Clinical Trial Protocol

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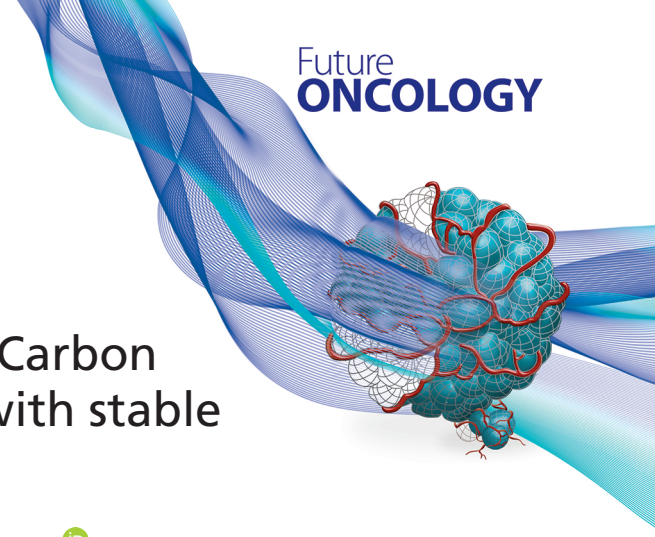

Immune checkpoint inhibitors and Carbon iON radiotherapy In solid Cancers with stable disease (ICONIC)

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ICONIC is a multicenter, open-label, nonrandomized phase II clinical trial aiming to assess the feasibility and clinical activity of the addition of carbon ion radiotherapy to immune checkpoint inhibitors in cancer patients who have obtained disease stability with pembrolizumab administered as per standard-of-care. The primary end point is objective response rate, and the secondary end points are safety, survival and disease control rate. Translational research is an exploratory aim. The planned sample size is 27 patients. The study combination will be considered worth investigating if at least four objective responses are observed. If the null hypothesis is rejected, ICONIC will be the first proof of concept of the feasibility and clinical activity of the addition of carbon ion radiotherapy to immune checkpoint inhibitors in oncology.

Plain language summary: ICONIC is a multicenter, open-label, nonrandomized, phase II clinical trial aiming to evaluate the feasibility and clinical activity of the addition of carbon ion radiotherapy to immune checkpoint inhibitors in cancer patients who have obtained disease stability with pembrolizumab administered as per standard-of-care. Considering that no clinical trials have been conducted thus far to assess the safety of the association between immune checkpoint inhibitors and carbon ion radiotherapy, the current clinical study will provide controlled data about the safety of this unprecedented therapeutic combination.

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Immune checkpoint inhibitors (ICIs) and Carbon iON radiotherapy In solid Cancers with stable disease (ICONIC) is a multicenter, open-label, nonrandomized phase II clinical trial aiming to assess the feasibility and clinical activity of the addition of carbon ion radiotherapy (CIRT) to ICIs in cancer patients who have obtained disease stability with pembrolizumab administered as per standard-of-care. ICONIC is the first clinical study conducted as a proof of concept of the feasibility and clinical activity of the addition of CIRT to ICIs in oncology.

Background & rationale

Immunotherapy has become the standard-of-care in different advanced malignancies. It is able to induce antitumor responses exploiting patients' immune system. Its effectiveness in the palliative setting has been demonstrated by several phase III trials [1]. Nevertheless, the response rate varies according to the cancer under study and the line of treatment. A potential way to improve the activity of single-agent ICIs is to enhance the clinical response with the use of further antitumor agents. This can be achieved through combination with other ICIs, chemotherapy or radiotherapy (RT). Activity related to the association of radiation and ICIs has been described in retrospective studies and explored in prospective trials [2]. Indeed, a significant milestone in oncology was progression-free survival (PFS) and overall survival (OS) improvement by adding durvalumab (an anti-PD-L1 agent) to chemoradiation in locally advanced, unresectable non-small-cell lung cancer (NSCLC) patients; these results suggested that the interaction between RT and ICIs is pivotal to improving response above the use of either immunotherapy or RT alone [3]. Furthermore, RT has the potential to induce tumor regression at nonirradiated, distant tumor sites – a phenomenon known as the 'abscopal effect' [4]. This means that potentially if a cancer patient has at least two disease sites, one of them could be irradiated, inducing immune-mediated antitumor activity, which might determine a dimensional reduction of non-irradiated other lesions. In other words, irradiating a metastatic lesion might induce a Response Evaluation Criteria in Solid Tumors (RECIST) 1.1 objective response in a different nonirradiated metastasis. In murine models, anti-CTLA-4 antibodies induce the abscopal effect when combined with fractionated photon beam RT; the same effect is not achieved when combined with an high single-dose RT [5]. Nevertheless, this phenomenon is rare, and the actual mechanisms are still unclear.

The ideal radiation able to induce an immunological boost should determine significant local damage, but avoiding both peritumoral tissues and draining lymph nodes and tumor-infiltrating lymphocytes. This goal can be reached with the most advanced radiation techniques, such as intensity-modulated RT or volumetric modulated arc therapy, whose ability to spare normal tissues is much higher than older methods (e.g., 3D or 2D RT). Moreover, evidence exists for the association between stereotactic body RT and immunotherapy [6].

Heavy particles, such as carbon ions and protons, have the ability to deliver high doses of energy, especially in target volumes (= tumor), with minimal post-transfer dose (Bragg peak). This advantageous dosimetric depth dose distribution is much different from the almost linear distribution typical of photons [7]. Among the charged particles, carbon ions may distinctly affect cell death pathways, leading to increased immunogenicity.

Post-CIRT immunogenic changes were evaluated in a human *in vitro* model [8]. Growth in the concentration of HMGB1, a crucial stakeholder in the immune response, was described in the culture supernatants of several cancer cell lines. Post-CIRT levels of HMGB1 were similar to iso-survival doses of x-rays, and the applied doses were consistent with those used in the clinic.

The higher linear energy transfer typical of particles induces clustered DNA lesions, which in turn elicit several DNA damage repairs [9]. dsDNA breaks lead to a complex biological pathway involving ATM kinase and to the upregulation of PD-L1 in the tumor microenvironment [10]. After extrusion of damaged dsDNA from nucleus to cytoplasm, the cGAS-STING pathway is activated and induction of an immune response mediated by IFN type I is thus elicited [11]. However, homeostasis is usually maintained because higher doses per fraction induce TREX, a DNA nuclease able to block the STING pathway, stopping activation of an IFN-mediated immune response [12]. Furthermore, anti-PD-L1 agents concomitant with photon beam RT reduce tumor infiltration of myeloid-derived suppressor cells and activate CD8+ T cells through TNF-α [13]. In this setting, preliminary *in vitro* studies showed an increased release of immune-stimulating cytokines after heavy ion exposure [14]. Recent literature reported that cytosolic DNA fuels an immunogenic response throughout micronuclei via cyclic guanosine monophosphate-adenosine monophosphate (cGMP-AMP) synthase/stimulator of interferon (IFN) gene pathway [15]. CIRT is more efficient in the production of micronuclei compared with fractionated photons at the same doses.

A further important actor in this context is the adenosine pathway. Adenosine is low in the interstitial fluid of unstressed tissue and is usually released during inflammation, trauma, hypoxia or ischemia [16]. ATP, the most relevant source of intracellular biological energy, is dephosphorylated into ADP by the CD39 enzyme, which dephosphorylates ADP into AMP as well. AMP is further dephosphorylated into adenosine, which can be deaminated by adenosine deaminase. Since adenosine is actively increased as a result of specific genetic alterations that occur during tumor progression, an *in vivo* study of the actors in this pathway might reveal a proimmunogenic antitumor microenvironment in patients responding to the association of hadrontherapy, especially carbon ions, and ICIs. As a proof-of-concept, soluble CD73, which catalyzes the dephosphorylation of AMP into adenosine, is associated with poor outcomes in terms of survival in metastatic melanoma patients treated with the anti-PD-1 agent nivolumab [17].

Furthermore, it is known that autophagy strengthens the processing and presentation of tumor antigens, arousing antitumor immunity. Although cancer cells can elude immunosurveillance, reducing autophagy, some treatments induce autophagic cell death, enhancing antitumor immunity. High linear energy transfer carbon ions have proved to be effective in inducing autophagy in various tumor cells in a dose- and linear energy transfer-dependent way by depressing the PI3K-Akt pathway [18–21].

There are uncertainties regarding which doses and fractionation schemes are more immunogenic, but preclinical studies, both *in vitro* and *in vivo*, seem to show a greater benefit in stimulating the immune system using a hypofractionated schedule. Vanpouille-Box *et al.* demonstrated that abscopal responses are not induced by high-dose radiation [12]. A total of 8 Gy delivered over 3 consecutive days was identified as an effective regimen. A single dose of up to 20 Gy was ineffective at causing T-cell-mediated rejection of an irradiated and synchronous nonirradiated mouse-derived poorly immunogenic mammary carcinoma cell line known as TSA with anti-CTLA-4. Furthermore, to note that the expression of IFN I-stimulated genes was upregulated by a treatment schedule of 8 Gy delivered over 3 consecutive days, but not with a single fraction of 20 Gy, and that this upregulation was a cancer cell-intrinsic response. Indeed, in a murine model of immune-resistant breast cancer, in the lack of tumor stroma, only virus infection and 8 Gy delivered over 3 consecutive days could induce the release of IFNb cytokines.

In addition, the timing between the administration of RT and immunotherapy seems to play an important role in achieving effective immune stimulation, which is strongly influenced by the mechanism of action of the immunotherapy. [22]. Nevertheless, as far as CIRT is concerned, only a few data are available *in vivo* regarding the association with ICIs, notably in an osteosarcoma mouse model [23]. This data are not definitive and not well defined. In a TSA breast cancer model, the highest abscopal effect was found when the first dose of CTLA-4 blockade was given during x-ray irradiation [5]. Golden *et al.* reported a clinical case of the abscopal effect in an NSCLC patient who underwent concurrent ipilimumab and photon beam RT [24]. In other animal models, Young *et al.* found that anti-CTLA-4 therapy was more effective when given after photon beam RT because of T-cell depletion [25]. Otherwise, immunological effects can be enhanced when anti-PD-1/PD-L1 therapy is administered concomitantly with or immediately after radiation [26].

It has been reported that the likelihood of inducing an abscopal effect varies among treatment targets. In a phase I trial of patients with metastatic solid malignancies who received stereotactic body RT with ipilimumab, targeting liver metastases resulted in greater activation of T cells compared with the use of RT to lung metastases [27]. However, any evidence in this setting is still contradictory [22].

The mathematical model by Poleszczuk *et al.* hypothesizes that an abscopal response might be obtained if an adequate number of T cells activated at the irradiated site (site of activation) arrive at each of the other tumor locations [28]. In addition to the physiological blood flow to the tissue, the traffic of the activated T cells will be caused by the starting imprinting of the T cells by tumor antigen-presenting dendritic cells, which award tissue tropism to the primed T cells. The limitations of the model have been fully discussed by Demaria and Formenti [29], and there is still a lot of uncertainty on this topic. Nonetheless, irradiating different organs, such as lymphatics, viscera, soft tissues and bones, has varying immunogenic potential [4,15].

Among possible predictive factors of the response to immunotherapy, baseline tumor burden was revealed to be an independent prognostic factor for PFS and OS in patients with metastatic renal cell carcinoma treated with target agents in prospective clinical trials [30]. However, a higher number of metastases did not prove to be associated with worse PFS. The main objective of the current study is to explore the feasibility and clinical activity of the addition of CIRT to ICIs in advanced metastatic malignancies in which immunotherapy is currently the standard-of-care.

Table 1. Frequency of grade ≥3 adverse events observed in pembrolizumab studies.

Cancer	Setting	Grade ≥3 AEs (%)	Ref.
Melanoma	First and second line	17	[31]
NSCLC	First line	26.6	[32]
	First line	18	[33]
HNSCC	First line	55	[34]
Urothelial	First line	15.7	[35]

AE: Adverse event; HNSCC: Head and neck squamous cell carcinoma; NSCLC: Non-small-cell lung cancer.

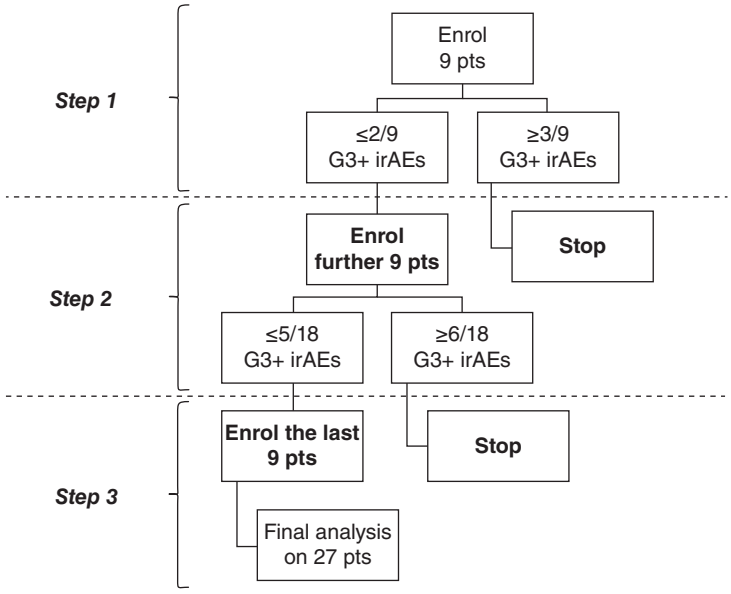

Figure 1. Safety run-in.
G3+ irAE: Grade ≥3 immune-related adverse event; Pt: Patient.

Materials & methods

Study design

This is a multicenter, open-label, nonrandomized, phase II clinical trial aiming to assess the feasibility and clinical activity of the addition of CIRT to pembrolizumab in advanced cancer patients who have obtained disease stability with single-agent pembrolizumab administered as per standard-of-care. To minimize the risk of toxicity in patients, a safety run-in phase will be conducted throughout the study.

The frequency of grade ≥3 treatment-related adverse events (AEs) varies according to the drug and disease under study. Table 1 reports the frequencies observed in clinical trials with pembrolizumab. Chemoimmunotherapy combinations are known to be related to higher frequencies of grade ≥3 treatment-related AEs. For this reason, to maximize patient safety, the reference for toxicity was measured considering first-line single-agent pembrolizumab anti-PD-1 immunotherapy (excluding combinations with chemotherapy or tyrosine kinase inhibitors and studies assessing treatment beyond progression, for which higher toxicity rates may have been expected). In these trials, the average frequency of grade ≥3 AEs was 26%.

The following steps will be followed. Nine patients will initially be enrolled. In the meantime, no further patients will be included. A disease assessment of these patients will be performed at least 8 weeks after the end of CIRT. If grade ≥3 immune-related AEs (irAEs) are observed in 33.3% (three of nine) of patients or more, the study will be stopped. If grade ≥3 irAEs are seen in 22.2% (two of nine) of patients or less, the study will be continued and a further nine patients will be enrolled. In the meantime, no further patients will be included. A disease assessment of these patients will be performed at least 8 weeks after the end of CIRT. If grade ≥3 irAEs are observed in 33.3% (six of 18) of patients or more, the study will be stopped. If grade ≥3 irAEs are seen in 27.8% (five of 18) of patients or less, the study will be continued and the remaining nine patients will be enrolled. A disease assessment will then be performed at least 8 weeks after the end of CIRT (Figure 1).

All patients will be followed up to 12 months after the end of CIRT. Follow-up visits will be performed by the referring medical oncologist according to standard-of-care procedures.

Eligibility criteria

General eligibility criteria for all cohorts

Inclusion criteria for the study are the following: signed written informed consent; histological confirmation of malignancy under treatment with single-agent anti-PD-1/PD-L1 immunotherapy per clinical practice (see cohort-specific inclusion criteria) with ICIs approved by Agenzia Italiana del Farmaco; disease stability as assessed by the Agenzia Italiana del Farmaco monitoring sheet; presence of at least two measurable target lesions (at least one that is to be followed up per RECIST and one that is suitable for CIRT); willing and able to comply with scheduled visits, treatment schedule, laboratory testing and other requirements of the study; 18 years of age or older (no upper limit); Eastern Cooperative Oncology Group performance status ≤2; measurable disease by CT scan or MRI per RECIST 1.1; and life expectancy >6 months.

Exclusion criteria for the study are the following: treatment with chemoimmunotherapy combinations; treatment with immunotherapy combinations (e.g., patients treated with anti-CTLA-4 + anti-PD-1/PD-L1 are excluded); receiving immunotherapy within clinical trials; receiving off-label immunotherapy or within expanded access programs or as compassionate use; high tumor burden (defined as more than ten lesions and/or sum of diameters >19 cm); distant metastases located in the CNS only; any serious or uncontrolled medical disorder that, in the opinion of the investigator, may increase the risk associated with study participation or study drug administration, impair the ability of the patient to receive protocol therapy or interfere with interpretation of the study results; autoimmune diseases, including local and systemic collagen vascular disease and inflammatory bowel disease; previous RT (regardless of energy) to the metastatic site selected for irradiation; any immune-related Common Terminology Criteria for Adverse Events grade 4 AE before study entry; any Common Terminology Criteria for Adverse Events grade ≥3 irAE observed within 3 weeks before start of CIRT; presence of metal prostheses or any other condition that would prevent adequate imaging for identification of the target volume and calculation of dose; and locoregional conditions not allowing hadrontherapy, such as active infections in RT target region or pre-irradiated lesions (CIRT will not be delivered to lesions previously treated with RT). In addition, prisoners or patients who are involuntarily incarcerated and patients who are compulsorily detained for treatment of a psychiatric or physical illness (e.g., infectious disease) are excluded from the study.

Cohort-specific inclusion criteria

Inclusion criteria for the melanoma cohort are pembrolizumab monotherapy, unresectable or metastatic melanoma and disease assessment (stable disease [SD] 12 weeks after treatment start or later). Inclusion criteria for the NSCLC cohort are pembrolizumab monotherapy, locally advanced or metastatic NSCLC with tumor proportion score ≥1% already treated with chemotherapy or untreated metastatic NSCLC with tumor proportion score ≥50% and disease assessment (SD 9 weeks after treatment start or later). Inclusion criteria for the head and neck squamous cell carcinoma cohort are pembrolizumab monotherapy, untreated recurrent/metastatic head and neck squamous cell carcinoma with combined positive score ≥1 and disease assessment (SD 9 weeks after treatment start or later). Inclusion criteria for the urothelial carcinoma cohort are pembrolizumab, locally advanced or metastatic urothelial carcinoma pretreated with platinum-based chemotherapy and disease assessment (SD 9 weeks after treatment start or later).

Planned sample size

After assessment of SD as the best response, the probability of achieving an objective response (either partial or complete response) to immunotherapy is deemed low (assumed <5%). Therefore, two options are possible during immunotherapy: progressive disease or SD. In case an objective response is observed after CIRT administration during immunotherapy maintenance in patients with SD, the combination of CIRT and immunotherapy could be deemed an active approach worthy of further investigation. The null hypothesis (p0) is the probability of achieving an objective response (either partial or complete response) after observing SD as the best response in patients receiving maintenance pembrolizumab as per clinical practice.

The alternative hypothesis (p1) is the probability of achieving an objective response (either partial or complete response) after observing SD as the best response in patients receiving CIRT followed by maintenance pembrolizumab as per clinical practice. Applying an A'Hern single-stage design for phase II clinical trials, assuming the parameters null hypothesis (p0) = 5%, alternative hypothesis (p1) = 20%, type I error (α) = 0.05 and power (1-β) = 0.8, a total of 27 patients should be enrolled. In this case, the combination of CIRT and pembrolizumab maintenance will be considered active and worth further investigation if at least four objective responses are observed.

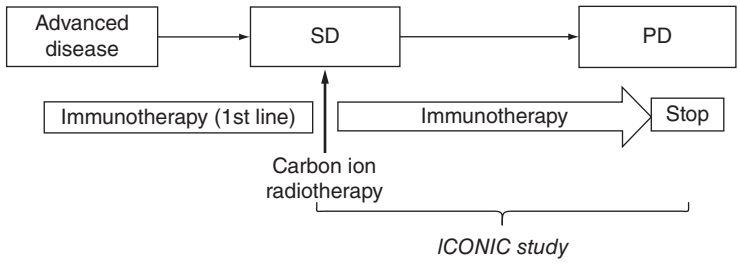

Figure 2. Study intervention.
ICONIC: Carbon iON radiotherapy In solid Cancers with stable disease; PD: Progressive disease; SD: Stable disease.

Study intervention

Immunotherapy

Only cancer patients under treatment with pembrolizumab monotherapy administered within clinical practice and according to the Italian Drug Agency (Agenzia Italiana del Farmaco) will be enrolled. If disease stability is observed as the best response at least 9–12 weeks from the start of treatment, patients can be included in the study as long as all eligibility criteria are met. Immunotherapy will be administered at each participating institution (Fondazione IRCCS Istituto Nazionale dei Tumori, Milan and Fondazione I.R.C.C.S. Policlinico San Matteo, Pavia) according to standard-of-care guidelines.

Carbon ion RT

After confirming disease stability and upon patient inclusion in the study, a hypofractionated carbon ion boost will be administered to one site of disease (Figure 2). CIRT will be delivered at the National Center for Oncological Hadrontherapy (CNAO).

Patients will receive radiation to a single lesion with a total dose of 24 Gy[relative biological effectiveness] delivered with the following schedule: 8 Gy[relative biological effectiveness]/fraction, one fraction/day, for 3 days. The biological efficacy and safety of the proposed regimen are, in principle, comparable to conservative fractionations (widely used in stereotactic body RT) for oligometastatic disease. In case of multiple metastases, the precedence for CIRT will be done to the symptomatic lesion followed by the potentially more immunogenic and feasible ones, with the following score of priority: lymphatic, visceral, soft tissue and bone locations. Brain metastases will not be considered for carbon ion irradiation. Further details regarding CIRT administration are reported in the Supplementary File 'Annex'.

Study procedures

Staging & response

The enrolled patient must have a baseline whole-body CT scan with contrast performed within 28 days prior to the start of CIRT. CT scan slice thickness must be <5 mm. To be considered pathologically enlarged and measurable, a lymph node must be ≥15 mm on the short axis. According to RECIST, only the short axis will be measured and followed after treatment. Only measurable lesions will be included in the baseline evaluation. For this reason, leptomeningeal disease, ascites, pleural or pericardial effusion, inflammatory, breast disease, lymphangitic involvement of skin or lung will be excluded from the analysis. Tumors located in a previously irradiated area or an area subjected to any other locoregional therapy are usually not considered measurable unless progression is demonstrated.

Follow-up

A disease assessment will be performed at least 8 weeks after CIRT. 1 year of follow-up is expected for each patient. Follow-up visits will be performed by the referring oncologist following standard-of-care procedures (every 3 or 6 weeks).

Translational research

Blood samples from patients receiving CIRT along with ICIs will be collected right before and after radiation therapy. These blood samples will then be subjected to analysis for characterization of cytokines (e.g., interleukins, IFNs), soluble markers known to be related to immune response (HMGB1, sPD-L1, tumor cfDNA) and immune cell composition (e.g., cytotoxic T cells, T-helper cells). Samples will be analyzed using multiplex ELISA, chip cytometry or a next-generation sequencing technique depending on the end point. The authors thus intend to

correlate responding patients with a certain composition of immune cells existing before therapy to find markers in liquid biopsies indicating which patients might benefit from the study treatment; investigate changes in the composition of immune cells in the blood as a result of the therapy, thus indicating advantages or risks of the study treatment association; and examine the correlation between clinical response and cytokines as well as soluble markers (HMGB1, sPD-L1, tumor cfDNA) known to be related to immune response.

Objectives

The authors hypothesize that adding CIRT to ICIs will increase clinical response in advanced malignancies in which immunotherapy is the standard-of-care. The primary objective is to estimate the effect, in terms of clinical response, of the combination of immunotherapy and CIRT in the palliative setting across different advanced malignancies for which pembrolizumab is currently the standard-of-care. The secondary objectives are: i) to describe the safety profile of the association of CIRT and pembrolizumab in the palliative setting across different advanced malignancies for which pembrolizumab is currently the standard-of-care; ii) to estimate the effect, in terms of survival, of pembrolizumab with the association of CIRT in the palliative setting across different advanced malignancies for which pembrolizumab is currently the standard-of-care; iii) to assess the response of the metastatic lesion treated with CIRT. The exploratory aim is to collect biological data in patients receiving CIRT and pembrolizumab.

End point measures

The primary end points are objective response rate (ORR) according to RECIST 1.1 [36], assessed at least 8 weeks after CIRT, and toxicity according to Common Terminology Criteria for Adverse Events version 5.0 [37]. The secondary end points are PFS, OS, ORR according to immune-related RECIST [38], percentage of patients with disease progression as best response, objective response of metastatic lesions treated with CIRT and disease control rate according to RECIST (defined as ORR + SD).

Statistics

With regard to statistical analysis for the primary end points, the ORR number, assessed at least 8 weeks after CIRT according to RECIST, will be evaluated for the whole population. The ORR proportion according to RECIST 1.1, together with the binomial 95% CI, will be estimated for the entire population. In a secondary analysis, the ORR proportion (95% CI) will be presented for each disease. In addition, at the end of the study, the proportion of grade $\geq$3 AEs, together with the binomial 95% CI, will be described for each malignancy. With respect to statistical analysis for the secondary end points, PFS will be calculated as the time between the start date of the study treatment combination and progression of disease, death or last follow-up. PFS will be estimated for each malignancy by the Kaplan–Meier product-limit method. OS will be calculated as the time between the start date of the study treatment combination and death from any cause or last follow-up. OS will be estimated for each malignancy by the Kaplan–Meier product-limit method. The ORR proportion according to immune-related RECIST, together with the binomial 95% CI, will be estimated for each disease. The proportion of patients with disease progression as the best response, together with the binomial 95% CI, will be estimated for each disease. The objective response of metastatic lesions, together with the binomial 95% CI, will be estimated for each disease. The disease control rate according to RECIST (defined as ORR + SD), together with the binomial 95% CI, will be estimated for each disease.

The significance level will be set to 0.05 (two-sided type I error α), and statistical analysis will be performed with Stata 16 (StataCorp, LLC, TX, USA). With regard to the handling of missing data and dropouts, the authors assume a 10% dropout rate. No data imputation will be performed. Dropouts (patients without measured ORR) will be replaced by recruiting new subjects. The cause of the dropout will be recorded to assess the influence of selection bias.

Discussion

RT initiates both immune-stimulating and immune-suppressive pathways, and for this reason, the opportunity to associate RT and ICIs is one of the emerging oncological treatment strategies. Considering the literature data on photon beam RT and ICIs and the well-known biological and physical peculiar hallmarks of heavy ions, the query is if there are extra advantages in the combination with particles. In fact, although basic science studies on this topic are thus far scant, several *in vitro*, *ex vivo* and *in vivo* studies have recently suggested that charged particles may be

more immunogenic than photons [9–12,14.,18,19,23,39]. Indeed, compared with photons, charged particles are able to interact differently with cellular molecules and activate different cell death pathways.

To note that the ballistic characteristics of particles are advantageous in sparing normal tissues and in this context more naive circulating T lymphocytes, memory T cells and other immune cells, essential to guide and maintain a tumor-specific immune response. Moreover, protracted fractionated regimens of RT have proved to induce different degree of lymphopenia because of the irradiation of circulating blood cells and active hematopoietic organs reduced in particle therapy treatment for its more favorable integral dose [39]. For instance, even though carbon ions are more efficient than photons in the inducement of chromosomal aberrations per unit dose on the tumor, considering patients treated on the same target volume, the peripheral blood of photon beam patients had higher levels of aberrations compared with the CIRT ones [40]. In addition, for comparable irradiated volumes, CIRT patients are less lymphopenic than patients who have undergone conventional photon beam RT also with modern and high collimate techniques [41].

With regard to translational research, the choice to focus this analysis on blood only is supported by the possibility of easily assessing the immunogenicity of particle therapy by analyzing circulating markers in the blood, which is regularly drawn in clinical practice. Moreover, on one hand, the heterogeneous set of diseases included in this trial are characterized by significant differences in terms of biological characteristics and tumor microenvironment that will not draw any reliable conclusions in a tumor tissue analysis, on the other, considering that the authors will deal with a palliative treatment in an advanced disease, the authors deemed unethical to propose additional biopsies that would not be performed in clinical practice deemed ethically acceptable. Despite the fact that blood analysis results cannot be directly transferred to clinics, any findings will serve as hypotheses for future research.

Thus far, there are no clinical trials assessing the safety of the combination of ICIs and CIRT. However, data concerning the sequential use of CIRT and ICIs have recently been published. In particular, in a retrospective study conducted at the National Center for Oncological Hadrontherapy (CNAO), the sequential use of CIRT and ICIs was followed by late AEs of any grade in the majority (82%) of patients, whereas the incidence of grade $\geq$3 AEs was in line with what was already reported in the scientific literature for CIRT and ICI alone [42].

Conclusion

Considering that no clinical trials have been conducted thus far to assess the safety of the combination of ICIs and CIRT, the current clinical study will provide controlled data regarding the safety of this unprecedented therapeutic combination.

Executive summary

State of the art

- Immunotherapy has become the standard-of-care in different advanced malignancies, and its effectiveness in the palliative setting has been demonstrated by several phase III trials.
- The response rate to immunotherapy varies according to the cancer under study and the line of treatment.
- A potential way to improve the activity of single-agent immune checkpoint inhibitors is to enhance the clinical response with the use of further antitumor agents, including radiotherapy.
- Studies have shown that carbon ions may lead to a broader immunogenic response; as a result of their dosimetric characteristics, it is possible to direct and sustain an immune response against tumors by reducing integral dose sparing immune cells.

Aim of the study

- The goal of the ICONIC trial is to explore the feasibility and clinical activity of the addition of carbon ion radiotherapy to immune checkpoint inhibitors in advanced malignancies in which immunotherapy is currently the standard-of-care.

Supplementary data

To view the supplementary data that accompany this paper please visit the journal website at: www.futuremedicine.com/doi/suppl/10.2217/fon-2022-0503

Author contributions

S Cavalieri, L Licitra and E Orlandi made substantial contributions to the conception and design of the trial. A Facoetti, M Durante, A Helm and C Fournier provided the translational research section. S Cavalieri performed the sample size calculation, wrote the

first draft of the study protocol and the first draft of the manuscript. C Klersy, VV Ferretti and A De Silvestri supervised the sample size calculation, and provided the statistical plan. All authors have contributed to the writing of the manuscript; substantively revised the manuscript; approved the submitted version; and agreed to be personally accountable for their own contributions and to ensure that questions related to the accuracy or integrity of any part of the work, even parts in which the author was not personally involved, are appropriately investigated and resolved and the resolution documented in the literature. All authors read and approved the final manuscript.

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Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

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