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Scientific Article

Soluble interleukin-6 receptor mediated fatigue highlights immunological heterogeneity of patients with early breast cancer who undergo radiation therapy

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Abstract

Purpose: This study aimed to explore the associations between dose-volume parameters of localized breast irradiation, longitudinal interleukin-6 soluble receptor (sIL-6R), and leukocyte counts as markers of an immune-mediated response and fatigue as a centrally-driven behavior.

Methods and Materials: This prospective cohort study recruited 100 women who were diagnosed with stage 0-IIIa breast cancer, prescribed 40 Gy in 15 fractions over 3 weeks adjuvant radiation therapy, and had no prior or concurrent chemotherapy. Dose-volume parameters were derived from treatment plans and related to serum sIL-6R concentrations, leukocyte counts, and a validated measure of self-reported fatigue at baseline, after 10 and 15 fractions, and 4 weeks after radiation therapy.

Results: sIL-6R concertation was significantly higher in patients with a total volume of tissue irradiated within the 50% isodose >2040 cm^3 ($P = .003$). When controlling for body mass index, this result only remained significant after treatment. The volume of liver irradiated within the 10% isodose correlated with the sIL-6R concentration during and after radiation therapy ($\rho = .3$-$.4$; $P = .03$-$.007$). The 38% of the cohort that was classified as fatigued had a higher mean sIL-6sR concentration at all observation points, but the differences were only statistically significant during radiation therapy: Mean (standard deviation [SD]) after 15 fractions for fatigued patients was 47.6 ng/dL (11.2 SD) versus 41.6 ng/dL (11.4 SD) for nonfatigued patients ($P = .01$). Cohort leukocyte counts and leukocyte subsets decreased consistently from baseline and the values for the fatigued group were 4% lower at baseline and between 7% and 9% lower during and after treatment compared with those of the nonfatigued group but the differences were not statistically significant.

Conflicts of interest: None.

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Conclusions: This is the first study to show that localized irradiation induces increased systemic sIL-6R during treatment in participants who reported elevated levels of fatigue before, during, and after treatment. This behavioral response appears to reflect a variation in innate host immunity, which then mediates the cellular and/or psychological stress of radiation therapy.

Introduction

Fatigue is a recognizable defense against overexertion. Cancer-related fatigue (CRF) also limits functioning but is more intense and has an unpredictable relationship with activity and rest.[1] Such characteristics suggest why fatigue is the predominant determinant of quality of life in women who undergo radiation therapy for breast cancer.[2] A key challenge to the effective management of radiation therapy-related fatigue (RRF) is an understanding of its biological basis.[3] Objective radiobiological markers of fatigue would help discriminate the component of RRF that is attributable to the effects of radiation therapy from numerous putative contributory factors.[4]

Therapeutic irradiation elicits a complex of inflammatory responses that involve interstitial infiltration, clearance of neutrophils, and consequential mononuclear cells and inflammatory proteins.[5] A principal mediator of this innate immune response is the cytokine interleukin 6 (IL-6). Raised levels of IL-6 have inconsistently been implicated in the precipitation and potentiation of CRF.[6] A limited range of leukocytes and hepatocytes express the membrane-bound receptor for IL-6 (mIL-6R).[7] However, immunological stress such as therapeutic irradiation readily induces the shedding of mIL-6R to form an agonistic soluble receptor, systemic interleukin-6 soluble receptor (sIL-6R).[8] Shed sIL-6R binds with high affinity to circulating IL-6 and enables intracellular signaling via the transmembrane glycoprotein gp130. As gp130 is ubiquitously expressed by parenchyma, stroma, endothelium, and nerves, almost universal systemic cell-signaling is enabled.

IL-6/sIL-6R trans-signaling establishes a theoretical link between immune response within the localized radiation portal and a centrally-perceived response such as fatigue. Pathological control of IL-6 ultimately depends on sIL-6R; therefore, RRF may be correlated with sIL-6R concentration or the volumes of tissue that are irradiated.[9] Increased irradiation of particular local structures have inconsistently been linked with increased fatigue in patients with breast[10] and head and neck[11] cancers.

The aim of this study is to explore the longitudinal relationships between radiation dose-volume parameters, leukocyte, and sIL-6R concentrations at an immunological level as well as fatigue at the behavioral level. The confounding effects of body mass index (BMI), anxiety, and depression are also considered.

Methods and materials

A local research ethics committee (07/WSE04/82) approved this longitudinal cohort study. All participants provided written informed consent.

Participants

Women who attended new patient clinics at the Velindre Cancer Centre were consecutively invited to achieve a sample size of 100. Eligible women were diagnosed with stage group 0-IIIa carcinoma of the breast and prescribed the UK adjuvant radiation therapy schedule of 40 Gy in 15 fractions over 3 weeks (±10 Gy/5 fractions boost). The principal exclusion criteria were thyroid dysfunction; inflammatory or autoimmune diseases; uncontrolled heart, lung, or liver disease; history of significant or untreated depression; prior or concurrent systemic endocrine or cytotoxic therapy; and evidence of locally advanced or metastatic disease. Prior systemic therapy was excluded to determine the pathophysiological effects of irradiation.

Observations

Sociodemographic, disease characteristic, and BMI data were extracted from clinical case records. Psychological mood was recorded using the hospital anxiety and depression scale.[12] Baseline data were collected between 10 and 22 days before radiation therapy. Three longitudinal observations were timed to capture changes in the reported course of RRF: after fraction 10, fraction 15, and 4 weeks after the completion of radiation therapy. Participants were asked to complete self-report questionnaires and provide 2 blood samples at each time point.

Fatigue measure

Fatigue intensity over the preceding 7 days was measured with the Functional Assessment of Chronic Illness Therapy Fatigue Scale (FACIT-F), which is a 13-item questionnaire that generates a cumulative score between 0 (maximum fatigue) and 52 (no fatigue).[13] Participants were categorized as fatigued if the mean of their FACIT-F scores at fractions 10 and 15 were ≤34.[14]

Immunological measures

Differential leukocyte counts were auto-analyzed (Pentra XL 80, Horiba ABX, Montpellier, France). The second blood sample was centrifuged at 1500xg for 15 minutes and the resultant sera stored at −70° C until analyzed. The concentration of sIL-6R was measured with enzyme-linked immunosorbent assay kits (R&D Systems, Minneapolis, MN).

Radiation therapy dose-volume parameters

A computed tomography planning scan with a 2-mm slice thickness was conducted for all participants. Tangential fields were optimized to create a nondivergent posterior border, constrain the central lung distance to ≤20 mm, minimize cardiac irradiation, and cover the planning target volume (PTV) with 95% to 107% of the International Commission on Radiation Units and Measurements prescription point dose.

The heart, liver, ipsilateral lung, and external outline were delineated on an anonymous copy of the plan. The heart was defined by the extent of the pericardial sac including the roots of the great vessels and coronary arteries, and the superior margin corresponded to the level of the left pulmonary artery. The volume of tissue within the 95% isodose was considered a proxy for the volume of the breast. An experienced oncology radiologist reviewed the first 5 cases prospectively to assess the reliability of structure delineation and a 5% random sample was selected retrospectively.

A collapsed cone convolution algorithm (Oncentra MasterPlan v3.1) calculated the dose to the regions of interest (ROIs). The minimum, maximum, and mean ROI doses and the absolute and percentage volume of ROIs irradiated to 10%, 50%, and 90% ($ROI_{10,\ 50,\ 90}$, respectively) of the prescription dose were recorded. If the full extent of the liver was not included in the planning scan, only absolute volumetric data (cm^3) could be generated.

Data analysis

Independent *t* tests compared longitudinal measures between the fatigued and nonfatigued groups. A linear fixed model with repeated measures was used to determine the impact of the overall PTV on sIL-6R concentration. Bivariate relationships between irradiation of individual organs and sIL-6R were evaluated with Pearson's correlations. Nonparametric equivalents were used where data was not normally distributed. Linear regression was used to investigate the association between irradiated volumes and sIL-6R when controlling for patient and clinical variables. Logistic regression was used to evaluate the odds of being in the fatigued group on the basis of sIL-6R and clinical variables. A significance level of $P < .01$ was adopted to mitigate against multiple testing.

Results

The sociodemographic and clinical characteristics of the 100 participants have been described previously.[14] Select patient and disease characteristics are summarized in Table 1. The course of the longitudinal measures for the fatigued (n = 38) and nonfatigued (n = 62) groups are summarized in Table 2. Independent sample *t* test comparisons were made between the groups at each time point. Eighty-eight patients had 4-week follow-up blood data because 12 women declined to return for a blood test after treatment.

Association between irradiation and interleukin-6 soluble receptor concentration

A linear model with repeated measures was conducted to evaluate the association between PTV irradiation and longitudinal sIL-6R development. Systemic sIL-6R concentration was included at the 4 time points, and ordinal

Table 1 Summary of patient and disease-related characteristics

Patient characteristics for sample (n = 100)	Fatigued group (n = 38)	Non-fatigued group (n = 62)
	Mean (SD)	
Age (years)	55.9 (9.3)	59.0 (8.5)
Body mass index (kg/m^2)	29.2 (4.8)	27.6 (4.6)
Time from surgery to radiation therapy (days)	61.5 (12.2)	61.0 (16.7)
Psychological mood		
Anxiety	7.6 (4.8)	4.3 (2.8)
Depression	4.8 (3.4)	1.9 (2.0)
Total HADS score	6.2 (4.1)	12.3 (6.9)
	Frequency n (%)	
Menopausal status		
Postmenopausal	25 (65)	48 (77.4)
Perimenopausal	7 (18.4)	9 (14.5)
Premenopausal	6 (15.8)	5 (8.1)
TNM stage		
0	7 (18.4)	6 (9.7)
I	25 (65.8)	45 (72.6)
IIA	6 (15.8)	11 (17.7)
Laterality		
Right	20 (52.6)	32 (51.6)
Left	18 (47.4)	30 (48.4)
Surgical procedure		
Wide local excision	38	60
Mastectomy	0	2
Electron breast boost 10 Gy/5#/1 week	1	1
SCF irradiation 40 Gy/15#/3 weeks	1	2

HADS, Hospital Anxiety & Depression Scale; TNM stage, tumor, node, metastases stage group; SCF, supra clavicular fossa; SD, standard deviation.

Table 2 Longitudinal measures for the fatigued and nonfatigued groups before radiation therapy (time point 1), after fraction 10 (time point 2), after fraction 15 (time point 3), and 4 weeks after completion of radiation therapy (time point 4)

Group	Measure *Mean (SD)*	Time point			
		1	2	3	4 (n = 88)
Nonfatigued	FACIT-F	45.5 (6.4)	46.0 (5.1)	43.6 (6.7)	44.3 (7.4)
(n = 62)	sIL-6R (ng/dL)	39.8 (11.3)	39.8 (10.7)	41.6 (11.4)	39.6 (12.2)
	Leukocytes (x10^9/L)	7.0 (1.4)	6.5 (1.4)	6.0 (1.5)	5.8 (1.7)
Fatigued	FACIT-F	33.9 (8.3)[a]	28.9 (8.0)[a]	23.1 (7.9)[a]	31.0 (10.9)[a]
(n = 38)	sIL-6R (ng/dL)	43.5 (11.6)	44.8 (10.1)[b]	47.6 (11.2)[c]	41.5 (10.1)
	Leukocytes (x10^9/L)	6.7 (1.6)	6.0 (1.6)	5.6 (1.7)	5.3 (1.4)

FACIT-F, Functional Assessment of Chronic Illness Therapy Fatigue scale; sIL-6R, soluble interleukin 6 receptor; SD, standard deviation.

[a] Significant at < .0005.

[b] Group difference significant at < .05.

[c] Significant at < .01.

thirds categories of PTV_{50} (≤1359 cm^3; 1360-2040; >2040 cm^3) were included as the between-subjects factor. There was a large effect for time (F = 10.8; $P < .001$; partial eta squared), which indicated a significant change in sIL-6R concentrations across the 4 time points. The main effect between the PTV groups was significant (F = 8.4; $P < .001$; moderate partial eta effect size = 0.3).

Post hoc comparisons using Tukey's honestly significant difference test indicated that the greatest difference in sIL-6R concentration was between the largest (>2040 cm^3) PTV_{50} group and the other two groups ($P = .0003$; Table 3). Statistically comparable results were found for PTV_{10} and PTV_{90}.

The Pearson's correlation between BMI and PTV_{50} was $\rho = .7$. When BMI was included as a covariate in the model, the effect for the PTV_{50} group was reduced to F = 3.9 ($P = .24$).

Correlations were performed between individual organ 10%, 50%, and 90% isodose values, mean organ dose, and sIL-6R concentrations at all time points. The only statistically significant result was between the liver subvolumes and sIL-6R concentration for right-sided patients only ($liver_{10}$ at time point 2: n = 52; $\rho = .29$; $P = .03$; time point 3: n = 52; $\rho = .33$; $P = .01$; 4-week follow-up: n = 49; $\rho = 0.40$; $P = .007$). After controlling for anxiety and depression, BMI, age, tumor stage, and time from surgery to radiation therapy, the volume of tissue within the 50% isodose remained statistically significant (beta = 0.43; $P = .008$). Overall, the model explained 53% of the variance in sIL-6R concentration at 4-weeks postradiation therapy.

Association between interleukin-6 soluble receptor and fatigue

A series of independent samples *t* tests revealed significant differences in the sIL-6R concentrations of the fatigued and nonfatigued groups at weeks 2 and 3 of radiation therapy but not pre- or post-treatment (Table 2). At week 3, fatigued participants had significantly higher sIL-6R concentrations (mean: 47.6 ng/dL [standard deviation: 11.2 ng/dL]) than nonfatigued participatns (mean: 41.6 ng/dL [standard deviation: 11.4 ng/dL]; t = 2.48'; $P = .01$; moderate eta squared effect size = 0.06).

Table 3 Fatigue scores and sIL-6R concentrations for ordinal thirds PTV_{50} categories

PTV_{50} group (cm^3)	Measure *Mean (SD)*	Time point			
		1	2	3	4 (n = 88)
≤1359	FACIT-F	43.3 (8.2)	41.7 (9.1)	38.7 (11.3)	42.9 (8.9)
(n = 34)	sIL-6R (ng/dL)	36.2 (10.5)	38.4 (10.1)	39.6 (9.21)	35.1 (10.06)
1360-2040	FACIT-F	40.8 (9.5)	38.6 (12.1)	34.3 (12.8)	37.5 (12.3)
(n = 33)	sIL-6R (ng/dL)	41.1 (11.1)	41.6 (10.7)	42.9 (12.4)	39.4 (10.0)
>2040	FACIT-F	38.9 (10.3)	38.3 (10.0)	34.3 (11.0)	37.3 (10.7)
(n = 33)	sIL-6R (ng/dL)	47.0 (10.2)[a]	47.2 (11.4)[a]	50.7 (11.5)[a]	46.4 (11.4)[a]

FACIT-F, Functional Assessment of Chronic Illness Therapy Fatigue scale; PTV_{50}, planning target volume irradiated within 50% isodose; sIL-6R, soluble interleukin 6 receptor; SD, standard deviation.

[a] Significant at < 0.01.

Association between irradiated volumes and fatigue

The group with PTV50 <1359 cm^3 remained the least fatigued group during treatment (Table 3). FACIT-F scores for the medium and large groups were almost identical during and after radiation therapy. The volume of the lung that was irradiated within the 10% and 50% isodose levels and the maximum lung dose all correlated negatively with FACIT-F score at week 3 and 4 weeks' post-treatment ($\rho = 0.22$-0.30; $P = .01$). At 4 weeks' follow-up, the maximum liver dose data revealed a small but statistically significant relationship with fatigue ($\rho = 0.24$; $P = .01$). All correlations remained significant when controlling for BMI.

Logistic regression was used to assess the likelihood of being in the fatigued group based on age, anxiety and depression, time from surgery to radiation therapy, tumor stage, and volumes of irradiation and sIL-6R concentration during radiation therapy. Only depression was a useful predictor (odds ratio: 1.4; 95% confidence interval, 1.2-1.8; $P = .03$).

Discussion

The repeated measures data indicated that larger volumes of irradiated thoracic tissue are associated with increased peripheral sIL-6R concentrations. The moderate effect size was comparable when considering the mean dose and volume of tissue within the 10%, 50%, and 90% isodose, which suggests that low dose effects are not a particular feature. How much of the increase in sIL-6R can be attributed to the direct effects of irradiation and how much is secondary to positive associations between irradiated volumes and BMI is complex because the latter 2 variables are strongly correlated.[11] Secondary associations with BMI (as a proxy for adiposity) are likely to account for any relationship between irradiated volumes and sIL-6R before radiation therapy commences. Significant positive associations between the volume of tissue irradiated within the primary beam and sIL-6R were evident during treatment and at 4 weeks post-treatment. Multivariable analysis suggests that the increase in sIL-6R relates to a larger treated volume.

There were no statistically significant associations between cardiac or pulmonary dose-volumetric parameters and sIL-6R concentration at any time point. Correlations between the volume of liver that was irradiated within the 10% isodose and sIL-6R concentration were statistically nonsignificant before radiation therapy but consistently significant during and after for participants with right-sided tumors. Medium-strength associations suggest that the irradiation of a sufficiently large volume of liver is associated with a peripherally detectable increase in sIL-6R concentration (hepatocytes are a main source of systemic sIL-6R).[15] No correlations existed for left-sided tumors, which supports the hypothesis that radiation dose to the liver was a substantive effect rather than a consequence of increased BMI. However, a higher BMI was clearly associated with both larger volumes of hepatic irradiation (for right-sided disease) and elevated peripheral sIL-6R.

The overall pattern of data supports the hypothesis of a probable causal link between targeted radiation and serum sIL-6R concentration. However, there was considerable heterogeneity within the sIL-6R data. The active role of the host immune system in mediating individual response to localized radiation therapy is a developing area, with particular focus on the so-called abscopal anti-tumor effects on distant systemic disease.[16,17] The idea that standard dose-volume parameters determine local tissue response but the host immune system mediates systemic effects has relevance for sensitivity to fatigue with a minority of patients suffering disproportionately. Therefore, the next consideration was whether sIL-6R concentration or volumes of irradiation related to fatigue at the behavioral level.

Relationship between interleukin-6 soluble receptor and fatigue

Before radiation therapy, there was no significant difference in sIL-6R concentrations between the fatigued and nonfatigued groups. By week 3 of radiation therapy, sIL-6R was significantly elevated within the fatigued but not in the nonfatigued group. Considering the extremes of fatigue, a subgroup of participants with fatigue scores below the bottom quintile recorded virtually no change in concentration (37.1-37.0 ng/mL) from baseline to week 3 compared with the most fatigued fifth (41.9-51.3 ng/mL). Both findings are consistent with the concept of a subgroup of participants who are defined by preexisting factors that are susceptible to an exaggerated inflammatory response during radiation therapy.

To our knowledge this is the first time sIL-6R has been related to acute fatigue toxicity and extends the limited data linking sIL-6R and chronic fatigue after treatment for breast cancer.[9] A noteworthy trend is that total leukocyte counts and all leukocyte subsets except basophils were lower in the fatigued group at baseline (–4.4%) and all subsequent points (–7% to –9%) compared with the nonfatigued group. This differential did not reach statistical significance, preceded radiation therapy, and is consistent with previously demonstrated innate immunological characteristics of CRF in survivors of breast cancer.[9] Increased cytokine expression and fatigue have also been linked with more severe skin reactions due to breast radiation therapy, which suggests a potential external indicator of inherited susceptibility.[18,19]

Volumes of irradiated tissue as a predictor of fatigue

This study extends the limited data that evaluate the link between radiation dose-volume parameters and increased

fatigue.[10,11] When controlling for psychological mood, the absolute volume of thoracic tissue that is irradiated by the primary beam was not a useful predictor of fatigue in women with breast cancer, which uniquely explains approximately 1% of the variance[14]

The group with the smallest volume irradiated were the least fatigued before, during, and after treatment. This relationship may be evident due to links between lower BMI and greater physical activity and improved psychological mood. However, a report that stated that patients undergoing partial breast radiation therapy experienced less fatigue and improved quality of life compared with whole breast radiation therapy[10] suggests that standard radiobiological principles and toxicity probability increase with volume of normal tissues irradiated and has clinical relevance for RRF.

The local recurrence risk-stratification approach that was tested in the IMPORT low trial[20] provides a partial breast planning approach with the potential to minimize RRF as well as other early and late treatment toxicity. More broadly, unanticipated fatigue toxicity that is associated with low dose effects should be considered[21] as the use of intensity modulated radiation therapy expands.

When considering individual organs, no significant associations were evident between any cardiac dose-volumetric parameter and fatigue. Cardiac irradiation does not appear to contribute to either sIL-6R concentration or fatigue during the acute period. The study findings were in accordance with an abstract presented at an American Society of Clinical Oncology conference that found no relationship between cardiac irradiated volumes and fatigue in 48 patients with early breast cancer.[22] The abstract did report significant correlations between the volume of lung irradiated and fatigue.

In the current study, the volume of lung that was irradiated within the 10% and 50% isodose levels and the maximum lung dose all correlated negatively with FACIT-F score at week 3 and 4 weeks posttreatment, which suggests a small-to-moderate association between larger volumes of lung irradiated and increased fatigue. These correlations remained significant when BMI was controlled for.

Finally, at 4-week follow-up, the maximum liver dose data revealed a small but statistically significant relationship fatigue ($\rho = 0.24$; $P = .01$). Hepatocytes are one of the few cells (other than leukocytes) to express the membrane bound IL-6 receptor. As the liver plays a central role in metabolizing cytotoxic agents, the clinical relevance of these finding may be increased when considering the scheduling of chemotherapy and radiation therapy treatments. In summary, lung and liver irradiation may contribute to the pathophysiology of RRF.

As the single largest study of its type, these results are a reliable basis for hypothesis generation. However, the multiple comparisons that were performed may yield statistically significant results with little or no clinical significance. A reduced significance level partially addressed this limitation. The use of a single cytokine and reliance on cross-sectional correlations may not reflect the wave-like release of cytokines in response to radiation.[5] Finally, the results should be applied with caution to patients who received prior chemotherapy.

Conclusions

The current study revealed an acute elevation in sIL-6R as a response to irradiation, which is of greater magnitude in more fatigued participants. These results were not confounded by the effects of chemotherapy. The respective courses of fatigue, sIL-6R, and leukocytes suggest that radiation therapy exacerbates a premorbid immunological state in fatigued participants. Susceptibility to debilitating systemic effects, such as fatigue via IL-6/sIL-6R regulated signaling, suggests a promising therapeutic target in addition to psychological and behavioral support. Larger BMI and/or breast size may be a useful external indicator of higher-risk patients.

References

1. Neefjes EC, van der Vorst MJ, Blauwhoff-Buskermolen S, Verheul HM. Aiming for a better understanding and management of cancer-related fatigue. *Oncologist*. 2013;18:1135-1143.
2. Noal S, Levy C, Hardouin A, et al. One-year longitudinal study of fatigue, cognitive functions, and quality of life after adjuvant radiotherapy for breast cancer. *Int J Radiat Oncol Biol Phys*. 2011;81:795-803.
3. Barsevick AM, Irwin MR, Hinds P, et al. Recommendations for high-priority research on cancer-related fatigue in children and adults. *J Natl Cancer Inst*. 2013;105:1432-1440.
4. Filler K, Saligan LN. Defining cancer-related fatigue for biomarker discovery. *Support Care Cancer*. 2016;24:5-7.
5. Herskind C, Bamberg M, Rodemann HP. The role of cytokines in the development of normal-tissue reactions after radiotherapy. *Strahlenther Onkol*. 1998;174:12-15.
6. Saligan LN, Olson K, Filler K, et al. The biology of cancer-related fatigue: A review of the literature. *Support Care Cancer*. 2015;23:2461-2478.
7. Knüpfer H, Preiss R. Lack of knowledge: Breast cancer and the soluble interleukin-6 receptor. *Breast Care*. 2010;5:177-180.
8. Rose-John S. IL-6 trans-signaling via the soluble IL-6 receptor: Importance for the pro-inflammatory activities of IL-6. *Int J Biol Sci*. 2012;8:1237-1247.
9. Collado-Hidalgo A, Bower JE, Ganz PA, Cole SW, Irwin MR. Inflammatory biomarkers for persistent fatigue in breast cancer survivors. *Clin Cancer Res*. 2006;12:2759-2766.
10. Albuquerque K, Tell D, Lobo P, Millbrandt L, Mathews HL, Janusek LW. Impact of partial versus whole breast radiation therapy on fatigue, perceived stress, quality of life and natural killer cell activity in women with breast cancer. *BMC Cancer*. 2012;12:251.
11. Powell C, Schick U, Morden JP, et al. Fatigue during chemoradiotherapy for nasopharyngeal cancer and its relationship to radiation dose distribution in the brain. *Radiother Oncol*. 2014;110:416-421.
12. Zigmond AS, Snaith RP. The hospital anxiety and depression scale. *Acta Psychiatr Scand*. 1983;67:361-370.
13. Cella D. *Manual of the Functional Assessment of Chronic Illness Therapy (FACIT) Measurement System*. Evanston, IL: Northwestern Healthcare and Northwestern University; 1997.

14. Courtier N, Gambling T, Enright S, Barrett-Lee P, Abraham J, Mason MD. Psychological and immunological characteristics of fatigued women undergoing radiotherapy for early-stage breast cancer. *Support Care Cancer*. 2013;21:173-181.
15. Lee EJ, Lee SJ, Kim JH, et al. Radiation inhibits interleukin-12 production via inhibition of C-rel through the interleukin-6/ signal transducer and activator of transcription 3 signaling pathway in dendritic cells. *PLoS ONE*. 2016;11:e0146463.
16. Reynders K, Illidge T, Siva S, Chang JY, De Ruysscher D. The abscopal effect of local radiotherapy: Using immunotherapy to make a rare event clinically relevant. *Cancer Treat Rev*. 2015;41:503-510.
17. Formenti SC, Demaria S. Systemic effects of local radiotherapy. *Lancet Oncol*. 2009;10:718-726.
18. De Sanctis V, Agolli L, Visco V, et al. Cytokines, fatigue, and cutaneous erythema in early stage breast cancer patients receiving adjuvant radiation therapy. *Biomed Res Int*. 2014;2014:523568.
19. Bower JE, Ganz PA, Irwin MR, Castellon S, Arevalo J, Cole SW. Cytokine genetic variations and fatigue among patients with breast cancer. *J Clin Oncol*. 2013;31:1656-1661.
20. Ciurlionis L, Tsang Y, Titley J, et al. Interim analysis of treatment plans in the IMPORT LOW Trial. Available at: http://abstracts.ncri.org.uk/abstract/interim-analysis-of-treatment-plans-in-the-import-low-trial-2/. Accessed January 19, 2018.
21. Gulliford SL, Miah AB, Brennan S, et al. Dosimetric explanations of fatigue in head and neck radiotherapy: An analysis from the PARSPORT Phase III trial. *Radiother Oncol*. 2012;104:205-212.
22. Rucinska M, Langkjer ST. Correlation between fatigue level and irradiated volume of the whole body, lung and heart in breast cancer patients receiving adjuvant radiotherapy after breast-conserving surgery. *J Clin Oncol*. 2007;25:19608.