University of Turin

UNIVERSITA
DEGLI STUDI
DI TORINO

Department of Oncology

Doctoral School in Life and Health Sciences

PhD Program in Biomedical Sciences and Oncology

Director: Prof. Emilio Hirsch

Title:

Stereotactic radiation therapy: clinical aspects, integration with new drugs and prognostic/predictive biomarkers.

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External reviewers: Prof.ssa Sara Ramella, Prof. Antonio Russo

PhD program cycle: XXIX

Academic years: 2014/2015-2015/2016-2016/2017-2017/2018

Settore scientifico disciplinare: MED/36

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Summary of the research activity during PhD and PhD project

Objective

The focus of this PhD research program was a) to evaluate the role of radiation therapy in different setting of oligometastatic cancer, through various approaches, including technical and clinical assessments, b) to study, with translational approach in a multidisciplinary setting, the link between immuno-modulation/inflammation and radiotherapy again with heterogeneous experimental strategies, including a main area of research on plasmatic changes of cytokines as potential prognostic/predictive biomarkers in lung stereotactic radiotherapy.

Background and Methodology

Due to advancement of imaging and diagnosis, a higher number of oligometastatic patients are identified. Nowadays oligometastatic patients are attracting the scientific international oncological community and it is a new and relevant challenge to establish what is the correct definition of oligometastasis.

In particular in non-small cell lung cancer setting, recent prospective studies are evaluating the possibility to combine a systemic treatment with the prescription of local radiotherapy. We tackled this issue, with an editorial, through a brief revision of current literature and on-going trials. Additionally, we reported our personal experience, publishing a retrospective study focused on the impact of a combination of tyrosine kinase in epidermal growth factor-mutant or anaplastic lymphoma kinase rearrangement-positive metastatic non-small cell lung cancer and radiotherapy. We observed interesting results in terms of survival and toxicity profile.

Indeed, in other histologies the definition of oligometastastic patient is unclear and an appropriate selection to local treatment is a crucial issue. For this reason, we evaluated the impact of local ablative radiotherapy in metastatic colon-rectal cancer

after systemic therapy and a prospective Phase II trial including metastatic breast cancer women, reporting good results in outcomes and tolerability.

In lung oligometastatic patients, a Biological Equivalent Dose (BED) of at least 100 Gy_{10} represents the unique predictor factor correlated to efficacy after an ablative radiation treatment. Thus, the radiation oncology community is wondering whether other parameters could be helpful to predict response to stereotactic ablative radiotherapy or to select the subset of patients appropriate for ablative treatment. For this reason, we started to explore different experimental scenarios including the metabolic profile and tumour volume changes of lung lesions by means of PET-CT and Cone-beam CT (Image Guided Radiotherapy) respectively, and translation research on immuno-modulation/inflammation and radiotherapy.

Image Guided radiotherapy based on daily patient set-up position verification not only allowed a better definition of tumour target in order to reduce and eliminate uncertainties, but as we reported in two distinct experiences, it may help to identify radio-sensitive oligometastatic patients.

In fact, we reported that complete response from lung metastasis at 6 months after stereotactic body radiation therapy was significantly associated to values (maximal and mean) of pre-stereotactic ablative radiotherapy PET-CT SUV. Additionally, lung lesion shrinkage of at least 20% at the last session of stereotactic radiotherapy could be predictable of complete response 6 months thereafter. Certainly, further investigations about this topic are needed.

The introduction of technological improvement such as Intensity-modulated radiotherapy and stereotactic ablative radiotherapy has allowed radiation oncologists to prescribe higher dose prescriptions to targets when useful or required. Intensity-modulated radiotherapy is an advancement of 3D-conformal radiotherapy that targets the radiation dose into the tumour, thus minimising the exposure of healthy tissue in several anatomical regions. Furthermore, stereotactic ablative radiotherapy is a novel radiotherapy method that delivers a very high dose of radiation (in a single or a few

fractions) with high precision to the tumour, thus maximising the sparing of surrounding normal tissues.

Additionally, ablative radiotherapy causes the disruption of the tissue architecture and alteration in tumour microenvironment. These events are associated with the proliferation of inflammatory signals detected by the immune system, with the production of cytokines, chemokines and the activation of immune system induces an immunomodulation process.

Hence, the promising modern techniques could improve radiotherapy tolerability, especially in challenging clinical situations, as well as in patients with connective tissue diseases and cancer as we reported in a recent review.

Furthermore, considerable evidence has shown that the risk of treatment-related side effects is higher in patients with HIV than in patients who are immunocompetent. New drugs, such as immunotherapy and targeted therapies, and improvements in radiotherapy technologies, are optimising the effectiveness and tolerability of cancer treatment. Despite these developments, the role of radiotherapy alone or in combination with drugs for HIV patient population remains to be defined. For this reason, a review discussing the role of radiotherapy, with or without chemotherapy or new drugs, in the treatment of cancer in patients with HIV, with a focus on the efficacy and tolerability of this approach on the basis of available evidence has been published.

Finally, an additional translational research issue and the main goal of the PhD program was developed back in 2014 as a multi-institutional scientific collaboration, between the University of Torino (Prof. Umberto Ricardi, Dr. Andrea Riccardo Filippi), Centro di Riferimento Oncologico (CRO) IRCCS – Aviano (Dr. Marco Trovò) and Radiation Oncology Department Sacro Cuore Don Calabria – Negrar (Prof. Filippo Alongi), allowed to develop the PhD project focused on translational approach in particular the association between cytokine concentration and radiotherapy treatment in non-small cell lung cancer.

This Research was conducted with the approval of Institutional Review Board, and funding has been obtained by CRO - Aviano.

In the first part, a pilot study focusing on the kinetic of multiple plasmatic cytokines has been conducted in patients treated with different schedules and radiation techniques (Intensity Modulated Radiotherapy and Stereotactic Ablative Radiotherapy). The aim of this research was to establish the different expression of inflammatory cytokines in early-stage and locally advanced non-small cell lung cancer after radiotherapy. These approaches induce distinct cytokine changes in non-small cell lung cancer patients, supporting the hypothesis which these two radiotherapy regimes (dose schedules and techniques) could have a different impact on the host immune activity. This study has been published as full paper.

In the second phase of the translational research, we concentrated our attention in the subgroup of patients with a diagnosis of early stage non-small cell lung cancer (Stage IA and IB) treated with stereotactic ablative radiotherapy. Aim of this study is to define a correlation between interleukin (IL)-13 concentrations measured pre and post stereotactic ablative radiotherapy treatment and cancer specific survival. Additionally, we evaluate the correlation between IL-13 level and the risk to develop severe radiological acute and late lung toxicity. IL-13 is a pleiotropic Th2 cytokine involved in the regulation of biological systems and it is one of the most recent and relevant cytokines currently under investigation for its possible role in cancer promotion. Here, we reported the unpublished of this preliminary pilot study results focused on primary non-small cell lung cancer.

Manuscript 1:

Ricardi U, **Giaj Levra N**, Badellino S, Alongi F.

Role of consolidative stereotactic ablative radiotherapy in patients with oligometastatic non-small cell lung cancer.

J Thorac Dis. 2017;9:2235-2237. doi: 10.21037/jtd.2017.06.133.

This editorial is a comment on the role of local stereotactic ablative consolidative therapy in patients with oligometastatic non-small cell lung cancer and a brief revision of current literature and ongoing trial.

Editorial

Role of consolidative stereotactic ablative radiotherapy in patients with oligometastatic non-small cell lung cancer

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Provenance: This is a Guest Editorial commissioned by the Section Editor Dr. JianJun Qin (Division of Thoracic Surgery, Henan Cancer Hospital, Zhengzhou University, Zhengzhou, China).

Comment on: Barton MK. Local consolidative therapy may be beneficial in patients with oligometastatic non-small cell lung cancer. CA Cancer J Clin 2017;67:89-90.

Submitted Jun 21, 2017. Accepted for publication Jun 22, 2017.
doi: 10.21037/jtd.2017.06.133
View this article at: http://dx.doi.org/10.21037/jtd.2017.06.133

Oligometastatic non-small cell lung cancer (NSCLC), presenting with one to five synchronous or metachronous metastatic lesions, has recently been considered a distinct disease state (1). In this setting, three different clinical conditions can be identified: (I) *de novo* oligometastatic—patients with a synchronous diagnosis of primary and metastatic lesions naive from oncological treatments; (II) oligorecurrent—patients with a controlled primary tumor after loco-regional treatment but with new and limited metastatic sites; (III) oligoprogressive—patients with a limited metastatic progression during systemic therapy (one or few sites), but with a control of the primary tumor and most of metastatic disease (2).

Locally ablative therapies are often used for such clinical presentations, alone or in combination with systemic chemotherapy/molecular target therapies/immunotherapy; however, the subset of patients who may benefit from these interventions at metastatic sites or at the primary lesion has not been conclusively identified. These issues are reflected by the heterogeneous survival outcomes reported in several retrospective and a limited number of prospective studies on oligometastatic lung cancer (3).

Stereotactic ablative radiotherapy (SABR) has been considered an emerging therapeutic approach: recent technological improvements, including high accuracy in patient positioning verification systems, image guidance and intensity modulated radiation delivery, allow clinicians to focus ablative radiation doses on small cancer volumes, maximising the sparing of surrounding normal tissues, and promote the potential role of SABR in oligometastatic settings, with high rates of local control for different anatomical districts from various primary tumor sites in absence of relevant toxicity (4).

A recent study in *Lancet Oncology*, reported a randomised, controlled, phase 2 trial, including patients with oligometastatic NSCLC (5). Patients were randomized to receive a local consolidative treatment to all metastatic sites (radiotherapy or surgery), followed by a maintenance systemic therapy or an exclusive maintenance systemic approach.

After a median follow-up of 12.4 months, median progression-free survival in the local consolidative therapy group was 11.9 *vs.* 3.9 months in the maintenance treatment group (HR =0.35; 90% CI, 0.18–0.66; P=0.0054).

Authors concluded that local consolidative treatments for metastatic NSCLC patients with limited number of metastatic sites is able to improve progression-free survival compared to exclusive maintenance therapy. Moreover, a phase 3 randomized clinical trial was recommended to confirm this hypothesis, encouraging a new therapeutic approach in oligometastatic NSCLC patients. In an editorial published by *CA: A Cancer Journal for Clinicians* in March 2017, Barton underlined the importance of this design that reflects real-world treatment approach, and will make clinicians and patients more comfortable with the approach of consolidative local therapy (6).

Several aspects should be considered when radiotherapy as local treatment is offered to oligometastatic patients: the appropriate patient selection, the radiation dose prescription

and the treatment tolerability. Moreover, clinical outcomes seem to be influenced by several factors, including a longer disease-free interval between cancer diagnosis and prescription of local treatment, adenocarcinoma histology, absence of lymph nodal involvement, lower overall tumor burden, and primary tumor control (7,8). Other additional elements could impact on OS in this scenario are a good performance status, limited nodal disease, presence of epidermal growth factor receptor (EGFR) mutation, and metastases limited to a single organ (9). Moreover, as reported by Rusthoven *et al.*, the predominant pattern of failure in advanced NSCLC after first-line systemic therapy is local recurrence, justifying SABR treatment to improve time to disease progression and postpone the prescription of second-line systemic therapies (10).

The patients enrolled in Gomez *et al.* study met several criteria, including the presence of three or less metastatic lesions, no progression after front-line chemotherapy, no malignant pleural effusion, and the ability to tolerate aggressive local treatment, representing ideal candidates for locally ablative therapy (5). Patients randomized to local consolidate therapy group were treated with various kind hypofractionated regimens, including from palliative schedules to ablative treatment, but specific details about biologically effective dose (BED), total dose prescription and fractionation have not been reported (5).

Another limitation of this study pointed out by Mary Kay Barton is the lacking of data about overall survival (OS). In fact, the marked PFS advantage led to early study closure with OS data not yet mature at the time of reporting (6).

In this study, no patients in either group had a grade 4 adverse event nor died from an adverse event (5). Nevertheless, local ablative treatment in combination with systemic therapy can increase severe toxicities; on the other hand, the probability to discontinue the maintenance therapies, promoting a potential disease progression, could certainly affect QoL. Unfortunately, in Gomez *et al.* study QoL data collection was lacking, limiting a critical opinion about this issue.

Currently, from similar ongoing trials focused on NSCLC and other primary histologies such as SARON (NCT02417662) and ROLE (NCT01796288), or inclusive of multiple oligometastatic tumor types, such as CORE (NCT02759783) and SABR-COMET (NCT01446744) results are awaited.

Finally, another intriguing prospective is represented by the combination of SABR and immunotherapies.

Historically, tumoricidal effect correlated to radiotherapy has been justified by a direct and non-repairable damage of DNA. Conversely, recent literature has started to report a relationship between ablative radiation doses, microenvironment alteration and immune system activation (11). Apparently, local and systemic tumor control seems to depend on a balance between immunosuppressive and immunostimulatory signals generated within the tumor and the immune surveillance. Immune surveillance system is a complex process concerning several immune system cells (i.e., CD8 and CD4 lymphocytic cells, natural killer cells, B lymphocytes and macrophages).

Specifically, radiation seems to be able to create an "in situ" vaccine phenomenon. In fact, it has been reported that different radiation techniques and dose schedules influenced immune system response to tumor through several pathways, including changes in different cytokine expressions, leading to alteration in tumor microenvironment (12). Theoretically, the combination of hypofractionated schedules and immune checkpoint inhibitors could contribute to tumor rejection (13), to prolong survival (14), and rarely to realize abscopal effect (15). Hence, a combination of immunotherapies and SABR may play a role in the treatment of metastatic NSCLC patients.

In conclusion the inclusion of local treatment, such as SABR seems to be a promising treatment option in oligometastatic NSCLC patients. Dr. Gomez says that planned expansion phase 3 studies trials will use OS as the primary endpoint, enroll a larger number of patients, and incorporate novel agents such as immunotherapy into the design (6). Strong coordination, interaction, and collaboration among all professional figures, including medical and radiation oncologists, are crucial to select patient eligible to local treatment in order to offer the most appropriate oncological perspective.

Acknowledgements

None.

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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Cite this article as: Ricardi U, Giaj Levra N, Badellino S, Alongi F. Role of consolidative stereotactic ablative radiotherapy in patients with oligometastatic non-small cell lung cancer. J Thorac Dis 2017;9(8):2235-2237. doi: 10.21037/jtd.2017.06.133

Manuscript 2:

Borghetti P, Bonù ML, Roca E, Pedretti S, Salah E, Baiguini A, Greco D, Triggiani L, Maddalo M, **Giaj-Levra N**, Alongi F, Magrini SM, Buglione M.

Radiotherapy and Tyrosine Kinase Inhibitors in Stage IV Non-small Cell Lung Cancer: Real-life Experience.

In Vivo. 2018;32:159-164. doi: 10.21873/invivo.11219

This respective study investigated the role of conventional radiotherapy and stereotactic body radiotherapy in patients with epidermal growth factor mutant or anaplastic lymphoma kinase rearrangement-positive metastatic non-small cell lung cancer.

in vivo *32*: 159-164 (2018)
doi:10.21873/invivo.11219

Radiotherapy and Tyrosine Kinase Inhibitors in Stage IV Non-small Cell Lung Cancer: Real-life Experience

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Abstract. *Aim: To investigate the role of conventional radiotherapy (RT) and stereotactic body radiotherapy (SBRT) in patients with epidermal growth factor (EGFR)-mutant or anaplastic lymphoma kinase (ALK) rearrangement-positive metastatic non-small cell lung cancer (NSCLC). Patients and Methods: Fifty patients with EGFR-mutated or ALK rearrangement-positive NSCLC were treated at our Institution. Radiotherapy was delivered before, after or concomitantly with tyrosine kinase inhibitors (TKIs). Acute toxicities and overall survival (OS) were assessed. Results: Radiotherapy was performed within 30 days before TKI, concomitantly with TKI and within 30 days after TKI in eight (16%), 33 (66%) and 9 (18%) cases, respectively. The median duration of TKI therapy in the whole series was 11.9 months. The median OS was 19.3 months and 1- and 2-year OS was 71.5% and 36.5%, respectively. The group treated with SBRT had a significant benefit in terms of OS (p=0.043). Only two grade 3 toxicities were reported. Conclusion: RT concomitantly or close to TKI administration in stage IV NSCLC was shown to be feasible and safe. Intriguing data on OS were also reported.*

The treatment-of-choice for patients with stage IV epidermal growth factor (*EGFR*)-mutant or anaplastic lymphoma kinase (*ALK*)-rearranged NSCLC consists of oral tyrosine kinase inhibitors, such as anti-EGFR (gefitinib, erlotinib, afatinib, osimertinib) or anti-ALK (crizotinib, ceritinib, alectinib) tyrosine kinase inhibitors(TKIs). (TKIs), which have replaced cytotoxic chemotherapy as first-line treatment (1, 2). In these patients, the adoption of TKIs allows for median progression-free (PFS) and overall (OS) survival ranging between 8-13 months and 18-25 months, respectively (3, 4).

Almost all patients eventually develop progressive disease, requiring for further treatment. The standard strategy is to switch to a second-line chemotherapy or, when indicated, to a new-generation TKI. Alternatively, in cases of oligoprogression, especially for asymptomatic cases and brain progression, it is now more and more accepted to continue with the first-line therapy and to treat the new sites of progression with a local therapy, such as radiotherapy (RT) (5-9). Oligoprogression represents a condition in which a large part of the disease burden is controlled by systemic therapy, except for a few small sites of involvement, which probably acquired resistance to the drug (10).

There is also a biological rationale underlying the association of local RT (possibly with ablative doses) with TKI inhibitors; some pre-clinical studies showed that TKI down-regulated the proliferative signals triggered by RT (such as radiation-induced autophosphorylation of EGFR. This suggests a potential radiosensitizing effect of TKIs (11).

Unfortunately, despite the great interest in recent years, there is still a lack of data on the benefits and potential side-effects of the association of the two treatments. We, therefore, conducted a retrospective analysis of patients treated at our Institution with conventional-RT or stereotactic body radiotherapy (SBRT) combined with TKIs for *EGFR*-mutant or *ALK* rearrangement-positive stage IV NSCLC.

Patients and Methods

Using an Institutional query system, we identified all patients treated with RT from January 2010 to December 2016 at our Institution concomitantly with TKIs for *EGFR*-mutant or ALK rearrangement-positive stage IV NSCLC. Inclusion criteria of the current retrospective analysis were: (i) patients with stage

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Key Words: NSCLC, TKI, stereotactic radiotherapy.

IV NSCLC receiving anti-EGFR or anti-ALK TKIs; (ii) oligoprogressive disease, defined as ≤4 new metastatic lesions at the time of presentation for radiotherapy; (iii) multi-progressive disease, defined as >4 new lesions, according to Institutional policy, despite some consideration of oligoprogressive disease as up to six new lesions (10).

EGFR and ALK status. Mutation analysis was conducted by extracting DNA from tissue biopsy and identifying *EGFR* exon 19 deletion and exon 21 L858R mutations by standard sequencing and fragment analysis, while fluorescence *in situ* testing was used to detect *ALK* gene translocations.

TKI administration. When RT was delivered concomitantly with TKIs, the dose prescriptions were as follows: Gefitinib: 250 mg/day, erlotinib: 150 mg/day, crizotinib: 500 mg/day, and osimertinib: 80 mg/day, and continued after RT until disease progression or unacceptable toxicity (considered grade 3).

Radiation treatment. We included patients treated with RT during TKI therapy or up to 30 days before or after TKI administration. According to these criteria, patients were divided into three groups: Group A: those who underwent RT no more than 30 days before the beginning of the drug; group B: those who underwent RT no more than 30 days after the definitive suspension of TKI therapy; and group C: those who underwent RT during the administration of TKI. Doses and fractionation of RT depended on the type of disease progression (oligoprogression *vs*. multi-progressive disease), sites of progression (brain *vs*. visceral) and clinical presentation (symptomatic *vs*. asymptomatic).

Outcomes and statistical analysis. To summarize the most relevant features of the clinical variables, descriptive statistics were calculated. The primary endpoint was therapy tolerability, the secondary endpoint was the OS, defined as the time from the date of the beginning of drug treatment to the date of death (any cause) or until the date of the last follow-up. All toxicities reported in the medical records were scored using the Common Terminology Criteria for Adverse Events rating scale (CTCAE 4.0) (11). Toxicity related to RT was defined as adverse events occurring within 90 days at the site of irradiation. Clinical and therapeutic characteristics were analyzed with Chi square test ($p<0.05$). OS was estimated with Kaplan–Meier curves and potential factors affecting OS were investigated at univariate analysis with the log-rank test ($p<0.05$). The statistical analysis was performed with SPSS (version 22.0; IBM Corp., Armonk, NY, USA).

Results

Between January 1 2010 and December 31 2016, 102 patients with a diagnosis of *EGFR*-mutant or *ALK*-rearranged NSCLC who underwent RT were identified. Fifty-two patients were excluded from the study because they had RT more than 30 days from the start or the end of TKI prescription. The total number of patients available for analysis was therefore 50. Their median age was 65 (range=30-84) years and Eastern Cooperative Oncology Group Performance Status was 0 or 1 in 16 (32%) and 29 (58%) cases, respectively. At the beginning of RT, the pattern of progression was defined as oligoprogression in 11 patients (22%), and multi-progression in 39 patients (78%). The majority of the patients (54%) were naïve to previous chemotherapy, 15 (30%) were treated with a first chemotherapy line, eight patients (16%) had received two chemotherapy lines before TKI. Patients characteristics are summarized in Table I.

Table I. *Description of the series*.

Characteristic	N (%)
Age (median 65 years)	
≤65 Years	26 (52)
>65 Years	24 (48)
Performance status	
0	16 (32)
1	29 (58)
2	5 (10)
No. of sites treated with RT	
≤4	11 (22)
>4	39 (78)
Previous CHT	
0	27 (54)
1	15 (30)
2	8 (16)
RT schedule	
SBRT	9 (18)
No SBRT	41 (82)
RT target	
Brain	27 (54)
Bone	19 (38)
Other	4 (8)
RT aim	
Symptomatic	28 (56)
Palliative	13 (26)
Ablative	9 (18)

RT: Radiation therapy, CHT: chemotherapy, SBRT: stereotactic body radiotherapy.

Thirty-four patients were treated with gefitinib, nine with crizotinib, four with erlotinib and three with other TKIs. SBRT was given in nine cases (18%) and mild hypofractionated RT in the remaining 41 patients (82%). RT-treated disease sites were the brain for 27 patients (57%), bone in 19 cases (38%) and other sites (only four cases). RT had ablative aim only for nine cases (18%).

In regard to the timing of RT, eight patients (16%) were classified into group A, nine (18%) in group B and 33 (66%) in group C. The median duration of TKI administration was 11.9 months (range=0.4-59.1 months). Analyzing the specific groups, the median duration of TKI administration was 9.7, 8.3s and 14.2 months, for groups A-C, respectively. Additionally, the median duration of administration of the drug after RT in group C was 4.4 months (range=0.3-49.4 months). Figure 1 summarizes the timing of TKI-RT in the series and in the three different groups.

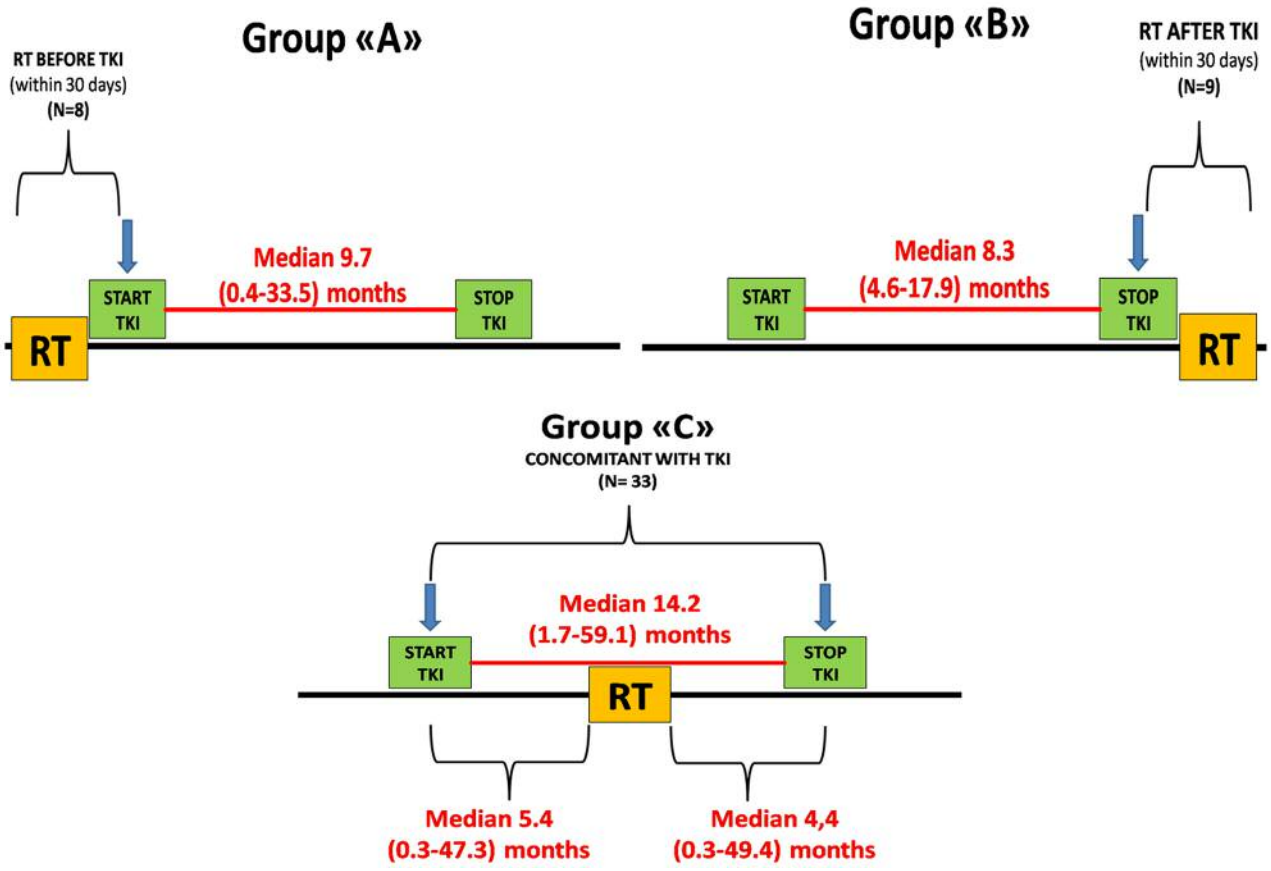

Figure 1. *Timing of radiotherapy (RT) and tyrosine kinase inhibitors (TKIs) in the three treatment groups: Group A: Those who underwent RT no more than 30 days before the beginning of TKI; group B: those who underwent RT no more than 30 days after the definitive suspension of TKI therapy; and group C: those who underwent RT during the administration of TKI.*

The median follow-up was 16 (range=1-58.9) months and was calculated from the beginning of systemic therapy to the last follow-up visit or death from any cause. The median OS was 19.3 months and 1- and 2-year OS was 71.5% and 36.5%, respectively. At univariate analysis, SBRT was associated with better OS (p=0.043). No other clinical or therapeutic variable significantly affected OS.

Tolerability outcomes. In twenty-nine patients (38%), no adverse event was recorded. In the remaining cases (31, 62%), the following toxicity was registered: 17 patients experienced exacerbation of pain during RT (grade 1-2); 14 patients experienced transitory exacerbation of neurological signs/symptoms of brain metastasis such as headache, drowsiness, confusion, nausea and emesis during RT treatment; only two events were grade 3. The treatment was never suspended because of an adverse event. No skin rash was observed. Adverse events are summarized in Table II.

Table II. *Acute toxicity (no. of events)*

Toxicity	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5
Neurological symptoms	5	7	2	0	0
Pain	10	7	0	0	0
Other	0	0	0	0	0

Discussion

There is emerging biological evidence, as described in pre-clinical studies, of the possible synergistic effect of TKIs given concurrently with RT at several levels, including cell-cycle kinetics, apoptosis induction, and the targeting of accelerated cellular repopulation. The potential relationship between EGFR signaling and DNA damage repair is also supported by new data regarding the inhibition of RAD51

recombinase expression. In detail, anti-EGFR and RT induce accumulation of tumor cells in G_1 and G_2-M phases, respectively, with a reduction of cells in S-phase. When combined with RT, TKIs promotes a further reduction in the S-phase fraction, enhances the induction of apoptosis, inhibits *EGFR* autophosphorylation and expression of RAD51 following radiation exposure, thus promoting an increase in radiosensitivity (12-14).

The combination treatment with radiotherapy and TKIs does not seem to be associated with a worse toxicity in relation to RT alone or TKI alone, as suggested by other authors (15). Currently the indications for this strategy are increasing, in particular, for patients with oligometastatic disease with visceral and brain metastasis, diffuse brain metastasis and symptomatic systemic progression. Moreover, in all cases of systemic asymptomatic progression, local treatments might be considered in order to limit the malignant potential of TKI-resistant sites as sources of further disease dissemination. This is coherent with the available evidence, as the updated 2017 National Comprehensive Cancer Network (NCCN) guidelines show (16).

Some studies have demonstrated the benefit of the association of local and systemic treatments in this clinical setting, the phase II trial conducted by Iyengar *et al*. (5) combined SBRT with erlotinib in patients with NSCLC and metastatic lesions investigating PFS at 6 months as the principal endpoint. PFS and OS were superior in the erlotinib/RT group compared to systemic therapy alone. In more than half of the patients, no *EGFR* mutations were found, leading the authors to conclude that the prolonged PFS was attributable to SBRT. Gan *et al*. proposed SBRT for all oligometastatic foci in patients with *ALK*-rearranged NSCLC in progression during crizotinib, finding no grade 3-5 toxicity, a mean crizotinib therapy duration of 28 months and a 1- and 2-year OS rates of 86 and 57%, respectively (6). Despite these encouraging results, it is still not clear if SBRT for oligoprogressive NSCLC disease could change the natural history of the disease or whether the oligometastatic state represents a manifestation of a less aggressive biological behavior itself. The ongoing SABR-Comet trial will try to answer this question randomizing patients with oligoprogressive disease to standard of care *versus* SRT to all oligometastatic foci (17).

A special consideration must be made for brain metastasis. About 20-40% of patients with NSCLC develop brain metastases during the history of their illness (18). Historically, whole-brain radiation therapy (WBRT) alone or in combination with surgery and stereotactic radiotherapy has been the standard of care for brain metastases. Recent data examining survival in patients with brain metastasis in a population selected for *EGFR* mutations showed survival rates of 14 to 17 months from the time of brain metastasis development. Yet the treatment of brain metastasis in patients with a driver mutation remains controversial. Despite the evidence of a lower rate of central nervous system (CNS) progression in patients treated with EGFR-TKI than in those receiving chemotherapy, brain progression is still the most common site of failure in patients with *ALK*-rearranged NSCLC during TKI therapy and remains the most important event with an impact on prognosis (19). Recent phase II trials, published in 2012 and 2013, demonstrated the activity of EGFR-TKIs against brain metastasis in a very selected group of patients with *EGFR*-mutant NSCLC without the upfront use of RT (20, 21). Nevertheless, it is important to underline that the cerebrospinal fluid (CSF) penetration ratio (defined as concentration in CSF/concentration in blood) of erlotinib ranges between 2.5% to 13% and for gefitinib from <1% to 10%. Thus, despite preclinical data showing encouraging results in terms of CNS efficacy of second- and third-generation TKIs, CNS penetration of first-generation inhibitors seems to remain suboptimal (22).

An important emerging issue regards the possibility of postponing RT at the time of diagnosis of brain metastasis in patients naïve to TKIs. A series compared upfront erlotinib *versus* upfront WBRT or SBRT (both eventually followed by erlotinib) in 110 patients affected by EGFR-mutated NSCLC naïve to TKI with newly diagnosed brain metastasis. OS did not differ significantly between the erlotinib-treated and WBRT-treated groups, with a median OS of 26 months and 35 months, respectively (p=0.62) The SBRT -treated group had significantly longer OS than the erlotinib-treated group, with a median of 64 months (p=0.006). Better local control was found in both groups treated with upfront RT. In the WBRT group, the administration of erlotinib within 2 months of WBRT was associated with improved intracranial control on univariate analysis (23). A recent publication compared treatment outcomes between TKI monotherapy *versus* RT plus TKIs in a cohort of 133 patients with a diagnosis of stage IV NSCLC with brain metastasis, finding a better median intracranial PFS (16.0 *vs*. 11.5 months, p=0.017) and a better OS (22 *vs*. 15 months p=0.015) in the RT plus TKI group. Interestingly, patients harboring *EGFR* exon 21 mutations seemed to benefit more from the association (24).

Finally, Magnusson *et al*. in a large multicenter retrospective series of 351 patients compared three different approaches for first diagnosis of brain metastasis in *EGFR*-mutant NSCLC naïve to TKIs: SBRT followed by TKI, WBRT followed by TKI and TKI alone with deferral of RT at progression. The median OS for the SBRT (n=100), WBRT (n=120), and EGFR-TKI (n=131) cohorts was 46, 30, and 25 months, respectively (p<0.001), leading to speculation that in oligoprogressive and multi-progressive settings, the major benefits are derived from the treatment associations, and that deferral of RT for brain metastasis could be associated with inferior OS (25).

Our series shows that RT combined with TKIs is a well-tolerated and promising treatment option in terms of survival, particularly when stereotactic RT with ablative aim is applied and when RT is given concomitantly with TKI. Oligoprogressive disease represents a relatively small fraction of the series and RT was proposed not only with an ablative intent, but also with a palliative-symptomatic one. For those reasons, our series represents a more "real-world" picture of the treatment of patients with stage IV NSCLC with a driver mutation. In addition, our data suggest that performing RT concomitantly and without suspension of TKI may extend the duration of drug administration, potentially leading to delaying the switch to a second-line systemic therapy. Despite most treatments being performed in a context of multi-progressive disease with palliative-symptomatic aim, OS was similar to that reported in the TKI registration studies, that notoriously consider a strictly selected group of patients (3, 4). This led us to speculate that this combination treatment could provide an advantage in terms of survival, without increasing acute toxicity, not only in those with a low burden of disease but also in a non-oligoprogressive disease setting.

Conclusion

Our study contributes to enrich the rapidly increasing literature about a very challenging setting. Local therapy such radiotherapy can contribute to optimizing the management of NSCLC with a driver mutation, in an ablative, but also palliative-symptomatic setting. Our series, in line with the current pre-clinical and clinical evidence, suggests that at progression, a strategy combining RT and systemic TKI therapy could provide major benefit and therefore must always be considered.

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Received October 15, 2017
Revised November 8, 2017
Accepted November 13, 2017

Manuscript 3:

Mazzola R, Tebano U, Aiello D, Di Paola G, **Giaj-Levra N**, Ricchetti F, Fersino S, Fiorentino A, Ruggieri R, Alongi F.

Increased efficacy of stereotactic ablative radiation therapy after bevacizumab in lung oligometastases from colon cancer

Tumori 2017; doi: 10.5301/tj.5000701 (in press)

This respective study evaluated the synergetic efficacy of stereotactic ablative radiotherapy in lung oligometastatic colon cancer patients and bevacizumab.

TJ
ISSN 0300-8916

Tumori 2017; 00(00): 000-000
DOI: 10.5301/tj.5000701

ORIGINAL RESEARCH ARTICLE

Increased efficacy of stereotactic ablative radiation therapy after bevacizumab in lung oligometastases from colon cancer

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ABSTRACT

Aim: Metastases from colorectal cancer are poorly responsive to stereotactic ablative radiation therapy (SABR) due to intratumoral hypoxia. Intratumoral oxygenation is improved by administration of angiogenesis inhibitors. Thus, there could be a clinical synergistic effect of SABR with bevacizumab on metastases from colorectal cancer. The aim of this study was to evaluate the feasibility and efficacy of SABR after bevacizumab in lung oligometastases from colon cancer.

Methods: The data of patients with lung metastases from colon cancer who underwent SABR were retrospectively evaluated according to the following inclusion criteria: number of metastases ≤3; lung oligometastases from colon cancer in patients who underwent SABR; patients receiving previous chemotherapy alone or in combination with bevacizumab; Karnofsky performance status >80; life expectancy >6 months; at least 6 months' follow-up after SABR; presence of KRAS mutation. The results were compared with those of a similar cohort of patients with irradiated lung lesions from colorectal cancer in whom bevacizumab was not previously administered.

Results: A total of 40 lung metastases were analyzed. The complete response rate after SABR was higher in patients who had received bevacizumab than in those who had not (p = 0.04). Additionally, in the bevacizumab group, a higher rate of post-SABR complete response was observed in case of oligopersistent versus oligorecurrent metastases (p = 0.001).

Conclusions: In the setting of lung oligometastases from colon cancer the present study attested the higher efficacy of SABR after bevacizumab administration. Further studies in this field of research are strongly advocated.

Keywords: Bevacizumab, Colorectal lung malignancies, Oligometastases, SABR

Introduction

The term "oligometastases" was coined and adopted to refer to a limited tumor burden that can be potentially cured with local therapies. More recently, "oligoprogression" has been used to denote a condition including few metastatic sites not responsive to systemic treatment (1). In these contexts, the efficacy and safety of stereotactic ablative radiation therapy (SABR) in improving local control has been documented in several settings (2). In oligometastatic and oligoprogressive selected patients, the rationale for introducing SABR in a multimodality treatment approach has been not only to optimize metastasis response by "local consolidation", but also to delay the start of a subsequent line of anticancer drugs. Oligometastatic lung lesions are potentially curable with different local treatments including SABR, surgery or radiofrequency (3-5). With regard to SABR, local response seemed to be related to a biologically equivalent dose (BED) ≥100 Gy_{10} (6). However, compared with metastases from other primary tumor types, metastases from colorectal cancer remain poorly responsive to SABR due to hypoxia (7).

Thus, a crucial question remains: Is it possible to improve the effectiveness of SABR in case of oligometastases from colon cancer? Preclinical studies have found that intratumoral oxygenation is improved by the administration of angiogenesis inhibitors due to so-called vascular normalization (8, 9), with subsequent normalization of the dysfunctional intratumoral vasculature. Bevacizumab is a humanized monoclonal

Accepted: September 26, 2017
Published online:

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antibody targeted against the activity of vascular endothelial growth factor A (VEGF-A); it has been investigated, combined with other anticancer drugs, in several advanced cancer types. In metastatic colon cancer, bevacizumab has been shown to improve progression-free survival (PFS) and overall survival (OS) when combined with cytotoxic agents both in first and subsequent lines of therapy (10-12).

Starting from this background we assumed that SABR might have clinical synergistic efficacy with bevacizumab and we hypothesized that this interaction could improve the outcome of selected colorectal cancer patients with oligometastatic/oligoprogressive disease in the lung. More specifically, in the present study we evaluated the feasibility and efficacy of SABR after bevacizumab in oligopersistent/oligoprogressive lung metastases from colon cancer. The results were compared with those of a similar cohort of patients with irradiated lung lesions from colorectal cancer in whom bevacizumab was not previously administered.

Materials and methods

Study design

The data of patients with lung metastases from colon cancer who underwent SABR were retrospectively evaluated according to the following inclusion criteria: a) number of metastases ≤3; b) oligopersistent disease defined as 1-3 metastatic lesions (in patients who were previously rendered free of gross metastatic disease) after at least 1 course of systemic therapy; c) oligoprogressive disease defined as progression of 1-3 metastases following or during treatment of metastatic disease with other therapies; d) previous chemotherapy alone or in combination with bevacizumab; e) Karnofsky performance status >80; f) life expectancy >6 months; g) at least 6 months of follow-up after SABR; h) KRAS mutation.

SABR procedures

All patients were planned and treated in the supine position with a Posirest™ (CIVCO® Medical Solutions) and a Vac-Lok™ cushion (CIVCO® Medical Solutions). A 4D-CT scan in the treatment position was acquired in all patients, and for each patient 10 phases were reconstructed with 3-mm slice thickness and interslice distance. The gross tumor volume (GTV) was equal to the clinical target volume (CTV). It consisted of the radiological lung lesion as identified by optimizing the Hounsfield unit window for the lungs and by repeating the delineation on each 4D-CT phase. The internal target volume (ITV) was defined as the boolean envelope of the GTVs from each respiratory phase. The planning target volume (PTV) was defined as ITV plus an isotropic margin of 5 mm in all directions. The organs at risk (OARs) were the ipsilateral and contralateral lung, heart, spinal cord, esophagus and chest wall.

The prescribed total dose of SABR varied according to the tumor site (central or peripheral) and the maximum diameter of the lesions; a strategy of risk-adapted dose prescription was adopted. We used 3-5 fraction schedules for peripheral lesions and 8-10 fraction schedules for central lesions. The dose prescription was at the median PTV dose, while assuring from optimization 95% of Dose Prescription (Dp) to at least 95% of the PTV, and a near-maximum target dose (D2%) not larger than 107% of Dp. All adopted schedules satisfied a BED_{10} ≥100 Gy at the isocenter, where α/β equal to 10 Gy was assumed for all metastatic lesions.

Constraints for nearby OARs varied according to the location of metastases (central versus peripheral) and the dose prescription. All plans were performed by RapidArc™ (v. 10.0.28, Varian Inc.) volumetric modulated arc therapy (VMAT) using 2 coplanar arcs of ≈ 200° with a single isocenter per metastatic lesion. Jaw tracking was used to minimize residual leaf transmission. The final dose distributions were computed by the Analytical Anisotropic Algorithm (AAA, v. 10.0.28) as implemented in the Eclipse™ (v. 10.0.28, Varian Inc.) treatment planning system. Patients were typically treated with 6 MV flattening filter free (FFF) photon beams by means of a True-Beam™ linac (Varian Inc.) equipped with a Millennium™ MLC (Varian Inc.) with leaf dimension at the isocenter of 5 mm. A maximum dose rate of 1,400 MU/min for 6 MV-FFF was used. Before each fraction, Image-guided Radiotherapy (IGRT) was performed by means of kV-Cone Beam CT.

Systemic therapy

Chemotherapy +/- bevacizumab was administered in accordance with the National Comprehensive Cancer Network guidelines (13), specifically for metastatic colon cancer with mutated KRAS. Bevacizumab was not allowed within 2 weeks of the beginning of SABR and it was considered contraindicated in accordance with the data sheet of the drug.

Follow-up and toxicity related to SABR

Tumor response was assessed by means of 18-FDG-PET/CT and according to the PET Response Criteria in Solid Tumors (PERCIST) (14) within 3 months of SABR and every 3 months thereafter. Toxicity related to SABR was assessed prospectively in accordance with the Common Terminology Criteria for Adverse Events (CTCAE) v. 4.0 during SABR and every 3 months thereafter. Toxicities occurring within 3 months of the beginning of SABR were defined as acute, those occurring after 3 months as late toxicity.

Statistical analysis

In order to summarize the most relevant features of the clinical variables, descriptive statistics were performed. All the categorical variables were analyzed with contingency tables using Fisher's exact test or Pearson's chi-squared test, while the continuous variables were analyzed with analysis of variance (1-way ANOVA), *t*-tests (with equal or unequal variance), or nonparametric Wilcoxon (Mann-Whitney) and Kruskal-Wallis tests.

Three clinical outcomes were defined: 1) local control as the absence of in-field local recurrence (in the prior radiation field); 2) distant-metastasis-free survival (DMFS); 3) OS from the end of SABR. These parameters were assessed using the Kaplan-Meier method. Complete response of lung metastases was considered as the primary endpoint. All patients

enrolled in the study achieved a post-SABR follow-up of at least 6 months. For this reason, analysis of the primary endpoint focused on 2 time points: a) 3 months post-SABR and b) 6 months post-SABR.

Logistic regression models were used to evaluate the impact of bevacizumab administration in combination with SABR on complete response of lung metastases during follow-up. Additionally, the following dependent variables were taken into account to estimate the possible correlation with complete metastasis response: oligopersistent versus oligorecurrent metastases, number of fractions, BED, tumor volume, lesion site (central versus peripheral), and number of metastatic lesions submitted to SABR.

P values ≤0.05 were considered statistically significant. Statistical analysis was performed using the SAS software v. 9.4.

Results

Patients and metastases

For the intent of the present analysis, 23 patients for a total of 40 metastatic lung lesions from colon cancer met the inclusion criteria. All analyzed patients had only lung oligometastases and no disease outside the lung.

The median follow-up was 18 months (range, 6-32 months). The median age of the patients was 70 years (range, 48-75). Concerning SABR, all lesions were treated with BED ≥100 Gy. The median diameter of the lung metastases submitted to SABR was 2.3 cm (range, 1-4). The metastases were metachronous, classified as oligopersistent (16/40 lesions) and oligoprogressive (24/40 lesions) after 1-2 schedules of systemic antiblastic therapies administered according to international guidelines (13).

Prior to SABR, bevacizumab was administered in combination with chemotherapy in 17/40 lesions (42.5%). Table I presents the characteristics of the 2 groups of metastases submitted to SABR, distinguishing a bevacizumab-group and a no-bevacizumab-group.

Analyzing the 2 subgroup of patients, we found that the median BED and delivered dose were statistically significantly lower in the bevacizumab group than the no-bevacizumab group (Fig. 1).

Clinical outcomes

For the entire study population, the 1-year OS and local control rates were 100% and 89.3%, respectively. The median DMFS was 6 months (range, 3-15 months). During follow-up, progression of distant metastases was recorded as follows: liver (7 cases), lymph nodes (5 cases), and lung – out of field (10 cases). Three cases of in-field failure were registered 12 months after SABR. Of these, only 1 lesion in the bevacizumab-group failed at the previous site of SABR.

At 12 months post-SABR, datasets were analyzed in 16/23 patients of the entire study population for a total of 28/40 metastases (14 lesions for both groups analyzed here). One-year local control in the bevacizumab group was 93% versus 86% in the no-bevacizumab group.

Analysis of complete response rates of lung metastases

In Table II we present the rates of complete response in the bevacizumab and no-bevacizumab groups 3, 6 and 12 months post-SABR. The rate of complete response 3 months after SABR was higher in the cohort of lesions previously submitted to bevacizumab than in the remaining lesions ($p = 0.04$). At this time point, complete response was registered in 11/17 (64%) metastases in the bevacizumab group and 10/23 (43%) in the lesions in which bevacizumab was not previously administered. The statistical significance was confirmed 6 months after SABR. In fact, at this last time point, complete response was registered in 16/17 (94%) metastases in the bevacizumab group and 8/23 (34%) in the remaining lesions ($p = 0.005$). Additionally, in the bevacizumab group a higher post-SABR complete response rate was observed in oligopersistent versus oligorecurrent metastases ($p = 0.001$).

No statistically significant difference was found between post-SABR complete response and BED, number of fractions, lesion volume prior to SABR, number of metastatic lesions undergoing SABR, and lesion site (central versus peripheral).

TABLE I - Characteristics of the two groups of metastases treated with SABR

Variables	No-bevacizumab group (23 metastases)	Bevacizumab group (17 metastases)
Previous lines of systemic therapy	None: 11	None: 7
	One: 5	One: 3
	Two: 7	Two: 7
Number of metastases submitted to SABR	One metastasis: 12	One metastasis: 9
	Two metastases: 7	Two metastases: 5
	Three metastases: 4	Three metastases: 3
Number of fractions of SABR (median, range)	6 (3-10)	5 (3-8)
Total dose of SABR (median, range)	55 Gy (50-70)	51 Gy (48-60)
Biologically effective dose (median, range)	110 (100-164)	103 (100-115)

SABR = stereotactic ablative radiation therapy.

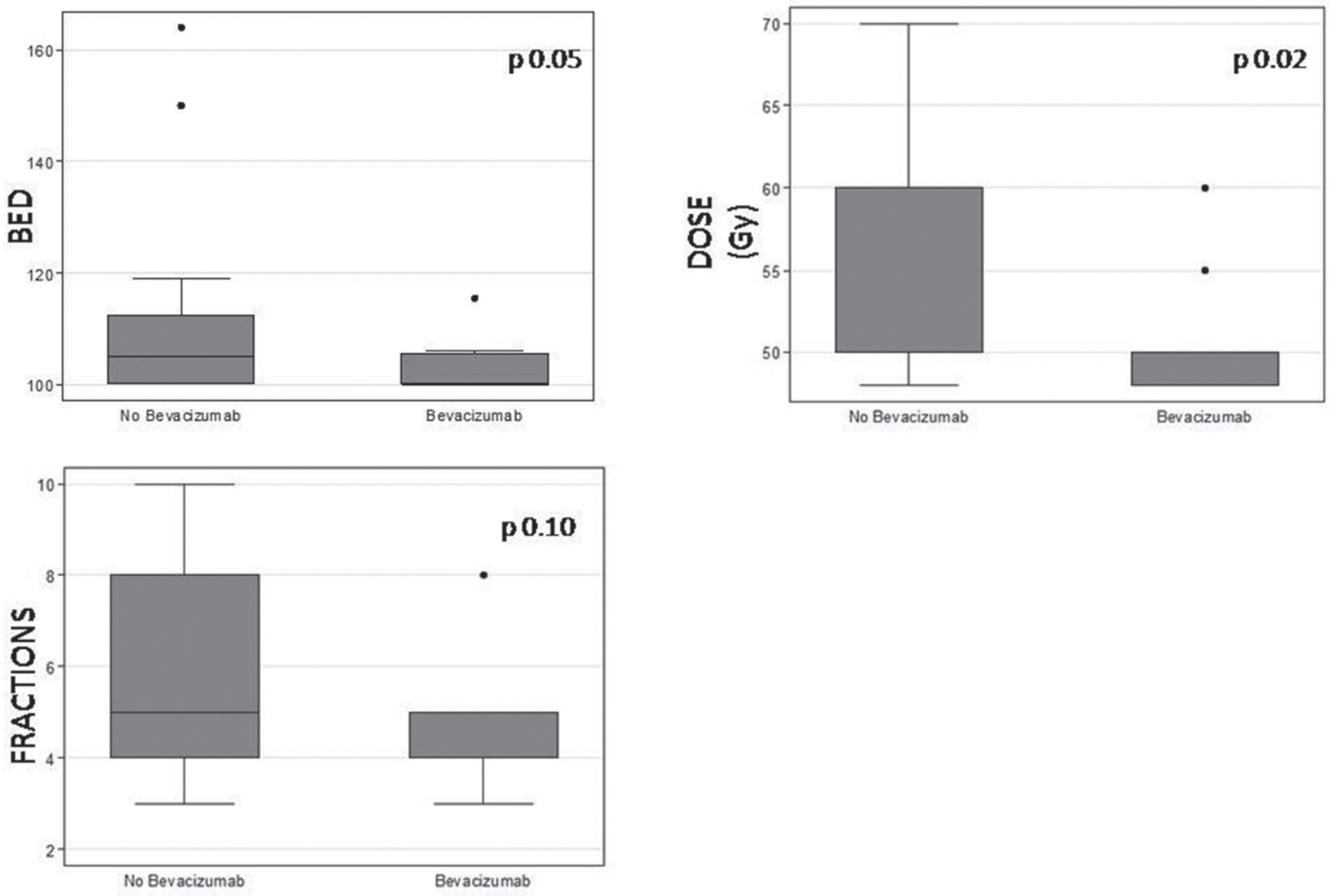

Fig. 1 - The box plots present the distribution of the biologically effective dose (BED), total dose delivered (DOSE) and number of fractions in the 2 groups of patients analyzed. In the bevacizumab group, BED and total dose were significantly lower than in the no-bevacizumab group.

TABLE II - Rates of complete response at 3, 6 and 12 months post-SABR in the bevacizumab and no-bevacizumab groups

Follow-up	Number of metastases analyzed	Bevacizumab group	No-bevacizumab group
Three months	40	11/17 metastases (64%)	10/23 metastases (43%)
Six months	40	16/17 metastases (94%)	8/23 metastases (34%)
Twelve months	28	13/14 metastases (92%)	8/14 metastases (57%)

SABR-related toxicity (CTCAE v. 4.0)

During SABR, 1 patient (4%) in the bevacizumab group experienced grade 2 pulmonary toxicity while 2 patients (6%) in the no-bevacizumab group experienced acute grade 1 toxicity. At the time of the analysis, no late toxicity equal to or higher than grade 3 was recorded in either group. Late adverse events included chest wall pain (2 patients with peripheral metastases; 6%) and asymptomatic pneumonitis (2 patients; 6%) without any difference between the 2 groups.

Discussion

The role of SABR for oligometastatic lung disease has been extensively investigated, including colorectal lung metastases (15-17). Some studies have suggested a sort of radioresistance affecting tumor response after SABR in comparison to other primary tumors (7), probably related to the high proportion of hypoxic cells characterizing metastases from colorectal cancer (18). Preclinical investigations have found that intratumoral oxygenation can be increased by angiogenesis inhibitors (8, 9). As a consequence, their administration could be followed by enhancement of the antitumor activity of irradiation (19, 20). However, the positive effect on radiation response is confined to a limited period after antiangiogenic drug administration (21). Thus, the possible synergistic effect of the combination of SABR and antiangiogenic molecules seems to be more complex. The real radiobiological target of SABR remains the object of debate (22). It is conceivable that a high radiation dose per fraction produces endothelial damage as a possible dependent factor of tumor response.

Several mechanisms could explain the possible synergistic effect when SABR is combined with bevacizumab: 1) enhancement of endothelial cell apoptosis after SABR due to inhibition of the protective cellular pathways (VEGF-mediated) that limit SABR-related endothelial cell damage (23); 2) reduction of proangiogenic growth factors by cancer cells after SABR delivery (24); 3) improvement of the immune response (25).

Based on this background, we speculated that there might be clinical synergistic efficacy of SABR with bevacizumab. In the present retrospective study in which we analyzed metachronous oligopersistent and oligoprogressive lung metastases from colon cancer, a statistically significantly higher rate of complete metastasis response 3 and 6 months after SABR (with previously administered bevacizumab) was observed. We assume that the higher rate of local response was independently related to SABR-related factors. In fact, when the 2 subgroups of patients were compared, the median BED and mean delivered dose were statistically significantly lower in the bevacizumab group than the no-bevacizumab group.

When we look at the literature, Agolli and colleagues (17) recently reported long-term SABR outcomes in colorectal lung oligometastases. Although in their experience most lesions were treated with a BED >100 Gy (range, 76-120 Gy), complete response occurred in 20% of lesions. In the current analysis, at 6 months post-SABR the complete response rate of metastases in the bevacizumab group was 94% as opposed to 34% in the non-bevacizumab group ($p = 0.005$). To better understand this result, we related other variables to the complete response rates with the intent to explore the role of possible confounding factors. In the group of lesions previously submitted to bevacizumab, oligopersistent lung metastases showed a higher complete response rate than oligoprogressive lesions ($p = 0.001$). No other statistically significant differences were found with respect to the remaining variables analyzed. Unlike other studies that analyzed the impact of SABR on lung oligometastases from colorectal cancer (7, 15-17), in the present study only the outcomes of lung metastases from colon cancer are reported. The radiosensitivity of colon or rectal cancer lung metastases treated with SABR is different, with worse local control in patients with rectal primary tumors (26). In the literature, the observed in-field failure was in the range of 7.5%-34% of colorectal lung lesions treated with SABR (7, 16). The findings regarding local control at the time of analysis in our experience seem intriguing compared with the data in the literature, although longer follow-up is needed to draw any conclusions. For the entire cohort of lesions analyzed, 1-year local control was 89.3%, whereas in the bevacizumab group it was 93%.

Tumor response was evaluated by means of 18-FDG-PET/CT. 18-FDG-PET/CT is strongly recommended by the Royal College of Radiologists in case of metastatic disease from colorectal cancer (27). At present more advanced image analysis methods, such as radiomics, are under investigation for treatment evaluation and response prediction or as potential biomarkers. Moreover, common measure parameters like SUV_{max}, SUV_{mean}, metabolic tumor volume and total lesion glycolysis derived from 18F-FDG PET scans could be adopted in lung oligometastases for monitoring tumor response (28).

A crucial issue of the present analysis regards adverse events to the lung when combining SABR with bevacizumab. No acute/late grade ≥3 toxicities were recorded, attesting the optimal tolerability profile of SABR for lung lesions even in combination with bevacizumab.

Obviously, we are conscious of the methodological limitations of the present analysis as well as the small sample size analyzed. However, to the best of our knowledge, no other experience exists in the literature evaluating the efficacy and safety of a combination including bevacizumab and SABR in the specific setting of lung metastases from colon cancer in KRAS-mutated patients. In fact, all lung metastases analyzed in the present study presented a KRAS mutation. Of note, in case of metastatic colorectal cancer a sort of lung tropism is recognized when KRAS is mutated. Based on the current state of knowledge, KRAS mutation does not represent a predictive factor for local control after SABR. It does, however, predict a worse 1-year metastasis-free survival after SABR when compared with KRAS wild-type colorectal lung metastases (29).

In summary, in the setting of oligopersistent/oligoprogressive lung metastases from colon cancer the present study attested the higher efficacy of SABR after bevacizumab administration. Further studies in this field of research are strongly advocated.

Compliance with ethical standards:

Informed consent: Informed consent was obtained from all patients.

Ethical standards: All procedures involving human participants were in accordance with the ethical standards of the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Abbreviations

AAA	Analytical Anisotropic Algorithm
BED	Biologically equivalent dose
CTCAE	Common Terminology Criteria for Adverse Events
CTV	Clinical target volume
DMFS	Distant-metastasis-free survival
FFF	Flattening filter free
GTV	Gross tumor volume
ITV	Internal target volume
OAR	Organ at risk
OS	Overall survival
PERCIST	PET Response Criteria in Solid Tumors
PTV	Planning target volume
SABR	Stereotactic ablative radiation therapy
VEGF	Vascular endothelial growth factor
VMAT	Volumetric modulated arc therapy

Disclosures

Financial support: None.
Conflict of interest: The authors have no conflicts of interest to disclose.

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Manuscript 4:

Trovo M, Furlan C, Polesel J, Fiorica F, Arcangeli S, **Giaj-Levra N**, Alongi F, Del Conte A, Militello L, Muraro E, Martorelli D, Spazzapan S, Berretta M.

Radical radiation therapy for oligometastatic breast cancer: Results of a prospective phase II trial.

Radiother Oncol. 2017. doi: 10.1016/j.radonc.2017.08.032. (in press)

This prospective phase II clinical trial determined the impact of radical radiation treatment to all metastatic sites in oligometastatic breast cancer.

Radiotherapy and Oncology xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Radiotherapy and Oncology

journal homepage: www.thegreenjournal.com

Original article

Radical radiation therapy for oligometastatic breast cancer: Results of a prospective phase II trial

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ARTICLE INFO

Article history:
Received 19 June 2017
Received in revised form 14 August 2017
Accepted 20 August 2017
Available online xxxx

Keywords:
SBRT
Oligometastases
Breast cancer

ABSTRACT

Background and purpose: We conducted a prospective phase II multicentric trial to determine if radical radiation therapy to all metastatic sites might improve the progression-free survival (PFS) in oligometastatic breast cancer patients. Secondary endpoints were local control (LC), overall survival (OS) and toxicity.

Methods and materials: Inclusion criteria were the following: oligometastatic breast cancer with ≤5 metastatic sites, FDG-PET/CT staging, no brain metastases, primary tumor controlled. Radiotherapy could be delivered using stereotactic body radiotherapy (SBRT) technique or fractionated intensity modulated radiotherapy (IMRT). SBRT consisted of 30–45 Gy in 3 fractions, while IMRT was delivered to a total dose of 60 Gy in 25 fractions. We hypothesized that radical radiation therapy could increase the PFS from 30% (according to the published literature) to 50% at two years.

Results: 54 Patients with 92 metastatic lesions were enrolled. Forty-four were treated with SBRT, and 10 with IMRT. Forty-eight (89%) patients received a form of systemic therapy concomitantly to radiation therapy. Sites of metastatic disease were the following: bones 60 lesions, lymph nodes 23 lesions, lung 4 lesions, liver 5 lesions. After a median follow-up of 30 months (range, 6–55 months), 1- and 2-year PFS was 75% and 53%, respectively. Two-year LC and OS were 97% and 95%, respectively. Radiation therapy was well tolerated, and no Grade ≥3 toxicity was documented. Grade 2 toxicity were pain and fatigue in 2 cases.

Conclusions: Patients with oligometastatic breast cancer treated with radical radiotherapy to all metastatic sites may achieve long-term progression-free survival, without significant treatment-related toxicity. While waiting for data from randomized trials, the use of radical radiation therapy to all metastatic sites in patients with oligometastatic breast cancer should be considered a valuable option, and its recommendation should be individualized.

The standard of care for metastatic breast cancer is systemic therapy, with radiation used for palliation of symptoms. Interestingly, a large proportion of patients enrolled in first-line metastatic breast cancer trials have a limited number of metastatic sites [1–6]. Despite this, the outcome in terms of progression-free survival (PFS) after a first line of systemic therapy is poor, ranging from 6 to 16 months [5,6]. Moreover, only 2% of patients that achieve a complete remission of disease after systemic therapy maintain a long-term response [7]. Treatment with the anti-human epidermal growth factor receptor 2 (HER) monoclonal antibodies in addition to chemotherapy dramatically improved survival in HER2-positive metastatic breast cancer patients, and the 2-year PFS reported in a recently published randomized trial was about 40% for those patients treated with the combination of pertuzumab, trastuzumab and docetaxel [8]. Unfortunately, the minority of breast cancers over-express HER2, and therefore the majority of patients do not benefit of anti-HER2 therapies.

A "clinical significant state of oligometastases" has been defined to describe a clinical scenario in which a limited number of meta-

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https://doi.org/10.1016/j.radonc.2017.08.032
0167-8140/

static sites might represent a state in which the full metastatic potential of cancer has not been achieved [9]. Based on this hypothesis, local treatments, including surgery or ablative radiotherapy, have been employed with the aim of achieving long term local control and possibly increasing the overall outcome [10].

Based on these observations, recent studies have investigated the possible role of ablative local therapies in oligometastatic breast cancer patients. However, the majority of the published studies were designed to assess the safety and efficacy of local radiotherapy only in terms of local control, without providing evidence of its effect on the overall outcome [11]. Therefore, we conducted a phase II multicentric prospective study to determine the progression-free survival (PFS) of oligometastatic breast cancer patients treated with radical radiotherapy to all metastatic sites. Secondary endpoints were local control (LC), overall survival (OS) and toxicity.

Methods and materials

Patients

Between January 2012 and December 2015, patients affected by oligometastatic breast cancer were enrolled in this phase 2 prospective study. The study was conducted with the approval of institutional review boards (trial number CRO 2012-47), and each patient signed an informed consent form.

To be included in this study patients had to be affected by metastatic breast cancer with $\leq$5 metastases. The extent of disease had to be assessed with FDG-PET/CT, and in case of liver metastases also with an MRI of the abdomen. Further eligibility criteria included: ECOG performance status <2, primary tumor controlled, absence of brain metastasis. The use of systemic therapies was allowed.

Treatment

Gross tumor volume (GTV) included only foci of PET uptake. No enlargement of the margin to account for presumed microscopic disease was permitted. Planning target volume (PTV) was delineated by uniform margins of 3–5 mm around the GTV. In case of lung or liver metastases, patients underwent simulation with four-dimensional CT (4DCT), with the aim of characterizing tumor motion for target delineation.

Radiotherapy could be delivered using both stereotactic body radiotherapy (SBRT) technique or fractionated intensity modulated radiotherapy (IMRT). SBRT consisted in 30–45 Gy in 3 fractions, while IMRT was delivered to a total dose of 60 Gy in 25 fractions. The spinal cord was the dose limiting tissue: for the three-fractions scheme and the 25-fraction scheme the maximum dose had to be <17 Gy and <46 Gy, respectively. The choice on the use of SBRT or IMRT was left to each treating physician.

Follow-up

Patients were seen in follow-up at regular intervals to determine tumor status and the presence of symptoms. The first clinical examination to determine toxicity was performed 1 month after treatment. Follow-up visits and CT scan or MRI were performed at 3, 9 and 15 months after radiotherapy, and then every 6 months. Follow-up FDG-PET/CT was performed every 6 months after treatment. Toxicities were scored according to the Common Toxicity Criteria Adverse Events version 4 (CTCAE v.4).

Statistical considerations and endpoints

The purpose of the present study was to assess the prognostic role, in terms of PFS, of radical radiation therapy delivered to all metastatic sites in oligometastatic breast cancer patients. A Simon 2-stage design was use to determine the sample size [12]. Considering a 2-year PFS of 30%, as reported by literature in metastatic breast cancer [1–6], and setting the hypothesis of a 2-year PFS of 50% with the new treatment, the required number of patients is 46 (with $\alpha = 0.05$ and $\beta = 0.20$).

Local control was defined as a lack of progression of the treated metastatic lesions (i.e., any response or stable disease). Distant failure was defined as any failure outside of the treated site. PFS was defined as the time from the end of treatment to local or distant progression, or death from any cause. The study endpoints, including PFS, LC and OS were estimated using the Kaplan–Meier method, starting from the end of radiotherapy to the event of interest or last available follow-up. The log-rank test (2-sided) was used to test the differences between the subgroups. In all cases, statistical significance was considered for $p < 0.05$. The effect of individual factors on PFS was assessed through hazard ratios and a corresponding 95% confidence interval (CI), estimated using the Cox proportional hazard model. The hazard ratios for potential risk factors predicting PFS included several patient, tumor and treatment characteristics. Mutual adjustment was performed through a multivariate model, in which all the variables were simultaneously included in the regression equation.

Results

In the study period, 54 patients with a combined 92 metastatic lesions were enrolled. Patient and tumor characteristics are

Table 1
Patient and tumor characteristics ($n = 54$).

Characteristics	No. of patients	%
Age, years		
Median	55	
Range	36–83	
Status at diagnosis		
Early-stage disease (stage I-II)	14	26
Locally-advanced disease (stage III)	27	50
Metastatic disease (stage IV)	13	24
Oligometastatic status		
At diagnosis	40	74
Induced	14	26
Histology		
Ductal	48	89
Lobular	6	11
Grade		
Well differentiated (G1)	3	6
Moderately differentiated (G2)	19	35
Poorly differentiated (G3)	28	52
Not described	4	7
Estrogen receptor		
Positive	43	80
Negative	11	20
Her2-neu		
Negative	41	76
Positive	11	20
Not described	2	4
Tumor phenotype		
Luminal A/B	43	80
Her-2 Rich	4	7
"Triple-negative"	7	13
Systemic treatment concomitant with radiation		
Hormonal therapy	9	17
Chemotherapy	33	61
Chemotherapy + Trastuzumab	2	4
Trastuzumab	4	7
None	6	11

Please cite this article in press as: Trovo M et al. Radical radiation therapy for oligometastatic breast cancer: Results of a prospective phase II trial. Radiother Oncol (2017), https://doi.org/10.1016/j.radonc.2017.08.032

reported in Table 1. Forty patients had oligometastatic disease at diagnosis and 14 had an induced oligometastatic status by effective systemic therapies (widespread metastases that responded to systemic therapy). Half of the patients ($n = 27$) had 1 metastatic site, while the other 27 had ≥2 metastases: 19 patients had 2 lesions, 6 patients had 3 lesions, 1 patient had 4 lesions, and 1 patient had 5 lesions. Sites of metastatic disease were the following: bones, 60 lesions; lymph nodes, 23 lesions; lung, 4 lesions; liver, 5 lesions. The majority of patients ($n = 44$) were treated with SBRT, and 10 with fractionated IMRT. SBRT schedules were the following: 30–36 Gy in 3 fractions for 46 (85%) patients, and 45 Gy in 3 fractions for 8 (15%) patients; fractionated IMRT consisted of 60 Gy in 25 fractions for all cases ($n = 10$). Median time from the diagnosis of oligometastatic status and radiotherapy was 5 months. Forty-eight (89%) patients received a form of systemic therapy concomitantly to radiation therapy. Hormonal therapy alone was administered in 9 (17%) patients, chemotherapy in 35 (65%) patients (taxanes or capcitabine in 30 cases), and Trastuzumab in 6 (11%) patients. Five patients who had a Her-2 positive breast cancer received hormonal therapy alone. Each of these five patients had one or two bone metastases only, which were treated with SBRT.

After a median follow-up of 30 months (range, 6–55 months), 1- and 2-year PFS was 75% and 53%, respectively (Fig. 1).

Only two patients experienced local failure. One of these two patients had an isolated local failure for a spinal lesion that was treated with a minimum dose of 17 Gy in 3 fractions (being the spinal cord constraint prior on the PTV coverage). Two-year LC and OS were 97% and 95%, respectively (Fig. 2).

Radiation therapy was well tolerated, and no Grade ≥3 toxicity was documented. Grade 2 toxicity included pain and fatigue in two cases. Four patients experienced Grade 1 pain.

Prognostic factors associated with increased PFS were not identified (Table 2). In particular, there was no difference in PFS for patients with 1 vs. ≥2 metastasis, for bone-only vs. visceral metastases, or for patients with metastatic disease at diagnosis vs. induced metastatic status.

Discussion

In the present paper we report the final results of a prospective phase II trial, in which oligometastatic breast cancer patients underwent radical radiation therapy to all metastatic sites. The majority (85%) of them had only one or two metastatic lesions, primarily to the bones or lymph nodes.

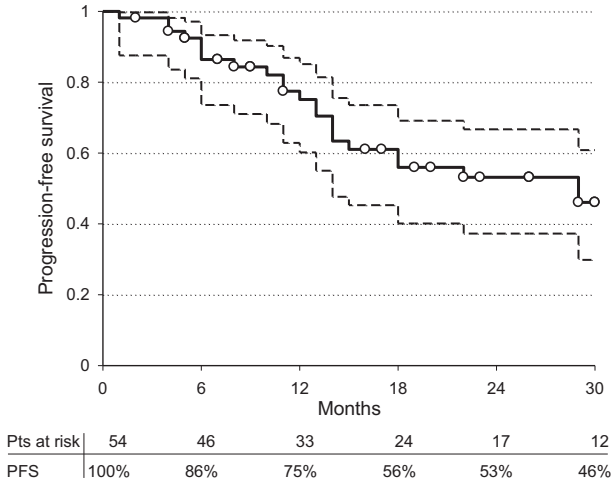

Fig. 1. Kaplan–Meier estimates of progression-free survival.

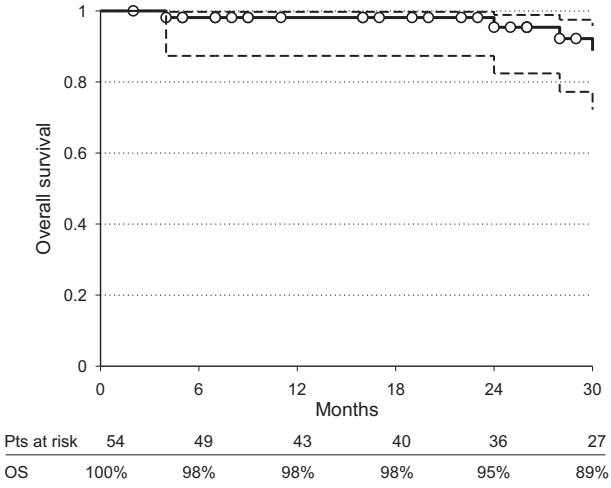

Fig. 2. Kaplan–Meier estimates of overall survival.

We tested the hypothesis that the treatment of all metastatic sites might have a prognostic meaning. The observed PFS at 2 years of 53% supports the hypothesis that metastasis-directed therapy may improve the outcome. We showed that the achievement of local control was associated with an good progression-free survival. More than half of the treated patients were free from local and distant progression at 2 years. These results support the hypothesis that oligometastatic disease is potentially curable with local therapies

Our results are comparable to those reported by Milano et al., who documented a 2-year PFS of 44% in a prospective pilot study, in which patients with a limited number of metastases from breast cancer were treated with radical SBRT [13]. Scorsetti et al. published a prospective observational study of SBRT for oligometastatic breast cancer patients, in which the majority (70%) of patients had liver or lung metastases and primary end point was local control [11]. The authors reported inferior outcome, with a PFS at 2 years of only 27%, although local control was 90% at 2 years. These data might reflect the heterogeneity of metastatic breast cancer, and the different prognostic values of the metastatization sites.

We were not able to identify any risk factors as predictors of survival. Particularly, there was no difference in PFS between "induced" or "de novo" oligometastatic disease, or between patients with metastatic disease at diagnosis vs. those who experience metastatic progression. Interestingly, the number of metastases (1 vs. ≥2) was also not associated with PFS. It is likely that a larger cohort of patients will be needed to identify prognostic factors.

One potential limitation of this study is the possible positive selection of patients, given that the majority of patients had only one or two metastases. Moreover, 40 (74%) patients were oligometastatic at diagnosis, and this may explain the high survival rate reported in the present trial. Another consideration is that we compared our results to those reported by historical cohorts of metastatic breast cancer patients treated with systemic chemotherapy alone, where not all patients had oligometastatic disease. Additionally, the majority of our patients had bone-only metastases, which is a factor associated with better outcomes compared to visceral metastases [14], although this was not documented in our cohort.

Determining the prognostic role of radical radiotherapy to all metastatic sites in a single arm phase 2 trial is complex and strongly linked to patient selection. For example, the Paloma-2 trial was a randomized phase 3 study designed to assess the efficacy of palbociclib plus letrozole vs. letrozole alone in postmenopausal women with ER-positive, Her-2 negative advanced breast cancer,

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Table 2
Hazard ratio (HR) and 95% confidence intervals for progression in 54 oligometastatic breast cancer patients.

	Patients	Relapses		HR (95% CI)[a]	HR (95% CI)[b]
		N	(%)		
Age					
<55 years	24	10	(41.7)	Reference	Reference
≥55 years	30	15	(50.0)	0.95 (0.42–2.12)	1.12 (0.38–3.33)
Metastatic disease at diagnosis					
No	41	20	(48.8)	Reference	Reference
Yes	13	5	(38.5)	0.86 (0.27–2.78)	0.72 (0.20–2.66)
ER					
Negative	11	3	(27.3)	Reference	Reference
Positive	43	22	(51.2)	2.22 (0.66–7.44)	3.68 (0.92–14.77)
Her2					
Negative	41	18	(43.9)	Reference	Reference
Positive	11	5	(45.5)	1.09 (0.37–3.20)	1.94 (0.52–7.19)
Missing	*2*	*2*	*(100.0)*		
Oligometastatic Status					
Induced	14	6	(42.9)	Reference	Reference
At diagnosis	40	19	(47.5)	0.98 (0.39–2.50)	0.58 (0.20–1.71)
Number of lesions					
1	27	12	(44.4)	Reference	Reference
≥2	27	13	(48.2)	1.15 (0.52–2.52)	0.55 (0.20–1.48)

[a] Adjusted for age.
[b] Mutually adjusted for all variables in the table.

half of whom had non-visceral disease [15]. The authors reported a 2-year PFS of 45% and 60% for patients treated with hormonal therapy alone and hormonal therapy plus palbociclib, respectively.

Also, it must be underlined that in the present cohort, the large majority (65%) of the patients were treated with chemotherapy, although the 80% of patients had a hormonal receptor positive breast cancer. It may be hypothesized that the intensity of systemic treatments is more important than the RT approach.

Despite these limitations, this study shows that patients with oligometastatic breast cancer treated with radical radiotherapy to all metastatic sites may achieve long-term progression-free survival, without significant treatment-related toxicity. However, there remains the need to identify which subgroups of patients might benefit more from aggressive local therapies in this clinical scenario. Whether radiotherapy should be routinely performed in such patients is not defined yet, due to the scarcity of quality published literature. It must be underlined that the true prognostic value of local therapies in terms of overall survival is not known.

There is ongoing research in this field: the NRG-BR002 is a randomized Phase II/III trial assessing the role of SBRT or surgical ablation for oligometastatic breast cancer patients [16]. The results of this trial will help determine the real impact of local therapies in this subgroup of patients, but itwill take time before a definitive answer is obtained. In the meantime, the use of radical radiation therapy to all metastatic sites in patients with oligometastatic breast cancer can be considered a valuable option and should be recommended to the appropriate candidates.

Conflict of interest

None.

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Please cite this article in press as: Trovo M et al. Radical radiation therapy for oligometastatic breast cancer: Results of a prospective phase II trial. Radiother Oncol (2017), https://doi.org/10.1016/j.radonc.2017.08.032

Manuscript 5:

Mazzola R, Fiorentino A, Di Paola G, **Giaj Levra N**, Ricchetti F, Fersino S, Tebano U, Pasetto S, Ruggieri R, Salgarello M, Alongi F.

Stereotactic Ablative Radiation Therapy for Lung Oligometastases: Predictive Parameters of Early Response by 18FDG-PET/CT.

J Thorac Oncol. 2017;12:547-555. doi: 10.1016/j.jtho.2016.11.2234.

The retrospective study investigated the use of PET-CT parameters as predictive of response after stereotactic ablative radiotherapy for lung oligometastases.

ORIGINAL ARTICLE

Stereotactic Ablative Radiation Therapy for Lung Oligometastases: Predictive Parameters of Early Response by ^{18}FDG-PET/CT

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Received 28 June 2016; revised 28 October 2016; accepted 15 November 2016
Available online - 15 February 2017

ABSTRACT

Objectives: The objective of this study was to investigate fludeoxyglucose F 18 positron emission tomography/computed tomography (^{18}FDG-PET/CT) parameters as predictive of response after stereotactic ablative radiotherapy (SABR) for lung oligometastases.

Methods: The inclusion criteria of the current retrospective study were as follows: (1) lung oligometastases treated by SABR, (2) presence of ^{18}FDG-PET/CT before and after SABR for at least two subsequent evaluations, (3) Karnofsky performance status higher than 80, and (4) life expectancy longer than 6 months. All patients were treated with a biologically equivalent dose of at least 100 Gy with an alpha/beta ratio of 10. The following metabolic parameters were semiquantitatively defined: maximum standardized uptake value (SUV_{max}), mean standardized uptake value (SUV_{mean}), metabolic tumor volume, and total lesion glycolysis.

Results: A total of 50 patients met the inclusion criteria, for a total of 70 lung metastases. The pre-SABR median SUV_{max} was 6.5 (range 4–17), the median SUV_{mean} was 3.7 (range 2.5–6.5), and the median metabolic tumor volume was 2.3 cm^3 (0.2–31 cm^3). The following metabolic parameters were significantly related to complete response at 6 months: SUV_{max} less than 5 ($p < 0.001$) and SUV_{mean} less than 3.5 ($p = 0.03$). ΔSUV_{max} at 3 to 6 months was +126% for lesions with in-field progression versus −26% for the remaining lesions ($p = 0.002$). ΔSUV_{mean} at 3 to 6 months was +15% for lesions with in-field progression versus −26% for the remaining metastases ($p = 0.008$).

Conclusions: In the current analysis, complete response from lung metastasis at 6 months after stereotactic body radiation therapy was significantly associated with both the maximum and mean values of pre-SABR ^{18}FDG-PET/CT SUV. Longer-term trials are strongly advocated to improve the personalization of the monitoring of tumor response in patients with lung oligometastases and, consequently, monitoring of the cost-effectiveness of the health care.

Keywords: SABR; Lung malignancies; Predictive factors; ^{18}FDG-PET/CT

Introduction

Stereotactic ablative radiotherapy (SABR) is an emerging therapeutic approach that involves the use of focused ablative radiation doses with a higher biological effect compared with conventional radiotherapy (RT). During the past few years, the efficacy and safety of SABR has been documented in several settings, including in a subset of selected patients with metastases, usually with one to five lesions, designated with the term

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Disclosure: The authors declare no conflict of interest.

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ISSN: 1556-0864
http://dx.doi.org/10.1016/j.jtho.2016.11.2234

oligometastases.[1–4] In lung oligometastases, SABR guarantees excellent local control (LC) rates with negligible toxicity.[5–8] Unacceptably increased levels of grade 3 to 5 pulmonary toxicity for centrally located lesions (i.e., tumors within 2 cm of the large bronchial tree) were initially reported for the stereotactic body radiation therapy (SBRT) schedule of 60 Gy in three fractions[9] and confirmed for schedules with 40 to 60 Gy given in three or four fractions.[10] Thus, the use of more fractionated schedules has been developed as an adequate approach to SBRT for centrally located tumors,[11–13] although caution according to patient's specificity is still necessary.[14]

Tumor control seems to be strictly related to a biologically equivalent dose (BED) of at least 100 Gy with an alpha/beta ratio of 10 (100 $Gy_{10,}$)[15] resulting in a high rate of cell killing owing to several biological effects (direct tumor cell death, vascular damage, indirect tumor cell death, and imunomodulation).[16] Fludeoxyglucose F 18 positron emission tomography integrated with computed tomography (^{18}FDG-PET/CT) is often adopted in the setting of lung metastases as an effective tool in staging and to monitor the response after systemic therapies. Additionally, disease assessment by means of ^{18}FDG-PET/CT could affect the management of patients with lung metastases who are candidates for local treatment with curative intent (lung metastasectomy), especially in metastatic colorectal cancer.[17] The evaluation of tumor response after SABR for lung malignancies by ^{18}FDG-PET/CT needs further validation; however, the metabolic features could be utilized as a surrogate for tumor response.[18]

Apart from a BED of at least 100 Gy_{10}, in lung SABR for oligometastases no factors to predict the efficacy of the treatment are available as yet. Thus, the radiation oncology community is wondering whether other parameters could be helpful to predict response to SABR or to select the subset of patients with oligometastases appropriate for SABR.[19] The metabolic profile of lung oligometastases, defined by means of ^{18}FDG-PET/CT, could represent a *piece of this puzzle* concerning the issue of predictive factors to customize SABR for this subset of patients.

The aim of the study was to assess ^{18}FDG-PET/CT results during the follow-up period and the difference from functional imaging before SABR.

Materials and Methods

Patients and SABR

Lung SABR for oligometastases was performed when the following criteria were satisfied: (1) controlled primary tumor, (2) absence of progressive disease for longer than 6 months, and (3) no more than five metastatic lesions.

Planning and treatment for all patients was performed while they were in a supine position with a Posirest (CIVCO Medical Solutions, Orange City, Iowa) and a Vac-Lok cushion (CIVCO Medical Solutions). A four-dimensional CT scan in the treatment position was acquired for all patients, and for each patient, 10 phases were reconstructed with 3 mm of slice thickness and interslice distance. Gross tumor volume was equal to clinical target volume. It consisted of the radiological lung lesion, as identified by optimizing the Hounsfield units (HU) window for lungs and by repeating the delineation on each four-dimensional CT phase. Internal target volume was defined as the Boolean envelope of the gross tumor volumes from each respiratory phase. Planning target volume (PTV) was defined as the internal target volume plus an isotropic margin of 5 mm in all directions. The conceived organs at risk (OAR) were the homolateral and contralateral lung, heart, spinal cord, esophagus, and chest wall.

The prescribed total dose of SABR was varied according to the tumor site (central or peripheral) and maximum diameter of the lesions by using a strategy of risk-adapted dose prescription. We used schedules of three to five fractions for peripheral lesions versus schedules of eight to 10 fractions for central lesions. Furthermore, schedules of four fractions of 12 Gy or five fractions of 11 Gy, instead of three fractions of 18 Gy, were selected for peripheral lesions of patients with larger tumors (>2 cm) and/or a higher risk profile. Similarly, 10 fractions of 7 Gy, instead of eight fractions of 7.5 Gy, were considered for centrally located lesions according to the potential presence of overlap between PTV and critical OAR (e.g., bronchial tree or esophagus). In the case of overlap, the sparing of the OAR was privileged with respect to the target dose coverage: 95% of the prescribed dose (Dp) was then optimized to at least 95% of the target volume, which was usually defined as PTV minus OAR, unless a further crop was necessary to ensure a within-tolerance maximum dose to the overlapping OAR. The dose prescription was at the median PTV dose with assurance from optimization to 95% of the Dp to at least 95% of the PTV and a near-maximum target dose not larger than 107% of the Dp.

By neglecting tumor repopulation, given the reduced number of fractions in SBRT schedules, BED was calculated by the formula $D \times [1 + d/(\alpha/\beta)]$,[20] where d is the dose per fraction, and D is the total dose. All adopted schedules satisfied a BED_{10} of a least 100 Gy at the isocenter, where α/β equal to 10 Gy was assumed for all metastatic lesions.

The constraints for OAR were a $D_{0.1cc}$ value of less than 20 Gy on the spinal cord planning risk volume (isotropically expanded by 4 mm from spinal cord) and

a D_{1cc} value less than 30 Gy for the heart and esophagus. For the total lungs minus PTV, the dose constraints were V_5 less than 30%, V_{10} less than 20%, and V_{20} less than 10% and mean lung dose less than 4 Gy. All plans were performed by RapidArc, version 10.0.28 (Varian Inc., Palo Alto, CA) volumetric modulated arc therapy by typically using two coplanar arcs of approximately 200 degrees with a single isocenter per metastatic lesion. Jaw tracking was used to minimize residual leaf transmission. The final dose distributions were computed with the analytical anisotropic algorithm (version 10.0.28), as implemented in the Eclipse treatment planning system, version 10.0.28 (Varian Inc.). Patients were typically treated with 6-MV flattening filter–free photon beams by means of a TrueBeam linac (Varian Inc.) equipped with a Millennium multileaf collimator (Varian Inc.) with a leaf dimension at the isocenter of 5 mm. A maximum dose rate of 1400 MU/min for the 6-MV flattening filter–free photon beam was used. Before each fraction, image-guided RT was performed by means of kV cone beam CT. Evaluation of tumor response was assessed by means of ^{18}FDG-PET/CT and according to the PET Response Criteria in Solid Tumors[21] within 3 months after SABR and every 3 months thereafter.

Study Design and Definition of the Metabolic Parameters

The inclusion criteria of the current retrospective study were as follows: (1) one to five lung oligometastases treated with SABR for each patient, (2) presence of ^{18}FDG-PET/CT before and after SABR for at least two subsequent evaluations, (3) Karnofsky performance status higher than 80, and (4) life expectancy longer than 6 months.

Pre-SBRT ^{18}FDG-PET/CT three-dimensional (3D) scans (i.e., without gating) were performed with the patient within the same fixation devices to be used for treatment, whereas in the post-SBRT PET/CT 3D-scans no fixation device was adopted. The scans were performed with a Siemens Biograph mCT-S(64) system (Siemens Knoxville, TN). Tomographic images were reconstructed by using the TrueX point spread function plus time of flight iterative reconstruction algorithm (three iterations, 21 subsets, and a 5-mm full-width at half-maximum Gaussian filter) and analyzed with the Siemens Syngo TrueD 3D VOI isocontour tool (Siemens). PET acquisitions were started 60 minutes after administration of 2.96 MBq/kg of ^{18}FDG; patients were enrolled if their blood glucose level was lower than 140 mg/dL. When lesions in the lower lung segment were detected, patients underwent a 30-second breath-hold acquisition to avoid or minimize movement issues.

For the intent of the analysis, the following ^{18}FDG metabolic parameters were retrospectively defined: (1) SUV_{max} (i.e., the highest uptake value over all pixels within the region of interest [ROI]), (2) SUV_{mean} (i.e., the mean uptake value within the ROI), (3) metabolic tumor volume (MTV) (i.e., the total volume with an SUV of 2.5 or greater), and (4) total lesion glycolysis (TLG) as an estimate of tumor metabolic rate (i.e., the product of SUV_{mean} and MTV). Both pre- and post-SBRT ^{18}F-FDG-PET/CT data sets were analyzed semiquantitatively with Syngo Multimodality Workplace software (Siemens AG, Erlangen, Germany) by two nuclear physicians who were blinded to all imaging studies and clinical and pathological results. For each lung lesion, the irregular isocontour ROI was determined on the basis of a fixed threshold for the ^{18}FDG SUV (e.g., SUV $\geq$ 2.5).[21] PET-CT SUV values were standardized according to the European Association for Nuclear Medicine procedure guidelines for tumor imaging, version 2.0.[22]

Statistical Analysis

To summarize the most relevant features of the clinical variables, descriptive statistics were performed. All the categorical variables were analyzed with contingency tables with Fisher's exact test or Pearson's chi-square test, whereas the continuous variables were analyzed by one-way analysis of variance, *t* tests (with equal or unequal variance), or nonparametric Wilcoxon (Mann-Whitney) and Kruskal-Wallis tests.

Three clinical outcomes were defined: (1) LC as the absence of local recurrence in field (in the prior radiation field), (2) distant metastases–free survival, and (3) overall survival from the end of SABR. These parameters were assessed by using Kaplan-Meier curves.

Logistic regression models were used to assess the relationship between the pre-SABR metabolic parameters (SUV_{max}, SUV_{mean}, MTV, TLG, ΔSUV_{max}, and ΔSUV_{mean} considering pre-SABR and post-SABR values) with local failure, distant metastatic progression, and complete response of lung metastasis during follow up. The following dependent variables were taken into account with the metabolic parameters to estimate the possible correlation with local failure and distant metastases: patient's age, number of fractions, BED, type of primary tumor, tumor volume, and number of metastatic lesions. These variables were dichotomized at the median value for the analysis.

The receiver operating characteristic curves were used to assess the sensitivity and specificity of the cutoff of the pre-SABR metabolic parameters in correlation with the probability of complete response of the lung lesion during follow-up after SABR. The area under the curve (AUC) was used to verify the accuracy; in the case

of a moderately accurate test (AUC> 0.7), the product of maximum sensitivity and specificity was chosen as the cutoff value.

A p value of 0.05 or less was considered statistically significant. Statistical analysis was performed with R software, version 3.1.2 (R Foundation for Statistical Computing, Vienna, Austria).

Results

Patients

From January 2012 to November 2015, 50 patients met the inclusion criteria of the present analysis, for a total of 70 lung metastatic lesions. Table 1 shows patient and lung metastases characteristics. All patients analyzed in the current study had only lung oligometastases with absence of disease outside the lung. The lesions were metachronous and classified as oligopersistent and/or oligorecurrent[23] in a scenario of metastatic disease after one or two schedules of systemic antiblastic therapies administered according to international guidelines,[24] taking into account the specific primary tumor.

All patients reached a follow-up after SABR of at least 6 months. The median follow-up was 18 months (range 6–53 months). The 1-year overall survival and LC (lack of any recurrence in field) rates were 86% and 78%, respectively. The median distant metastases–free survival was 6 months (range 3–15 months). During the follow-up, the distant metastases sites were the brain (two), liver (four), lymph nodes (two), bone (one), and lung out of field (two). There was an in-field disease progression in seven lesions.

Table 1. Characteristics of Patients (n = 50) and Lung Metastases (n = 70)

Parameter	n	%
Sex		
Male	34	68
Female	16	32
Median age	70 y (range 48-85)	
Primary lesion site		
Lung	34	49
Colon	28	41.5
Corpus uteri	6	8.7
Larynx	1	1.5
Lesion histologic subtype		
Adenocarcinoma	50	71
Squamous	20	29
Lung lesion side		
Right	44	63
Left	26	37
SABR, no. fractions		
3	7	10
4	11	16
5	28	40
8	10	14
10	14	20
Lesion diameter, maximum		
Median 2.3 cm (range 1-5)		
Biologically equivalent dose		
Median 110 Gy (range 100-164)		
Gross tumor volume		
Median 3.8 cm^3 (range 0.3-33)		
Internal target volume		
Median 7.5 cm^3 (range 0.6-35.5)		
Planning target volume		
Median 26 cm^3 (range 5.5-78.5)		

SABR, stereotactic ablative radiotherapy.

Pre-SABR Metabolic Findings

The median interval between pre-SABR ^{18}FDG-PET/CT and the first fraction of SABR was 5 days (range 3–7 days). Before treatment, the median SUV_{max} was 6.5 (range 4–17), the median SUV_{mean} was 3.7 (range, 2.5–6.5), and the median MTV was 2.3 cm^3 (range 0.2–31 cm^3). For lesions with in-field disease progression, the median TLG was 17.4 (range 2–52.8); for the remaining lesions, the median value was 170.6 (0.5–171).

Post-SABR Metabolic Findings

Table 2 details the post-SABR median metabolic findings within 3 months after treatment and at 6, 9, 12 and 18 months of follow-up for all the lesions analyzed. Figure 1 shows the SUV_{max} and SUV_{mean} behavior curves during follow-up for lesions with in-field and distant failures.

For lesions without in-field failure (n = 63), an increase in SUV_{max} and SUV_{mean} values was registered at 9 to 12 months after SABR in comparison with the control at 6 months of follow-up after SABR. In particular, SUV_{max} has been estimated at +5.4%, whereas SUV_{mean} has been estimated at +1.6%. This phenomenon was no longer evident in the subsequent metabolic imaging.

Table 2. Post-SBRT Metabolic Findings at 3, 6, 9, 12, and 18 Months of Follow-up

Follow-up	No. Lesions Analyzed	Median Value of SUV_{max} (Range)	Median Value of SUV_{mean} (Range)	Median Value of MTV (Range)
3 mo	70	3.8 (1.9-14)	3 (1.9-6.5)	3.9 (0.25-50)
6 mo	51	2.8 (2-20)	2.7 (1-5)	5 (1-18)
9 mo	24	2.5 (2-11)	2.5 (2-4)	7 (0.05-10)
12 mo	18	2.6 (1.7-11.5)	2.5 (2-4)	7.8 (0.05-10)
18 mo	6	2.4 (2-3.7)	2.4 (2-2.7)	Not evaluable

SBRT, stereotactic ablative radiotherapy; SUV_{max}, maximum standardized fludeoxyglucose F 18 uptake value; SUV_{mean}, mean standardized fludeoxyglucose F 18 uptake value; MTV, metabolic tumor volume, defined as total volume with a standardized uptake value of 2.5 or greater.

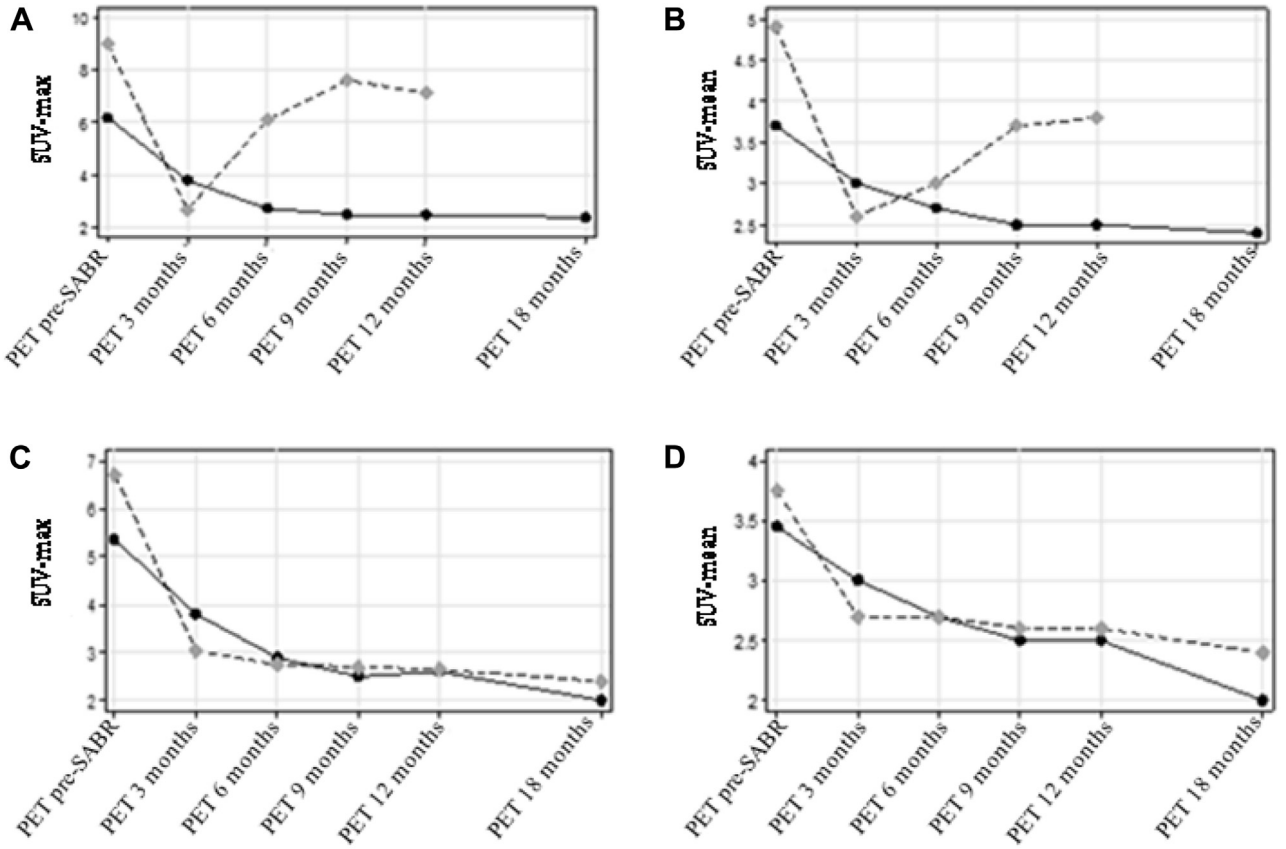

Figure 1. Maximum standardized uptake value (SUV_{max}) (*A*) and mean standardized uptake value (SUV_{mean}) (*B*) curves for patients with local failure (*dashed line*), with solid line representative of patients without local failure after stereotactic ablative radiotherapy (SABR). SUV_{max} (*C*) and SUV_{mean} (*D*) curves for patients with distant metastases after SABR (*dashed line*), with the solid line representative of patients without distant metastases after SABR. PET, positron emission tomography.

Metabolic Parameters Predictive of SABR Outcomes

No statistical correlation was observed between the pre-SABR metabolic variables (SUV_{max} and SUV_{mean}, MTV and TLG, and ΔSUV_{max} and ΔSUV_{mean}) and clinical parameters (patient's age, number of fractions, BED, type of primary tumor, tumor volume, and number of metastatic lesions) with local failure or distant progression. Conversely, a complete lung lesion response at 6 months after SABR was related to the pre-SABR SUV_{max} and SUV_{mean} values.

In fact, at this time point a complete response was observed in 94% of lesions if a pre-SABR SUV_{max} value less than 5 was registered ($p = 0.001$, AUC = 0.90, sensitivity = 88%, and specificity = 94%).

Table 3 showed statistical correlations between pre-SABR metabolic parameters (SUV_{max}, SUV_{mean}, MTV, and TLG) with in-field failure, distant metastatic progression and response of the lung metastasis 6 months after SABR. Figure 2 shows the receiver operating characteristic curve for a pre-SABR SUV_{max} value less than 5 in correlation with complete lung lesion response at 6 months after SABR. Similarly, a pre-SABR SUV_{mean} value less than 3.5 was related to complete response at 6 months after SABR ($p = 0.03$, sensitivity = 31%, specificity = 34%, and AUC = 0.32).

Findings of the Analysis of In-Field Recurrences

Considering the seven lung metastases with in-field failure, a pre-SABR SUV_{max} value greater than 8 was related to a higher increase in SUV_{max} at 6 months of follow-up (in terms of absolute value) compared with a pre-SABR SUV_{max} value less than 8 ($p = 0.005$). Although there is no statistically significant relation (because of the sample size), an OR of 1.89 for in-field recurrence was found in the case of a pre-SABR SUV_{mean} value of at least 4. Only two of seven lesions with in-field relapse were centrally located. The dichotomization of the sample in terms of tumor location did not give statistically significant results. The 86% of patients with local failure had distant progression versus a rate of only 19% in cases without local failure ($p = 0.004$, OR = 25).

Table 3. Correlations between Pre-SABR Metabolic Parameters with Local Failure, Distant Metastatic Progression, and Lung Metastasis Response

	Local Failure (In-Field)			Distant Metastatic Progression			Lung Metastasis Complete Response (6 mo after SABR)		
Parameter	OR	95% CI	*p* Value	OR	95% CI	*p* Value	OR	95% CI	*p* Value
SUV_{max} (for values ≥5)	2.93	0.52-5.11	0.219	1.98	0.66-5.91	0.221	0.313	0.09-0.99	**0.05**
SUV_{mean} (for values ≥ 5)	1.06	0.22-5.16	0.936	1.85	0.61-5.68	0.281	0.237	0.06-0.84	**0.026**
MTV	1.01	0.89-1.14	0.855	1.04	0.96-1.14	0.281	1.01	0.91-1.11	0.946
TLG	1.01	0.97-1.02	0.897	1.01	0.99-1.02	0.294	0.99	0.97-1.02	0.791

Note: Boldface indicates statistically significant *p* values.
SABR, stereotactice ablative radiotherapy; CI, confidence interval; SUV_{max}, maximum standardized fludeoxyglucose F 18 uptake value; SUV_{mean}, mean standardized fludeoxyglucose F 18 uptake value; MTV, metabolic tumor volume, defined as total volume with a standardized uptake value of 2.5 or greater; TLG, total lesion glycolysis.

Findings on the Δ Values between PET Scans

A ΔSUV_{max} between the pre-SABR and first control values (here defined as ΔSUV_{max} at 0–3 months) was −65% for lesions with in-field progression versus −22.5% for the remaining metastases. Conversely, the ΔSUV_{max} at 3 to 6 months was +126% for lesions with in-field progression versus −26% for the remaining metastases ($p = 0.002$, two-sample Wilcoxon rank sum test). The Δ SUV_{mean} at 0 to 3 months was −39% for lesions with in-field progression versus −17% for the remaining metastases. Δ SUV_{mean} at 3 to 6 months was +15% for lesions with in-field progression versus −26% for the remaining metastases ($p = 0.008$, two-sample Wilcoxon rank sum test).

Discussion

In the past few years, a growing interest in the use of SABR as a therapeutic option for lung oligometastases has arisen. Post-SABR radiological changes are frequently detected on diagnostic CT scan imaging.[25,26]

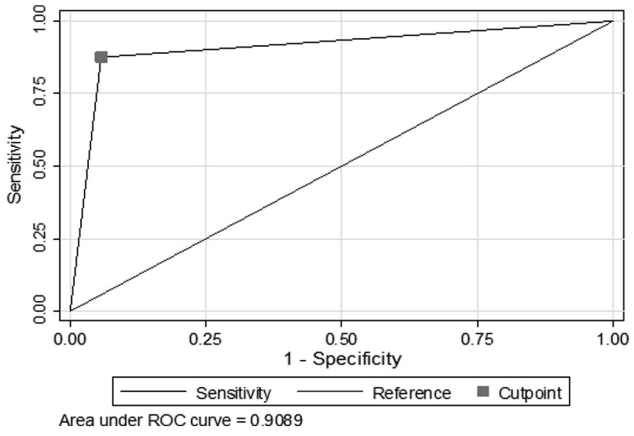

Figure 2. Receiver operating characteristic (ROC) curve for pre-stereotactic ablative radiotherapy maximum standardized uptake value less than 5 as a factor predictive of complete lung lesion response 6 months after stereotactic ablative radiotherapy.

In case of mass-like patterns on CT scans after SABR, it is difficult to differentiate between radiation fibrosis or tumor recurrence. Thus, ^{18}FDG-PET/CT may be utilized as an important tool to monitor tumor response by means of semiquantitative metabolic parameters.[21,27] Furthermore, the role of ^{18}FDG-PET/CT as a predictor of outcome in patients with primary lung malignancies treated with SABR has been investigated.[28,29]

In a retrospective study, lung lesion volume variations were analyzed by contouring on cone beam CT images to evaluate early predictive parameters of response to SABR. At the last session of SABR, a lung lesion shrinkage of at least 20% was revealed to be predictable of complete response 6 months thereafter.[30] Several metabolic predictive factors for recurrence and survival after SABR for primary lung cancer have already been investigated by several studies.[31–33] Similarly, the present study was designed to investigate the role of ^{18}FDG-PET/CT parameters as predictive of early response after SABR in the setting of lung oligometastases. In the current analysis, a complete lung lesion response at 6 months after SABR was related to pre-SABR SUV_{max} and SUV_{mean} values. Lung oligometastases with a pre-SABR SUV_{max} value less than 5 as well as a SUV_{mean} value less than 3.5 was revealed to be related to complete response at 6 months.

The issue of pre-SABR FDG uptake as a predictive factor is not new, especially in the setting of primary lung cancer. In a large patient population affected by primary lung cancer, a pre-treatment SUV_{max} value greater than 3 was associated with worse survival and a greater propensity for local recurrence and distant metastasis after SABR.[31] These findings may mean that a low metabolic activity in lung malignancies could identify patients who would benefit from an SABR-approach alone. On the other hand, the present findings could assume more relevance in the scenario of a multidisciplinary approach in lung oligometastases: in the case of pre-SABR high metabolic uptake, a sequential approach with systemic therapies could be

evaluated early. Although these hypotheses need future evaluations, these arguments could appear intriguing in terms of (1) customizing therapeutic management after SABR (adding cytotoxic drugs), (2) monitoring patients with oligometastases according to the probability of tumor response, and (3) adapting the SABR dose prescription according to SUV stratification. PET-SUV thresholds, if standardized, might be helpful for decision making regarding stratification of patients with oligometastases into slowly progressing patients and rapidly progressing patients. The exact therapeutic implication for intervention remains to be determined, and the primary use of systemic therapy in patients with high PET SUV could be an option. Clinical trials with stratification based on SUV PET are needed to justify the different treatment strategies. On the other hand, in this setting of disease, PET could influence the frequency or imaging strategies during follow-up to create a sort of personalization of follow-up allowing for possible health care cost benefits.

Besides the well-recognized and common measurement parameters such as SUV_{max}, SUV_{mean}, MTV, and TLG derived from ^{18}FDG-PET/CT scans, more advanced image analysis methods such as radiomics are currently under investigation for evaluation of treatment and prediction of response or as potential biomarkers to adopt in clinical interpretation of molecular images. These radiomics applications could provide promising findings to integrate with the conventional parameters for imaging measurements. Nevertheless, no robust and reliable models seem to be available as yet and no large consensus has been achieved by nuclear medicine physicians, especially in this context.[34] Thus, radiomics features were not used in this study.

The role of ^{18}FDG-PET/CT in the detection of lung tumor response after conventional RT is well recognized. In this setting, 18-FDG-PET/CT showed high rates of sensitivity and specificity, estimated at 100% and 92%, respectively.[35] In the scenario of patients with lung oligometastases who underwent SABR, it was shown that ^{18}FDG-PET/CT is effective in detecting responses.[36] However, some concerns remain about the role of ^{18}FDG-PET/CT versus CT scan alone after lung SABR. First of all, differentiating tumor recurrence from radiation fibrosis remains challenging in lung SABR scenario. Moreover, in the absence of morphological change on a CT scan, ^{18}FDG-PET/CT allows for a better understanding of tumor response. A decrease in metabolic uptake would indicate a decreased tumor activity and possible response to treatment. Compared with CT scan alone, fused ^{18}FDG-PET/CT images may allow differentiation of metabolically active recurrent tumor from metabolically inactive radiation-induced fibrosis. ^{18}FDG uptake after SABR for lung malignancies could be moderate early after treatment. A pathological confirmation of malignancy is generally preferred before the initiation of any curative-intent therapy. Many candidates for SABR have comorbidities, including compromised pulmonary and cardiac function, that could increase the risks associated with transthoracic biopsy or repeated biopsy if the initial attempt is not conclusive.[37] In lung malignancies, a study[38] found that a PET-directed SABR strategy (without prior biopsy) could be warranted thanks to a point estimate of malignancy of 85%. Again, in a Dutch study[39] the use of PET scans has made it possible to obtain a probability of malignancies of 92%. Thus, in the current study ^{18}FDG-PET/CT parameters were used to evaluate the response rates. Additionally, in the case of metastatic disease, we are reluctant to promote an invasive procedure except in those cases that are really difficult to evaluate and in which histological subtype is easy to obtain (no contraindications to surgery). In a systematic review, an SUV_{max} value of 5 or greater was identified as highly suggestive of recurrence.[40] However, the metabolic uptake usually decreases at 12 months and longer without clear images of mass-like shape uptake.[41] Strangely, in our experience, MTV, which is a metabolic biomarker defined as total volume with an SUV of at least 2.5, increased over the follow-up without statistically significant correlations with local failure or distant progression, as well as with the other pre-SABR metabolic variables here analyzed. From our point of view, the increase in MTV could be related to the enlargement of the phlogistic area in the lung parenchyma after SABR with an SUV of at least 2.5. However, this last aspect needs specific further investigation.

In the case of centrally located lesions that overlap with crucial OAR, the reduced target dose coverage might determine an increased risk of local failure for such centrally located lesions. In the present study population, only two of seven lesions with in-field relapse were centrally located. The dichotomization of the sample in terms of tumor location did not give statistically significant results. A Δ $SUV_{max/mean}$ value for 0 to 3 months was revealed to be more marked in terms of SUV reduction for patients in which *in-field* progression during follow-up was registered. Conversely, the $\Delta SUV_{max/mean}$ value in the interval from 3 to 6 months was increased for the same patients. These findings could attest that an early ^{18}FDG-PET/CT evaluation after SABR may be not as necessary for all the patients. Although the identification of the subgroup of patients in whom ^{18}FDG-PET/CT could be delayed after SABR remains not investigated in the present study, longer-term trials are strongly advocated to improve the personalization of tumor response monitoring in patients with oligometastases and, subsequently, the cost-effectiveness of health care.

Finally, our findings confirm the role of lung SABR in the metastatic setting. In fact, 86% of patients with local failure had distant progression versus only 19% of those without local failure. These results could reflect the postulate by Hellman and Weichselbaum according to which a state of tumor dormancy with reduced ability to metastasize could exist in patients with oligometastases.[42] Thus, ablation of macroscopic *foci* of disease could favorably modify the natural history and management of the oligometastatic phase.

Acknowledgments

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The authors alone are responsible for the content and writing of this article.

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Manuscript 6:

Mazzola R, Fiorentino A, Ricchetti F, **Giaj Levra N**, Fersino S, Di Paola G, Lo Casto A, Ruggieri R, Alongi F.

Cone-beam computed tomography in lung stereotactic ablative radiation therapy: predictive parameters of early response.

Br J Radiol. 2016:20160146. doi: 10.1259/bjr.20160146 [Epub ahead of print]

This retrospective study analysed the lung lesion volume variations by contouring on Cone beam CT during stereotactic ablative radiotherapy as early predictive parameters of radiation response.

BJR

Received: 12 February 2016 | Revised: 19 April 2016 | Accepted: 31 May 2016

http://dx.doi.org/10.1259/bjr.20160146

Cite this article as:
Mazzola R, Fiorentino A, Ricchetti F, Giaj Levra N, Fersino S, Di Paola G, et al. Cone-beam computed tomography in lung stereotactic ablative radiation therapy: predictive parameters of early response. *Br J Radiol* 2016; **89**: 20160146.

FULL PAPER

Cone-beam computed tomography in lung stereotactic ablative radiation therapy: predictive parameters of early response

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Objective: To analyze lung lesion volume variations by contouring on cone-beam CT (CBCT) images to evaluate the early predictive parameters of stereotactic ablative radiation therapy (SABR) treatment response.
Methods: The prescribed dose of SABR was varied according to the tumour site (central or peripheral) and maximum diameter of the lesions by using a strategy of risk-adapted dose prescription with a dose range between 48 and 70 Gy in 3–10 consecutive fractions. For the purpose of the analysis, the gross tumour volume (GTV) was recontoured for each patient at first and last CBCT using two lung levels/windows: (a) −600/1000 HU and (b) −1000/250 HU. Univariate analysis was performed to evaluate a correlation between lung lesion variations on CBCT using the two levels/windows and treatment response 6 months after SABR. Independent variables were the number of fractions, time between initial and final fraction, biologically effective dose and pre-SABR GTV. Cut points of lesion volume reduction were evaluated to determine the correlation with complete response 6 months after SABR.
Results: 41 lung lesions were evaluated. 82 lung lesions were recontoured for each CBCT level/window. A lung lesion shrinkage of at least 20% was revealed to be statistically related to complete response 6 months after SABR for both the CBCT levels/windows used. The probability of complete response ranged between six and eight times higher in respect to CBCT levels/windows −600/1000 HU and −1000/250 HU, respectively, compared with patients without a lesion shrinkage of 20% at the last session of SABR.
Conclusion: According to current findings, a lung lesion shrinkage of at least 20% at the last session of SABR could be predictable of complete response 6 months thereafter. Further investigations about this topic are needed.
Advances in knowledge: Prediction of the early tumour response could be useful to personalize imaging restaging after the completion of SABR or to incorporate additional therapies in case of poor responders to improve clinical outcomes.

INTRODUCTION

In lung malignancies, precise delivery of high radiation doses in a small number of fractions by means of stereotactic ablative radiotherapy (SABR) guarantees excellent local control (LC) rates with negligible toxicity.[1–5] The high rate of tumour control by SABR seems to be strictly related to a biologically effective dose (BED) ≥100 Gy.[6] Moreover, a new biologic effect with a high dose per fraction, resulting in immediate vascular damage, seems to play a crucial role in the so-called "indirect cell death".[7]

SABR has become the standard treatment for medically inoperable Stage I non-small-cell lung cancer (NSCLC),[8,9] although in a pooled analysis of two randomized trials, SABR has been recently defined as a promising option for treating operable Stage I NSCLC.[10] Similarly, during the past years, the efficacy and safety of SABR has been documented in a subset of selected patients with lung metastasis, usually with 1–5 lesions, recognized with the term of "oligometastases".[11–13]

Three-dimensional conformal radiation therapy (RT) or intensity-modulated RT, including volumetric-modulated arc therapy, has been used for SABR plans.[14,15] Image-guided RT, by means of on-board imaging including cone-beam CT (CBCT), can minimize setup uncertainties for more accurate focal treatments.[14] In addition, a potential benefit of CBCT could be the ability to assess tumour volume changes during SABR.[16,17]

Based on this background, the aim of the present study was to analyze the lung lesion volume variations by contouring on CBCT images to identify early predictive parameters of SABR response.

METHODS AND MATERIALS

Patients

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

From January 2012 to March 2015, 31 consecutive patients for a total of 41 lung lesions were treated with SABR at our institution. Lung SABR was performed in medically inoperable Stage I NSCLC and in patients with oligometastasis. In the latest group, SABR was specifically indicated when the following criteria were satisfied: (a) controlled primary tumour, (b) the absence of progressive disease longer than 6 months and (c) the number of metastatic lesions ≤5. In case of patients with Stage I NSCLC, a pathological confirmation was performed in 7/10 cases; in the remaining 3 patients, biopsy was not performed because of compromised pulmonary and cardiac function. For these last patients, contrast CT scan and positron emission tomography (PET)/CT features were considered as surrogates of malignancy diagnosis. Lung lesion characteristics are detailed in Table 1.

Table 1. Lung lesions characteristics ($n = 41$)

Parameters	Numbers	(%)
Type of lesions		
Primitive	10	(24)
Metastases	31	(76)
Lesion histology		
Adenocarcinoma	36	(88)
Squamous	1	(2.5)
Urotelial	1	(2.5)
Unknown	3	(7)
Primitive lesion site		
Lung	22	(56)
Colon	17	(39)
Bladder	1	(2.5)
Larynx	1	(2.5)
Lung lesion side		
Right	31	(76)
Left	10	(24)
Number of fractions in SABR		
3	4	(10)
4	4	(10)
5	13	(32)
8	12	(30)
10	8	(18)
Maximum lesion diameter		
Median 1.75 cm (range, 0.6–5 cm)		
BED		
Median 105 Gy (range, 95–150 Gy)		
GTV		
Median 3.8 cc (0.3–33 cc)		
ITV		
Median 7.5 cc (0.6–35.5 cc)		
PTV		
Median 26 cc (5.5–78.5 cc)		

BED, biologically effective dose; GTV, gross tumour volume; ITV, internal target volume; PTV, planning target volume; SABR, stereotactic ablative radiotherapy.

Planning and treatment

All patients were planned and treated in supine position with a Posirest™ (CIVCO® Medical Solutions, Orange City, IA) and a Vac-Lok™ cushion (CIVCO® Medical Solutions, Orange City, IA). A four-dimensional CT (4D-CT) scan in treatment position was acquired for all patients, and for each patient, 10 phases were reconstructed with 3 mm of slice thickness and interslice distance. Gross tumour volume (GTV) was equal to clinical target volume (CTV). It consisted of radiological lung lesion as identified by optimizing the Hounsfield units (HU) window for lungs and by repeating the delineation on each 4D-CT phase. To privilege accuracy over rapidity in the estimate of potential tumour volume variations during treatment, the internal target volume was defined as the Boolean envelope of the GTVs from each respiratory phase, instead of by means of maximum intensity projections. The GTV–CTV margin and the stability of the breathing cycle were not taken into account. Planning target volume (PTV) was defined as internal target volume plus an isotropic margin of 5 mm in all directions. Organs at risk were: homolateral and contralateral lung, heart, spinal cord, oesophagus and chest wall.

The prescribed total dose of SABR was varied according to the tumour site (central or peripheral) and maximum diameter of the lesions using a strategy of risk-adapted dose prescription with a range of doses between 48 and 70 Gy in 3–10 consecutive fractions.[1,2,18–20]

All patients were treated with a BED ≥100 Gy, assuming an α/β value of 10 Gy for the tumour, according to literature,[21] except for one case in which a schedule with a BED of 95 Gy was delivered because of prior contralateral pneumonectomy.

The objective of the plan was to cover at least 95% of the PTV volume with 95% of the prescribed dose. At the same time, a near-maximum target dose ($D_{2\%}$) not larger than 107% of the prescribed dose had to be assured. Constraints for organs at risk were: maximum dose in 0.1 cc ($D_{0.1cc}$) < 20 Gy on spinal cord planning risk volume (isotropically expanded by 4 mm) and D_{max} 1 cc (D_{1cc}) < 30 Gy for the heart and oesophagus. For ipsilateral or homolateral, contralateral and sum of the volume of

both lungs excluding PTV, the dose constraints were: volume of lung that receives 5 Gy (V_5) < 30%, volume of lung that receives 10 Gy (V_{10}) < 20% and V_{20} < 10% and mean lung dose <4 Gy. All volumetric-modulated arc therapy plans were designed and optimized with rapid arc technique v. 10.28 (Varian, Palo Alto, CA) using two partial and coplanar arcs of approximately 200°, with a single isocentre in most of the cases. Jaw tracking was used to reduce the leaf residual transmission. The final dose distributions were computed with the analytical anisotropic algorithm implemented in the Eclipse planning system (Varian, Palo Alto, CA). Patients were treated with 6 or 10 MV using flattening filter-free (FFF) beams by means of a TrueBeam™ linear accelerator (Varian, Palo Alto, CA) equipped with the millennium multileaf collimator with a leaf dimension of 5 mm at the isocentre. A maximum dose rate of 1400 μ min^{-1} for the 6-MV FFF beam and of 2400 μ min^{-1} for the 10-MV FFF beam was used. As a result, the use of 10-MV FFF beams for plans with a prescribed dose per fraction (d) equal to 15–20 Gy translated into an appreciable reduction in beam-on time of about 2 min, from 4.5 to 2.5 min roughly, and, thus, in a likely reduced risk of intrafraction target uncertainties. Whereas, for $d \leq 10$ Gy, we privileged the use of 6-MV FFF beams which are associated with a slightly improved superficial target dose coverage at target lung interfaces; at these d values, the potential reduction in the beam-on time from the use of the 10-MV FFF beams is as small as 0.5 min.

The median interval between the 4D-CT scan in the treatment position and the first fraction of SABR was 5 days (range, 3–7 days). The median time between initial and final SABR fraction was 5 days (range, 3–12 days).

Before each fraction, image-guided RT was performed by means of kilovoltage CBCT. The CBCT acquisition protocol (125 kV, 270 mA s^{-1} and weighted CT dose index of 0.36 cGy) was optimized for thorax imaging with a field of view of 42 cm. The CBCT acquisition time was approximately 2 min. The reconstructed volume from CBCT was converted to 2.5-mm slices and transferred in the digital imaging and communications in medicine format to the treatment planning system.

Evaluation of tumour response

Tumour response was evaluated by thoracic and abdominal CT scan with contrast and fluorine-18 fludeoxyglucose (^{18}F-FDG) PET integrated with CT (PET/CT) before and after treatment (3 and 6 months later). Complete response (CR) was defined as the disappearance of the lesions at CT scan; a reduction of >30% was considered partial remission (PR); any growing lesion not imputable clearly to fibrosis was reported as progression of disease; stable disease (SD) was defined as neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for progression of disease, taking as reference the smallest sum of the diameters while on study, according to the response evaluation criteria in solid tumours.[22]

Study methods

All patients analyzed had at least 6 months of follow-up. Thus, for the purpose of the present retrospective study, analysis objectives were evaluated at 6 months. For every patient, each lung lesion was manually recontoured at the first and last CBCT using two lung levels/windows: (a) −600/1000 HU, according to literature,[16] and (b) −1000/250 HU, which is the default level/window for lung parenchyma in the Eclipse system (Varian). Lung lesions were recontoured retrospectively by a single radiation oncologist (RM) and reviewed simultaneously by another radiation oncologist (AF). In Figure 1, an example of lung lesion CBCT definition at the first and last CBCT using both lung levels/windows is shown.

Statistical analysis

In order to summarize the most relevant features of the clinical variables, descriptive statistics were performed. All the variables were analyzed with Pearson's χ^2 or Fisher's exact tests and contingence tables. Univariate analysis was performed to evaluate a correlation between lung lesion variations on CBCT using the two levels/windows and the treatment response 6 months after SABR. Independent variables were: the number of fractions, time between initial and final fraction, BED and pre-SABR GTV volume. The independent variables were dichotomized at the median value. Cut points of lesion volume reduction were evaluated to determine the correlation with CR 6 months after SABR, using steps of 5% from the minimum of the lesion shrinkage until the significant value. The area under the curve (AUC) was used to assess accuracy; if a test was judged at least moderately accurate (AUC >0.7), the maximum product of sensitivity and specificity was chosen as the cut-off value. Two sample t-tests were performed to evaluate the impact of the accuracy of the CBCT volume definition—tumour located closer to the mediastinum, large bronchi or ribs and secondly, the peripheral tumours with a large motion amplitude which probably impacts the size of the 4D-CT-based CTV. Three clinical outcomes were defined: local control as lack of any recurrence in field, distant metastasis-free survival (DMFS) as the period until metastasis is detected and overall survival (OS) after SABR, all estimated using Kaplan–Meier and cumulative incidence methods. A p-value ≤0.05 was considered significant. Statistical analyses were performed using R-software v. 3.1.2.

RESULTS

Patients

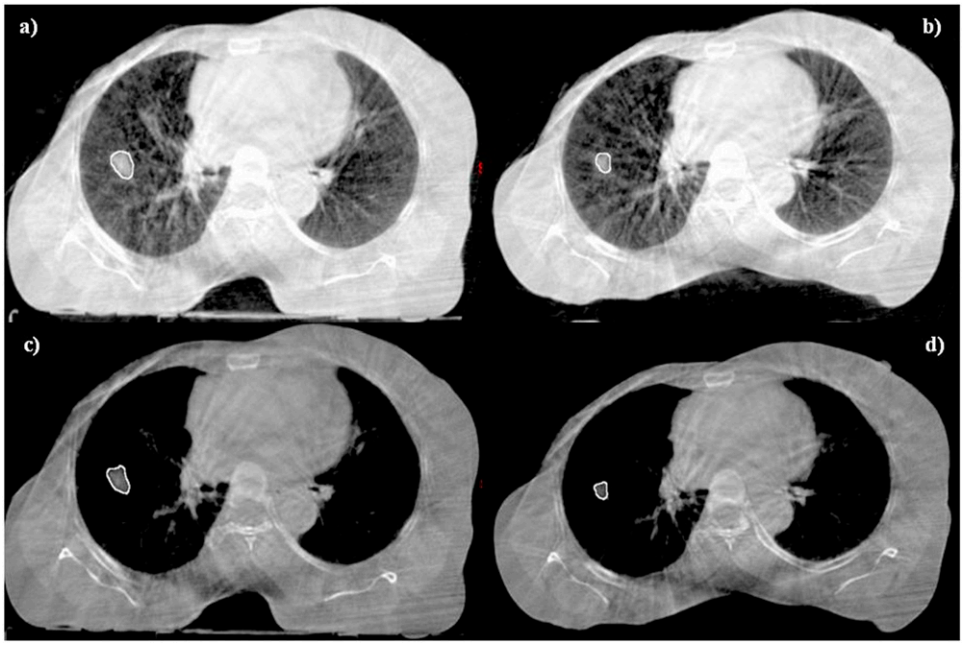
Median follow-up was 16 months (range, 9–43 months). The median age of patients was 68 years (range, 44–83 years). All patients completed the treatment without interruptions. In both patients with primary disease and those with metastasis, 1-year OS and LC (as lack of any recurrence in field) were 100%. The median DMFS was 13 months (range, 4–42 months). In six patients with oligometastasis, distant recurrences were recorded (four in the lung—out of field and two in the liver).

CR and PR, evaluated by CT scan and ^{18}F-FDG-PET/CT at 6 months, were recorded in 18/41 (44%) lesions and 15/41 (37%) lesions, respectively. An SD was recorded in the remaining 8/41 (19%) lesions. No in-field progression was registered 6 months after SABR.

Findings on cone-beam CT level/window −600/1000 HU

At the CBCT level/window −600/1000 HU, the median initial GTV was 4.3 cc (range 0.2–26 cc); at the last treatment session, the median GTV was 3.8 cc (range 0.3–28 cc). The average rate

Figure 1. An example of lung lesion cone-beam CT (CBCT) definition: (a, b) first and last CBCT lung level/window of −1000/250 HU; (c, d) first and last CBCT lung level/window of −600/1000 HU.



of GTV shrinkage was 12% (range, 0–31%). Tumour shrinkage at the last session of SABR was statistically related to CR/PR *vs* SD 6 months after the end of the treatment (p 0.04), with a nine times higher probability of obtaining a CR/PR in the presence of tumour volume reduction (p 0.01). Similarly, considering the CR *vs* PR/SD outcomes, the probability of observing a CR was 6 times higher (p 0.01) compared with patients without a tumour volume reduction at last session of SABR.

Findings on cone-beam CT level/window −1000/250 HU

At the CBCT level/window −1000/250 HU, the median initial GTV was 4 cc (range, 0.4–29 cc) while the median final GTV was 3.1 cc (range, 0.4–28 cc). The average rate of GTV shrinkage was 22.5% (range, 4–30%). Tumour shrinkage was statistically related to CR/PR *vs* SD 6 months after the end of the treatment (p 0.007). When analyzing the rate of CR *vs* PR/SD no statistic significance was found. The probability to obtain a CR/PR was 11 times higher (p 0.009) compared with cases without tumour shrinkage at the last session of SABR.

Findings on gross tumour volume definition variability

Comparing the two CBCT levels/windows, no difference was observed in terms of GTV variability (p 0.5 for initial GTVs and p 0.9 for final GTVs). Similarly, no statistical difference was noted in terms of variability in regard to lung lesion volumes between planning CT definition and first CBCT contouring (p 0.5 and p 0.2 for −600/1000 HU and −1000/250 HU, respectively).

Tumour shrinkage cut-off value as a predictive parameter of early response

A GTV shrinkage of at least 20% revealed to be statistically correlated to lung lesion CR 6 months after SABR (p 0.05), leading to the largest odds ratios for both the CBCT levels/windows used. In detail, the probability of CR ranged between six and eight times higher (for levels/windows −600/1000 HU and −1000/250 HU, respectively) compared with patients without a GTV decrease of at least 20% at the last session of SABR. The time between initial and final fraction did not statistically influence this finding (p 0.5).

Values of sensitivity and specificity of the cut-off value of 20% analyzing all the study population and other variables (number of fractions >5, BED ≤110, BED >110 and GTV dimension pre-SABR >6 cc) are shown in Tables 2 and 3. In Table 4, the detailed sensitivity, specificity and AUC values of the various tumour shrinkage thresholds analyzed in all the study populations, using the CBCT levels/windows of −600/1000 HU and −1000/250 HU, are shown.

DISCUSSION

In the past years, a growing interest in the use of SABR as a therapeutic option for lung malignancies has arisen. Post-SABR radiological changes are commonly found on diagnostic CT scan imaging.[23] Moreover, PET/CT after SABR for lung malignancies may be utilized as a surrogate of tumour response, according to available literature data.[24–26]

On-board CBCT has been recently implemented in clinical practice for precise treatments. Another potential application of CBCT could be the ability to assess tumour volume changes during RT.[16,17] The issue of tumour volume reduction during conventional RT is well recognized in the scenario of adaptive strategy in head and neck cancer. In fact, this topic was largely investigated to minimize healthy tissue toxicity, focalizing the high dose to a smaller tumour volume.[27–31] Concerning lung RT, in a small series of 38 patients affected by unresectable

Table 2. Sensitivity and specificity of tumour shrinkage cut-off of 20% using the cone-beam CT level/window of −600/1000 HU

Parameters	Complete response odds ratio (*p*-value) [95% CI]	Sensitivity (%)	Specificity (%)	Positive-predictive value (%)	Negative-predictive value (%)	Cases correctly classified (%)	AUC
All populations of study	8.3 (0.007) [1.8–38]	87	56	72	76	73	0.71
Number of fractions >5	28 (0.001) [4.02–80.2]	87	82	91	75	85	0.84
BED ≤110	7 (0.03) [1.2–40.8]	80	64	75	70	73	0.71
BED >110	7 (n.v.) [n.v.]	100	43	67	100	73	0.71
GTV dimension pre-SABR >6 cc	n.v.	100	50	73	100	79	0.75

AUC, area under the curve; BED, biologically effective dose; CI, confidence interval; GTV, gross tumour volume; n.v., not valuable; SABR, stereotactic ablative radiotherapy.

NSCLC and treated with concomitant chemo-conventional RT, tumour volume response evaluated by means of weekly CBCT was associated with a longer OS.[17] Unfortunately, no measured CBCT cut-off tumour response was determined for a better understanding of the behaviour or prediction of therapeutic outcomes. Conversely, Brink et al[32] showed a rather controversial result that greater tumour reduction during RT is unfavourable in terms of locoregional control and OS for non-adenocarcinoma histologies, supposing that the rapid tumour shrinkage during RT could be an indicator of tumour aggressiveness (in terms of high kinetic proliferative).

To our knowledge, no data are available in the setting of lung SABR on the use of CBCT as a surrogate modality imaging to identify early tumour response. This is the first study in which tumour volume reduction, quantified by means of CBCT images, has been evaluated as a predictive parameter of early efficacy (6 months after SABR completion).

Lung lesion contouring was performed retrospectively at the first and last CBCT of each treatment using specific windows/levels given in HU, although it is known that CT numbers of CBCT images may not represent the real HU because of various artefacts including body scattering. This choice was related to the possibility of avoiding uncertainties between planning CT contours and CBCT volume estimation. Moreover, negligible volumetric modifications of the lesions between the planning CT data *vs* the first CBCT were found. This result could be related to the start of SABR with respect to the simulation phase. In fact, in our department, the median time between the simulation phase and the first fraction of SABR was 5 days (range 3–7 days). Conversely, the two window/level settings used for CBCT lung

Table 3. Sensitivity and specificity of tumour shrinkage cut-off of 20% using the cone-beam CT level/window of −1000/250 HU

Parameters	Complete response odds ratio (*p*-value) [95% CI]	Sensitivity (%)	Specificity (%)	Positive-predictive value (%)	Negative-predictive value (%)	Cases correctly classified (%)	AUC
All populations of study	6 (0.01) [1.43–22.2]	78	61	72	69	71	0.70
Number of fractions >5	9 (0.009) [1.72–47.6]	77	73	85	61	76	0.75
BED ≤110	7 (0.03) [1.2–40.8]	80	64	75	70	73	0.71
BED >110	7 (0.2) [0.44–65.7]	75	57	67	67	67	0.66
GTV dimension pre-SABR >6 cc	35 (0.02) [1.7–302]	87	83	87.5	83	85	0.85

AUC, area under the curve; BED, biologically effective dose; CI, confidence interval; GTV, gross tumour volume; SABR, stereotactic ablative radiotherapy.

Table 4. Sensitivity, specificity and area under the curve (AUC) values of the various tumour shrinkage cut-offs analyzed in all the populations the of study using the cone-beam CT (CBCT) levels/windows of −600/1000 HU and −1000/250 HU

CBCT level/window of −600/1000 HU				CBCT level/window of −1000/250 HU			
Cut-off of tumour reduction between first and last CBCT (%)	Sensitivity (%)	Specificity (%)	AUC	Cut-off of tumour reduction between first and last CBCT (%)	Sensitivity (%)	Specificity (%)	AUC
5	10	44	0.31	5	13	42	0.29
10	17	45	0.44	10	19	49	0.49
15	68	45	0.61	15	69	54	0.63
20	87	56	0.71	20	78	61	0.70

tumour contouring resulted in quite large differences in tumour volumes as well as during-treatment tumour shrinkage. In fact, depending on the window/level used, an average tumour volume reduction from 12% to 22.5% was recorded at last CBCT treatment. The early therapeutic response reported here could be hypothesized by the radiobiological assumption that the ablative doses could lead to an indirect cell death, overstepping the "4Rs of radiobiology" biological effects of conventional RT.[7,33] Interestingly, in the present analysis, tumour shrinkage noted at the last session of SABR was statistically related to lesion CR/PR *vs* SD and/or CR *vs* PR/SD after treatment. It is well known that distinguishing between recurrence and radiation-induced density changes in the lung after SABR is difficult.[34] However, high-risk features on serial CT scans are suggestive of recurrence such as enlarging opacity, sequential enlargement, bulging margin, linear margin disappearance, loss air bronchogram and cranio-caudal growth.[26] In addition, ^{18}F-FDG-PET/CT, even though it needs further validations, could be of some utility to distinguish between recurrence and the radiation induced. In fact, a maximum standardized uptake value above 5 is considered suspicious for recurrence.[35] Both imaging evaluation (CT scans and ^{18}F-FDG-PET/CT) were performed in all patients here analyzed to validate SABR efficacy.

In lung SABR, no factors are still available to predict the efficacy, apart from a BED ≥100 Gy. For this reason, the present findings appear very intriguing. In fact, the rate of tumour reduction, estimated in 20%, reported here was associated with a high probability of obtaining a CR 6 months after SABR. Obviously, a cut-off of 20% should not be considered a definitive value because of the small sample size analyzed here. At best, the findings of the present analysis could be hypothesis generating for further investigations. In fact, critical issues of the present study including the retrospective nature and the short follow-up could affect the results. Again, the accuracy of the obtained values related to the different slice thicknesses used in planning CT and CBCT reconstruction, the lack of estimation of CBCT HUs depending on the scatter related to patient diameter and the potential limit of CBCT in soft-tissue contrast compared with CT scan could introduce uncertainties and, thus, represent the limitations of the analysis. In a phantom study, the consistency of the estimated size of lung lesions between CBCT and CT scan was reported without significant variability.[36] Nevertheless, based on the findings reported here, CBCTs performed as part of routine care may be used to evaluate tumour changes and to predict treatment efficacy early. CBCT lung lesion variations were evaluated using a level/window suggested by Altorjai et al.[16] These authors analyzed the interobserver and intra observer target variations on CBCT images compared with CT-based delineation in case of Stage I–II NSCLC or lung metastases SABR. The authors concluded that a window/level setting of −600/1000 HU for CBCT images could be utilized for target volume delineation purposes. In the present analysis, we started from this assumption and, to give robustness to findings or avoid bias, the default level/window for lung parenchyma in the Eclipse system (Varian) was used to further validate the lesion size changes at the last fraction of SABR. Interestingly, a tumour shrinkage cut-off of at least 20% was confirmed as predictable for CR for both the lung levels/windows used.

CONCLUSION

Early predictive parameters of SABR efficacy could be helpful for clinicians in order to improve patient-tailored surveillance and management. Prediction of the early tumour response could be useful to personalize imaging restaging after the completion of therapy or to incorporate additional therapies in case of poor responders to improve clinical outcomes. Obviously, the current paradigm of early assessment of SABR efficacy for lung malignancies using the CBCT images warrants additional prospective studies in this direction with a longer follow-up and homogeneous population both in terms of primary or metastatic lesions as well as in fractionation schemes used.

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Manuscript 7:

Giaj-Levra N, Sciascia S, Fiorentino A, Fersino S, Mazzola R, Ricchetti F, Roccatello D, Alongi F.

Radiotherapy in patients with connective tissue diseases.
Lancet Oncol. 2016;17:e109-17. doi: 10.1016/S1470-2045(15)00417-9.

In this review, we analyse evidence and discuss the available data for radiotherapy in patients with connective tissue diseases in terms of immune system activation, toxicity and impact of new radiation technology.

Radiotherapy in patients with connective tissue diseases

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The decision to offer radiotherapy in patients with connective tissue diseases continues to be challenging. Radiotherapy might trigger the onset of connective tissue diseases by increasing the expression of self-antigens, diminishing regulatory T-cell activity, and activating effectors of innate immunity (dendritic cells) through Toll-like receptor-dependent mechanisms, all of which could potentially lead to breaks of immune tolerance. This potential risk has raised some debate among radiation oncologists about whether patients with connective tissue diseases can tolerate radiation as well as people without connective tissue diseases. Because the number of patients with cancer and connective tissue diseases needing radiotherapy will probably increase due to improvements in medical treatment and longer life expectancy, the issue of interactions between radiotherapy and connective tissue diseases needs to be clearer. In this Review, we discuss available data and evidence for patients with connective tissue diseases treated with radiotherapy.

Lancet Oncol 2016; 17: e109–17

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Introduction

Connective tissue diseases are a heterogeneous group of autoimmune rheumatic diseases characterised by immune system dysregulation and the development of autoantibodies. Patients typically alternate between active or symptomatic periods and non-active or quiescent phases. Connective tissue diseases have historically been considered an absolute or relative contraindication to radiotherapy because of the hypothesis of a greater risk of severe radiotherapy-related acute and late complications.

Few reports have been made of the outcomes of patients with newly diagnosed connective tissue diseases (or exacerbation of pre-existing disease) who need radiotherapy (table 1, 2).[1–21] Although an analysis of the little available data shows that risk of radiotherapy toxicity in patients with connective tissue diseases seems to be based largely on anecdotal evidence, radiation oncologists remain hesitant. In 1998, the American College of Radiology[22] concluded that, "a history of collagen vascular disease is a relative contraindication to breast conservation treatment because published reports indicate that such patients tolerate irradiation poorly. Most radiation oncologists will not treat patients with scleroderma or active systemic lupus erythematosus, considering either an absolute contraindication." Thus, radiotherapy has been underused in patients with connective tissue diseases who have cancer.[16]

With improved medical treatments, prognosis for patients with connective tissue diseases has improved. The 5-year survival in systemic lupus erythematosus has increased from about 40% in the 1950s, to 90% in the 1980s, to more than 90–95% nowadays.[23] Therefore, a higher number of patients with connective tissue diseases are expected to be diagnosed with cancer and will potentially be eligible for oncological treatment, including radiotherapy. Substantial improvements have been made in radiation technology, including the development of intensity-modulated radiotherapy and image-guided radiotherapy. These techniques are available in clinical practice, potentially minimising acute and late local side-effects. Thus, new radiotherapy techniques could be considered feasible even in patients with connective tissue diseases who have cancer. In this Review, we analyse evidence and discuss the available data for radiotherapy in patients with connective tissue diseases.

Connective tissue diseases, cancer environments, and radiation interactions

Connective tissue diseases are chronic and debilitating autoimmune disorders that cause substantial morbidity and mortality and disproportionately affect women. These diseases include rheumatoid arthritis, systemic sclerosis, scleroderma, systemic lupus erythematosus, dermatomyositis, and vasculitis. Connective tissue diseases often develop after environmental triggering via cellular pathways in genetically susceptible individuals with disease-associated polymorphisms.[24] However, the specific cellular and molecular mechanisms leading to connective tissue diseases, and factors that establish involved organs are involved, are poorly understood.

Associations between connective tissue diseases and cancer are being increasingly investigated. Links between them are multifaceted and have different relationships in terms of frequency, timing, and type of cancers. Several studies have highlighted the dynamic and bidirectional interactions occurring at the cancer–immune system interface that might be relevant to the origins of autoimmunity.[25] Data for patients with systemic sclerosis and concomitant cancer suggest that, in some cases, autoimmunity might be triggered by an autoantigen mutation in the patient's cancer.[26,27] Also, connective tissue diseases might cause changes in immune function that could be affected by immunosuppressive therapy.[24] Although the evidence was not overwhelming, some investigators have reported that these changes in immune function did affect radiotherapy toxicity.[28] This bidirectional hypothesis was based on the idea that some connective tissue diseases share a common pathological pathway of vascular obliteration and fibrosis due to heightened inflammation and a clinical pattern of possible systemic involvement. The potential for radiotherapy to augment these pathological changes became a topic of investigation.

	Tumour type	Patients with connective tissue disease (n)	Type of connective tissue disease	Increase in severe acute toxicity	Increase in severe late toxicity	Treatment	Conclusion
Teo et al, 1989[1]	Head and neck	10	Dermatomyositis	Yes	Yes	External-beam radiotherapy	Effect
Fleck et al, 1989[2]	Breast	9	Mixed	Yes	Yes	External-beam radiotherapy	Effect
Varga et al, 1991[3]	Mixed	4	Progressive systemic sclerosis	No	Yes	External-beam radiotherapy	Effect
Hareyama et al, 1995[4]	Head and neck	2	Mixed	Yes	No	Concurrent chemotherapy and external-beam radiotherapy	Inconclusive*
Bliss et al, 1996[5]	Cervix	5	Mixed	Yes	No	External-beam radiotherapy and brachytherapy	Effect
Turesson et al, 1996[6]	Breast	35	NA	NA	No	NA	No effect
Rakfal and Deutsch, 1998[7]	Mixed	6	Systemic lupus erythematosus, discoid lupus erythematosus	No	No	External-beam radiotherapy	No effect
Khoo et al, 2004[8]	Anal cancer	2	Systemic lupus erythematosus	No	No	Concurrent chemotherapy and external-beam radiotherapy	No effect
Dragun et al, 2011[9]	Breast	9	Mixed	No	No	Intraoperative radiotherapy and brachytherapy	No effect
Lowell et al, 2011[10]	Brain metastases	14	Mixed	No	No	Gamma knife	No effect

NA=not available. *Inconclusive effect based on presented data.

Table 1: **Patient characteristics and findings from selected case studies of patients with connective tissue diseases and cancer reporting toxicity**

Radiotherapy acutely affects early responding tissues, such as the basal dermis and oral and gastric mucosa, by reducing proliferation. Radiation-induced obliteration of capillaries and small vessels is also well documented.[28] In patients with connective tissue diseases, these acute effects might act in conjunction with immune-related damage caused by immune complex deposition, complement cascade activation, and infiltrating inflammatory cells (figure 1). Such common targeting might be additive to typical radiation-induced acute tissue injuries.[11] The additive injury induced by both radiation and the pre-existing connective tissue diseases might also help to explain the potentially increased late effects noted in some of these patients after radiotherapy.[3] Radiotherapy might trigger the onset of connective tissue diseases by enhancing the expression of self-antigens (eg, from apoptotic cell debris), diminishing regulatory T-cell activity, and activating effectors of innate immunity such as dendritic cells through Toll-like receptor-dependent mechanisms, all of which could potentially lead to a break of immune tolerance.[25] This potential mechanism has raised a debate among radiation oncologists about whether patients with connective tissue diseases tolerate radiation as well as people with no connective tissue disease.[29]

Experimental evidence supports the hypothesis that the immune system is able to repress tumour cells and that immune surveillance has a key role in the identification and elimination of cancer cells.[30] Three different phases have been described in the interaction between cancer cells and the immune system: elimination (which is still considered the cornerstone in the immune surveillance process), equilibrium between the immune system and cancer cells, and escape.[30] Immune surveillance is considered a complex process involving different immune system cells—ie, CD8 cells, natural killer cells, CD4 cells, macrophages, and B lymphocytes.[30] After radiotherapy, the disruption of the tissue architecture is associated with changes in blood flow (zones with hyperperfusion and hypoxia) and lymphatic function and an increase in interstitial pressure.[31] Additionally, irradiation of the tumour and its microenvironment is associated with the proliferation of inflammatory signals detected by the immune system.[32] The resulting production of cytokines and chemokines then attracts antigen-presenting cells (dendritic cells) that, after uptake of tumour-associated antigens, cause CD8 activation involved in tumour killing (figure 1).[33,34]

Evidence is also increasing that inflammation contributes to cancer development and that cancer cells use inflammatory mechanisms to prevent immune-system activation and to protect the tumour from immune attack (equilibrium and escape phases).[35] Moreover, inflammatory elements (such as chemokines and interleukins) released by tumour cells promote infiltration, progression of disease, and metastases (figure 2).[36]

Various mechanisms might exist that exacerbate the pathophysiological response induced by radiation exposure in patients with connective tissue diseases. One potential mechanism includes the overexpression of profibrotic cytokines, such as transforming growth factor β

	Primary tumour site	Patients with connective tissue disease (n)	Type of connective tissue disease (n)	Study design	Increase in severe acute toxicity	Increase in severe late toxicity	Median radiotherapy dose	Radiotherapy technique	Conclusion
Ross et al, 1993[11]	Mixed	61	Rheumatoid arthritis (n=39), systemic lupus erythematosus (n=13), other (n=9)	Matched pair analysis	No	No	56 Gy	External-beam radiotherapy, brachytherapy	No effect
Morris et al, 1997[12]	Mixed	209	Rheumatoid arthritis (n=131), systemic lupus erythematosus (n=25), other (n=53)	Retrospective	No	Yes	45 Gy	External-beam radiotherapy	Inconclusive*
Chen et al, 2001[13]	Breast	36	Rheumatoid arthritis (n=17), systemic lupus erythematosus (n=5), scleroderma (n=4), other (n=10)	Matched pair analysis	Yes	Yes	64 Gy	External-beam radiotherapy, brachytherapy	No effect (effect in scleroderma)
Phan et al, 2003[14]	Mixed	38	Systemic lupus erythematosus (n=21), scleroderma (n=2), other (n=15)	Matched pair analysis	No	No	55·17 Gy	External-beam radiotherapy, brachytherapy	No effect (effect in scleroderma)
Liu et al, 2004[15]	Prostate	15	NA	Prospective	No	Yes	66 Gy	External-beam radiotherapy	Effect
Benk et al, 2005[16]	Mixed	38	Systemic lupus erythematosus (n=38; 4 radiotherapy treated)	Retrospective	No	No	NA	NA	No effect
Gold et al, 2007[17]	Mixed	20	Scleroderma (n=20)	Retrospective	No	No	36 Gy	External-beam radiotherapy, brachytherapy	No effect
Lin et al, 2008[18]	Mixed	73	Rheumatoid arthritis (n=33), systemic lupus erythematosus (n=13), scleroderma (n=9), other (n=18)	Retrospective	No	Yes	NA	External-beam radiotherapy	No effect (effect unknown in pelvic site systemic lupus erythematosus or scleroderma)
Gold et al, 2008[19]	Mixed	41	Progressive systemic sclerosis (n=20), systemic lupus erythematosus (n=21)	Retrospective	NA	No	NA	External-beam radiotherapy, brachytherapy	Inconclusive*
Pinn et al, 2008[20]	Mixed	21	Systemic lupus erythematosus (n=21)	Retrospective	Yes	No	49·75 Gy	External-beam radiotherapy, brachytherapy, intensity-modulated radiotherapy	No effect
Patel et al, 2012[21]	Mixed	12	Discoid lupus erythematosis (n=12)	Retrospective	No	No	69 Gy	External-beam radiotherapy, brachytherapy	No effect

NA=not available. *Inconclusive effect based on presented data.

Table 2: **Effect of connective tissue diseases on toxicity after cancer treatments reported in retrospective and matched pair studies**

(TGFβ) and interleukin 1. Radiation injury in healthy tissues is usually characterised by the appearance of a fibrinous exudate within the stroma and by deposition of extracellular matrix components, including collagen, through myofibroblasts produced by fibroblast activation and differentiation.[37] In some connective tissue diseases (such as systemic sclerosis) in which TGFβ concentrations are already increased, late effects after radiotherapy might be more evident.[3] Another potential mechanism involves radiation microvascular damage in a context of vasculitis, leading to increased late effects and reduced tolerance to treatment. After radiation, endothelial cell injury and tissue hypoxia stimulate the recruitment into the tissue of inflammatory circulating cells, such as macrophages, which are a source of profibrotic mediators, including TGFβ1.[38,39] Additionally, increased concentrations of proangiogenesis factors (eg, VEGF) as a result of vascular damage and leakage of vessels in response to radiotherapy could exacerbate late effects such as dermal atrophy, telangectasia, necrosis, and fibrosis.[40] Finally, radiation-induced damage to basement membranes causes this to become a target tissue, leading to increased autoimmunity.[12,28]

Preclinical studies and case reports

Some studies have used in-vitro sensitivity to radiation in lymphocytes from patients with connective tissue diseases to assess risk indicators for radiation-related

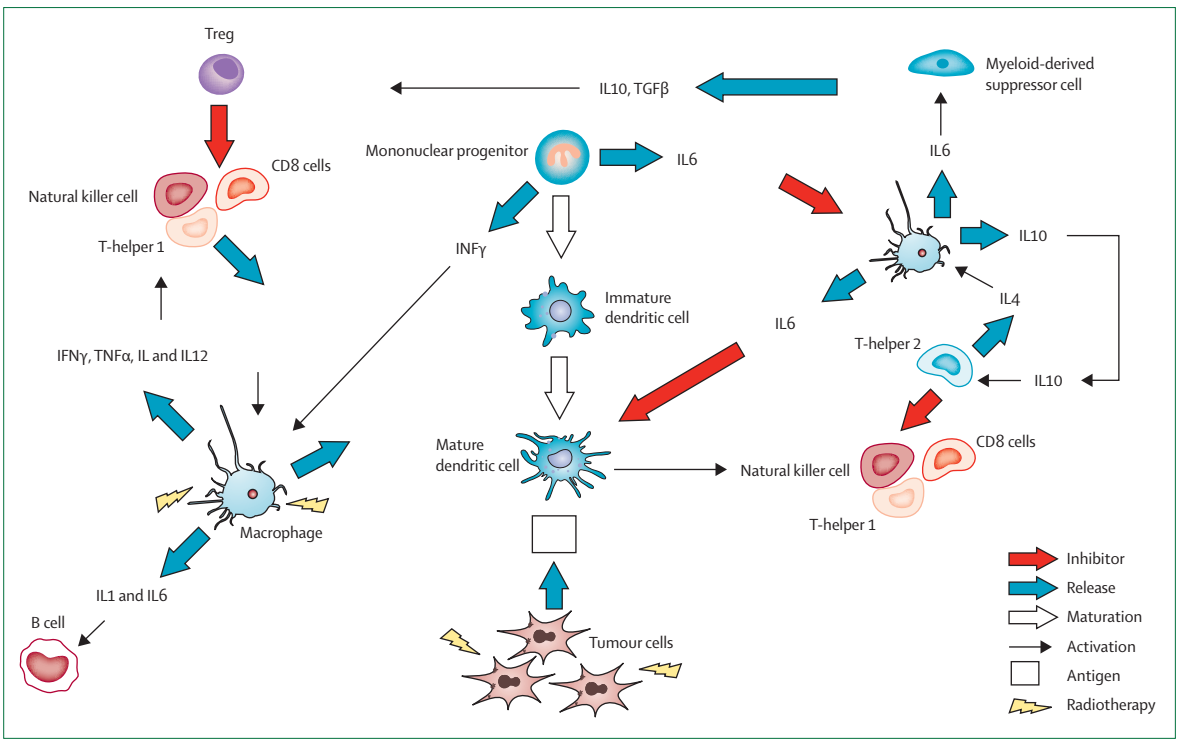

Figure 1: **Main immune cells, interleukins, and cytokines involved in immune surveillance**
TGF=transforming growth factor. IFN=interferon. IL=interleukin. TNF=tumour necrosis factor.

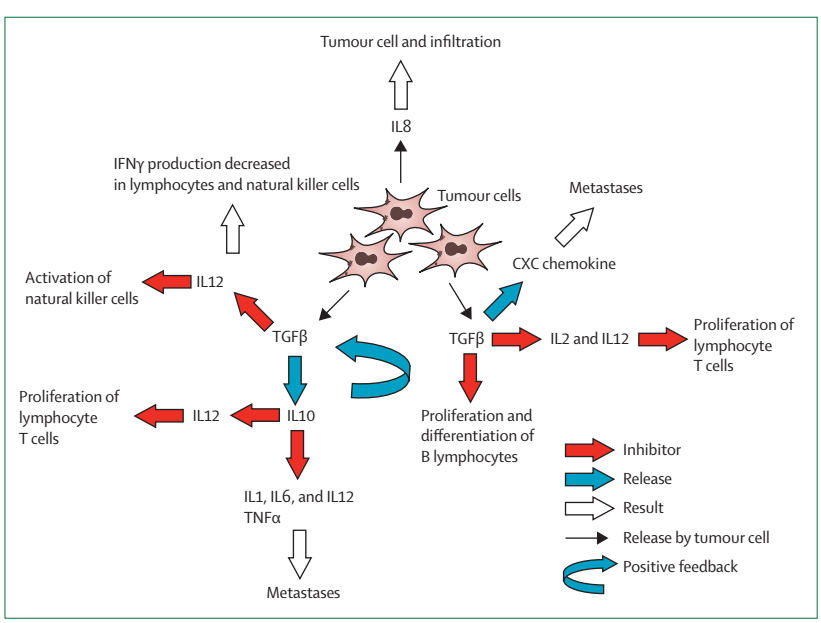

Figure 2: **Tumour-cell mechanisms against the immune system**
TGF=transforming growth factor. CXC=CXC chemokine. IFN=interferon. IL=interleukin. TNF=tumour necrosis factor.

side-effects.[41–43] Carrillo-Alascio and colleagues[41] used pulsed-field gel electrophoresis to quantify the initial radiation-induced DNA double-strand breaks in peripheral lymphocytes from 52 patients with systemic lupus erythematosus. Systemic lupus erythematosus did not confer a higher intrinsic risk of radiosensitivity when compared with 48 healthy participants without connective tissue diseases.[41] In another study,[43] the same investigators carried out an in-vitro evaluation of the repair of mainly single-stranded DNA breaks after peripheral blood radiation of 48 children with systemic lupus erythematosus, systemic sclerosis, juvenile rheumatoid arthritis, and dermatomyositis. Greater DNA damage and a delay in DNA repair were noted in the children with connective tissue diseases group than in healthy children.[43] Another in-vitro study that used tritiated thymidine incorporation assays showed that patients with active systemic lupus erythematosus had increased radiotherapy-related lymphocytic sensitivity when compared with healthy patients when irradiated with ^{60}Co-γ photons between 0 Gy and 10 Gy, resulting in a potentially higher probability of radiation toxicity.[42]

Similarly, immune system changes, which can affect radiosensitivity, are being investigated. Among others, Budach and colleagues[44] investigated the possibly abnormal reaction to high radiation doses in two groups of germline mutation-carrying mice, one with severe combined immunodeficiency (SCID; even though it is not classified as a connective tissue disease) and one that had normal radiation sensitivity (C3H). The lethal dose for 50% of the irradiated animals after single-dose whole-body irradiation was lower for SCID mice than for C3H mice, as was the radiation dose that was needed to achieve 50% local control and tumour growth delay,

thus confirming that abnormal radiation sensitivity was observed in SCID mice.[44] A possible mechanism correlated with increased sensitivity of SCID tumour cell lines is the inability of the tumour cells to overcome their genetic deficiency in DNA double-strand break repair in SCID fibroblasts.[45]

More than 300 cases involving patients with connective tissue diseases have been published reporting toxicity after radiotherapy and several early and late radiotherapy-related complications, including some deaths, have also been reported.[2,5,7,10,46] The first two severe events in patients with connective tissue diseases given radiotherapy were noted in the late 1960s.[47,48] In one case, a patient with systemic lupus erythematosus who had lymphoma died of heart failure 1 year after radiotherapy to the mediastinal and retroclavicular nodes (20 Rad [20 Gy] and 39 Rad [39 Gy], respectively, with ^{60}Co),[47] whereas the second patient, who had facial lupus, developed radiotherapy-correlated osteomyelitis of the maxilla.[48] However, no data about radiotherapy dose or modality were provided. Teo and colleagues[1] assessed the radiation toxicity profiles of ten patients with a diagnosis of early-stage nasopharyngeal carcinoma and dermatomyositis (table 1). At a median follow-up of 51·8 months, all patients had subcutaneous fibrosis and xerostomia, two patients had radiation skin necrosis, and one patient had a VI and XII cranial nerve deficit.[1] However, no information was provided about radiotherapy dose and techniques.

Fleck and colleagues[2] published a study of nine patients with breast cancer (four women with a pre-existing connective tissue disease and five who developed a connective tissue disease after radiotherapy). Eight received radiotherapy using ^{60}Co with a prescription dose of 40–50 Gy and an electron boost on the tumour bed of 5–15 Gy. Three patients with a pre-existing connective tissue disease reported a severe toxicity profile: the first case involved moist desquamation and brachial plexopathy; the second case showed soft-tissue necrosis needing chest-wall resection, rib fractures, and pulmonary fibrosis; and the third patient had soft-tissue necrosis, bronchopleural–cutaneous fistula, and osteonecrosis of the clavicle, sternum, and rib. None of the patients with a new diagnosis of connective tissue diseases after radiotherapy had severe complications.[2]

According to McCormick,[49] to reduce the side-effects in patients with connective tissue disease and breast cancer, a more aggressive local surgery and systemic therapy, in particular for younger women (<40 years), was better than breast-conserving surgery followed by radiation. More recently, accelerated partial breast irradiation by either brachytherapy or intraoperative radiotherapy has been considered an alternative experimental option for the treatment of early-stage breast cancer in women with a history of connective tissue diseases. Dragun and colleagues[9] published a report of nine patients with connective tissue diseases with breast cancer given accelerated partial breast irradiation via high-dose brachytherapy; toxicity and cosmetic profiles were reported as satisfactory. Indeed, the authors concluded that it might not be necessary to exclude patients with connective tissue diseases from clinical trials of accelerated partial breast irradiation. As confirmation, Turesson and colleagues[6] reported that autoimmune disease did not increase the risk of skin teleangectasia in 35 patients who received radiotherapy for breast cancer. Finally, Lowell and colleagues[10] published data on the use of a very high dose of radiation delivered with gamma knife for brain metastases in 14 patients with connective tissue diseases, and reported no grade 3 or 4 toxicity (table 1).

In conclusion, in-vitro studies and clinical case reports describe a narrow and heterogeneous picture for patients with connective tissue diseases who receive radiotherapy. Despite these data limitations, more recently published data show that patients with connective tissue diseases seem to be less affected by toxicity than are healthy individuals and case reports (table 1).

Retrospective and controlled studies

To our knowledge, no randomised controlled study has assessed whether patients with connective tissue diseases are more likely to develop acute or late radiotherapy-related toxicity. However, we retrieved 11 case series.[11–21] In a retrospective analysis, Morris and Powell[12] reported a large series of 209 patients with connective tissue diseases given radiotherapy with a median radiation dose of 45 Gy (range 13–82) between 1960 and 1995. After a median follow-up of 6 years, clinically significant acute side-effects (Radiation Therapy Oncology Group/Eastern Cooperative Oncology Group RTOG/ECOG Early Morbidity Scoring Scale of more than three) were similar in patients with and without rheumatoid arthritis (both 12%). At 5 years, the risk of late morbidity for patients with rheumatoid arthritis was 6%, similar to the rate for the healthy population generally, whereas for patients without rheumatoid arthritis it was 21% (p=0·0002). The most highly represented connective tissue disease after rheumatoid arthritis was systemic lupus erythematosus, with 25 patients (12%). No correlation between dose, fraction size, irradiated volume, and late effects were reported.[12]

Similar results were reported in a matched-control study of 61 patients with connective tissue diseases.[11] The number of acute reactions after radiotherapy in the connective tissue diseases group was only slightly higher than in the matched-control group, with grade 3 or greater acute toxicity noted in seven patients in the connective tissue diseases group and four in the matched-control group. Patients with systemic lupus erythematosus had an increase in the number of acute reactions due to radiation (36% of patients with systemic lupus erythematosus *vs* 18% in the control group, p=0·5), whereas patients with rheumatoid arthritis had

an increase in late complications (24% *vs* 5%; p=0·125). Nevertheless, the study showed no significant differences in acute and late toxicity complications between groups.[11]

Chen and colleagues[13] reported no significant differences in acute complications after breast cancer radiotherapy between a group of 36 women with connective tissue diseases and a matched-control group (14% *vs* 8%, respectively; p=0·40), but did note a significant difference in late toxicity in those patients with connective tissue diseases (17% *vs* 3%; p=0·0095). However, when the investigators stratified patients by specific autoimmune disease, they found a significant difference only in four patients with scleroderma.[13] Phan and colleagues[14] assessed 76 patients who received radiation for cancer (38 patients with connective tissue diseases and 38 in the control group) and did not show any significant differences in terms of acute or late complications between groups. However, increased risk of radiation complications was reported in patients with scleroderma (n=4).

In another study, Lin and colleagues[18] reported toxic effects in 73 patients with connective tissue diseases given radiotherapy. No differences were noted in acute toxicity between patients with connective tissue diseases and those in the control group. However, patients with a diagnosis of connective tissue diseases had a significantly higher incidence of late toxicity compared with the control group (29% *vs* 14%, respectively; p=0·001), with a non-significant increase in severe late toxicity (9% *vs* 4%; p=0·079). Patients with diagnosed connective tissue diseases who received radiation to the pelvis had a higher probability of severe toxicity reactions (grade 3 or higher); furthermore, the incidence of severe late toxicity was higher in patients with a diagnosis of systemic lupus erythematosus and scleroderma than in the control group.[18]

Gold and colleagues[19] retrospectively analysed the toxicity profile of 41 patients with connective tissue diseases given radiation for cancer (20 patients with systemic sclerosis and 21 patients with systemic lupus erythematosus). Patients were divided into high-severity and low-severity connective tissue diseases on the basis of the number of involved organs. Univariate analysis showed a significant increase in the risk of any grade toxicity for patients with high-severity connective tissue diseases compared with those with low-severity connective tissue diseases (p=0·006), although no differences in grade 3 or higher toxicity were found between the two groups (p=0·56). Despite the small number of enrolled patients, the severity of connective tissue diseases could be considered as an important factor in the prediction of treatment tolerability. Nonetheless, the severity of connective tissue diseases was not a clear contraindication to radiotherapy.[19]

Varga and colleagues[3] reported on the toxicity profile of four patients with systemic sclerosis who were given radiotherapy.[3] All patients had cutaneous and subcutaneous late toxicity, visceral fibrotic reactions at the radiation site, and severe skin toxicity and fibrosis extending beyond the radiation field involving internal organs. Three of the four patients subsequently died, two from bowel obstruction and one from pneumonia.[3]

Liu and colleagues[15] planned a prospective study to investigate the effect of neoadjuvant androgen-deprivation therapy and radiotherapy in men with prostate cancer. A subanalysis showed that 15 of the men had a connective tissue disease and that these patients had a greater frequency of late genitourinary grade 2 toxicities compared with healthy men (relative risk 3·98; p=0·007).[15]

As previously stated, several studies have reported radiotherapy-related toxicity profiles in patients with a range of connective tissue diseases (tables 1, 2). Nevertheless, only a few of the studies[7,8,17,20] focused on patients with scleroderma and systemic lupus erythematosus, with contentious conclusions about radiotherapy toxicity.

Gold and colleagues[17] assessed the toxicity profiles of 20 patients with scleroderma and cancer who had been treated with radiotherapy or brachytherapy or both, with or without concurrent chemotherapy. Univariate analysis showed a significant association between acute toxicity, radiotherapy dose, and increased scleroderma involvement of organs. For late side-effects, negative antinuclear antibody serology was correlated with a higher probability of toxicity. None of the analysed pretreatment and treatment variables were correlated with severe acute and late toxicity.[17] There have been no further reports to confirm severe acute and late complication profiles in this specific setting.[7,8,10]

Rakfal and Deutsch[7] described data for six patients who had a diagnosis of systemic lupus erythematosus and different malignancies with various radiotherapy doses, reporting no unexpected severe acute or late side-effects. Khoo and colleagues[8] reported no relevant acute or late complications in two patients with anal cancer with systemic lupus erythematosus taking concomitant immunosuppressive therapy who were treated with combined chemoradiotherapy (^{60}Co and external-beam radiotherapy).

One of the most important reports was published by Pinn and colleagues,[20] which included 21 patients with systemic lupus erythematosus who received a total of 35 consecutive courses of radiotherapy. Of the 17 patients who were evaluable for late toxicity, four patients (24%) had a grade 3 or higher toxicity. The presence of renal involvement according to the American Rheumatism Association criteria was correlated with an increased risk of any grade of late toxicity (p<0·006). Univariate analysis established a correlation between acute toxicity and total dose (>49·8 Gy), treatment sites, and curative intent for treatment. Brachytherapy was used in one treatment course, 2D radiotherapy in 30 courses, 3D conformal radiotherapy in three, and intensity-modulated radiotherapy in one. Moreover, absence of photosensitivity (p<0·02), absence of arthritis (p<0·03), and presence of a malar rash (p<0·04) were correlated

with an increased risk of grade 3 or greater acute toxicity. No specific association between technique and late toxicity was noted. Radiation dose prescription, radiation techniques, and anatomical site (ie, abdomen, pelvis, breast, brain, neck, and chest) were associated with a high risk of any late toxicity.

In conclusion, the small number of described cases and the heterogeneity of the connective tissue disease seem to strongly affect the statistical power of these studies, thus limiting the possibility to show any robust association between radiation toxicity and connective tissue diseases, and confirming that radiotherapy is frequently withheld unjustly to treat patients with connective tissue diseases.[16,19,21]

Clinical solutions and future perspectives

Various treatment strategies have been considered for patients with connective tissue diseases to reduce the risk of toxicity during or after radiotherapy such as avoiding concomitant treatment or reducing dose prescription. Although the use of chemoradiotherapy is considered the gold standard in many cases, multimodality treatment in patients with connective tissue diseases could be correlated with a more severe toxicity profile than single-modality treatment, thereby affecting its feasibility.[4,12,19,50] In radiotherapy, the radiation dose could be reduced to lower the toxicity profile, but this could impair effectiveness.[12,28,44,51] However, Delanian and colleagues[52] reported that reducing radiation dose (from 65 Gy to 40 Gy) in patients with connective tissue diseases (one with lung cancer and two with anal–rectal cancer) resulted in complete remission, although side-effects were observed at the radiation site. Some investigators have postulated that hyperactivation of the immune system by tumour cells makes patients with connective tissue diseases more sensitive to radiation than others.[53,54] Another strategy is changing dose fractionation schedules or reducing treatment volume, which might decrease toxicity complications.[2,12,28,40,51,52,54] Nevertheless, a crucial question still remains—is it really necessary to modify radiotherapy features to decrease toxicity in patients with connective tissue diseases?

The most common radiotherapy approach is to use external beams to deliver ionising radiation. In the past few decades, most departments have replaced their ^{60}Co machines with the more precise linear accelerator. Despite modern radiotherapy now being available, most reports of patients with connective tissue diseases involve obsolete and unsatisfactory technologies including 2D radiotherapy. Intensity-modulated radiotherapy and stereotactic ablative radiotherapy have allowed radiation oncologists to prescribe higher dose prescriptions to targets when useful or required. Intensity-modulated radiotherapy is considered an advancement of 3D-conformal radiotherapy that targets the radiation dose into the tumour, thus minimising the exposure of healthy tissue in several anatomical regions. Intensity-modulated radiotherapy is considered the most appropriate technique in head and neck cancers and in most pelvic tumours, including prostate cancer. In this disease, intensity-modulated radiotherapy decreased long-term toxicity with no negative effect on overall survival when compared with 3D-conformal radiotherapy.[54–66]

Stereotactic ablative radiotherapy is a novel radiotherapy method that delivers a very high dose of radiation (in a single or a few fractions) with high precision to the tumour, thus maximising the sparing of surrounding normal tissue. Several retrospective and prospective stereotactic ablative radiotherapy studies have shown promising results in terms of local tumour control and survival in some settings, including in early non-small-cell lung cancer.[67] Moreover, image-guided radiotherapy based on daily patient set-up position verification allowed better definition of the tumour target to reduce and ultimately eliminate uncertainties. To our knowledge, no randomised controlled trials using image-guided radiotherapy have assessed toxicity and efficacy in patients with connective tissue disease. Hence, the promising, modern techniques could improve radiotherapy tolerability, especially in challenging clinical situations, as well as in patients with connective tissue diseases and cancer.[68,69]

Search strategy and selection criteria

We searched Medline, Google Scholar, PubMed, and the ProQuest Dissertation, and Theses databases for reports published in English from June, 1946, to Jan 1, 2015. Our detailed search algorithm is shown in the appendix. We identified additional references with a manual review of the reference lists of included articles. Two independent reviewers (NGL and SS) identified potential studies and exported them to an electronic reference management software program (RefWorks version 2.0). NGL and SS determined eligibility by reviewing first the title and abstract and then the full paper. Disagreements were resolved by consensus; if consensus was not achieved, then a third author (FA) provided an assessment of eligibility. Because the data for eligibility were dichotomous (yes *vs* no), we established inter-rater agreement at both the title and abstract review and the full article review stages by calculating Cohen's κ coefficient. A study was included when it reported on cancer-related radiotherapy and included patients with connective tissue diseases. A study was excluded when no detailed information (eg, outcome of radiotherapy, clinical manifestations related to the underlying connective tissue diseases, solid evidence of diagnosis of connective tissue diseases) was reported. Review articles were excluded from the analysis. For data extraction, all the papers were scrutinised for the following information: study design (retrospective, prospective, case-control, cross-sectional and case series, or case report); number of patients, sex, and age (mean, range); type of radiotherapy; type of underlying connective tissue disease; type of underlying cancer; definition of radiotherapy acute and late toxicity profile; outcome in terms of toxicity profile; and timing of connective tissue diseases onset or exacerbation.

See **Online** for appendix

For more on **Cohen's κ coefficient** see http://facultyvassaredu/lowry/kappa.html

Conclusion

The data that are currently available from case series and a few retrospective studies are still not enough to support a specific contraindication for radiotherapy in patients with connective tissue diseases. Nevertheless, a cautious approach for patients with active connective

tissue diseases seems to be reasonable. Moreover, the recent implementation of new radiotherapy approaches could be promising to improve the feasibility and tolerability of radiotherapy in some patients with cancer, including those with connective tissue diseases. Further well designed prospective studies, which also assess the most appropriate total dose and fractionation schedules, will probably help to overcome the unresolved concerns about radiotherapy indication for patients with connective tissue diseases.

Contributors

FA, NGL, and SS searched the literature, assisted with the organisation of the manuscript, interpreted and collected data, and wrote and edited the Review. AF and DR assisted with the organisation of the manuscript, interpreted and collected data, and wrote and edited the Review. SF, RM, and FR interpreted and collected data, helped to design the figures and panel, and wrote and edited the Review.

Declaration of interests

We declare no competing interests.

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Manuscript 8:

Alongi F, **Giaj-Levra N**, Sciascia S, Fozza A, Fersino S, Fiorentino A, Mazzola R, Ricchetti F, Buglione M, Buonfrate D, Roccatello D, Ricardi U, Bisoffi Z.

Radiotherapy in patients with HIV: current issues and review of the literature.
Lancet Oncol. 2017;18:e379-e393. doi: 10.1016/S1470-2045(17)30440-0.

In this Review, we discussed the role of radiotherapy, with or without chemotherapy or new drugs, in the treatment of cancer in patients with HIV, in terms of efficacy and tolerability of this approach. Moreover, we analysed the biological basis of interactions between HIV and radiotherapy, evidence from preclinical studies, and immunomodulation by radiotherapy in the HIV setting.

Radiotherapy in patients with HIV: current issues and review of the literature

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Although the introduction of highly active antiretroviral therapy has radically improved the life expectancy of patients with HIV, HIV positivity is still considered a major barrier to oncological treatment for patients with cancer because of their worse prognosis and increased susceptibility to toxic effects compared with patients who are immunocompetent. The use of radiotherapy with or without chemotherapy, immunotherapy, or molecular targeted therapy is the standard of care for several cancers. These new drugs and substantial improvements in radiotherapy techniques, including intensity-modulated radiotherapy, image-guided radiotherapy, and stereotactic ablative radiotherapy, are optimising the feasibility of such anticancer treatments and are providing new opportunities for patients with cancer and HIV. In this Review, we discuss the role of radiotherapy, with or without chemotherapy or new drugs, in the treatment of cancer in patients with HIV, with a focus on the efficacy and tolerability of this approach on the basis of available evidence. Moreover, we analyse and discuss the biological basis of interactions between HIV and radiotherapy, evidence from preclinical studies, and immunomodulation by radiotherapy in the HIV setting.

Lancet Oncol 2017; 18: e379–93

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Introduction

According to estimates from the 2015 Global Burden of Disease Study,[1] more than 38·8 million people worldwide are affected by HIV/AIDS. Several approaches have been implemented to control HIV infection, including educational programmes about sexual health, specific programmes targeting key populations, and more widespread access to antiretroviral therapy for treatment and prevention.[1] Indeed, the decrease in the incidence of HIV, along with the reduction in HIV-related deaths, is closely associated with the introduction of highly active antiretroviral therapy (HAART) in 1996.[2] By contrast, an increased incidence of cancer has been reported since 1996.[3] Specifically, in the era before HAART, the incidence of cancer in patients with HIV was 31% compared with 58% after the introduction of HAART.[3] HAART has contributed to improved immune system competence and has prolonged life expectancy in patients with HIV, thus increasing the probability of these patients developing cancer.[4]

Although mortality for people living with HIV remains much lower in high-income countries than in other areas of the world, some countries with scarce resources have shown encouraging outcomes for HAART coverage and viral suppression.[1] Hence, access to adequate care and radiotherapy or chemotherapy to treat cancer in patients with HIV should be expanded on a global level. Historically, HIV has been considered a contraindication to cancer treatment because of the worse prognosis of patients with HIV compared with those without, and because of their increased susceptibility to toxic effects. Nevertheless, most of these studies were done before the widespread use of HAART. New drugs, such as immunotherapy and targeted therapies, and improvements in radiotherapy techniques, including intensity-modulated radiotherapy and image-guided radiotherapy, are optimising the effectiveness and tolerability of cancer treatment.[5] Despite these developments, the role of radiotherapy alone or in combination with drugs for this patient population remains to be defined.

In this Review, we selected studies that discuss the role of radiotherapy, with or without chemotherapy or new drugs, in the treatment of cancer in patients with HIV, with a focus on the efficacy and tolerability of this approach on the basis of available evidence (figure 1). Moreover, we analyse and discuss the biological basis of interactions between HIV and radiotherapy, evidence from preclinical studies, and immunomodulation by radiotherapy in the HIV setting.

Antiretroviral HIV therapy, immune system response, and cancer

HAART has revolutionised the survival of patients with HIV by guaranteeing CD4 count normalisation and reducing the viral load. Despite these therapeutic improvements, HAART is considered a lifelong treatment because it is unable to eliminate HIV, even in patients with a negative viral load.[6]

Prolonged use of HAART has been shown to cause viral resistance, especially in the advanced stages of infection, leading to cancer in some patients.[7] Several DNA and RNA viruses have been associated with human cancers, and a number of distinct mechanisms have been described to explain the oncogenic role of these viruses. For example, viruses might directly induce transformation of infected cells; host cell growth and survival can be deregulated by integration of the virus into the host's genome or by the establishment of a stable episome after viral infection. Alternatively, recognition of viral genes by host cells might initiate DNA damage responses, which many viruses require for replication. Additionally, viral infection might lead to cancer by inducing chronic inflammation, thus encouraging carcinogenic transformation.[8] HIV represents a unique situation in that it is not itself oncogenic but it does inhibit the patient's immune system, leading to the

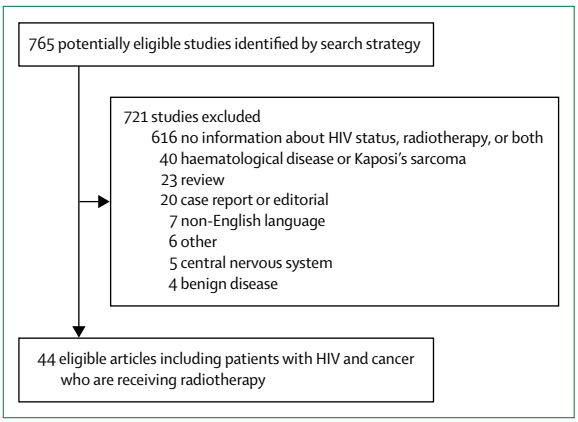

Figure 1: **Study selection**

disruption of immunosurveillance and allowing hypermutated malignant cells to emerge.

A meta-analysis[9] has shown that HIV-related immune depression confers an increased risk of malignancy, similar to what is observed in solid organ transplantation recipients. Moreover, a possible association has been proposed between various non-AIDS-defining malignancies and HIV in a mechanism whereby suppressed cell-mediated immunity, impaired immune surveillance, angiogenesis, and reduced apoptosis provide a prolific environment for aggressive tumorigenesis.[10] In one study,[11] HIV induced an irreversible alteration in the innate and adaptive immune system of affected individuals by infecting CD4-positive T cells, which were progressively destroyed while CD8-positive T cells were chronically activated. This destruction of CD4-positive T cells in patients with HIV might be due to various HIV proteins (gp120, Tat, and Nef) that have been shown to induce an apoptotic process in uninfected CD4-positive T cells.[12] An alternative hypothesis is that CD4-positive T cells might be killed by natural killer cells.[13] Therefore, new immunological strategies are needed to improve the efficacy of HAART by preventing development of HAART resistance.

Oncological drugs are being investigated for use in patients with HIV to deplete infected cells. In particular, immunotherapies are being studied for their potential to induce an immune response against both HIV and cancer antigens. Inhibitor signals through immune checkpoints on CD4-positive and CD8-positive T cells allow tumour cells to avoid immunosurveillance. A similar process is used by HIV, which increases the expression of immune checkpoints, in particular programmed cell death protein-1, thereby promoting disease progression[14] and immune escape[15] (figure 2). A 2015 study[16] reported that immune checkpoint expression is associated with persistence of HIV activity. In that study,[16] ipilimumab (an anti-cytotoxic T-lymphocyte protein-4 human immunoglobulin G1 antibody) increased the CD4-positive T-cell count in a patient with metastatic melanoma. Two ongoing phase 1 clinical trials are investigating the use of immunotherapies in patients with HIV and cancer (NCT02408861 and NCT02595866).

Biological basis of interactions between HIV and radiotherapy

For patients with HIV and cancer, radiotherapy represents an important local treatment option. Considerable evidence has shown that the risk of treatment-related side-effects is higher in patients with HIV than in patients who are immunocompetent.[17,18] These clinical observations are probably related to the direct or indirect effects of HIV infection that might enhance the effect of ionising radiation.

Reductions in the concentrations of both glutathione and related endogenous thiols, as well as in the concentrations of superoxide dismutase and catalase, have been reported in patients with HIV.[19] These impairments in the endogenous antioxidant systems enhance oxidative stress, resulting in an increase in the production of reactive oxygen species.[20] Any stimulation of polymorphonuclear cells, monocytes, macrophages, or T cells, as occurs in patients with HIV, increases the production of reactive oxygen species.[20] Increased oxidative stress has an important role in cell death, including apoptosis or necrosis of epithelial cells, melanocytes, endothelial cells, and stromal cells through various mechanisms, including both direct and indirect DNA damage.[20] Thus, the state of chronic immune activation and the various drugs that are used in patients with HIV lead to a constant state of oxidative stress, which is further exacerbated by the upregulation of tumour necrosis factor-α (TNF-α) by HIV itself.[21] Moreover, reactive oxygen species, HIV, and TNF-α activate the transcription of nuclear factor κ-B, which further increases the concentrations of TNF-α and reactive oxygen species.

Several nutrients, including vitamins, flavonoids, minerals, and aminoacids, are important scavengers of reactive oxygen species, acting to maintain the redox potential within cells and protect them from the damaging effects of electrophiles and reactive oxygen species.[20] Alterations in the bowel mucosa of patients with HIV affect the absorption of these nutrients, thus contributing to the depletion of the scavenger system.[20]

All of these direct or indirect mechanisms trigger an increase in the production of reactive oxygen species, which themselves are mediators of the damaging effects of radiation, and also lead to the depletion of radioprotective thiols.[22]

Preclinical studies of radiotherapy for patients with HIV and cancer

In-vivo and in-vitro studies[17,23–30] have shown some evidence of increased sensitivity to radiotherapy in patients with HIV and cancer. Formenti and colleagues[17] showed that, in Kaposi's sarcoma, fibroblasts derived from the skin biopsy samples of patients with HIV were more radiosensitive than fibroblasts derived from patients without HIV.

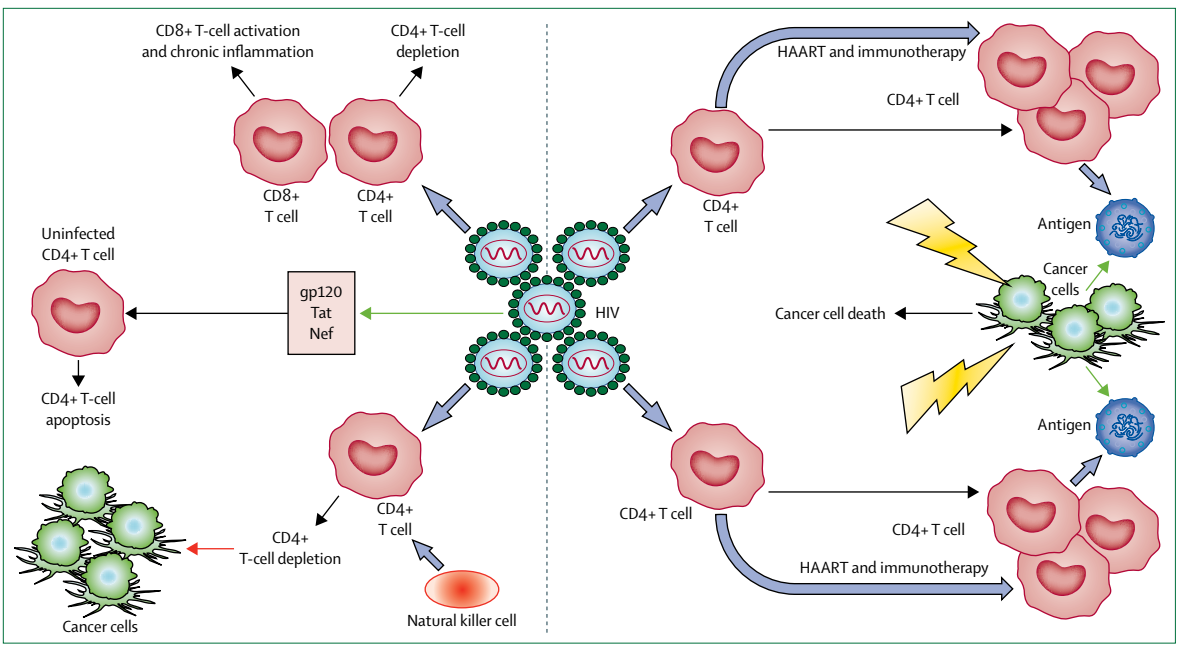

Figure 2: **Effect of HIV on CD4 count and effect of HAART and immunotherapy on HIV and cancer cells**
The black arrows indicate outcomes, the blue arrows indicate attachment, the green arrows represent the release of something from a cell, the red arrow indicates proliferation, and the lightning bolts indicate radiotherapy. The black dotted line separates two different conditions: on the left is HIV-mediated immune suppression and promotion of cancer proliferation; on the right is use of the combination of HAART, immunotherapy, and radiotherapy against HIV infection and cancer. HAART=highly active antiretroviral therapy.

However, the mechanism underlying the increased radiosensitivity of patients with HIV and cancer is still not well defined. Several preclinical studies[23,31] have shown that the metabolites of clindamycin and sulphonamides have increased toxicity in Tat-expressing or HIV-infected Jurkat cells, resulting in a deficiency in intracellular glutathione, which was suggested as an explanation for the enhanced radiosensitivity of these cells.

Sun and colleagues[24] analysed the effects of the HIV-1 Tat protein on cellular responses to ionising radiation in two Tat-expressing cell lines (TT2 and TE671-Tat) derived from human rhabdomyosarcoma cells. The authors of that study[24] concluded that the HIV-1 Tat protein sensitises rhabdomyosarcoma cells to radiation by dysregulating cell-cycle checkpoints and reducing the cellular capacity to repair radiation-induced damage. These results suggest that radiotherapy for any type of cancer could be more effective in patients with HIV than in those without.

Other preclinical reports[25,26] have suggested that HIV protease inhibitors, typically components of antiretroviral therapy, have an important role in the radiosensitisation of normal tissue and tumour cells. HIV protease inhibitors might inhibit the PI3K–Akt pathway, which is considered an important survival mechanism in some tumour cells. In these cells, PI3K is expressed, resulting in radiotherapy resistance.[32] The effect of HIV protease inhibitors on the PI3K pathway has been observed both in vivo and in vitro.[27] Gupta and colleagues[27] tested two of the most common HIV protease inhibitors (amprenavir and nelfinavir) in vivo as adjuvant antitumour drugs. The authors concluded that the combination of HIV protease inhibitors and radiotherapy increased synergistic effects compared with either treatment alone. Another study done by Pajonk and colleagues[30] concluded that the HIV protease inhibitor, saquinavir, is a radiation sensitiser that inhibits proteasome activity in mammalian cells. HIV protease inhibitors might also act as sensitisers to radiotherapy or chemotherapy by triggering other molecular processes, such as proteasome inhibition, endoplasmic reticulum stress, the unfolded protein response, and autophagy.[33,34]

Several studies[35,36] have shown that HIV protease inhibitors induce cell apoptosis via activation of endoplasmic reticulum stress. Liu and colleagues[29] analysed the association between radiosensitivity induced by HIV protease inhibitors and endoplasmic reticulum stress in patients with head and neck squamous cell carcinoma. They showed that the HIV protease inhibitors, lopinavir and ritonavir, dose-dependently sensitised head and neck squamous cell carcinoma cells to irradiation and inhibited cell growth. Additionally, lopinavir and ritonavir induced the activation of endoplasmic reticulum stress, which was associated with the downregulation of cyclin D1 expression and cell arrest in the G0/G1 phase. HIV protease inhibitors also activated the unfolded protein response in head and neck squamous cell carcinoma cells. One of the three main branches of the unfolded protein response identified to date includes PERK, in addition to IRE1 and ATF6. PERK activation allows phosphorylation of eIF2α, which then leads to ATF4 expression.

	Study design	Histology	Patients with HIV (n)	Antiretroviral therapy	CD4 count	Radiotherapy responsible for CD4 count reduction?	Effect of pre-radiotherapy CD4 count on prognosis?	Effect of CD4 count on survival?
Holland et al, 1994[40]	Retrospective	Anal cancer	7	NA	<200 cells per mL in four patients, ≥300 cells per mL in three patients	NA	Yes	Low CD4 count had a detrimental effect on survival
Kao et al, 1999[41]	Retrospective	Head and neck cancer	8	NA	NA	No	No	No
Hoffman et al, 1999[42]	Retrospective	Anal cancer	17	NA	<200 cells per mL in eight patients, ≥200 cells per mL in nine patients	NA	Yes	No
Tirelli et al, 2000[43]	Retrospective	Lung cancer	36	HAART	150 cells per mL*	NA	No	No
Place et al, 2001[44]	Retrospective	Anal cancer	23	With or without HAART	200 cells per mL in patients with in-situ squamous cell carcinoma and 222 cells per mL in patients with infiltrating squamous cell carcinoma*	NA	Yes	Yes
Spano et al, 2004[45]	Retrospective	Lung cancer	22	HAART	<200 cells per mL in two patients, 200–500 cells per mL in 15 patients, ≥500 cells per mL in five patients	NA	Yes	Yes
Blazy et al, 2005[46]	Retrospective	Anal cancer	9	HAART	<200 cells per mL in four patients, 200–500 cells per mL in four patients, >500 cells per mL in one patient	NA	NA	No
Wexler et al, 2008[47]	Retrospective	Anal cancer	32	HAART	350 cells per mL*	Yes	Yes	Yes
Seo et al, 2008[48]	Prospective	Anal cancer	17	HAART	190 cells per mL†	NA	No	No
Oehler-Janne et al, 2008[25]	Retrospective	Anal cancer	40	HAART	321 cells per mL*	NA	No	No
Ng et al, 2008[49]	Retrospective	Prostate cancer	14	HAART	523 cells per mL†	NA	No	No
Abramowitz et al, 2009[50]	Retrospective	Anal cancer	44	HAART	NA	NA	No	No
Fraunholz et al, 2010[51]	Retrospective	Anal cancer	21	HAART	347·5 cells per mL*	Yes	NA	No
Hauerstock et al, 2010[52]	Retrospective	Anal cancer	34	HAART	<350 cells per mL in 19 patients, ≥350 cells per mL in 11 patients, unknown in four patients	NA	No	No
Kahn et al, 2012[53]	Match pair analysis	Prostate cancer	13	HAART	<300 cells per mL in four patients, ≥300 cells per mL in eight patients	Yes	No	No
Alfa-Wali et al, 2012[54]	Prospective	Anal cancer	60	With or without HAART	305 cells per mL for all patients, 289 cells per mL for all patients who received chemoradiotherapy, 209 cells per mL for patients who received chemoradiotherapy and no HAART, and 332 cells per mL for patients who received chemoradiotherapy and HAART*	Yes	No	Yes
Martellotta et al, 2012[55]	Retrospective	Anal cancer	65	With (96·8%) or without (3·2%) HAART	<200 cells per mL in 24 patients, 200–400 cells per mL in 14 patients, >400 cells per mL in 21 patients, unknown in six patients	NA	No	No
Sankatsing et al, 2013[56]	Prospective	Mixed	90	cART	400 cells per mL in the radiotherapy group and 471 cells per mL in the non-radiotherapy group	Yes	NA	NA
Fraunholz et al, 2014[57]	Retrospective	Anal cancer	36	HAART	367 cells per mL*	Yes	NA	No
White et al, 2014[58]	Retrospective	Anal cancer	53	HAART	455 cells per mL*	NA	No	Inconclusive‡
Grew et al, 2015[59]	Retrospective	Anal cancer	39	HAART	381 cells per mL*	NA	No	No
Simonds et al, 2015[60]	Retrospective	Cervical cancer	36	HAART	341 cells per mL*	NA	No	No
Sparano et al, 2016[61]	Prospective	Anal cancer	45	HAART	401 cells per mL*	Yes	No	No

HAART=highly active antiretroviral therapy. cART=combination antiretroviral therapy. NA=not available. *Data are median count. †Data are mean count. ‡Overall survival (95% CI 0·32–0·97; p=0·06).

Table 1: **Associations between CD4 count, HIV, radiotherapy tolerability, and outcomes in included studies**

The activation of PERK, eIF2α, and ATF4 represses global protein translation, reduces concentrations of cyclin D1, and induces cell-cycle arrest. ATF4 also induces CHOP expression, which inhibits cell growth.[29] The results of the study by Liu and colleagues[29] suggest that activation of the endoplasmic reticulum stress response is one of the principle mechanisms underlying radiosensitivity induced by HIV protease inhibitors.

Given the safety of HIV protease inhibitors, these drugs are excellent candidates to test as radiation sensitisers in clinical trials, even for patients without HIV.[27]

CD4 counts in patients with HIV and cancer

CD4-positive T cells are directly involved in the adaptive immune response,[37] helping to promote the activation and proliferation of CD8-positive T cells,[38] the generation

of CD8-positive T cell memory,[39] and the activation of macrophages and eosinophils.[37]

Anecdotal evidence suggests that patients with a pre-treatment CD4 count of fewer than 200 cells per µL (ie, patients with AIDS) have an increased probability of developing toxicity when treated with chemotherapy and radiotherapy. Conversely, patients with HIV who have a CD4 count of greater than 200 cells per µL, a good performance status score, and who have been treated with HAART, show tolerability and outcomes similar to patients without HIV. Table 1 summarises the results of studies of CD4 counts in patients with cancer and HIV.

One of the first studies to analyse the effect of radiotherapy on CD4 count, tolerability, and outcomes in patients with HIV was done by Holland and colleagues,[40] who concluded that patients with HIV should be considered for palliative treatment because of their worse outcomes and significantly increased probability of side-effects compared with those without HIV. Similar results were obtained by Hoffmann and colleagues,[42] who observed that the toxicity profile of patients with immunodeficiency (CD4 count of fewer than 200 cells per µL) was severe. Other clinical studies[44,46,55,57] confirmed these findings in terms of clinical outcomes and tolerability.

In one study,[44] HAART influenced clinical outcomes and patients appeared to have died of HIV and not of cancer progression. Alfa-Wali and colleagues[54] investigated the effect of concurrent chemotherapy and radiotherapy on CD4 count during the follow-up of patients with HIV and anal cancer. A median CD4 count of 305 cells per µL was measured at diagnosis, but patients showed a progressive reduction in CD4 count during follow-up. The authors of that study[54] concluded that being immunosuppressed might be associated with an increased probability of AIDS-related death. Wexler and colleagues[47] found that patients with a median CD4 count of fewer than 350 cells per µL and a median viral load of 700 copies per mL have an increased risk of hospital admission and haematological toxicity. That study[47] also reported a decrease in CD4 count after chemotherapy and radiotherapy in one (6%) of 16 patients, which persisted for at least 8 months after radiotherapy. A comparison of CD4 counts of patients before and after radiotherapy revealed that only five (28%) of 18 patients with pretreatment CD4 determination showed a reduction of more than 10% on the CD4 count.[47] The authors of that study[47] concluded that a low CD4 count or high viral load at disease presentation were associated with increased haematological toxicity and decreased tolerability of treatment. Moreover, irradiation of pelvic bone marrow, tumour site, and radiation dose can affect the delay in CD4 recovery.[47,56] A reduction in the CD4 count during follow-up was shown in other studies,[51,57] although slow CD4 recovery had no effect on clinical outcomes. More recently, an innovative approach was published that included treating patients with HIV and anal cancer with chemotherapy, radiotherapy, and cetuximab.[61] An analysis of the CD4 count confirmed that it was significantly decreased between baseline and the end of treatment. Nevertheless, during follow-up, some recovery of the CD4 count was achieved after the end of treatment without any effect on the HIV viral load.[61]

Other studies focusing on prostate cancer, cervical carcinoma, head and neck cancer, and lung cancer have assessed the association between CD4 counts and clinical outcomes. Few studies[41,43,49,53,60,62,63] have shown an effect for CD4 counts on the efficacy of treatment with chemotherapy and radiotherapy, although one study[45] of lung cancer showed worse survival outcomes that were associated with CD4 count. Data on the association between CD4 count and treatment toxicity remain insufficient, and the role of CD4 count continues to be a highly debated issue for patients with HIV and cancer that needs further investigation.

Clinical studies of radiotherapy in patients with HIV and cancer

Anal cancer

Anal cancer is 80–120 times more common in patients with HIV/AIDS than in the general population and the incidence in that population is still increasing.[25] Randomised trials[64,65] have established the combination of radiotherapy and chemotherapy with fluorouracil and mitomycin as the standard treatment for patients with anal canal cancer because it can cure many patients and guarantees preservation of anal sphincter function.

More than 20 clinical reports[25,26,40,42,44,46–48,50–52,55,58,59,66–76] of the use of radiotherapy and chemotherapy for patients with HIV and anal cancer have been published, which show heterogeneous results in terms of outcomes and toxicity (table 2). Studies[40,66] published before the introduction of HAART reported that patients with HIV/AIDS and anal cancer responded poorly to conventional chemoradiation. In those studies,[40,66] patients with HIV were more prone to treatment discontinuation, admission to hospital, and were more likely to receive reduced radiotherapy and chemotherapy doses. Additionally, several studies[25,26,42,44,46–48,51,52,54,67,69,71,73] showed that concurrent chemotherapy and radiotherapy was associated with an increased probability of acute and late cutaneous, gastrointestinal, and myelosuppressive toxicity in patients with HIV compared with those without. These toxic effects were associated with reduced overall survival and cancer-free survival,[59,70,75] particularly in patients with a CD4 count of fewer than 200 cells per µL.[42,44] The best treatment approach for patients with anal cancer and HIV/AIDS is still under debate and a multidisciplinary discussion is required. One trial[61] investigated the use of cetuximab (an anti-EGFR antibody) in combination with radiotherapy for the treatment of anal carcinoma in patients with HIV. Good results in terms of locoregional control were observed, with a locoregional recurrence probability of 20%. Nevertheless, grade 4 toxicity was reported in 26% of patients.[61]

Toxicity remains a relevant issue in the management of patients with anal cancer and HIV because low tolerability to radiotherapy is considered to be predictive of cancer progression.[48] Intensity-modulated radiotherapy is currently under investigation for patients with anal cancer to determine its effect in terms of quality of life and tolerability.[77]

The results of a previously published series[66–68] confirmed that the use of concurrent chemoradiation with curative intent should be considered for patients with HIV and anal cancer. Furthermore, despite the potentially increased risk of toxicity, treatment de-intensification was not recommended.[66–68]

Cervical cancer

Cervical cancer is a common malignancy in women with HIV and is considered an AIDS-defining cancer.[78] The increased incidence of cervical cancer in women with HIV can be explained by the fact that genital human papillomavirus infection is more common in these patients (63% *vs* 30% in those without).[78] Concomitant radiotherapy and chemotherapy is the gold standard for the treatment of locally advanced cervical carcinoma.

No published randomised clinical trials have compared the outcomes of patients with HIV and cervical cancer with those patients without HIV. The only available data are

	Study design	Patients with HIV (n)	Treatment	Follow-up	Acute toxicity	Late toxicity	Outcomes	Effect of HIV on outcomes
Chadha et al, 1994[66]	Retrospective	9	Concurrent chemotherapy and radiotherapy (40 Gy plus 10 Gy boost)	9 months	Yes	Yes	NA	Detrimental in patients with low CD4 count
Holland et al, 1994[40]	Retrospective	7	Concurrent chemotherapy and radiotherapy, chemotherapy alone, or radiotherapy alone (50·4 Gy)	NA	Yes*	Yes*	NA	Detrimental in patients with low CD4 count
Peddada et al, 1997[67]	Retrospective	8	Concurrent chemotherapy and radiotherapy (30 Gy of conformal radiotherapy)	41 months	Yes	NA	NA	Inconclusive
Hoffman et al, 1999[42]	Retrospective	17	Concurrent chemotherapy and radiotherapy (51·8 Gy of conformal radiotherapy)	17 months	Yes (<200 CD4-positive T cells per mL)	NA	Median disease-free survival of 13·5 months	Detrimental in patients with HIV
Cleator et al, 2000[68]	Retrospective	12	Concurrent chemotherapy and radiotherapy (38–51 Gy plus boost with 10–18 Gy conformal radiotherapy)	4·8 years	No	NA	60% overall survival at 5 years	Not detrimental
Kim et al, 2001[69]	Retrospective	13	Concurrent chemotherapy and radiotherapy (50–54 Gy conformal radiotherapy)	25·4 months	Yes	Yes	Median overall survival of 3·1 years	Detrimental in patients with HIV in terms of overall survival
Place et al, 2001[44]	Retrospective	23	Concurrent chemotherapy and radiotherapy (30–60 Gy)	5 years	Yes	NA	NA	Detrimental in patients with low CD4 count who have not received HAART
Stadler et al, 2004[70]	Retrospective	14	Concurrent chemotherapy and radiotherapy (54 Gy conformal radiotherapy)	NA	NA	NA	40% overall survival at 5 years	Detrimental in patients with HIV treated with HAART in terms of overall survival
Blazy et al, 2005[46]	Retrospective	9	Concurrent chemotherapy and radiotherapy (60 Gy)	36 months	Yes	No	NA	Not detrimental
Edelman and Johnstone, 2006[71]	Retrospective	17	Concurrent chemotherapy and radiotherapy (50·4–59·4 Gy)	25·6 months	Yes	Yes	67% overall survival at 18 months	Not detrimental
Oehler-Janne et al, 2006[26]	Retrospective	10	Concurrent chemotherapy and radiotherapy (53·6 Gy plus boost with 14 Gy brachytherapy)	44 months	Yes	Yes	70% overall survival at 5 years	Detrimental
Wexler et al, 2008[47]	Retrospective	32	Concurrent chemotherapy and radiotherapy (54 Gy conformal radiotherapy)	35 months	Yes	No	65% overall survival at 5 years	No difference between patients with and without HIV
Oehler-Janne et al, 2008[25]	Retrospective	40	Concurrent chemotherapy and radiotherapy (52–60 Gy) with or without brachytherapy	36 months	Yes	No	61% overall survival at 5 years	Detrimental
Chiao et al, 2008[72]	Retrospective	175	Chemotherapy, radiotherapy	32 months	NA	NA	77% overall survival at 2 years	No difference between patients with and without HIV
Seo et al, 2008[48]	Prospective	17	Concurrent chemotherapy and radiotherapy(56·3–58·8 Gy conformal radiotherapy)	3·1 years	Yes	NA	91·7% overall survival at 3 years	No difference between patients with and without HIV

(Table 2 continues on next page)

from low-quality, observational, retrospective studies[60,79–82] done in low-income countries where access to chemotherapy and radiotherapy or brachytherapy is poor (table 3). In those studies,[60,79–82] information about treatment compliance and treatment methods (ie, radiotherapy dose or brachytherapy use) was scarce. Additionally, most of those studies[79–81] showed a detrimental effect in terms of survival in patients with HIV.

A possible explanation for the worse outcome of cervical cancer in patients with HIV is that the HIV infection might lead to microsatellite instability and loss of heterozygosity, which enhances the aggressiveness of virus-related

	Study design	Patients with HIV (n)	Treatment	Follow-up	Acute toxicity	Late toxicity	Outcomes	Effect of HIV on outcomes
(Continued from previous page)								
Abramowitz et al, 2009[50]	Retrospective	44	Radiotherapy (45 Gy conformal radiotherapy plus brachytherapy or boost to 60–65 Gy)	27 months	No difference between patients with and without HIV	No difference between patients with and without HIV	85% overall survival at 3 years	No difference between patients with and without HIV
Hauerstock et al, 2010[52]	Retrospective	34	Concurrent chemotherapy and radiotherapy (54 Gy 3D conformal or intensity-modulated)	25·2 months	Yes	NA	69% overall survival at 3 years	Not detrimental
Fraunholz et al, 2010[51]	Retrospective	21	Concurrent chemotherapy and radiotherapy (54 Gy plus boost of 5·4–10·8 Gy conformal radiotherapy)	53 months	Yes	Yes	67% overall survival at 5 years	Not detrimental
Hammad et al, 2011[73]	Retrospective	13	Concurrent chemotherapy and radiotherapy (45–63 Gy)	NA	Yes	NA	Median overall survival of 33·5 months	No difference between patients with and without HIV
Munoz-Bongrand et al, 2011[75]	Retrospective	20	Concurrent chemotherapy and radiotherapy(60–70 Gy conformal radiotherapy)	32·5 months	NA	NA	39% overall survival at 5 years	Detrimental in patients with HIV in terms of overall survival and local control
Martellotta et al, 2012[55]	Retrospective	65	Concurrent chemotherapy and radiotherapy (53·9%)	NA	No difference between patients with and without HIV	No difference between patients with and without HIV	Median overall survival of patients with HIV was 106 months	No difference between patients with and without HIV
Alfa-Wali et al, 2012[54]	Prospective	60	Concurrent chemotherapy and radiotherapy (50·4–60·0 Gy)	6·5 years	Yes (30% of patients grade 3)	NA	64% overall survival at 5 years	No difference between patients with and without HIV
White et al, 2014[58]	Retrospective	53	Concurrent chemotherapy and radiotherapy (54 Gy conformal or intensity-modulated)	34 months	No difference between patients with and without HIV	No difference between patients with and without HIV	72% overall survival at 3 years	No difference between patients with and without HIV
Fraunholz et al, 2014[57]	Retrospective	36	Concurrent chemotherapy and radiotherapy (54 Gy conformal radiotherapy)	66 months	No difference between patients with and without HIV	NA	74% overall survival at 5 years	No difference between patients with and without HIV
Grew et al, 2015[59]	Retrospective	39	Concurrent chemotherapy and radiotherapy (54 Gy conformal or intensity-modulated)	15 months	No difference between patients with and without HIV	NA	76% overall survival at 3 years	Detrimental in patients with HIV in terms of overall survival and colostomy-free survival
Wieghard et al, 2016[74]	Retrospective	14	Concurrent chemotherapy and radiotherapy (45–54 Gy intensity-modulated)	29·2 months	No difference between patients with and without HIV	No difference between patients with and without HIV	Median overall survival was 68·8 months in patients with HIV and 110·9 months in those without	No difference between patients with and without HIV
Sparano et al, 2017[61]	Prospective	45	Concurrent chemotherapy and radiotherapy plus cetuximab (45–54 Gy conformal or intensity-modulated)	56 months	Yes	NA	79% overall survival at 3 years	Not detrimental
Martin et al, 2017[76]	Retrospective	42	Concurrent chemotherapy and radiotherapy (50·4 Gy conformal or intensity-modulated)	51 months	No difference between patients with and without HIV	No difference between patients with and without HIV	Overall survival at 5 years was 70·7% of patients with HIV and 78·4% of those without	No difference between patients with and without HIV

NA=not available. HAART=highly active antiretroviral therapy. *Unclear grade toxicity.

Table 2: **Associations between HIV status and outcomes in studies of patients with anal cancer**

	Study design	Histology	Patients with HIV (n)	Treatment	Follow-up	Acute toxicity	Late toxicity	Outcomes	Effect of HIV on outcomes
Shrivastava et al, 2005[79]	Retrospective	Cervical carcinoma	42	Radiotherapy (external beam radiotherapy, intracavitary radiotherapy)	12 months	Yes	Yes	NA	Detrimental
Gichangi et al, 2006[80]	Prospective	Cervical carcinoma	41	Radiotherapy (external beam radiotherapy)	NA	Yes	NA	NA	Detrimental
Kigula-Mugambe and Kavuma, 2006[81]	Retrospective	Cervical carcinoma	7	Radiotherapy (external beam radiotherapy, intracavitary radiotherapy)	NA	NA	NA	0% overall survival at 4 years	Detrimental
Simonds et al, 2012[82]	Retrospective	Cervical carcinoma	59	Chemotherapy, radiotherapy (conformal radiotherapy plus high-dose-rate brachytherapy)	NA	Yes	NA	NA	NA
Simonds et al, 2015[60]	Retrospective	Cervical carcinoma	36	Chemotherapy, radiotherapy (external beam radiotherapy)	NA	Yes	NA	NA	NA
Tirelli et al, 2000[43]	Retrospective	Lung cancer	36	Surgery, chemotherapy, radiotherapy	NA	Yes	NA	Median overall survival of 5 months	Detrimental
Spano et al, 2004[45]	Retrospective	Lung cancer	22	Surgery, chemotherapy, radiotherapy	NA	No	No	Median overall survival of 7 months	Not detrimental
Suneja et al, 2013[83]	Retrospective	Lung cancer	337	Surgery, chemotherapy, radiotherapy	NA	NA	NA	Poorer for patients with HIV	Inconclusive

NA=not available.

Table 3: **Associations between HIV status and outcomes in studies of patients with gynaecological or lung cancers**

cancers.[84] Another possible explanation is that HIV infection is associated with anaemia. It is well known that lack of oxygenation affects tumour radiosensitivity and is an adverse prognostic factor, especially in cervical cancer.[85]

Several studies[86,87] have confirmed the effectiveness of new radiation techniques, including intensity-modulated radiotherapy and image-guided radiotherapy, in reducing pelvic toxicity compared with conformal techniques. These preliminary findings could be promising, even when such technologies are used in the treatment of patients with cervical cancer and HIV.

Although data from the literature suggest that patients with HIV and cervical cancer have a poor prognosis, international guidelines recommend treating these patients with curative intent, similar to their HIV-seronegative counterparts. Moreover, starting HAART before commencing radiotherapy or chemotherapy is important because HAART enhances the efficacy and tolerability of anticancer treatment.[88]

Lung cancer

Radiotherapy in combination with chemotherapy is the treatment of choice for locally advanced lung cancer. No published prospective clinical trials have specifically assessed the efficacy and toxicity of radiotherapy and chemotherapy regimens in patients with HIV and this cancer. The only available data are from a case-control series and case reports (table 3).[43,45,83] Toxicity from radiotherapy is higher in patients with HIV and lung cancer, with the proportion of patients with grade 3–4 oesophageal toxicity being as high as 31%, and as high as 80% for radiation-induced oesophagitis.[89] These results might be possible because of increased mucosal vulnerability and concurrent opportunistic oesophageal infections in this patient population.[89] These data must be considered with caution because they are from studies in which outdated radiotherapy techniques (eg, conformal radiotherapy) were used. The more recent techniques, such as intensity-modulated radiotherapy can effectively reduce toxicity by minimising the dose to organs at risk, such as the oesophagus and lungs.[90–92] Use of highly conformal radiotherapy techniques in these patients is therefore crucial, particularly considering the fact that pulmonary function can be compromised by opportunistic pulmonary infection with subsequent fibrosis.[45]

One study[93] compared the outcomes of 64 patients with lung cancer and HIV before and after the beginning of treatment with HAART. Investigators found that the median overall survival was 3·8 months for the pre-HAART population compared with 7 months for the post-HAART population (p=0·01), and that cancer-related mortality at 1 year was 85% for the pre-HAART population versus 67% for the post-HAART population (p=0·02). In that study,[93] almost all patients had locally advanced disease (79% of patients treated in the pre-HAART era and 91% of patients treated in the post-HAART era) and were therefore treated with chemotherapy with or without radiotherapy, although chemotherapy was more commonly used in the post-HAART patients (79% *vs* 48%). These data confirm that specific antineoplastic treatments and HAART have a synergistic effect and can be feasibly and safely administered together.[93]

In the absence of definitive data, lung cancer in patients with HIV should be treated in the same way as in the general population, with particular attention to the management of side-effects. In particular,

	Study design	Histology	Patients with HIV (n)	Treatment	Follow-up	Acute toxicity	Late toxicity	Outcomes	Effect of HIV on outcomes
Kao et al, 1999[41]	Retrospective	Head and neck cancer	8	Conformal radiotherapy	NA	No	No	NA	Not detrimental
Levinson et al, 2005[10]	Retrospective	Prostate cancer	5	Radiotherapy (brachytherapy and conformal radiotherapy)	NA	NA	NA	NA	NA
Oluwole et al, 2005[94]	Retrospective	Breast cancer	5	Radiotherapy (one patient)	NA	NA	NA	NA	NA
Ng et al, 2008[49]	Retrospective	Prostate cancer	14	Radiotherapy (palladium-103 with or without external beam radiotherapy)	26 months	No	NA	NA	NA
Sanfilippo et al, 2010[95]	Retrospective	Head and neck cancer	13	Radiotherapy, chemotherapy (66·4 Gy)	22 months	No	No	NA	Not detrimental
Kahn et al, 2012[53]	Match pair analysis	Prostate cancer	13	Radiotherapy (conformal radiotherapy or intensity-modulated radiotherapy)	39 months	No	No	Overall survival no difference between patients with and without HIV	No difference between patients with and without HIV
Mourad et al, 2013[96]	Retrospective	Head and neck cancer	71	Surgery, chemotherapy, radiotherapy (70 Gy)	47 months	Yes	Yes	55% overall survival at 4 years	Detrimental
Phakathi et al, 2016[97]	Prospective	Breast cancer	14	Surgery, chemotherapy, radiotherapy	NA	NA	NA	NA	No difference between patients with and without HIV

NA=not available.

Table 4: **Associations between HIV status and outcomes in studies of patients with prostate cancer, head and neck cancer, or breast cancer**

intensity-modulated radiotherapy should be used to minimise treatment-related toxicity.

Head and neck cancer

Radiotherapy alone or in combination with drugs is the mainstay of treatment for most head and neck cancers. At present, little information is available about head and neck cancer in patients with HIV (table 4).[95,96,98] Patients with a diagnosis of head and neck cancer and HIV generally show a poor tumour response to treatment and extensive skin or mucosal toxic effects because of their immunocompromised status.

In a retrospective analysis,[98] eight patients with HIV and either head and neck carcinoma, squamous cell carcinoma, or Kaposi's sarcoma received radiotherapy, of which all patients received antiretroviral therapy and antifungal drugs. An analysis of clinical outcomes showed that all patients either had a partial (patients without Kaposi's sarcoma) or complete (all patients with Kaposi's sarcoma) response. The authors concluded that HIV is not a contraindication to radiotherapy and that select patients with HIV and non-Kaposi's-sarcoma malignant neoplasms could benefit from radiotherapy.

Mourad and colleagues[96] published the largest retrospective single-centre investigation of definitive radiotherapy with or without chemotherapy in patients with head and neck cancer and HIV. They found that definitive radiotherapy with or without chemotherapy was less effective in terms of the observed outcomes in patients with HIV than in those without.[96]

Therefore, the available evidence suggests that head and neck cancer in patients with HIV should be treated according to evidence-based medicine. In patients who are immunocompetent, the use of new radiotherapy techniques, such as intensity-modulated radiotherapy, is the standard of care to spare critical organs and subsequently reduce acute and late side-effects.[99] This technological approach should also be used for the treatment of head and neck cancer in patients with HIV.

Breast cancer

Although breast cancer is the most common oncological disease in women, the incidence of breast cancer in women with HIV is no higher than in the general population. Only a few studies[94,97] have investigated the association between HIV status and outcomes in patients with breast cancer (table 4).

Voutsadakis and colleagues[100] discussed the specific pathophysiological mechanism in patients with HIV and breast cancer and reported data concerning women with HIV who were treated with surgery, radiotherapy, and systemic therapy. The HIV population in that study[100] predominantly consisted of young women, which could partially explain the more aggressive biology of breast cancer in young women enrolled in that study. Oestrogen concentrations in women with HIV who are premenopausal have been found to be lower than in patients without HIV. Women with HIV often experience an early, substantial loss of fat, which is an essential tissue involved in the production of oestrogen. Reduced oestrogen concentrations might place breast cancer cells at a survival disadvantage and decrease their malignant latent capability. Nevertheless, patients with HIV and breast cancer have a poor prognosis, consistent with their younger age,[100] although another report did not confirm this hypothesis.[94] It remains unclear whether the

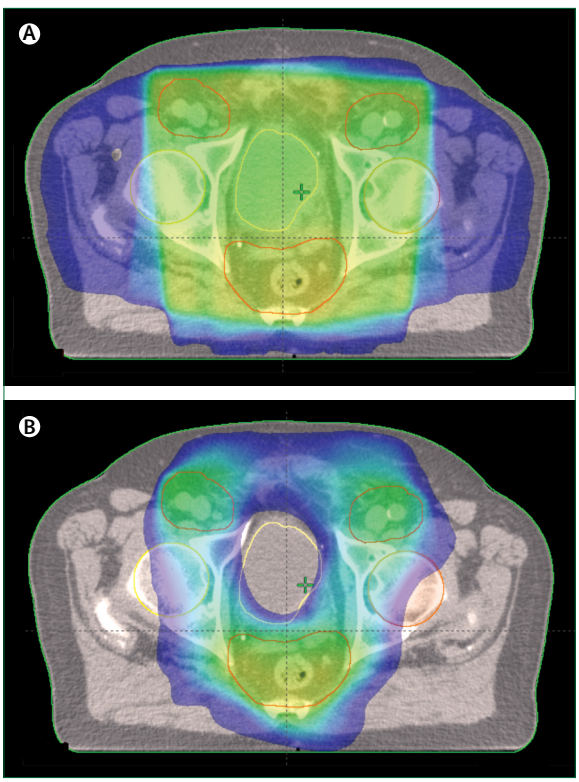

Figure 3: **Comparison of the dose distribution between conformal radiotherapy and volumetric-modulated arc therapy in a patient with HIV and anal cancer**
(A) Conformal radiotherapy. (B) Volumetric-modulated arc therapy.

presence of HIV in tumour cells has a role in breast cancer pathogenesis or whether the virus only has a role when immunosurveillance is labile.[101]

The available evidence suggests that patients with breast cancer and HIV should be treated according to the guidelines for those who are immunocompetent. To date, conformal radiotherapy (tangential fields) has been considered the standard approach. Additionally, the use of intensity-modulated radiotherapy or rotation techniques (ie, volumetric-modulated arc therapy) is usually recommended in selected patients, including those with unfavourable clinical conditions (ie, pectus excavatum and bilateral breast cancer) for whom a decrease in heart, lung, and contralateral breast dose is necessary.

Prostate cancer

The incidence of prostate cancer in men with HIV is unknown and data on this patient population are scarce. Patients with HIV and prostate cancer often have rapid disease progression because of their severely compromised immune system and poor response to androgen deprivation therapy due to their hypogonadism baseline status. The cause of hypogonadism is not completely understood, but it would appear that multifactorial elements are involved (HIV status, malnutrition, HAART therapy, and infections).[102]

Preliminary results of radiotherapy for prostate cancer in patients with HIV were reported by Ng and colleagues.[49] In that study,[49] 14 patients were treated with brachytherapy, external beam radiotherapy, or a combination of both and, in four patients, elective nodal irradiation was done. During follow-up, prostate-specific antigen concentrations for most patients were under biochemical control. No unusual urinary or rectal toxicities were observed, and treatment complications were congruent with patients without HIV. Moreover, radiotherapy did not appear to have a long-term negative effect on the immune system; the average CD4 count remained stable and the viral load increased in only two of 14 patients.[49]

Kahn and colleagues[53] did a matched-cohort analysis of definitive radiotherapy for prostate cancer in patients with HIV. They reported the biochemical outcome and toxicity of patients treated with radiotherapy (intensity-modulated radiotherapy or conformal radiotherapy) to the prostate, with or without whole-pelvis irradiation, and compared the results to a matched control population including patients without HIV and those with an unknown HIV status. Acute and late genitourinary and gastrointestinal toxicities were lower in patients with HIV than in those without, and a similar probability of biochemical control was observed. Additionally, viral loads before and after radiotherapy were found to be predictive of biochemical failure. Patients with HIV developed an average decline in CD4 count of 193 cells per μL, although CD4 counts were not predictive of biochemical failure (table 4).[53]

Patients with HIV and prostate cancer would appear to be eligible for all therapeutic options. As previously described, when pelvic irradiation is provided, a reduction in CD4 count is observed. Intensity-modulated radiotherapy is an innovative technique to increase treatment tolerability and to reduce bone marrow irradiation.[103]

Clinical solutions and future directions

The use of radiotherapy with or without chemotherapy or new drugs is considered the standard of care for several cancers. We might assume that CD4-positive T-cell counts could have an effect on tolerability and, in some cases, clinical outcomes in patients with HIV, especially in those treated in the era before HAART treatment. HAART has undoubtedly revolutionised survival in patients with HIV, guaranteeing the normalisation of CD4 counts and reducing viral loads, although viral resistance associated with the use of HAART remains an area of debate. Therefore, this issue needs to be taken into account in the cancer treatment strategy. In the past few decades, massive technological improvements in radiotherapy, and the introduction of immunotherapy and targeted therapy based on genomic and mutational cancer profiles, have improved cancer-specific survival and treatment tolerability.

To date, the most common cancer diagnosis in patients with HIV has been anal cancer, often involving treatment

of large volumes of tumour and healthy tissue. As described in the literature,[47,56] exposure of high volumes of bone marrow reserve to radiation is associated with reduced and persistently low CD4 counts after the end of radiotherapy, and pelvic bone marrow sparing is strongly suggested. Therefore, the introduction of intensity-modulated radiotherapy and stereotactic ablative radiotherapy has allowed radiation oncologists to apply increased conformal doses to target tumours and to minimise the involvement of nearby healthy tissues (figure 3). Intensity-modulated radiotherapy is considered an advancement of three-dimensional conformal radiotherapy, allowing for a decrease in the exposure of normal tissue to radiation, particularly in anal, cervical, or prostate cancer for which pelvic irradiation is frequently used. Similarly, intensity-modulated radiotherapy in the treatment of head and neck cancer has clearly shown the possibility to substantially reduce the dose to functional organs, including salivary glands, mucosa, and swallowing structures, thereby allowing treatment to be completed without discontinuation due to side-effects. This approach could be crucial to vulnerable patient populations, including patients with HIV.

Stereotactic ablative radiotherapy is an innovative approach that allows the delivery of a very high conformal dose to the tumour, with rapid dose fall-off to healthy surrounding tissue (figure 4). Patients with non-small-cell lung cancer who are immunocompetent but not eligible for surgery because of comorbidities would benefit from stereotactic ablative radiotherapy,[104] thus representing a new standard curative option for these patients. Several studies[105] have shown that stereotactic ablative radiotherapy can guarantee excellent results, and stereotactic ablative radiotherapy is under investigation for use in operable early-stage non-small-cell lung cancer, with promising preliminary results.[106] Specifically, stereotactic ablative radiotherapy might provide a non-invasive and appealing alternative curative approach for patients with HIV in whom comorbidities (ie, concurrent pulmonary infection) can affect the feasibility of surgical resection.

Evidence suggests that radiotherapy might be both immunostimulating and immunosuppressive. Both radiation-induced direct cell death and proinflammatory cytokines are responsible for dendritic-cell activation and for the promotion of T-cell (CD8-positive and CD4-positive) activation.[107] T cells are essential for tumour regression after irradiation with an ablative dose (15–20 Gy). An in-vivo study[108] showed that nude mice with a low concentration of T cells and B cells and wild-type mice without CD8-positive T cells did not respond to tumour cell irradiation. Chemotherapy agents, such as paclitaxel and dacarbazine, have been shown to suppress T-cell activity, thereby decreasing the radiation-induced suppression of a tumour.[109] Conversely, cyclophosphamide was shown to promote T-helper-17 differentiation, thereby improving radiation-induced tumour suppression.[110] Hence, these studies show the potential interaction of radiotherapy or chemotherapy and immune system modulation in patients with cancer. Additionally, one study[111] has suggested that the presence of tumour-infiltrating T cells is correlated with improved clinical outcomes in several cancers.

One of the most intriguing clinical approaches is the combination of radiotherapy and immune-checkpoint inhibitors in patients with cancer (figure 2). One study[112] showed that patients with an adequate immune system and pre-existing tumour-specific T cells had improved outcomes when treated with immune-checkpoint inhibitors. Moreover, use of localised radiotherapy appeared to promote both the induction of tumour-specific T cells and the response to immune therapies.[113] Additionally, initial-experience in-vivo studies[114,115] reported that the combination of immunotherapy and radiotherapy induced immune infiltration into the cancer microenvironment and promoted the abscopal effect of radiotherapy.

To our knowledge, only one study[116] has investigated the use of stereotactic intracranial radiotherapy and ipilimumab in a patient with metastatic melanoma and HIV. Hence, the combined use of immunotherapy and radiotherapy in patients with HIV opens up a new research field to establish the effect of these therapies on improving cancer survival and controlling HIV infection.

Conclusion

In most patients with HIV and cancer, radiotherapy alone or in combination with chemotherapy seems to be feasible and leads to similar clinical outcomes as in patients with

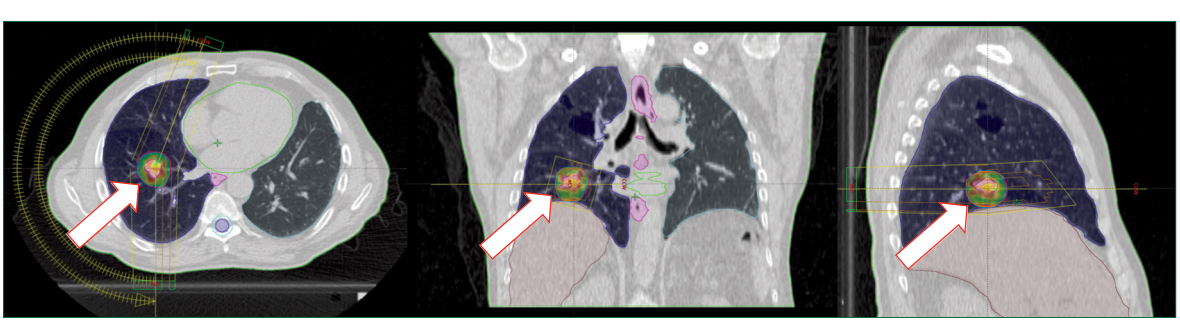

Figure 4: **Patient with HIV and early-stage non-small-cell lung cancer (white arrow) treated with stereotactic ablative radiotherapy**
The patient received 54 Gy radiotherapy in three fractions. The colour wash indicates the high dose distribution focused on the tumour lesion.

Search strategy and selection criteria

We searched MEDLINE, Google Scholar, PubMed, the ProQuest Dissertation, and Theses databases for reports published in English between June 1, 1946, and Jan 31, 2017. Search terms included ("hiv"[MeSH Terms] OR "hiv"[All Fields]) AND ("radiotherapy"[MeSH Terms] OR "radiotherapy"[All Fields] OR ("cancer"[All Fields] AND "radiotherapy"[All Fields]) OR "cancer radiotherapy"[All Fields]). We identified additional references by doing a manual search of the references of all included articles. Two independent reviewers (NGL and SS) identified potential studies and exported them to an electronic reference management software program (Ref Works, version 2.0). NGL and SS determined eligibility by first reviewing the title and abstract and then the full article. Disagreements were resolved by consensus; if consensus was not achieved, then a third author (FA) provided an assessment of eligibility. A study was included if it reported on cancer-related radiotherapy and included patients with HIV. A study was excluded when no detailed information (eg, outcome of radiotherapy, clinical manifestations related to the underlying HIV) was reported. Studies of haematological diseases, Kaposi disease, and brain tumours, and review articles, were also excluded. With regard to data extraction, all studies were analysed for study design, number of patients, sex, age (mean and range), type of radiotherapy, radiation dose, type of antiretroviral therapy, type of underlying solid cancer, outcome in terms of toxicity profile, CD4 count, and viral load.

cancer who are immunocompetent, despite evidence to suggest increased toxicity for this patient population. The introduction of immunotherapy represents an emerging tool to improve survival in the oncological setting and to enhance the efficacy of HAART. Moreover, the most up-to-date technological treatments (intensity-modulated radiotherapy and stereotactic ablative radiotherapy) allow clinicians to reduce irradiation to healthy tissue. Radiotherapy itself has also been suggested to be a potential promoting factor for immune system activation (ie, immunomodulation and the abscopal effect). Although modern radiotherapy techniques are emerging as the new standard in most disease sites because of their proven advantages in terms of reduced side-effects, prospective clinical studies are needed to confirm the effect of immunotherapy and targeted drugs on immunomodulation in combination with this approach.

Contributors

FA, NGL, SS, and ZB searched the literature, assisted with the organisation of the manuscript, interpreted and collected data, and wrote and edited the Review. DB, UR, and DR assisted with the organisation of the manuscript, interpreted and collected data, and wrote and edited the Review. AFo, RM, AFi, FR, MB, and SF interpreted and collected data, helped to design the figures and panel, and wrote and edited the Review.

Declaration of interests

We declare no competing interests.

Acknowledgments

We would like to thank Gianluisa Sicignano for her valuable help regarding planning and preparation of the radiation treatment figures.

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Manuscript 9:

Trovo M, **Giaj-Levra N**, Furlan C, Bortolin MT, Muraro E, Polesel J, Minatel E, Tedeschi R, Filippi AR, Alongi F, Ricardi U.

Stereotactic body radiation therapy and intensity modulated radiation therapy induce different plasmatic cytokine changes in non-small cell lung cancer patients: a pilot study.

Clin Transl Oncol. 2016;18:1003-10. doi: 10.1007/s12094-015-1473-x.

This prospective pilot study investigated kinetics of multiple plasmatic cytokines in patients who underwent radiotherapy with different schedules and techniques in order to establish the correlation between cytokine expression in early-stage or locally advanced non-small cell lung cancer and radiotherapy.

Clin Transl Oncol
DOI 10.1007/s12094-015-1473-x

RESEARCH ARTICLE

Stereotactic body radiation therapy and intensity modulated radiation therapy induce different plasmatic cytokine changes in non-small cell lung cancer patients: a pilot study

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Received: 28 October 2015 / Accepted: 8 December 2015

Abstract
Purpose To assess kinetics of plasmatic cytokines during radiation therapy (RT) for locally advanced and early-stage non-small cell lung cancer (NSCLC).
Methods This prospective study was conducted on 15 early-stage NSCLC underwent to extreme hypofractionated regimen (52 Gy in 8 fractions) with stereotactic body RT (SBRT), and 13 locally advanced NSCLC underwent to radical moderated hypofractionated regimen (60 Gy in 25 fractions) with intensity modulated RT (IMRT). For patients undergoing SBRT, peripheral blood samples were collected on the first day of SBRT (TFd), the last day (TLd) and 45 days (T45d) after the end of SBRT. For patients undergoing IMRT, blood samples were collected at: TFd, 2 weeks (T2w), 4 weeks (T4w), TLd, and T45d. The following cytokines were measured: IL-1, IL-1ra, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12, IL-13, IL-15, IL-17A, EGF, FGF-2, INF-γ, MIP-1α, MIP-1β, TGF-α, TNF-α, and VEGF. Cytokine levels measured in different RT time and compared.
Results No difference in baseline levels of cytokines was documented between patient radiation approaches (except for MIP-1α). For SBRT patients, a mean reduction of IL-10 and IL-17 plasma level was documented between TLd and TFd, respectively ($p < 0.05$). For IMRT patients, a statistically significant ($p < 0.05$) mean plasma level reduction was documented between T4w and TFd for all the following cytokines: IL-1, IL-1ra, IL-2, IL-12, FGF-2, MIP-1α, MIP-1β, TGF-α, TNF-α, VEGF.
Conclusions SBRT and IMRT induce different plasmatic cytokine changes in NSCLC patients, supporting hypothesis that RT regimes of dose schedules and techniques have different impacts on the host immune response.

Keywords Non-small cell lung cancer · Stereotactic ablative radiotherapy · Intensity modulated radiotherapy · Cytokines

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Introduction

Radiation therapy (RT) has a central role in the treatment of non-small cell lung cancer (NSCLC). In early-stage NSCLC or oligometastatic lung patients, the use of stereotactic body radiation therapy (SBRT) is becoming progressively a relevant therapeutic treatment option. SBRT assumes the use of extreme hypofractionated schedules (3–10 fractions) to limited target volume achieving local control rates between 80 and 95% [1–3].

In locally advanced NSCLC, the use of definitive thoracic RT with concurrent chemotherapy is considered the standard approach in patients not eligible to surgical resection [4, 5]. However, results in locally advanced NSCLC, remain unsatisfactory; to improve outcomes in this setting, various advancements, including four-

Published online: 21 December 2015

dimensional RT planning, image guidance RT, IMRT, protons, moderate hypofractionated schedules, and extreme fractionation by SBRT for an integrated boost, are currently under evaluation [6].

In order to decrease acute and late lung toxicity, the identification of the proper patient who might be at high risk for radiation-induced lung injury (RILI) after RT seems to be crucial, particularly for those with poor lung function at the beginning of the treatment. In fact, the prediction of lung toxicity derived from solely dosimetric models has not shown to be sufficiently reliable, especially in more aggressive radiation regimens and/or during concomitant chemo-radiation protocols [7, 8].

It is well recognize that radiation triggers a wide range of cellular and stromal effects in addition to direct cell death effects [9, 10]. Conventional fractionated and hypofractionated radiation treatments are associated with repetitive stimuli that induce initial injuries in lung parenchyma cells. Each radiation insult activates a multiple system interacting within a network of cellular and subcellular signaling. These events induce immune system activation, with a persistent elevation of cytokines, and mediate the cellular response of normal tissue to radiation [11]. Several studies supported the hypotheses that blood-borne biomarkers can be used as surrogates of early tissue injury and subsequently they can be used possible prognostics and predictors for the clinical onset of pneumonitis and fibrosis [12–14].

On these grounds, we have conducted a pilot prospective study to investigate the kinetics of multiple plasmatic cytokines in patients who underwent RT with different schedules and techniques (IMRT and SBRT) in order to establish the correlation between cytokine expression in early-stage or locally advanced NSCLC and RT.

Methods and materials

Patient characteristics

Between May and December 2011, 28 consecutive patients, 18 male and 10 women with a median age of 69 (range 51–83) were treated. 15 of 28 were classified as early-stage NSCLC patients and underwent extreme hypofractionated regimen by means of SBRT consisting of 52 Gy in 8 fractions; 13 of 28 were locally advanced NSCLC patients underwent radical moderated hypofractionated regimen by means of IMRT consisting of 60 Gy in 25 fractions. Patients and tumor characteristics are listed in Table 1. In all cases, during the lung tumor board consultation, the thoracic surgeon with a high level of experience in non-small cell lung cancer surgery excluded any surgical approach. In the 11 cases without a histological confirmation, the so-called proof of malignancy criterions was used for diagnosis. This consists of repeated computed tomography to reveal any new growing lesions and 18F-fluorodeoxyglucose-positron emission tomography positivity, with a SUVmax value over 2.5 [15].

Table 1 Patient and tumor characteristics ($n = 28$ in RT and $n = 40$ in control)

Age median (years) control	70 (66–79)
Age median (years) RT	69 (51–83)
Gender (control/RT)	
Male	25/18
Female	15/10
Smoker (control/RT)	
Yes	19/23
No	21/5
Performance status in RT patients	
0–1	22
2	6
Histology	
Squamous carcinoma	8
Adenocarcinoma	9
Unknown	11
Stage	
I	15
IIIA	6
IIIB	7
Radiation technique for primary treatment	
SBRT	15
IMRT	13

SBRT stereotactic body radiation therapy, *IMRT* intensity modulated radiation therapy, *RT* radiotherapy

Cytokine levels of 40 asymptomatic healthy adult blood donors were also considered as control group values for a comparative analysis with the 28 NSCLC patients treated with RT. In this healthy group, 15 male and 25 women with a median age of 70 years (range 66–79) were randomly selected for the purpose of the comparative analysis.

Radiation treatment

All 28 NSCLC patients were treated with Helical Tomotherapy, a technique that allows the delivery of Image-Guided—IMRT, resulting in a highly conformal radiation dose delivered.

SBRT schedules consisted of 52 Gy in 8 consecutive daily fractions (6.5 Gy per fraction), prescribed to the 80–85% isodose-line and dose calculation was corrected for tissue inhomogeneity.

IMRT schedules consisted of moderated hypofractionated treatment of 60 Gy in 25 fractions (2.4 Gy per

fraction) prescribed at the 95–98% of the Planning Target Volume. Concomitant chemotherapy, consisting of weekly docetaxel, was administered to 9 of the 13 patients (69.2%).

Cytokine measurement and blood samples

Twenty-one cytokines in the serum were simultaneously measured: interleukin (IL)-1, IL-1ra, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12, IL-13, IL-15, IL-17A, epidermal growth factor (EGF), fibroblast growth factor (FGF)-2, interferon (INF)-γ, macrophage inflammatory protein (MIP)-1α, MIP-1β, tumor growth factor (TGF)-α, tumor necrosis factor(TNF)-α, and vascular endothelial growth factor (VEGF).

To determine cytokine profile the multiplex platform MilliplexxMAP human cytokine/chemokine immunoassay (Merk-Millipore, Milano, Italy) was used. Median fluorescence intensity, calculated from duplicates for each sample, was collected using the Luminex200 system (Luminex Corporation, Austin, TX). This is a bead array coupled with discrete fluorescent molecules to detect multiple soluble analyses.

Most of the serum and plasma cytokines showed a significant signal-to-noise ratio at the minimum standard concentration of (3.2 pg/ml). For this reason, the value of 3.2 pg/ml was considered the lower limit sensitivity of the standard curve.

Ethylenediaminetetraacetic acid (EDTA)-peripheral blood samples were collected at the following time:

Patients undergoing SBRT, first day of SBRT (TFd), last day of SBRT (TLd) and 45 days (T45d) after the end of SBRT.

Patients undergoing IMRT at TFd, at 2 weeks (T2w), at 4 weeks (T4w), at TLd, and T45d (Fig. 1).

Cytokine levels measured at different RT time were compared using a two-tailed Student's t test; statistical significance was claimed for $p < 0.05$.

This prospective study was conducted with the approval of our Institutional Review Board, and written informed consent was obtained from all the patients.

Results

Cytokine serum levels in NSCLC patients and control patients

At the baseline, comparing control population and NSCLC patients, a significantly elevated serum levels of five cytokines (IL-1ra, IL-12, IL-17, INF-γ and FGF-2) were found in NSCLC patients than in control group ($p < 0.05$). Conversely, plasma levels of EGF, MIP-1β, TGF-α, TNF-α and VEGF were significantly lower in NSCLC patients compared to controls ($p < 0.05$) as reported in Table 2.

Cytokine serum levels in early stage compare to locally advanced NSCLC

At the baseline mean serum cytokines levels were comparable in early-stage patients and locally advanced patients group (Table 3), except for MIP-1α ($p = 0.03$). RT affected the plasmatic levels of multiple cytokines.

For early-stage NSCLC patients treated with SBRT, a statistically significant ($p < 0.05$) plasma level reduction of IL-10 and IL-17 was documented between TFd and TLd

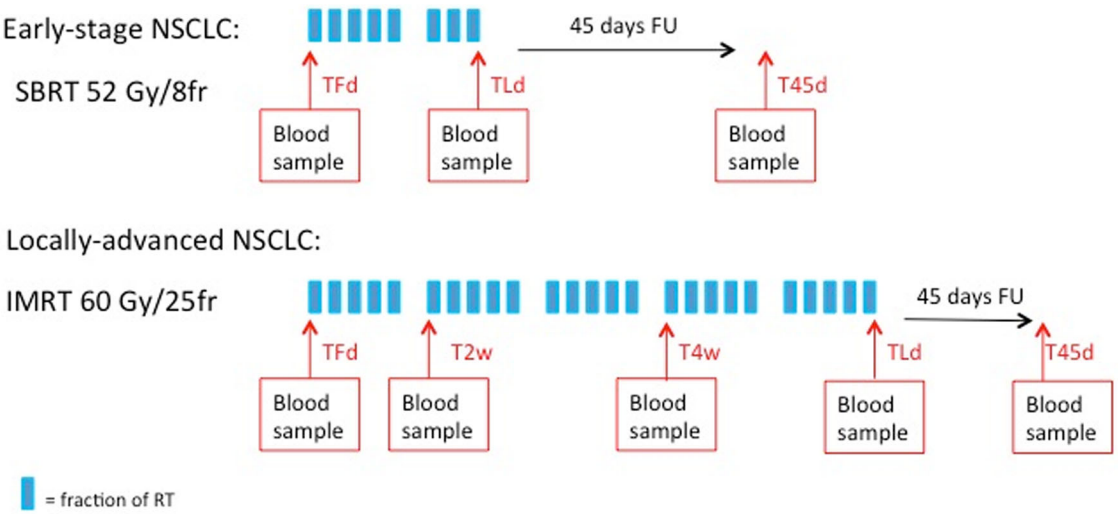

Fig. 1 Blood sample schedules for SBRT and IMRT treatment

Table 2 Baseline cytokine levels (mean values in pg/ml) in controls and in NSCLC patients

Cytokine measured	Controls (pg/ml) (range)	NSCLC patients (pg/ml) (range)	*p* value
IL-1	6.3 (3.2–35.6)	9.5 (3.2–28.6)	NS
IL-1ra	22.1 (3.2–197.9)	70.0 (3.2–234.7)	<0.05
IL-2	10.4 (3.2–88.5)	12.7 (3.2–39.8)	NS
IL-4	11.0 (3.2–131.6)	13.3 (3.2–52.0)	NS
IL-5	5.9 (3.2–92.5)	3.9 (3.2–15.9)	NS
IL-6	31.2 (3.2–134.5)	11.1 (3.2–36.8)	NS
IL-7	21.6 (3.2–55.6)	40.3 (3.2–85.1)	NS
IL-8	11.8 (3.2–40.7)	15.9 (3.2–81)	NS
IL-10	36.2 (3.2–742.7)	22.4 (3.2–133.0)	NS
IL-12	10.4 (3.2–63.7)	64.9 (3.2–231.7)	<0.05
IL-13	13.8 (3.2–214.2)	9.0 (3.2–38.5)	NS
IL-15	7.0 (3.2–34.2)	16.1 (3.2–58.7)	NS
IL-17	6.9 (3.2–34.7)	9.7 (3.2–40.6)	<0.05
EGF	204.8 (10.3–428.1)	87.0 (5.3–823.8)	<0.05
FGF-2	72.3 (3.2–567.4)	131.0 (46.7–581.0)	<0.05
INF-γ	9.3 (3.2–58.5)	26.1 (3.2–103.4)	<0.05
MIP-1α	17.8 (3.2–51.0)	8.1 (3.2–25.9)	NS
MIP-1β	93.3 (3.2–148.1)	57.7 (22.0–117.1)	<0.05
TGF-α	23.7 (3.2–112.5)	6.9 (3.2–24.0)	<0.05
TNF-α	21.0 (3.2–45.1)	16.0 (6.4–48.5)	<0.05
VEGF	452.6 (3.2–1120.3)	173.3 (3.2–842.4)	<0.05

NS not significant

(Fig. 2). No differences in the levels of all the other cytokines analyzed were found.

For locally advanced NSCLC patients, the effects induced by radiation on the kinetics of cytokine levels were more complex. RT led to a plasmatic level reduction of the following cytokines: IL-1, IL-1ra, IL-2, IL-12, FGF-2, MIP-1β, TGF-α, TNF-α, and VEGF. Such reduction became statistically significant ($p < 0.05$) at week 4 (T4w) of the RT course (Fig. 3). All these cytokines returned to pre-RT levels the last day (TLd) of RT, and remained stable within 45 days after the completion of RT.

Discussion

Several studies focused on the correlations between the risk of radiation-induced lung injury and variations in pro-fibrogenic and pro-inflammatory cytokines [12–14]. These markers can be a useful tool in early diagnosis of radiation pneumonitis or in the differentiation between radiation fibrosis and disease recurrence. In NSCLC the use of plasmatic cytokine has been evaluated, especially in the locally advanced setting for dose escalation; however, only data about single cytokines, or few molecules are still available and the reported findings are considered not conclusive [16–18].

In the present study the kinetic behavior of multiple plasmatic cytokines in patients with a diagnosis of NSCLC treated RT has been analyzed.

The immunosurveillance in lung cancer is complex and it declined during the course of the disease with an unresponsiveness of the immune system to specific antigen develops [19–21]. The increase of several cytokines level in NSCLC can be justified by an activation of immune system, as pro-inflammatory and growth factors, in order to neutralize cancer cell. On the other side the reduction of EGF, MIP-1β, TGF- α and TNF- α can be justified by a physiological down regulation of the immune system and by the release of cytokines (as TGF-β) from cancer cells to suppress immune system efficacy in the microenvironment [22].

Cytokines can be also in part produced in tumors, having a crucial role in promoting tumor cell growth, facilitating angiogenesis, invasion, and metastases. Therefore, tumor-derived cytokines might decrease according to tumor response during the RT course. This assumption can support the hypothesis of the use of plasmatic cytokines as tumor markers during RT to monitor disease response. Different studies assessed the relationship between RT and levels of plasmatic cytokines for different primary tumors, including lung cancer. Nevertheless, the results of these studies remain controversial.

Table 3 Baseline cytokine levels (mean values in pg/ml) in early-stage and in locally advanced non-small cell lung cancer patients

Cytokine measured	Early-stage patients (pg/ml) (range)	Locally advanced patients (pg/ml) (range)	*p* value
IL-1	8.2 (3.4–28.6)	11.6 (3.2–22.6)	NS
IL-1ra	55.5 (3.2–214)	86.6 (3.2–234.7)	NS
IL-2	9.5 (3.2–37.3)	16.5 (3.2–39.8)	NS
IL-4	10.8 (3.2–52.0)	16.3 (3.2–49.5)	NS
IL-5	4.3 (3.2–9.0)	4.8 (3.2–15.9)	NS
IL-6	9.7 (3.2–30.4)	12.6 (3.2–36.8)	NS
IL-7	34.6 (14.1–58.8)	46.6 (3.2–85.1)	NS
IL-8	12.7 (3.2–29.5)	19.5 (3.2–81)	NS
IL-10	14.4 (3.2–41.7)	31.7 (3.2–133.0)	NS
IL-12	55.9 (3.2–182.5)	75.1 (3.2–231.7)	NS
IL-13	8.7 (3.2–38.5)	9.3 (3.2–23.9)	NS
IL-15	11.7 (3.2–58.7)	21.2 (3.2–51.6)	NS
IL-17	9.7 (3.2–40.6)	10.2 (3.2–38.3)	NS
EGF	52.1 (3.2–222.4)	127.2 (5.3–823.8)	NS
FGF-2	107.8 (3.2–296.0)	157.5 (46.7–581.0)	NS
INF-γ	23.8 (3.2–80.3)	28.6 (3.2–103.4)	NS
MIP-1α	5.8 (3.2–15.8)	10.8 (3.2–25.9)	0.03
MIP-1β	55.3 (24.6–106.5)	60.4 (22.0–117.1)	NS
TGF-α	6.1 (3.2–15.4)	8.1 (3.2–24.0)	NS
TNF-α	13.5 (3.2–31.2)	18.8 (6.4–48.5)	NS
VEGF	118.5 (3.2–375.5)	236.5 (3.2–842.4)	NS

NS not significant

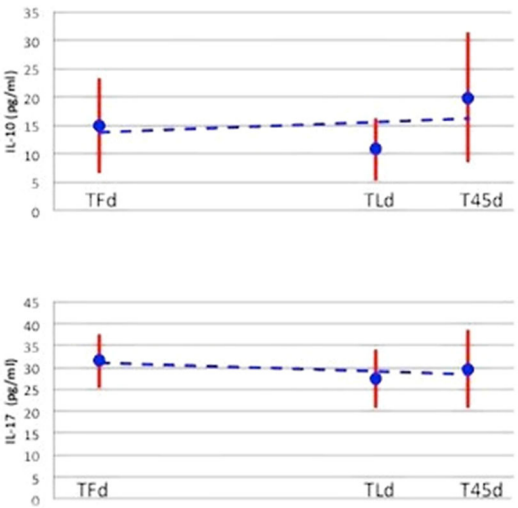

Fig. 2 SBRT leads to a plasmatic level reduction of IL-10 and IL-17

RT has dual effects through tumor immunogenicity stimulation and a suppressive response to immune system T-cell. As reported in our experience, patients treated with RT presented an increased level of interferon type γ (IFNγ) compared to control patients. This data confirmed that RT plays a relevant role to stimulate IFN production, using the STING-mediated pathway [23] and this improves dendritic Cell [24] recruitment and prime T cells activity [25].

Another relevant cytokine is represented by IL-12. IL-12 is one of essential pro-inflammatory cytokines involved in Th1 and natural killer cells stimulations, promotion in dendritic cell maturation, and finally macrophages activation [26]. Conversely in large cancer presentation, advanced stage of the disease and specific histology (i.e., Colorectal, Gastric, Malignant, melanoma, Malignant glioma Hepatocellular Renal cell Head and neck) a down regulation in IL-12 levels has been reported with a negative impact in innate and adaptive immune systems compared to a promotion in cancer progression and distant metastases dissemination [26]. In our experience, a statistical difference in IL-12 ($p = 0.05$) levels in patients treated with RT has been observed compared to control group, demonstrating a potential role of ionizing radiation to stimulate immune system in NSCLC setting.

Moreover, different immune system activations and cytokine kinetics expressions have been found with the two different RT dose schedules and techniques evaluated. SBRT schedules used in early-stage NSCLC patients appears to induce a reduction of plasmatic levels limited to only two cytokines: IL-10 and IL-17 without affecting any other cytokine including those mainly involved in the development of lung injury, as IL-1α, IL-6, MIP-1α, and TNF-α [27–31]. By contrast, patients with locally advanced NSCLC treated with moderate hypofractionated IMRT showed a more complex cytokine kinetic behavior induced by radiation.

Some studies reported a correlation between IL-10 expression and a poorer prognosis in NSCLC, reporting a decrease in IL-10 levels in those patients responding to RT [32, 33]. IL-10 is an anti-inflammatory cytokine produced by monocytes and macrophages, with pleiotropic effects relative to immune-regulation. IL-10 down-regulates inflammation by blocking the production of pro-inflammatory cytokines, such as IL-6, and reduces the function of antigen-presenting cells, thus suppressing the anti-tumor immune response [34, 35]. A primary effect of IL-10 on lung cancer cells may be to increase their metastatic potential by promoting angiogenesis, through the increase of vascular density, and resistance to apoptosis [17]. In this respect, the reduction of IL-10 observed during SBRT treatment may suggest a decreased risk of metastasis in patients treated with this approach and may favor an anti-tumor immune response. Conversely, low levels of IL-10 were documented in patients developing radiation pneumonitis throughout RT treatment, whereas in the absence of this side effect circulating IL-10 remained consistently elevated [36]. This observation prompts to extend IL-10 monitoring after RT and to investigate its potential association with the development of lung injury. Furthermore,

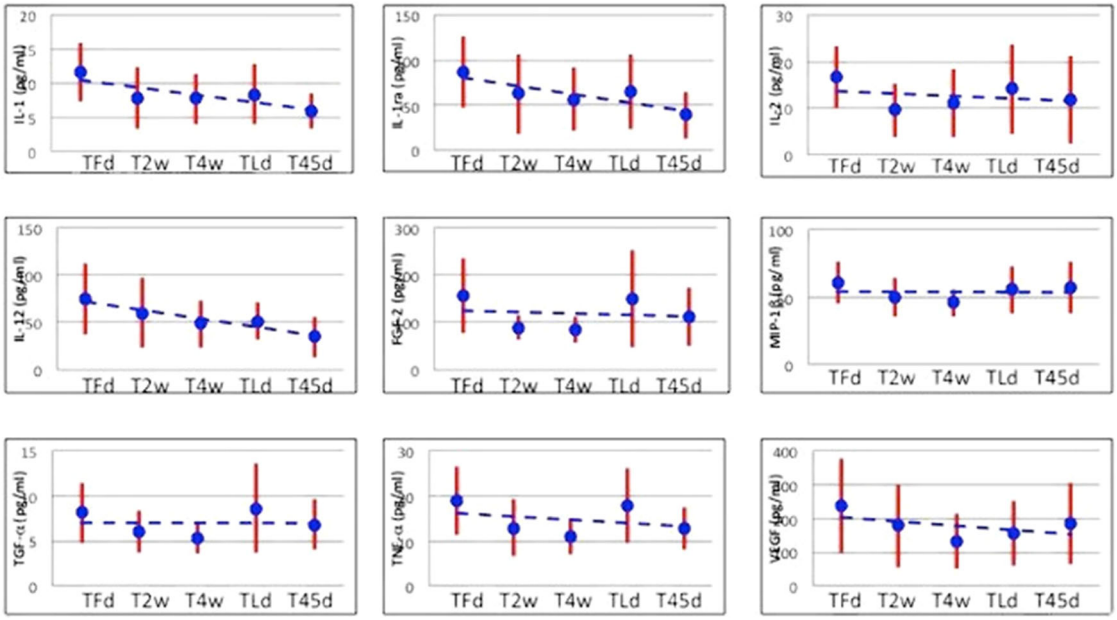

Fig. 3 IMRT induces a plasmatic level reduction of multiple cytokines

studies investigating single nucleotide polymorphisms in the promoter region of the IL-10 gene, reported an association between poor prognosis in late-stage NSCLC patients and the haplotypes responsible for higher IL-10 mRNA expression levels [35].

IL-17 is a pro-inflammatory cytokine produced by activated CD4+ T cells distinct from T helper (Th) 1 and Th2 cells. Whereas Th1 cells have been identified as important regulators of IFN-γ-driven anti-tumor immune response, IL-17-producing Th17 cells have been found to promote tumor growth and enhance neoplastic cell proliferation in NSCLC [37]. In vivo experiments suggested that IL-17 produced by lung CD4+ Th17 cells plays an important role in animal models of NSCLC in favoring tumor development [12]. Moreover, IL-17 may play a role in the metastasis of lung cancer by promoting lymphangiogenesis through the induction of VEGF production by tumor cells [38]. These observations, therefore, together with our results, stimulate further studies aimed at prospectively investigate IL-10 and IL-17 levels and their kinetics as potential markers of clinical response in early-stage NSCLC patients treated with SBRT.

Cytokine levels modulations observed in locally advanced NSCLC patients throughout radical IMRT was much more complex and involved both cytokines usually associated with an anti-tumor immune response (IL-1, IL-2, IL-12, MIP-1α, TNF-α) and those traditionally related to a tumor-promoting environment (IL-1ra, FGF-2, TGF-α, and VEGF) [39–41]. Interestingly, moderate hypofractionated IMRT for locally advanced NSCLC seemed to induce a reduction in the levels of several cytokines associated with radiation-induced lung injury, as IL-1, MIP-1α, and TNF-α [27–31]. However, this RT approach led also to a decrease in the levels of IL-1ra, which was previously associated with a decreased risk of lung injury [42]. On these grounds, a more careful investigation of the kinetics of these cytokines is required to determine the potential role of their modulations in the development of lung injury after IMRT treatment.

We herein clearly demonstrate that SBRT and IMRT treatments induce different plasmatic cytokine changes, which probably reflect distinct effects on tumor cells and on tumor microenvironment.

A different fractionation and radiation dose prescription can have an impact in immune system activation and indirect tumor cell death. In "in vitro", cancer cell irradiation determined a proimmunogenic activation and a stimulation to immune system responses; this phenomena is correlated with radiation dose (ablative dose prescription, use of concurrent chemo-radiotherapy approach) [43].

Whereas, in "in vivo" studies, radiation dose and delivery schedule, required to determine immunogenic cancer death, is influenced by the microenvironment and remain not completely established [44].

The use of high dose (a large single dose of 15 Gy) can be considered more effective than compared to 3 Gy given in 5 consecutive days, but a comparable priming immune system activation has been detected with both regimens [45]. Another group of investigators, using a different model antigen, showed an induction of anti-tumor T-cell responses by a single 20-Gy dose but not by 5-Gy doses given 4 times over 2 weeks, demonstrating that both dose

and the interval between radiation fractions may be important [45].

In conventional treatments, the use of 2 Gy fractionation is able to induce T-cell infiltration and to reprogram macrophages activate, reducing angiogenesis promotion [46]. Inducible nitricoxide synthetase (iNOS) expression by the macrophages is required for this effect, which occurred after a conventional fractionation [46]. Conversely, in hypofractionated treatment, a radiation dose of 10 Gy caused relevant vascular flow impairing effector T-cell recruitment to the tumor, and enhances a hypoxia-driven immunosuppressive environment. Whereas, radiation delivery at doses of 8–10 Gy is likely to induce vascular damage and change intratumor microenvironment, leading to indirect tumor cell death, release of antigens and immune system activation [47].

Nevertheless, the present study discloses two limitations that may affect the general cytokine impairment observed in the IMRT arm. First, the concomitant chemotherapy with docetaxel, administered in 9 of 13 locally advanced NSCLC patients, may affect cytokine plasmatic levels, due to the immune-modulating effects attributed to this drug and, generally, to taxanes, which are able for example to induce a reduction of plasmatic IL-1β and TNF-α in breast cancer patients [48]. Second, the difference in the tumor stage of the two groups of treatment may influence the immune impairment induced by the tumor itself, leading to a more profound immunosuppression in the locally advanced cases [49], which may be therefore more sensitive to a radiation-induced decrease of cytokine levels. In conclusion, we strongly favor the possibility that the limited cytokine changes induced by extreme hypofractionated schedules SBRT in early-stage NSCLC patients may reflect a minor impact of this treatment on patients' immune proficiency as compared to the moderate hypofractionated schedules—IMRT approach, thus favoring the generation of potentially effective host anti-tumor responses. According to the "in vitro" experiences, we confirm, with an "in vivo" study, that the use of ablative radiation dose and concurrent chemo-radiotherapy (used in 70 % of locally advanced NSCLC) determined an immune system activation when compared with control group. Apparently, comparing SABR and IMRT groups, only MIP-1α demonstrated a statistical difference. This difference, in favor to conventional fractionation could, pilot, confirm the reprogram macrophages activate, reducing angiogenesis promotion, and the induction on T-cell infiltration.

This is a pilot experience evaluating the variability levels of cytokines in patients with a diagnosis of early-stage and locally advanced NSCLC treated with RT. The next step, ongoing, will be to evaluate the correlation between cytokine and radiological imaging response after RT with a larger sample size to confirm our hypotheses.

Compliance with ethical standards

Informed consent Informed consent was obtained from all individual participants included in the study.

Ethical approval The current study has been performed in accordance with the ethical standards laid down in the 1964 declaration of Helsinki and its later amendments. All persons gave their informed consent prior to their inclusion in the study.

Conflict of interest The authors have declared that no conflict of interest exists.

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Manuscript 10:

Title: Preliminary report of a pilot study on IL-13 as prognostic biomarker in patients with early stage lung cancer treated with stereotactic ablative radiotherapy.

Abstract

Purpose: To estimate the prognostic role of plasmatic levels of IL-13 in patients with early stage non-small cell lung cancer (NSCLC) treated with stereotactic ablative radiation therapy (SABR).

Method: Fifteen patients were prospectively enrolled in this pilot study from January 2010 to December 2012. Blood samples were collected at the following time: first day of SABR (TFd), last day of SABR (TLd) and 45 days (T45d) after the end of SABR. Firstly, we aimed to investigate whether IL-13 levels were associated with cancer specific survivals (CSS). Secondly, we tested if different IL-13 levels might identify specific subgroups of patients at higher risk of radiation-induced lung toxicity.

Results: All patients received a radiation dose prescription of 52 Gy in 8 fractions for stage IA-B NSCLC. IL-13 levels, measured at TFd ($p=0.038$) and T45d, resulted significantly associated with lower CSS ($p=0.045$). Additionally, a trend of correlation between IL-13 levels at T45d and late moderate-severe chronic radiological lung injury was also observed ($p= 0.06$)

Conclusions: The results of this pilot study suggest that IL-13 levels may correlate with lower CSS and a higher incidence of late moderate-severe lung toxicity. A larger sample size is needed to confirm or not this hypothesis.

Introduction

Patients affected with early stage (IA-B) non-small cell lung cancer (NSCLC) not eligible for surgery are ideal candidate for stereotactic ablative radiotherapy (SABR). From the first anecdotal experiences to randomized trials, SABR have demonstrated excellent results in terms of local control, tolerability [1] with a cancer specific survival (CSS) of about 70% at 3 years [2]. The highest local control rates seem to be directly associated to the delivery of higher Biologically Effective Dose (BED) ≥ 100 Gy [3].

Despite these results, a substantial proportion of patients still relapse and die due to systemic disease progression. For selected patients, adjuvant systemic treatment should possibly be used in order to improve CSS, however prognostic factors are lacking.

In tumour microenvironment, different interleukins (ILs) are released, as a typical feature of cancer-related inflammation and immune system activation [4]. Several ILs are also released by cancer cells to induce tumour growth and invasion, while anti-tumor immunity is focused on cancer cell elimination through the release and interaction of different ILs [5].

In a previous experience, our group preliminarily evaluated the kinetic of multiple plasmatic cytokines in patients with early and locally advanced NSCLC, who received radiotherapy with different schedules, dose prescription and techniques [6]. This experience demonstrated that the levels of different plasmatic cytokine may be influenced and changed after radiotherapy at different time-points. Despite these results, influence of cytokine levels on clinical outcomes has not been explored.

IL-13 is a pleiotropic Th2 cytokine that plays an important role in the regulation of biological systems [7]. In particular, IL-13 is one of the most recent and relevant cytokines currently under investigation for its possible role in cancer promotion. IL-13, binding to IL-13 receptor α2 (IL13Rα2), may induce tumour cell migration and invasion [8]. Initial *"in vitro"* and *"in vivo"* experiences have demonstrated that IL-

13 receptor activation is correlated to a poorer prognosis for lung cancer patients [8] and for breast cancer with lung metastases setting [9]. Hence, IL-13 may be considered a promising prognostic marker and a potential therapeutic target in lung cancer.

On this background, we conducted a secondary analysis on our previous consecutive and prospective patient series collected blood samples, with the aim to investigate for a possible correlation between IL-13 plasmatic levels and CSS in order to identify a new prognostic factor and hypothetically a subgroup of patients eligible to adjuvant systemic therapy.

Methods and materials

Study design

This is a secondary analysis on blood samples from patients enrolled in a previous pilot study including patients consecutively treated with SABR for primary lung cancer at Department of Radiation Oncology, Centro di Riferimento Oncologico (CRO) between January 2010 and December 2012 [6]. Clinical data were prospectively collected and periodically updated. Informed consent and protocol consent for the study was obtained from all patients.

Primary and secondary endpoints of the study were to investigate for a correlation between plasmatic levels of IL-13 and CSS and radiation-induced lung toxicity, respectively.

Details on patient inclusion criteria and radiation technique are specified in our previous publication [6]. Briefly, 15 consecutive patients with stage IA-B NSCLC were included in the study. The total dose prescription was 52 Gy in 8 consecutive daily fractions (6.5 Gy per fraction), prescribed to 80–85% isodose-line and dose calculation was corrected for tissue inhomogeneity. All patients were treated with a BED >100Gy, assuming an α/β value of 10_{Gy} for the tumour.

Inflammatory level measurement and blood samples

As previously described [6], ethylenediaminetetraacetic acid peripheral blood samples were collected at the following time: first day of SABR (TFd), last day of SABR (TLd), and 45 days (T45d) after the end of SABR. Cytokine levels (including IL-13) were measured in all patients via multiplex platform MilliplexxMAP human cytokine/chemokine immunoassay (Merk-Millipore®, Milano, Italy). Median fluorescence intensity, calculated from duplicates for each sample, was collected using the Luminex200 system (Luminex Corporation™, Austin, TX). This is a bead array coupled with discrete fluorescent molecules to detect multiple soluble analyses.

In particular, IL-13 showed a significant signal-to-noise ratio at the minimum standard concentration of 3.2 pg/ml. For this reason, the value of 3.2 pg/ml was considered the lower limit sensitivity of the standard curve. Radiographic follow-up and evaluation of lung acute and late toxicity were performed. All alive patients had a minimum of 6 months follow-up at the time of this report. Radiological alterations have been assessed at 45 days, 3 months, and 6 months by the end of SABR. Images were obtained by means of Computed Tomography (CT) helical scanning with the following parameters: 3 mm slice thickness, 120 kV tube voltage, 250 mA tube current and 0.75 s/slice as scan time.

Evaluation of parenchimal fibrosis in SABR treatment

All CT-scans (pre and post-SABR) were compared and analysed in order to identify any increase and/or change in lung parenchyma density during the follow-up. In order to differentiate radiation induced parenchymal changes from other disorders, the radiological analysis was strictly focused to the irradiated volume. The abnormalities were classified according to Ikezoe et al. criteria [10]. Early radiological abnormalities, defined at 45 days and 3 months by the end of SABR were classified as follows: (1) no radiological changes, (2) Patchy ground glass opacity, (3) Patchy consolidation and ground glass opacity, (4) Diffuse ground glass

opacity and (5) Diffuse Consolidation. The late radiological abnormalities, defined at 6 months by the end of SABR, were classified as: (1) no radiological changes, (2) Scar-like pattern, (3) Mass-like pattern, (4) Modified conventional pattern.
A diagnostic radiologist and a radiation oncologist reviewed all CT images in blinded modality. Disagreements were resolved by consensus; if consensus was not achieved, then a third senior radiation oncologist with a high expertise in SABR treatment provided an assessment of imaging radiological toxicity interpretation.
Clinical toxicity was evaluated according to Common Terminology Criteria for Adverse Events (CTCAE) version 4.0. (https://ctep.cancer.gov)

Outcomes

CSS was defined as the time between SABR and death from progression disease. Radiological lung injury evaluation and scoring was previously described. 18F-PET/CT was additionally performed to confirm any local or distant progression, while bronchoscopy and biopsy were performed if feasible or indicated.

Statistical analyses

For the comparison of variables at baseline and follow-up, Student's t-test was used for normally distributed parameters, and the non-parametric Mann-Whitney test was used for non-normally distributed parameters. Correlations were calculated and significance was determined by Fisher's test. Multivariable logistic regression analysis was used to identify any independent predictors of CSS and radiation-induced lung toxicity. Progression free survival and overall survival were not defined as endpoints of this study, given the high rate of non-cancer related deaths and the strong correlation between progression and cancer-specific survival in this setting. Moreover, progression-free survival may suffer from biases in evaluating local relapse or distant progression, while cause of death was prospectively collected and

CSS is a robust endpoint. Kaplan-Meier curves were not generated and analysed due to the limited number of patients enrolled.

SPSS (IBM Corporation, NY, USA) software program was used for these analyses. $p<0.05$ was considered statistically significant**.**

Results

Patient characteristics are summarized in **Table 1**. One patient was excluded from the analysis as lost at follow-up < 6 months after SABR. Among the 14 patients included, 13 (92.8%) were excluded from surgery approach due to comorbity (COPD and/or cardiovascular disease) and one patient (7.2%) refused resection. Six patients (42.8%) had a histological diagnosis of non-small cell lung cancer, while in 8 cases (57.2%) a clinical diagnosis of lung cancer was made according to previously reported specified criteria [6]. In all cases, dose-constraints to the organs at risk have been considered acceptable and all patients completed radiation treatment without interruption.

Median follow-up was 31 months (range 6-63 months). At the time of analysis, 10 patients were dead, with a median survival time of 27 months (range 6-63 months). In 4 patients (28.6%) out of 10 a progressive disease, with either systemic and/or local progression and/or primary uncontrolled tumour, occurred at a median interval of 14 months (range, 8-20 months). Failure at SABR site was observed in 2 patients (14.3%). In 4 patients (28.6%) death was correlated to disease and median CSS time was 27 months; while 6 cases (42.8%) dead for other causes. At the time of progression, 3 patients received a systemic treatment and in 1 case was offered an adrenal gland resection. In **Table 2** we resumed clinical outcomes of all 14 patients

and in **Table 3** we reported characteristics of 4 patients died after disease progression.

Lung Toxicity

At 45 days and 3 months by the end of SABR, early radiological abnormalities were identified as follows: no changes or mild radiological acute toxicity in 7 patients (50%); while a moderate or severe radiological toxicity was detected in 7 patients (50%). At 6 months by the end of SABR, no changes or mild alteration were identified in 8 patients (57.1%), and a moderate or severe radiological toxicity in 6 patients (42.9%). Late clinical pulmonary toxicity was recorded in 4 patients (28.6%), with 3 grade 1 (21.4%) and 1 grade 2 (7.1%). One case of grade 2 chest wall toxicity, and 2 patients developed grade 1 cutaneous erythema.

CSS and IL-13 plasmatic levels

When analysing IL-13 level measured at TFd, we observed a negative correlation with CSS. Patients dead after disease progression had a mean pre-treatment IL-13 level of 18.65 pg/ml (SD 18.19) compared to 5.25 pg/ml (SD 3.94) in remaining population (HR 4.1; 95% CI 2.01-7.08; p=0.038), **Figure 1**.

This negative correlation was also confirmed at T45d; patients who died after disease progression had a mean IL-13 level of 11.07 pg/ml (SD 8.68) compared to 5.52 pg/ml (SD 3.82) in remaining population (HR 2.1; 95% CI 1.7-11.01; p=0.045), **Figure 2**.

Comparing mean IL-13 level measured between patients death after disease progression and death for other causes at TFd we observed the following value: 15.3 pg/ml and 7.5 pg/ml, as reported in **Figure 3**; while at T45d, mean IL-13 level were 11.1 pg/ml and 6.0 pg/ml, respectively (**Figure 4**).

Severe late radiological lung toxicity and IL-13.

It has been observed a statistical trend of correlation between IL-13 levels measured at Td45 and the occurrence of late moderate-severe radiological lung injury. Patients with no radiological or Scar-like pattern had a mean IL-13 level of 4.85 pg/ml compared to 10.1 pg/ml in patients with Mass-like pattern or Modified conventional pattern (HR 2.7; 95% CI 0.89-13.01; p=0.06) **Figure 5**.

Discussion

Despite the efficacy of SABR for early stage non-surgical NSCLC, CSS is still unsatisfactory. For this reason, it would be rational to add systemic treatments for selected patients at higher risk of relapse and death, but prognostic factors have not still been identified.

It is well known that in tumour microenvironment, cancer cells use inflammatory mechanisms to prevent immune system activation and to promote infiltration and progression of the disease [5]. In fact, the dissemination of cancer cells and the development of metastatic lesions required the acquisition of genetic and epigenetic alterations [11]. Additionally, anti-tumor immunity releases ILs (IFN-gamma, TNF-alpha, IL-2) in order to promote cancer cell elimination [4].

ILs are involved in a variety of immunomodulatory functions including immune cell maturation, proliferation and migration [12], however ILs pattern expression on cancer cells may modified response to the tumor microenvironment, promoting local and distant progression. In particular, IL-13 is a Th2 cell derived cytokine involved in the inflammatory process and immune system regulation [7].

Recent publications started to consider IL13Rα2 and IL-13 as potential biomarkers and therapeutic targets *"in vivo"* and *"in vitro"* experiences [8,9,15].

Papageorgis et al. published an *"in vivo"* experience with the aim to identify novel genes that regulate metastatic progression in breast cancer setting [9]. In particular, breast cancer with high-grade tumours and increased IL13Rα2 levels was associated with a worse prognosis for metastatic free survival. In fact, depletion of IL-13 pattern expression in metastatic breast cancer was associated with a delay in tumour growth and a suppression in the development of lung metastases, due to a weak tumour cell migration and metastatic capacity. Authors concluded that IL-13 pattern could be used as a promising biomarker and anti-IL13Rα2 therapies should be explored in breast cancer patients to improve metastatic-free survival [9].

Focusing on human lung cancer, an "*in vivo"* study explored the role of IL13Rα2 as prognostic factor [8]. IL-13 pattern positive expression was detected in 79 patients out of 181 resected NSCLC patients. Authors reported that a positive expression of IL-13 pattern was correlated to a worse overall survival (p=0.001) and disease free survival (p=0.006) in lung cancer patients. Additionally, as demonstrated in *"in vivo"*, IL-13 patterns promoted lung cancer cell growth by activating the transcriptional co-activator with PDZ-binding motif (TAZ) pathway through phosphatidylinositol 3 kinase (PI3K). In fact, IL13Rα2 silencing was associated with suppression in lung cancer growth, invasion and metastasis. Hence, an inhibition of IL-13 pattern is a potential therapeutic approach in lung cancer.

In our pilot experience, we observed a high mortality rate, with only 4 patients alive at the time of the analysis (**Table 2**); this can be justified by an unfavourable patient selection, as median age was 75 years, only 6 patients (42.8%) had a histological malignant proof of lung cancer and all patients recorded significant comorbidities, as reported in **Table 1**. Nevertheless, IL-13 plasmatic levels were significantly associated to CSS in patients with a diagnosis of early stage NSCLC undergoing SABR, when measured at TFd and after 45 days by the end of treatment (T45d). Additionally, mean IL-13 levels was higher in patients dead after disease progression compared to patients dead for other causes. This result may initially confirm the

potential prognostic biomarker role of IL-13 in early stage NSCLC. This preliminary analysis certainly suffers from the small sample size and from other potential biases, such as the high rate for non-cancer related deaths in this cohort. Despite this limit, the higher levels of IL-13 detected in patients who had disease progression and subsequently died in comparison with surviving patients or patients dying for other causes suggest that IL-13 could be considered as a good candidate biomarker for further studies.

In our study, patients with higher level of IL-13 seem to have also a major risk of developing moderate-severe chronic lung injury, with potential effects in terms of lung function, quality of life and potentially survival. During follow-up a complete radiological response is a rarely detectable circumstance in patients with early stage NSCLC treated with SABR [13,14], being lung injury a very frequent event as the primary tumour may be surrounded by inflammatory post-radiation changes. The role of Th2 cytokines in favouring fibrosis development (e.g. IL-4, IL-10, IL-13) is unclear, but it is under investigation in *"in vivo"* studies. In particular a recent experience using a mouse model, evaluated the correlation between IL-13 and the progression of radiation-induced pulmonary fibrosis. Authors observed that irradiated lungs (6 Gy in 5 fraction) in wild-type c57BL/6NcR mice was associated with an accumulation of activated macrophages, displayed elevated levels of IL-13 and extensive parenchymal fibrosis compared to IL-13 deficient mice. Furthermore, therapeutic neutralization of IL-13, was sufficient to protect mice from lung fibrosis [15]. Moreover, for the treatment of asthma, a monoclonal anti IL-13 and anti IL-4 drug targeting the shared receptor of IL-4/IL-13, dupilumab, was recently introduced [16].

Conclusions

In this pilot study, we preliminarily showed the potential role of IL-13 as prognostic marker in early stage NSCLC patients treated with SABR. Nevertheless, the small number of patients enrolled limits our conclusions and a higher accrual of patients is recommended to confirm this finding. We are planning to expand this prospective training cohort and, if the finding will be confirmed, to further validate IL-13 as prognostic biomarker in two separate cohorts of surgical and SABR patients with stage I NSCLC.

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Tables

Table 1. Patient characteristics

Age (y), median (range)	**75 (60-86)**
Sex	
Male	9 (64.3%)
Female	5 (35.7%)
Performance Status (ECOG)	
0	4 (28.6%)
1	5 (35.7%)
2	5 (35.7%)
Smoke	
Never smoker	4 (28.6%)
Former smoker	6 (42.8%)
Smoker	4 (28.6%)
Comorbidity	
COPD	14 (100%)
Hypertension	8 (57.1%)
Diabetes	5 (35.7%)
Other	3 (21.4%)
Lung site	
Left	7 (50%)
Right	7 (50%)
Lobe	
Upper	7 (50%)
Lower	7 (50%)
Stage	
IA	7 (50%)
IB	7 (50%)
Pathological diagnosis	
Bronchoscopy/FNA	6 (42.8%)
Not done	8 (57.2%)
Histology	
Adenocarcinoma	4 (66.7%)
Squamous cell	1 (16.7%)

Other	1 (16.7%)

Table 2. Clinical outcomes of enrolled patients

Patient	Age	Sex	Stage	DDP	DOR	Alive
1	80	Male	IA	**Y**	N	N
2	77	Female	IB	N	**Y**	N
3	68	Female	IA	**Y**	N	N
4	70	Female	IB	**Y**	N	N
5	73	Male	IA	N	N	**Y**
6	86	Male	IB	N	**Y**	N
7	84	Male	IB	N	N	**Y**
8	81	Male	IB	**Y**	N	N
9	80	Male	IB	N	**Y**	N
10	60	Male	IA	N	**Y**	N
11	78	Male	IA	N	**Y**	N
12	68	Female	IB	N	N	**Y**
13	67	Female	IA	N	**Y**	N
14	74	Male	IA	N	N	**Y**

DDP: dead after progression; DOR: dead for other causes; Y: yes; N: no

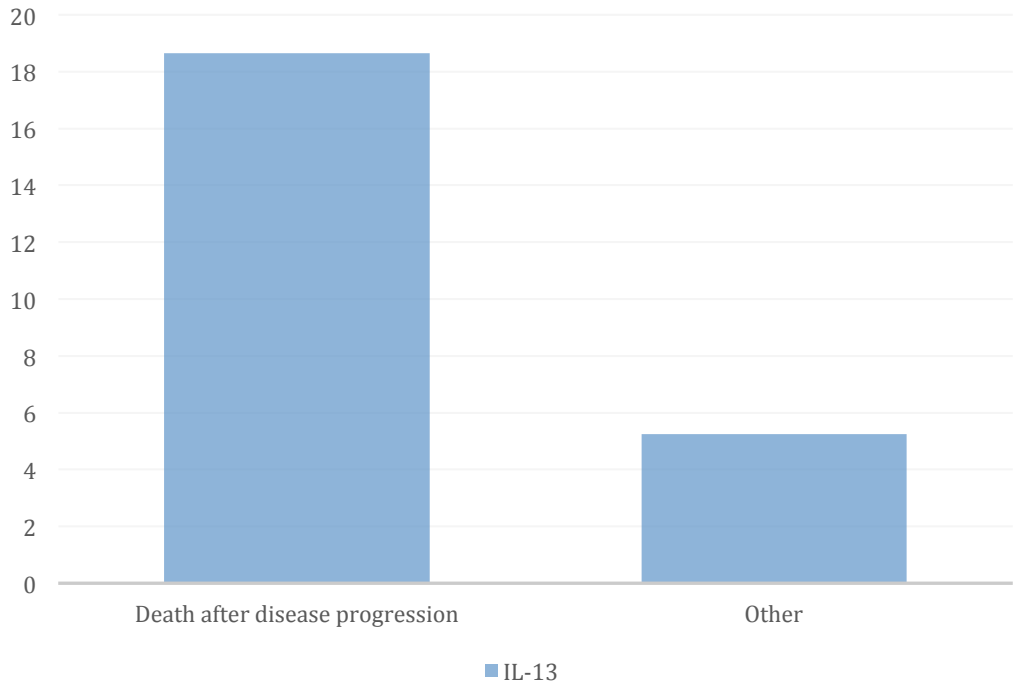
Table 3. Patient died after disease progression

Patient	Stage	Sex	Time progression (months)	Site progression	Treatment	CSS (months)
1	IA	Male	20	Local, contralateral lung	ST	37
3	IA	Female	19	Adrenal gland	S	33
4	IB	Female	8	Mediastinal lymph node	ST	11
8	IB	Male	9	Local, contralateral lung	ST	23

ST: systemic therapy; S: surgery

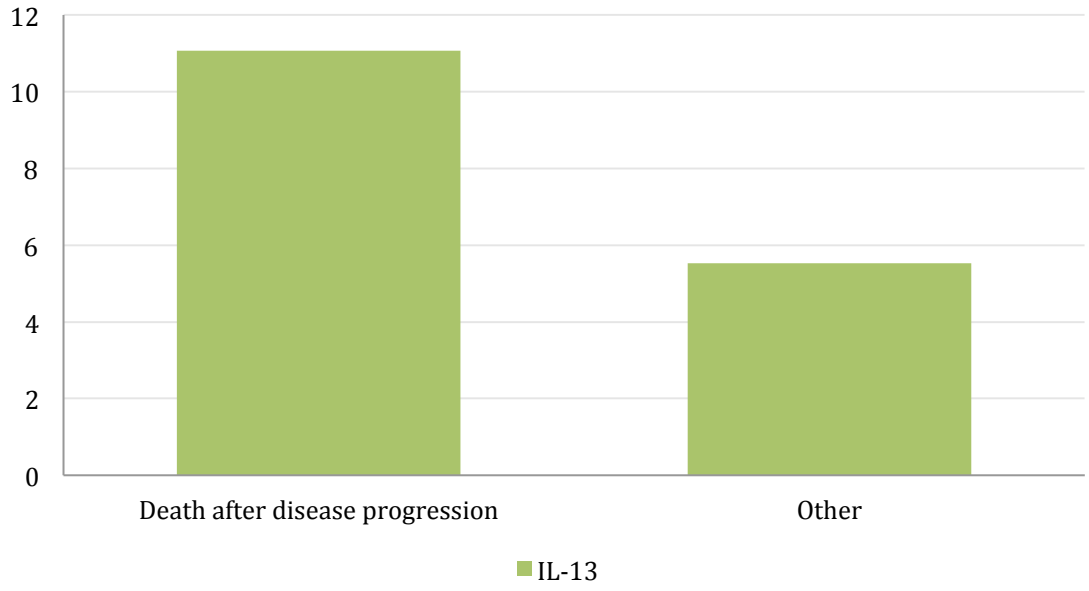
Figures

Figure 1. Correlation between IL-13 level (pg/ml) and CSS at TFd



p=0.038

Figure 2. Correlation between IL-13 level (pg/ml) and CSS at TF45



p= 0.045

Figure 3. Mean IL-13 level (pg/ml) at first days of SABR in patients death after disease progression and death for other causes.

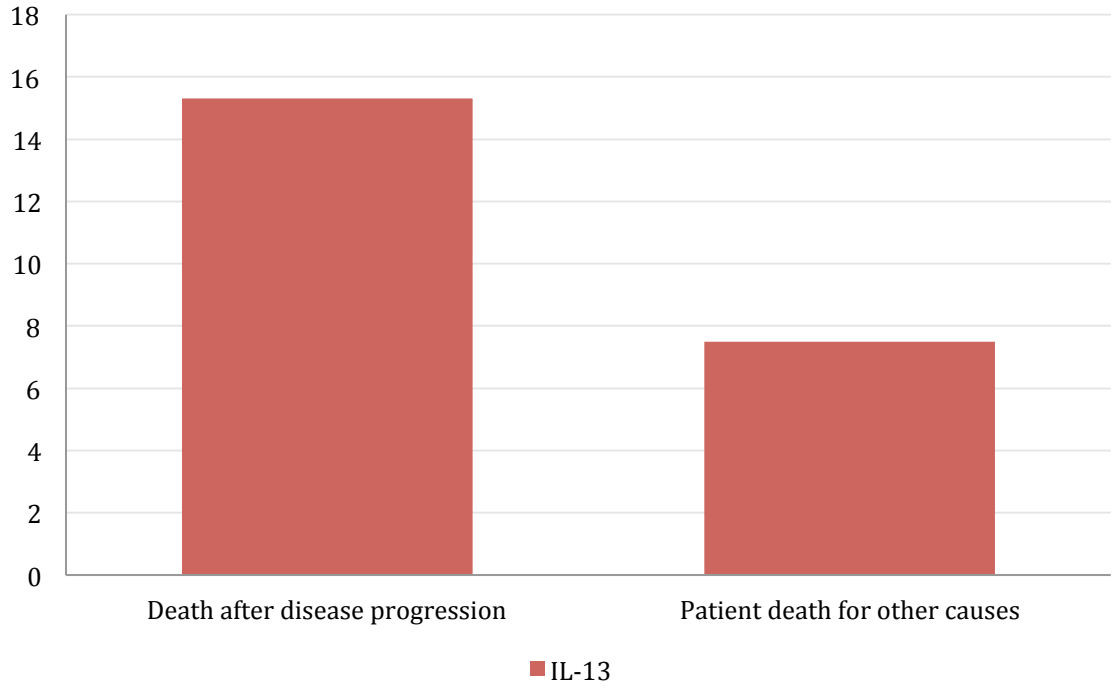


Figure 4. Mean IL-13 level (pg/ml) at 45 days by the end of SABR in patients death after disease progression and death for other causes.

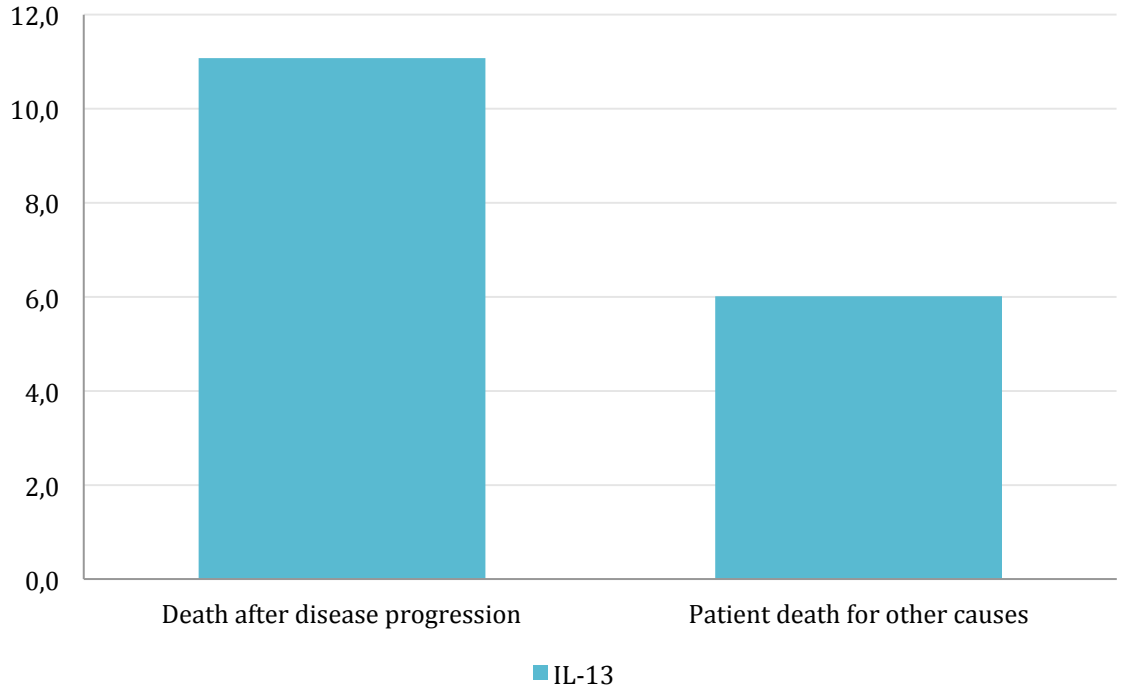
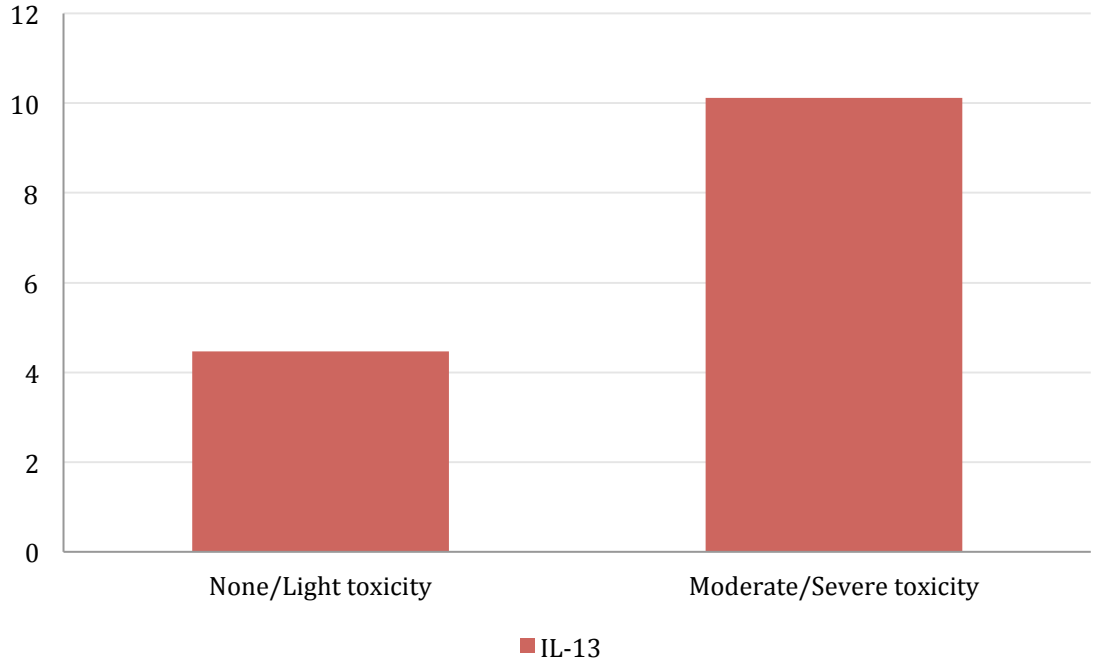


Figure 5. Correlation between IL-13 level (pg/ml) and late radiological toxicity at 45 day from SABR



p=0.06

Final consideration and future prospective

During these last years, we are observing a rapid development in the oncological management of cancer patient. Recently, the scientific community is focusing its attention on the definition and treatment of oligometastatic patients.

This current oncological "revolution" is leaded by significant improvement in medical oncology therapies, in particular after the introduction of new generation of target therapies and more recently immunotherapy. In addition, advances in radiation technologies such as the introduction of intensity-modulated radiotherapy, stereotactic ablative treatment and more recently proton therapy, are improving the tolerability and efficacy of radiation treatments. Moreover, the use of imaged guided radiotherapy, including radiological (e.g. PET-CT) and Linac based on-board imaging (e.g. Cone-beam CT), is mandatory to increase the accuracy in order to spare healthy tissues.

In particular, in the recent years what the Radiation Oncology community have learn is that stereotactic ablative radiotherapy is an effective and safe treatment and it is becoming a relevant oncological strategy in several oncological scenario.

At the same time, medical oncologists are observing significant and promising results in stage IV patients treated with new generation of drugs.

Certainly, the future challenge that will require a multidisciplinary approach, is to comprehend not only which patients can really benefit from aggressive and combined therapy, but also to understand the correct integration and treatment time in order to maximise the oncological benefit.

From our point of view, we have decided to follow different research fields, focusing not only on clinical and technical aspects but in a translation perspective as well.

In particular, the research of prognostic/predictive markers of radio-sensitivity has been considered a crucial aspect.

This propensity allowed us to publish different experiences to evaluate the role of PET-CT and Cone Beam-CT in order to predict an efficacy of ablative radiation treatment in oligometastatic cancer patients.

Finally, focusing on PhD project, the research of haematological biomarkers was an alternative research field in cancer. In the first part of our research, we selected patients with a diagnosis of early stage and locally advanced non-small cell lung cancer, in order to explore the kinetic of several citokines using different radiation dose prescription and techniques. Several limitations of this study can be underline, including the selection of excessive number of citokines and other cofounding factors as the chemotherapy use in locally advance setting. This decision was justified by the need to identify potential prognostic biomarkers for additional studies. Nevertheless, this experience demonstrated that the levels of different plasmatic cytokine may be influenced and changed after radiotherapy at different time-points. In order to avoid potential biases, we have decided to focus our research in the early stage non-small cell lung cancer population. In fact, as we reported in our unpublished data, stereotactic ablative radiotherapy have demonstrated excellent results in terms of local control, tolerability and a cancer specific survival of about 70% at 3 years. Hence, a substantial proportion of patients still relapse and dies due to systemic disease progression and probably an adjuvant systemic treatment should prescribe in order to improve cancer specific survival.

In the treatment of early stage non-small cell lung cancer, a Biological Equivalent Dose (BED) of at least 100 Gy represents the unique predictor factor correlated to efficacy and the need to identify potential prognostic biomarker is a challenge.

As we reported in two different Reviews, radiotherapy causes the disruption of the tissue architecture and alteration in tumour microenvironment. These events are associated with the proliferation of inflammatory signals detected by the immune system, production of cytokines, chemokines and the activation of immune system, inducing an immunomodulation process. Evidence is also increasing that

inflammation contributes to cancer development and that cancer cells use inflammatory mechanisms to prevent immune-system activation and to protect the tumour from immune attack and promoting tumour cells infiltration, progression of disease, and metastases.

Hence, the identification of prognostic inflammatory biomarkers could be considered a significant element to identify unfavourable early stage non-small cell lung cancer.

As we reported in our unpublished data, IL-13 is one of the most relevant biomarker and therapeutic target. In fact, initial laboratory experiences demonstrated in lung metastatic breast cancer setting and non-small cell lung cancer, an increased IL13Rα2 (receptor) levels was associated with a worse prognosis for metastatic free survival and overall survival. Additional a laboratory study in lung cancer setting demonstrated that higher level of IL-13 seems to have also a major risk of developing moderate-severe chronic lung injury after radiotherapy.

Despite the limited number of patients enrolled in our study, we preliminarily demonstrated on non-small cell lung cancer patients a correlation between pre-radiotherapy and post-radiotherapy (45 days after radiotherapy) IL-13 level and cancer specific survival.

Moreover, we observed initial promising data on the correlation between IL-13 at 45 days and risk to develop severe lung fibrosis after radiotherapy.

In future prospective, the identification of inflammatory biomarkers could be evaluated as an additional element that needs to be evaluated also in oligometastatic setting.

We strongly support the multidisciplinary approach not only to reach the most appropriate oncological treatment, but also for economic viability. In fact, we are able to offer several oncological approach, according to the genetic profile, but we need to learn the most appropriate treatment for every oncological patient. In conclusion, I do believe that the multidisciplinary approach is the only key liason to pursue these objectives.

Selected abstracts

- **American Society for Radiation Oncology (ASTRO) Congress – San Diego, USA – 24th-27th September 2017 – Electronic poster -** Increased efficacy of stereotactic ablative radiation therapy after bevacizumab in lung oligometastases from colon cancer.
- **European Society of Radiotherapy and Oncology (ESTRO) 36° Congress – Vienna, Austria – 5th-9th May 2017 – Electronic poster -** Stereotactic ablative radiation therapy for brain metastases with volumetric modulated arc therapy and flattening filter free delivery: feasibility and early clinical results.
- **European Society of Radiotherapy and Oncology (ESTRO) 36° Congress – Vienna, Austria – 5th-9th May 2017 – Electronic poster -** Stereotactic Ablative Radiation Therapy for Lung Oligometastases: Predictive Parameters of Early Response by 18FDG-PET/CT.
- **European Society of Radiotherapy and Oncology (ESTRO) 36° Congress – Vienna, Austria – 5th-9th May 2017 – Electronic poster -** Hippocampal dose during Linac-based stereotactic radiotherapy for brain metastases: An observational study.
- **European Society of Radiotherapy and Oncology (ESTRO) 35° Congress – Turin, Italy – April 29th – May 3rd 2016 – Electronic poster -** Cone-beam computed tomography in lung stereotactic ablative radiation therapy: predictive parameters of early response.
- **European Society of Radiotherapy and Oncology (ESTRO) 34° Congress – Barcelona, Spain – April 24th – 28th 2015 - Electronic poster -** Whole brain radiotherapy with hippocampal avoidance and simultaneous integrated boost for brain metastases: a dosimetric volumetric-modulated arc therapy study.

Citation

- **ESTRO newsletters – November – December 2016 – Section Read it before your patients - Radiotherapy in patients with connective tissue diseases.** *Lancet Oncol. 2016;17:e109-17. doi: 10.1016/S1470-2045(15)00417-9*

My Thanks

A conclusione del percorso di Dottorato di Ricerca desidero ringraziare il Prof. Filippo Alongi, Direttore U.O.C. Radioterapia Oncologica - Ospedale Sacro Cuore Don Calabria, Cancer Care Center - Negrar (Verona) e Università di Brescia, Struttura nella quale esercito, per il sostegno dimostrato nello sviluppare, durante questi 4 anni, molti dei temi di Ricerca qui citati. Lo ringrazio per la Sua attenzione e aiuto nella crescita personale, professionale e scientifica.

Un particolare ringraziamento anche al Dott. Marco Trovò, Direttore Radioterapia Oncologica - U.O.C. Ospedale di Udine, il quale ha contribuito assieme alla Dott.ssa Elena Muraro, Centro di Riferimento Oncologico (CRO) di Aviano (Pordenone), all'arruolamento, allo sviluppo e al finanziamento del Progetto di Dottorato di Ricerca. Ringrazio i miei Colleghi e tutti i Co-Autori, assieme ai quali con grande entusiasmo sempre portiamo avanti idee e progetti.

Ringrazio il Prof. Umberto Ricardi e il Dott. Andrea Riccardo Filippi - Università degli Studi di Torino - Dipartimento Oncologico per aver creduto e sostenuto questo progetto e contributo alla revisione della tesi di Dottorato.

Un particolare grazie al Dott. Savino Sciascia – Università degli Studi di Torino per il suo contributo nella elaborazione dei dati raccolti.

Da ultimo, ma non meno prezioso, vorrei dedicare questa tesi ai miei Genitori Franco e Claudia, a mio fratello Matteo, ad Elisa, a Savino, ad Erika, a Sergio e a Pulli. Grazie per avermi sempre ascoltato ogni singolo giorno.