HHS Public Access

Author manuscript

*Int J Radiat Oncol Biol Phys*. Author manuscript; available in PMC 2016 April 27.

Published in final edited form as:

*Int J Radiat Oncol Biol Phys*. 2016 March 1; 94(3): 571–579. doi:10.1016/j.ijrobp.2015.11.026.

# Lymphocyte-Sparing Effect of Stereotactic Body Radiation Therapy in Patients With Unresectable Pancreatic Cancer

**Aaron T. Wild, MD**[*], **Joseph M. Herman, MD, MSc**[†], **Avani S. Dholakia, MD**[†], **Shalini Moningi, BA**[†], **Yao Lu, ScM**[‡], **Lauren M. Rosati, BS**[†], **Amy Hacker-Prietz, PA-C**[†], **Ryan K. Assadi, BS**[†], **Ali M. Saeed, PhD**[†], **Timothy M. Pawlik, MD, MPH, PhD**[§], **Elizabeth M. Jaffee, MD**[‖], **Daniel A. Laheru, MD**[‖], **Phuoc T. Tran, MD, PhD**[†], **Matthew J. Weiss, MD**[§], **Christopher L. Wolfgang, MD, PhD**[§], **Eric Ford, PhD**[¶], **Stuart A. Grossman, MD**[‖], **Xiaobu Ye, MD**[‡], and **Susannah G. Ellsworth, MD**[†]

[*]Department of Radiation Oncology, Memorial Sloan-Kettering Cancer Center, New York, New York [†]Department of Radiation Oncology, Johns Hopkins University School of Medicine, Baltimore, Maryland [‡]Department of Oncology Biostatistics, Johns Hopkins University School of Medicine, Baltimore, Maryland [§]Department of Surgery, Johns Hopkins University School of Medicine, Baltimore, Maryland [‖]Department of Medical Oncology, Johns Hopkins University School of Medicine, Baltimore, Maryland [¶]Department of Radiation Oncology, University of Washington School of Medicine, Seattle, Washington

## Abstract

**Purpose—**Radiation-induced lymphopenia (RIL) is associated with inferior survival in patients with glioblastoma, lung cancer, and pancreatic cancer. We asked whether stereotactic body radiation therapy (SBRT) decreases severity of RIL compared to conventional chemoradiation therapy (CRT) in locally advanced pancreatic cancer (LAPC).

**Methods and Materials—**Serial total lymphocyte counts (TLCs) from patients enrolled in a prospective trial of SBRT for LAPC were compared to TLCs from an existing database of LAPC patients undergoing definitive CRT. SBRT patients received 33 Gy (6.6 × Gy 5 fractions). CRT patients received a median dose of 50.4 Gy (1.8 Gy × 28 fractions) with concurrent 5-fluorouracil (77%) or gemcitabine (23%) therapy. Univariate and multivariate analyses (MVA) were used to identify associations between clinical factors and post-treatment TLC and between TLC and survival.

**Results—**Thirty-two patients received SBRT and 101 received CRT. Median planning target volume (PTV) was smaller in SBRT (88.7 $cm^3$) than in CRT (344.6 $cm^3$; $P<.001$); median tumor

Reprint requests to: Susannah G. Ellsworth, MD, Department of Radiation Oncology and Molecular Radiation Sciences, Sidney Kimmel Comprehensive Cancer Center, Johns Hopkins Hospital, c/o Susan Pass-waters, 1550 Orleans St, Ste 1M16, Baltimore, MD 21287. Tel: (410) 955-8837; sbatkoy2@jhmi.edu.

Data were presented previously at the American Society of Clinical Oncology Gastrointestinal Cancers Symposium, San Francisco, CA, Jan 19–21, 2012; and at the 55th Annual Meeting of the American Society for Radiation Oncology, Atlanta, GA, Sept 22–25, 2013.

Conflict of interest: none.

Supplementary material for this article can be found at www.redjournal.org.

diameter was larger for SBRT (4.6 cm) than for CRT (3.6 cm; *P*=.01). SBRT and CRT groups had similar median baseline TLCs. One month after starting radiation, 71.7% of CRT patients had severe lymphopenia (ie, TLC <500 cells/mm$^3$ vs 13.8% of SBRT patients; *P*<.001). At 2 months, 46.0% of CRT patients remained severely lymphopenic compared with 13.6% of SBRT patients (*P*=.007). MVA demonstrated that treatment technique and baseline TLCs were significantly associated with post-treatment TLC at 1 but not 2 months after treatment. Higher post-treatment TLC was associated with improved survival regardless of treatment technique (hazard ratio [HR] for death: 2.059; 95% confidence interval: 1.310–3.237; *P*=.002).

**Conclusions—**SBRT is associated with significantly less severe RIL than CRT at 1 month in LAPC, suggesting that radiation technique affects RIL and supporting previous modeling studies. Given the association of severe RIL with survival in LAPC, further study of the effect of radiation technique on immune status is warranted.

## Introduction

Recent studies have demonstrated a link between treatment-induced lymphopenia and inferior survival in glioblastoma, non-small cell lung cancer (NSCLC), and both resected and unresectable pancreatic cancer (1–4). Radiation therapy (RT) probably contributes directly to treatment-induced lymphopenia in cancer patients, as lymphopenia occurs after RT regardless of whether other lymphotoxic agents, such as corticosteroids or certain chemotherapeutics (eg, temozolomide) are given (5–7). A proposed mechanism for radiation-induced lymphopenia (RIL) is irradiation of circulating blood, because lymphopenia occurs even after irradiation of tissues such as the breast and the brain, which contain little bone marrow or lymphatic tissue, respectively (6, 8). Furthermore, irradiation of circulating blood alone via a radioactive source embedded within a shielded dialysis unit can also cause prolonged, severe lymphopenia (9).

Our group previously published a model that calculates radiation dose received by circulating blood during external beam RT (10), which suggested that circulating lymphocytes receive potentially lymphotoxic doses of radiation during a typical RT course. For example, during a 30-fraction treatment (2 Gy/fraction) to an 8-cm–diameter planning target volume (PTV), 95% of circulating blood receives >0.5 Gy, with mean dose to circulating blood of >2 Gy. In vitro data show that 2 Gy kills approximately 60% of lymphocytes (11). The model also suggested that decreasing target volume and fraction number would significantly reduce circulating blood dose (Fig. 1). We, therefore, sought clinical data to support the model's predictions.

Stereotactic body radiation therapy (SBRT) for pancreatic cancer provided an ideal setting in which to test our hypothesis that smaller target volumes and hypofractionation would lower dose to circulating blood and spare circulating lymphocytes. SBRT is a novel technique that uses respiratory motion correction and daily image guidance to enable delivery of large radiation doses to highly focused extracranial targets (12). Herein, we report the comparison of post-treatment total lymphocyte counts (TLCs) among patients with locally advanced pancreatic cancer (LAPC) who were treated either with induction chemotherapy and SBRT

on a prospective clinical trial or with conventional external beam RT and concurrent chemotherapy.

## Methods and Materials

### Patient selection

The SBRT group included 32 patients treated in a multi-institutional prospective phase 2 trial (13). All patients provided informed consent, and the protocol was approved by each institutional review board (IRB). Although 49 patients were treated in the protocol, only the 32 who were treated at our institution with TLCs available for review were included in the present study. The second group was derived from a previously established retrospective database of 101 patients consecutively treated with definitive conventional chemoradiation therapy (CRT). In that group, all patients had provided informed consent for treatment, and our IRB approved the review of medical records. In both groups, unresectable disease was defined as (*1*) superior mesenteric artery and/or celiac axis tumor encasement; or (*2*) superior mesenteric-portal vein confluence occlusion.

SBRT patients were required to be >18 years of age with Eastern Cooperative Oncology Group (ECOG) performance status ≤1, nonmetastatic, biopsy-confirmed locally advanced pancreatic cancer (LAPC), and no previous abdominal irradiation. Tumors had to be <7.5 cm in greatest axial dimension. CRT patients had to fulfill the same criteria, except for tumor size restrictions, as this was a specific eligibility criterion for the phase 2 SBRT clinical trial, and had to have received definitive, conventionally fractionated CRT (fraction size ≤3 Gy/day) to ≥30 Gy.

### Therapeutic interventions

Figure E1 (available online at www.redjournal.org) summarizes therapies administered to both cohorts. SBRT patients received 6.6 Gy × 5 fractions [total 33 Gy; early ($\alpha/\beta$ =10) and late ($\alpha/\beta$ =3) biological effective doses (BED) of 54.8 and 105.6 Gy, respectively] over 1 to 2 weeks. Patients could receive ≤3 weekly doses of gemcitabine (1000 mg/m$^2$) before SBRT and maintenance weekly gemcitabine beginning ≥1 week after SBRT. In both groups, patients could receive second-line chemotherapy for recurrent disease at the discretion of the treating medical oncologist.

All SBRT patients underwent endoscopic ultrasonography-guided implantation of up to 5 gold fiducials within the pancreatic tumor. Patients underwent computed tomography (CT) simulation in supine position in custom-fitted immobilization devices with oral or intravenous contrast agent and 4-dimensional (4D) assessment of tumor motion. If ≥5 mm of tumor motion was present, measures to correct for respiratory motion were used, usually active breathing control (ABC). Patients with respiratory tumor motion of <5 mm were treated using free breathing with an internal target volume defined based on respiratory motion. Final PTV included a 2- to 3-mm margin on gross tumor volume for ABC cases or a 2- to 3-mm margin on the internal target volume for free-breathing cases.

CRT patients received a median 1.8 Gy × 28 fractions (total 50.4 Gy; early or late BED of 59.5 or 80.6 Gy) with concurrent 5-fluorouracil (5-FU) or gemcitabine. Concurrent 5-FU

was administered by continuous infusion (200–250 mg/m$^2$/day) or as capecitabine (800–1000 mg/m$^2$ twice daily) Monday through Friday. Concurrent gemcitabine was administered weekly at 300 to 600 mg/m$^2$.

CRT patients underwent CT simulation in the supine position in custom-fitted immobilization devices with oral or IV contrast; 4D simulations and ABC were not used for CRT planning. The clinical target volume included the gross tumor volume plus regional lymph nodes and was expanded from 1.5 to 2.5 cm to generate the PTV. Radiation was delivered using either 3D conformal or intensity modulated RT.

### Data collection

TLCs were collected from complete blood counts at baseline (before starting RT) and monthly intervals after starting RT and are reported for all patients with available data at each time point. Patients were classified as having severe (grade 3–4) lymphopenia (<500 cells/mm$^3$) or milder (grade 0–2) lymphopenia (≥500 cells/mm$^3$) based on National Cancer Institute's Common Terminology Criteria for Adverse Events, version 4.0.

### Statistical analysis

Demographic, baseline, and treatment characteristics were summarized using descriptive statistics. Means were compared parametrically, including the Student *t* test for intergroup comparisons and paired *t* test for intragroup comparisons. Proportions and medians were compared between groups using Fisher exact and Mann-Whitney U tests, respectively. Survival was calculated from histopathologic diagnosis until death; patients lost to follow-up were censored at the last follow-up. Survival probability was estimated using Kaplan-Meier statistics. Univariate Cox regression analysis was used to test for associations between potential prognostic factors and survival. Factors that were significantly associated with or exhibited a trend toward significant association ($P \leq .15$) with survival on univariate analysis and factors of accepted clinical importance (specified in the results section) were entered as covariates in a multivariate proportional hazards regression model for survival. Using this model, the hazard ratio (HR) for death ascribable to each covariate was estimated with backward elimination. A logistic regression model was also used to analyze factors potentially associated with severe posttreatment lymphopenia at the first and second months after therapy. The a priori level of statistical significance was a *P* level of <.05, with all *P* values 2 sided. SPSS version 20.0 software (IBM Corp, Armonk, NY) was used for statistical analyses.

## Results

### Patients

Table 1 compares demographics, baseline disease, and treatment characteristics between the SBRT and CRT groups. Groups were similar in median age, sex, race, performance status, tumor location, histologic grade, and median baseline carbohydrate antigen 19–9 (CA19–9) concentration. More SBRT than CRT patients received induction gemcitabine (84% vs 15%, respectively; $P < .001$), yet median baseline TLC was not significantly different (1320 vs 1455 cells/mm$^3$, respectively, $P = .29$). PTV (volume of tissue receiving ≥95% of prescribed

radiation dose), was markedly smaller in the SBRT group than in the CRT group (88.7 vs 344.6 $cm^3$, respectively, $P<.001$). Although the median PTV was nearly 4 times smaller in the SBRT group, SBRT patients had a larger median tumor diameter than CRT patients (4.6 vs 3.6 cm, respectively, $P=.01$). More SBRT patients received maintenance gemcitabine (97 vs 52%, respectively, $P<.001$).

### Radiation-induced lymphopenia

Table 2 and Figure 2 summarize changes in TLC following RT. Median baseline TLC for SBRT patients was 1320 (interquartile range [IQR]: 920–1710) versus 1455 cells/$mm^3$ (IQR: 1115–1712) for CRT patients ($P=.29$) (Fig. 2A). In the SBRT group, 19 or 27 patients (70%) had normal baseline TLCs (≥1000 cells/$mm^3$) versus 86 or 100 CRT patients (86%) ($P=.08$). None of the SBRT patients (0%) and 1 CRT patient (1%) were severely lymphopenic (TLC<500 cells/$mm^3$) at baseline ($P=1.0$). No significant differences in median baseline TLCs existed between patients who did or did not receive induction chemotherapy (1340 vs 1455 cells/$mm^3$, respectively; $P=.11$).

One month after patients began RT, median TLC was 690 cells/$mm^3$ (IQR: 625–940) and 358 cells/$mm^3$ (IQR: 250–525) for SBRT and CRT patients, respectively ($P<.001$) (Fig. 2A). Median decrease in TLC from baseline per patient at 1 month was 35.0% (IQR: 10.9–51.6) among SBRT patients versus 74.0% (IQR: 62.5–84.3) among CRT patients ($P<.001$) (Fig. 2B). In the SBRT group, 4 of 29 patients (13.8%) experienced severe lymphopenia at 1 month; however, 71 of 99 patients (71.7%) in the CRT group were severely lymphopenic at 1 month ($P<.001$) (Fig. 2C).

Two months after patients began RT, median TLC was 780 cells/$mm^3$ (IQR: 653–898) for SBRT patients versus 560 cells/$mm^3$ (IQR 360–920) for CRT patients ($P=.11$) (Fig. 2A). Median decrease in TLC from baseline per patient at 2 months was 32.1% (IQR: 13.1–52.4) among SBRT patients versus 60.7% (IQR: 38.8–75.2) among CRT patients ($P=.02$) (Fig. 2B). In the SBRT group, 3 of 22 patients (13.6%) with 2-month TLC data available were severely lymphopenic at 2 months versus 46 of 101 CRT patients (45.5%; $P=.007$) (Fig. 2C). Multivariate analysis showed that treatment group, lack of induction chemotherapy, and baseline TLC were significantly associated with the risk of severe lymphopenia at 1 month ($P=.002$ and $P=.01$, respectively); at 2 months, however, none of the analyzed clinical factors were significantly associated with severe post-treatment lymphopenia. Table 3 presents the results of the logistic regression analysis of factors associated with lymphocyte counts at 1 and 2 months after treatment.

To account for differing RT course lengths for the 2 groups, resulting in different time intervals between end of RT and 1- to 2-month TLCs, long-term TLC data were examined for the CRT group (Fig. E2; available online at www.redjournal.org). Unfortunately, long-term laboratory data were unavailable at our institution for SBRT patients, as most patients pursued further therapy locally. Over 12 months of follow-up, median TLCs for CRT patients remained low and failed to recover to a normal range (>1000 cells/$mm^3$). Furthermore, when the distribution of TLCs at each of months 3 to 12 was compared to month 2 by Mann-Whitney U test, no significant differences were found (all $P>.05$) (Fig. E2; available online at www.redjournal.org), suggesting that time interval after RT likely

does not account for the differences in TLC observed between CRT and SBRT groups at 1 and 2 months.

### Survival

Median follow-up was 13.9 months (range: 3.9–45.2) for SBRT patients and 12.4 months (range, 2.5–38.7) for CRT patients ($P$=.09), with a median follow-up for all patients of 12.7 months. Median survival from diagnosis for all patients was 13.9 months (95% confidence interval [CI]: 12.0–17.1). There was a trend toward longer median survival for SBRT patients (18.8 months [95% CI: 13.6–23.8] vs 13.6 months for CRT [95% CI: 10.8–15.8]; $P$=.09); 1-year survival was higher for SBRT patients (72.1 vs 56.7%), as was 2-year survival (18.1 vs 13.0%). At the time of analysis, 104 of 133 patients (70.7%) had died. Four of the 104 deaths (3.8%) were due to infection (2 SBRT patients [6.3%] and 2 CRT patients [2.0%]; $P$=.24). The remaining deaths resulted from disease progression.

Univariate Cox regression analyses were used to assess associations between potential prognostic factors and survival (Table 4), which showed that 6 factors had a significant association or trend toward significant association ($P \leq .15$) with survival: (*1*) baseline CA19-9; (*2*) severe lymphopenia; (*3*) induction chemotherapy; (*4*) treatment group (SBRT vs CRT); (*5*) PTV; and (*6*) maintenance chemotherapy. Table 4 shows HRs, 95% confidence limits, and $P$ values associated with each factor. Median survival for patients with severe lymphopenia at 2 months after starting RT was 12.4 months (95% CI: 8.7–16.1) versus 15.2 months (95% CI: 12.7–17.9) for patients with TLC >500 cells/mm$^3$ ($P$=.055) (Fig. 3). Overall survival was lower in the severely lymphopenic group at both 1 (51.4% vs 63.9%, respectively) and 2 years (8.4% vs 17.3%, respectively).

These 6 factors, along with factors of established clinical importance (age, ECOG performance status, histologic grade, tumor diameter, baseline TLC), were used to construct a multivariate proportional hazards model for survival. Backward elimination identified 4 factors as significantly predictive of inferior survival, listed on HR magnitude: lack of maintenance chemotherapy, baseline CA19-9 >90 U/mL, severe lymphopenia at 2 months, and older age (Table 4).

## Discussion

Results of this study suggest that smaller target volumes and hypofractionated regimens may be associated with higher post-treatment lymphocyte counts in patients undergoing RT for LAPC. Additionally, RIL is associated with inferior survival in these patients regardless of treatment technique (3, 4). Although baseline lymphopenia is a poor prognostic factor in several solid tumors, the association between treatment-induced lymphopenia and inferior cancer survival has only recently been described (1–3). Of particular interest is a recently published analysis that identified RIL as a prognostic factor for survival in LAPC (4). That study demonstrated a significantly increased risk of death (HR =2.87, $P$=.002) in patients with TLC <500 cells/mm$^3$ at 2 months after RT began.

The immune system is known to play an important role in cancer control; this task requires functional lymphocytes, which are capable of identifying and destroying cancerous cells (14,

15). The amount of tumor-infiltrating lymphocytes correlates with prognosis for various cancers, including pancreatic cancer (16–19). Given that standard chemoradiation treatment regimens for LAPC induce some degree of lymphopenia in nearly all patients, with almost half of patients experiencing severe lymphopenia, it is important to investigate strategies that aid in sparing these important effector cells (4). As demonstrated in this study, SBRT both improves local tumor control and may spare circulating lymphocyte populations. We hypothesize that the increased number of circulating lymphocytes may aid in tumor control at the primary site and possibly at distant metastatic lesions.

RIL was first described in 1916 and can occur after irradiation of any anatomic site (6–8, 20, 21). It is thought to be due to irradiation of lymphocytes circulating through the target field during treatment; irradiation of circulating blood alone via a cesium source mounted inside a shielded dialysis unit causes a 60% to 80% drop in circulating lymphocytes that lasts for years following RT exposure (9). Changes in RT technique may reduce exposure of circulating blood, and previous studies have suggested that reducing fraction number and/or shrinking fields may preserve circulating lymphocytes. MacLennan and Kay (8) performed a prospective study in children with acute lymphocytic leukemia undergoing prophylactic cranial irradiation. Total RT dose was held constant at 24 Gy, but fraction number was left to investigator discretion, ranging from 5 to 15 fractions (8). Posttreatment TLCs were inversely proportional to fraction number, with each additional fraction causing a further 7% to 8% drop in post-radiation TLC. In breast cancer, treating only the breast resulted in higher post-treatment TLCs than treating a larger volume including comprehensive nodal fields (22). Larger field sizes also increased chromosomal aberrations in circulating lymphocytes in a prospective series of lung cancer patients treated with carbon-ion RT and were associated with lower post-treatment TLC in another recent NSCLC study (23, 24).

In addition to direct toxicity to circulating lymphocytes, RT may affect lymphocyte homeostasis via cytokines. Increased circulating levels of interleukin-7 (IL-7) stimulate lymphocyte proliferation in patients with lymphopenia due to human immunodeficiency virus infection or chemotherapy (25). However, glioma patients treated with RT and temozolomide were unable to mount the expected increase in IL-7, despite severe lymphopenia (26). Irradiation also increases galectin-1 (Gal-1) secretion, leading to decreased TLCs, suppression of the antitumor response, and promotion of aggressive tumor growth in NSCLC and head and neck cancers (27).

Our group modeled RT dose received by circulating blood (10) in order to calculate changes in blood dose when treatment-related parameters (target size, dose rate, total RT dose, and number of fractions) are altered. Circulating blood dose appears to depend upon target volume and fraction number (Fig. 1) early in treatment; as treatment progresses, the circulating blood dose increases exponentially, approaching 100% of circulating blood by approximately the fifth week of RT. SBRT decreases both target volume and fraction number and, as such, was predicted to spare circulating lymphocytes. Our clinical observations provide preliminary corroboration of the model, although further investigations in larger prospective cohorts are necessary to validate these findings.

Despite major alterations in target volumes and fractionation scheme, SBRT does not appear to compromise disease control in LAPC. Historic standard targets for pancreatic tumors included both the primary tumor and the regional lymph node fields (28), but previous experiences with SBRT for LAPC and results of our phase 2 protocol showed that target volume can be decreased without increasing locoregional failure (13, 29). As shown in previous trials, the present series demonstrated that SBRT was not associated with inferior local control or survival compared to conventional CRT (13, 30, 31).

One limitation of this retrospective study is the lack of data for lymphocyte subpopulations; however, we plan prospective research to address this gap in our knowledge. The study was also limited by relatively small numbers of patients in the SBRT group; although absolute differences in the median TLC and risk of severe lymphopenia persisted at 1 and 2 months after treatment, these differences were only statistically significant at 1 month after treatment. This finding may be due at least in part to the relatively small number of data points available in the SBRT cohort 2 months after therapy. Additionally, although baseline clinical characteristics were similar, most SBRT patients received gemcitabine-based induction chemotherapy and were more likely to receive maintenance chemotherapy. Lymphopenia is a known toxicity of gemcitabine, particularly in combination with other agents, and the possibility of synergistic lymphotoxicity when gemcitabine is added to RT cannot be ruled out (32, 33). Nevertheless, we believe that these variations are unlikely to be responsible for the observed differences in circulating lymphocyte counts between the 2 groups, particularly since the risk of lymphopenia was lower in the SBRT patients, despite treatment with induction/maintenance gemcitabine at higher rates than the CRT patients. Of the CRT patients who received induction chemotherapy, no changes in TLC were observed until RT began. Similar findings have been observed in patients with NSCLC receiving chemotherapy followed by chemoradiation (2). Furthermore, in our study, RIL remained a significant adverse prognostic factor in a multivariate analysis that adjusted for various chemotherapy regimens.

## Conclusions

In conclusion, our comparison of patients treated on a phase 2 trial of SBRT for LAPC with a large retrospective cohort treated with conventional CRT suggests that decreasing fraction number and shrinking target volumes may spare circulating lymphocytes in patients at high risk of treatment-induced lymphopenia following conventional CRT. Additionally, patients with higher post-treatment TLCs had longer survival. Further research is needed to better understand the relationship between TLCs and clinical outcomes in LAPC.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## Acknowledgments

This study was funded by the Viragh Family Foundation, the Claudio X. Gonzalez Family Foundation, the Keeling Family Foundation, the DeSanti Family Foundation, and the McKnight Family.

## References


1. Grossman SA, Ye X, Lesser G, et al. Immunosuppression in patients with high-grade gliomas treated with radiation and temozolomide. Clin Cancer Res. 2011; 17:5473–5480. [PubMed: 21737504]
2. Campian JL, Ye X, Brock M, et al. Treatment-related lymphopenia in patients with stage III non-small-cell lung cancer. Cancer Invest. 2013; 31:183–188. [PubMed: 23432821]
3. Balmanoukian A, Ye X, Herman J, et al. The association between treatment-related lymphopenia and survival in newly diagnosed patients with resected adenocarcinoma of the pancreas. Cancer Invest. 2012; 30:571–576. [PubMed: 22812722]
4. Wild AT, Ye X, Ellsworth SG, et al. The association between chemoradiation-related lymphopenia and clinical outcomes in patients with locally advanced pancreatic adenocarcinoma. Am J Clin Oncol. 2015; 38:259–265. [PubMed: 23648440]
5. Hughes MA, Parisi M, Grossman S, et al. Primary brain tumors treated with steroids and radiotherapy: Low CD4 counts and risk of infection. Int J Radiat Oncol Biol Phys. 2005; 62:1423–1426. [PubMed: 16029802]
6. Meyer KK. Radiation-induced lymphocyte-immune deficiency. A factor in the increased visceral metastases and decreased hormonal responsiveness of breast cancer. Arch Surg. 1970; 101:114–121. [PubMed: 5451189]
7. Onsrud M, Grahm I, Gaudernack G. Early stage carcinoma of the uterine cervix. Effects of intracavitary radium treatment on lymphoid cells in blood and pelvic lymph nodes. Acta Radiol Oncol. 1986; 25:127–130. [PubMed: 3012956]
8. MacLennan IC, Kay HE. Analysis of treatment in childhood leukemia. IV. The critical association between dose fractionation and immunosuppression induced by cranial irradiation. Cancer. 1978; 41:108–111. [PubMed: 272224]
9. Weeke E. The development of lymphopenia in uremic patients undergoing extracorporeal irradiation of the blood with portable beta units. Radiat Res. 1973; 56:554–559. [PubMed: 4767134]
10. Yovino S, Kleinberg L, Grossman SA, et al. The etiology of treatment-related lymphopenia in patients with malignant gliomas: Modeling radiation dose to circulating lymphocytes explains clinical observations and suggests methods of modifying the impact of radiation on immune cells. Cancer Invest. 2013; 31:140–144. [PubMed: 23362951]
11. Nakamura N, Kusunoki Y, Akiyama M. Radiosensitivity of CD4 or CD8 positive human T-lymphocytes by an in vitro colony formation assay. Radiat Res. 1990; 123:224–227. [PubMed: 2117766]
12. Potters L, Kavanagh B, Galvin JM, et al. American society for therapeutic radiology and oncology (ASTRO) and American College of Radiology (ACR) practice guideline for the performance of stereotactic body radiation therapy. Int J Radiat Oncol Biol Phys. 2010; 76:326–332. [PubMed: 20117285]
13. Herman JM, Chang DT, Goodman KA, et al. Phase II multi-institutional trial evaluating gemcitabine and stereotactic body radiation therapy for locally advanced unresectable pancreatic adenocarcinoma. Cancer. 2015; 121:1128–1137. [PubMed: 25538019]
14. Hanahan D, Weinberg RA. Hallmarks of cancer: The next generation. Cell. 2011; 144:646–674. [PubMed: 21376230]
15. Schreiber RD, Old LJ, Smyth MJ. Cancer immunoediting: Integrating immunity's roles in cancer suppression and promotion. Science. 2011; 331:1565–1570. [PubMed: 21436444]
16. Zhang L, Conejo-Garcia JR, Katsaros D, et al. Intratumoral T cells, recurrence, and survival in epithelial ovarian cancer. N Engl J Med. 2003; 348:203–213. [PubMed: 12529460]
17. Loi S, Sirtaine N, Piette F, et al. Prognostic and predictive value of tumor-infiltrating lymphocytes in a phase III randomized adjuvant breast cancer trial in node-positive breast cancer comparing the addition of docetaxel to doxorubicin with doxorubicin-based chemotherapy: BIG 02–98. J Clin Oncol. 2013; 31:860–867. [PubMed: 23341518]
18. Fukunaga A, Miyamoto M, Cho Y, et al. $CD8^+$ tumor-infiltrating lymphocytes together with $CD4^+$ tumor-infiltrating lymphocytes and dendritic cells improve the prognosis of patients with pancreatic adenocarcinoma. Pancreas. 2004; 28:26–31.

19. Ino Y, Yamazaki-Itoh R, Shimada K, et al. Immune cell infiltration as an indicator of the immune microenvironment of pancreatic cancer. Br J Cancer. 2013; 108:914–923. [PubMed: 23385730]
20. Shohan J. Some theoretical considerations on the present status of roentgen therapy. The Boston Medical and Surgical Journal. 1916; 175:321–327.
21. Raben M, Walach N, Galili U, et al. The effect of radiation therapy on lymphocyte subpopulations in cancer patients. Cancer. 1976; 37:1417–1421. [PubMed: 1083285]
22. Idestrom K, Petrini B, Blomgren H, et al. Changes of the peripheral lymphocyte population following radiation therapy to extended and limited fields. Int J Radiat Oncol Biol Phys. 1979; 5:1761–1766. [PubMed: 160901]
23. Lee R, Yamada S, Yamamoto N, et al. Chromosomal aberrations in lymphocytes of lung cancer patients treated with carbon ions. J Radiat Res. 2004; 45:195–199. [PubMed: 15304960]
24. Tang C, Liao Z, Gomez D, et al. Lymphopenia association with gross tumor volume and lung V5 and its effect on non-small cell lung cancer patient outcomes. Int J Radiat Oncol Biol Phys. 2014; 89:1084–1091. [PubMed: 25035212]
25. Mackall CL, Fry TJ, Gress RE. Harnessing the biology of IL-7 for therapeutic application. Nat Rev Immunol. 2011; 11:330–342. [PubMed: 21508983]
26. Ellsworth S, Balmanoukian A, Kos F, et al. Sustained CD4 T cell-driven lymphopenia without a compensatory IL-7/IL-15 response among high-grade glioma patients treated with radiation and temozolomide. Oncoimmunology. 2014; 3:27357.
27. Kuo P, Bratman SV, Shultz DB, et al. Galectin-1 mediates radiation-related lymphopenia and attenuates NSCLC radiation response. Clin Cancer Res. 2014; 20:5558–5569. [PubMed: 25189484]
28. Regine WF, Winter KA, Abrams RA, et al. Fluorouracil vs gemcitabine chemotherapy before and after fluorouracil-based chemoradiation following resection of pancreatic adenocarcinoma: A randomized controlled trial. JAMA. 2008; 299:1019–1026. [PubMed: 18319412]
29. Chang DT, Schellenberg D, Shen J, et al. Stereotactic radiotherapy for unresectable adenocarcinoma of the pancreas. Cancer. 2009; 115:665–672. [PubMed: 19117351]
30. Herman JM, Wild AT, Wang H, et al. Randomized phase III multi-institutional study of TNFerade biologic with fluorouracil and radiotherapy for locally advanced pancreatic cancer: Final results. J Clin Oncol. 2013; 31:886–894. [PubMed: 23341531]
31. Loehrer PJ, Feng Y, Cardenes H, et al. Gemcitabine alone versus gemcitabine plus radiotherapy in patients with locally advanced pancreatic cancer: An Eastern Cooperative Oncology Group trial. J Clin Oncol. 2011; 29:4105–4112. [PubMed: 21969502]
32. Mackenzie MJ, Saltman D, Hirte H, et al. A phase II study of 3-aminopyridine-2-carboxaldehyde thiosemicarbazone (3-AP) and gemcitabine in advanced pancreatic carcinoma. A trial of the Princess Margaret Hospital Phase II consortium. Invest New Drugs. 2007; 25:553–558. [PubMed: 17585372]
33. Kindler HL, Ioka T, Richel DJ, et al. Axitinib plus gemcitabine versus placebo plus gemcitabine in patients with advanced pancreatic adenocarcinoma: A double-blind randomised phase 3 study. Lancet Oncol. 2011; 12:256–262. [PubMed: 21306953]

## Summary

This study compared post-treatment total lymphocyte counts (TLC) in patients treated with stereotactic body radiation therapy to TLC with conventional chemoradiation; multivariate analysis demonstrated that treatment technique and baseline TLC were significantly associated with post-treatment TLC at 1 but not 2 months post treatment, providing preliminary support for a model predicting that fraction number and field size are associated with post-treatment TLC. Additionally, severe radiation-induced lymphopenia was associated with inferior survival regardless of treatment technique.

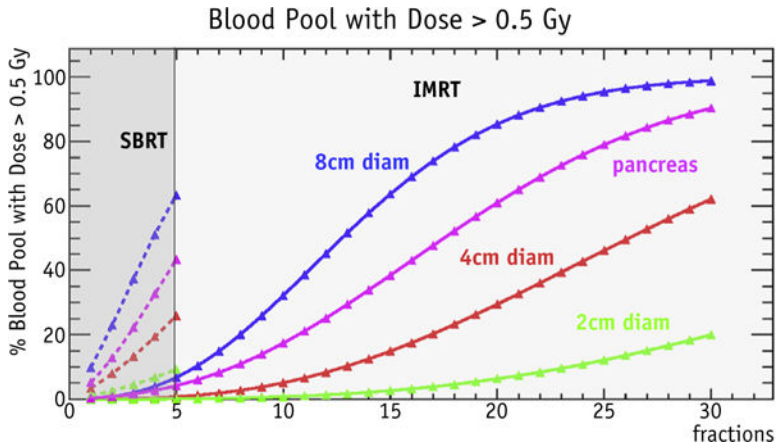


**Fig. 1.**

Percentage of blood exposed to >0.5 Gy as a function of fraction number and planning target volume (PTV). Dose to circulating blood increases with fraction number and PTV diameter. *Abbreviations:* diam = diameter; IMRT = intensity modulated radiation therapy; SBRT = stereotactic body radiation therapy.

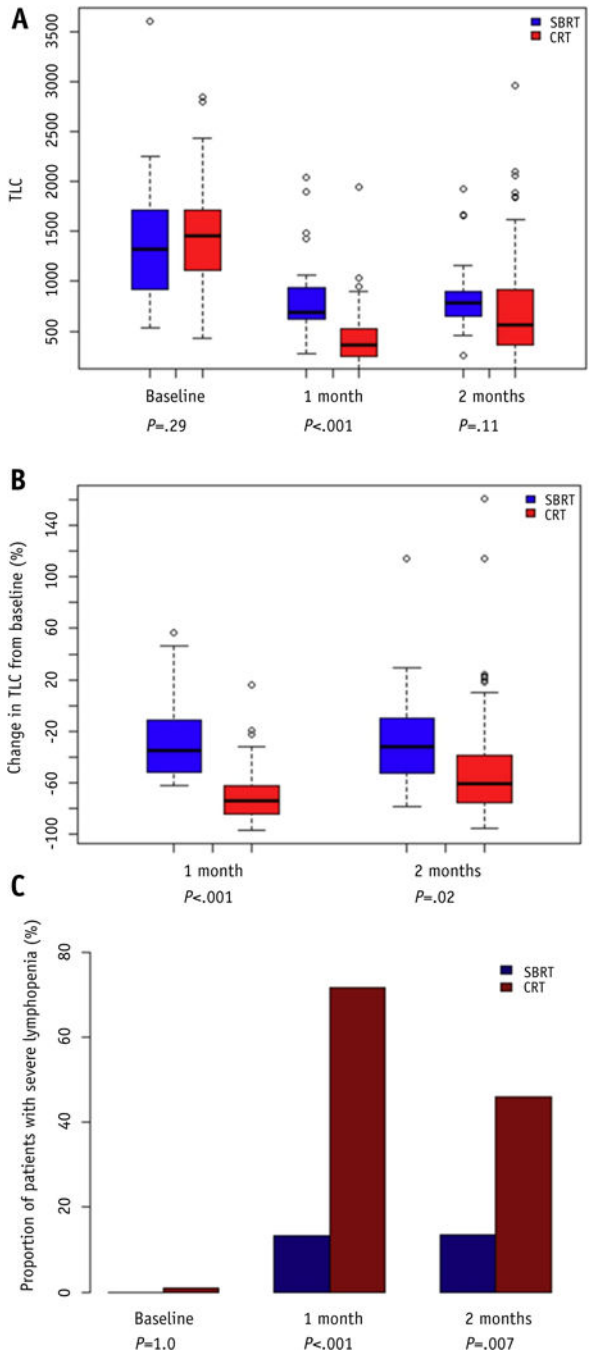


**Fig. 2.**

Graphic summary of the development of lymphopenia in the SBRT and CRT cohorts. All box-and-whisker plots show median (middle horizontal line), 75th percentile (top horizontal line), 25th percentile (bottom horizontal line), 91st percentile (top whisker), and 9th percentile (bottom whisker) for TLC obtained at baseline and at 1 and 2 months after starting radiation therapy. (A) TLC; (B) percentage of change in TLC per patient; (C) percentage of patients with severe lymphopenia. *Abbreviations:* CRT = chemoradiation therapy; SBRT = stereotactic body radiation therapy; TLC = total lymphocyte count.

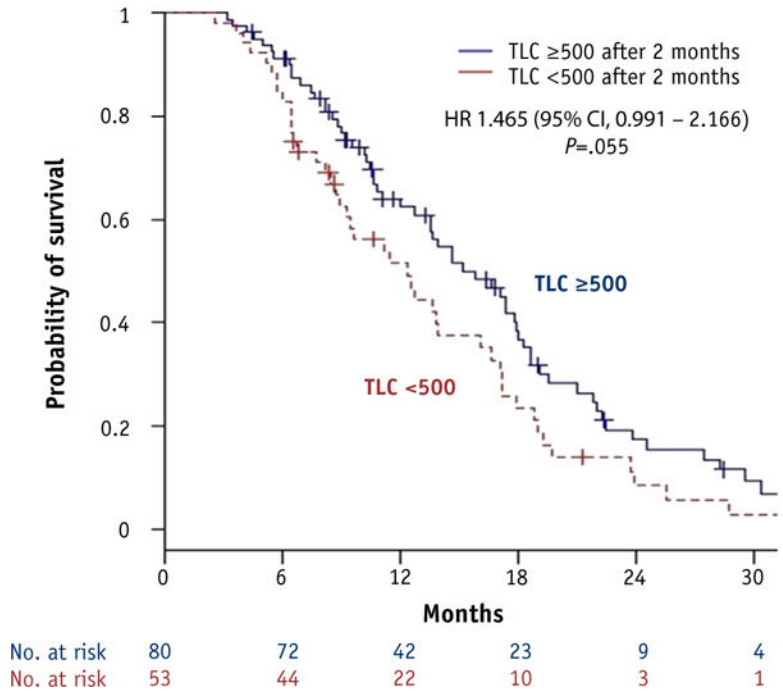


**Fig. 3.**

Kaplan-Meier curve showing survival for all patients (n=133), stratified by severe lymphopenia (TLC: <500 cells/mm$^3$) 2 months after starting radiation therapy. HR and *P* values are derived from univariate Cox regression analysis. Censored patients are represented by +. *Abbreviations:* CI = confidence interval; HR = hazard ratio; TLC = total lymphocyte count.

## Table 1

Demographic, disease, and treatment characteristics for the SBRT (n=32) and CRT groups (n=101)

| Characteristic | SBRT group (n=32) | CRT group (n=101) | *P* value |
|---|---|---|---|
| Demographic | | | |
| Median age (IQR) (y) | 67 (59–74) | 63 (55–69) | .06 |
| Females (%) | 13 (41) | 44 (44) | .84 |
| No. with ECOG PS 0 * (%) | 15 (47) | 43 (43) * | .69 |
| No. with ECOG PS 1 (%) | 16 (50) | 43 (43) * | .54 |
| No. with ECOG PS 2 (%) | 1 (3) | 4 (4) * | 1 |
| No. of whites (%) | 28 (88) | 76 (75) | .22 |
| Baseline disease | | | |
| No. of tumors at pancreatic head (%) | 27 (84) | 76 (75) | .49 |
| Median tumor diameter (IQR) (cm) | 4.6 (3.8–5.7) | 3.6 (2.9–4.7) | .01 |
| Histologic grade: no. of adenocarcinoma NOS (%) | 18 (56) | 68 (67) | .29 |
| Median baseline serum CA19-9 (IQR) (U/mL) | 215 (63–670) | 184 (46–716) | .96 |
| Median baseline TLC (IQR) (cells/mm$^3$) | 1320 (920–1710) | 1455 (1115–1712) | .29 |
| Therapy | | | |
| Median radiation dose (IQR) (Gy) | 33.0 (33.0–33.0) | 50.4 (50.0–50.4) | <.001 |
| Median daily radiation fraction size (IQR) (Gy) | 6.6 (6.6–6.6) | 1.8 (1.8–2.0) | <.001 |
| Median PTV (IQR) (cm$^3$) | 88.7 (62–119) | 344.6 (295–541) | <.001 |
| No. of patients who received induction chemotherapy (%) | 27 (84) | 15 (15) | <.001 |
| No. of patients who received concurrent chemotherapy (%) | 0 (0) | 101 (100) | <.001 |
| No. of patients who received maintenance chemotherapy (%) | 31 (97) | 53 (52) | <.001 |
| No. of patients who required unplanned treatment breaks (%) | 1 (3) | 9 (9) | .45 |

*Abbreviations:* CA19-9 = carbohydrate antigen 19–9; CRT = chemoradiation therapy; ECOG PS = Eastern Cooperative Oncology Group performance status; IQR = interquartile range; NOS = not otherwise specified; PTV = planning target volume; SBRT = stereotactic body radiation therapy; TLC = total lymphocyte count.

* Data for ECOG PS was missing in 7 patients in the CRT group.

## Table 2

Comparison between SBRT and CRT lymphocyte counts before and after RT

| Lymphocyte count | SBRT group (n=32)* | CRT group (n=101)* | *P* value |
|---|---|---|---|
| Baseline | n=27* | n=100* | |
| Median TLC (IQR) (cells/mm$^3$) | 1320 (920–1710) | 1455 (1115–1712) | .29 |
| No. of patients with severe lymphopenia (TLC: <500/mm$^3$) (%) | 0 (0) | 1 (1) | 1.00 |
| One month after starting radiation therapy | n=29* | n=99* | |
| Median TLC (IQR) ((cells/mm$^3$) | 690 (625–940) | 358 (250–525) | <.001 |
| No. of patients with severe lymphopenia (%) | 4 (13.8) | 71 (71.7) | <.001 |
| Reduction in median TLC % per patient (IQR) | 35.0 (10.9–51.6) | 74.0 (62.5–84.3) | <.001 |
| Two months after starting radiation therapy | n=22* | n=101 | |
| Median TLC (IQR) (cells/mm$^3$) | 780 (653–898) | 560 (360–920) | .11 |
| No. of patients with severe lymphopenia (%) | 3 (13.6) | 46 (46.0) | .007 |
| Reduction in median TLC % per patient (IQR) | 32.1 (13.1–52.4) | 60.7 (38.8–75.2) | .02 |

*Abbreviations:* CRT = chemoradiation therapy; IQR = interquartile range; SBRT = stereotactic body radiation therapy; TLC = total lymphocyte count.

*Not all patients in the SBRT and CRT groups had TLC data available at each time point; therefore, the number of patients from each group contributing to the tabulated data is listed for each time point.

## Table 3

Multivariate analysis (logistic regression approach) of predictors of severe lymphopenia (TLC < 500 cells/mL) at 1 month after radiation

| Characteristic | Hazard ratio (95% CI) | *P* value |
|---|---|---|
| Multivariate associations 1 month after RT | | |
| CRT vs SBRT | 13.92 (21.5–90.22) | .006 |
| Baseline TLC | 1.00 (1.00–1.00) | .03 |
| Females vs males | 0.50 (0.16–1.54 | .23 |
| Race: white vs other | 2.48 (0.67–9.15) | .17 |
| Tumor location (body/tail vs other) | 1.61 (0.43–6.00) | .48 |
| Received induction chemotherapy (yes vs no) | 0.30 (0.08–1.06) | .06 |
| Multivariate association 2 months after RT | | |
| CRT vs SBRT | 4.00 (0.58–27.62) | .16 |
| Baseline TLC | 1.00 (1.00–1.00) | .23 |
| Race: white vs other | 0.62 (0.19–2.06) | .44 |
| ECOG performance status (1 or higher vs other) | 1.93 (0.71–5.20) | .20 |
| Tumor location (body/tail vs other) | 1.67 (0.49–5.69) | .42 |
| Maximum tumor diameter | 1.14 (0.82–1.60) | .43 |
| Received induction chemotherapy (Yes vs no) | 0.32 (0.08–1.25) | .10 |

*Abbreviations:* CI = confidence interval; CRT = conventional chemoradiation therapy; ECOG = Eastern Cooperative Oncology Group; CA19-9 = carbohydrate antigen 19-9; TLC = total lymphocyte count; SBRT = stereotactic body radiation therapy.

## Table 4

Univariate and multivariate associations between patient characteristics and survival

| Characteristic | Hazard ratio (95% CI) | *P* value |
|---|---|---|
| Univariate associations | | |
| Age ≥65 vs <65 | 1.083 (0.731–1.602) | .69 |
| Age: continuous | 1.009 (0.992–1.026) | .29 |
| No. of males vs females | 1.001 (0.677–1.481) | .99 |
| Race: other vs Caucasian | 1.146 (0.720–1.823) | .56 |
| ECOG: ≥1 vs 0 | 1.328 (0.894–1.974) | .16 |
| Tumor location: body/tail vs head/uncinate | 1.206 (0.758–1.918) | .43 |
| Histologic grade: poorly differentiated vs other | 1.119 (0.673–1.862) | .66 |
| Tumor diameter: maximum (cm) | 1.033 (0.907–1.175) | .63 |
| Baseline CA19-9 >90 U/mL vs ≤90 U/mL | 1.939 (1.215–3.094) | .005 |
| Baseline lymphocyte count: continuous | 1.000 (1.000–1.000) | .98 |
| Baseline lymphocyte count: ≥ 1000 vs < 1000 * | 1.136 (0.682–1.894) | .62 |
| Severe lymphopenia (TLC <500 cells/mm$^3$) at 2 months † | 1.465 (0.991–2.166) | .055 |
| Induction chemotherapy received prior to radiation: no vs yes | 1.432 (0.909–2.255) | .12 |
| Treatment group: CRT vs SBRT | 1.578 (0.925–2.691) | .09 |
| PTV: continuous (cm$^3$) ‡ | 1.002 (1.001–1.003) | <.001 |
| Radiation treatment break required: yes vs no | 1.657 (0.825–3.331) | .16 |
| Maintenance chemotherapy after radiation: no vs yes | 2.001 (1.352–2.963) | <.001 |
| Multivariate associations | | |
| Maintenance chemotherapy after radiation: no vs yes | 3.171 (1.941–5.181) | <.001 |
| Baseline CA19-9 >90 U/mL | 2.246 (1.387–3.637) | .001 |
| Severe lymphopenia (TLC <500 cells/mm$^3$) at 2 months | 2.059 (1.310–3.237) | .002 |
| Age: continuous | 1.029 (1.007–1.051) | .009 |

*Abbreviations:* CA19-9 = carbohydrate antigen 19-9; CI = confidence interval; CRT = conventional chemoradiation therapy; ECOG = Eastern Cooperative Oncology Group; PTV = planning target volume; SBRT = stereotactic body radiation therapy; TLC = total lymphocyte count.

* Pretreatment lymphocyte count is dichotomized at 1000 cells/mm$^3$ per NCI-CTC threshold for abnormal versus normal lymphocyte counts.

† Lymphocyte count at 2 months was dichotomized at 500 cells/mm$^3$ according to the NCI Common Terminology Criteria for Adverse Events threshold for grades 3 to 4 lymphopenia.

‡ Planning target volume is the total volume of tissue (cm$^3$) receiving ≥95% of the prescribed radiation dose.