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Review Article

The Role of Combined Radiotherapy and Immunotherapy in Locally Advanced Non-small Cell Lung Cancer

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Abstract

Immune checkpoint inhibitors (ICIs) have recently transformed the landscape for patients with locally advanced non-small cell lung cancer (NSCLC). The addition of consolidation durvalumab after definitive chemo-radiotherapy improved progression-free and overall survival in unresectable stage III NSCLC patients. Experimental evidence of the potential of radiotherapy to enhance anticancer immunity has increased interest in combining radiotherapy and immunotherapy, particularly in the concurrent treatment modality. Being a complex setting, stage III NSCLC treatment cannot be separated from the involvement of a multidisciplinary team for the adequate identification and management of patients. Trials on the efficacy and safety of therapeutic strategies combined with radiotherapy, immunotherapy and chemotherapy for stage III disease are ongoing. Future studies should answer current questions about radiotherapy (timing, sequencing, dose and fractionation) and biomarkers in order to achieve better patient selection and more favorable survival outcomes.

Keywords: Combination treatment; Immunotherapy; Locally advanced stage III NSCLC; PD-1/PD-L1 inhibitors; Radio-immunotherapy; Radiotherapy; Synergy

1. Introduction

Lung cancer is the second most common cancer and the main cause of cancer death for both sexes [1]. NSCLC is the most common type of lung cancer, since includes about 85% of all lung cancers. Historically, the standard of care for patients with locally advanced unresectable NSCLC has been concurrent chemo-radiotherapy (CCRT) [2, 3]. However, outcomes remain poor, with 5-year survival rates of 15-20%. Immunotherapy is a relatively new therapeutic approach in thoracic oncology and is considered the "new weapon" for cancer treatment along with surgery, chemotherapy and radiotherapy. The purpose of this paper is to introduce the new therapeutic potential emerging from the research regarding the combinations of ionizing radiation with new immunotherapeutic drugs in order to obtain new possibilities of treatment in neoplasms such as lung cancer with a severe prognosis. A brief presentation will be provided below of the modern and complex radio-biological concepts that opened the research on the effect of ionizing radiation in promoting the activation of the immune system on neoplasms by creating conditions similar to an "in situ vaccination". Innovative studies using combinations of radiation therapy with immunotherapeutic agents in the treatment strategy of locally advanced NSCLC will also be presented.

2. Synergy between radiotherapy and immunotherapy

The main cellular target of ionizing radiation is DNA where single or double helix breaks occur with direct or indirect mechanisms [4]. The radio-induced effect on cells varies on the basis of the specificity of the cell population, the type of ionizing radiation used, and the mode of temporal delivery of the radiation [4, 5]. Radiation can awaken lymphocyte cells responsible for immunosurveillance. These biological evidences have stimulated the study of the efficacy of new combinations of radiotherapy and immunotherapy in order to obtain a new antitumor synergy. Historically, radiotherapy has been considered an immunosuppressive agent due to the cytocidal effect on cell populations responsible for immunosurveillance (T lymphocytes, dendritic cells (DCs), Treg cells) [6]. Recently, some surprising immune-mediated processes have been progressively clarified. It has been shown that tumor cell histotypes subjected to radio-induced death processes expose their own antigens on the cell surface activating DCs; DCs mature into antigen presenting cells (APCs) having a primary role in activating cytotoxic T cells responsible for immunogenic death [7]. The molecular mechanisms that, after exposure to radiation, activate DCs are represented by the extracellular release of calreticulin (endoplasmic reticulum protein), of the High Mobility Group Box B1 (HMGB) proteins, of cellular energy in the form of ATP and of heat shock proteins (HSP) [6]. These endogenous elements, through the Toll-like receptors (TLRs), activate the DCs that bind to naïve CD8+ lymphocytes and send stimulating signals to the cytotoxic T lymphocytes (CTLs) that induce the immunogenic death of the tumor cell [7]. CTLs can act on tumor cells not present in the irradiation site but located at a distance; the immunological cytocidal effect on micro or macroscopic metastases is called the "abscopal effect" [8]. The technological mode of delivery of the radiotherapy dose appears to influence the immune-mediated response: low doses of radiation (<1Gy) appear to primarily activate innate immunity cells that do not induce immunogenic cell death [8]; on the other hand, higher radiation doses (>2Gy) can induce processes of immune-mediated tumor cell death [9]. One of the

main combined modalities of radiotherapy and immunotherapy inhibits suppressive antitumor immunity receptors. The cytotoxic T-lymphocyte antigen 4 (CTLA-4) receptor has a regulatory role in maintaining tolerance towards endogenous intra-tissue antigens including cellular antigens. The CTLA-4 present on the membrane of the T cell competes with the CD28 receptor of the DC that presents the antigen, preventing the activation process of the CTLs. The CTLA-4 receptor itself is overexpressed in the Treg cell, enhancing its immunosuppressive action [10]. The use of anti-CTLA-4 drugs, such as ipilimumab, has opened the way to a wide field of research and the synergy of action of CTLA-4 receptor inhibiting drugs associated with radiotherapy has been proven in multiple clinical experiences that have legitimized the biological rationale (6). Another receptor with an inhibitory role is programmed death-1 (PD-1). Its overexpression prevents the expansion of CTLs after interaction with activated DCs. Drugs inhibiting the PD-1 receptor or its ligands such as PD-L1 (pembrolizumab, nivolumab, atezolizumab and durvalumab) can release the brake of the immune-mediated response and promote immunogenic cell death [7]. The CTLA-4 and PD-1 receptors therefore appear to be important targets to be neutralized with drugs in order to enhance immunity: ionizing radiations appear to enhance the action of drugs inhibiting the suppressive function of these receptors. If, on the one hand, the immunomodulation mechanisms of radiotherapy include the upregulation of the expression level of immunogenic cell markers such as intercellular adhesion molecule (ICAM)-1, major histocompatibility complex (MHC)-1 and first apoptosis signal (Fas), the induction of immunogenic cell death, the release of tumor antigens and cytokines (interferon (IFN), tumor necrosis factor (TNF)α, interleukin (IL)-1, IL-6 etc.) as well as the enhanced homing of immune cells in the tumor (11-19), on the other hand radiotherapy can also induce immunosuppressive effects involving the increased release of negative regulatory cytokines such as transforming growth factor (TGF)-β, the accumulation of radioresistant suppressor cells and the upregulation of PD-L1 expression [20-27]. The integration of PD-1/PD-L1 inhibitors with radiotherapy could not only enhance positive immunoregulation but also attenuate negative immune resistance, thus achieving better antitumor immunity.

3. The radio-immunotherapy combination: clinical evidence and ongoing studies

Based on the biological mechanisms illustrated so far and the data emerging from preclinical studies, an attempt was made to assess whether these results could be transferred to the clinic environment. In reality, the clinical studies concluded for the combination of radiotherapy and immunotherapy are currently very limited, although innovative.

3.1 Studies on the combination with PD-L1 inhibitors

Durvalumab is currently approved for the curative treatment of unresectable stage III NSCLC. A human monoclonal antibody directed against PD-L1, it blocks the interaction of PD-L1 with PD-1 and CD80, counteracting the immune evasion mechanisms implemented by the tumor and enabling the reactivation of the immune system. The PACIFIC study is a multicentre, randomized, double-blind, placebo-controlled, phase III practice-changing trial with durvalumab as the treatment of all-comer patients (unselected based on PD-L1 expression) with unresectable NSCLC stage III (locally advanced) whose disease has not progressed after

platinum-based CCRT (total radiotherapy dose between 54 and 66 Gy). The study involved 713 patients randomized to receive immunotherapy treatment with durvalumab or placebo (2:1) 10 mg/kg intravenous (IV) every 2 weeks between 1 and 42 days after the last dose of radiotherapy, up to a maximum of 12 months of treatment. The co-primary endpoints of the trial were progression-free survival (PFS) and overall survival (OS). At the first planned interim analysis, the PFS was significantly higher in durvalumab-treated patients compared to placebo recipients (median PFS: 16.8 versus 5.6 months, hazard ratio (HR) 0.52, 95% confidence interval (CI) 0.42-0.65, p = 0.001) [28]. After a median follow-up of 25.2 months, the OS in the general population was in favour of the durvalumab immunotherapy arm (median OS: not achieved versus 28.7 months, HR 0.68, 95% CI 0.53-0.87; survival rate at 2 years: 66.3% versus 55.6%, p = 0.005), as well as the updated PFS (HR 0.51, 95% CI 0.41-0.63, p = 0.001) [29]. A pre-planned subgroup analysis initially demonstrated a survival advantage in favour of durvalumab regardless of PD-L1 expression levels at the predetermined cut-off of 25%, measured on archival tissue, available in approximately 63% of patients: cut-off ≥ 25% (HR 0.46) and cut-off <25% (HR 0.92). Based on this data, durvalumab has been approved by the Food and Drug Administration (FDA) as a consolidation therapy in patients with unresectable stage III NSCLC, in response to or stable disease following radically dosed chemo-radiotherapy, regardless of the tumor expression levels of PD-L1. However, a subsequent unplanned exploratory post-hoc analysis, requested by the European regulatory authorities, aimed at exploring the predictive role of PD-L1 at a different cut-off than the pre-established one (cut-off: 1% versus 25%), highlighted a significant correlation between PD-L1 expression levels and survival benefit in patients treated with durvalumab: ≥1% cut-off (HR 0.53) and cut-off <1% (HR 1.36). Based on this evidence, durvalumab was approved by the European Medicines Agency (EMA) as consolidation therapy after radical intent CCRT in stage III patients with ≥1% PD-L1 expression. A 4-year update of the study was recently presented, confirming a clinically relevant and long-lasting survival benefit in durvalumab-treated patients with a median OS of 47.5 months versus 29.1 months in the control arm (HR 0.71, 95 % CI 0.57-0.88) and a PFS of 35.3% versus 19.5%, respectively. Survival update based on PD-L1 expression confirms a significant benefit limited to the subgroup of patients with PD-L1 ≥1%, although the immunohistochemical expression of this biomarker was evaluated only in 63% of patients included in the study [30].

As part of a sequential treatment, durvalumab is being studied in a number of other clinical trials. The single-arm phase II PACIFIC 6 (NCT03693300) trial uses durvalumab (1500 mg IV every 4 weeks) after sequential chemotherapy and radiotherapy (60 Gy/30 fx) in patients with unresectable stage III NSCLC up to a maximum of 24 months of treatment. The randomized phase III PACIFIC 5 study (NCT03706690) uses durvalumab (1500 mg IV every 4 weeks) after chemo-radiotherapy in the same patient setting until radiological or clinical disease progression. The single-arm phase II NCT03589547 study investigates the combination of durvalumab 10 mg/kg IV every 2 weeks and stereotactic body radiation therapy (SBRT) after chemo-radiotherapy in patients with stage III NSCLC. In this study, the radiotherapy dose of 60 Gy will be followed by 20 Gy/2-3 fx of SBRT in combination with durvalumab as consolidation therapy. A study of a different nature is the multicentre, randomized, double-blind,

placebo-controlled phase III PACIFIC 2 trial (NCT03519971), which assesses whether durvalumab (1500 mg IV every 4 weeks) concomitant with chemo-radiotherapy provides an additional benefit in terms of PFS and objective response rate (ORR), compared with chemo-radiotherapy alone. The patient setting is the same as in the PACIFIC study but with a different radiotherapy and immunotherapy timing and with immunotherapy also provided as consolidation until disease progression in patients showing response or disease stability after concomitant treatment [31]. This study is limited by the fact that the comparator arm does not use the current standard of care (CCRT and durvalumab consolidation). The randomized phase III ECOG-ACRIN 5181 (NCT04092283) study will compare the current standard of care with the addition of concomitant durvalumab (750mg IV every 2 weeks) compared to chemo-radiotherapy followed by consolidation durvalumab. Durvalumab with platinum doublet concurrent chemo-radiation is still being studied in the phase I CLOVER (NCT03509012) trial in patients with unresectable stage III NSCLC.

Some trials are exploring whether platinum-based chemotherapy can be replaced by immunotherapy in locally advanced NSCLC. The single-arm phase I ARCHON-1 study (NCT03801902) is currently recruiting patients with PD-L1 expression ≥50% to be treated with definitive thoracic radiotherapy (60 Gy in 30 or 15 fractions) and concomitant durvalumab. In the phase I study CASE1518 (NCT03818776) radio-immunotherapy with durvalumab is being evaluated in patients unsuitable for chemo-radiotherapy. Others studies are testing neoadjuvant strategies in resectable locally advanced NSCLC. The CHIO3 (NCT04062708) study is a single-arm phase II trial for resectable stage IIIA/B NSCLC that combines pre-surgery chemotherapy with durvalumab followed by postoperative radiotherapy (54 Gy) and adjuvant durvalumab for 13 cycles. The phase II NCT03871153 study evaluates CCRT plus durvalumab followed by surgery and adjuvant durvalumab for 6 cycles in patients with resectable stage III NSCLC and N2 lymph node involvement. In stage IIIA N2 patients, durvalumab as a neoadjuvant and adjuvant treatment is being studied in the single-arm phase II trial NCT02572843, which provides conventional radiotherapy in cases of incomplete R1/R2 surgical resection. A phase II study, NCT03237377, will look at the effects of radio-immunotherapy (45 Gy/25 fx) neoadjuvant (durvalumab or durvalumab plus the CTLA-4 tremelimumab inhibitor) for resectable stage IIIA NSCLC. Patients will receive standard adjuvant chemotherapy if indicated. In studies of this type it will be possible to obtain tumor samples before and after radio-immunotherapy treatment, which can be used for correlative studies.

Atezolizumab is a humanized monoclonal antibody directed against PD-L1. DETERRED is a non-randomized phase II study for unresectable stage III NSCLC and involves two treatment groups: group 1 with chemo-radiotherapy (60-66 Gy) with carboplatin/paclitaxel followed by adjuvant atezolizumab and group 2 with chemo-radiotherapy and concomitant and then adjuvant atezolizumab in 10 and 30 patients, respectively. Atezolizumab is administered at 1200 mg IV every 3 weeks. According to a first analysis, the 1-year PFS is 50% and the OS is 79% in group 1 (median follow-up time of 22.5 months and 27.4 months for survivors); 1-year PFS is 57% and OS is 79% in group 2 (median follow-up time of 11.8 months and 13.7 months for survivors). PD-L1 status at baseline

tumor biopsy evaluable for 34 patients showed no significant difference in cancer recurrence for PD-L1 <1% (7/16 = 44%) versus ≥1% (6/18 = 33%) or for the cut-off of PD-L1 <50% (11/26 = 42%) versus ≥50% (2/8 = 25%) [32]. AFT-16 (NCT03102242) is a phase II single-arm study with induction atezolizumab (1200 mg IV every 3 weeks) followed by chemo-radiotherapy (60 Gy) then adjuvant atezolizumab for up to 1 year for the treatment of unresectable stage IIIA/B NSCLC. Table 1 summarizes current PD-L1 inhibitors and radiation clinical trials for locally advanced NSCLC.

3.2 Studies on the combination with PD-1 inhibitors

Pembrolizumab is a humanized monoclonal antibody that binds to the PD-1 receptor preventing its negative regulation of T cell activity. Further support for the combination of immunotherapy and definitive treatment of locally advanced NSCLC comes from the single-arm phase II study HCRN LUN14-179, which examines pembrolizumab (200 mg IV every 3 weeks) as consolidation immunotherapy for up to 1 year for the treatment of unresectable stage III NSCLC that has not progressed after CCRT (59.4-66.6 Gy). The median follow-up for 93 patients was 32.2 months with a median time to metastatic disease or death of 30.7 months (95% CI 18.7 months - not reached). The median PFS was 18.7 months (95% CI 12.4-33.8 months) and the median OS 35.8 months (95% CI 24.2 months - not reached). The 1-2 and 3-year OS estimates were 81.2%, 62%, and 48.5%, respectively (33). NCT03053856 is a single-arm phase II study with adjuvant pembrolizumab in patients with N2 positive NSCLC treated with neoadjuvant CCRT (44 Gy) followed by surgery.

KEYNOTE-799 is a non-randomized, open-label phase II study evaluating pembrolizumab (200 mg IV every 3 weeks) concomitant with chemo-radiotherapy (60 Gy/30 fx) in patients with unresectable stage III NSCLC. To conclude the study treatments, participants will receive 14 additional cycles of consolidation pembrolizumab. In cohort A (squamous and nonsquamous NSCLC) carboplatin/paclitaxel is the chemotherapy regimen of choice while in cohort B (nonsquamous NSCLC only) it is cisplatin/pemetrexed. As at January 2020, 112 and 73 patients were enrolled in cohorts A and B, respectively. Median follow-up was 8.3 months (0.7-14) in cohort A and 5.8 months (0.2-13.7) in cohort B. ORR (90% CI) was 67% (58.9-74.3%) in cohort A and 56.6% (44.4-68.2%) in cohort B. The 6-month (Kaplan-Meier estimate) PFS and OS rates were 81.4% and 87.2% and 85.2% and 94.8% in cohorts A and B, respectively. Enrollment is complete for cohort A and ongoing for cohort B [34]. SPRINT (NCT03523702) is a phase II trial that studies the combination of radiotherapy and pembrolizumab in patients with locally advanced disease and high PD-L1 expression (≥50%). Patients with PD-L1 expression <50% will be enrolled and treated with standard concurrent chemo-radiotherapy. Both pembrolizumab and durvalumab are used in the randomized phase III KEYLYNK-012 study comparing pembrolizumab plus chemo-radiotherapy (60 Gy) followed by pembrolizumab with or without the poly (ADP-ribose) polymerase (PARP) inhibitor olaparib versus chemo- radiotherapy followed by durvalumab in patients with unresectable stage III NSCLC. PFS and OS are the two primary endpoints. The study is currently recruiting patients [35]. In the phase I study CASE4516 (NCT02987998), 20 patients with resectable stage IIIA NSCLC will receive neoadjuvant chemo-radiotherapy (45 Gy) along with pembrolizumab and subsequent surgery, followed by consolidation pembrolizumab.

The PD-1 inhibitor nivolumab is being investigated by two phase II studies: one is ETOP NICOLAS, a safety study of nivolumab (360 mg IV every 3 weeks) combined with chemo-radiotherapy (66 Gy) and subsequent consolidation nivolumab (480 mg IV every 4 weeks) for up to 1 year. 21 patients with unresectable stage IIIA/B NSCLC received this treatment and, after the first safety analysis, a total of 80 patients were enrolled. The 1-year OS rate was 79% (95% CI 68-87%) while the median OS was not reached [36]. The other study is BTCRC LUN 16-081 (NCT03285321) with nivolumab or nivolumab/ipilimumab as consolidation treatment after chemo-radiotherapy (59.4-66.6 Gy) for unresectable stage IIIA/B NSCLC. The study is interesting because the safety of consolidation nivolumab/ipilimumab after chemo-radiotherapy has not been previously assessed. Furthermore, nivolumab is being evaluated as a consolidation treatment after chemo-radiotherapy (60 Gy) plus ipilimumab in the phase I/II study NCT03663166 for patients with unresectable stage III NSCLC. Phase III studies include RTOG 3505 (NCT02768558) which evaluates consolidative nivolumab against observation after 60 Gy of radiotherapy given concurrently with cisplatin-etoposide chemotherapy. The randomized phase III CheckMate73L trial, undergoing enrollment, compares nivolumab (360 mg every 3 weeks) plus CCRT followed by nivolumab (360 mg every 3 weeks in combination or 480 mg every 4 weeks alone) ± ipilimumab (1 mg/Kg every 6 weeks) versus chemo-radiotherapy followed by durvalumab (10 mg/kg every 2 weeks) for locally advanced stage III NSCLC. 888 patients will be randomized and the primary evaluated endpoints will be PFS and OS (nivolumab plus ipilimumab arm versus durvalumab for both endpoints) [37]. As for other immunotherapeutic agents, a phase I study (NCT04013542) using radio-immunotherapy without chemotherapy in patients with stage II/III NSCLC is underway for nivolumab and specifically for nivolumab/ipilimumab. Table 2 shows the clinical trials with PD-1 inhibitors and radiotherapy for locally advanced NSCLC described so far.

Study	Phase	Stage	RT Dose (Gy)	ICI Agent	Experimental Arm (ICI Sequence)	Status	Results
NCT02125461 PACIFIC	III	Unresectable stage III	54-66	Durvalumab	Consolidation	Active, not recruiting	PFS (stratified HR 0.55, 95% CI 0.44–0.67; median 17.2 vs 5.6 months) OS (stratified HR 0.71, 95% CI 0.57–0.88; median 47.5 vs 29.1 months)
NCT03706690 PACIFIC 5	III	Unresectable stage III	Conventional RT	Durvalumab	Consolidation	Recruiting	-
NCT03693300 PACIFIC 6	II	Unresectable stage III	Conventional RT	Durvalumab	Consolidation	Recruiting	-
NCT03589547	II	Stage III	60 Gy→20 Gy/2-3 fx SBRT	Durvalumab	Durvalumab and consolidation SBRT following chemoradiation	Recruiting	-
NCT03519971 PACIFIC 2	III	Unresectable stage III	Conventional RT	Durvalumab	Concurrent and consolidation	Active, not recruiting	-
NCT04092283 ECOG-ACRIN 5181	III	Unresectable stage III	Conventional RT	Durvalumab	Concurrent and consolidation	Recruiting	-
NCT03509012 CLOVER	I	Unresectable stage III	Conventional RT	Durvalumab	Concurrent	Active, not recruiting	-
NCT03801902 ARCHON-1	I	Unresectable stage II-III with PD-L1 ≥50%	Conventional or hypofractio-nated RT	Durvalumab	Concurrent	Recruiting	-
NCT03818776 CASE1518	I	Unresectable stage II-III	Proton beam therapy RT	Durvalumab	Concurrent	Recruiting	-
NCT04062708 CHIO3	II	Resectable stage IIIA/B	54	Durvalumab	Neoadjuvant and adjuvant	Not yet recruiting	-
NCT03871153	II	Resectable Stage III (N2)	45-61.2 Gy/25-34 fx	Durvalumab	Neoadjuvant and adjuvant	Recruiting	-
NCT02572843	II	Resectable stage IIIA (N2)	Conventional RT if R1-2	Durvalumab	Neoadjuvant and adjuvant	Active, not recruiting	-
NCT03237377	II	Resectable stage IIIA	45 Gy/25 fx	Durvalumab ± tremelimumab	Neoadjuvant	Recruiting	-
NCT02525757 DETERRED	II	Unresectable stage II-III	60-66	Atezolizumab	Consolidation ± concurrent	Active, not recruiting	1-year PFS (57% and 50%) 1-year OS (79% and 79%)
NCT03102242 AFT-16	II	Unresectable IIIA/B	60 Gy/30 fx	Atezolizumab	Neoadjuvant and adjuvant	Active, not recruiting	-

NCT04513925 SKYSCRAPER-03	III	Unresectable stage III	54-66	Atezolizumab + tiragolumab	Consolidation	Recruiting	-
NCT03822351 COAST	II	Unresectable stage III	Conventional RT	Durvalumab ± oleclumab or monalizumab	Consolidation	Active, not recruiting	-
NCT03840902 INTR@PID LUNG 005	II	Unresectable stage III	IMRT	M7824	Concurrent and consolidation	Recruiting	-

Table 1: Current PD-L1 inhibitors and radiation therapy clinical trials for locally advanced NSCLC.

Fx: fractions; Gy: Gray; ICI: immune checkpoint inhibitor; IMRT: intensity modulated radiation therapy; NCT: national clinical trial; OS: overall survival; PD-L1: programmed death-ligand 1; PFS: progression-free survival; RT: radiotherapy; SBRT: stereotactic body radiation therapy.

Study	**Phase**	**Stage**	**RT Dose (Gy)**	**ICI Agent**	**Experimental Arm (ICI Sequence)**	**Status**	**Results**
NCT02343952 HCRN LUN14-179	II	Unresectable stage IIIA/B	59.4-66	Pembrolizumab	Consolidation	Active, not recruiting	PFS (95% CI 12.4–33.8; median 18.7 months) OS (95% CI 24.2 months to not reached; median 35.8 months)
NCT03053856	II	Resectable stage IIIA (N2)	44 Gy/22 fx	Pembrolizumab	Adjuvant	Not yet recruiting	-
NCT03631784 KEYNOTE-799	II	Unresectable stage III	60 Gy/30 fx	Pembrolizumab	Concurrent and consolidation	Active, not recruiting	6-months PFS (81.4% cohort A and 85.2% cohort B) 6-months OS (87.2% cohort A and 94.8% cohort B)
NCT02987998 CASE4516	I	Resectable stage IIIA	45 Gy/25 fx	Pembrolizumab	Neoadjuvant and adjuvant	Active, not recruiting	-
NCT03523702 SPRINT	II	Unresectable stage II-III with PD-L1 ≥50%	Accelerated, dose-painted RT	Pembrolizumab	Sequential	Recruiting	-
NCT04380636 KEYLYNK-012	III	Unresectable stage III	60 Gy over 6 weeks	Pembrolizumab ± olaparib	Concurrent and consolidation	Recruiting	-
NCT02434081	II	Unresectable	66	Nivolumab	Concurrent and	Completed	1-year PFS

ETOP NICOLAS		stage IIIA/B			consolidation		(54%) 1-year OS (79%)
NCT03285321 BTCRC LUN 16-081	II	Unresectable stage IIIA/B	59.4-66.6	Nivolumab ± ipilimumab	Consolidation	Recruiting	-
NCT03663166	I/II	Unresectable stage III	60 Gy/30 fx	Ipilimumab vs nivolumab	Concurrent vs consolidation	Recruiting	-
NCT02768558 RTOG 3505	III	Unresectable IIIA/B	60 Gy/30 fx	Nivolumab	Consolidation	Recruiting	-
NCT04026412 CheckMate73L	III	Unresectable stage III	Conventional RT	Nivolumab ± ipilimumab	Concurrent and consolidation	Recruiting	-
NCT04013542	I	Unresectable stage II-III	60 Gy over 6 weeks	Nivolumab + ipilimumab → nivolumab	Concurrent and consolidation	Active, not recruiting	-

Table 2: Current PD-1 inhibitors and radiation therapy clinical trials for locally advanced NSCLC.

Fx: fractions; Gy: Gray; ICI: immune checkpoint inhibitor; NCT: national clinical trial; OS: overall survival; PD-L1: programmed death-ligand 1; PFS: progression-free survival; RT: radiotherapy.

Study	**Phase**	**Stage**	**RT Dose (Gy)**	**ICI Agent**	**Experimental Arm (ICI Sequence)**	**Status**	**Results**
NCT03999710 DART	I/II	Unresectable stage III non suitable for CCRT	60 Gy/30 fx	Durvalumab	Concurrent and consolidation	Recruiting	-
JMA-IIA00434 SPIRAL-RT	II	Unresectable stage III non suitable for CCRT	54-66	Durvalumab	Consolidation	Active	-
JRCTs031190070 NEJ039A	II	Unresectable stage III in PS 2 and/or > 75 years old patients	60 Gy/30 fx	Durvalumab	Consolidation	In progress	-
NCT04351256 TRADE-hypo	II	Unresectable stage III in elderly and/or frail patients	Conventional or hypofractionated RT	Durvalumab	Concurrent	Recruiting	-

Table 3: Current ICIs and radiation therapy clinical trials for locally advanced NSCLC unsuitable for standard CCRT.

CCRT: concurrent chemo-radiotherapy; fx: fractions; Gy: Gray; ICI: immune checkpoint inhibitor; JMA: Japan Medical Association; JRCT: Japan Registry of Clinical Trials; NCT: National Clinical Trial; PS: performance status; RT: radiotherapy.

4. Safety

The issues surrounding the safety and toxicity of concomitant radio-immunotherapy constitute a relevant consideration and will be addressed in many of the studies reported above. To date, little is known about the toxicity of the combination of radiotherapy and immunotherapy, especially with regard to pneumonitis. It should not be forgotten that the new immunotherapics have side effects in the clinic that are closely linked to the mechanism with which they activate the immune system, causing autoimmune effects. These are increased by the effects of radiotherapy; hence the concern that the two types of treatment, given in close sequence, may increase the risk of toxicity such as pneumonitis compared to when administered separately [38].

Data from available sequential radiotherapy and immunotherapy studies to date have shown similar rates of grade 3-5 toxicity. In the phase I KEYNOTE-001 study in the metastatic setting (melanoma or NSCLC) to evaluate whether patients receiving radiotherapy and pembrolizumab had different outcomes than those receiving pembrolizumab alone, 63% of 24 patients treated with previous thoracic radiotherapy had toxicity pulmonary (versus 40% in patients without prior radiotherapy). There were no differences between the two groups in patients with grade ≥3 pulmonary toxicity [39]. Similarly, in the PACIFIC trial, although rates of pneumonitis were higher in the durvalumab cohort (33.9% versus 24.8%), there was no difference in grade 3-4 pneumonitis rates (3.4% versus 2.6%, no p value) [28]. In the NICOLAS trial, with reference to pulmonary toxicity, the percentage of pneumonitis of each grade was 42.5% with 10% of them grade ≥3 [40]. In the DETERRED and KEYNOTE-799 trials, grade ≥2 pneumonitis occurred in 10% (group 1) and 16% (group 2) in the atezolizumab study and grade ≥3 pneumonitis in 8% and 5.5% in cohorts A and B of the pembrolizumab study [41, 34].

A multi-center safety and toxicity analysis showed that grade ≥3 subacute adverse effects in patients with pulmonary SBRT and concomitant immunotherapy or with SBRT alone were 26.8% and 2.9%, respectively. The risks of all grade pneumonitis were similar in the two groups (33.9% versus 27.9%, $p = 0.47$) with a significant difference in grade ≥3 pneumonitis (10.7% versus 0%, $p<0.01$) [42]. In a work by Balasubramanian et al., concomitant versus sequential immunotherapy is associated with similar rates of pneumonitis in stage III NSCLC. The authors observed no differences between PD-1 or PD-L1 inhibitors regardless of whether treatment was given concomitantly or sequentially to chemo-radiotherapy. In the cited systematic review, 12 studies were identified (8 with immunotherapy after chemo-radiotherapy, 2 with concomitant immunotherapy and 2 with both approaches). Pooled rates of grade ≥3 pneumonitis were 8.3% (95% CI 5.6-12.1%) in the concomitant regimen versus 7% (95% CI 4-11.9%) in the sequential regimen. Rates of grade ≥3 pneumonitis were similar between patients receiving PD-L1 inhibitors (6.1%, 95% CI 2.9-12.4%) or PD-1 inhibitors (8.7%, 95% CI 6.3-11.9%) (43). However, the toxicity rates associated with concomitant radio-immunotherapy have not been fully investigated and this topic remains an area of active interest [44].

Several published trials have shown that the occurrence of immune-related adverse events (irAEs) may be associated with substantially improved ORR, PFS and OS in NSCLC patients treated with PD-1/PD-L1 inhibitors alone [45-47]. This predisposition is also found in combination treatment. In a

retrospective analysis of 201 patients treated with nivolumab combined with prior thoracic radiotherapy, improved PFS and lower rates of disease progression occurred in patients with a history of treatment-related pneumonitis compared to those without such a history (3.6 versus 2.3 months, p = 0.023; 29.4% versus 47.9%, p = 0.059) [48]. Similarly, Hwang et al. demonstrated that patients with grade ≥2 irAEs have superior survival benefits [45]. It could be speculated that the development of irAEs is related to an overactive immune response, which partially indicates that the combined treatment evokes potent antitumor immunity and therefore the irAEs are not only related to toxicity overlap but have a predictive role in improved PFS and OS. This aspect remains controversial and some large retrospective studies have failed to demonstrate a relationship between irAEs and clinical benefits [49, 50]. In addition, a proportion of patients with severe irAEs may even die. On the other hand, discontinuation of immunotherapy upon the onset of severe irAEs may influence therapeutic efficacy [28]. According to the current standard of treatment, PD-1/PD-L1 inhibitors should be discontinued for grade ≥3 irAEs (51). Uncertainty remains as to whether immunotherapy can be resumed after recovery from an irAE. If not, a better therapeutic alternative needs to be explored. More robust randomized clinical trials with longer follow-up are needed to gain a comprehensive understanding of the toxicities of combination treatments.

The single-arm phase II DART study (NCT03999710) is currently enrolling patients with locally advanced stage II/III NSCLC who are not eligible for CCRT (due to medical comorbidities, neuropathy, renal dysfunction, etc.) but who will be treated with radiotherapy (60 Gy/30 fx) and concomitant durvalumab, followed by 1 year of consolidation durvalumab. Durvalumab after radiotherapy is being studied in a single-arm phase II trial (SPIRAL-RT) in which patients with stage III NSCLC who are ineligible for chemo-radiotherapy will receive the drug after undergoing conventional radiotherapy alone [52]. A trial in progress is NEJ039A, a phase II study of daily carboplatin plus radiotherapy followed by maintenance durvalumab for patients with stage III NSCLC and performance status (PS) 2 or age >75 years [53]. For elderly and frail patients with unresectable stage III NSCLC unfit for chemotherapy, the phase II TRADE-hypo study speculates that durvalumab in combination with hypofractionated radiotherapy is safe and effective given the feasibility and activity of this regimen have been demonstrated in combination with chemotherapy in stage III patients [54, 55]. Taking into account the concern of a cumulative risk of severe pneumonitis resulting from the application of both thoracic radiotherapy and immunotherapy, the TRADE-hypo study will examine two regimens of conventionally fractionated and hypofractionated radiotherapy in combination with concomitant durvalumab [56]. The aim of the study is to provide an additional and optimized therapeutic option for a potentially undertreated cohort of patients.

5. Beyond chemo-radiotherapy in locally advanced NSCLC

The complex management of locally advanced lung neoplasms and the need for multimodal therapies require these cases to be managed in all therapeutic decision-making phases by a multidisciplinary team and, where possible, also in the context of clinical trials. Stage III NSCLC represents a heterogeneous group of patients with variable prognosis. For locally advanced resectable NSCLC (20-30% of patients)

[57], surgery is the primary curative treatment, which is generally accompanied by neoadjuvant and/or adjuvant chemotherapy and radiotherapy, resulting in 5-year OS rates of 50 -70% [58]. However, in more advanced cases, surgery is rarely feasible and definitive chemo-radiotherapy is the standard of care [59], with 5-year OS rates of approximately 15-20% [60]. An updated meta-analysis of 6 prospective randomized studies (1024 patients with locally advanced unresectable disease) compared CCRT with the sequential modality [61]. There was a 13% difference in the 2-year risk of death in the two groups. For the survival analysis, the study by Curran et al. was excluded, as only the abstract is available and published. A significant benefit of concomitant treatment was reported in terms of both OS (HR 0.74, 95% CI 0.62-0.89; 702 patients) and 2-year survival (RR 0.87, 95% CI 0.78-0.97; 937 patients). Although not statistically significant, an increase in treatment-related mortality (4% versus 2%) was confirmed, as well as an increased incidence of severe esophagitis (RR 4.96, 95% CI 2.17-11.37; 947 patients) to the disadvantage of the concomitant arm. Another meta-analysis [3] compared concomitant versus sequential treatment strategy in this patient setting. In this revision, 6 trials and 1205 patients were included, and a meta-analysis was conducted on individual data to better assess the effect on OS and acute toxicity, with a mean follow-up time of approximately 6 years. Concomitant treatment is associated with improved survival (HR 0.84, 95% CI 0.74-0.95, p = 0.004) with an absolute benefit of 5.7% at 3 years and 4.5% at 5 years. It is also highlighted that the effect of concomitant treatment is mainly expressed in the local control of the disease (HR 0.77, 95% CI 0.62-0.95, p = 0.01), while there are no differences in the two groups regarding distant progression. The most relevant toxicity in the concomitant arm is grade 3-4 acute esophagitis, reported in 4-18% of cases. The studies included in the meta-analysis [61] show a moderate overall risk of selection and attrition bias, which could affect the confidence in the results obtained. Regarding the assessment of the risk of bias, a complete analysis of selection bias is not possible in three of the studies included in the meta-analysis [3]. Furthermore, the study by Furuse et al. had been excluded from the meta-analysis due to a slight discrepancy in the radiotherapy treatment program, while that of Ulutin et al. was likewise excluded because it was not randomized and with a limited number. The number of trials and patients included in the analysis, together with the reported survival benefit, support a clinical benefit in favour of concomitant treatment. Prior to the approval of durvalumab, chemo-radiotherapy was the only treatment option available for patients with unresectable stage III NSCLC for decades. The 4-year update of the PACIFIC study results demonstrating the prolonged and clinically significant benefit of OS and PFS in this complex setting of patients treated with durvalumab after CCRT [30], confirms the possibility of pursuing a curative intent in this desease setting and the importance of strong collaboration between various specialists. Of course, clinical trials with concomitant and/or adjuvant immunotherapy in which the comparator arm is the treatment under the PACIFIC regimen are ongoing (Table 1). The phase II INTR@PID LUNG 005 trial (NCT03840902) uses the anti-TGF-β and anti-PD-L1 agent M7824 (1200 mg every 2 weeks) according to a strategy of concomitant plus adjuvant versus chemo-radiotherapy plus placebo followed by durvalumab (10 mg/kg IV every 2 weeks). The phase II COAST trial (NCT03822351) evaluates durvalumab alone or with oleclumab (monoclonal anti-CD73 antibody

inhibiting the production of immunosuppressive adenosine) or monalizumab (anti-CD94/NK group 2 member A (NKG2A) monoclonal antibody, inhibitory checkpoint receptor for human leukocyte antigens (HLA)-E) in patients who have not previously progressed following CCRT. The phase III SKYSCRAPER-03 trial (NCT04513925) is comparing the anti-T cell immunoglobulin and ITIM domain (TIGIT) antibody tiragolumab (840 mg IV every 4 weeks) plus atezolizumab (1680 mg every 4 weeks) with durvalumab (10 mg/kg IV every 2 weeks) in patients with locally advanced, unresectable stage III NSCLC who have not progressed after CCRT. Also in the phase III studies CheckMate73L, KEYLYNK-012 and ECOG-ACRIN 5181 already mentioned above, new combined immunotherapy strategies versus the PACIFIC regimen are being investigated.

6. Radio-immunotherapy: challenges and directions

Experimental research has recently highlighted a radiobiological effect consisting in the potential of ionizing radiation to control the growth of a tumor mass through the induction of immunogenic cell death [6]. It has been shown that radiotherapy can promote the release of tumor antigens that induce maturation of DCs, cross-priming of CTLs and tumor lymphocyte recruitment, in this way immunologically converting “cold” tumors into “hot” tumors [62]. Consequently, poorly immunogenic tumors can overcome immunoescape and resistance to PD-1/PD-L1 inhibitors through the priming effects of radiotherapy [63]. Furthermore, the increased expression of PD-L1 induced by radiotherapy could make patients more susceptible to PD-1/PD-L1 inhibitors. The recent success of ICIs in metastatic NSCLC [64-66] has led to interest in moving ICIs into the curative setting and, after the PACIFIC trial, to examine their effects in conjunction with radiotherapy. A series of studies are now underway, for which the results are awaited. It must be considered that many aspects still need to be clarified in order to introduce these new strategic modalities into daily clinical practice. In particular, a factor to be evaluated is represented by the total dose of radiotherapy and its fractioning: the goal is to administer a dose of radiation sufficient not only to trigger an effective anti-inflammatory response but above all to activate a tumor-specific immune response by minimizing adverse events as much as possible. For this latter aspect, the tendency is to select sequential rather than concurrent combination schedules more often. Some publications have also suggested that sequencing may depend on the type of ICI [67]. Anti-PD-L1 therapy appears to be more effective when administered concurrently with radiotherapy [22], while anti-CTLA-4 therapy appears to have better synergy when administered earlier [68]. These differences could be explained by the mode of action of the drugs, considering that CTLA-4 acts early in inducing tolerance and PD-1 acts late in maintaining long-term tolerance [69, 70]. Some of these uncertainties could be clarified by ongoing clinical trials such as SABRseq (NCT03307759), in which patients will be assigned to a stereotactic ablative radiotherapy (SABR) regimen followed by pembrolizumab or pembrolizumab followed by SABR. In addition, the NCT02400814 trial will divide patients into three treatment arms, in which atezolizumab will be administered before, after or concomitant with SABR (the two mentioned studies are conducted in patients with metastatic NSCLC). Regarding the optimal dose, as we have seen, several combination studies

use radiotherapy at conventional doses in multiple fractions although some are testing different modalities. To date, available SBRT regimens for early-stage NSCLC include 30-34 Gy x 1 fraction, 15-20 Gy x 3 fractions, 12 Gy x 4 fractions and 10-12 Gy x 5 fractions [71]. A randomized phase II study in stage I/II disease showed 30 Gy in 1 fraction to be equivalent to 60 Gy in 3 fractions in terms of toxicity, local control, PFS and OS [72]. Similarly, the phase II study RTOG 0915 has found that 34 Gy in 1 fraction and 48 Gy in 4 fractions achieved similar tumor control rates [73, 74]. In the retrospective study by Stephans et al., a SBRT dose of 54-60 Gy in 3 fractions was associated with a statistically significant lower rate of local failure compared to 30-34 Gy in 1 fraction, 48-50 Gy in 4-5 fractions and 50-60 Gy in 8-10 fractions [75]. Pulmonary toxicity was slightly higher with the 3 fractions than with the other regimens. In addition, limited data has shown that when combined with PD-1/PD-L1 inhibitors, SBRT doses range from 30 to 50 Gy in 3-5 fractions with acceptable toxicity [76]. The randomized phase II PEMBRO-RT study demonstrated that a SBRT dose of 8 Gy x 3 fractions could significantly potentiate the effects of pembrolizumab with improved ORR, PFS and OS [77]. It is clear that the discrepancy in optimal radiotherapy dose and fractioning can be partly attributed to different pathological types of tumor, tumor size and location, intrinsic radiosensitivity and host characteristics, all of which make it difficult to compare different studies and determine a standard regimen.

The other important question concerns the potential predictive biomarkers of response to combination therapy. Since the introduction of PD-1/PD-L1 inhibitors in cancer treatment, research has been looking for response predictors. Intratumor PD-L1 expression emerged as the first predictive biomarker [78] and, independently, tumors with high mutation burden have the potential to generate a larger number of neoantigens, making them more immunogenic [79]. In addition, mismatch repair (MMR) deficiency and microsatellite instability have shown some predictive power [80, 81] but also tumor-infiltrating lymphocytes (TILs), especially CTLs and cytokines, could potentially act as promising biomarkers [82, 83]. Regarding SBRT, the neutrophil-lymphocyte, platelet-lymphocyte and lymphocyte-monocyte ratios could play predictive roles in identifying patients for SBRT treatment [84]. Furthermore, some evidence suggests that high IFN-I/γ could influence radiotherapy efficacy and that lymphopenia could negatively impact the immunogenicity of radiotherapy [85-88]. The search for predictors responding to the radio-immunotherapy combination remains an unexplored field and it is plausible to think that a single biomarker is not enough to effectively predict the response to treatment [89]. Unresolved challenges remain (indefinite cut-off thresholds, different testing assays, unrepresentative biopsy samples, non-feasible biopsy repeats) and push to probe new approaches such as liquid biopsy on the levels of circulating tumor DNA (ctDNA) and other circulating molecules. In the PEMBRO-RT study of pembrolizumab after high dose radiation (SBRT) versus pembrolizumab alone in patients with advanced NSCLC, PD-L1 negative patients had a higher response rate to combined treatment than PD-L1 positive ones [77]. Statistically significant differences in OS were found only in the negative PD-L1 subgroup compared to the positive PD-L1 subgroup (HR 0.48, 95% CI 0.24-0.99, p = 0.046 and HR 1.4, 95% CI 0.42-4.66, p = 0.58 respectively) [77] indicating that PD-L1 negativity could be an effective biomarker for screening patients most

suitable for SBRT treatment combined with PD-1/PD-L1 inhibitors. A possible explanation is that negative PD-L1 expression can be converted to positive during treatment with SBRT with sensitizing effect on PD-1/PD-L1 inhibitors [22, 90]. Currently, there are no validated predictive biomarkers that can improve patient selection and outcomes and this is still an area to be fully investigated. Practical considerations to be addressed also include the optimal duration of immunotherapy when combined with radiotherapy, the safest dose to use in combination, and whether patients with autoimmune diseases, chronic viral infections or organ dysfunction should be evaluated more carefully to understand the resulting risks and benefits from combination treatments.

7. Conclusion

ICIs have greatly changed the scenario for patients with unresectable stage III NSCLC. The addition of consolidation durvalumab after definitive chemo-radiotherapy resulted in the first improvement in PFS and OS seen in many decades for these patients and sparked interest in examining concomitant radio-immunotherapy. Studies on the efficacy and safety of this combined therapeutic modality for stage III disease are ongoing and the results are expected to provide solid evidence and solve the challenges discussed above. More consolidated confirmations on large groups of patients are necessary in order to validate the biological rationale of these innovative radio-immunological strategies in the clinic. Furthermore, personalized therapy is essential to cope with the heterogeneity of locally advanced NSCLC and hypothetical differentiated strategies may be the right way to go. Among them, neoadjuvant immunotherapy (plus chemotherapy if large tumor), surgery ± radiotherapy and consolidation immunotherapy could be considered for patients with N2 disease with metastatic involvement of a single lymph node station. Neoadjuvant chemo-immunotherapy, concurrent radio-immunotherapy and consolidation immunotherapy could be indicated for large or N3 or bulky or N2 (with multiple lymph node stations involved) tumors. Lastly, concurrent radiotherapy with immunotherapy or chemotherapy and subsequent consolidation immunotherapy could be considered for small tumors with N2 lymph node involvement in multiple stations, respectively with high and low PD-L1 expression.

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