ORIGINAL ARTICLE

Changes in systemic immune response after stereotactic ablative radiotherapy

Preliminary results of a prospective study in patients with early lung cancer

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KEY WORDS

immune system, lung cancer, SABR, stereotactic ablative radiotherapy, T cells

ABSTRACT

INTRODUCTION Non-small cell lung cancer (NSCLC) is the most common lung tumor. Conventional conservative treatment in medically inoperable patients with early stage NSCLC has poor outcome. To improve treatment efficacy, stereotactic ablative radiotherapy (SABR) has been developed, which enables the delivery of high-dose radiation to the tumor.

OBJECTIVES This prospective study was conducted to confirm the hypothesis that a sudden death of cancer cells after SABR may lead to changes in systemic immune response.

PATIENTS AND METHODS We enrolled 89 treatment-naive patients with stage T1/2aN0 NSCLC. All patients received SABR, in accordance with treatment standards at our department. Blood samples were collected 3 times: before treatment (n = 89), and then at 2 (n = 86) and 12 weeks (n = 75) after treatment completion to assess the proportion of CD4(+) and CD8(+) T cells, and the expression of T-lymphocyte transcription factors: T-bet, GATA-3, ROR-γt, and FoxP3. Serum C-reactive protein (CRP) levels, absolute neutrophil count (ANC), absolute lymphocyte count, and white blood cell (WBC) count were measured to exclude the impact of nonspecific inflammatory reaction. The expression levels of lymphocyte antigens were measured by flow cytometry.

RESULTS Serum CRP levels, ANC, and WBC count remained stable during the study. We observed slight lymphopenia, which correlated with irradiated lung volume. After SABR, the proportion of CD8(+), CD4(+), as well as the proportion of CD4(+) T cells expressing GATA-3(+), T-bet(+), or ROR-γt(+) increased, while the number of CD4(+)FoxP3(+) cells (specific for regulatory T cells) decreased.

CONCLUSIONS Our findings may suggest that SABR enhances the systemic immune response by increasing the proportion of proinflammatory T-cell subpopulations.

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INTRODUCTION Lung cancer is the leading cause of cancer death worldwide.[1] Non-small cell lung cancer (NSCLC) is the most common lung tumor. Lobectomy with mediastinal lymph node dissection offers a 5-year overall survival rate of about 50% in early stages of NSCLC, and is considered as the treatment of choice.[2,3] The 5-year overall survival rate after combined radical chemoradiotherapy in medically inoperable patients with the earliest stages of NSCLC is about 15%.[4,5] To improve treatment efficacy, stereotactic ablative radiotherapy (SABR) has been developed and adopted during the last decade to enable the delivery of high-dose conformal radiotherapy in a short time of 1 to 5 fractions. It is believed that higher doses are biologically more effective.[6]

Indeed, SABR has shown promising results in medically inoperable NSCLC.[7,8] Unfortunately, 2 independent randomized phase 3 trials, STARS/NCT00840749 and ROSEL/NCT00687986, were terminated prematurely owing to poor accrual. The recently published results of a pooled analysis of those trials have shown the absolute improvement in overall survival of 16% at 3 years, and better treatment tolerance with high-grade toxicity of 10% and 48% for SABR and lobectomy, respectively.[9,10] The risk of nodal or distal metastases in patients treated with SABR is about

15%. The use of SABR made it possible to significantly increase the biologically equivalent dose to achieve a local control rate of 95%, without compromising the tolerance.[6] SABR is nowadays considered a highly efficient treatment modality compared with radical surgery, despite the fact that a direct comparison of the 2 methods is impossible without randomized trials.[11,12]

Cell death induced by high-dose X-ray triggers the occurrence of many new epitopes.[13] The signals generated by irradiated dying tumor cells contribute to radiation-induced antitumor immune response.[14] Radiation-induced antitumor T-cell priming has been described elsewhere.[15-17] A single dose of 10 Gy upregulates CD86 and CD70—the markers of dendritic cell activation.[15,16] Antitumor immune response is a consequence of upregulation of chemokines and cell surface receptors, as well as vascular damage. The optimal radiation regimen to induce such changes has not been defined. Data from in vitro studies have shown proimmunogenic effects of doses varying from 2 to 30 Gy. The assessment of this process in vivo is complicated by immune changes caused by irradiation of the tumor and its microenvironment.[18] Kuo et al[19] has shown that radiation-related induction of galectin 1 is associated with more infiltrating intratumoral CD8(+) T cells, less intratumoral CD4(+), and CD8(+) T-cell apoptosis, and lower microvessel density.

The aim of this prospective study was to confirm the hypothesis that SABR-induced cancer cell death in vivo impacts the systemic immune response profile manifested as alterations in the ratio of helper CD4(+) and cytotoxic CD8(+) lymphocytes, decreased level of T lymphocytes with the expression of the forkhead box P3 (FoxP3) protein (a master transcription factor for regulatory T cells), or changed the proportions of T cells expressing the T-box transcription factor (T-bet), transacting T–cell-specific transcription factor 3 (GATA-3), or retinoic acid-related orphan receptor γt (ROR-γt) antigens (master transcription factors related to Th1-, Th2-, or Th17-type immune responses, respectively) in peripheral blood mononuclear cells (PBMCs). C-reactive protein (CRP) levels and peripheral blood count were measured to exclude the influence of nonspecific systemic reaction to acute inflammation or hematologic suppression on changes in the immune system. Moreover, it has been shown that even small field hypofractionated radiotherapy may cause lymphopenia, which is a well-known negative prognostic factor.[19]

PATIENTS AND METHODS

The study protocol was approved by a local ethics committee. All medically inoperable patients with stage T1/2aN0 NSCLC (according to the 7th edition of the American Joint Committee on Cancer Staging System), excluded from surgery owing to the high risk of the procedure related to comorbidities, treated with SABR in the Department of Clinical Oncology and Radiotherapy, University Clinical Centre in Gdańsk, Poland, were eligible after they provided written informed consent. Blood samples, disease characteristics, and treatment-related data were collected. In accordance with institutional standards and national treatment guidelines, patients were screened with the use of a 18-fluorodeoxyglucose positron emission tomography scan combined with computed tomography (PET-CT) before entering the study. Information about the project was given after referral for SABR following a consultation with a multidisciplinary tumor board.

Study group Patients above 18 years of age, with histopathologically proven NSCLC stage T1/2aN0M0 were eligible. The inclusion of patients without histological proof of NSCLC was possible provided there was a PET-CT scan and clinical confirmation of the disease.

The key exclusion criteria included a history of previous anticancer treatment (chemotherapy, radiotherapy, small molecule inhibitors, immunotherapy), histological diagnosis of small cell lung cancer, a history of, or active, autoimmune disease or allergies requiring immunosuppression, acute and chronic inflammatory diseases, severe asthma, severe chronic obstructive pulmonary disease, concomitant second cancer, and previous organ allograft or allogeneic bone marrow transplantation.

Study flow To assess changes in immune response during the treatment, peripheral blood samples were collected from each patient 3 times: on the first day of treatment (shortly before SABR), and then 2 weeks and 3 months after finishing radiotherapy. At each time point, we checked: 1) the proportion of different subpopulations of T cells in PBMCs by flow cytometry; 2) peripheral blood count: white blood cells (WBCs), absolute neutrophil count (ANC), absolute lymphocyte count (ALC); and 4) serum CRP levels.

Each patient was followed in accordance with the local standard of care.

Radiotherapy treatment SABR was delivered using the linear accelerator (Clinac ® 2300 linear accelerator, Varian Medical Systems Inc. ©, Palo Alto, California, United States), in accordance with the approved institutional standards for radiotherapy, planned and carried out by a specialist in radiation oncology. The protocol allowed the use of 3 equally acceptable techniques: 3-dimensional conformal radiotherapy, intensity-modulated radiotherapy, or volumetric-modulated arc therapy. The total dose and fractionation were selected on the basis of localization of the primary tumor (proximity to critical organs such as the bronchi, big vessels, heart, spinal cord, and ribs). Possible fractionation schedules included: 54 Gy in 3 fractions, 55 Gy in 5 fractions, or 60 Gy in 8 fractions. Treatment plans were evaluated by 2 experienced medical physicists, 2 specialists in

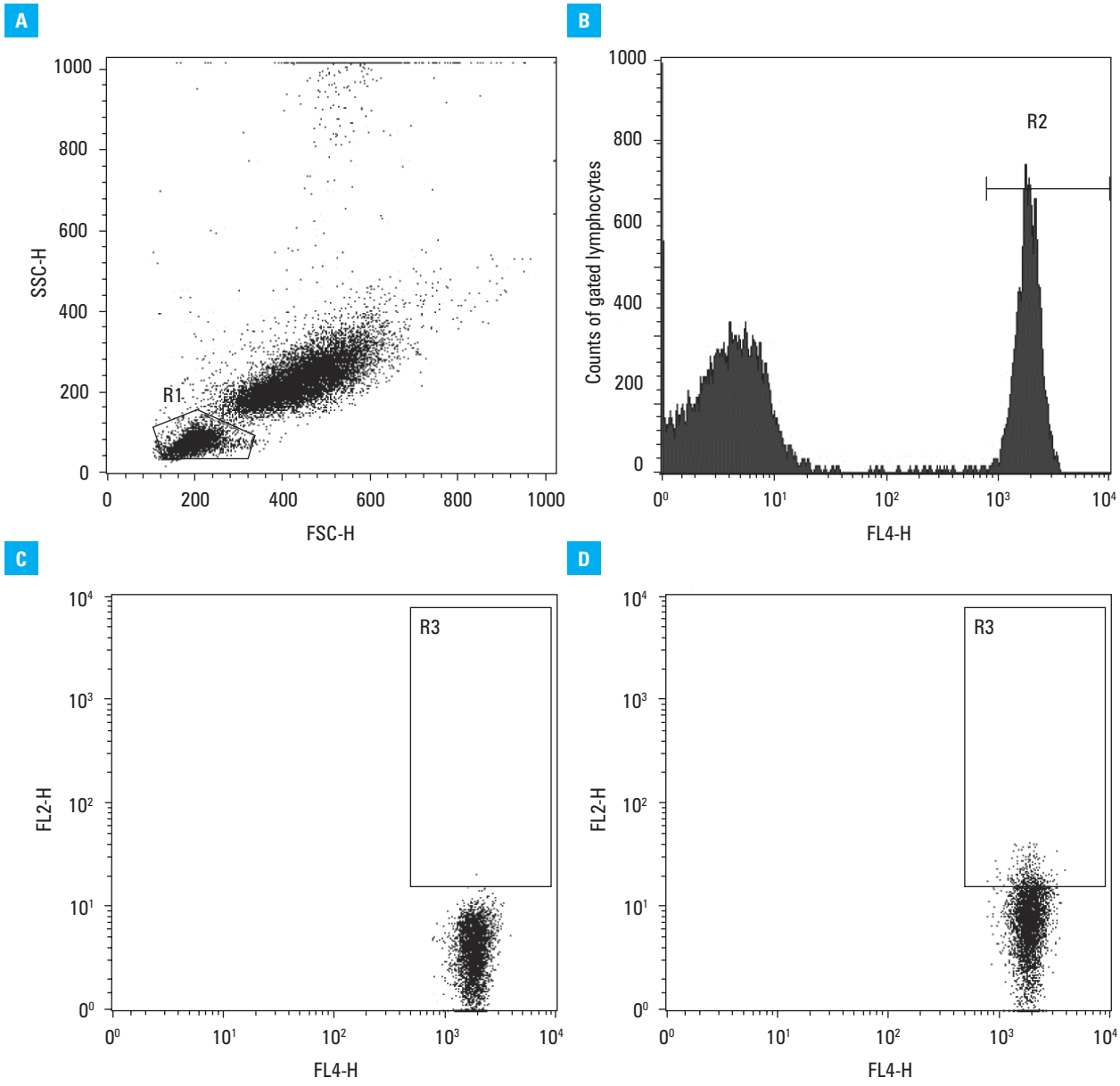

FIGURE 1 Representative flow cytometry plots showing gating strategy and expression of FoxP3 in CD4(+) lymphocytes; **A** – lymphocytes were gated (R1) from the whole population of cells based on forward vs side scatter; **B** – CD4(+) cells were gated (R2) from lymphocyte population; **C** – CD4(+) lymphocytes (gates R1 and R2) stained with isotype control; **D** – CD4(+) lymphocytes (gates R1 and R2) stained with anti-FoxP3 antibody
Abbreviations: FLx-H, fluorescent label channel height; FSC-H, forward scatter height; SSC-H, side scatter height

radiation oncology, and were finally dosimetrically verified. The patients' set-ups were checked before each fraction using cone beam computed tomography and portal imaging.

Flow cytometry Blood samples were processed within 4 hours after collection from patients. A total of 300 µl of EDTA-2K-anticoagulated peripheral blood was stained with allophycocyanin-conjugated antihuman CD4 and CD8 antibodies for 30 minutes at +4°C. Subsequently, red blood cells were lysed with BD PharmLyse buffer, and intracellular staining with phycoerythrin-conjugated antibodies directed against specific human transcription factors or appropriate isotype control antibodies was performed using the Transcription Factor Buffer Set in accordance with the manufacturer's instructions. Separate staining was performed for each investigated antigen, and isotype controls were used to distinguish positive and negative cells. The cells were analyzed on a FACSCalibur flow cytometer using the CellQuest analysis software (equipment, software, and all reagents were sourced from Becton Dickinson Company, San Jose, California, United States). The analyzed cells were gated on lymphocyte population on forward versus side scatter dot-plot. According to intracellular antigens, the data were presented as the percentage of CD4(+) cells. Data for total CD4(+) or CD8(+) cells were presented as the proportion of all gated lymphocytes (FIGURE 1).

The following intracellular antigens (differentiation-inducing transcription factors; BD Biosciences antibodies)[20] were used: 1) T-bet, specific for Th1-cell population; 2) GATA-3, Th2-cell

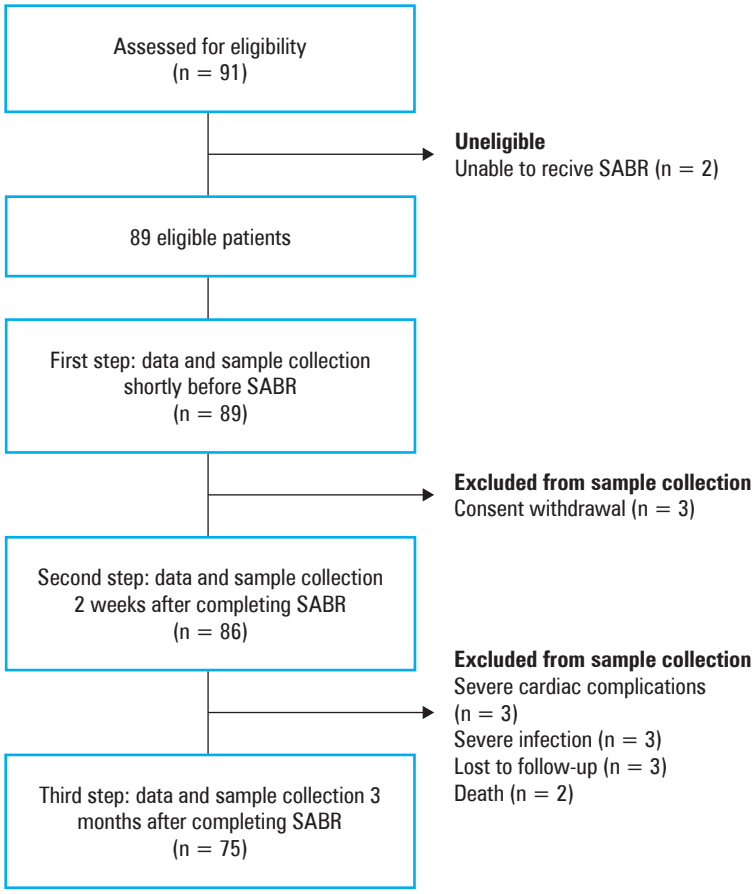

FIGURE 2 Flow chart

TABLE 1 Characteristics of patients (n = 89)

Parameter		Value
Age, y, median (range)		74 (53–87)
Sex, male/female, no. of patients		60/29
Type of cancer, no. of patients	Squamous cell carcinoma	27
	Adenocarcinoma	37
	Unspecified NSCLC	15
	Radiologic diagnosis only	10
TNM, no. of patients	T1	50
	T2a	39
	N0/M0	89
Comorbidities, % of patients	COPD	64
	Congestive heart failure	30.3
	Myocardial infarct (>6 mo before)	25
	Peripheral vascular disease	17.2
Fractionation schedule, no. of patients	54 Gy / 3 fractions	24
	55 Gy / 5 fractions	42
	60 Gy / 8 fractions	23
GTV, cm^3, mean (range)		15.8 (0.9–82.4)
V20 Gy, %, mean		6.1
Mean lung dose, Gy, mean		4.3
Esophagus mean dose, Gy, mean		2.0

Abbreviations: COPD, chronic obstructive pulmonary disease; GTV, gross tumor volume; NSCLC, non-small cell lung cancer, V20 Gy, 20-Gy isodose volume of the lungs; TNM, TNM Classification of Malignant Tumours according to the 7th edition of the American Joint Committiee on Cancer Staging System

master transcription factor; 3) ROR-γt, typical for Th17-type cells; and 4) FoxP3, T–regulatory-cell master differentiation factor. The following surface antigens were used: 1) CD4, specific marker for T-helper subpopulation of lymphocytes; and 2) CD8, specific marker for effector and cytotoxic T lymphocytes.

Serum CRP levels and peripheral blood count were measured using standard laboratory methods.

Statistical analysis The data were analyzed using the StatSoft Statistica 10 software (www.statsoft.com). The normality of the data distribution was tested using the Kolmogorov–Smirnov test. Nonparametric tests were used to analyze variables with nonnormal distribution. Dependent variables were evaluated using the Freedman analysis of variance (ANOVA) test with the Kendal coefficient of concordance. To identify significant correlations between the analyzed parameters, we calculated Spearman rank correlation. A *P* value of less than 0.05 was considered statistically significant.

RESULTS

Baseline characteristics of the study group Between November 2013 and January 2016, 89 of 91 eligible patients were enrolled to the study. The study flow is shown in FIGURE 2. All included patients received SABR: 24 patients in 3 fractions; 42, in 5; and 23, in 8. PBMCs were collected according to the study flow: before SABR (n = 89), and at 2 weeks (n = 86) and 3 months (n = 75) after SABR. Baseline clinical characteristics of patients are outlined in TABLE 1. The median follow-up after treatment was 17.4 months. There were only 12 clinical events (progression or death). Statistical analysis of clinical outcome was impossible due to too short follow-up of the enrolled patients at the time of the study.

Evaluation of intracellular transcription factors The analysis of intracellular transcription factors is summarized in TABLE 2. Flow cytometry assays showed an early (at 2 weeks after SABR) 3-fold increase in the peripheral blood fraction of GATA-3(+)CD4(+) lymphocytes, which are specific for Th2-type immune response. A further increase in GATA-3(+)CD4(+) among PBMCs was detected 3 months after SABR.

Similarly, 2 weeks after SABR, a 2-fold increase in the level of lymphocytes with the expression of T-bet transcription factor, specific for Th1--cell population, was detected; however, this rise was immediately followed by slow, steady decline (FIGURE 3).

During the study period, the ratio of FoxP3(+) to ROR-γt(+)CD4(+) cells has changed significantly. Three months after SABR, a reduction in the percentage of FoxP3(+)CD4(+) cells and a simultaneous increase in the Th17-type cells (ROR-γt(+)) resulted in a significant decrease of the mean ratio of FoxP3(+) to ROR-γt(+)CD4(+),

TABLE 2 Overall results of the expression of lymphocyte transcription factors, surface antigens, peripheral blood count, and serum C-reactive protein level in all patients at 3 timepoints

Parameter	Baseline	2 weeks after SABR	3 months after SABR	*P* value	*W*
Transcription factors, % of CD4(+) cells[a]					
GATA-3	5.25 (3.5–7.0)	12.48 (10.0–14.9)	22.61 (19.9–25.3)	<0.00001	0.65
T-bet	4.50 (3.3–5.6)	8.90 (7.4–10.3)	7.0 (6.2–7.8)	<0.00001	0.44
ROR-γt	3.50 (3.0–4.0)	4.02 (3.3–4.7)	7.10 (6.2–7.8)	<0.00001	0.40
FoxP3	6.75 (6.1–7.4)	8.40 (7.3–9.5)	5.70 (5.2–6.3)	0.0001	0.20
FoxP3/ROR-γt ratio	3.42 (1.7–5.2)	4.56 (3.1–6.0)	1.12 (0.8–1.5)	<0.00001	0.50
Surface antigens, % of all gated lymphocytes[a]					
CD4	37.80 (36.4–39.2)	40.08 (37.9–42.3)	37.88 (36.2–39.5)	0.032	<0.1
CD8	22.60 (21.5–23.8)	26.65 (24.7–28.5)	24.70 (23.5–25.9)	0.001	0.1
Peripheral blood count and serum CRP level[b]					
WBC count	7.87 (7.2–8.5)	7.03 (6.4–7.6)	7.57 (6.9–8.2)	0.001	<0.1
ALC	1.96 (1.7–2.1)	1.49 (1.4–5.4)	1.65 (1.5–1.8)	<0.00001	0.25
ANC	4.88 (4.4–5.4)	4.58 (4.1–5.1)	4.90 (4.4–5.4)	0.13	NA
CRP	5.54 (3.4–7.1)	5.8 (4.4–8.9)	7.1 (4.0–11.2)	0.7	NA

Data are presented as:
a mean % of cells expressing given antigen
b mean level of blood cells (10^9/l) or CRP (mg/l). 95% CI shown in brackets. Friedman analysis of variance test with Kendall coefficient of concordance was used (*W*).

Abbreviations: ALC, absolute lymphocyte count; ANC, absolute neutrophil count; CRP, C-reactive protein; WBC, white blood cells

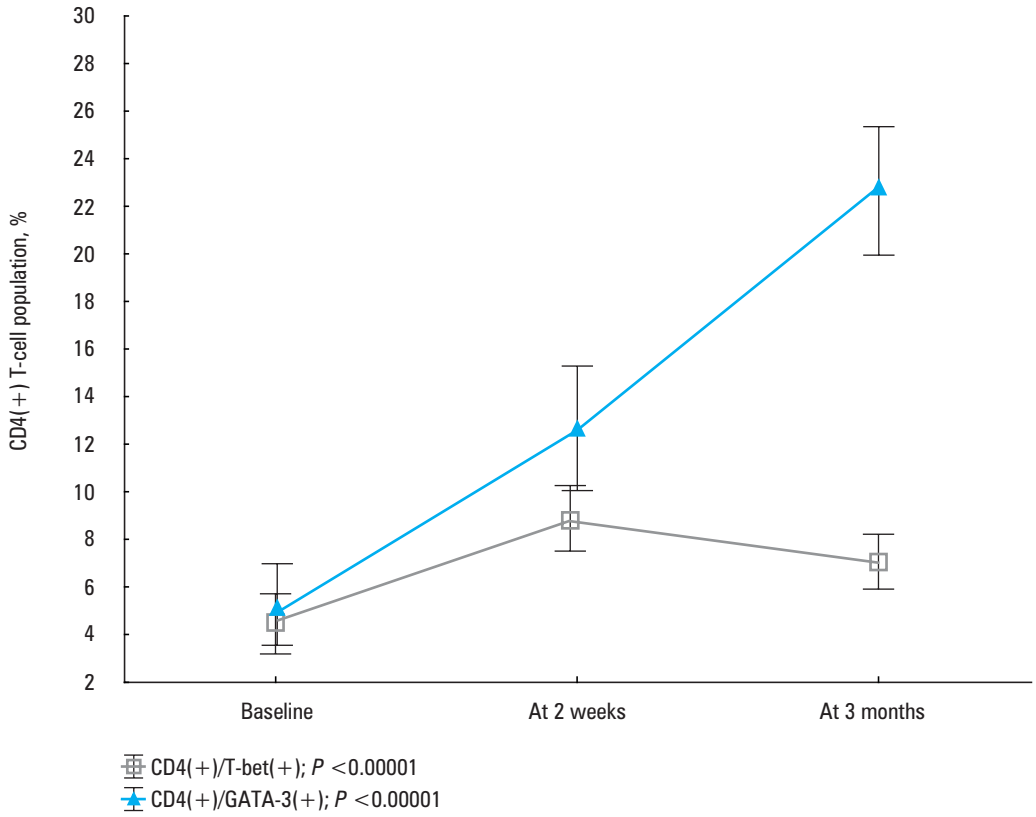

FIGURE 3 Percent of GATA-3 (Th2) positive or T-bet (Th1) positive cells in the whole CD4(+) T-cell population at baseline and at 2 weeks and 3 months after radiotherapy; symbol, mean count; error bars, mean ±95% CI

from 3.42 before treatment to 1.12 (TABLE 2, FIGURE 4).

CD4(+) and CD8(+) lymphocyte count The analysis of PBMCs showed a significant increase in CD4(+) and CD8(+) cells 2 weeks after SABR. At 3 months, only CD8(+) lymphocytes remained elevated (TABLE 2).

Peripheral blood count and serum C-reactive protein Serum CRP level, WBC count, and ANC remained stable in all patients during the study period. However, 2 weeks after SABR, we observed a slight, but significant, lymphopenia ($P < 0.001$), followed by full recovery to normal values at 3 months (TABLE 2).

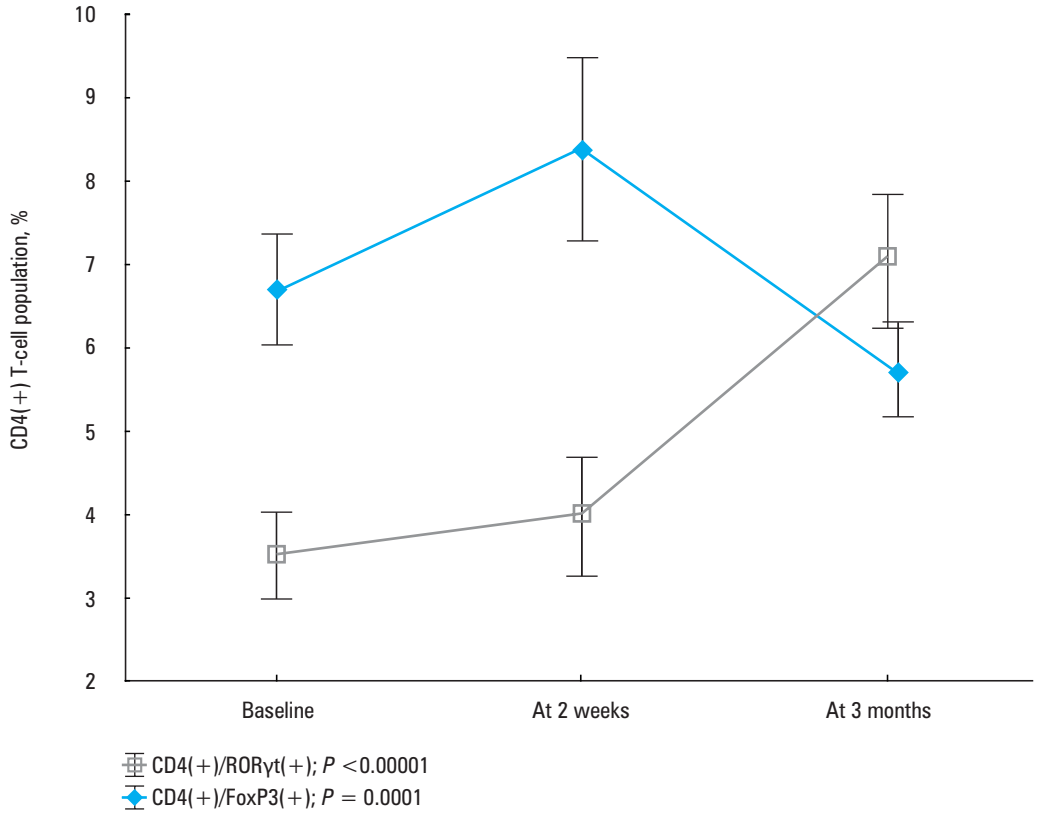

FIGURE 4 Percent of ROR-γt (Th17) positive or FoxP3 (T-reg) positive cells in whole CD4 (+) T-cell population at baseline and at 2 weeks and 3 months after radiotherapy; symbol, mean count; error bars, mean ±95% CI

Dosimetric parameters of healthy tissues and selected immune factors The exposure of healthy tissue to X-rays was small (TABLE 1). None of the variables: the SABR schedule, dosimetric parameters such as lung volume exposed to ≥20 Gy (V20 Gy, 20-Gy isodose volume of the lungs), mean lung dose (MLD), esophagus mean dose, as well as other radiotherapy parameters correlated with the immune system parameters, serum CRP levels, or WBC count and ANC. The MLD significantly correlated with lymphopenia (r = 0.47, P <0.0002; FIGURE 5), which was more frequent in the group of patients with an MLD above 3.7 Gy (P = 0.005, χ^2 test, and Kruskal–Wallis ANOVA test; FIGURE 6). Moreover, the most serious and early grade 1 lymphopenia (lymphocyte count $<0.8 \times 10^9$ /l at 2 weeks; National Cancer Institute, Common Toxicity Criteria for Adverse Events v. 4.03) was detected in 6 patients with an MLD higher than 3.7 Gy.

Correlations of immune system parameters with clinical characteristic of patients We did not find any significant correlations between the analyzed immune system parameters and any of the disease-related characteristics or nononcological comorbidities such as chronic obstructive pulmonary disease or cardiovascular diseases (Spearman ANOVA and Kruskall–Wallis tests were performed; data not shown).

DISCUSSION

Radiation-induced changes in the immune system have been investigated for many years, and postradiation alterations of different immune mechanisms have been described in multiple in vitro studies. Little is known about the role of immune mechanisms in the progression of NSCLC, and data on changes in the immune system in patients treated with radiotherapy are anecdotal.

High efficacy of SABR in lung cancer cannot be explained by direct effect of X-rays, and many other mechanisms, including immune response, may be involved.[21,22] The advantage of radiosurgery, such as that used in our study, is the delivery of very high doses of radiation in a relatively short time, only to the gross tumor surrounded by a very small margin of healthy tissue. This technique improves treatment tolerance and, at least in theory, significantly reduces the risk of a decreased immune response.[23] The formation of free radicals and decreased hypoxia following tumor shrinkage are frequently observed in addition to direct effects of ionizing radiation on DNA. Sudden death of cancer cells is accompanied by a release of large amounts of multipeptide cancer-specific antigens (CSAs). When combined with the above effects, it may affect the secretion of immunomodulating cytokines and chemokines.[16]

Rapid release of antigens induced by very high ablative doses of radiation might trigger a chain of reactions resembling vaccination.[24] As showed by Dewan et al,[25] the described phenomenon may be used to improve response to immunotherapies. Animal studies conducted by Huang et al[26] and Filatenkov et al[27] indicated that radiotherapy damages the immunosuppressive tumor microenvironment and subsequently leads to the recruitment of immune cells such as antigen-presenting cells, T cells, and NK cells, which are crucial in antitumor reactions.[28] Bernstein et al[29] have shown that a single dose of 5 Gy, 10 Gy, or 15 Gy increases the expression of costimulatory molecules and

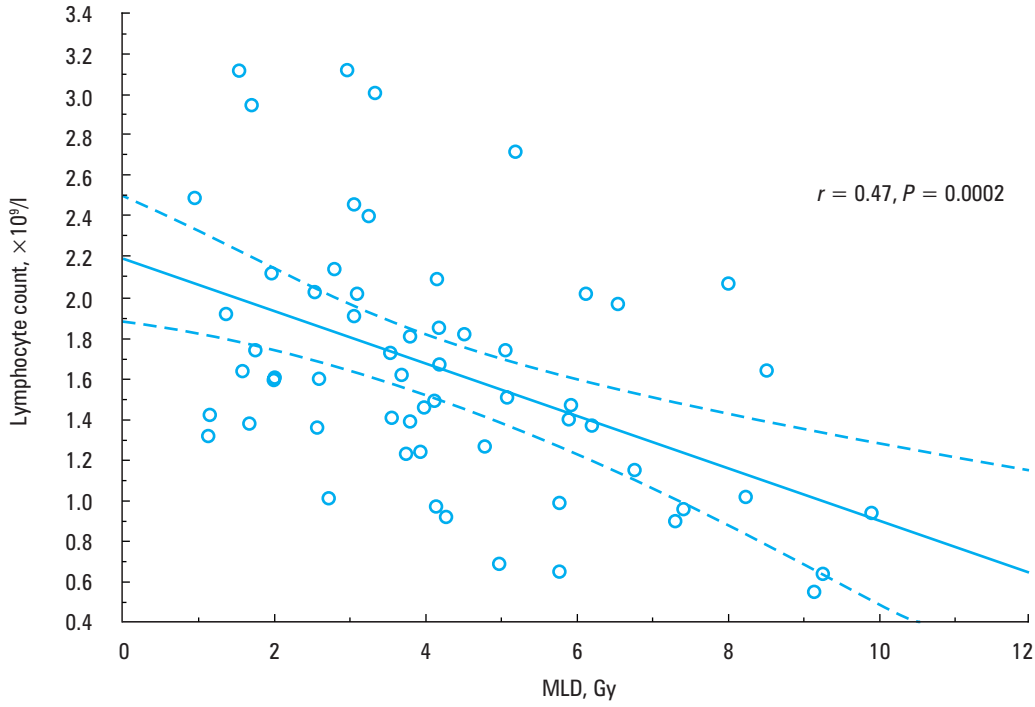

FIGURE 5 Correlation between absolute lymphocyte count 3 months after stereotactic ablative radiotherapy and mean lung dose (MLD)

decreases coinhibitory signaling proteins in prostate cancer cells in vivo, leading to the activation of cytotoxic T lymphocytes and increased production of interferon γ. Such mechanisms could be effective even in the absence of immunogenic cell death, and would be useful against radioresistant cancer cells. The combination of SABR with immunotherapy has been termed ISABR.[30,31] Prospective trials on radiotherapy with immune checkpoint blockade have been started (ClinicalTrials.gov: NCT02 303 990).[32]

The results of our prospective study show that SABR leads to a variety of changes in systemic immune response in patients with NSCLC. In treatment-naive patients, similar peripheral blood levels of CD4(+)T-bet(+) and CD4(+)GATA-3(+) cells (characteristic for Th1- and Th2-type cells, respectively) with a higher proportion of T cells expressing the FoxP3 transcription factor (polarized towards regulatory T cells) and a low level of cells with expression of the Th17 master transcription factor ROR-γt were detected.

Three months after treatment completion, the mean ratio of FoxP3(+) and ROR-γt subpopulations was 1.12, compared with 3.42 before irradiation. We believe that reversing the ratio of FoxP3(+) to ROR-γt(+) CD4(+) T-cell percentage might be important in the immune-related mechanism of SABR. Experimental data published by Zhao et al[33] and Zhang et al[34] show that a high ratio of FoxP3(+)/ROR-γt(+) CD4(+) T cells correlates with more advanced stage of NSCLC. Since the elevated ratio of FoxP3(+) to ROR-γt(+) ratio predicts poor prognosis, its reduction may improve the clinical outcome of patients treated with SABR.[35]

In our study, the most significant change after SABR was observed in the expression of Th2-type CD4(+) T-cell lymphocyte transcription factor, GATA-3, which is a master regulator of humoral immune reactions.[36] The count of Th1-polarized T cells with the expression of transcription factor T-bet, which is known to stimulate an adaptive cytotoxic cell response, has also been elevated after SABR.[37] The growth arrest in the percentage of T-bet(+) cells after 3 months is probably connected with the excess of GATA-3(+) cells, which are known to suppress the growth of Th1–T--cell line.[38] Hernandez et al[39] described a phenomenon of a positive feedback loop between the GATA-3 transcription factor and the activated T-lymphocyte receptor, whose activation depends on the presence of antigens. Thus, the accumulation of GATA-3(+) cells may be connected with the overexpression of various CSAs. This accumulation suggests long-term systemic immune effects of SABR on the gross lung tumor.

Another systemic immune reaction might be reflected by simultaneous elevation of CD4(+) and CD8(+) cells 2 weeks after SABR completion. This may suggest rapid stimulation of adoptive, especially cellular, immune response triggered by CSAs and enabled by the decrease of suppressive reactions of the tumor microenvironment.[40] Early enhancement of CD4(+) cells is essential for durable immunity, especially in accordance with dendritic cell stimulation by CSAs.[41,42] In contrast to decreased level of FoxP3(+) cells, these results suggest SABR stimulation of cellular immune response. Long-lasting elevation of CD8(+) cells 3 months after SABR may suggest slight but prolonged promotion of cytotoxic cell production.[40,43,44] These findings are supported by increased expression of transcription factors, which may lead to polarization towards more proinflammatory cell types like Th1, Th2, and Th17, observed after SABR in our study.

As mentioned earlier, we did not observe any correlation between the volume of irradiated healthy tissue or nononcological comorbidities

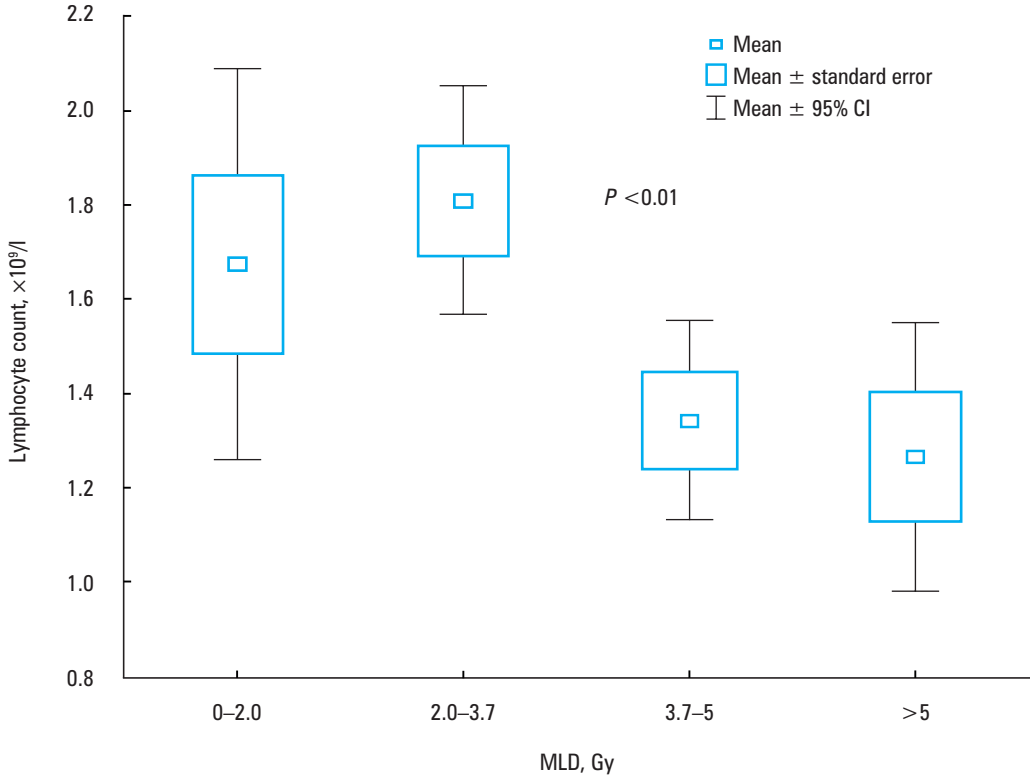

FIGURE 6 Absolute lymphocyte count 2 weeks after stereotactic ablative radiotherapy in patient subgroups divided according to the mean lung dose (MLD); χ^2 test and Kruskal–Wallis analysis of variance test, both $P < 0.01$

and immune changes. This suggests that observed immunological phenomena were directly related to SABR-induced changes in cancer cells.

It is believed that high exposure to radiotherapy triggers systemic immunosuppression and uncontrollable inflammation. A highly increased level of CRP, as well as decreased ALC and ANC, can be detected very early, within days after total- or half-body irradiation, indicating radiation-induced damage of hematopoietic progenitor cells with simultaneous acute immune system disruption.[45,46] Our study results show that SABR does not cause such severe hematologic and immune disorders reflected by CRP level, WBC count, and ANC. However, ALC significantly decreased early after starting the treatment. The risk of lymphopenia correlated with MLD. Kuo et al[19] showed a similar effect in a small study of patients with stage I–II NSCLC treated definitively with SABR.[19] Lymphopenia detected in 15 of 20 patients (75%) was associated with worse overall survival (hazard ratio, 1.148) and disease-free survival (hazard ratio, 1.134). This explains the utmost importance of lung tissue sparing. Data from small studies suggest that lymphopenia and elevation of CRP levels after radical radiotherapy in lung cancer patients are negative predictive factors, but this finding warrants further research.[47-49]

Considering all the observed changes of the percentage of PBMC subpopulations, such as the increased proportion of CD4(+), CD8(+) cells, and proinflammatory type of T helpers expressing the major transcription factors of Th1-, Th2-, or Th17-type cells, while reducing the number of FoxP3(+) Th-type cells (polarized towards regulatory T-cell subpopulation) indirectly prove that SABR may enhance the immune system by changing the systemic immune profile towards more active types of adaptive response.[50] Interestingly, we observed that SABR does not induce nonspecific acute inflammatory reaction or severe hematologic toxicity. The results of our study confirm the presence of immune changes after SABR in patients with early lung cancer.

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Contribution statement JR conceived the idea of the study and performed formal analysis of the data. JR and TS contributed to the design of the study, methodology, and software. JR, TS, and RZ contributed to original draft preparation. RZ coordinated funding for the project. RZ and ZK reviewed the manuscript and supervised the project. All authors edited and approved the final version of the manuscript.

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