses at all times and CD4% at 12 months. In contrast, radiotherapy had
23 significant selective immunosuppressive impact on the recovery of IL-4 responses.
24 Immunosuppression from both chemo- or radiotherapy has been reported by others, including a
25 depletion of CD4+, CD8+ and CD56+ subsets, impaired NK cell activity and LP in various cancer
26 patients (Santin et al., 2000; Steele, 2002; Verastegui, Morales, Barrera-Franco, Poitevin, &

1 Hadden, 2003). NK activity has been found to decrease significantly during and following
2 chemotherapy in BC patients, although NK numbers were less affected and tended to return to
3 baseline before subsequent cycles of chemotherapy (Beitsch, Lotzova, Hortobagyi, & Pollock, 1994;
4 Bonilla et al., 1990; Sewell et al., 1993).

5 Direct comparison of three different types of cancer adjuvant therapy on immune responses
6 has rarely been conducted. Santin et al. (Santin et al., 2000) reported no significant differences in
7 immune suppression between radiotherapy alone and radiotherapy combined with cisplatin in
8 cervical cancer patients, but the sample size was extremely small (N = 8). While time between
9 completion of adjuvant therapy and blood draw for immune function measurement differed among
10 adjuvant therapy types in this study, those differences are an unlikely explanation for the differential
11 responses, because the patterns of IL-2 and IL-4 responses to therapy types remained similar
12 across the timepoints. It appears that chemotherapy or combination of chemo- and radiotherapy
13 suppresses Th1-type cytokine response, whereas radiotherapy suppresses Th2-type cytokine
14 response.

15 Cancer stage had no significant effects on immune recovery in this study. Findings from prior
16 studies have been mixed in that some found incrementally lower NK cell activity with advances in
17 BC stage particularly among those with metastatic BC (Konjevic & Spuzic, 1993), whereas others
18 did not find stage-dependent reductions in immune responses (Campbell, Scott, Maecker, Park, &
19 Esserman, 2005). One likely reason for not observing cancer stage effect is that advanced stage
20 metastatic BC patients were not included in this study.

21 Clinical significance of differential and delayed immune recovery is unclear at present and
22 warrants long-term in-depth investigations. The impact of delayed immune recovery in early stage
23 BC patients may be more subtle than clinical outcomes documented in advanced metastatic BC
24 patients. In advanced BC patients, early immune recovery of lymphocyte count or proliferative
25 responses predicted better survival and a lower recurrence of disease in those who received stem
26 cell transplant (Nieto et al., 2004; Porrata et al., 2001). Similarly, advanced BC patients with lower

1 levels of IL-2 had a significantly shorter survival time than others with higher IL-2 levels (7.2 months
2 vs. 16.6 months) (Lissoni et al., 1991), and higher levels of pre-chemotherapy IL-6 significantly
3 predicted shorter disease-free and overall survival (Bachelot et al., 2003; Bozcuk et al., 2004), thus
4 also indicating a differential predictive role by different immune measures. Even in early BC patients,
5 lower LP was correlated with greater tumor burden (tumor size and axillary lymph node involvement)
6 and those who had not recovered their LP response to baseline values in 12 months from surgery
7 developed a far greater rate of metastatic disease (61%) within the subsequent 2 years than those
8 who had recovered LP above the baseline (2%) (Verastegui et al., 2003; Wiltschke et al., 1995).
9 Similarly, low baseline NK cell activity was associated with greater numbers of positive axillary
10 lymph nodes (Levy et al., 1985), and lower baseline IL-2 levels were associated with a greater tumor
11 relapse rate, 33.3% vs. 4.7% in early stage BC patients (Arduino et al., 1996).

12 Although clinical outcomes from delayed immune recovery in early stage BC patients might
13 be mostly non-life threatening outcomes, such as infections, they can compromise patients' quality
14 of life. Such infections were documented in 11-17% of early BC patients (median follow-up of 69
15 months) (Henderson et al., 2003) and 47% of metastatic BC patients (median follow-up of 40 weeks)
16 (Slamon et al., 2001). Thus, research needs to be extended to determine clinical significance of
17 delayed and poor immune recovery in early stage BC cancer patients employing large sample sizes
18 and longer follow-up periods to gain sufficient statistical power. In addition, investigators need to
19 simultaneously assess potential psychosocial, behavioral, disease-related and biological factors that
20 might contribute to variability in immune recovery. These findings may provide a basis for future
21 interventions to facilitate a timely recovery of immune responses and perhaps improve clinical
22 outcomes in early stage BC patients.

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Table 1. Demographic characteristics of participants (N = 80)

Variable	Mean (SD)	n (%)
Age:	49.5 (8.7)	
Range	32-70	
Years of Education:		
Less than high school		1 (1.3)
Graduated from High School		13 (16.3)
Trade School/some college		28 (35.0)
Bachelor's degree		17 (21.3)
Some Graduate school/Graduate degree		15 (18.8)
Other		6 (7.5)
Employment Status:		
Not employed		15 (18.8)
Part time		12 (15.0)
Full time (40 hr/week)		53 (66.3)
Body Mass Index	28.8 (6.29)	
19 -24.9		28 (35.0)
25 – 29.9		17 (21.3)
30 – 46.6		31 (38.8)
Missing		4 (5.0)
Ethnicity:		
African American		18 (22.5)
Native American		2 (2.5)
Caucasian		60 (75.0)
Religion		
Protestant		62 (77.5)
Catholic		12 (15.0)
Jewish		1 (1.3)
None/Missing		4 (5.0)
Marital Status:		
Single		5 (6.3)
Married or living as married		58 (72.5)
Separated/Divorced/Widowed		16 (20.0)
Missing		1 (1.3)
Menopausal Status:		
Pre-menopause		22 (27.5)
Post-menopause		46 (57.5)
Peri-menopause		12 (15.0)

Table 2. Medical information of participants (N = 80)

Variable	N	n (%)
Stage (TNM classification)	80	
0 (*1)		1 (1.3)
I		21 (26.3)
II		45 (56.3)
III		11 (13.8)
Unknown		2 (2.5)
Estrogen & Progesterone Receptor	80	
Positive/positive		59 (73.8)
Negative/negative		17 (21.3)
Mixed		1 (1.3)
Unknown		3 (3.8)
Adjuvant Therapy	80	
Chemotherapy		20 (25.0)
Radiotherapy		26 (32.5)
Chemo + radiotherapy		33 (41.3)
No adjuvant therapy		1 (1.3)
Hormonal therapy		60 (75.0)
Chemotherapy Regimen	53	
AC		21 (39.6)
ACT/ATC		28 (52.8)
Other (e.g., CMF)		4 (7.6)
Recurrence	80	
Yes		3 (3.8)
No		74 (92.5)
Unknown		3 (3.8)

AC = Adriamycin 60mg/m² + Cytosin 600 mg/m² together every 2-3 weeks for 4 doses; ATC = Adriamycin

60mg/m², Taxol 145mg/m², and Cytosin 600 mg/m² sequentially every 2-3 weeks for 3 doses each;

ACT = Adriamycin 50mg/m² + Cytosin 500 mg/m² together every 2-3 weeks for 4 doses followed by Taxotere

75mg/m² every 2-3 weeks for 4 doses

Table 3. Regression weight and probability of immune recovery to baseline or above over time by type of cancer adjuvant therapy

Variable	Treatment Group	Months Post Baseline						p value
		2		6		12		
		β' (SE) ¹	Probability	β' (SE)	Probability	β' (SE)	Probability	
CD4	Chemo	-0.199 (0.53)	0.45	-0.170 A (0.49)	0.46	-1.544 B (0.65)	0.18	0.04
	Radiation	0.622 (0.54)	0.65	0.423 (0.57)	0.60	1.225 (0.61)	0.778	0.28
	Combined	-0.182(0.44)	0.46	0.318 (0.43)	0.58	0.470 (0.48)	0.62	0.36
CD56	Combined	0.399 A (0.33)	0.60	0.883 A (0.34)	0.71	1.156 B (0.34)	0.76	0.13
CD8	Combined	0.392 (0.32)	0.60	0.912 (0.30)	0.71	0.914 (0.31)	0.71	0.18
IFN- γ	Chemo	-0.721 (0.53)	0.33	-1.057 (0.51)	0.26	-1.213 (0.561)	0.23	0.83
	Radiation	-1.358 A (0.67)	0.21	0.104 B (0.55)	0.53	-0.810 (0.60)	0.31	0.05
	Combined	0.027 A (0.43)	0.51	-0.300 (0.43)	0.43	-1.30 B (0.54)	0.21	0.04
IL-1 α	Combined	-0.680 (0.30)	0.34	-0.5681 (0.28)	0.36	-0.307 (0.31)	0.42	0.43
IL-2	Combined	-0.101 A (0.37)	0.48	-0.671 B (0.38)	0.34	-1.486 C (0.44)	0.19	<.001
IL-4	Combined	-1.272 (0.36)	0.22	-1.253 (0.35)	0.22	-1.283 (0.34)	0.22	0.99
IL-6	Combined	-0.222 A (0.34)	0.45	0.526 B (0.38)	0.63	0.726 B (0.42)	0.67	0.04
NK (MV)	Combined	-0.674 (0.29)	0.34	-0.625 (0.26)	0.35	-0.708 (0.27)	0.33	0.92
LAK (MV)	Chemo	-0.716 (0.53)	0.33	-0.525 (0.47)	0.37	-1.170 (0.52)	0.24	0.26
	Radiation	-0.034 (0.54)	0.49	-0.213 (0.52)	0.45	-0.204 (0.58)	0.45	0.91
	Combined	-0.722 A (0.44)	0.33	-0.022 (0.40)	0.50	0.340 B (0.39)	0.58	<0.02
LP (MV)	Combined	-0.249 A (0.38)	0.44	-0.774 B (0.40)	0.32	-0.886 B (0.35)	0.29	0.03

Pairs of regression weights with different letters are significantly different at $p < 0.05$.

Combined: Collapsed over types of cancer adjuvant therapy, as interaction with time was not statistically significant

MV: Multivariate model tested.

Table 4. Regression weight and probability of immune recovery to baseline or above by the type of cancer adjuvant therapy

Variable	Timepoint (month)	Treatment Group						p value
		Chemo		Radiation		Combined		
		β' (SE) ¹	Probability	β' (SE)	Probability	β' (SE)	Probability	
CD4	2	-0.199 (0.53)	0.45	0.622 (0.54)	0.65	-0.182 (0.44)	0.46	0.39
	6	-0.170 (0.49)	0.45	0.423 (0.57)	0.60	0.318 (0.44)	0.58	0.59
	12	-1.544 A (0.65)	0.18	1.225 B (0.61)	0.77	0.470 B (0.48)	0.62	0.002
CD56	Combined	0.648 (0.34)	0.66	0.822(0.48)	0.70	0.969 (0.37)	0.73	0.77
CD8	Combined	0.514 (0.36)	0.63	0.741 (0.39)	0.68	0.963 (0.37)	0.59	0.66
IFN-r	2	-0.721 (0.53)	0.33	-1.358(0.67)	0.21	0.027 (0.42)	0.51	0.17
	6	-1.057 (0.51)	0.25	0.104 (0.55)	0.53	-0.300 (0.43)	0.43	0.27
	12	-1.213 (0.56)	0.23	-0.810(0.60)	0.31	-1.30 (0.54)	0.21	0.82
IL-1 α	Combined	0.221 (0.43)	0.56	-1.086 (0.51)	0.25	-0.691 (0.28)	0.33	0.10
IL-2	Combined	-2.140 A (0.70)	0.11	0.979 B (0.70)	0.73	-0.895 A (0.39)	0.29	0.004
IL-4	Combined	-0.379 A (0.39)	0.41	-2.369 B (0.50)	0.09	-1.059 A (0.39)	0.26	0.002
IL-6	Combined	0.684 (0.51)	0.67	0.120(0.53)	0.53	0.225 (0.31)	0.56	0.56
NK (MV)	Combined	-0.976 (0.43)	0.27	-0.525 (0.44)	0.37	-0.506 (0.3124)	0.38	0.65
LAK (MV)	2	-0.716 (0.53)	0.33	-0.525(0.47)	0.37	-1.170 (0.52)	0.24	0.54
	6	-0.034 (0.54)	0.49	-0.213 (0.52)	0.45	-0.204 (0.58)	0.45	0.66
	12	-0.722 A (0.44)	0.33	-0.022 (0.40)	0.50	0.340 B (0.39)	0.58	0.05
LP (MV)	Combined	-0.878 (0.55)	0.29	-0.528 (0.59)	0.37	-0.504 (0.42)	0.38	0.82

Pairs of regression weights with different letters are significantly different at $p < 0.05$.

Combined: Collapsed over timepoints, as interaction with type of cancer adjuvant therapy was not statistically significant

MV: Multivariate model tested.

Table 5. Regression weight and probability of immune recovery to baseline or above by cancer stage

Variable	Timepoint (month)	Cancer Stage				p value
		Stage 1-2		Stage > 2		
		β' (SE)	Probability	β' (SE)	Probability	
CD4	Combined	0.153 (0.31)	0.54	0.061 (0.44)	0.52	0.86
CD56	Combined	1.061 (0.30)	0.74	0.564 (0.42)	0.64	0.31
CD8	Combined	0.838 (0.27)	0.70	0.640 (0.41)	0.66	0.69
IFN-r	Combined	-0.955 (0.30)	0.28	-0.518 (0.35)	0.37	0.31
IL-1 α	Combined	-0.570 (0.30)	0.36	-0.468 (0.36)	0.39	0.82
IL-2	Combined	-1.387 (0.39)	0.20	0.016 (0.62)	0.50	0.07
IL-4	Combined	-0.916 (0.30)	0.29	-1.622 (0.43)	0.17	0.11
IL-6	Combined	0.611 (0.40)	0.65	0.076 (0.39)	0.52	0.2
NK (MV)	Combined	-0.566 (0.27)	0.36	-0.772 (0.37)	0.32	0.63
LAK (MV)	Combined	-0.294 (0.31)	0.43	-0.432 (0.38)	0.39	0.74
LP(MV)	Combined	-0.815 (0.38)	0.31	-0.458 (0.50)	0.39	0.52

Combined: Collapsed over time periods, as interaction with cancer stage was not statistically significant

MV: Multivariate model tested.