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Pulsar to Overcome Acquired Resistance (AR) to Immunocheck-Point Inhibitors (ICIs) in Oligometastastic Cancer Progression: A Clinical and Translational Research

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ABSTRACT

Design: *Acquired Resistance (AR) to immuno-check point inhibitors (ICIs) still remains a common and insufficiently studied clinical challenge in most metastatic tumors which have been initially responder to immunotherapy. In attempt to overcome this phenomenon, PULSAR in conjunction with immuno-checkpoint inhibitors (ICIs) might be a reasonable field of research.*

"Personalized, ultrahypofractionated stereotactic adaptive radiation therapy" (PULSAR) is a new SBRT modality delivering pulsed fractions during ICIs therapy. The rationale of this research is to induce the PD-L1 over expression by the tumor in order to overcome the AR to ICIs and reverse the tumor microenviroment (TME) from refractory cold tumor to responder hot tumor. To assess this effect the lymphocytes populations and the PD-L1 expression on tumoral circulating extracellular vescicles (EVs) will be investigated.

As primary end point of this multicenter observational prospective trial is to assess the usefulness of PULSAR on Metastatic Progression Free Survival in oligometastatic patients developing AR to ICIs in terms of clinical response and immunoresponse by immunophenotype and PD-L1 expression on circulating tumoral EVs. The secondary end point is to assess the optimal timing between the pulsed fractions and the response among different metastatic anatomical sites (liver, lung, nodes, bones).

Method: *Patients suffering from oligometastic cancer who progress under ICIs therapy showing de novo lesions or refractory lesions in liver, lungs, nodes and bones will be prospectively enrolled in this study. ICIs therapy will be not discontinued during PULSAR and delivered as per protocol according the cancer hystology. A 6 Gy pulse fraction will be given every week (group A) or every two weeks (group B) to a total 3-6 fractions, depending on the volume. One-three sites will be treated concurrently. Clinical response will be evaluated with FDG-Pet before and after 3 weeks off PULSAR according i-RECIST criteria. As surrogate of TME response, the lymphocyte population and the PD-L1 expression on tumoral circulating EVs in peripheral blood will be analyzed. Peripheral blood will be collected at T0 (at baseline before the first pulse), at T1-T3-6 and T 21 (3 weeks after PULSAR off).*

The promoter centre will collect, analyze the blood samples and then elaborate the statistical data.

The Metastatic Progression Free Survival will be estimated with Kaplan -Maier test. The Shapiro-Wilk test will be used to verify all data distribution. Irradiated lesions, ICI type, cancer type will be related to the clinical and immunological outcomes. The differences among data will be carried out using t-test o test di Wilcoxon. The frequences difference will be assessed with Chi-square or Fisher exact test. The statistical significance will be setted at 0.05. To achieve a 0.95 statistic power, 210 patients will be enrolled within in 3 years.

Conclusion: *By this multicenter observational prospective study, information on the effictiveness of PULSAR in overcoming AR during ICI are expected; information on effective pulsed fractions timing and response by anatomical sites are also awaited.*

Keywords
PD-L1, SBRT, TME, Immunotherapy, Radiotherapy.

Introduction
Immunotherapy with Programmed Cell Death Protein 1 / Programmed Death-Ligand 1 [PD-(L)1] blockade also known as immuno-checkpoint inhibitor has revolutionized cancer treatment, leading to remarkable response mainly in locally advanced and metastatic cancer [1]. However at a certain point of the ICIs therapy course, acquired resistance may occur and alternative approaches are required to take a control on a new progressive disease.

Acquired Resistance (AR) to immuno-check point inhibitors (ICIs) is defined as progression of cancer after an initial antitumor response and still remains a common and insufficiently studied clinical challenge in most metastatic tumors during immunotherapy [2].

This phenomenon is more frequently recorded in patients with lung cancer, where AR occurs at higher rates than many other tumor types sensitive to PD-(L)1 blockade [3]. Nevertheless, all tumors may be involved. Various mechanisms in incoming resistance to ICIs have been hypothesized like mutations in the cancer genome or immonological changes in tumor microenvironment (TME) [4,5]. Further, the anatomical site of progression seems to play a role because among metastatic sites (liver, bones, nodes) a different prevalence of programmed death-ligand 1 (PD-L1) and tumor-infiltrating lymphocytes (TILs) distribution have been recorded. Indeed, liver metastases seem to express a more immunosuppressive microenvironment while nodes and lung metastases show a more sensible TME to ICIs due to a highest prevalence of PD-L1 and TILs quote [6]. It is well aknowledged that radiotherapy leads to an immune re-modulation in the TME by influencing almost all steps of the cancer cross talk and the adaptive immune system [7,8]. This interaction allows to an "in situ vaccination" effect reversing a cold tumor into a hot tumor thus enhancing the efficacy of immuno-check point inhibitors [9-13]. In AR, by delivering a higher dose per fraction with SBRT, it is reasonable to induce and amplify the immunogenic cancer death. Further, extending the time between fractions, it could be effective in modulating the TME and stimulating the immune system thus leading to tumor control without adverse events as aimed in the "personalized, ultrahypofractionated stereotactic adaptive radiation therapy" (PULSAR) [14]. With this modality, a pulse of SBRT often ≥ 5 Gy is delivered every 2–4 weeks for 2–5 pulses, dependent on normal tissue toxicity, timed within 48 h of checkpoint-inhibitor infusions [15]. The targets are adapted at each pulse on CT- or MR-guided. Thus PULSAR is able to deliver ablative doses in conjunction with checkpoint inhibitors, without a pause in systemic therapy, allowing for an immune response and adaptation of radiation fields based upon tumor reshaping. Our hypothesis is to apply this modality to overcome AR to ICIs in progressive oligometastatic disease by enriching the TME with tumoral antigen expression like th PD-L1 over expression and activation of tumor-specific CD8+ T cells as described in several studies. Murine models reported by Moore and Morris have shown evidences of an effective immune response induced with PULSAR on metastatic sites during ICIs therapy with a deleyed time between fractions [15,16]. In the study of Moore, PULSAR was tested in combination with α-PD-L1 therapy in immune activated and resistant syngeneic immunocompetent mouse models of cancer. As a result, the effectiveness of systemic administration of the α-PD-L1 therapy was primarily dependent on how the radiation was sequenced. Interstingly a lower benefit was seen when ICI and current radiotherapy was given daily or every 4 days. On the contrary, a highest effectiveness was observed when radiation pulse was given every 10 days or more [15]. Among clinical experiences, it should be reminded the case report on a patient with metastatic renal cancer showing a12 cc volume on a hilar mass in the left lung treated with 12 Gy pulses each spaced 1 month apart. The mass decreased to a 3 cc volume following this modality [16].

A normal tissue recovery is another point to focus on. To this regard, the PATRIOT trial has confirmed an improved toxicity and outcome giving SBRT one weekly instead of every other day [17]. If so , the optimal timing between fractions to achieve the TME sensivity reversion needs to be investigated [18]. To test the TME reprogramming effect, flow cytometry assessment of lymphocyte population on peripheral blood could be a valid surrogate together to PD-L1 over-expression on liquid biopsy. The PD-L1 over expression could be evaluated on circulating tumoral EVs also known as exosomes. This extracellular vesicles (EVs) are lipid bilayer particles released by normal and neoplastic cells, that have been identified in different body fluids in addition to blood (eg saliva, urine, cerebrospinal fluid and breast milk) and have been reported to increase in patients with various types of tumors, including hematological malignancies [19]. They have different size ranging from small (sEVs; ~30–200 nm) to medium/large (m/lEVs; ~200–10,000 nm). Interestingly, EVs carry a selected cargo in terms of lipids, proteins and nucleic acids, and

show specific surface antigens deriving from their parental cells [20]. Notably, the EV lipid bilayer protects their molecular content from degradation of proteases and nucleases, thus providing well defined genetic/protein/lipid signatures associated with specific phenotypes. Numerous studies have reported that PD-L1 is also found on tumor-derived EV membranes, especially exosomal PD-L1, which contributes significantly to immunosuppression through CD8+ T cell deactivation [21]. Exosomal PD-L1 has been shown to be associated with tumor growth, progression, and metastasis [22]. Moreover, that total exosomal PD-L1 seem to increases through either escalated exosome secretion or enhanced PD-L1 synthesis via multiple pathways activation, including the cGAS-STING pathway which is exploited by SBRT [23]. Taking into account the evidence reported in the literature on the effect of PULSAR with ICIs and the role of the tumoral exosomes expressing PD-L1, the aim of the present study is to investigate the role of PULSAR in cases of AR to ICIs therapy through the TME reprogramming by clinical and immunological information.

Design

Aim of this prospective multicentre observational and translational research is to assess the combination of PULSAR and ICIs therapy in overcoming AR in oligometastic patients defined by the ESTRO-ASTRO consesus document [24]. Patients showing oligometastatic cancer like head and neck, melanoma, lung, renal, bladder cancer who develop AR to ICIs therapy in their course are elegible as reported below. AR to ICIs therapy will be defined on the CT scan and FDG-Pet imaging describing de novo lesions or progression in older lesion not responding to ICIs therapy.

Patients will be treated with PULSAR 6 Gy/fr /week to a total 3-6 fractions according to irradiated volumes and involved sites. More lesions will be treated concurrently. Two groups will be identified according pulses delivery timing. Pulses will be delivered once a week (group A) or once every 2 weeks (group B). At every pulse, based upon the findings on CB-CT, a replan will be performed.

Patient will continue to receive the same ICIs therapy as per protocol. To study the effect on TME reprogramming and the PD-L1 tumor over expression, samples of blood and serum will be collected and analysed in flow-cytometry and nanoparticle tracking. Blood will be collected at T0 and before every pulse (T1-T6) and 3 weeks off therapy. The study design is reported in Figure 1.

Treatment Description

Patients selection

Patients is described as below:

Inclusion Criteria

Patients with oligometastatic cancer developing AR to ICI with de novo or not responding lesions, asymptomatic for spinal cord compression or pain refractory to medical therapy.
Informed consent obtained
ECOG 0-1
Age ≤ 80 years
Hystology allowed to ICIs
Sites : liver, nodes, bones, lung

Exclusion Criteria

Patients with multimetastatic cancer disease and symptomatic lesions
ECOG > 2
Age > 80 years
No informed consent obtained

Clinical Staging and Restaging

Diagnosis of AR will be recorded on CT and FDG-Pet imaging detected on the basis of SUV, TLG and MTV; clinical response to PULSAR on FDG-Pet will be scored according i-RECIST criteria before and 3 weeks off the entire PULSAR course [25].

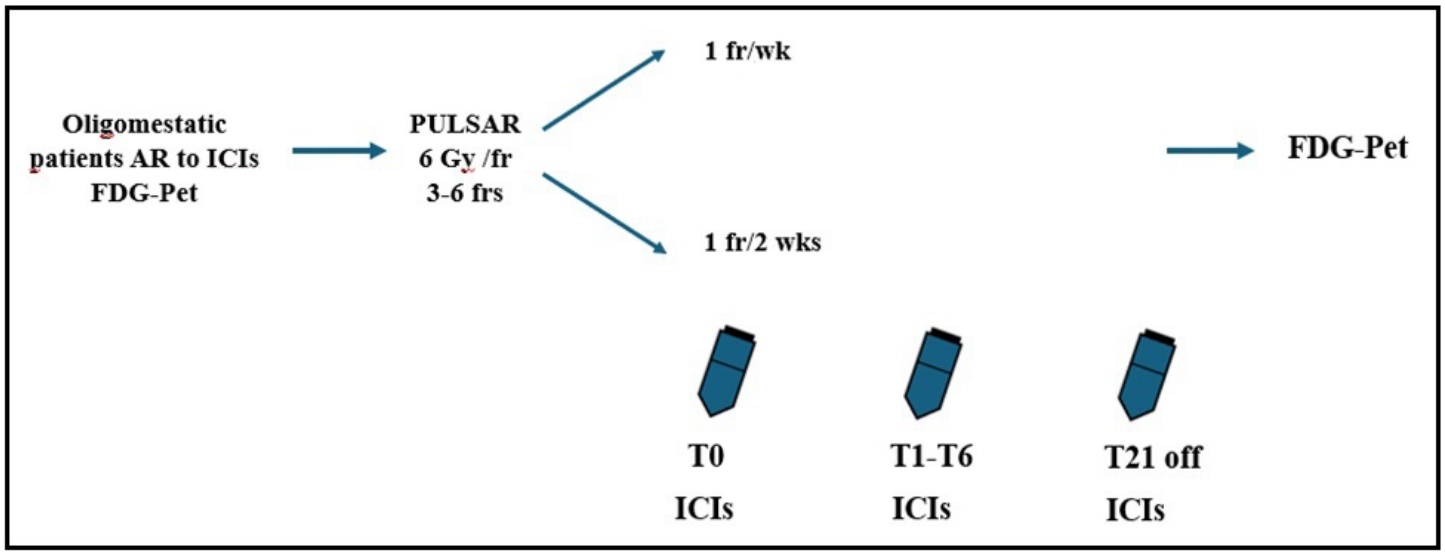

Figure 1: Study design. PULSAR: Personalized, ultrahypofractionated stereotactic adaptive radiation therapy; AR: Acquired resistance; ICIs: Immunocheck point inhibitors.

Immunological Response Assessment

As surrogate of TME toning, peripheral blood will be runned by flow cytometry assessing total T lymphocytes (CD3+), T helper (CD3+ CD4+), T cytotoxics (CD3+ CD8+), T regolators (Tregs: CD4+ CD25+ CD127low), T double negative (DNT: CD3+ CD4- CD8- CD16- CD56-), T double positives (DPT: CD3+ CD4+ CD8+), T natural killer: CD3± CD16+ CD56+) and B (CD19+) using fluorochromes monoclonal antibodies. The isolation of extracellular vescicles (cEVs), the analysis of size distribution and concentration by nano-particle tracking analysis will follow in our laboratory. Thereafter the quantification and the PD-L1 phenotyping of cEVs by flow cytometer will be performed [19].

Sample Collection

Peripheral blood on EDTA and serum will be collected at T0 (before the first fraction) and before each pulse ant then after 3 weeks off therapy.

Radiation treatment PULSAR

SBRT will be applied to each pulsed fraction according ICRU-SBRT criteria [26]. Prescription dose will be 6 Gy/pulse for 3/6 total fractions debending by the site and the volume dimension. PTV will be defined on CT and PET imaging at diagnosis. Contraints to OAR's will be observed on the basis of TG-101 [27]. Treatment will be delivered with IGRT linac based. Replanning will be defined on the basis of cone beam informations during every pulse. Pulses will be delivered according two timing: A = one pulse every week; B = one pulse every two weeks.

Treatment Volumes

The clinical target volume (CTV) should include the significative GTV as defined on imaging plus a 0.3-0.5 mm margins. Planning target volume (PTV) will correspond to the CTV with a variable 0.5 -0.6 mm margins. The organs at risk (OARs) will be defined by the irradiated anatomical site.

Systemic Treatment

ICIs like nivolumab, avalumab, atezolizumab, pembrolizumab according cancer type will be delivered per protocol within the PULSAR programme.

Endpoints

The primary endpoint of this study is to evaluate the outcome in terms of Metastatic Progression free survival in patients with oligometastatic cancer developing AR to ICIs therapy the effect of PULSAR to overcome AR in terms of the metastatic progression free survival. The surrogate of the TME toning effect will be carried out by flow cytometry and PD-L1 expression on circulant Evs. **The secondary endpoint** is to evaluate the effective timing between pulses in one fraction /one week or one fraction / two weeks and the response according the irradiated of anatomical metastatic sites.

Statistic

Sample size

Metastatic progression free survival will be carried out with Kaplan -Maier test. PD-L1 expression on EVs from T0 al T3/6 PULSAR + T21 will be related to lymphocyte flow cytometry and clinical outcome. Irradiated lesions, ICI type, cancer type will be related to the clinical and immunological outcomes. The Shapiro-Wilk test will be used to verify all data distribution. The differences among data will be analyzed with t-test o test di Wilcoxon. The frequences difference will be assessed with Chi-square or Fisher exact test. The statistical significance will be setted at 0.05. To achieve a power 0.95 statistical power, 210 patients will be enrolled within in 3 years. Data analysis will last 3 years.

Data Collection Procedure

Data from each center will be collected in electronic case report forms (CRFs) and transfered into a single cloud-based database. Subsequently, the aggregated data will be processed by the promoter center.

Planned Timeline

It is scheduled as follows: 0-3 months: project organization; 18-36 months: patient enrolment; 48-60 months: laboratory work assessment statistical analysis and publication of data about primary end-point.

Ethics Committee Approval for Ongoing Research

The protocol has been written according to the principles of good clinical practice (GCP). This study is conducted in accordance with the most recent version of the Declaration of Helsinki and with the Italian laws and regulations. The study protocol was approved by the ethics committee of promoter center CEUR (ethics committee identifier code CEUR 20240013204; n.23/2024). Approval by the respective ethics committee relevant to each site will be collected before opening new sites. Written informed consent, signed and personally dated will be obtained from each patient before inclusion in the study.

References

1. Alsaab HO, Sau S, Alzhrani R, et al. PD-1 and PD-L1 checkpoint signaling inhibition for cancer immunotherapy: mechanism, combinations, and clinical outcome. Front Pharmacol. 2017; 8: 1-15.
2. Schoenfeld AJ, Antonia SJ, Awad MM, et al. Clinical definition of acquired resistance to immunotherapy in patients with metastatic non-small-cell lung cancer. Ann Oncol. 2021; 32: 1597-1607.
3. Black J, Wojas Krawczyk C, Nicòs M, et al. Failure of Immunotherapy-The Molecular and Immunological Origin of Immunotherapy Resistance in Lung Cancer. Int J Mol Sci. 2021; 22: 9030.
4. Gettinger S, Choi J, Hastings K, et al. Impaired HLA class I antigen processing and presentation as a mechanism of acquired resistance to immune checkpoint inhibitors in lung cancer. J Cancer Discov. 2017; 7: 1420-1435.
5. Anagnostou V, Smith KN, Forde PM, et al. Evolution of neoantigen landscape during immune checkpoint blockade in non-small cell lung cancer. J Cancer Discover. 2017; 7: 264-276.

6. Botticelli A, Cirillo A, Scagnoli S, et al. The Agnostic Role of Site of Metastasis in Predicting Outcomes in Cancer Patients Treated with Immunotherapy. Vaccines. 2020; 8: 203-221.
7. Monjazeb AM, Schalper KA, Villarroel Espindola F, et al. Effects of Radiation on the Tumor Microenvironment. Semin Radiat Oncol. 2020; 30: 145-157.
8. Arnold KM, Flynn NJ, Raben A, et al. The Impact of Radiation on the Tumor Microenvironment: Effect of Dose and Fractionation Schedules. Cancer Growth Metastasis. 2018; 11: 1179064418761639.
9. Demaria S, Bruce NG, Devitt ML, et al. Ionizing radiation inhibition of distant untreated tumors (abscopal effect) is immune mediated. Int J Radiat Oncol Biol Phys. 2004; 58: 862-870.
10. Formenti SC, Demaria S. Radiation therapy to convert the tumor into an in situ vaccine. Int J Radiat Oncol Biol Phys. 2012; 84: 879-880.
11. Formenti SC, Demaria S. Combining radiotherapy and cancer immunotherapy: a paradigm shift. J Natl Cancer Inst. 2013; 105: 256-265.
12. Dovedi SJ, Cheadle EJ, Popple AL, et al. Fractionated Radiation Therapy Stimulates Antitumor Immunity Mediated by Both Resident and Infiltrating Polyclonal T-cell Populations when Combined with PD-1 Blockade. Clin Cancer Res. 2017; 23: 5514-5526.
13. Sharabi AB, Lim M, DeWeese TL, et al. Radiation and checkpoint blockade immunotherapy: Radiosensitisation and potential mechanisms of synergy. Lancet Oncol. 2015; 16: 498-509.
14. Miljanic M, Montalvo S, Aliru M, et al. The Evolving Interplay of SBRT and the Immune System, along with Future Directions in the Field. Cancers. 2022; 14: 4530.
15. Moore C, Hsu CC, Chen WM, et al. Personalized Ultrafractionated Stereotactic Adaptive Radiotherapy (PULSAR) in Preclinical Models Enhances Single-Agent Immune Checkpoint Blockade. Int J Radiat Oncol Biol Phys. 2021; 110: 1306-1316.
16. Morris Z, Dohopolski M, Rahimi A, et al. Future Directions in the Use of SAbR for the Treatment of Oligometastatic Cancers. Semin Radiat Oncol. 2021; 31: 253-262.
17. Quon HC, Ong A, Cheung P, et al. Once-weekly versus every-other-day stereotactic body radiotherapy in patients with prostate cancer (PATRIOT): A phase 2 randomized trial. Radiother Oncol. 2018; 127: 206-212.
18. Huang NH, Lei Z, Yang HN, et al. Radiation-induced PD-L1 expression in tumor and its microenvironment facilitates cancer-immune escape: a narrative review. Ann Transl Med. 2022; 10: 1406.
19. Laurenzana I, Trino S, Lamorte D, et al. Analysis of Amount, Size, Protein Phenotype and Molecular Content of Circulating Extracellular Vesicles Identifies New Biomarkers in Multiple Myeloma. Int J Nanomedicine. 2021; 16: 3141-3160.
20. Chen G, Huang AC, Zhang W, et al. Exosomal PD-L1 contributes to immunosuppression and is associated with anti-PD-1 response. Nature. 2018; 560: 382-386.
21. Kim DH, Kim H, Choi YJ, et al. Exosomal PD-L1 promotes tumor growth through immune escape in non-small cell lung cancer. Exp Mol Med. 2019; 51: 1-13.
22. Liu J, Peng X, Yang S, et al. Extracellular vesicle PD-L1 in reshaping tumor immune microenvironment: biological function and potential therapy strategies. Cell Commun Signal. 2022; 20: 14.
23. Timaner M, Kotsofruk R, Raviv Z, et al. Microparticles from tumors exposed to radiation promote immune evasion in part by PD-L1. Oncogene. 2020; 39: 187-203.
24. Lievens Y, Guckenberger M, Gomez D, et al. Defining oligometastatic disease from a radiation oncology perspective: An ESTRO-ASTRO consensus document. Radiother Oncol. 2020; 148: 157-166.
25. Carter BW, Bhosale PR, Yang WT. Immunotherapy and the role of Imaging. Cancer. 2018; 124: 2906-2922.
26. Wilke L, Andratschke N, Blanck O, et al. ICRU Report 91, Prescribing, Recording, and Reporting of Stereotactic Treatments with Small Photon Beams: Statement from the DEGRO/DGMP working group stereotactic radiotherapy and radiosurgery. Strahlenther Onkol. 2019; 195: 193-198.
27. Benedict SH, Yenice KM, Followill D, et al. Stereotactic body radiation therapy: The report of AAPM Task Group 101. Med Phys. 2010; 37: 4078-4101.