Lung cancer: the immune system and radiation

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ABSTRACT

Lung cancer has a known relationship with smoking and is one of the leading causes of cancer-related death worldwide. Although the number of studies discussing lung cancer is vast, treatment efficacy is still suboptimal due to the wide range of factors that affect patient outcome. This review aims to collect information on lung cancer treatment, specially focused on radiation therapy. It also compiles information regarding the influence of radiotherapy on the immune system and its response to tumour cells. It evaluates how immune cells react after radiation exposure and the influence of their cytokines in the tumour microenvironment. The literature analysis points out that the immune system is a very promising field of investigation regarding prognosis, mostly because the stromal microenvironment in the tumour can provide some information about what can succeed in the future concerning treatment choices and perspectives. T cells ($CD4^+$ and $CD8^+$), interleukin-8, vascular endothelial growth factor and transforming growth factor-β seem to have a key role in the immune response after radiation exposure. The lack of large scale studies means there is no common consensus in the scientific community about the role of the immune system in lung cancer patients treated with radiotherapy. Clarification of the mechanism behind the immune response after radiation can lead to better treatments and better quality life for patients.

KEY WORDS: Carcinoma, non-small-cell lung.
Immune system.
Lung neoplasms.
Radiotherapy.
Small cell lung carcinoma.

Introduction

Lung cancer (LC) is a disease with a poor prognosis once diagnosed, making it a very aggressive cancer with high mortality. The latest global statistics (GLOBOCAN 2012), show that LC was the most often diagnosed cancer, with 1.82 million diagnosed cases (13% of all cancers) and 1.59 million deaths (19.4% of all cancers).[1,2] It mostly affects males (68.4%), but in recent years the percentage has been declining, with the increase in females especially in developed countries.

Smoking is the most well-known risk factor, where we can find over 60 carcinogenic substances in cigarette smoke. Some 85–90% of LC cases involve a history of current or past smoking habit.[3,4] The remaining 10–15% of non-smoker LC cases are a combination of genetic alterations caused by pollution, passive smoking and gene mutations in epidermal growth factor receptor (*EGFR*), human epidermal growth factor receptor 2 (*HER2*) and in the protein B-Raf (BRAF), usually more related to cases of LC in younger people.[5,6] Exposure to specific substances can be an oncogenic factor, such as asbestos and radon gas.[7,8] The fact that the majority of LC cases appear at older ages makes the patient's age a background factor.[6]

Classification and staging of LC are critical for definitive diagnosis, treatment strategy and to predict the patient's outcome. As 70% of patients are diagnosed in advanced stages, the pathologist has a crucial role in the classification of LC, for which the diagnosis is established by histopathology and molecular analysis of tissue biopsies. In general, LC can be divided into two main types: non-small-cell (NSCLC) and small-cell (SCLC) tumours.

The NSCLC type includes adenocarcinoma, squamous cell carcinoma, and large cell carcinoma, each with its subtypes. They represent nearly 85% of LC cases and are typically unresponsive to treatment protocols.[8] Adenocarcinoma is the most common type of LC (40%) and the bronchioloalveolar carcinoma subtype is more associated with women and non-smokers, in contrast to squamous cell carcinoma that is linked to tobacco consumption.[9]

On the other hand, SCLC tends to affect the neuroendocrine system and is related to smoking habit (only 1% of non-smokers). They are likely to have a good response to radiotherapy (RT) and chemotherapy but there is often extensive lymph node metastasis.[10–14] The staging of LC is a way of assessing the extent of the disease, and has a major role in prognosis and treatment.[12]

Treatment

Therapy for LC depends on several factors such as cell type, extent of spread, and the person's performance status (PS). The main local treatments include surgery and RT, while at the systemic level there is chemotherapy. In the last years an

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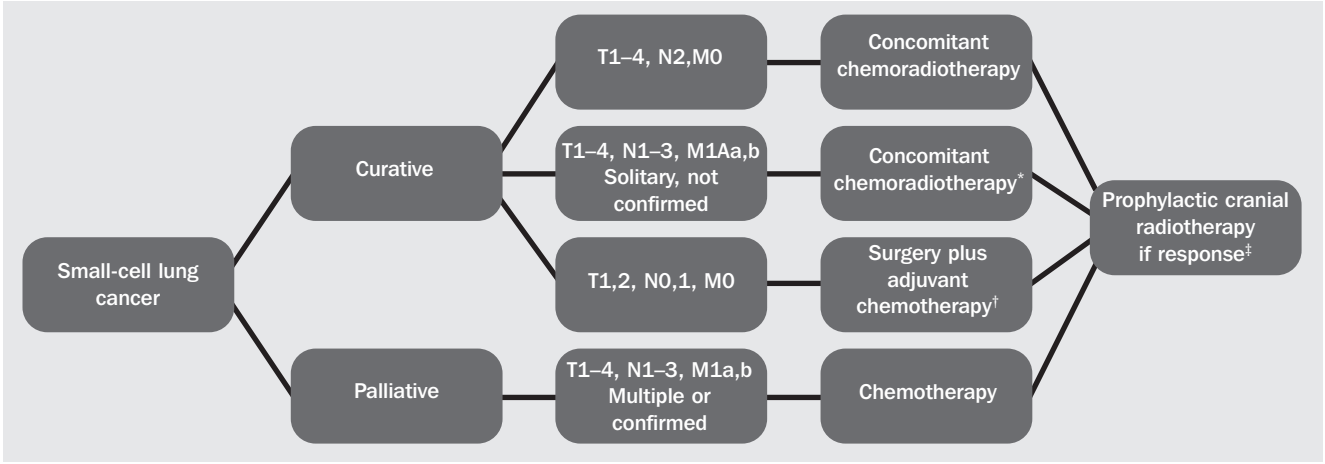

Fig. 1. Small-cell lung cancer treatment algorithm (Adapted from Ref 25).
*If no confirmation of solitary metastasis is obtained, radiotherapy may be added after first response evaluation and is omitted in case of obvious metastatic involvement; †concomitant chemotherapy as an alternative option; ‡or stable disease in case of localised disease.

increase of targeted based therapies has been noticed, in order to achieve tumour-specific structures at the molecular level.[15–17] In most cases, treatment of LC encompasses a set of some of these therapies. Radiotherapy and chemotherapy may precede surgery (neoadjuvant therapy) or may follow it (adjuvant therapy).[9,18–20]

Resection is the treatment of choice for patients with early diagnosed NSCLC (stage I or II) who are able to withstand surgery. Platinum-based adjuvant chemotherapy and targeted therapies are recommended for some patients (stage II) to assist the treatment and lower the risk of metastasis. Radiotherapy is not suggested in stage I or II LC, being used in the later stages when the tumour is unresectable. Patients with stage III LC have different treatment perspectives depending on which lymph node chains are affected by tumour spread (if any).

If the malignant cells have not spread to other lobes and the lymphatic system is only minimally affected (stage IIIA), treatment is similar to that for stage I/II disease but includes adjuvant chemotherapy. Otherwise, if there is involvement of lymph nodes and surgery is not recommended, platinum-based radio/chemotherapy should be applied. Patients with stage IIIB or IV disease have considerable involvement of lymph nodes with large tumour masses, and therapy is based on local RT with chemotherapy. Owing to the extent of such lesions, palliative treatment is offered in most cases.[9,15,19,21,22]

Small-cell lung cancer is known to be aggressive with a tendency to grow and spread quickly; this explains why surgery is performed in very few cases, especially in stage I, followed by chemotherapy or radiotherapy, or both (Fig. 1). The patients with stage II or III disease usually are treated with a combination of platinum-based chemotherapy and radiotherapy if the patient is able to tolerate both at the same time. In stage IV SCLC, palliative platinum-based chemotherapy is indicated, if the patient's general condition allows.[19,23,24] Targeted therapies have been well studied, with the subsequent release of drugs that block tumour vascularisation (angiogenesis) or interfere with the activity of growth factor receptors and molecular pathways triggered downstream.[15–17]

Radiotherapy has an important role because it can provide quick symptom control, and usually the indications are the pain due to chest mass, presence of bone metastases or neural compression, presence of haemoptysis, presence of cough and dyspnoea due to local obstruction of airways, signs of superior vena cava syndrome or spinal cord compression. If there are pathological bone fractures (or risk of bone fractures) the post-operative RT after stabilising should be considered.[21]

The use of RT has increased in the treatment of patients due to the improvement in high-precision RT equipment as well as the development of medical imaging, in particular the widespread use of three-dimensional imaging systems. It is possible to target the oncogenic lesion with high doses of radiation with great accuracy. As a consequence, better tumour control rates make RT an alternative to surgery for some tumour stages.[25]

Radiotherapy: physical and biological basis

Applying radiation to a tumour is one of the most important therapies for LC, being a option at different tumour stages, although the goal is always to deliver a high dose to the whole tumour and produce as much cell destruction as possible while simultaneously reducing injury to surrounding normal tissue.[14,18,26]

Radiation is delivered primarily with high-energy photons (gamma rays or X-rays) or charged particles (electrons or protons). The interaction of a photon beam with matter results in the attenuation of the beam. The photoelectric effect (Compton Effect) and pair production are the main forms of interaction between photons and the tissue. As a result, tissue electrons are ejected causing ionisation and excitation of atoms or molecules of the medium to which radiation is applied, in a cascade reaction. The exposure describes the amount of X-ray or gamma radiation that travels through a volume of air at 0°C, producing pairs of ions. The units for exposure are the Roentgen (R) and coulomb/kilogram (C/kg).[27]

When ionising radiation interacts with the human body, it gives energy to the body tissues. The amount of energy absorbed per unit weight of the organ or tissue is called the absorbed dose (Gy). One Gy corresponds to the release of

the energy of one joule per kilogram of organ or tissue weight (1 Gy = 1J/kg).[27]

Considering tissue irradiation with high-energy photons, there are diverse possibilities of interaction, resulting in various possible molecular lesions. Those effects are developed in three phases: physical, chemical and biological. Initially, a few milliseconds after interaction, the electrons from atoms of the involved tissue are ejected (ionisation) or they pass into a higher energy level (excitation). These ejected electrons can ionise or excite other nearby atoms or molecules, resulting in an ionisation cascade. Chemical reactions occur almost instantly both in a direct or indirect way. If the photon directly interacts with DNA, changes in the ability of the cell to divide can occur with eventual induction of cell death. However, as most of the cell is water the possibility of DNA damage is fairly low.

When radiation interacts with water, a process named water radiolysis occurs. In this process, an electron is extracted from the water molecule, initiating a chemical reaction that ends with the production of free radicals and ions that are highly reactive. These free radicals and ions produced form hydrogen peroxide (H_2O_2), which is responsible for approximately two-thirds of the destructive effect on the lesion. Among the free radicals produced, the hydroxyl radical ($^{\bullet}HO$) is especially aggressive to DNA, being responsible for 60–70% of the damage. After a short period, biological effects begin, with aggression and repair mechanism involvement. Enzymes begin to repair damaged DNA in order to restore stability. In some cases, however, they fail to repair some lesions and it is the accumulation of pathogenic mutations that lead, eventually, to cell death.[9,28,29]

One characteristic of radiation effects is latency time, which means that the effects begin sometime after radiation exposure. This is why RT requires several sessions for its effects to be noticed. Also, due to the biological effects of radiation, adverse effects can be observed some weeks or months after irradiation caused by injuries to stem cells and cells of the surrounded normal tissues. Damage to the intestinal mucosa or haematopoietic cells are examples of lesions caused by radiation exposure during treatment.[30]

Radiation treatments are usually given in several sessions per week in small doses, called fractions. The total dose is normally fractionated in small doses given five or six days per week, over several weeks. After interactions by ionising photons, cells are redistributed into different phases of the cell cycle into to attempt repair of the damage caused. Solid tumours are frequently hypoxic, which reduces the sensitivity to radiation; however, if the radiation is given in fractions, tumour cells may have time to reoxygenate between sessions, increasing the sensitivity to RT of the residual tumour cells.[6]

Radiotherapy: dose and fractionation

Although LC is the most prevalent cancer among tumour types, there are no global official guidelines to apply radiation. This can be explained by the fact that the treatments are highly variable, depending on tumour characteristics, on the number and localisation of lymph nodes affected, and on the presence or absence of metastasis.[9,31,32]

As previously mentioned there are two main histological types of LC, NSCLC and SCLC, for which treatment guidelines vary. Radiotherapy has a potential role in all stages of NSCLC, as either definitive or palliative therapy. The critical goals of modern RT are to maximise tumour control with minimal treatment toxicity,[19] When applying RT, three-dimensional imaging techniques are recommended in order to avoid collateral damage to normal tissue.[33]

Non-small-cell LC is the most common type, but only 15–20% of the cases are potentially curable, the majority of them at early stages. In these cases, radical RT also know as continuous hyperfractionated accelerated radiotherapy (CHART) is recommended but the prognosis is still not very satisfying, with low five-year overall survival rates.

According to UK guidelines (2006) there are three main schedules of RT with curative intent: accelerated hypofractioned RT, conventional radiotherapy and CHART. In accelerated hypofractioned RT the dose of 52.5–55 Gy is delivered in 20 daily fractions over four weeks, with or without chemotherapy. In conventional radiotherapy a dose of 60–66 Gy is delivered in 2-Gy fractions over 6.5 weeks and run usually with chemotherapy. In the CHART option, more than one fraction is given each day, delivering 54 Gy in 36 fractions. Each fraction is applied three times per day over 12 days.[19]

Table 1. Conventionally fractionated and palliative RT for non-small-cell lung cancer treatment combinations.

Treatment type	Total dose	Fraction size	Treatment duration
Definitive RT with or without chemotherapy	60–74 Gy	2 Gy	6–7.5 weeks
Pre-operative RT	45–50 Gy	1.8–2 Gy	6–7.5 weeks
Post-operative RT			
Negative margins	50–54 Gy	1.8-2 Gy	5–6 weeks
Extracapsular nodal extension or microscopic positive margins	54–60 Gy	1.8-2 Gy	6 weeks
Gross residual tumor	60–70 Gy	2 Gy	6–7 weeks
Palliative RT			
Obstructive disease (superior vena cava syndrome or obstructive pneumonia)	30–45 Gy	3 Gy	2–3 weeks
Bone metastases with soft tissue mass	20–30 Gy	4–3 Gy	1–2 weeks
Bone metastases without soft tissue mass	8–30 Gy	8–3 Gy	1 day–2 weeks
Brain metastases	CNS GLs	CNS GLs	CNS GLs
Symptomatic chest disease in patients with poor PS	17 Gy	8.5 Gy	1–2 weeks
Any metastasis in patients with poor PS	8–20 Gy	8–4 Gy	1 day–1 week

CNS-GLs: Central nervous system – guidelines; PS: Performance status. Adapted from ref 22.

Radiotherapy for NSCLC is applied with palliative intent whenever specific conditions are present; for example, obstructive disease such as superior vena cava syndrome or an obstructive pneumonia, bone metastases with or without soft tissue masses, brain metastases, presence of symptomatic chest disease or any metastasis. For this approach the patients are divided in two main groups according to PS. For patients with NSCLC and poor PS, a dose of 10 Gy applied once is suggested. In patients with medium or good PS, a dose of 39 Gy distributed over 13 sessions with spinal cord protection, or 20 Gy in five fractions, is recommended.[31,32,34–36]

Currently, and according to the National Comprehensive Cancer Network guidelines (2013), in early-stage NSCLC (stage I), stereotactic ablative RT (SABR), also known as stereotactic body RT (SBRT), is recommended for patients who have inoperable tumour or who refuse surgery. For locally advanced NSCLC (stage II-III) in patients with inoperable tumour, the standard of care is RT concurrent with chemotherapy. In advanced/metastatic NSCLC (stage IV), RT is recommended for local palliation or relief of symptoms such as pain, bleeding or obstruction. Definitive local therapy to isolated or metastatic sites (oligometastases) including but not limited to brain, lung and adrenal gland, achieves prolonged survival in a small number of well selected patients with good PS who have received radical therapy to the intrathoracic disease.[19]

Patients with node-negative early-stage disease, SABR is recommended.[18,34] For centrally located tumours, defined as within 2 cm of the proximal bronchial tree, an adapted SABR regimens of 4–10 fractions appears to be effective and safe.[18,19,37–40] Table 1 summarised the most commonly used doses and types of treatment combinations with RT for NSCLC.

Until recently, SCLC patients were grouped into limited SCLC and extensive SCLC depending on the area affected by the tumour. Currently, and according to ESMO clinical practice guidelines and National Comprehensive Cancer Network (version 2. 2013) guidelines, SCLC is classified under the tumour-node-metastasis (TNM) version 7 staging system.[19,21] In this group, patients usually receive cycles of chemotherapy as a firstline treatment. Radiotherapy only has a role in SCLC when applied after, or simultaneously with, chemotherapy, depending on patient performance and acceptable response to chemotherapy.[41,42] In these circumstances, RT is delivered as either 1.5 Gy twice daily up to a total dose of 45 Gy, or as fractions of 1.8–2.0 Gy once daily up to a total dose of 60–70 Gy.[19] Cranial irradiation also has a prophylactic role, with a dose of 25 Gy given in 10 fractions, or 30 Gy in 10–15 fractions, in order to reduce the probability of brain metastasis (Fig 1).[32,35,41–43]

Table 2. Tumour node metastasis classification.

TX	Positive cytology only
T1	≤3 cm
T1a	≤2 cm
T1b	>2–3 cm
T2	Main bronchus ≥2 cm from carina, invades visceral pleura, partial atelectasis
T2a	>3–5 cm
T2b	>5–7 cm
T3	>7 cm chest wall, diaphragm, pericardium, mediastinal pleura, main bronchus <2 cm from carina, total atelectasis, separate nodule(s) in the same lobe
T4	Mediastinum, heart, great vessels, carina, trachea, oesophagus, vertebra; separate tumour nodule(s) in a different ipsilateral lobe
N1	Ipsilateral peribronchial, ipsilateral hilar
N2	Subcarinal, ipsilateral mediastinal
N3	Contralateral mediastinal or hilar, scalene or supraclavicular
M1	Distant metastasis
M1a	Separate tumour nodule(s) in a contralateral lobe; pleural nodules or malignant pleural, or pericardial effusion
M1b	Distant metastasis

Immune system and radiation

Living cells have different sensitivities to ionising radiation exposure, but, in general, actively reproducing cells are more radiosensitive. This behaviour reflects the Bergonie-Tribondeau Law which states that the radiosensitivity of a cell, tissue or organs increases with its reproductive capacity and decreases with its degree of differentiation. Oncogenic cells are constantly reproducing and are thus much more sensitive to radiation effects; however, blood cell precursors are also constantly reproducing and are eliminated as a collateral damage, what leads to leucopenia.[32,44]

Lymphocytes are known to be vulnerable to radiation, due to induced apoptosis in mature T cells and B cells, lowering their levels in peripheral blood. This can also be detected in bone marrow where radiation damages precursors of monocytes and granulocytes as well as natural killer (NK) cells, compromising innate immunity.[24,26,45]

Previous studies have shown that subpopulations of T lymphocytes ($CD4^+$ and $CD8^+$) tend to reduce in number after RT, and in such cases patients have a poor prognosis; if $CD4^+/CD8^+$ count is normal the five-year survival rate is better.[26,28,46] The most plausible reason is that, in the immune response to cancer cells, the tumour-infiltrating $CD8^+$ T cells have a crucial role, recognising TAA peptides. These peptides are associated with major histocompatibility complex class I molecules (MHC1), which can be expressed on the cancer cell surface and recognised by tumour-specific $CD8^+$ T cells.[24,26,45]

In NSCLC the presence of infiltrating $CD8^+$ cells alone does not result in a better prognosis, as $CD4^+$ T cells have a key role in this particular carcinoma. The synergistic effect of high number of $CD4^+$ and $CD8^+$ T cells infiltrating the cancer stroma illustrates cooperation between these two cell lines, as activation of $CD4^+$ T cells is required for immunisation of $CD8^+$ cells against the tumour cells. Cytokines such as interleukin-2 (IL-2) released by $CD4^+$ T cells are important in growth and proliferation of $CD8^+$ cells.[26,46]

In RT there are sequences of reactions that follow after the injury to cells, which involve multiple immune mechanisms that can boost immune feedback against the tumour. The tumour tissue responds to ionising photons by the upregulation of acute-phase proteins such as tumour necrosis factor-α (TNFα), IL-1 and IL-6. MHC 1 and adhesion molecules such as intercellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule-1

(VCAM-1) or the E-selectin are also upregulated in endothelial cells and can assist in leucocyte passage across endothelial barriers near the tumour. Dead cells release ATP and high-mobility group protein 1 (HMGB1) to the extracellular space, increasing the inflammatory process. Subsequently, the inflammatory process recruits macrophages and dendritic cells to the tumour, which in turn will present antigens to T lymphocytes and stimulate T-cell responses, resulting in death of the previously damaged malignant cells.[29,31]

During the inflammatory process caused by radiation, cytokines are released and have a key role in the physiopathology of LC. The tumour microenvironment has its own specific cytokine concentrations and these can influence the outcome of RT. The inflammation caused by radiation can change these concentrations, leading also to different treatment outcomes.[31,36]

As mentioned by Crohns *et al*. IL-6, IL-8 and VEGF are elevated both locally and systemically in patients with LC of squamous cell type.[35] Interleukin-6 is a primary inflammation protein and is produced as an immune response by the tumour to radiation damage, and may be found mostly in sputum. Interleukin-8 and VEGF are strongly associated with patients who have a shorter survival time.[34,47]

Macrophages can also have impact on prognosis in NSCLC, where their high tumour infiltration and high IL-8 production lead to poor prognosis. Interleukin-8 and VEGF are known to be potent promoters of angiogenesis, which in turn leads to faster tumour growth and lower average survival.[37,48]

The microenvironment changes caused by ionising photon damage can boost the immunological response but the cytokines released can benefit the growth of the tumour. Further studies are needed to assess the prognostic value of these data.[29,31,32] The main information obtained from the literature is compiled in Table 4.

Hald *et al*.[46] attempted to understand why RT can stimulate the immune cells and facilitate an antitumour immune response. They analysed several immune cell markers in 55 patients with NSCLC treated with RT after surgical resection. After construction of tissue microarrays (TMAs), the expression of the markers $CD1a^+$, $CD3^+$, $CD4^+$, $CD8^+$, $CD20^+$, $CD56^+$, $CD68^+$, $CD117^+$, $CD138^+$ were studied by immunohistochemistry in the tumour and stroma. This was the first published paper that studied these markers in stroma, and it showed that $CD4^+$ and $CD8^+$stroma co-expression can be a relevant prognostic factor.[46] Lower levels of $CD4^+/CD8^+$ co-expression lead to poor prognosis, and the benefit from RT is lower. However, the authors advised caution in the interpretation of the results as only a small population (n=12) presented with elevated $CD4^+/CD8^+$.

Crohns *et al.* designed a study to understand the role of several cytokines in LC. For this they collected bronchoalveolar lavage (BAL) fluid and blood serum from 36 patients with NSCLC, and 36 controls at baseline.[35] Blood samples were collected during RT and again three months later, in patients and controls. They measured TNFα, IL-1α, IL-6, IL-8, IL-12, IL-18 and VEGF using a sandwich enzyme-linked immunosorbent assay (ELISA) kit. The authors observed higher levels of IL-6 and IL-8 in patients than in controls, both in BAL and blood, and concluded that these two cytokines are upregulated in LC. After radiation the IL-6 increased in BAL, mostly because of oxidative stress caused by the radiation, resulting in inflammation. The authors also concluded that higher basal levels of IL-8 and VEGF determine a decrease in survival rates. These two

Table 3. Tumour stage grouping.

Occult carcinoma	TX	N0	M0
Stage 0	Tis	N0	M0
Stage IA	T1a,b	N0	M0
Stage IB	T2a	N0	M0
Stage IIA	T2b	N0	M0
	T1a,b	N1	M0
	T2a	N1	M0
Stage IIB	T2b	N1	M0
	T3	N0	M0
Stage IIIA	T1a,b,T2a, b	N2	M0
	T3	N1,N2	M0
	T4	N0,N1	M0
Stage IIIB	T4	N2	M0
	Any T	N3	M0
Stage IV	Any T	Any N	M1

Adapted from Früh *et al*., 'Small-Cell Lung Cancer (SCLC): ESMO Clinical Practice Guidelines for Diagnosis, Treatment and Follow-Up'.

Table 4. Summary of studies used from literature review.

	Hald *et al.* (2013)	Crohns *et al.* (2010)	Wang *et al.* (2009)	Zhao *et al.* (2008)	Gridley *et al.* (2004)	Novakova-Jiresova *et al.* (2004)
Patients (*n*)	55	36	23	26	12	46
Stage	NSCLCI–IIIA	NSCLCI–IV	NSCLCI–IIIB	NSCLCI–III	NSCLCI–IIIA	NSCLCIIIA–IIIB
Chemotherapy (CHT)	None	14% received CHT	78% CHT	65% treated with carboplatin and taxol	None	Carboplatin
Radiotherapy (total dose and fractions)	≥50 Gy (various Fx regimes)	30–60 Gy (Fx not mentioned)	>50 Gy (Fx: 25–27)	64–70.1 Gy (various Fx regimes)	51 Gy (Fx: 20)	60 Gy (Fx: 30)
Surgery	Surgical resection	Not mentioned	Inoperable	Inoperable	Inoperable	Unresectable
Median overall survival	24 months	9.9 months	Not mentioned	Not mentioned	Not Mentioned	Not mentioned
Immune cells or cytokines studied	T cell (CD4+ and CD8+)	IL-8 and VEGF	TGFβ1	TGFβ1	bFGF, TNFα, IL-1β, IL-6, IL-10	TGFβ

molecules are related to angiogenesis and may play a role in assessing LC prognosis through serum and BAL testing.[35]

Gridley *et al*. studied cytokine change during RT in 12 patients with inoperable NSCLC. Basic fibroblast growth factor (bFGF), TNFα, IL-1β, IL-6, IL-10 and procollagen III peptide (P III P) were studied in serum.[38] Levels of these cytokines were analysed at baseline and 15, 30, 45, 60, 90, 120, 150, 180 and 210 days after the beginning of RT. This study also compared patients treated with proton treatment alone with those who received combined photon and proton therapy. bFGF, TNFα, and IL-6 were significantly higher in patients receiving the combination RT mainly because tissue damage is greater. No other significant data were presented by the authors.[38]

Transforming growth factor (TGF) is one the best studied cytokine in LC and has an important role in immunity. Some studies attempted to find a correlation between TGFβ and radiation pneumonitis (RP), and found a predictive role in the quantification of this molecule.[39,40] As RP is one of the most predominant secondary effects of RT, it is important to evaluate any tendency for this to develop.[37,40–42]

Wang *et al*. aimed to clarify the predictive role of TGFβ1 in RP. They assessed TGFβ1 level both in blood serum and induced sputum, and quantified them before and near the end of RT. Twenty-three patients with NSCLC were enrolled and the levels of serum TGFβ1 were analysed by double antibody sandwich ELISA. During the RT sessions, nine of the 23 patients (39%) had RP and the patients with higher concentrations of TGFβ1 showed a greater tendency to RP, although the results were not statistically significance. TGFβ1 was detected in macrophages and epithelial cells by immunohistochemistry techniques. The authors concluded that use of TGFβ1 alone was not a good predictor of RP, but could have some importance combined with tumour response.[40]

Novakova-Jiresova *et al*. studied 46 subjects, 24% of whom developed symptoms of RP. After analysis of the TGFβ levels during the six weeks of RT sessions, the patients who did not developed RP showed a tendency to lower TGFβ levels. However, levels of TGFβ in patients who developed RP increased mid way through the therapy. The difference in the TGFβ dynamics between the groups reached marginal statistical significance only in the third week of treatment, and subsequently the correlation diminished.[41,42]

Zhao *et al.* aimed to investigate the role of circulating TGFβ1 during radiation therapy in predicting radiation-induced lung toxicity (RILT). TGFβ1 level was determinate before radiation therapy and at two and four weeks after the beginning of RT; by the end, 23% of patients had symptoms of RILT. The results showed a radiation-induced elevation of circulating TGFβ1 after four weeks of RT. No correlation was found between the levels of this cytokine and patients with and without RILT.[38]

After analysis of the literature regarding cytokines and immune cells, it is highly suggestive that the immune system response is strongly correlates with radiation effects. Although the scientific community generally associates RT with immunosuppressive effects, some new information shows the opposite, and RT could have a stimulating effect on the immune system.[38] Understanding how the immune system behaves in response to radiation and in tumour tissue, it is possible that these cells, or their cytokines, can be used as prognostic factors. However, these processes are complex and further research is needed.[35,46,49]

Pro-angiogenic molecules stimulated by radiation, such as IL-8 and VEGF, indicate a poor prognosis, although the presence of T cells indicates a positive response by the immune system against tumour tissue. Clearly, the effect of RT is a balance of several factors that can lead to different outcomes.[35,46]

Radiation has many benefits in the treatment of LC, but adverse effects such as RP and RILT can diminish the final result.[33] To help predict the appearance of these effects in NSCLC patients treated with RT, TGFβ is already being studied widely.[40–42] The results show that TGFβ1 has some relevance in predicting radiation-induced side-effects, as levels of this cytokine increase in the third or fourth week after commencement of RT in patients who later suffer these effects.[39–41,43,44]

Conclusions

The link between radiation therapy and the immune system is noticeable. T cells, macrophages, NK cells and other immune cells have a key role in the control of the tumour microenvironment. The lack of large-scale clinical trials makes the data unreliable, but potentially contain promising information. These difficulties aside, there are too many variables in the studies related to radiation and the immune system. Among the variables, RT treatment, stages of disease, patient characteristics and the small amount of studied subjects are factors that make it difficult for the scientific community to use these data in the routine diagnosis, treatment and prognosis of LC. Clarification of the mechanism behind the immune response after radiation exposure can help to predict the reactions to the treatment of LC and result in better therapy results. This improvement could lead to a better quality of life and a better prognosis for patients. □

References

1 Internation Agency for Researc h on Cancer. Lung Cancer Estimated Incidence, Mortality and Prevalence Worldwide. Lyon: IARC, 2014.

2 Siegel R, Naishadham D, Jemal A. Cancer statistics, 2013. *CA Cancer J Clin* 2013; **63** (2): 11–30.

3 Gaughan EM, Cryer SK, Yeap BY, Jackman DM, Costa DB. Family history of lung cancer in never smokers with non-small-cell lung cancer and its association with tumors harboring EGFR mutations. *Lung Cancer* 2013; **79** (3): 193–7.

4 Barnard S. Lung cancer: epidemiology, treatment. *Surgery* 2004; **22** (5): 97–100.

5 Toloza EM, Harpole L, Detterbeck F, McCrory DC. Invasive staging of non-small cell lung cancer: a review of the current evidence. *Chest* 2003; **123** (1 Suppl): 157S–166S.

6 Stewart DJ ed. *Lung cancer: prevention, management and emerging therapies*. Chichester: Humana, 2010.

7 Peto R, Lopez AD, Boreham J, Thun M, Heath C Jr, Doll R. Mortality from smoking worldwide. *Br Med Bull* 1996; **52** (1): 12–21.

8 Molina JR, Yang P, Cassivi SD, Schild SE, Adjei AA. Non-small cell lung cancer: epidemiology, risk factors, treatment, and survivorship. *Mayo Clin Proc* 2008; **83** (5): 584–94.

9 Goldstraw P, Ball D, Jett JR *et al*. Non-small-cell lung cancer. *Lancet* 2011; **378** (9804): 1727–40.

10 Wistuba II, Gazdar a F. Molecular pathology of lung cancer. *Verh Dtsch Ges Pathol* 2000; **84**: 96–105.
11 Rosti G, Bevilacqua G, Bidoli P, Portalone L, Santo a, Genestreti G. Small cell lung cancer. *Ann Oncol* 2006; **17** (Suppl 2): ii5–10.
12 Saeed I, Anderson J. Cancer of the lung: staging, radiology, surgery. *Surgery* 2011; **29** (5): 221–6.
13 Bearz A, Berretta M, Lleshi A, Tirelli U. Target therapies in lung cancer. *J Biomed Biotechnol* 2011; **2011**: 921231.
14 Ray M, Jablons D, He B. Lung cancer therapeutics that target signaling pathways: an update. *Expert Rev Respir Med* 2010; **4** (5): 631–45.
15 Kwak EL, Bang YJ, Camidge DR *et al.* Anaplastic lymphoma kinase inhibition in non–small-cell lung cancer. *N Engl J Med* 2010; **363** (18): 1693–703.
16 Cameron SE, Andrade RS, Pambuccian SE. Endobronchial ultrasound-guided transbronchial needle aspiration cytology: a state of the art review. *Cytopathology* 2010; **21** (1): 6–26.
17 Husain AN, Colby T, Ordonez N *et al*.; International Mesothelioma Interest Group. Guidelines for pathologic diagnosis of malignant mesothelioma: 2012 update of the consensus statement from the International Mesothelioma Interest Group. *Arch Pathol Lab Med* 2013; **137** (5): 647–67.
18 Rekhtman N, Ang DC, Sima CS, Travis WD, Moreira AL. Immunohistochemical algorithm for differentiation of lung adenocarcinoma and squamous cell carcinoma based on large series of whole-tissue sections with validation in small specimens. *Mod Pathol* 2011; **24** (10): 1348–59.
19 National Comprehensive Cancer Network. *Non-small cell lung cancer*. NCCN Clinical Practice Guidelines. Fort Washington: NCCN, 2013 (Version 2).
20 Rossi A, Martelli O, Di Maio M. Treatment of patients with small-cell lung cancer: from meta-analyses to clinical practice. *Cancer Treat Rev* 2013; **39** (5): 498–506.
21 Früh M, De Ruysscher D, Popat S *et al*. Small-cell lung cancer (SCLC): ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol* 2013; **24** (Suppl 6): vi99–105.
22 Paumier A, Le Péchoux C. Radiotherapy in small-cell lung cancer: where should it go? *Lung Cancer* 2010; **69** (2): 133–40.
23 Formenti SC, Demaria S. Systemic effects of local radiotherapy. *Lancet Oncol* 2009; **10** (7): 718–26.
24. Schumacher K, Haensch W, Roefzaad C, Schlag PM. Prognostic significance of activated CD8 (+) T cell infiltrations within esophageal carcinomas. *Cancer Res* 2001; **61** (10): 3932–6.
25 Ikushima H. Radiation therapy: state of the art and the future. *J Med Invest* 2010; **57** (1–2): 1–11.
26 Hiraoka K, Miyamoto M, Cho Y *et al*. Concurrent infiltration by CD8+ T cells and CD4+ T cells is a favourable prognostic factor in non-small-cell lung carcinoma. *Br J Cancer* 2006; **94** (2): 275–80.
27 de Lima JJ. *Biofísica médica* 3rd edn (in Portuguese). University of Coimbra: 2014, 873.
28 Maehata Y, Onishi H, Kuriyama K *et al*. Immune responses following stereotactic body radiotherapy for stage I primary lung cancer. *Biomed Res Int* 2013; **2013**: 731346.
29 Lumniczky K, Sáfrány G. The impact of radiation therapy on the antitumor immunity: local effects and systemic consequences. *Cancer Lett* 2015; **356** (1): 114–25.
30 Joiner M, van der Kogel A eds. *Basic clinical radiobiology* 4th edn. Boca Raton: CDC, 2009.
31 Shiao SL, Coussens LM. The tumor-immune microenvironment and response to radiation therapy. *J Mammary Gland Biol Neoplasia* 2010; **15** (4): 411–21.
32 Dienstmann R, Martinez P, Felip E. Personalizing therapy with targeted agents in non-small cell lung cancer. *Oncotarget* 2011; **2** (3): 165–77.
33 Jeremic B ed. *Advances in radiation oncology in lung cancer* 2nd edn. Heidelberg: Springer-Verlag, 2005.
34 Yuan A, Yu CJ, Chen WJ *et al*. Correlation of total VEGF mRNA and protein expression with histologic type, tumor angiogenesis, patient survival and timing of relapse in non-small-cell lung cancer. *Int J Cancer* 2000; **89** (6): 475–83.
35 Crohns M, Saarelainen S, Laine S, Poussa T, Alho H, Kellokumpu-Lehtinen P. Cytokines in bronchoalveolar lavage fluid and serum of lung cancer patients during radiotherapy – association of interleukin-8 and VEGF with survival. *Cytokine* 2010; **50** (1): 30–6.
36 Durante M, Reppingen N, Held KD. Immunologically augmented cancer treatment using modern radiotherapy. *Trends Mol Med* 2013; **19** (9): 565–82.
37 Chen JJ, Yao P, Yuan A *et al*. Up-regulation of tumor interleukin-8 expression by infiltrating macrophages: its correlation with tumor angiogenesis and patient survival in non-small cell lung cancer. *Clin Cancer Res* 2003; **9** (2): 729–37.
38 Gridley DS, Bonnet RB, Bush DA *et al*. Time course of serum cytokines in patients receiving proton or combined photon/proton beam radiation for resectable but medically inoperable non-small-cell lung cancer. *Int J Radiat Oncol Biol Phys* 2004; **60** (3): 759–66.
39 Rübe CE, Palm J, Erren M *et al*. Cytokine plasma levels: reliable predictors for radiation pneumonitis? *PLoS One* 2008; **3** (8): e2898.
40 Wang J , Qiao XY, Lu FH *et al*. TGF-beta 1 in serum and induced sputum for predicting radiation pneumonitis in patients with non-small cell lung cancer after radiotherapy. *Chin J Cancer* 2010; **29** (3): 325–9.
41 Zhao L, Sheldon K, Chen M *et al*. The predictive role of plasma TGF-beta1 during radiation therapy for radiation-induced lung toxicity deserves further study in patients with non-small cell lung cancer. *Lung Cancer* 2008; **59** (2): 232–9.
42 Novakova-Jiresova A, Van Gameren MM, Coppes RP, Kampinga HH, Groen HJ. Transforming growth factor-beta plasma dynamics and post-irradiation lung injury in lung cancer patients. *Radiother Oncol* 2004; **71** (2): 183–9.
43 Anscher MS. Targeting the TGF-beta1 pathway to prevent normal tissue injury after cancer therapy. *Oncologist* 2010; **15** (4): 350–9.
44 Marks LB, Bentzen SM, Deasy JO *et al*. Radiation dose-volume effects in the lung. *Int J Radiat Oncol Biol Phys* 2010; **76** (3 Suppl): S70–6.
45 Nakakubo Y, Miyamoto M, Cho Y *et al*. Clinical significance of immune cell infiltration within gallbladder cancer. *Br J Cancer* 2003; **89** (9): 1736–42.
46 Hald SM, Bremnes RM, Al-Shibli K *et al*. CD4/CD8 co-expression shows independent prognostic impact in resected non-small cell lung cancer patients treated with adjuvant radiotherapy. *Lung Cancer* 2013; **80** (2): 209–15.
47 Yuan A, Yang PC, Yu CJ *et al*. Interleukin-8 messenger ribonucleic acid expression correlates with tumor progression, tumor angiogenesis, patient survival, and timing of relapse in non-small-cell lung cancer. *Am J Respir Crit Care Med* 2000; **162** (5): 1957–63.
48 Zhu YM, Bagstaff SM, Woll PJ. Production and upregulation of granulocyte chemotactic protein-2/CXCL6 by IL-1beta and hypoxia in small cell lung cancer. *Br J Cancer* 2006; **94** (12): 1936–41.
49 Xu QY, Gao Y, Liu Y, Yang WZ, Xu XY. Identification of differential gene expression profiles of radioresistant lung cancer cell line established by fractionated ionizing radiation *in vitro*. *Chin Med J (Engl)* 2008; **121** (18): 1830–7.