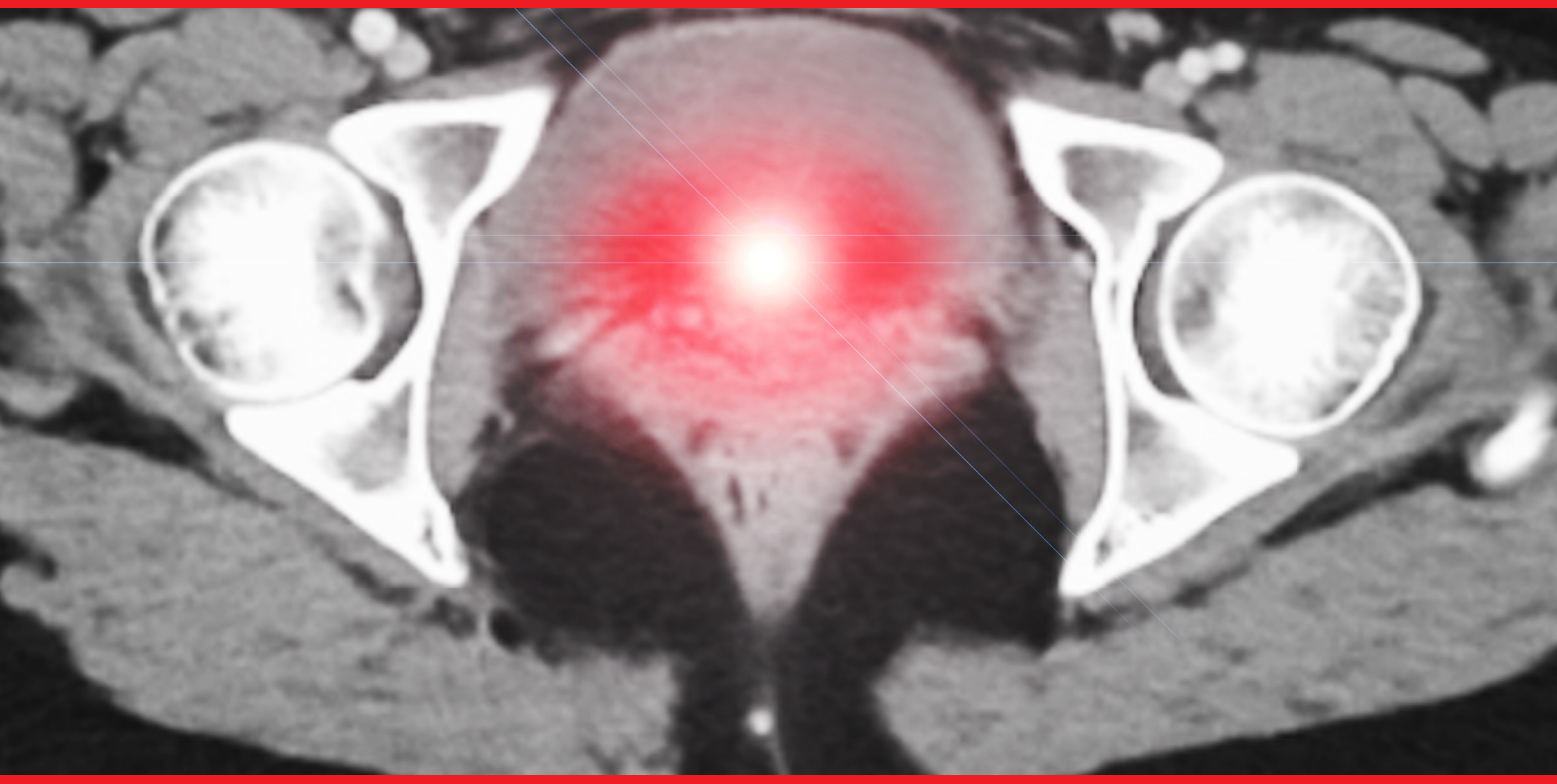
June 2025
Volume 14, Number 2

Applied Radiation Oncology™



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Scotch Plains, NJ 07076
Tel: 908-301-1995
Fax: 908-301-1997
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ESSN: 2334-5446 (online)

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Applied Radiation Oncology Print ISSN: 3065-4467, Online ISSN: 2334-5446, USPS 25688 is published quarterly by Anderson Publishing, LTD at 180 Glenside Avenue, Scotch Plains, NJ 07076. Readers can renew or subscribe at appliedradiationoncology.com/subscribe.

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Applied Radiation Oncology™

June 2025, Vol 14, No 2

FOCUS: HEAVY PARTICLE TREATMENT

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Radiobiology and Physics of Heavy Particles

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Dr Suh is the editor-in-chief of *Applied Radiation Oncology*, and professor and chairman, Department of Radiation Oncology, at the Taussig Cancer Institute, Rose Ella Burkhardt Brain Tumor and Neuro-Oncology Center, Cleveland Clinic, Cleveland, OH.

In this issue of *Applied Radiation Oncology*, we turn our focus to the radiobiology and physics of heavy particles, which provide another option to treat various malignancies.

Dr Daniel Koffler and his co-authors cover the subject in a comprehensive two-part review. *Eradicating Cancer Stem Cells Using High Linear Energy Transfer Radiation Therapy Part 1: Physics and Radiobiology* offers a primer on the fundamental physics and radiobiology that distinguish high linear energy transfer (LET) particles from conventional photon and proton therapies. Part 2, *LET Painting and Other Advanced Techniques*, delves into LET painting, spatial fractionation, and multi-ion strategies as promising tools to selectively target cancer stem cells. Together, these articles present the possibility that high LET may offer more than local control—it may enable systemic disease modification.

This month's research article, *The Effect of Pentoxifylline and Vitamin E in Preventing Grade 3 Radiation Pneumonitis: A Single Arm, Phase II Prospective Study*, presents a phase II study evaluating the combination of pentoxifylline and vitamin E in preventing grade three pneumonitis in patients undergoing thoracic reirradiation with stereotactic ablative body radiation therapy. The results showed that the pneumonitis incidence was significantly reduced at all time points, and the intervention was well tolerated, making it a promising adjunct in this clinical scenario.

Malignant Melanotic Nerve Sheath Tumor of the Neck: A Case Report details the rare presentation of a malignant melanotic nerve sheath tumor in the carotid space, underscoring the critical importance of genomic profiling and multidisciplinary management for these aggressive, anatomically challenging tumors. It also highlights the evolving role of proton therapy in head and neck reirradiation.

June's Resident Voice editorial, *Entrustable Professional Activities in Radiation Oncology: A Framework for Competency-Based Training*, discusses the implementation of entrustable professional activities (EPAs) as a model for evaluating competency in radiation oncology education, which is rapidly evolving. The editorial underscores the current gaps in training, particularly in brachytherapy, and offers EPAs as a structured solution for assessing readiness for real-world clinical practice.

The June issue captures a central theme: bridging the gap between the current and future state of heavy particles and residency training. By redefining how we train the next generation of radiation oncologists and by refining our approach to radioresistant tumors, each article invites us to reconsider established paradigms and embrace innovation to provide better care of patients.

As always, I thank you for your continued support of *Applied Radiation Oncology*!

Published: June 1, 2025. https://doi.org/10.37549/ARO-D-25-00018

Eradicating Cancer Stem Cells Using High Linear Energy Transfer Radiation Therapy Part 1: Physics and Radiobiology

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Abstract

Linear energy transfer (LET), a measurement of ionization density, tracks the radiobiological potency of any given course of therapeutic radiation and its efficacy in killing cancer cells. As opposed to the low LET of photon and proton therapy, high LET charged particle therapy can overcome multiple mechanisms of resistance to effectively treat radioresistant tumors. In part 1 of this two-part series, we review the physics and radiobiology of high LET to demonstrate its unique capability to address the problem of cancer stem cells (CSCs), which remain largely impervious to conventional therapies and are the ultimate explanation of progression and metastasis as well as the dire prognosis of malignancies such as pancreatic cancer and glioblastoma at any stage.

Keywords: LET, linear energy transfer, particle therapy, carbon ion therapy, heavy particle therapy, radiobiology, radiation physics, spatial fractionation, ion therapy

Introduction

Heavy particle therapy (HPT), including carbon ion radiation therapy (CIRT), exhibits high linear energy transfer (LET) and offers a distinct mode of energy deposition compared with conventional photon therapy as well as proton therapy. Instead of diffusing ionization events over wide spatial geometries, HPT delivers dense ionization along the beam pathway, producing clustered and complex damage to cancer-cell DNA that is exponentially more lethal to these directly targeted cells than low LET radiation. The direct effects of HPT on cancer-cell DNA are also responsible for enhanced immune activation and clinically validated abscopal responses, suggesting a greater role for HPT in augmenting systemic disease control than conventional radiation therapy.

In particular, HPT shows promise in the treatment of glioblastoma (GBM) and pancreatic ductal adenocarcinoma (PDAC), both of which are marked by recalcitrant cancer stem cell (CSC) biology that is substantially responsible for their resistance, recurrence profiles, and overall grim clinical outcomes. Preclinical data indicate

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Disclosures: The authors have no conflicts of interest to disclose. None of the authors received outside funding for the production of this original manuscript and no part of this article has been previously published elsewhere.

Published: June 1, 2025. https://doi.org/10.37549/ARO-D-25-00003P1

that high LET radiation can overcome CSC resistance mechanisms in general, including in GBM and PDAC, by halting or reversing stemness-promoting processes such as epithelial-mesenchymal transition (EMT) that low-LET radiation tends to induce.

New approaches such as LET painting and spatial fractionation enable delivery of high LET radiation selectively to high-biological-risk tumor subvolumes. Such techniques are necessary for the clinical implementation of HPT that is both safe and effective at improving outcomes in resistant cancers, as the same augmented lethality that is welcome when delivered to cancer cells poses a greater danger to nearby healthy tissue than low LET exposure. Additionally, biologically selective LET deposition may enhance the immunogenic/abscopal properties of HPT.

Here, we review the current status and approaching frontiers of high LET radiation therapy with a focus on its application to the often underappreciated, pressing clinical problem of CSC biology, as well as clinical and logistical barriers to the expansion of HPT beyond the niches it currently occupies.

Basic Science of Heavy Particle Therapy and Suitability in Treatment-Resistant Cancers

Physics and Biology of Heavy Particles: Local Structure Defines Radiobiology and Trumps Dose

LET can be defined as average ionizing energy transferred to medium per length of radiation track, typically in keV/μm. It is a dynamic property of charged particles over the course of a beam path, increasing with mass and decreasing with kinetic energy.[1-4] More specifically, LET is proportional to $\frac{Z_{eff}^2}{\beta^2}$, where Z_{eff} is effective charge and β is velocity.[5] The relationship of these properties with medium stopping power defines the distinct Bragg peak depth dose profile for all charged particles.[1,2]

Clinical Relevance of LET: Protons Vs Heavy Ions

By convention, "low LET" charged particles have locally restricted averaged $LET_\Delta \leq 10$ keV/μm and "high LET" charged particles have $LET_\Delta \geq 20$ keV/μm, respectively, in the Spread Out Bragg Peak (SOBP).[6-8] The category of HPT applies to all charged particles satisfying this definition, which in practice means all charged particles that have greater mass than protons. The conventional exclusion of protons from HPT reflects the proportional relationship between LET and Z_{eff}^2, that is, even the smallest possible increase in effective charge —from 1 (protons) to 2 (helium ions) —results in a 4-fold increase in LET. Carbon ions, the most common heavy charged particles in clinical use, have a +6 charge and an LET that is 36 times greater than that of protons.

Limitations of Proton Therapy in Resistant Disease

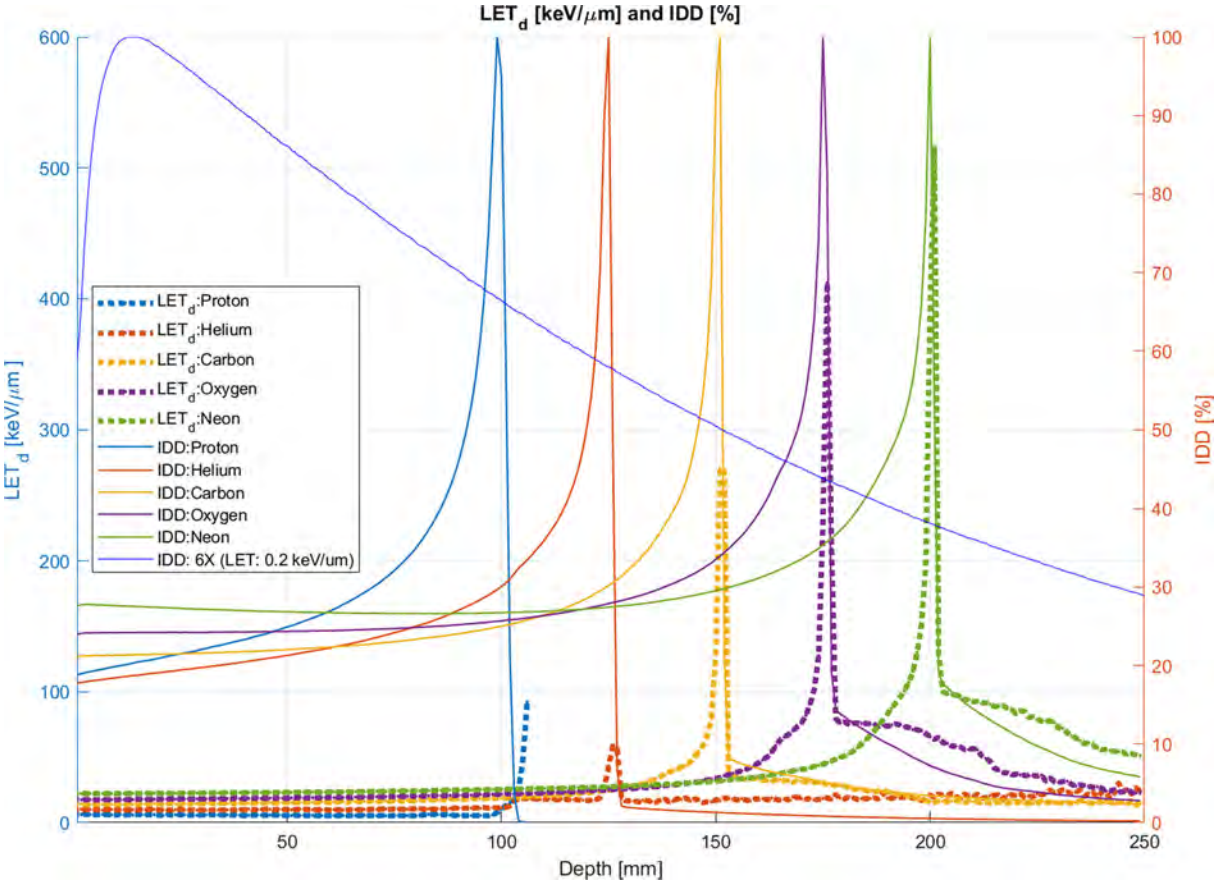
Low Z_{eff} sustains a photon-like low LET close to ~0.5-1 keV/μm throughout the proton beam range, including most of the SOBP.[7-13] Clinical proton irradiation, like photon irradiation, is sparsely ionizing and ill-suited to radioresistant targets. Rapid deceleration in the final few microns of the proton SOBP causes LET to rise as high as 80 keV/μm due to its inverse proportionality to β^2. However, in standard proton beam therapy (PBT), the need for good coverage of large, 3-D targets requires a broad SOBP, rendering the high LET distal edge clinically insignificant in terms of dose contribution (**Figure 1**).[7,8,14]

Despite efforts to improve PBT, the fundamental differences between proton beams and heavier ions render them physically and radiobiologically distinct therapeutic modalities.[10,15-18] From a purely dosimetric perspective, HPT offers advantages over PBT, including more robust optimization, smaller spot sizes, reduced lateral and longitudinal scatter, and steeper distal fall-off, though this is tempered by the presence of fragmentation tails beginning with particles as massive as carbon ions.[1,2,19] Along with its physical advantages, HPT may affect the clinical outcome of aggressive cancers such as PDAC and GBM, specifically with respect to the CSC biology driving them, due to its radiobiological properties.

Biological Effects of LET at the Molecular Level

Tracks of low LET radiation in biological matter are characterized by sparse, diffuse ionization events.[1,14,20-22] Each Gray of photon and proton radiation at therapeutic acceleration energies induces approximately 30-40 lethal double-stranded breaks (DSBs) at wide distances, facilitating easy repair compared with 1000 sublethal, single-stranded breaks (SSBs) and a greater number of nonlethal lesions to base pairs.[6,9,23-25] DNA damage from photon and proton therapy arises primarily through indirect ionization of reactive oxygen species (ROS) generated through water radiolysis, rather than through direct interaction with beam energy or δ-rays.[26-28] This indirect ionization requires cellular oxygen to "fix" lesions, which explains the relative radioresistance of hypoxic tissues to photon and proton radiation

Figure 1. Dose-averaged linear energy transfer (LET) and integrated depth-dose (IDD) per clinically relevant ion species. Note the increasing absolute LET, sharpness of distal fall-off, and fragmentation tails with increasing particle mass/effective charge.



and the similarity of their oxygen enhancement ratios (OERs), both approaching the maximum value of 3.[23,26-32] Sensitivity to photon and proton radiation is also modulated by the cell cycle. It is diminished during the G1 and S phases of the standard cell cycle when DSBs are more isolated due to chromatin condensation. Cell cycle radiosensitivity reaches its lowest point in the G0 phase, associated with stem-like cell states, when DNA repair mechanisms are at their most robust.[23,33-36]

Conventional radiation therapy (RT) is susceptible to synergistic cellular and microenvironmental resistance mechanisms caused by the structural dispersion of photon and proton beam energy, leading to entropy directly or through an intermediary of clonogenically insignificant isolated interactions. At the extreme end of contumacious CSC biology in PDAC and GBM, limited successes and serial failures of radical dose escalation of conventional RT may be caused by the microenvironmental CSC niche acting effectively as an energy sink. Higher doses in proximity to critical organs-at-risk (OARs) and/or exceeding volumetric thresholds threaten to narrow to the point of futility or even invert the therapeutic ratio, thereby allowing CSCs to escape any genuine clonogenic peril posed by conventional radiation.[37] LET escalation, conversely, obviates the futility of dose escalation and holds promise as a clinical solution to the CSC problem.

LET-Induced DNA Complexity and Repair Disruption

High LET radiation therapy differs from conventional RT at all physical doses. At the 10^0-10^1 nm scale of single DNA molecules, increasing ionization density brings a higher overall yield of DSB, a higher yield of clustered DSB—that is, 2 or more DSB produced by a single radiation track up to ~25 bp or 1-2 helical turns apart on a single DNA molecule—and a higher probability of direct rather than ROS-dependent indirect damage.[2,23,38,39] Whereas sparse DSB promote anti-apoptotic pathways,

particularly in p53-mutated stem-associated phenotypes typical of high-grade cancers, accumulation of clustered DSB reverses this pattern, suppressing anti-apoptotic BCL family factors prominently, and enabling non-p53-mediated apoptosis where the p53 pathway has been lost.[40-44] Experimental studies show that sufficiently high LET can efficiently induce apoptosis irrespective of p53 status.[45]

Clustered DSBs in proximity to each other create complex, multi-track lesions causing deletions upwards of 1000 bp in size, compared with <100 bp typical for low LET modalities. DNA repair mechanisms become less efficient and more error-prone, specifically by exhaustion of the high-fidelity homologous recombination mechanism forcing reliance on low-fidelity non-homologous end joining; SSB and base damages along the same track as DSB augment lethality as shown by XRCC1 recruitment at DSB foci.[1,38,46-48] High LET lesions also slow down the DNA repair process, shown by more durable γH2AX phosphorylation, inducing failed repair of potentially lethal lesions and sublethal lesions made lethal by second hits.[49]

Chromosomal Catastrophe and Multiple Cell Death Pathways

Frequent geometric variation of DSB promotes lethal mutations within chromosomes and, along with the fragmentation of large intact segments >1000 bp, exposes lesioned strands on different chromosomes to each other, inducing aberrant, nonviable chromosomal rearrangements such as rings, dicentrics, and micronuclei.[1,46,50-52] The volume and variability of lesion geometry, chromosomal rearrangement, and iteratively misrepaired DSB trigger cell death by mechanisms within and beyond the apoptotic spectrum, including autophagy, senescence, necroptosis, and necrosis.[48,51,53-60] Notably, impairment and delay of DNA repair cause cells with accumulated unrepaired DSBs escaping apoptosis and G2/M checkpoint arrest to form micronuclei upon entry into mitosis.[61-63]

In contrast to the stemness-promoting EMT often induced by conventional low LET irradiation, high LET irradiation tends to reverse EMT. Low LET promotes diffuse ROS and stimulates HIF complex stabilization with downstream EMT products, whereas high LET causes HIF destabilization and downregulation of its products.[64,65] Both high and low LET can produce ROS efficiently, yet the expected signaling pathways triggered by ROS, such as PI3K/Akt and EGFR, are suppressed under high LET irradiation.[66-70] Due to the density of indirect and direct ionization, structured propagation of ROS and oxidative stress occurs only locally along the track, evading the spatial distribution element required to trigger the usual signaling pathways.[71-73]

Multiscale Damage: From DNA to Tissue Architecture

High ionization density of heavy particles leads to intrinsically more lethal DNA lesions, overwhelms multiple DNA repair pathways, sensitizes hypoxic cells through various mechanisms, and activates cell death pathways that counteract resistance. These processes propagate over increasingly large structural scales. Clustered DSBs from separate tracks can aggregate into more complex lesions affecting structures from the level of nucleosomes (10^1 nm), chromatin loops (10^2 nm), entire chromosomes (10^3 nm), and multiple chromosomes across whole nuclei (10^4 nm), up to the architectural scale of tissues, including extranuclear organelles, neighboring cells, ECM, and stroma (10^5 nm).

At sufficiently high ionization density, a single radiation track can cause complex damage. Additionally, the extension of single-track clustered lesions across the chromatin ultrastructure multiplies the formation of lethal chromosomal aberrations and radiosensitizes resistant cell cycle phases as the shielding effect of chromatin packing and coiling is lost.[47,51,74]

Cytosolic DNA Exposure, Innate Immunity, and Abscopal Effects

As LET rises, progressively complex DNA damage leads to the formation of increasingly small, cleaved DNA fragments (<40 bp) capable of diffusing into the cytosol through intermittent nuclear membrane disruption. In parallel, persistent unrepaired or aberrantly repaired chromosomal lesions can rupture into the cytosol from unstable micronuclei—sometimes undergoing explosive fragmentation, as seen in chromothripsis.[61,62,75-77] Neoantigenic recognition in the extranuclear environment by damage-associated molecular pattern-sensing mechanisms triggers cascades of pro-immune cytokines stimulating MHC-1 upregulation and surface expression, $T_H1 > T_H2$ and M1 > M2 polarizations, dendritic cell maturation and phagocytosis, CD4 and CD8 lymphocyte infiltration, antitumoral natural killer (NK) cell priming, etc, culminating in immune cell death through both innate and adaptive immunity.[1,3,78-84] These immune effects may extend beyond the local tumor microenvironment to produce abscopal effects—immune-mediated regression of distant, untreated lesions.[85-100]

These features help explain the unique efficacy of high LET

irradiation in targeting CSCs, as demonstrated in basic research. In both in vitro and in vivo settings, alone and in combination with amplifying systemic agents, CIRT has been shown to reduce or eliminate the clonogenically viable subset of CD44-marked PDAC-CSCs.[101-103] In the GBM setting, HPT has shown even more robust findings in multiply reproduced culture and xenograft experiments. Both CIRT and high LET neutron therapy eradicate GBM-CSCs expressing the major CSC marker CD133 and shift the transcriptional profile of GBM cells away from pathways associated with stemness, invasion, angiogenesis, hypoxia, chemoradioresistance, and proliferation, including specific suppression of major stemness/EMT signaling pathways, including Akt and EGFR.[67,68,104-109] CSC sensitivity to CIRT has been shown to extend to multiple cancers in individual studies and systematic reviews.[110-113]

Toward Optimal LET for CSC Eradication

Within the typical LET range of the carbon ion SOBP of roughly 40-80 keV/µm, the OER is reduced but not fully abrogated, meaning some CSC-associated radioresistance mechanisms may persist. To achieve complete hypoxia-independent CSC sterilization, more massive ions such as oxygen, nitrogen, or neon —capable of generating LET ≥ 100 keV/µm within SOBP—may be required. However, practical application of ions with effective charge numbers (Z_{eff}) > 10 is likely limited by unmitigable toxicity to normal tissue.[1,29,72,114-119]

Conclusion

The physical and biological underpinnings of HPT, especially its ability to target CSCs via high LET-induced damage, provide a compelling foundation for its clinical application in aggressive and treatment-resistant tumors. GBM and PDAC, relatively common cancers with stubbornly poor prognoses, are auspicious use cases for CSC-directed therapy. In Part 2, we apply the foregoing principles to advanced techniques such as LET painting, biologically optimized spatial fractionation, and multi-ion therapy. These strategies are necessary to allow for the safe delivery of HPT, enabling the realization of its biological potential. We further explore the logistical barriers to wide implementation of HPT and prospects for overcoming them.

References

1) Tinganelli W, Durante M. Carbon ion radiobiology. *Cancers (Basel)*. 2020;12(10):3022. doi:10.3390/cancers12103022

2) Durante M, Loeffler JS. Charged particles in radiation oncology. *Nat Rev Clin Oncol*. 2010;7(1):37-43. doi:10.1038/nrclinonc.2009.183

3) Helm A, Fournier C. High-LET charged particles: radiobiology and application for new approaches in radiotherapy. *Strahlenther Onkol*. 2023;199(12):1225-1241. doi:10.1007/s00066-023-02158-7

4) Tommasino F, Scifoni E, Durante M. New ions for therapy. *Int J Part Ther*. 2016;2(3):428-438. doi:10.14338/IJPT-15-00027.1

5) Johnson D, Chen Y, Ahmad S. Dose and linear energy transfer distributions of primary and secondary particles in carbon ion radiation therapy: a monte carlo simulation study in water. *J Med Phys*. 2015;40(4):214-219. doi:10.4103/0971-6203.170785

6) Brahme A. Recent advances in light ion radiation therapy. *Int J Radiat Oncol*. 2004;58(2):603-616. doi:10.1016/j.ijrobp.2003.09.034

7) Kempe J, Gudowska I, Brahme A. Depth absorbed dose and LET distributions of therapeutic 1H, 4He, 7Li, and 12C beams. *Med Phys*. 2007;34(1):183-192. doi:10.1118/1.2400621

8) Kantemiris I, Karaiskos P, Papagiannis P, Angelopoulos A. Dose and dose averaged LET comparison of 1H. *Med Phys*. 2011;38(12):6585-6591. doi:10.1118/1.3662911

9) Tommasino F, Durante M. Proton radiobiology. *Cancers (Basel)*. 2015;7(1):353-381. doi:10.3390/cancers7010353

10) Smith EAK, Winterhalter C, Underwood TSA, et al. A monte carlo study of different LET definitions and calculation parameters for proton beam therapy. *Biomed Phys Eng Express*. 2021;8(1). doi:10.1088/2057-1976/ac3f50

11) Murshed H. Chapter 3—radiation biology. In: Murshed H, ed. *Fundamentals of Radiation Oncology*. 3rd ed. Academic Press. 2019:57-87. doi:10.1016/B978-0-12-814128-1.00003-9

12) Podgoršak EB, International Atomic Energy Agency. *Radiation Oncology Physics: A Handbook for Teachers and Students*. International Atomic Energy Agency; 2005.

13) Orton CG, ed. *Radiation Dosimetry: Physical and Biological Aspects*. Plenum Press; 1986. doi:10.1007/978-1-4899-0571-0

14) Byun HK, Han MC, Yang K, et al. Physical and biological characteristics of particle therapy for oncologists. *Cancer Res Treat*. 2021;53(3):611-620. doi:10.4143/crt.2021.066

15) Giovannini G, Böhlen T, Cabal G, et al. Variable RBE in proton therapy: comparison of different model predictions and their influence on clinical-like scenarios. *Radiat Oncol*. 2016;11(1):68. doi:10.1186/s13014-016-0642-6

16) McNamara A, Willers H, Paganetti H. Modelling variable proton relative biological effectiveness for treatment planning. *Br J Radiol*. 2020;93(1107):20190334. doi:10.1259/bjr.20190334

17) Underwood TSA, McNamara AL, Appelt A, et al. A systematic review of clinical studies on variable proton Relative Biological Effectiveness (RBE). *Radiother Oncol*. 2022;175:79-92. doi:10.1016/j.radonc.2022.08.014

18) McMahon SJ, Paganetti H, Prise KM. LET-weighted doses effectively reduce biological variability in proton radiotherapy planning. *Phys Med Biol*. 2018;63(22):225009. doi:10.1088/1361-6560/aae8a5

19) Knäusl B, Fuchs H, Dieckmann K, Georg D. Can particle beam therapy be improved using helium ions? - a planning study focusing on pediatric patients. *Acta Oncol*. 2016;55(6):751-759. doi:10.3109/0284186X.2015.1125016

20) Durante M, Debus J, Loeffler JS. Physics and biomedical challenges of cancer therapy with accelerated heavy ions. *Nat Rev Phys*. 2021;3(12):777-790. doi:10.1038/s42254-021-00368-5

21) Schlaff CD, Krauze A, Belard A, O'Connell JJ, Camphausen KA. Bringing the heavy: carbon ion therapy in the radiobiological and clinical context. *Radiat Oncol*. 2014;9(1):88. doi:10.1186/1748-717X-9-88

22) Weber U, Kraft G. Comparison of carbon ions versus protons. *Cancer J*. 2009;15(4):325-332. doi:10.1097/PPO.0b013e3181b01935

23) Hill MA. Radiation track structure: how the spatial distribution of energy deposition drives biological response. *Clin Oncol*. 2020;32(2):75-83. doi:10.1016/j.clon.2019.08.006

24) Hall EJ, Giaccia AJ. *Radiobiology for the Radiologist*. 8th ed. Wolters Kluwer; 2019.

25) Kinner A, Wu W, Staudt C, Iliakis G. Gamma-H2AX in recognition and signaling of DNA double-strand breaks in the context of chromatin. *Nucleic Acids Res*. 2008;36(17):5678-5694. doi:10.1093/nar/gkn550

26) Desouky O, Ding N, Zhou G. Targeted and non-targeted effects of ionizing radiation. *J Radiat Res Appl Sci*. 2015;8(2):247-254. doi:10.1016/j.jrras.2015.03.003

27) Lee KB, Lee JS, Park JW, Huh TL, Lee YM. Low energy proton beam induces tumor cell apoptosis through reactive oxygen species and activation of caspases. *Exp Mol Med*. 2008;40(1):118-129. doi:10.3858/emm.2008.40.1.118

28) Rakotomalala A, Escande A, Furlan A, Meignan S, Lartigau E. Hypoxia in solid tumors: how low oxygenation impacts the "six Rs" of radiotherapy. *Front Endocrinol*. 2021;12. doi:10.3389/fendo.2021.742215

29) Scifoni E, Tinganelli W, Weyrather WK, et al. Including oxygen enhancement ratio in ion beam treatment planning: model implementation and experimental verification. *Phys Med Biol*. 2013;58(11):3871-3895. doi:10.1088/0031-9155/58/11/3871

30) Stewart RD, Streitmatter SW, Argento DC, et al. Rapid MCNP simulation of DNA Double Strand Break (DSB) Relative Biological Effectiveness (RBE) for photons, neutrons, and light ions. *Phys Med Biol*. 2015;60(21):8249-8274. doi:10.1088/0031-9155/60/21/8249

31) Bouleftour W, Rowinski E, Louati S, et al. A review of the role of hypoxia in radioresistance in cancer therapy. *Med Sci Monit*. 2021;27. doi:10.12659/MSM.934116

32) Crabtree HG, Cramer W, Murray JA. The action of radium on cancer cells. II—some factors determining the susceptibility of cancer cells to radium. *Proc R Soc Lond B*. 1933;113(782):238-250. doi:10.1098/rspb.1933.0044

33) Pawlik TM, Keyomarsi K. Role of cell cycle in mediating sensitivity to radiotherapy. *Int J Radiat Oncol Biol Phys*. 2004;59(4):928-942. doi:10.1016/j.ijrobp.2004.03.005

34) Krude T, Keller C. Chromatin assembly during s phase: contributions from histone deposition, DNA replication and the cell division cycle. *Cell Mol Life Sci*. 2001;58(5-6):665-672. doi:10.1007/pl00000890

35) Ma Y, Kanakousaki K, Buttitta L. How the cell cycle impacts chromatin architecture and influences cell fate. *Front Genet*. 2015;6:19. doi:10.3389/fgene.2015.00019

36) Zhao L, Wu D, Mi D, Sun Y. Radiosensitivity and relative biological effectiveness based on a generalized target model. *J Radiat Res*. 2017;58(1):8-16. doi:10.1093/jrr/rrw062

37) Chan JL, Lee SW, Fraass BA, et al. Survival and failure patterns of high-grade gliomas after three-dimensional conformal radiotherapy. *J Clin Oncol*. 2002;20(6):1635-1642. doi:10.1200/JCO.2002.20.6.1635

38) Liu W, Wu R, Guo J, et al. High turnover and rescue effect of XRCC1 in response to heavy charged particle radiation. *Biophys J*. 2022;121(8):1493-1501. doi:10.1016/j.bpj.2022.03.011

39) Hirayama R, Ito A, Tomita M, et al. Contributions of direct and indirect actions in cell killing by high-LET radiations. *Radiat Res*. 2009;171(2):212-218. doi:10.1667/RR1490.1

40) Lips J, Kaina B. DNA double-strand breaks trigger apoptosis in p53-deficient fibroblasts. *Carcinogenesis*. 2001;22(4):579-585. doi:10.1093/carcin/22.4.579

41) Roos WP, Kaina B. DNA damage-induced cell death by apoptosis. *Trends Mol Med*. 2006;12(9):440-450. doi:10.1016/j.molmed.2006.07.007

42) Zhang Y, Dube C, Gibert M, et al. The p53 pathway in glioblastoma. *Cancers*. 2018;10(9):297. doi:10.3390/cancers10090297

43) Weissmueller S, Manchado E, Saborowski M, et al. Mutant p53 drives pancreatic cancer metastasis through cell-autonomous PDGF receptor β signaling. *Cell*. 2014;157(2):382-394. doi:10.1016/j.cell.2014.01.066

44) Li S, Huang H, Xing M, et al. Carbon ion induces cell death and G2/m arrest through pRb/E2F1Chk2/Cdc2 signaling pathway in x-ray resistant B16F10 melanoma cells. *Dose Response*. 2022;20(2). doi:10.1177/15593258221092364

45) Takahashi A, Matsumoto H, Furusawa Y, et al. Apoptosis induced by high-LET radiations is not affected by cellular p53 gene status. *Int J Radiat Biol*. 2005;81(8):581-586. doi:10.1080/09553000500280484

46) Asaithamby A, Hu B, Chen DJ. Unrepaired clustered DNA lesions induce chromosome breakage in human cells. *Proc Natl Acad Sci USA*. 2011;108(20):8293-8298. doi:10.1073/pnas.1016045108

47) Mladenova V, Mladenov E, Stuschke M, Iliakis G. DNA damage clustering after ionizing radiation and consequences in the processing of chromatin breaks. *Molecules*. 2022;27(5):1540. doi:10.3390/molecules27051540

48) Hagiwara Y, Oike T, Niimi A, et al. Clustered DNA double-strand break formation and the repair pathway following heavy-ion irradiation. *Journal of Radiation Research*. 2019;60(1):69-79. doi:10.1093/jrr/rry096

49) Lopez Perez R, Nicolay NH, Wolf J-C, et al. DNA damage response of clinical carbon ion versus photon radiation in human glioblastoma cells. *Radiother Oncol J Eur Soc Ther Radiol Oncol*. 2019;133:77-86. doi:10.1016/j.radonc.2018.12.028

50) Ward JF. The complexity of DNA damage: relevance to biological consequences. *Int J Radiat Biol*. 1994;66(5):427-432. doi:10.1080/09553009414551401

51) Wilkinson B, Hill MA, Parsons JL. The cellular response to complex DNA damage induced by ionising radiation. *Int J Mol Sci*. 2023;24(5):4920. doi:10.3390/ijms24054920

52) Singleton BK, Griffin CS, Thacker J. Clustered DNA damage leads to complex genetic changes in irradiated human cells. *Cancer Res*. 2002;62(21):6263-6269.

53) Borges HL, Linden R, Wang JY. DNA damage-induced cell death. *Cell Res*. 2008;18(1):17-26. doi:10.1038/cr.2007.110

54) Wang JYJ. DNA damage and apoptosis. *Cell Death Differ*. 2001;8(11):1047-1048. doi:10.1038/sj.cdd.4400938

55) Jinno-Oue A, Shimizu N, Hamada N, et al. Irradiation with carbon ion beams induces apoptosis, autophagy, and cellular senescence in a human glioma-derived cell line. *Int J Radiat Oncol*. 2010;76(1):229-241. doi:10.1016/j.ijrobp.2009.08.054

56) Sai S, Koto M, Yamada S. Basic and translational research on carbon-ion radiobiology. *Am J Cancer Res*. 2023;13(1):1-24.

57) Wang X, Chen X, Li G, et al. Application of carbon ion and its sensitizing agent in cancer therapy: a systematic review. *Front Oncol*. 2021;11:708724. doi:10.3389/fonc.2021.708724

58) Koom WS, Sai S, Suzuki M, et al. Superior effect of the combination of carbon-ion beam irradiation and 5-fluorouracil on colorectal cancer stem cells in vitro and in vivo. *Onco Targets Ther*. 2020;13:12625-12635. doi:10.2147/OTT.S276035

59) Chaurasia M, Bhatt AN, Das A, Dwarakanath BS, Sharma K. Radiation-induced autophagy: mechanisms and consequences. *Free Radic Res*. 2016;50(3):273-290. doi:10.3109/10715762.2015.1129534

60) Bao C, Sun Y, Dwarakanath B, et al. Carbon ion triggered immunogenic necroptosis of nasopharyngeal carcinoma cells involving necroptotic inhibitor BCL-x. *J Cancer*. 2021;12(5):1520-1530. doi:10.7150/jca.46316

61) Roberts CJ, Morgan GR, Holt PD. A critical comparison of the micronucleus yield from high and low LET irradiation of plateau-phase cell populations. *Mutat Res*. 1986;160(3):237-242. doi:10.1016/0027-5107(86)90133-8

62) Durante M, Formenti SC. Radiation-induced chromosomal aberrations and immunotherapy: micronuclei, cytosolic DNA, and interferon-production pathway. *Front Oncol*. 2018;8:192. doi:10.3389/fonc.2018.00192

63) Akudugu JM, Slabbert JP, Roos WP, Böhm L. Micronucleus response of human glioblastoma and neuroblastoma cells toward low-LET photon and high-LET p(66)/be neutron irradiation. *Am J Clin Oncol*. 2003;26(3):e1-6. doi:10.1097/01.COC.0000072500.18954.2D

64) Subtil FSB, Wilhelm J, Bill V, et al. Carbon ion radiotherapy of human lung cancer attenuates HIF-1 signaling and acts with considerably enhanced therapeutic efficiency. *FASEB J*. 2014;28(3):1412-1421. doi:10.1096/fj.13-242230

65) Wozny A-S, Lauret A, Battiston-Montagne P, et al. Differential pattern of HIF-1α expression in HNSCC cancer stem cells after carbon ion or photon irradiation: one molecular explanation of the oxygen effect. *Br J Cancer*. 2017;116(10):1340-1349. doi:10.1038/bjc.2017.100

66) Konings K, Vandevoorde C, Baselet B, Baatout S, Moreels M. Combination therapy with charged particles and molecular targeting: a promising avenue to overcome radioresistance. *Front Oncol*. 2020;10:128. doi:10.3389/fonc.2020.00128

67) Stahler C, Roth J, Cordes N, Taucher-Scholz G, Mueller-Klieser W. Impact of carbon ion irradiation on epidermal growth factor receptor signaling and glioma cell migration in comparison to conventional photon irradiation. *Int J Radiat Biol*. 2013;89(6):454-461. doi:10.3109/09553002.2013.766769

68) Jin X, Li F, Zheng X, et al. Carbon ions induce autophagy effectively through stimulating the unfolded protein response and subsequent inhibiting akt phosphorylation in tumor cells. *Sci Rep*. 2015;5:13815. doi:10.1038/srep13815

69) Shi X, Zhang Y, Zheng J, Pan J. Reactive oxygen species in cancer stem cells. *Antioxid Redox Signal*. 2012;16(11):1215-1228. doi:10.1089/ars.2012.4529

70) Checa J, Aran JM. Reactive oxygen species: drivers of physiological and pathological processes. *J Inflamm Res*. 2020;13:1057-1073. doi:10.2147/JIR.S275595

71) Tsao D, Kalogerinis P, Tabrizi I, et al. Induction and processing of oxidative clustered DNA lesions in 56fe-ion-irradiated human monocytes. *Radiat Res*. 2007;168(1):87-97. doi:10.1667/RR0865.1

72) Stewart RD, Yu VK, Georgakilas AG, et al. Effects of radiation quality and oxygen on clustered DNA lesions and cell death. *Radiat Res*. 2011;176(5):587-602. doi:10.1667/rr2663.1

73) Wozny AS, Rodriguez-Lafrasse C. The "stealth-bomber" paradigm for deciphering the tumour response to carbon-ion irradiation. *Br J Cancer*. 2023;128(8):1429-1438. doi:10.1038/s41416-022-02117-6

74) Lorat Y, Timm S, Jakob B, Taucher-Scholz G, Rübe CE. Clustered double-strand breaks in heterochromatin perturb DNA repair after high linear energy transfer irradiation. *Radiother Oncol J Eur Soc Ther Radiol Oncol*. 2016;121(1):154-161. doi:10.1016/j.radonc.2016.08.028

75) Gekara NO. DNA damage-induced immune response: micronuclei provide key platform. *J Cell Biol*. 2017;216(10):2999-3001. doi:10.1083/jcb.201708069

76) Pang D, Winters TA, Jung M, et al. Radiation-generated short DNA fragments may perturb non-homologous end-joining and induce genomic instability. *J Radiat Res*. 2011;52(3):309-319. doi:10.1269/jrr.10147

77) Pang D, Chasovskikh S, Rodgers JE, Dritschilo A. Short DNA fragments are a hallmark of heavy charged-particle irradiation and may underlie their greater therapeutic efficacy. *Front Oncol*. 2016;6:130. doi:10.3389/fonc.2016.00130

78) Huang Y, Dong Y, Zhao J, et al. Comparison of the effects of photon, proton and carbon-ion radiation on the ecto-calreticulin exposure in various tumor cell lines. *Ann Transl Med*. 2019;7(20):542. doi:10.21037/atm.2019.09.128

79) Yoshimoto Y, Oike T, Okonogi N, et al. Carbon-ion beams induce production of an immune mediator protein, high mobility group box 1, at levels comparable with x-ray irradiation. *J Radiat Res*. 2015;56(3):509-514. doi:10.1093/jrr/rrv007

80) Ebner DK, Tinganelli W, Helm A, et al. The immunoregulatory potential of particle radiation in cancer therapy. *Front Immunol*. 2017;8:99. doi:10.3389/fimmu.2017.00099

81) Zhou H, Tu C, Yang P, et al. Carbon ion radiotherapy triggers immunogenic cell death and sensitizes melanoma to anti-PD-1 therapy in mice. *Oncoimmunology*. 2022;11(1):2057892. doi:10.1080/2162402X.2022.2057892

82) Onishi M, Okonogi N, Oike T, et al. High linear energy transfer carbon-ion irradiation increases the release of the immune mediator high mobility group box 1 from human cancer cells. *J Radiat Res*. 2018;59(5):541-546. doi:10.1093/jrr/rry049

83) Guo Y, Shen R, Wang F, et al. Carbon ion irradiation induces DNA damage in melanoma and optimizes the tumor microenvironment based on the cgas–STING pathway. *J Cancer Res Clin Oncol*. 2023;149(9):6315-6328. doi:10.1007/s00432-023-04577-6

84) Pompos A, Durante M, Choy H. Heavy ions in cancer therapy. *JAMA Oncol*. 2016;2(12):1539-1540. doi:10.1001/jamaoncol.2016.2646

85) Matsunaga A, Ueda Y, Yamada S, et al. Carbon-ion beam treatment induces systemic antitumor immunity against murine squamous cell carcinoma. *Cancer*. 2010;116(15):3740-3748. doi:10.1002/cncr.25134

86) Lussier DM, Alspach E, Ward JP, et al. Radiation-induced neoantigens broaden the immunotherapeutic window of cancers with low mutational loads. *Proc Natl Acad Sci U S A*. 2021;118(24):e2102611118. doi:10.1073/pnas.2102611118

87) Ando K, Fujita H, Hosoi A, et al. Intravenous dendritic cell administration enhances suppression of lung metastasis induced by carbon-ion irradiation. *J Radiat Res*. 2017;58(4):446-455. doi:10.1093/jrr/rrx005

88) Ogata T, Teshima T, Kagawa K, et al. Particle irradiation suppresses metastatic potential of cancer cells. *Cancer Res*. 2005;65(1):113-120.

89) Akino Y, Teshima T, Kihara A, et al. Carbon-ion beam irradiation effectively suppresses migration and invasion of human non-small-cell lung cancer cells. *Int J Radiat Oncol Biol Phys*. 2009;75(2):475-481. doi:10.1016/j.ijrobp.2008.12.090

90) Takahashi Y, Yasui T, Minami K, et al. Carbon ion irradiation enhances the antitumor efficacy of dual immune checkpoint blockade therapy both for local and distant sites in murine osteosarcoma. *Oncotarget*. 2019;10(6):633-646. doi:10.18632/oncotarget.26551

91) Fujita M, Imadome K, Shoji Y, et al. Carbon-ion irradiation suppresses migration and invasiveness of human pancreatic carcinoma cells miapaca-2 via rac1 and rhoa degradation. *Int J Radiat Oncol Biol Phys*. 2015;93(1):173-180. doi:10.1016/j.ijrobp.2015.05.009

92) Harding SM, Benci JL, Irianto J, et al. Mitotic progression following DNA damage enables pattern recognition within micronuclei. *Nature*. 2017;548(7668):466-470. doi:10.1038/nature23470

93) Katsuki S, Takahashi Y, Tamari K, et al. Radiation therapy enhances systemic antitumor efficacy in PD-L1 therapy regardless of sequence of radiation in murine osteosarcoma. *PLOS ONE*. 2022;17(7):e0271205. doi:10.1371/journal.pone.0271205

94) Zhou H, Yang P, Li H, et al. Carbon ion radiotherapy boosts anti-tumour immune responses by inhibiting myeloid-derived suppressor cells in melanoma-bearing mice. *Cell Death Discov*. 2021;7(1):332):332:. doi:10.1038/s41420-021-00731-6

95) Iijima M, Okonogi N, Nakajima NI, et al. Significance of PD-L1 expression in carbon-ion radiotherapy for uterine cervical adeno/adenosquamous carcinoma. *J Gynecol Oncol*. 2020;31(2):e19. doi:10.3802/jgo.2020.31.e19

96) Hartmann L, Schröter P, Osen W, et al. Photon versus carbon ion irradiation: immunomodulatory effects exerted on murine tumor cell lines. *Sci Rep*. 2020;10(1):21517. doi:10.1038/s41598-020-78577-8

97) Du J, Kageyama SI, Hirata H, et al. Comparative analysis of the immune responses in cancer cells irradiated with x-ray, proton and carbon-ion beams. *Biochem Biophys Res Commun*. 2021;585:55-60. doi:10.1016/j.bbrc.2021.11.004

98) Wang J, Dai Z, Miao Y, et al. Carbon ion (12C6+) irradiation induces the expression of klrk1 in lung cancer and optimizes the tumor microenvironment based on the NKG2D/NKG2D-ls pathway. *Cancer Letters*. 2021;521:178-195. doi:10.1016/j.canlet.2021.09.003

99) Moncharmont C, Guy JB, Wozny AS, et al. Carbon ion irradiation withstands cancer stem cells' migration/invasion process in Head and Neck Squamous Cell Carcinoma (HNSCC). *Oncotarget*. 2016;7(30):47738-47749. doi:10.18632/oncotarget.10281

100) Tubin S, Yan W, Mourad WF, Fossati P, Khan MK. The future of radiation-induced abscopal response: beyond conventional radiotherapy approaches. *Future Oncol*. 2020;16(16):1137-1151. doi:10.2217/fon-2020-0063

101) Sai S, Kim EH, Koom WS, et al. Carbon-ion beam irradiation and the mir-200c mimic effectively eradicate pancreatic cancer stem cells under in vitro and in vivo conditions. *OncoTargets Ther*. 2021;14:4749-4760. doi:10.2147/OTT.S311567

102) Sai S, Wakai T, Vares G, et al. Combination of carbon ion beam and gemcitabine causes irreparable DNA damage and death of radioresistant pancreatic cancer stem-like cells in vitro and in vivo. *Oncotarget*. 2015;6(8):5517-5535. doi:10.18632/oncotarget.3584

103) Oonishi K, Cui X, Hirakawa H, et al. Different effects of carbon ion beams and x-rays on clonogenic survival and DNA repair in human pancreatic cancer stem-like cells. *Radiother Oncol*. 2012;105(2):258-265. doi:10.1016/j.radonc.2012.08.009

104) Baek S-J, Ishii H, Tamari K, et al. Cancer stem cells: the potential of carbon ion beam radiation and new radiosensitizers (review). *Oncol Rep*. 2015;34(5):2233-2237. doi:10.3892/or.2015.4236

105) Chiblak S, Tang Z, Lemke D, et al. Carbon irradiation overcomes glioma radioresistance by eradicating stem cells and forming an antiangiogenic and immunopermissive niche. *JCI Insight*. 2019;4(2):e123837. doi:10.1172/jci.insight.123837

106) Chiblak S, Tang Z, Campos B, et al. Radiosensitivity of patient-derived glioma stem cell 3-dimensional cultures to photon, proton, and carbon irradiation. *Int J Radiat Oncol*. 2016;95(1):112-119. doi:10.1016/j.ijrobp.2015.06.015

107) Hirota Y, Masunaga SI, Kondo N, et al. High linear-energy-transfer radiation can overcome radioresistance of glioma stem-like cells to low linear-energy-transfer radiation. *J Radiat Res*. 2014;55(1):75-83. doi:10.1093/jrr/rrt095

108) Ciamarone F, Meister S, Furkel J, et al. Abstract 1105: carbon ion radiotherapy eradicates glioblastoma via tumor immune environment reprograming. *Cancer Res*. 2023;83(7_supplement):1105-1105. doi:10.1158/1538-7445.AM2023-1105

109) Valable S, Gérault AN, Lambert G, et al. Impact of hypoxia on carbon ion therapy in glioblastoma cells: modulation by LET and hypoxia-dependent genes. *Cancers (Basel)*. 2020;12(8):2019. doi:10.3390/cancers12082019

110) Vares G, Ahire V, Sunada S, et al. A multimodal treatment of carbon ions irradiation, mirna-34 and mtor inhibitor specifically control high-grade chondrosarcoma cancer stem cells. *Radiother Oncol*. 2020;150:253-261. doi:10.1016/j.radonc.2020.07.034

111) Sai S, Vares G, Kim EH, et al. Carbon ion beam combined with cisplatin effectively disrupts triple negative breast cancer stem-like cells in vitro. *Mol Cancer*. 2015;14(1):166. doi:10.1186/s12943-015-0429-7

112) Wang Q, Liu R, Zhang Q, et al. Biological effects of cancer stem cells irradiated by charged particle: a systematic review of in vitro studies. *J Cancer Res Clin Oncol*. 2023;149(9):6625-6638. doi:10.1007/s00432-022-04561-6

113) Cui X, Oonishi K, Tsujii H, et al. Effects of carbon ion beam on putative colon cancer stem cells and its comparison with x-rays. *Cancer Res*. 2011;71(10):3676-3687. doi:10.1158/0008-5472.CAN-10-2926

114) Lee SH, Mizushima K, Kohno R, et al. Estimating the biological effects of helium, carbon, oxygen, and neon ion beams using 3D silicon microdosimeters. *Phys Med Biol*. 2021;66(4):045017. doi:10.1088/1361-6560/abd66f

115) Sokol O, Scifoni E, Tinganelli W, et al. Oxygen beams for therapy: advanced biological treatment planning and experimental verification. *Phys Med Biol*. 2017;62(19):7798-7813. doi:10.1088/1361-6560/aa88a0

116) Kase Y, Kanai T, Matsumoto Y, et al. Microdosimetric measurements and estimation of human cell survival for heavy-ion beams. *Radiat Res*. 2006;166(4):629-638. doi:10.1667/RR0536.1

117) Inaniwa T, Suzuki M, Hyun Lee S, et al. Experimental validation of stochastic microdosimetric kinetic model for multi-ion therapy treatment planning with helium-, carbon-, oxygen-, and neon-ion beams. *Phys Med Biol*. 2020;65(4):045005. doi:10.1088/1361-6560/ab6eba

118) Tessonnier T, Mairani A, Brons S, et al. Experimental dosimetric comparison of 1H, 4He, 12C and 16O scanned ion beams. *Phys Med Biol*. 2017;62(10). doi:10.1088/1361-6560/aa6516

119) Tran LT, Bolst D, Guatelli S, et al. The relative biological effectiveness for carbon, nitrogen, and oxygen ion beams using passive and scanning techniques evaluated with fully 3D silicon microdosimeters. *Med Phys*. 2018;45(5):2299-2308. doi:10.1002/mp.12874

Eradicating Cancer Stem Cells Using High Linear Energy Transfer Radiation Therapy Part 2: LET Painting and Other Advanced Techniques

Description

The authors describe advanced high linear energy transfer (LET) techniques, including LET painting, biologically guided spatial fractionation, and multi-ion therapy, as potential methods to selectively ablate cancer stem cells while sparing normal tissue. The article highlights recent imaging advances that could help define high-risk tumor subvolumes and outlines logistical and clinical challenges to widespread implementation, making a compelling case for heavy particle therapy as a means of augmenting systemic cancer control.

Learning Objectives

Upon completing this activity:

- Clinicians will be able to discuss the principles of LET painting and multi-ion heavy particle therapy as emerging strategies to selectively target cancer stem cell–rich tumor subvolumes.
- Clinicians will be prepared to utilize functional imaging modalities to target both hypoxia and the underlying biological properties of cancer stem cells.
- Clinicians will be able to incorporate PATHY and grid/lattice spatial fractionation techniques into clinical practice while understanding the relative advantages and disadvantages of each approach.

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Target Audience

- Radiation oncologists
- Related oncology professionals

Commercial Support

None

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Eradicating Cancer Stem Cells Using High Linear Energy Transfer Radiation Therapy Part 2: LET Painting and Other Advanced Techniques

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Abstract

Linear energy transfer (LET), a measurement of ionization density, tracks the radiobiological potency of any given course of therapeutic radiation and its efficacy in killing cancer cells. As opposed to the low LET of photon and proton therapy, high LET charged particle therapy can overcome multiple mechanisms of resistance to effectively treat radioresistant tumors. A robust basic science literature demonstrates enhanced direct cancer stem cell (CSC) sterilization with increasing LET along with indirect mechanisms of tumor control such as immunogenesis. Such a strategy has yet to be implemented in clinical practice in the absence of an effective means of targeting CSCs without risking unacceptable harms to patients. In Part 2 of this 2-part series, we review newly emergent functional imaging technologies in conjunction with existing techniques of spatial fractionation and capabilities for multi-ionic therapy that hold promise as a means of translating the biological potential of high LET therapy into clinical protocols for effective anti-CSC therapy.

Keywords: LET, linear energy transfer, particle therapy, carbon ion therapy, heavy particle therapy, radiobiology, radiation physics, spatial fractionation, ion therapy

Introduction

Building upon the physical and radiobiological advantages of high linear energy transfer (LET) discussed in "Eradicating Cancer Stem Cells Using High Linear Energy Transfer Radiation Therapy Part 1: Physics and Radiobiology," this article examines the clinical translation of heavy particle therapy (HPT), especially via LET painting, to overcome cancer stem

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Disclosures: The authors have no conflicts of interest to disclose. None of the authors received outside funding for the production of this original manuscript and no part of this article has been previously published elsewhere.

Published: June 18, 2025. https://doi.org/10.37549/ARO-D-25-00003P2

cell (CSC)-related resistance. It explores how multi-ion strategies, spatial fractionation, and biologically guided planning enable targeted dose intensification to resistant subregions while sparing normal tissues. In doing so, it addresses not only technical implementation but also economic, logistical, and immunological challenges and opportunities.

Clinical Implementation of HPT

LET Painting for Treatment Planning Adaptive to Tumor Heterogeneity

The peak-to-plateau ratios of HPT, based on changes in LET over the course of the beam path, enable "LET painting" or "kill painting"--that is, the conformal and selective escalation of LET to a defined subvolume within a larger target volume that is otherwise treated with lower LET.[1-6] LET painting was developed initially to overcome the radioresistance of hypoxic tumor subregions using functional tracers such as ^{18}F-FMISO for guidance.[3] More recently, ionic copper-based hypoxia tracers such as ^{64}Cu-ATSM and ^{64}Cu-NOTA have been well-validated in cell and animal experiments. Though still early in their clinical development, these agents may allow for a more direct means of CSC targeting, not merely via hypoxia surrogacy, but through biological affinity for stemness markers such as CD133.[7-10] This shift suggests the potential for LET painting to transition from targeting microenvironmental resistance (i.e., hypoxia) to directly mapping and ablating CSC populations.

Conjugation of ^{64}CuNOTA to antibodies targeting the AC133 epitope of CD133 has enabled high-contrast detection of CD133-expressing gliomas in murine xenografts using both PET and near-infrared fluorescence molecular tomography.[11] Direct detection of CSCs using superparamagnetic iron oxide nanoparticles conjugated to appropriate homing moieties to CSC biomarkers such as AC133, AC141, CD44v6, and CD109 is under preclinical investigation by the present authors. This approach may allow for CSC delineation at the spatial resolution conditions of MRI.[12]

If successfully translated to the clinic, such functional imaging could support the identification of both high-biological risk tumor volumes (HRTVs) for targeted LET escalation, while defining low-biological risk tumor volumes (LRTV = GTV – HRTVs) for standard or de-escalated treatment. The most precise and conformal LET painting strategies may ultimately rely on treatment plans incorporating combinations of different ionic beams.[10,13] The addition of multiple ion species to a plan augments LET ranges for given doses, volume sizes and shapes, and desired gradients.

The potential for de-escalated therapy to LRTVs is no less important than escalated therapy to HRTVs in establishing HPT as a clinically viable strategy for improving outcomes in resistant cancers. Just as HPT is uniquely capable of sterilizing the most resistant malignant cells, its radiobiological potency creates peril for even those normal tissues capable of resisting injury by high doses of conventional radiation therapy (RT). Moreover, normal tissue injury as a result of HPT is more likely to be irreparable than that caused by conventional therapy.

A cautionary precedent can be found in a clinical trial in neon ion radiation therapy (NIRT) for glioblastoma (GBM) conducted at the Lawrence Berkeley Laboratory (LBL). Although the trial was terminated prematurely due to the facility's closure, early outcomes included tumor control and survival nominally comparable or superior to that seen in the modern treatment of GBM. However, these were accompanied by high grade late toxicities including potential treatment-related grade 5 toxicity in patients whose tumors had been controlled.[14] LET painting is thus an essential treatment planning strategy for safe clinical implementation of HPT with significantly augmented therapeutic ratio vs that of conventional RT.

Apart from protons and carbon ions already established in clinical use, research has been done on other species, including helium, lithium, oxygen, and neon ions.[10,13-19] Clinical helium ion therapy has commenced at the Heidelberg Ion Therapy Center (HIT, Germany), with clinical oxygen ion therapy under development.[20] Mayo Clinic Florida likewise plans to attain capacity for combination heavy ion therapy. Unlike other oncologic therapies, HPT offers modularity in dose distribution and potency, facilitating the individualization of therapeutic prescription, including at the level of intra-tumor heterogeneity.[21-24] Even before the clinical availability of multi-ionic radiation therapy (MIRT), the intensity-modulated composite particle therapy (IMPACT) and spot-scanning hadron arc (SHArc) models at the National Institute of Radiological Sciences (NIRS, Japan) and HIT, respectively, have been validated in Monte Carlo simulation for multi-ion treatment planning using LET painting and direct LET-based optimization extended to cover treatment with any combination of protons, helium ions, carbon ions, oxygen ions, and neon ions (**Figure 1**).[4-625-31] The greater the variety of LET spectra from different ions for treatment, the steeper the achievable LET gradients, the less the LET delivered to one voxel forces the LET range deliverable to adjacent voxels. Improved conformality of LET distributions to irregularly shaped targets such as those delineated by functional imaging

enables painting of LET gradients onto planning imaging at will.[3-510,13,,31] Preclinical and clinical studies of MIRT are summarized in **Table 1**.

HPT Planning: Spatial Fractionation to Maximize CSC Kill and Minimize Toxicity

LET painting with MIRT has particularly favorable properties for targeting CSCs in pancreatic ductal adenocarcinoma (PDAC) and GBM. In GBM, radiation volumes often extend to near-hemispheric dimensions due to the tumor's spread, while in PDAC, the proximity of critical organs at risk (OARs), particularly the duodenum, necessitates strict dose limitations or reductions.[34-36]

Although mono-ionic beams at ultra-high LET can sterilize CSCs throughout tumor organoids or xenografts without spatial discrimination, achieving uniform coverage to a CSC-ablative LET level across the gross tumor volume (GTV) in patients is substantially more challenging. The CLEOPATRA phase II randomized clinical trial, aiming to improve overall survival in GBM using carbon ion radiation therapy (CIRT), underscores this limitation. Of the total prescribed RT dose, only 18 Gy (RBE)—roughly 25%—was delivered with CIRT, with the remaining dose administered using conventional low LET photons, most likely due to increasing rates of toxicities such as high-grade radiation necrosis increasing with irradiated volume and dose, even at low LET.[37,38]

Although the final results from CLEOPATRA have not yet been published, it is plausible that the CIRT component was insufficient to yield a measurable survival benefit. Considering the standard curative-intent dose of 60 Gy, it is probable that, even if a signal exists, too little CIRT was given to capture it. In other words, directing HPT to entire GTVs frequently necessitates compromising dose and/or LET below the necessary threshold for optimal biological effect on the tumor and CSCs.

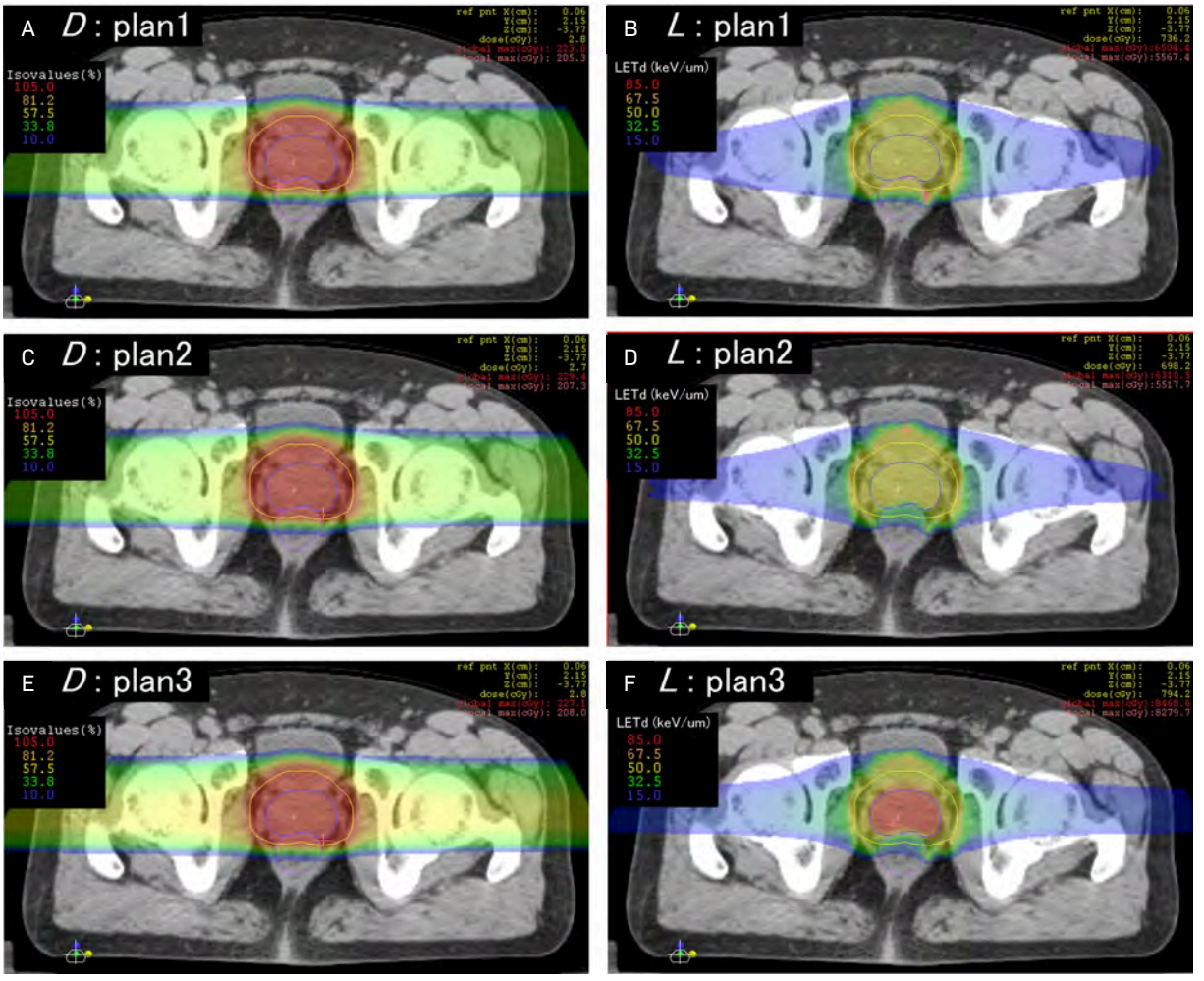
In our view, the superior strategy is the one that LET painting enables, namely partial volume ablation at high dose/high LET combinations, with the remainder of relatively radiosensitive and non-clonogenic volumes treated with a lower intensity/less toxic intervention, or even none at all. The concept of therapeutic modulation across tumor subregions—central to LET painting—already has a precedent in clinical practice through spatial fractionation, which is employed in the palliative setting for refractory, unresectable disease.[39] Though not technically a novel approach, spatially fractionated radiation therapy has resurged in the literature with improved delivery mechanisms and the utilization of immunotherapy in cancer care. Multiple techniques appear today; most prevalent is grid or lattice radiation therapy, painting a milieu of dose upon a tumor with focal peaks in dose surrounded by low-dose valleys, demonstrating marked response.[40] This approach potentially generates an immunological reaction to targeted tumor tissue, but the impact on CSCs is unknown. The grid is characterized by dose spheres with relatively random distribution within a larger, bulkier tumor, allowing the tumor tissue to provide a safe margin between dose peaks and OARs.

Focusing on immunological development and enhanced dose deposition in hypoxic CSC regions, the PArtial Tumor Irradiation Targeting HYpoxic Segment (PATHY) approach aims to selectively irradiate tumors in a more directed manner. PATHY is directed to one or more "Bystander Tumor Volume" (BTVs) defined as marginal reductions within the GTV, with the aim of sparing the surrounding peritumoral immune microenvironment (PIM) by subjecting it to tight formal constraints as a contoured OAR.[41,42] PATHY using photon stereotactic body radiation therapy (SBRT) and proton beam therapy (PBT) has demonstrated success at dramatic volumetric reductions of the GTV through bystander effects.[42-44] Refined by Tubin and colleagues at the MedAustron Ion Therapy Centre (Wiener Neustadt, Austria), PATHY is currently administered exclusively as a technique of 3-fraction daily CIRT.

A transition is now underway from simple Boolean-based geometric reductions of the GTV to biologically informed target delineation, with the BTV increasingly defined through functional hypoxia tracer imaging, such as with ^{64}Cu-ATSM PET.[45] Carbon-PATHY achieves significant treated tumor response and demonstrates methodologically validated macroscopic abscopal responses of unirradiated nodal and distant metastases.[42,46] At the preclinical stage, a recent experiment of murine xenografts of breast cancer showed that microtargeted partial CIRT fields determined by hypoxia PET imaging demonstrated an equivalent abscopal response to that of whole-volume carbon irradiation on non-irradiated grafts.[47]

Collectively, these murine and human data support the potential immunogenicity of confining HPT target volumes to select HRTVs. Similar minibeam techniques have been developed for use with heavier ions, particularly neon. Spatially fractionated NIRT has succeeded in vivo in murine models at inducing brisk reoxygenation and tumor-killing within hypoxic regions while sparing severe skin

Figure 1. Representative linear energy transfer (LET) painting case reprinted with permission from Inaniwa et al.[6] The left column represents RBE-weighted dose (D), and the right column represents dose-averaged LET (L) for 3 prostate cancer plans generated via the intensity-modulated composite particle therapy technique, with panels A-B, C-D, and E-F representing the dose and LET variables for the same plans, respectively. All plans are isodosimetric with variations in LET distribution. Note in plan 1 the uniformly higher LET in the anterior rectum with an aberrant LET hot spot along the left anterolateral rectal wall, outside the target, that would go undetected on pure dosimetric analysis. The LET distribution to the organs at risk is corrected in plans 2 and 3, and LET to the target is successfully escalated isodosimetrically in plan 3.



toxicity compared with broad-beam NIRT.[48-51] These promising early findings raise the prospects of immediate, definitive clinical applications for revived very high LET therapy, approaches that avoid the prohibitive late toxicities observed in the initial NIRT experiments at LBL, which curtailed its clinical adoption.[14,17-19] Such therapy would involve LET values exceeding conventional relative biological effectiveness (RBE)-based optimization thresholds, but in doing so may approach, and help define, the LET levels required for optimized anti-CSC therapy.

HPT and Immunotherapy: Turning Cold Tumors Hot

HPT may play a vital role in achieving more favorable outcomes for immunotherapy in cancers such as PDAC and GBM for which efforts in immunotherapy to this point have yielded frustratingly little benefit. PDAC is an archetypal immunologically "cold" tumor, demonstrating poor response to immune checkpoint inhibitors (ICIs) despite overexpression of PD-L1 and CD47. This resistance stems from complex genetic and epigenetic feedback mechanisms that collectively promote an immunosuppressive tumor microenvironment (TME); enhance clearance of cytotoxic agents and repair of cytotoxic damage; block activation, expansion, and infiltration of cytotoxic and pro-immune lymphocytes; suppress antigen recognition; and enable evasion and suppression of peripheral

Table 1. Studies of Multi-Ionic Radiation Therapy

STUDY	INSTITUTION	ION SPECIES	VARIABLE(S) UNDER STUDY
Inaniwa et al [27]	NIRS (Chiba, Japan)	Helium, carbon, oxygen, neon	Validation of SMK relative biological effectiveness (RBE) model on undifferentiated carcinoma and PDAC cells
Sokol et al[10]	GSI (Darmstadt, Germany)	Helium, oxygen	Multi-ionic LET painting for overcoming hypoxia radioresistance effects
Inaniwa et al[6]	NIRS (Chiba, Japan)	Proton, helium, carbon, oxygen	LET optimization via intensity-modulated multi-ionic radiation therapy (IMPACT)
Mein et al[25]	HIT (Heidelberg, Germany)	Helium, carbon, oxygen, neon	LET optimization via multi-ionic hadron arc therapy (SHArc)
Inaniwa et al[26]	National Institutes for Quantum Science and Technology (QST) (Chiba, Japan)	Helium, carbon, neon	Adaptation of multi-ionic therapy planning via SMK to account for oxygen-dependent cell responses (OSMK)
Sakata et al[28]	QST (Chiba, Japan)	Combination helium + oxygen vs carbon + neon	Silicon microdosimetric validation validation of MIRT planning
Inaniwa et al[29]	QST (Chiba, Japan)	Helium, carbon, oxygen, neon, silicon	Refinement of OSMK accounting for greater range/variation in LET over additional cell lines
Inaniwa et al[30]	NIRS (Chiba, Japan)	Helium, carbon, oxygen, neon	Correction of dose calculations for MIRT
Kopp et al[32]	HIT (Heidelberg, Germany)	Proton, helium, carbon	Initiation development of treatment planning system for MIRT
Inaniwa et al[26]	NIRS (Chiba, Japan)	Helium, carbon, oxygen, neon	Validation of MIRT optimization via lung substitute material
Masuda et al, 2025[33]	QST (Chiba, Japan)	Carbon, oxygen, neon	Retrospective validation of LET optimization to GTV for MIRT in patients with head and neck cancer (n = 16)

Abbreviations: GSI, GSI Helmholtzzentrum für Schwerionenforschung; GTV, gross tumor volume; HIT, Heidelberg Ion Therapy Center; IMPACT, intensity-modulated composite particle therapy; LET, linear energy transfer; MIRT, multi-ionic radiation therapy; NIRS, National Institute of Radiological Sciences; OSMK, oxygen-effect-incorporated stochastic microdosimetric kinetic model; PDAC, pancreatic ductal adenocarcinoma; RBE, relative biological effectiveness; SHArc, spot-scanning hadron arc model; SMK, stochastic microdosimetric kinetic model.

phagocytes.[52,53] Likewise, GBM appears to be immunologically cold with expression of PD-L1 found in a majority of tumor specimens and correlated with M2-polarized peripheral tumor-associated macrophages (TAMs), reduced lymphocyte infiltration, chemoresistance, and overall poor prognosis, ultimately failing in clinical evaluation to yield the hypothesized therapeutic gains for ICI based on crude PD-L1 expression on pathology.[54-57]

Emerging data suggest that the failure of immunotherapy in PDAC and GBM may reflect, at least in part, the central role of CSCs. In GBM, PD-L1 overexpression is driven by β-catenin—the terminal transcription factor of the canonical Wnt pathway and a key mediator of CSC–epithelial-to-mesenchymal transition (EMT) crosstalk in both PDAC and GBM. β-Catenin functions ubiquitously as an effector of stemness and EMT programs. Experimental data in GBM demonstrate strong co-expression of CD133 and β-catenin, with CD133 knockout resulting in the suppression of β-catenin, supporting a causal link downstream of CD133.[58-62]

Moreover, GBM cell culture analysis has found an inverse correlation between CD133 expression and CD4/CD8 infiltration while surgical specimen analyses have found M2 polarization and immunosuppressive microglia induction in GBM to be a product of the same CD133-activated Akt-Wnt interchange with redundant additional promotion by the CSC-associated TGF-β pathway.[63-65] Similarly, analysis of PDAC resection specimens has demonstrated a correlation between high PD-L1 expression and expression of both CD133 and CD44 that is lost when CD8 lymphocyte infiltration is high.[66] PDAC data further demonstrate an immunosuppressive M2 polarization of TAMs, low TME levels of CD4 and DC infiltration, relatively high T_H17 levels, high T_H2:T_H1 ratio, and an anti-immune cytokine balance.[67-70]

Collectively, these findings point to a deep relationship between the phenomenon of immunological coldness and the PDAC- and GBM-CSC biology mediated by CD133 and CD44. Likewise, the circulating tumor cells (CTCs) responsible for metastasis are the result of EMT-mediated transformation and extravasation;

the mobile immune-privileged circulatory routes, docking and colonization of distant organs necessary to complete the metastatic arc are only possible for CTCs acquiring a sufficient range and degree of stemness properties.[71-78] Preclinical evidence of the unique efficacy of high LET therapy in curbing CSC biology suggests the very same mechanisms may overcome native tumor resistance to immunotherapy, while the clinical demonstration of abscopality in the PATHY experience points to spatially fractionated HPT as the technical means by which local RT can be harnessed to achieve systemic tumor control effects.[42] In this context, maximizing the immunological benefits of HPT may require more than ICI alone. Recently developed CAR NK cell therapies not only yield more efficient direct tumor cell killing than CAR T cells, particularly in solid tumors, but also mimic the action of HPT in turning immunologically cold TMEs hot via activation of cytotoxic T cells and induction of M1 polarization, all without the inflammatory toxicities associated with CAR T therapies and which might be feared to result from combining HPT and ICIs.[79-84] Finally, the immunogenic mechanism of HPT, that is, cytosolic exposure of high volumes of tumor DNA, lends itself well to ongoing work in the development of personalized cancer vaccines, showing promising results in GBM and PDAC in early-stage clinical trials.[85,86]

Biological Optimization of HPT Physics: Calibrating the Instruments of CSC Eradication

Currently, the LET thresholds required for complete CSC sterilization remain unknown. Available data consistently demonstrate that CIRT enhances CSC killing, suppresses EMT, and reduces the oxygen enhancement ratio (OER), and they suggest a general relationship between LET, OER, and clonogenic survival. However, none of these studies were designed to achieve, nor did they achieve, complete CSC eradication. Instead, they relied on traditional RBE determination based on D values (the dose required to achieve 90% cell kill), which is useful for comparing CSC sensitivity between CIRT and photon or proton radiation but insufficient for defining the LET and dose conditions necessary to achieve CSC extinction. Moreover, these studies did not develop a framework to characterize clonogenic extinction in terms of changes in CSC biomarker expression as a function of LET; they simply reported expression changes at a limited number of dose–LET combinations.[87-106] Notably, they were also silent on non-local, indirect mechanisms of CSC or tumor control, such as immunogenic self-potentiation.

Most importantly, the LET range across these studies is wider than the delta between proton/photon LET and the lowest LET values of the carbon ion Spread Out Bragg Peak (SOBP) by multiples still inadequate to the task even at its upper boundary; the aggregate modal LET is 50 keV/µm but its selection is never justified for the treatment of CSCs within published material—50 keV/µm is a typical central tendency of whole tumor coverage by a carbon ion SOBP—and it is associated only with enhanced CSC killing, taking clear surviving fractions to be a given a priori.[88,90,93-96102,104,,106] One significant report, drawing upon a model determining an LET_U value of maximum kill efficiency, insists that LET should not exceed 100 keV/µm because of overkill effects and diminishing RBE.[101,107] Conversely, in preclinical settings, outright CSC eradication has been achieved by carbon ion irradiation at an arbitrarily determined 120 keV/µm delivered over clinically irrelevant dose/fraction regimens, illustrating the vast gap between the prominence of overkill in the literature and its actual clinical impact.[98,99]

Underkill of CSCs, unlike overkill, is an active clinical issue with implications for the prognosis of PDAC or GBM at any stage of disease and the expected 100% rate of failure for every prescription of (rightfully) de rigueur targeted therapy. Based on the data on LET/dose/CSC survival relationships, it is evident that HPT is a uniquely potent tool for CSC extirpation, that partial volume NIRT represents its maximum killing potency within safety limits, and that spatial fractionation may induce immunogenesis further augmented to abscopality by biologically guided partial volume selection and tightly constraining the PIM as an OAR.

Spatially fractionated and temporally ultrahypofractionated combination heavy ion therapy using the entire arsenal from helium to neon to ablate HRTVs defined by biological imaging as regions enriched with CSCs is the next logical progression following PATHY. As shown in SHArc and IMPACT Monte Carlo modeling, LET full-spectrum gradients are achievable and enable dose and/or LET de-escalation throughout the LRTV to mitigate the risk of compromising the PIM.[25,29,42,49,50] In turn, LRTV sparing is the critical limiting step in determining dose and LET combinations necessary for achieving clinical CSC extinction.[6,25,27-2932,,49] Moreover, only the delivery of such LET gradients may potentially extinguish the biological possibility of tumor recurrence through the combined effects of focal, direct CSC killing, and induction of bystander and immune secondary mechanisms transmitted over large relatively spared volumes.[42,46,91-94108-118]

Economics of HPT: Challenges of Upfront Capital Allocation, Potential for Long-Term Return, and Alternatives

A persistent perception that HPT facilities are prohibitively expensive to construct and operate remains a major barrier to the broader adoption, expansion, and patient access to this highly promising treatment modality. Though the capital requirements are undeniably high, the overall economics of HPT is more nuanced, and often more favorable, than commonly perceived. A comparative analysis of the cost-effectiveness of CIRT vs SBRT for the treatment of stage I non-small cell lung cancer at Gunma University found that the bulk of the difference was accounted for by costs of hospitalizations and ancillary studies rather than by technical fees for CIRT, the former of which could be mitigated or made more efficient.[119] The same institution found the mean total cost of CIRT was lower than that of chemoembolization in the treatment of hepatocellular carcinoma (¥4,974,278 vs ¥5,284,524).[120] A multi-institutional Japanese analysis found comparable total costs of treatment for locally recurrent rectal cancer using CIRT vs multimodality conventional treatment (¥4,803,946 vs ¥4,611,100).[121] A multi-institutional European analysis found that the average cost per fraction delivered was €1128 at combined CIRT/PBT centers, €743 at PBT-only centers, and €233 at photon-only centers.[122] These differences should be interpreted in light of HPT's unique suitability for hypofractionation, owing to its biological insensitivity to fractionation, as well as the potential for HPT-driven improvements in local control to reduce health care costs.

Notably, capital costs and yearly operational costs for combined CIRT/PBT, PBT-only, and photon facilities were respectively found to be €138.6 million, €94.9 million, and €23.4 million, and €36.7 million, €24.9 million, and €9.6 million.[122] These differences in initial and operational outlays are undoubtedly too much to bear for many health systems, even when factoring in cost-reducing public investment.[123] The expansion of particle therapy capabilities to include oxygen, neon, and other heavy ion species not yet in routine clinical use is sure to exacerbate cost differences, at least in the short term. Both the public health benefit of improved oncologic outcomes and the potential for long-term cost savings support a strong case for increased public investment in HPT, as well as for international cooperation and cost-sharing to facilitate broader access.

However, strategies that enable functional delivery of high LET therapy to larger populations than can be served by HPT are in the early phases of clinical development and deserve recognition. A conjugate of the high LET α-particle emitting radionuclide actinium-225 (Ac-225) to the somatostatin receptor binding complex DOTATATE has been used in the early stage trials of gastrointestinal (GI) origin neuroendocrine tumors and metastatic paragangliomas, in the latter of which, in an admittedly small data set, the ^{225}Ac-DOTATATE conjugate successfully controlled disease that had failed β-particle emitting lutetium-177 therapy.[124-126]

A clinical trial is underway investigating the ^{225}Ac-DOTATATE conjugate for metastatic or unresectable somatostatin receptor-expressing breast cancers.[127] Alpha emitters are a promising low-barrier means of delivering high LET therapy to appropriately selected patients, albeit limited anatomically to tumors to which they can be feasibly and reliably distributed, as well as far simpler than β emitters in terms of radiation safety precautions.

Another approach utilizes intratumoral infusion of nanoparticles composed of high Z materials with high electron density to increase the probability of ionization events for cells exposed to low LET radiation, functionally creating a field of high LET radiation delimited by the natural boundaries of gross tumor without risk of distribution to surrounding normal tissues. The phase II/III Act.In.Sarc. trial randomized 180 patients with locally advanced soft-tissue sarcoma indicated for neoadjuvant RT to intratumoral injection of the hafnium oxide-based NBTXR3 nanoparticle a week prior to RT to 50 Gy in 25 fractions followed in 5 weeks by surgical resection vs neoadjuvant monotherapy with RT and surgical resection after 5 weeks. The primary endpoint of pathologic complete response was doubled in the NBTXR3 arm over the RT-only arm (16% vs 8%) with all grade 3+ acute toxicity <10% and nearly identical rates of grade 3-4 wound complications.[128,129] The same agent has been incorporated into a pilot phase I trial for borderline resectable and unresectable non-metastatic PDAC, in which patients undergo intratumoral infusion of NBTXR3 prior to a 15-fraction course of RT.[130] Finally, clinical exploration of boron neutron capture therapy, which uses intravenous administration of $^{10}B_5$ preferentially taken up by tumor cells then bombarded with slow neutrons to induce cell killing by high LET α-particles and lithium ions, is active and expanding.[131-133]

Strategies such as those just described cannot recapitulate all of the benefits and versatility of HPT, but are nevertheless a promising means of bridging the

gap in access to offer some patients with aggressive/resistant cancers the opportunity for high LET therapeutic impact. These efforts should not be seen as exclusive of or in competition with HPT, but rather as complementary and potentially synergistic.

Conclusion

As discussed in Part I, high LET HPT has been shown in reproducible, basic research to be effective in the sterilization of CSCs. Consequently, it holds considerable promise as a means of significantly improving the prognostic paradigms of seemingly intractable human cancers whose outcomes are driven by their CSC biology. These findings are supported by early clinical studies demonstrating improved outcomes through crude deployment of HPT in the treatment of PDAC and GBM.[14,134] Moreover, the benefits of HPT appear to be synergistic with conventional and next-generation systemic therapies, though the ideal combinations and sequencing are yet to be determined. Economic and logistical challenges to expanding the reach of HPT are real but not insuperable, and the reduction it could entail in terms of reduced cancer recurrences may yield reductions in overall health care costs in the long run.

Literature to this point has downplayed the need for particles heavier than carbon ions to achieve true CSC eradication and overemphasized the so-called "overkill" problem. Safe implementation of oxygen or NIRT, however, necessitates selective targeting of high biological risk subvolumes within gross tumor to ensure critical OARs are not subjected to LET overdose. Multi-ionic LET painting models have been developed and validated as accomplishing this task. Likewise, clinically validated, state-of-the-art functional imaging technologies capable of detecting surrogates for CSC biology are already available, but have yet to be recognized for their potential utility in radiation oncology, let alone in defining HRTVs. Intriguingly, preclinical evidence suggests that selectively targeting CSC biology for high LET ablation may be the most promising approach to realizing the broader clinical potential of HPT, including the induction of immunogenic and abscopal cancer-killing effects. In this way, HPT may function not merely as a localized treatment but serve as an instrument of systemic cancer control.

Taken together, these advances suggest that the physics of high LET HPT may offer a radiobiological solution to the problem of CSCs and a tangible opportunity to alter the natural history of aggressive and treatment-refractory malignancies.[135]

References

1) Rajendran JG, Krohn KA. F-18 fluoromisonidazole for imaging tumor hypoxia: imaging the microenvironment for personalized cancer therapy. *Semin Nucl Med*. 2015;45(2):151-162. doi:10.1053/j.semnuclmed.2014.10.006

2) Hendrickson K, Phillips M, Smith W, et al. Hypoxia imaging with [f-18] FMISO-PET in head and neck cancer: potential for guiding intensity modulated radiation therapy in overcoming hypoxia-induced treatment resistance. *Radiother Oncol*. 2011;101(3):369-375. doi:10.1016/j.radonc.2011.07.029

3) Bassler N, Toftegaard J, Lühr A, et al. LET-painting increases tumour control probability in hypoxic tumours. *Acta Oncol*. 2014;53(1):25-32. doi:10.3109/0284186X.2013.832835

4) Kohno R, Koto M, Ikawa H, et al. High-linear energy transfer irradiation in clinical carbon-ion beam with the linear energy transfer painting technique for patients with head and neck cancer. *Adv Radiat Oncol*. 2024;9(1):101317. doi:10.1016/j.adro.2023.101317

5) Bassler N, Jäkel O, Søndergaard CS, Petersen JB. Dose- and LET-painting with particle therapy. *Acta Oncologica*. 2010;49(7):1170-1176. doi:10.3109/0284186X.2010.510640

6) Inaniwa T, Kanematsu N, Noda K, Kamada T. Treatment planning of intensity modulated composite particle therapy with dose and linear energy transfer optimization. *Phys Med Biol*. 2017;62(12):5180-5197. doi:10.1088/1361-6560/aa68d7

7) Liu T, Karlsen M, Karlberg AM, Redalen KR. Hypoxia imaging and theranostic potential of [64cu][cu(ATSM)] and ionic cu(II) salts: a review of current evidence and discussion of the retention mechanisms. *EJNMMI Res*. 2020;10(1):33. doi:10.1186/s13550-020-00621-5

8) Yoshii Y, Furukawa T, Kiyono Y, et al. Internal radiotherapy with copper-64-diacetyl-bis (N4-methylthiosemicarbazone) reduces CD133+ highly tumorigenic cells and metastatic ability of mouse colon carcinoma. *Nucl Med Biol*. 2011;38(2):151-157. doi:10.1016/j.nucmedbio.2010.08.009

9) Dehdashti F, Mintun MA, Lewis JS, et al. In vivo assessment of tumor hypoxia in lung cancer with 60cu-ATSM. *Eur J Nucl Med Mol Imaging*. 2003;30(6):844-850. doi:10.1007/s00259-003-1130-4

10) Sokol O, Krämer M, Hild S, Durante M, Scifoni E. Kill painting of hypoxic tumors with multiple ion beams. *Phys Med Biol*. 2019;64(4):045008. doi:10.1088/1361-6560/aafe40

11) Gaedicke S, Braun F, Prasad S, et al. Noninvasive positron emission tomography and fluorescence imaging of CD133 + tumor stem cells. *Proc Natl Acad Sci*. 2014;111(6). doi:10.1073/pnas.1314189111

12) Koffler D, Fekrmandi F, Ebner D, et al. Chimeric immunomagnetic nanoimaging of pancreatic and glioblastoma cancer stem cells for multi-ionic ablation. *Int J Part Ther*. 2024;12:100427. doi:10.1016/j.ijpt.2024.100427

13) Ebner DK, Frank SJ, Inaniwa T, Yamada S, Shirai T. The emerging potential of multi-ion radiotherapy. *Front Oncol*. 2021;11:624786. doi:10.3389/fonc.2021.624786

14) Castro JR, Phillips TL, Prados M, et al. Neon heavy charged particle radiotherapy of glioblastoma of the brain. *Int J Radiat Oncol*. 1997;38(2):257-261. doi:10.1016/S0360-3016(97)00039-4

15) Kempe J, Gudowska I, Brahme A. Depth absorbed dose and LET distributions of therapeutic 1H, 4He, 7Li, and 12C beams. *Med Phys*. 2007;34(1):183-192. doi:10.1118/1.2400621

16) Kantemiris I, Karaiskos P, Papagiannis P, Angelopoulos A. Dose and dose averaged LET comparison of 1H. *Med Phys*. 2011;38(12):6585-6591. doi:10.1118/1.3662911

17) Castro JR, Quivey JM, Lyman JT, et al. Current status of clinical particle radiotherapy at lawrence berkeley laboratory. *Cancer*. 1980;46(4):633-641. doi:10.1002/1097-0142(19800815)46:43.0.CO;2-O

18) Castro JR. Results of heavy ion radiotherapy. *Radiat Environ Biophys*. 1995;34(1):45-48. doi:10.1007/BF01210545

19) Linstadt DE, Castro JR, Phillips TL. Neon ion radiotherapy: results of the phase I/II clinical trial. *Int J Radiat Oncol*. 1991;20(4):761-769. doi:10.1016/0360-3016(91)90020-5

20) Tessonnier T, Ecker S, Besuglow J, et al. Commissioning of helium ion therapy and the first patient treatment with active beam delivery. *Int J Radiat Oncol*. 2023;116(4):935-948. doi:10.1016/j.ijrobp.2023.01.015

21) Ahmed KA, Liveringhouse CL, Mills MN, et al. Utilizing the Genomically Adjusted Radiation Dose (GARD) to personalize adjuvant radiotherapy in triple negative breast cancer management. *EBioMedicine*. 2019;47:163-169. doi:10.1016/j.ebiom.2019.08.019

22) Ho E, De Cecco L, Cavalieri S, et al. Genomic Adjusted Radiation Dose (GARD) predicts overall survival and outperforms AJCC 8th edition in prognostication of HPV-positive oropharyngeal squamous cell carcinoma. *Int J Radiat Oncol Biol Phys*. 2022;114(3):S143. doi:10.1016/j.ijrobp.2022.07.611

23) Kaidar-Person O, Poortmans P, Salgado R. Genomic-adjusted radiation dose to personalise radiotherapy. *Lancet Oncol*. 2021;22(9):1200-1201. doi:10.1016/S1470-2045(21)00411-3

24) Scott JG, Sedor G, Ellsworth P, et al. Pan-cancer prediction of radiotherapy benefit using Genomic-Adjusted Radiation Dose (GARD): a cohort-based pooled analysis. *Lancet Oncol*. 2021;22(9):1221-1229. doi:10.1016/S1470-2045(21)00347-8

25) Mein S, Kopp B, Tessonnier T, et al. Spot-scanning hadron arc (sharc) therapy: a proof of concept using single- and multi-ion strategies with helium, carbon, oxygen, and neon ions. *Med Phys*. 2022;49(9):6082-6097. doi:10.1002/mp.15800

26) Inaniwa T, Kanematsu N, Shinoto M, Koto M, Yamada S. Adaptation of stochastic microdosimetric kinetic model to hypoxia for hypo-fractionated multi-ion therapy treatment planning. *Phys Med Biol*. 2021;66(20). doi:10.1088/1361-6560/ac29cc

27) Inaniwa T, Suzuki M, Hyun Lee S, et al. Experimental validation of stochastic microdosimetric kinetic model for multi-ion therapy treatment planning with helium-, carbon-, oxygen-, and neon-ion beams. *Phys Med Biol*. 2020;65(4):045005. doi:10.1088/1361-6560/ab6eba

28) Sakata D, Lee SH, Tran LT, et al. Microdosimetric investigation for multi-ion therapy by means of Silicon On Insulator (SOI) microdosimeter. *Phys Med Biol*. 2022;67(21). doi:10.1088/1361-6560/ac8968

29) Inaniwa T, Kanematsu N. Event-by-event approach to the oxygen-effect-incorporated stochastic microdosimetric kinetic model for hypofractionated multi-ion therapy. *J Radiat Res*. 2023;64(4):685-692. doi:10.1093/jrr/rrad049

30) Inaniwa T, Lee SH, Mizushima K, et al. Nuclear-interaction correction for patient dose calculations in treatment planning of helium-, carbon-, oxygen-, and neon-ion beams. *Phys Med Biol*. 2020;65(2):025004. doi:10.1088/1361-6560/ab5fee

31) Tinganelli W, Durante M, Hirayama R, et al. Kill-painting of hypoxic tumours in charged particle therapy. *Sci Rep*. 2015;5:17016. doi:10.1038/srep17016

32) Kopp B, Mein S, Dokic I, et al. Development and validation of single field multi-ion particle therapy treatments. *International Journal of Radiation Oncology*Biology*Physics*. 2020;106(1):194-205. doi:10.1016/j.ijrobp.2019.10.008

33) Masuda T, Koto M, Ikawa H, et al. Design of multi-ion therapy for head and neck cancers using carbon-, oxygen-, and neon-ion beams: potential efficacy against tumor hypoxia. *Phys Med Biol*. 2025;70(8):40138796. doi:10.1088/1361-6560/adc5d6

34) Zaorsky NG, Lehrer EJ, Handorf E, Meyer JE. Dose escalation in stereotactic body radiation therapy for pancreatic cancer: a meta-analysis. *Am J Clin Oncol*. 2019;42(1):46-55. doi:10.1097/COC.0000000000000472

35) Chang DT, Schellenberg D, Shen J, et al. Stereotactic radiotherapy for unresectable adenocarcinoma of the pancreas. *Cancer*. 2009;115(3):665-672. doi:10.1002/cncr.24059

36) Hoyer M, Roed H, Sengelov L, et al. Phase-II study on stereotactic radiotherapy of locally advanced pancreatic carcinoma. *Radiother Oncol*. 2005;76(1):48-53. doi:10.1016/j.radonc.2004.12.022

37) Combs SE, Kieser M, Rieken S, et al. Randomized phase II study evaluating a carbon ion boost applied after combined radiochemotherapy with temozolomide versus a proton boost after radiochemotherapy with temozolomide in patients with primary glioblastoma: the CLEOPATRA trial. *BMC Cancer*. 2010;10(1):478. doi:10.1186/1471-2407-10-478

38) Harrabi SB, von Nettelbladt B, Gudden C, et al. Radiation induced contrast enhancement after proton beam therapy in patients with low grade glioma—how safe are protons?. *Radiother Oncol*. 2022;167:211-218. doi:10.1016/j.radonc.2021.12.035

39) Tubin S, Vozenin MC, Prezado Y, et al. Novel unconventional radiotherapy techniques: current status and future perspectives - report from the 2nd international radiation oncology online seminar. *Clin Transl Radiat Oncol*. 2023;40:100605. doi:10.1016/j.ctro.2023.100605

40) Grams MP, Deufel CL, Kavanaugh JA, et al. Clinical aspects of spatially fractionated radiation therapy treatments. *Phys Med*. 2023;111:102616. doi:10.1016/j.ejmp.2023.102616

41) Ebner DK, Kamada T, Yamada S. Abscopal effect in recurrent colorectal cancer treated with carbon-ion radiation therapy: 2 case reports. *Adv Radiat Oncol*. 2017;2(3):333-338. doi:10.1016/j.adro.2017.06.001

42) Tubin S, Fossati P, Carlino A, et al. Novel carbon ion and proton partial irradiation of recurrent unresectable bulky tumors (particle-PATHY): early indication of effectiveness and safety. *Cancers (Basel)*. 2022;14(9):2232. doi:10.3390/cancers14092232

43) Belyakov OV, Mitchell SA, Parikh D, et al. Biological effects in unirradiated human tissue induced by radiation damage up to 1 mm away. *Proc Natl Acad Sci USA*. 2005;102(40):14203-14208. doi:10.1073/pnas.0505020102

44) Tubin S, Gupta S, Grusch M, et al. Shifting the immune-suppressive to predominant immune-stimulatory radiation effects by SBRT-partial tumor irradiation targeting hypoxic segment (SBRT-PATHY). *Cancers (Basel)*. 2020;13(1):50. doi:10.3390/cancers13010050

45) Tubin S. Current and future practice patterns in PATHY at Med Austron; 2023.

46) Tubin S, Yan W, Mourad WF, Fossati P, Khan MK. The future of radiation-induced abscopal response: beyond conventional radiotherapy approaches. *Future Oncol*. 2020;16(16):1137-1151. doi:10.2217/fon-2020-0063

47) Huang Q, Sun Y, Wang W, et al. Biological guided carbon-ion microporous radiation to tumor hypoxia area triggers robust abscopal effects as open field radiation. *Front Oncol*. 2020;10:597702. doi:10.3389/fonc.2020.597702

48) Peucelle C, Martínez-Rovira I, Prezado Y. Spatial fractionation of the dose using neon and heavier ions: a monte carlo study. *Med Phys*. 2015;42(10):5928-5936. doi:10.1118/1.4930960

49) Prezado Y, Hirayama R, Matsufuji N, et al. A potential renewed use of very heavy ions for therapy: neon minibeam radiation therapy. *Cancers*. 2021;13(6):1356. doi:10.3390/cancers13061356

50) Imaizumi A, Hirayama R, Ikoma Y, et al. Neon ion (20 Ne10 +) charged particle beams manipulate rapid tumor reoxygenation in syngeneic mouse models. *Cancer Sci*. doi:10.1111/cas.16017

51) Furusawa Y, Fukutsu K, Aoki M, et al. Inactivation of aerobic and hypoxic cells from three different cell lines by accelerated (3)He-, (12)C- and (20)Ne-ion beams. *Radiat Res*. 2000;154(5):485-496. doi:10.1667/0033-7587(2000)154[0485:ioaahc]2.0.co;2

52) Gao S, Yang X, Xu J, Qiu N, Zhai G. Nanotechnology for boosting cancer immunotherapy and remodeling tumor microenvironment: the horizons in cancer treatment. *ACS Nano*. 2021;15(8):12567-12603. doi:10.1021/acsnano.1c02103

53) Kolbeinsson HM, Chandana S, Wright GP, Chung M. Pancreatic cancer: a review of current treatment and novel therapies. *J Invest Surg*. 2023;36(1):2129884. doi:10.1080/08941939.2022.2129884

54) Ricklefs FL, Alayo Q, Krenzlin H, et al. Immune evasion mediated by PD-L1 on glioblastoma-derived extracellular vesicles. *Sci Adv*. 2018;4(3):eaar2766. doi:10.1126/sciadv.aar2766

55) Nduom EK, Wei J, Yaghi NK, et al. PD-L1 expression and prognostic impact in glioblastoma. *Neuro Oncol*. 2016;18(2):195-205. doi:10.1093/neuonc/nov172

56) Lee GA, Lin W-L, Kuo D-P, et al. Detection of PD-L1 expression in temozolomide-resistant glioblastoma by using PD-L1 antibodies conjugated with lipid-coated superparamagnetic iron oxide. *Int J Nanomedicine*. 2021;16:5233-5246. doi:10.2147/IJN.S310464

57) Zhu Z, Zhang H, Chen B, et al. PD-L1-mediated immunosuppression in glioblastoma is associated with the infiltration and M2-polarization of tumor-associated macrophages. *Front Immunol*. 2020;11:588552. doi:10.3389/fimmu.2020.588552

58) Aguilera KY, Dawson DW. WNT ligand dependencies in pancreatic cancer. *Front Cell Dev Biol*. 2021;9:671022. doi:10.3389/fcell.2021.671022

59) Zhong Z, Yu J, Virshup DM, Madan B. Wnts and the hallmarks of cancer. *Cancer Metastasis Rev*. 2020;39(3):625-645. doi:10.1007/s10555-020-09887-6

60) Du L, Lee JH, Jiang H, et al. β-catenin induces transcriptional expression of PD-L1 to promote glioblastoma immune evasion. *J Exp Med*. 2020;217(11):e20191115. doi:10.1084/jem.20191115

61) Kim YM, Kahn M. The role of the Wnt signaling pathway in cancer stem cells: prospects for drug development. *Res Rep Biochem*. 2014;4:1-12. doi:10.2147/RRBC.S53823

62) Manoranjan B, Chokshi C, Venugopal C, et al. A CD133-AKT-Wnt signaling axis drives glioblastoma brain tumor-initiating cells. *Oncogene*. 2020;39(7):1590-1599. doi:10.1038/s41388-019-1086-x

63) Wu A, Wei J, Kong L-Y, et al. Glioma cancer stem cells induce immunosuppressive macrophages/microglia. *Neuro Oncol*. 2010;12(11):1113-1125. doi:10.1093/neuonc/noq082

64) Khan I, Mahfooz S, Karacam B, et al. Glioma cancer stem cells modulating the local tumor immune environment. *Front Mol Neurosci*. 2022;15:1029657. doi:10.3389/fnmol.2022.1029657

65) Li D, Zhang Q, Li L, et al. B2-microglobulin maintains glioblastoma stem cells and induces M2-like polarization of tumor-associated macrophages. *Cancer Res*. 2022;82(18):3321-3334. doi:10.1158/0008-5472.CAN-22-0507

66) Hou Y-C, Chao Y-J, Hsieh M-H, et al. Low $CD8^+$ T cell infiltration and high PD-L1 expression are associated with level of $CD44^+/CD133^+$ cancer stem cells and predict an unfavorable prognosis in pancreatic cancer. *Cancers (Basel)*. 2019;11(4):541. doi:10.3390/cancers11040541

67) Poh AR, Ernst M. Tumor-associated macrophages in pancreatic ductal adenocarcinoma: therapeutic opportunities and clinical challenges. *Cancers (Basel)*. 2021;13(12):2860. doi:10.3390/cancers13122860

68) Zhang M, Pan X, Fujiwara K, et al. Pancreatic cancer cells render tumor-associated macrophages metabolically reprogrammed by a GARP and DNA methylation-mediated mechanism. *Signal Transduct Target Ther*. 2021;6(1):366. doi:10.1038/s41392-021-00769-z

69) Hegde S, Krisnawan VE, Herzog BH, et al. Dendritic cell paucity leads to dysfunctional immune surveillance in pancreatic cancer. *Cancer Cell*. 2020;37(3):289-307. doi:10.1016/j.ccell.2020.02.008

70) Shimoda Y, Shibaki R, Yoshida T, et al. Concurrent high PD-L1 expression and $CD8^+$ immune cell infiltration predict PD-1 blockade efficacy in advanced EGFR-mutant NSCLC patients. *Clin Lung Cancer*. 2022;23(6):477-486. doi:10.1016/j.cllc.2022.04.001

71) Satelli A, Mitra A, Brownlee Z, et al. Epithelial-mesenchymal transitioned circulating tumor cells capture for detecting tumor progression. *Clin Cancer Res*. 2015;21(4):899-906. doi:10.1158/1078-0432.CCR-14-0894

72) Tinganelli W, Durante M. Tumor hypoxia and circulating tumor cells. *Int J Mol Sci*. 2020;21(24):9592. doi:10.3390/ijms21249592

73) Tinhofer I, Saki M, Niehr F, Keilholz U, Budach V. Cancer stem cell characteristics of circulating tumor cells. *Int J Radiat Biol*. 2014;90(8):622-627. doi:10.3109/09553002.2014.886798

74) Toloudi M, Apostolou P, Chatziioannou M, Papasotiriou I. Correlation between cancer stem cells and circulating tumor cells and their value. *Case Rep Oncol*. 2011;4(1):44-54. doi:10.1159/000324403

75) Chistiakov DA, Chekhonin VP. Circulating tumor cells and their advances to promote cancer metastasis and relapse, with focus on glioblastoma multiforme. *Exp Mol Pathol*. 2018;105(2):166-174. doi:10.1016/j.yexmp.2018.07.007

76) Lin D, Shen L, Luo M, et al. Circulating tumor cells: biology and clinical significance. *Sig Transduct Target Ther*. 2021;6(1):404. doi:10.1038/s41392-021-00817-8

77) Li X-Y, Dong M, Zang X-Y, et al. The emerging role of circulating tumor cells in cancer management. *Am J Transl Res*. 2020;12(2):332-342.

78) Sutton TL, Patel RK, Anderson AN, et al. Circulating cells with macrophage-like characteristics in cancer: the importance of circulating neoplastic-immune hybrid cells in cancer. *Cancers (Basel)*. 2022;14(16):3871. doi:10.3390/cancers14163871

79) Zhang L, Meng Y, Feng X, Han Z. CAR-NK cells for cancer immunotherapy: from bench to bedside. *Biomark Res*. 2022;10(1):12. doi:10.1186/s40364-022-00364-6

80) Morgan MA, Büning H, Sauer M, Schambach A. Use of cell and genome modification technologies to generate improved "off-the-shelf" CAR t and CAR NK cells. *Front Immunol*. 2020;11:1965. doi:10.3389/fimmu.2020.01965

81) Marofi F, Rahman HS, Thangavelu L, et al. Renaissance of armored immune effector cells, CAR-NK cells, brings the higher hope for successful cancer therapy. *Stem Cell Res Ther*. 2021;12(1):200. doi:10.1186/s13287-021-02251-7

82) Li H, Song W, Li Z, Zhang M. Preclinical and clinical studies of CAR-NK-cell therapies for malignancies. *Front Immunol*. 2022;13:992232. doi:10.3389/fimmu.2022.992232

83) Hermanson DL, Kaufman DS. Utilizing chimeric antigen receptors to direct natural killer cell activity. *Front Immunol*. 2015;6:195. doi:10.3389/fimmu.2015.00195

84) Crespo J, Sun H, Welling TH, Tian Z, Zou W. T cell anergy, exhaustion, senescence, and stemness in the tumor microenvironment. *Curr Opin Immunol*. 2013;25(2):214-221. doi:10.1016/j.coi.2012.12.003

85) Rojas LA, Sethna Z, Soares KC, et al. Personalized RNA neoantigen vaccines stimulate T cells in pancreatic cancer. *Nature*. 2023;618(7963):144-150. doi:10.1038/s41586-023-06063-y

86) Ahluwalia MS, Reardon DA, Abad AP, et al. Phase IIa study of SurVaxM plus adjuvant temozolomide for newly diagnosed glioblastoma. *J Clin Oncol*. 2023;41(7):1453-1465. doi:10.1200/JCO.22.00996

87) Paz AE, Yamamoto N, Sakama M, Matsufuji N, Kanai T. Tumor control probability analysis for single-fraction carbon-ion radiation therapy of early-stage non-small cell lung cancer. *Int J Radiat Oncol*. 2018;102(5):1551-1559. doi:10.1016/j.ijrobp.2018.07.2009

88) Koom WS, Sai S, Suzuki M, et al. Superior effect of the combination of carbon-ion beam irradiation and 5-fluorouracil on colorectal cancer stem cells in vitro and in vivo. *Onco Targets Ther*. 2020;13:12625-12635. doi:10.2147/OTT.S276035

89) Subtil FSB, Wilhelm J, Bill V, et al. Carbon ion radiotherapy of human lung cancer attenuates HIF-1 signaling and acts with considerably enhanced therapeutic efficiency. *FASEB J*. 2014;28(3):1412-1421. doi:10.1096/fj.13-242230

90) Wozny A-S, Lauret A, Battiston-Montagne P, et al. Differential pattern of HIF-1α expression in HNSCC cancer stem cells after carbon ion or photon irradiation: one molecular explanation of the oxygen effect. *Br J Cancer*. 2017;116(10):1340-1349. doi:10.1038/bjc.2017.100

91) Matsunaga A, Ueda Y, Yamada S, et al. Carbon-ion beam treatment induces systemic antitumor immunity against murine squamous cell carcinoma. *Cancer*. 2010;116(15):3740-3748. doi:10.1002/cncr.25134

92) Fujita M, Imadome K, Shoji Y, et al. Carbon-ion irradiation suppresses migration and invasiveness of human pancreatic carcinoma cells MIAPaCa-2 via Rac1 and RhoA degradation. *Int J Radiat Oncol Biol Phys*. 2015;93(1):173-180. doi:10.1016/j.ijrobp.2015.05.009

93) Wang J, Dai Z, Miao Y, et al. Carbon ion (12C6+) irradiation induces the expression of klrk1 in lung cancer and optimizes the tumor microenvironment based on the NKG2D/NKG2D-ls pathway. *Cancer Letters*. 2021;521:178-195. doi:10.1016/j.canlet.2021.09.003

94) Moncharmont C, Guy J-B, Wozny A-S, et al. Carbon ion irradiation withstands cancer stem cells' migration/invasion process in Head and Neck Squamous Cell Carcinoma (HNSCC). *Oncotarget*. 2016;7(30):47738-47749. doi:10.18632/oncotarget.10281

95) Sai S, Wakai T, Vares G, et al. Combination of carbon ion beam and gemcitabine causes irreparable DNA damage and death of radioresistant pancreatic cancer stem-like cells in vitro and in vivo. *Oncotarget*. 2015;6(8):5517-5535. doi:10.18632/oncotarget.3584

96) Oonishi K, Cui X, Hirakawa H, et al. Different effects of carbon ion beams and x-rays on clonogenic survival and DNA repair in human pancreatic cancer stem-like cells. *Radiother Oncol*. 2012;105(2):258-265. doi:10.1016/j.radonc.2012.08.009

97) Baek S-J, Ishii H, Tamari K, et al. Cancer stem cells: the potential of carbon ion beam radiation and new radiosensitizers (review). *Oncol Rep*. 2015;34(5):2233-2237. doi:10.3892/or.2015.4236

98) Chiblak S, Tang Z, Lemke D, et al. Carbon irradiation overcomes glioma radioresistance by eradicating stem cells and forming an antiangiogenic and immunopermissive niche. *JCI Insight*. 2019;4(2):e123837. doi:10.1172/jci.insight.123837

99) Chiblak S, Tang Z, Campos B, et al. Radiosensitivity of patient-derived glioma stem cell 3-dimensional cultures to photon, proton, and carbon irradiation. *Int J Radiat Oncol*. 2016;95(1):112-119. doi:10.1016/j.ijrobp.2015.06.015

100) Hirota Y, Masunaga S-I, Kondo N, et al. High linear-energy-transfer radiation can overcome radioresistance of glioma stem-like cells to low linear-energy-transfer radiation. *J Radiat Res*. 2014;55(1):75-83. doi:10.1093/jrr/rrt095

101) Valable S, Gérault AN, Lambert G, et al. Impact of hypoxia on carbon ion therapy in glioblastoma cells: modulation by LET and hypoxia-dependent genes. *Cancers (Basel)*. 2020;12(8):2019. doi:10.3390/cancers12082019

102) Sai S, Vares G, Kim EH, et al. Carbon ion beam combined with cisplatin effectively disrupts triple negative breast cancer stem-like cells in vitro. *Mol Cancer*. 2015;14(1):166. doi:10.1186/s12943-015-0429-7

103) Wang Q, Liu R, Zhang Q, et al. Biological effects of cancer stem cells irradiated by charged particle: a systematic review of in vitro studies. *J Cancer Res Clin Oncol*. 2023;149(9):6625-6638. doi:10.1007/s00432-022-04561-6

104) Cui X, Oonishi K, Tsujii H, et al. Effects of carbon ion beam on putative colon cancer stem cells and its comparison with x-rays. *Cancer Res*. 2011;71(10):3676-3687. doi:10.1158/0008-5472.CAN-10-2926

105) Dokic I, Mairani A, Niklas M, et al. Next generation multi-scale biophysical characterization of high precision cancer particle radiotherapy using clinical proton, helium-, carbon- and oxygen ion beams. *Oncotarget*. 2016;7(35):56676-56689. doi:10.18632/oncotarget.10996

106) Sokol O, Durante M. Carbon ions for hypoxic tumors: are we making the most of them. *Cancers (Basel)*. 2023;15(18). doi:10.3390/cancers15184494

107) Jones B. A simpler energy transfer efficiency model to predict relative biological effect for protons and heavier ions. *Front Oncol*. 2015;5:184. doi:10.3389/fonc.2015.00184

108) Lussier DM, Alspach E, Ward JP, et al. Radiation-induced neoantigens broaden the immunotherapeutic window of cancers with low mutational loads. *Proc Natl Acad Sci USA*. 2021;118(24):e2102611118. doi:10.1073/pnas.2102611118

109) Ando K, Fujita H, Hosoi A, et al. Intravenous dendritic cell administration enhances suppression of lung metastasis induced by carbon-ion irradiation. *J Radiat Res*. 2017;58(4):446-455. doi:10.1093/jrr/rrx005

110) Ogata T, Teshima T, Kagawa K, et al. Particle irradiation suppresses metastatic potential of cancer cells. *Cancer Res*. 2005;65(1):113-120.

111) Akino Y, Teshima T, Kihara A, et al. Carbon-ion beam irradiation effectively suppresses migration and invasion of human non-small-cell lung cancer cells. *Int J Radiat Oncol Biol Phys*. 2009;75(2):475-481. doi:10.1016/j.ijrobp.2008.12.090

112) Takahashi Y, Yasui T, Minami K, et al. Carbon ion irradiation enhances the antitumor efficacy of dual immune checkpoint blockade therapy both for local and distant sites in murine osteosarcoma. *Oncotarget*. 2019;10(6):633-646. doi:10.18632/oncotarget.26551

113) Harding SM, Benci JL, Irianto J, et al. Mitotic progression following DNA damage enables pattern recognition within micronuclei. *Nature*. 2017;548(7668):466-470. doi:10.1038/nature23470

114) Katsuki S, Takahashi Y, Tamari K, et al. Radiation therapy enhances systemic antitumor efficacy in PD-L1 therapy regardless of sequence of radiation in murine osteosarcoma. *PLOS ONE*. 2022;17(7):e0271205. doi:10.1371/journal.pone.0271205

115) Zhou H, Yang P, Li H, et al. Carbon ion radiotherapy boosts anti-tumour immune responses by inhibiting myeloid-derived suppressor cells in melanoma-bearing mice. *Cell Death Discov*. 2021;7(1):332. doi:10.1038/s41420-021-00731-6

116) Iijima M, Okonogi N, Nakajima NI, et al. Significance of PD-L1 expression in carbon-ion radiotherapy for uterine cervical adeno/adenosquamous carcinoma. *J Gynecol Oncol*. 2020;31(2):e19. doi:10.3802/jgo.2020.31.e19

117) Hartmann L, Schröter P, Osen W, et al. Photon versus carbon ion irradiation: immunomodulatory effects exerted on murine tumor cell lines. *Sci Rep*. 2020;10(1):21517. doi:10.1038/s41598-020-78577-8

118) Du J, Kageyama S-I, Hirata H, et al. Comparative analysis of the immune responses in cancer cells irradiated with x-ray, proton and carbon-ion beams. *Biochem Biophys Res Commun*. 2021;585:55-60. doi:10.1016/j.bbrc.2021.11.004

119) Okazaki S, Shibuya K, Takura T, et al. Cost-effectiveness of carbon-ion radiotherapy versus stereotactic body radiotherapy for non-small-cell lung cancer. *Cancer Sci*. 2022;113(2):674-683. doi:10.1111/cas.15216

120) Okazaki S, Shibuya K, Shiba S, Takura T, Ohno T. Cost-effectiveness comparison of carbon-ion radiation therapy and transarterial chemoembolization for hepatocellular carcinoma. *Adv Radiat Oncol*. 2024;9(4):101441. doi:10.1016/j.adro.2024.101441

121) Mobaraki A, Ohno T, Yamada S, Sakurai H, Nakano T. Cost-effectiveness of carbon ion radiation therapy for locally recurrent rectal cancer. *Cancer Sci*. 2010;101(8):1834-1839. doi:10.1111/j.1349-7006.2010.01604.x

122) Peeters A, Grutters JPC, Pijls-Johannesma M, et al. How costly is particle therapy? Cost analysis of external beam radiotherapy with carbon-ions, protons and photons. *Radiother Oncol*. 2010;95(1):45-53. doi:10.1016/j.radonc.2009.12.002

123) Vanderstraeten B, Verstraete J, De Croock R, De Neve W, Lievens Y. In search of the economic sustainability of hadron therapy: the real cost of setting up and operating a hadron facility. *Int J Radiat Oncol Biol Phys*. 2014;89(1):152-160. doi:10.1016/j.ijrobp.2014.01.039

124) Shi M, Jakobsson V, Greifenstein L, et al. Alpha-peptide receptor radionuclide therapy using actinium-225 labeled somatostatin receptor agonists and antagonists. *Front Med (Lausanne)*. 2022;9:1034315. doi:10.3389/fmed.2022.1034315

125) Satapathy S, Chandekar KR, Bal C. Gastro-enteric-pancreatic neuroendocrine tumor treatment: actinium-225-DOTATATE and combined therapies. *PET Clin*. 2023;18(2):215-221. doi:10.1016/j.cpet.2022.11.004

126) Yadav MP, Ballal S, Sahoo RK, Bal C. Efficacy and safety of ^{225}Ac-DOTATATE targeted alpha therapy in metastatic paragangliomas: a pilot study. *Eur J Nucl Med Mol Imaging*. 2022;49(5):1595-1606. doi:10.1007/s00259-021-05632-5

127) National Cancer Institute. Trial of 225Ac-DOTATATE (RYZ101) in Subjects with ER+, HER2-negative Unresectable or Metastatic Breast Cancer Expressing SSTRs; 2016. Accessed April 24, 2025. https://www.cancer.gov/about-cancer/treatment/clinical-trials/search/v?id=NCI-2024-08462

128) Bonvalot S, Le Pechoux C, De Baere T, et al. First-in-human study testing a new radioenhancer using nanoparticles (NBTXR3) activated by radiation therapy in patients with locally advanced soft tissue sarcomas. *Clin Cancer Res*. 2017;23(4):908-917. doi:10.1158/1078-0432.CCR-16-1297

129) Bonvalot S, Rutkowski PL, Thariat J, et al. NBTXR3, a first-in-class radioenhancer hafnium oxide nanoparticle, plus radiotherapy versus radiotherapy alone in patients with locally advanced soft-tissue sarcoma (act.in.sarc): a multicentre, phase 2-3, randomised, controlled trial. *Lancet Oncol*. 2019;20(8):1148-1159. doi:10.1016/S1470-2045(19)30326-2

130) M.D. Anderson Cancer Center. Phase I Study of NBTXR3 Activated by Radiotherapy for Locally Advanced or Borderline Resectable Pancreatic Ductal Adenocarcinoma; 2023. Accessed December 31, 2022. https://clinicaltrials.gov/study/NCT04484909

131) He H, Li J, Jiang P, et al. The basis and advances in clinical application of boron neutron capture therapy. *Radiat Oncol*. 2021;16(1):216. doi:10.1186/s13014-021-01939-7

132) Malouff TD, Seneviratne DS, Ebner DK, et al. Boron neutron capture therapy: a review of clinical applications. *Front Oncol*. 2021;11:601820. doi:10.3389/fonc.2021.601820

133) Nedunchezhian K, Aswath N, Thiruppathy M, Thirugnanamurthy S. Boron neutron capture therapy—a literature review. *J Clin Diagn Res*. 2016;10(12):ZE01-ZE04. doi:10.7860/JCDR/2016/19890.9024

134) Kawashiro S, Yamada S, Okamoto M, et al. Multi-institutional study of carbon-ion radiotherapy for locally advanced pancreatic cancer: Japan Carbon-ion Radiation Oncology Study Group (J-CROS) study 1403 pancreas. *Int J Radiat Oncol Biol Phys*. 2018;101(5):1212-1221. doi:10.1016/j.ijrobp.2018.04.057

135) Kong L, Gao J, Hu J, et al. Carbon ion radiotherapy boost in the treatment of glioblastoma: a randomized phase I/III clinical trial. *Cancer Commun (Lond)*. 2019;39(1):5):5:. doi:10.1186/s40880-019-0351-2

The Effect of Pentoxifylline and Vitamin E in Preventing Grade 3 Radiation Pneumonitis: A Single-Arm, Phase II Prospective Study

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Abstract

Objective/Hypothesis Stereotactic ablative radiation therapy (SABR) is becoming an increasingly popular treatment for patients with recurrent non-small cell lung cancer. Thoracic reirradiation, however, can be toxic, with some institutions reporting grade 3 pneumonitis in upward of 30% of reirradiated patients. Pentoxifylline (PTX) and vitamin E (VE) have mitigated toxicity in standard breast treatment and may be beneficial in the reduction of radiation-induced pneumonitis. The objective of this study is to prospectively evaluate the efficacy of PTX and VE in reducing grade 3 pneumonitis in patients undergoing SABR with locoregionally recurrent lung cancer or new lung primary tumors in the setting of prior thoracic radiation. We hypothesize that these patients will experience rates of grade 3 pneumonitis lower than 30% at 3, 6, and 12 months post-treatment.

Materials and Methods Patients who received radiation for a prior thoracic malignancy with a diagnosis of a recurrent or new NSCLC were recruited from our institution. PTX and VE were administered at the time of simulation, approximately 1 week prior to starting treatment, and were continued for 12 weeks post-treatment. SABR was delivered using standard stereotactic techniques to a dose of 50 Gy at 10 Gy per fraction over 2 weeks. Clinical and radiographic assessment of pneumonitis was conducted at 3, 6, and 12 months post-treatment. Demographic information was collected before treatment.

Results The rate of grade 3 pneumonitis in our PTX- and VE-treated cohort was significantly lower than 30% at 3 months (0%, 95% CI 0%-11%, $P = .001$), 6 months (5%, 95% CI 0%-20%, $P = .004$), and 12 months (0%, 95% CI 0%-21%, $P = .010$) post-treatment. Also, 92% of participants were medication compliant.

Conclusion PTX and VE are safe interventions that may reduce rates of grade 3 pneumonitis for patients undergoing reirradiation for locoregionally recurrent and/or new lung primary tumors.

Keywords: non-small cell lung cancer, stereotactic ablative radiation therapy, vitamin E, pentoxifylline, reirradiation, pneumonitis

Introduction

Stereotactic ablative radiation therapy (SABR) is a versatile treatment for patients with non-small cell lung cancer (NSCLC) in the settings of operable early stage disease and inoperable late-stage disease.[1] Despite advancements in this radiotherapeutic technique, recurrence is not uncommon, with studies suggesting in-field failure rates of up to 30% at 2 years post-treatment, particularly in the setting of concurrent chemoradiation for stage 3 NSCLC.[2] Traditionally, systemic therapy was the

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Disclosure: The authors have no conflicts of interest to disclose. None of the authors received outside funding for the production of this original manuscript and no part of this article has been previously published elsewhere.

Published: June 1, 2025. https://doi.org/10.37549/ARO-D-25-00012

choice approach for instances of loco-recurrent relapse; however, poor response rates prompted investigations regarding the safety and efficacy of reirradiation.

In addition to high rates of local failure, patients undergoing reirradiation with fractionated external beam radiation therapy have also experienced toxicities such as esophagitis, dry desquamation, and symptomatic pneumonitis, arguing for the need to dose-escalate using conventionally fractionated regimens.[3] Institutional data evaluating SABR to treat locoregional lung cancer recurrences and new lung primaries in patients who have received prior thoracic radiation therapy is promising, with in-field local tumor control >90% at 2 years and 2-year overall survival (OS) and progression-free survival of 59% and 26%, respectively.[4] Unfortunately, rates of grade 3 toxicities were 30%.[4] A more recent review article by the International Association for the Study of Lung Cancer Advanced Radiation Technology Committee agrees that SABR is efficacious, with rates of 2-year local control ranging from 75% to 100%, despite persistence of toxicities.[5]

Prior studies have attempted to mitigate the effects of radiation on normal lung by using radioprotective agents such as pentoxifylline (PTX).[6-8] PTX is thought to help prevent radiotoxicity by inhibiting platelet aggregation and adhesion, allowing for optimal blood flow and increased tissue oxygenation.[9] Adding PTX to the retreatment of thoracic malignancies may aid in further reducing radiation-induced effects on normal adjacent tissue. A recent randomized study in breast cancer demonstrated that PTX in combination with vitamin E (VE) resulted in reduced rates of breast fibrosis after radiation therapy.[10] A randomized controlled trial evaluating VE and PTX in primary lung radiation showed increased rates of radiation-induced lung toxicity in the control group.[11] However, to date, no studies have assessed the efficacy of PTX and VE in the prevention of toxicities in the setting of reirradiation using SABR for lung malignancies.

Our phase II prospective trial addresses this gap in the literature by (1) evaluating the use of SABR in treating patients with locoregionally recurrent lung cancer or new lung primary tumors in the setting of prior thoracic radiation therapy and (2) establishing the efficacy of PTX in combination with VE in reducing the rates of grade 3 or 4 toxicities. Each patient was treated with VE and PTX before, during, and after reirradiation for lung malignancy via SABR. We hypothesize that SABR will be a viable treatment modality for these patients and that PTX and VE will reduce the rate of grade 3 pneumonitis in the cohort.

Materials and Methods

Patients were enrolled in a prospective, single-arm trial at our institution to assess the effects of PTX and VE on the rates of toxicity while using SABR in patients with recurrent or new lung primary after receiving prior thoracic radiation therapy. The criterion for patient eligibility consisted of previous radiation therapy for a thoracic malignancy with a diagnosis of a recurrent or new NSCLC. Patients either underwent a biopsy to confirm the diagnosis or demonstrated a strong clinical suspicion for new or recurrent cancer based on the recommendations of a multidisciplinary thoracic oncology team. Patients were excluded for the following reasons: <30 Gy of overlap from prior radiation treatment, poor pulmonary function at baseline (FEV1 < 20% and/or diffusing capacity of the lungs for carbon dioxide <20% predicted), chemotherapy within 4 weeks of SABR initiation, and a plan to initiate chemotherapy or immunotherapy concurrent with SABR.

Patients were administered PTX 400 mg 3 times daily. This was the standard starting dose for the drug as recommended by the manufacturer. Patients were given VE 400 IU once daily. Dosing began 1 week prior to treatment, approximately at the time of simulation. The drugs were continued for a period of 3 months after completion of radiation therapy. Dose delays or modifications were based on toxicity and made at the discretion of the principal investigator.

All patients were prescribed 50 Gy delivered to the planned target volume (PTV) in 5 fractions at 10 Gy per fraction. Fractions were administered at least 36 hours apart, and therapy was completed within 14 days of initiation. Daily image guidance with cone beam CT was required. Respiratory gating or abdominal compression was utilized as deemed appropriate by the treating physician and physicist.

Treatment planning was performed using either 3-dimensional conformal therapy or intensity- modulated radiation therapy. Any combination of coplanar or noncoplanar fields designed to cover the target volumes while limiting dose to critical structures was allowed. If prior dosimetry was available, a composite of the dosimetry plans of the prior treatment volume and the new treatment plan was generated. Standard SBRT treatment planning was utilized. Successful treatment planning was designed to meet the following:

1. Normalization: The plan was normalized such that

100% of the dose delivered corresponded to the center of mass of the PTV.

2. Prescription isodose surface coverage: The prescription isodose surface was chosen such that 95% of the PTV was conformally covered by the prescription isodose surface, and 99% of the PTV received at least 90% of the dose.
3. High-dose spillage: Any dose >105% of the prescription isodose surface occurred primarily within the PTV itself and not within normal tissue. The cumulative volume of all tissue outside the target received no more than 105% of the prescription to 15% of the volume. PTV conformality was judged such that the ratio of the volume of the prescription isodose to the volume of the PTV was ideally <1.2 as outlined by the Radiation Therapy Oncology Group.
4. Standard dose constraints to critical structures: Outlined in accordance with the Quantitative Analyses of Normal Tissue Effects in the Clinic.

The Common Terminology Criteria for Adverse Events (v. 4.0) was used to assess radiation- and PTX-related toxicity. Standard radiation-related toxicities were expected. Toxicity was defined as acute (<3 mo from completion), subacute (3-12 mo), and late (>12 mo). Standard consent forms for lung irradiation were used for informed consent. Specifically, radiation pneumonitis and fibrosis were assessed. The CT appearance of the consolidation was classified as either diffuse or mass-like according to published criteria.[12] Diffuse consolidation was defined as consolidation occurring outside of the 50% isodose line. Mass-like consolidation was defined as a new or enlarging solid opacity occurring within or directly adjacent to the PTV.

Standard post-treatment follow-up occurred at 6 weeks and then every 3 months after the completion of radiation therapy. A complete history and physical examination were performed at each follow-up visit to assess for treatment-related toxicity. Pulmonary function tests with lung diffusion testing were ideally obtained at 6 months post-radiation and yearly as determined by the treating physician. Follow-up imaging took place 8-12 weeks post-radiation and then every 3 months thereafter for 2 years. CT of the chest was performed using intravenous contrast. Surveillance CT imaging of the chest was obtained as recommended by the treating physician. F-18 fluorodeoxyglucose-positron emission tomography was obtained at 6 months post-SABR and then when determined clinically relevant by the treating physician.

Statistical Design

The primary endpoint was to estimate overall treatment-related toxicity and assess the efficacy of PTX in reducing grade 3 or 4 toxicity. The study was designed to estimate grade 3 treatment-related toxicity. Reports from prior retreatment series estimate >grade 3 pulmonary toxicities to be approximately 30%.[4] Our goal was to reduce this rate to 15%.

The cumulative number of grade 3 or 4 toxic events was monitored after each patient was enrolled. If the cumulative number of toxic events produced enough evidence to conclude that the true toxicity rate is ≥30% (Pt0 = 0.30), then the trial was planned to stop early for safety reasons.

Descriptive statistics were used to summarize the demographic and clinical features of the cohort. Toxicity was analyzed by taking the highest/most severe score within the first 3 months post-treatment, between 3 and 6 months post-treatment, and between 6 and 12 months post-treatment. At each point, the proportions for each toxicity score were computed, and the primary analysis was based on a one-sided test that the rate of grade 3+ toxicity is <30%. To assess the relationship between the ordinal toxicity scores and demographic and clinical characteristics, we considered proportional odds regression. We included time (3 mo, 6 mo, 12 mo) as a predictor in all models and otherwise included predictors one at a time. As we used multiple scores from each individual, we included a random intercept term to introduce correlation between the repeated measures.[13] Odds ratios were rescaled using the random effect variance to provide an approximately marginal interpretation.[14] Kaplan-Meier curves of OS and for recurrence-free survival (RFS) were considered. Missing data were addressed by performing an available case analysis. Statistical significance was defined at the alpha = 0.05 level, and all data analysis was performed using R statistical software, v.4.3.3.[15]

Of note, noncompliant patients were included in all analyses for which they had available data to contribute. One noncompliant patient died before post-treatment data were collected and was not included in our results; 2 other noncompliant patients contributed toxicity and survival data at each timepoint.

Results

Demographics

Demographic information about our cohort of 40 patients, all recruited

Table 1. Participant Demographics

COLUMN1	MEAN/COUNT	STANDARD DEVIATION	RANGE	MISSING
Patients	40			
ECOG				0
0	15	38%		
1	24	60%		
2	1	3%		
Previous radiation dose (Gy)	56.9	10.8	30-73.5	6
Interval between doses (mo)	Median 12	IQR 12–27	8-120	4
>12 mo	17	47%		4
Dose at retreatment (Gy)				0
50	37	93%		
25	1	3%		
40	2	5%		
Central tumor (vs peripheral)	6	16%		3
Noncompliant	3	8%		0
Pretreatment FEV1	1.52	0.65	0.78-3.66	20

Eastern Cooperative Oncology Group (ECOG) performance status scale, patients scoring 0 are fully independent, patients scoring 1 are restricted in strenuous activity but able to perform basic ADLS, patients scoring 2 are ambulatory however limited in daily work activities.

Forced expiratory lung volumes (FEV1) as measured on pulmonary function tests (PFTs).

from our institution, is displayed in **Table 1**. Of those patients, 98% had a baseline Eastern Cooperative Oncology Group (ECOG) status of ≤1. The average previous radiation dose prior to enrollment was 56.9 Gy; individual prior radiation doses and fractionations are listed in **Table 2**. 93% of participants were able to receive the anticipated reirradiation dose of 50 Gy. Overall, PTX and VE were well tolerated, with only 3 patients reporting noncompliance during the study.

Grades of Radiation Pneumonitis and Drug-Related Toxicities

Toxicity results are outlined in **Table 3** and graphically displayed in **Figure 1**. Of note, only one patient experienced grade 3 pneumonitis, which occurred at 6 months post-treatment. Furthermore, there is statistically significant evidence that rates of grade 3 pneumonitis are less than 30% at 3 months (0%, 95% CI 0%-11%, $P = .001$), 6 months (5%, 95% CI 0%-20%, $P = .004$), and 12 months (0%, 95% CI 0%-21%, $P = .010$) post-reirradiation in our VE- and PTX-treated cohort. Following analysis of patient data, the lowest incidence of pneumonitis was experienced at 3 months post-treatment, with 11% of our cohort experiencing grade 1 pneumonitis and 7% experiencing grade 2 pneumonitis. No participants reported post-treatment toxicities associated with either PTX or VE during post-treatment follow-up visits. In accordance with proportional odds regression (**Table 4**), toxicity was significantly higher at 6 months than at 3 months (proportional odds ratio = 2.64, $P = .029$), but no statistically significant associations between toxicity and other demographic/clinical factors were found. The model used for **Table 4** uses the ordinal grade values (0 vs 1 vs 2 vs 3), not a binary cut-off. The proportional odds effect reflects the odds of an increase in grade.

Tumor Recurrence and Overall Survival

Of our initial cohort of 40 patients, 5 were lost to follow-up after treatment completion and were excluded from the survival analysis. Of the remaining 35 patients, 14 (40%) had an observed recurrence, 2 of which were local, 6 of which were regional recurrences, and 6 of which were distant. 27 (77%) patients died during the study. **Figure 2** reports the Kaplan-Meier plots for RFS and OS. Median RFS was 10 months (95% CI 6-30 mo) and median OS was 20 months (95% CI 12-50 mo). The 1-year RFS rate was 44% (31%-55%) and 1-year OS was 66% (51%-85%).

Discussion

SABR is a valuable reirradiation modality for patients with new or recurrent lung malignancies. VE and PTX are well-tolerated medications that are potentially efficacious in preventing grade 3 and higher rates of radiation-induced pneumonitis. 92% of our patients were medication-compliant and reported little or no side effects associated with VE and PTX. Of our cohort of 40 patients, only one experienced grade 3 pneumonitis, with most experiencing grade 0 pneumonitis. Median OS was 20 months, and median RFS was 10 months. To date, our trial is the first to assess the utility of these agents for patients being reirradiated via SABR for locoregionally recurrent disease or new primary malignancies.

Table 2. Individual Prior Radiation Doses and Fractionation

NUMBER OF PATIENTS	PREVIOUS RADIATION DOSE (GY)	PREVIOUS FRACTIONS	PREVIOUS BIOLOGICAL EQUIVALENT DOSE
1	30	10	39
1	33	10	43.8
1	37.5	15	46.8
1	45	15	58.5
1	45	25	53.1
3	48	4	105
1	50	5	100
2	50.4	28	59
3	54	3	151
2	59.4	33	70
5	60	30	72
1	61.2	30	71
1	63	30	73
1	65	33	71
4	66	33	72
1	66.4	35	74
1	66.4	33	72
1	66.6	33	72
1	68.4	38	80
1	73.5	35	74

Table 3. Toxicity Results at 3, 6, and 12 Mo Post-Treatment

	GRADE 0		GRADE 1		GRADE 2		GRADE 3		GRADE 3 < 30%	MISSING
	N	%	N	%	N	%	N	%		
3 mo	22	79	3	11	2	7	0	0	$P = .001$	13
6 mo	12	55	5	23	4	18	1	5	$P = .004$	18
12 mo	7	54	6	46	0	0	0	0	$P = .010$	27

Data regarding the rates and predictors of radiation-induced pneumonitis in the setting of primary radiation vs reirradiation have been published previously. In the primary setting, Schallenkamp et al[16] reported a rate of 13% in grade 2 or greater pneumonitis in the setting of conventional radiation, which may be related to the percentage of total lung volume minus gross tumor volume receiving ≥10 Gy. Accordingly, lower radiation doses to larger volumes of lung may induce a significant, symptomatic, and more aggressive inflammatory response than that experienced with higher doses of radiation. Patients with subclinical interstitial lung disease (ILD) have been found to be at greater risk for higher grades of pneumonitis —of 87 patients with subclinical ILD, 11.5% experienced grade 3 pneumonitis, 3.4% experienced grade 4 pneumonitis, and 5.7% experienced grade 5 pneumonitis, and the cumulative incidence of these high grades of pneumonitis was significantly higher in patients with subclinical ILD involving ≥25% of the lungs (46.1% vs 16.3%, $P = .004$).[17]

Systemic therapies may similarly influence the rates of grade 3 or higher primary radiation-induced pneumonitis. Neoadjuvant therapy with gemcitabine has been associated with a significantly higher incidence of grade 3 or higher pneumonitis (32.3% vs 13.3%, $P = .023$).[17] Of 106 patients treated with concurrent chemoradiation, pneumonitis ≥grade 2 occurred in 47 patients (44.3%), and pneumonitis ≥grade 3 occurred in 6 (5.7%).[18] Among this cohort, adjuvant therapy with immune checkpoint inhibitors (durvalumab, atezolizumab, pembrolizumab, or nivolumab) significantly influenced the rate of grade 2 or higher pneumonitis but not rates of grade 3 or higher pneumonitis.[18]

Understandably, toxicities are more prominent in the setting of reirradiation with SBRT. Data from the MD Anderson Cancer Center suggest a rate of grade 3 pneumonitis ranging from 18.9%[19] to 30%.[4] Rates of toxicity may be influenced by the following factors: ECOG score of 2 or 3, an FEV1 ≤ 65%, a previous PTV spanning the bilateral mediastinum, and V20 ≥ 30% on composite (previous RT + SABR) plans.[19] Others report comparable rates of toxicity, with 26% of a cohort experiencing grade 3 or greater toxicity. However, among this same cohort, 14% ultimately died from a post-treatment lung-related event other than bacteria-associated pneumonitis or disease progression. Subsequent chemotherapy, rather than radiation therapy factors such as total dose, lung dose, or interval between doses, was significantly related to such lethal events ($P = .009$).[20] Finally, out of a cohort

Figure 1. Trends in grades of pneumonitis at 3, 6, and 12 mo post-treatment.

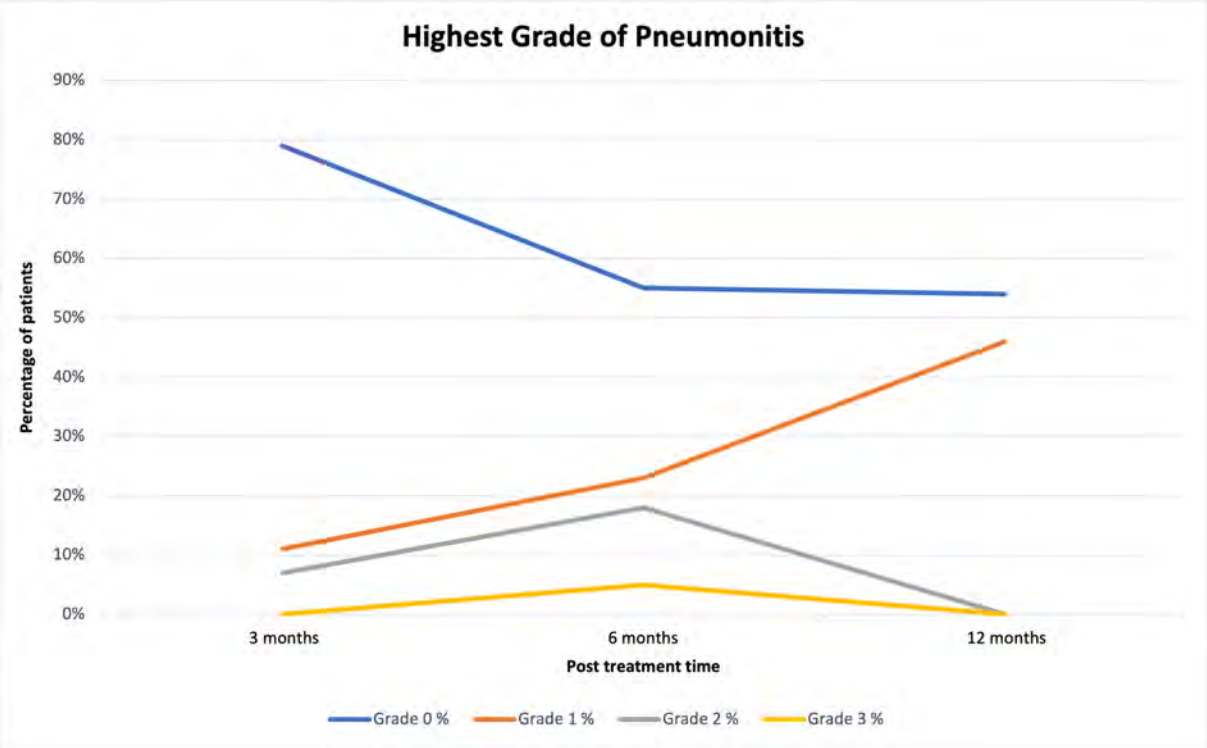


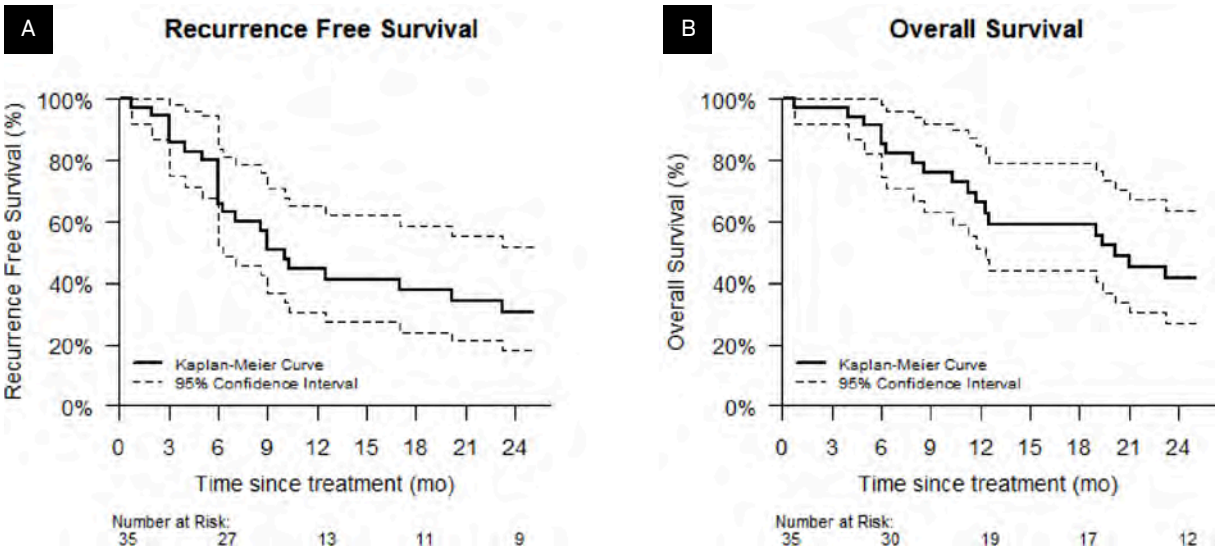
Table 4. Results From Proportional Odds Model on Toxicity Grade

	ODDS RATIO	CONFIDENCE INTERVAL	*P* VALUE	MISSING OBSERVATIONS
Time (mo)			.005	
3	Ref			
6	2.64	1.10–6.31	.029	
12	2.63	0.97–7.14	.057	
ECOG				
0	Ref			
1 or higher	0.87	0.38-1.97	.733	
Previous radiation dose (Gy)	0.84 (10-unit change)	0.53-1.35	.477	10
Interval between doses >12 mo	0.56	0.22-1.53	.226	8
Central tumor (vs peripheral)	1.97	0.62-6.25	.249	4
Noncompliant	2.22	0.57-8.65	.251	
Pretreatment FEV1	2.00 (1-unit change)	0.38-10.40	.410	27

Model is fit using all available toxicity grades (n = 62), and random effects are used to control for correlation between multiple scores from the same individual. The effect for each variable is assessed by controlling for time (3 mo, 6 mo, 12 mo).

Abbreviations: ECOG, Eastern Cooperative Oncology Group; FEV, forced expiratory lung volume.

Figure 2. Kaplan-Meier estimates for (A) recurrence-free survival and (B) overall survival.



of 70 patients followed throughout their primary and reirradiation treatments, 15 (21.4%) developed pneumonitis ≥grade 3 in the reirradiation setting.[21]

Given the prominence of symptomatic pneumonitis, particularly in the setting of reirradiation, clinicians should aim to limit such toxicities while maintaining ideal dose prescription and treatment parameters. VE and PTX are tools that can potentially help treating physicians achieve this goal. PTX is a nonselective phosphodiesterase inhibitor that increases intracellular cyclic GMP levels, resulting in greater flexibility of the red blood cell and leukocyte membrane. This permits greater passage of oxygen and nutrients through microvessels and, ultimately, into tissue. VE is a fat-soluble vitamin principally found in the cell membrane. It is also thought to contribute to membrane flexibility and may offer vascular protection and anti-inflammatory and antifibrosis capabilities.[22] Notably, they are well tolerated; the most common side effects of each medication are nausea, vomiting, and diarrhea, none of which were experienced by patients in our cohort.[9,23] Several studies have evaluated the efficacy of these agents, albeit outside of the setting of reirradiation with SABR for thoracic malignancy. For patients with breast cancer, randomized controlled trials suggest that VE and PTX lessen the degree of radiation-induced fibrosis but may not significantly impact OS or progression-free survival.[10,24] They are similarly beneficial in the setting of primary radiation for lung cancer, with PTX and VE patients experiencing a lower burden of lung toxicities compared with placebo-treated patients.[11]

Of note, a brief discussion is warranted regarding the concerns that have arisen from recent studies over the detrimental effects of VE supplementation. A meta-analysis of 19 trials found increased overall mortality in patients taking high doses of VE (over 400 IU daily for at least 1 y).[25] Another analysis indicated increased mortality from VE and β-carotene supplementation in the prevention of gastrointestinal cancers.[26] Additionally, the Selenium and Vitamin E Cancer Prevention Trial demonstrated that 400 IU VE supplementation raised prostate cancer rates in men after a follow-up of at least 7 years.[27] However, these findings are based on long-term and/or high-dose VE use, contrasting with our trial's short-term, standard supplementation. Moreover, our cohort differs significantly from those studied, possibly explaining the benefits associated with supplementation for patients undergoing reirradiation who are under significant physiological stress, which in turn may help explain the pneumo-protective impact of short-term antioxidant use. Lastly, variations in supplement quality, alternative isoforms of VE, patient-to-patient CYP450 metabolism, and drug-drug interactions impacting VE metabolism—none of which have been accounted for in these studies—may also influence individual VE susceptibilities and outcomes, further complicating interpretations.

Future studies are warranted to further address the tolerability and effectiveness of VE and PTX. Double-blind, randomized

controlled trials will help better elucidate the clinical utility of these pharmaceutical agents. Additionally, studying these agents in the setting of other radiotherapeutic treatments for other malignancies such as gastrointestinal cancers, head and neck cancers, and/or gynecologic and urologic cancers could be beneficial.

Strengths and Limitations

Given the simple design of this prospective study, we were able to clearly identify and accurately evaluate our principal outcome of rates of grade 3 pneumonitis in our cohort of patients with recurrent NSCLC. VE and PTX are well tolerated, allowing for a high compliance rate and suggesting they can be safely and similarly studied in the setting of reirradiation for other disease sites. Regarding limitations, as this was a single-arm clinical trial, we are unable to compare the utility and efficacy of VE and PTX compared with placebo, which would be afforded in the design of a randomized, double-blind, placebo-controlled trial. Our sample size is small and from a single institution; recruiting a more diverse cohort from across the United States would be beneficial. Also, some participants were lost to follow-up at different times post-treatment, possibly introducing a bias in our results if those participants ultimately developed different health outcomes secondary to late toxicities associated with the study drugs and/or radiation course compared with the participants who remained in the study.

Finally, further collecting pretreatment physiological and radiotherapeutic data to help better identify predictors of toxicity, a topic that was briefly explored in our discussion, would be warranted. Particularly, obtaining accurate pretreatment isodose volumes in addition to pretreatment dose/fractionation would be warranted. Many of the patients in this study were referrals from rural communities, where they may have received incomplete treatment for their primary malignancies, thus explaining the wide range of previously administered radiation dose. We did not have access to data for the specific circumstances dictating original radiotherapeutic dose selection or other pretreatment factors that may have influenced each patient's response and/or compliance to the study at hand. We also do not have pretreatment radiotherapeutic data beyond that of prior dose and fractionation to correlate with reirradiation toxicity.

Conclusion

PTX and VE are safe interventions that may prophylactically reduce rates of grade 3 pneumonitis for patients receiving subsequent SABR for recurrent NSCLC. Additional studies should be performed to evaluate the use of PTX and VE in the retreatment setting.

References

1) Chang JY, Mehran RJ, Feng L, et al. Stereotactic ablative radiotherapy for operable stage i non-small-cell lung cancer (revised STARS): long-term results of a single-arm, prospective trial with prespecified comparison to surgery. *Lancet Oncol*. 2021;22(10):1448-1457. doi:10.1016/S1470-2045(21)00401-0

2) Curran WJ Jr, Paulus R, Langer CJ, et al. Sequential vs. concurrent chemoradiation for stage III non-small cell lung cancer: randomized phase III trial RTOG 9410. *J Natl Cancer Inst*. 2011;103(19):1452-1460. doi:10.1093/jnci/djr325

3) Wu K-L, Jiang G-L, Qian H, et al. Three-dimensional conformal radiotherapy for locoregionally recurrent lung carcinoma after external beam irradiation: a prospective phase i-II clinical trial. *Int J Radiat Oncol Biol Phys*. 2003;57(5):1345-1350. doi:10.1016/s0360-3016(03)00768-5

4) Kelly P, Balter PA, Rebueno N, et al. Stereotactic body radiation therapy for patients with lung cancer previously treated with thoracic radiation. *Int J Radiat Oncol Biol Phys*. 2010;78(5):1387-1393. doi:10.1016/j.ijrobp.2009.09.070

5) Brooks ED, Verma V, Senan S, et al. Salvage therapy for locoregional recurrence after stereotactic ablative radiotherapy for early-stage NSCLC. *J Thorac Oncol*. 2020;15(2):176-189. doi:10.1016/j.jtho.2019.10.016

6) Dion MW, Hussey DH, Doornbos JF, et al. Preliminary results of a pilot study of pentoxifylline in the treatment of late radiation soft tissue necrosis. *Int J Radiat Oncol Biol Phys*. 1990;19(2):401-407. doi:10.1016/0360-3016(90)90549-y

7) Dion MW, Hussey DH, Osborne JW. The effect of pentoxifylline on early and late radiation injury following fractionated irradiation in C3H mice. *Int J Radiat Oncol Biol Phys*. 1989;17(1):101-107. doi:10.1016/0360-3016(89)90376-3

8) Ozturk B, Egehan I, Atavci S, Kitapci M. Pentoxifylline in prevention of radiation-induced lung toxicity in patients with breast and lung cancer: a double-blind randomized trial. *Int J Radiat Oncol Biol Phys*. 2004;58(1):213-219. doi:10.1016/s0360-3016(03)01444-5

9) Ward A, Clissold SP. Pentoxifylline. a review of its pharmacodynamic and pharmacokinetic properties, and its therapeutic efficacy. *Drugs*. 1987;34(1):50-97. doi:10.2165/00003495-198734010-00003

10) Jacobson G, Bhatia S, Smith BJ, et al. Randomized trial of pentoxifylline and vitamin E vs standard follow-up after breast irradiation to prevent breast fibrosis, evaluated by tissue compliance meter. *Int J Radiat Oncol Biol Phys*. 2013;85(3):604-608. doi:10.1016/j.ijrobp.2012.06.042

11) Misirlioglu CH, Demirkasimoglu T, Kucukplakci B, Sanri E, Altundag K. Pentoxifylline and alpha-tocopherol in prevention of radiation-induced lung toxicity in patients with lung cancer. *Med Oncol*. 2007;24(3):308-311. doi:10.1007/s12032-007-0006-z

12) Dunlap NE, Yang W, McIntosh A, et al. Computed tomography-based anatomic assessment overestimates local tumor recurrence in patients with mass-like consolidation after stereotactic body radiotherapy for early-stage non-small cell lung cancer. *Int J Radiat Oncol Biol Phys*. 2012;84(5):1071-1077. doi:10.1016/j.ijrobp.2012.01.088

13) Tutz G, Hennevogl W. Random effects in ordinal regression models. *Comput Statist Data Anal*. 1996;22(5):537-557. doi:10.1016/0167-9473(96)00004-7

14) Zeger SL, Liang KY. An overview of methods for the analysis of longitudinal data. *Stat Med*. 1992;11(14-15):1825-1839. doi:10.1002/sim.4780111406

15) R Core Team. *A Language and Environment for Statistical Computing*; 2024. https://www.R-project.org/

16) Schallenkamp JM, Miller RC, Brinkmann DH, Foote T, Garces YI. Incidence of radiation pneumonitis after thoracic irradiation: dose-volume correlates. *Int J Radiat Oncol Biol Phys*. 2007;67(2):410-416. doi:10.1016/j.ijrobp.2006.09.030

17) Li F, Liu H, Wu H, Liang S, Xu Y. Risk factors for radiation pneumonitis in lung cancer patients with subclinical interstitial lung disease after thoracic radiation therapy. *Radiat Oncol*. 2021;16(1):70. doi:10.1186/s13014-021-01798-2

18) Jang JY, Kim SS, Song SY, et al. Radiation pneumonitis in patients with non-small-cell lung cancer receiving chemoradiotherapy and an immune checkpoint inhibitor: a retrospective study. *Radiat Oncol*. 2021;16(1):231. doi:10.1186/s13014-021-01930-2

19) Liu H, Zhang X, Vinogradskiy YY, et al. Predicting radiation pneumonitis after stereotactic ablative radiation therapy in patients previously treated with conventional thoracic radiation therapy. *Int J Radiat Oncol Biol Phys*. 2012;84(4):1017-1023. doi:10.1016/j.ijrobp.2012.02.020

20) Yang WC, Hsu FM, Chen YH, et al. Clinical outcomes and toxicity predictors of thoracic re-irradiation for locoregionally recurrent lung cancer. *Clin Transl Radiat Oncol*. 2020;22:76-82. doi:10.1016/j.ctro.2020.03.008

21) Patel R, Rezaei A, Zhang Y, Podder T, Biswas T. EP08.04-02 evaluating safety profiles of radiation-related toxicities in thoracic re-irradiation. *J Thorac Oncol*. 2023;18(11):S591. doi:10.1016/j.jtho.2023.09.1111

22) Pareek P, Sharma A, Thipparampalli JR, et al. Pentoxifylline and vitamin E alone or in combination for preventing and treating side effects of radiation therapy and concomitant chemoradiotherapy. *Cochrane Database Syst Rev*. 2016;2021(10):CD012117. doi:10.1002/14651858.CD012117

23) Rodrigo R, Guichard C, Charles R. Clinical pharmacology and therapeutic use of antioxidant vitamins. *Fundam Clin Pharmacol*. 2007;21(2):111-127. doi:10.1111/j.1472-8206.2006.00466.x

24) Magnusson M, Höglund P, Johansson K, et al. Pentoxifylline and vitamin e treatment for prevention of radiation-induced side-effects in women with breast cancer: a phase two, double-blind, placebo-controlled randomised clinical trial (ptx-5). *Eur J Cancer*. 2009;45(14):2488-2495. doi:10.1016/j.ejca.2009.05.015

25) Miller ER 3rd, Pastor-Barriuso R, Dalal D, et al. Meta-analysis: high-dosage vitamin e supplementation may increase all-cause mortality. *Ann Intern Med*. 2005;142(1):37-46. doi:10.7326/0003-4819-142-1-200501040-00110

26) Bjelakovic G, Nikolova D, Simonetti RG, Gluud C. Antioxidant supplements for prevention of gastrointestinal cancers: a systematic review and meta-analysis. *Lancet*. 2004;364(9441):1219-1228. doi:10.1016/S0140-6736(04)17138-9

27) Klein EA, Thompson IM Jr, Tangen CM, et al. Vitamin E and the risk of prostate cancer: the selenium and vitamin E cancer prevention trial (SELECT). *JAMA*. 2011;306(14):1549-1556. doi:10.1001/jama.2011.1437

Malignant Melanotic Nerve Sheath Tumor of the Neck: A Case Report

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Abstract

Malignant melanotic nerve sheath tumors (MMNSTs) are rare, aggressive tumors with significant potential for distant metastasis. This case report describes an atypical presentation of MMNST in a middle-aged patient, arising in the carotid space rather than in the more commonly reported paraspinal regions. The report details clinical presentation, diagnostic evaluation, and the planned course of adjuvant radiation therapy after surgical excision. Comprehensive genomic profiling and multidisciplinary treatment planning were key components of management. Given the rarity of MMNST and its potential for recurrence and metastasis, vigilant, long-term follow-up is essential. This case underscores the importance of recognizing uncommon anatomical presentations that may pose unique diagnostic and therapeutic challenges.

Keywords: nerve sheath neoplasm, malignant, melanotic, adjuvant treatment, case report

Introduction

Melanotic nerve sheath tumors (MMNSTs) are exceptionally rare neoplasms, comprising less than 1% of all nerve sheath tumors, with fewer than 200 cases described in the literature.[1] Owing to their rarity, most of the existing knowledge about MMNSTs comes from individual case reports or small case series.[2] Management can be particularly challenging owing to the tumors' potential for aggressive behavior, including local and distant metastasis in over one-third and approximately 44% of cases, respectively.[1]

Patient History and Surgical Treatment

A middle-aged patient presented to a hospital in September 2023 with complaints of drooping of the right eyelid, neck pain, sore throat, and dull pain in and behind the right ear. The patient had an unremarkable medical history but reported a history of melanoma in the patient's maternal grandfather. The patient had a 1.25 pack-year smoking history, having smoked 0.25 packs/day for 5 years and last smoking 16 years previous. They reported drinking "socially."

Right-sided Horner syndrome was confirmed by a positive apraclonidine test. The patient underwent complete resection of the right parapharyngeal space tumor and a right neck dissection. This consisted of transcervical excision of a 4 cm right parapharyngeal space mass seemingly arising from the cervical sympathetic trunk. The mass extended to within 10 mm of the base of the skull and was described as "darker than expected with significant pigment" per surgical note. The mass was resected without complications.

Imaging Findings

A contrast-enhanced CT neck was significant for a 3.9 × 1.7 × 1.6 cm heterogeneous, partially calcified enhancing

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Disclosure/informed consent: The authors have no conflicts of interest to disclose. None of the authors received outside funding for the production of this original manuscript and no part of this article has been previously published elsewhere. The patient involved in this case report provided informed written consent to participate in and publish the results of this case study. Ethics approval was waived.

Published: June 1, 2025. https://doi.org/10.37549/ARO-D-25-00007

Figure 1. Preoperative MRI of the neck showing axial and coronal postcontrast, fat-saturated T1 sequences and demonstrating an enhancing lesion in the right carotid space.

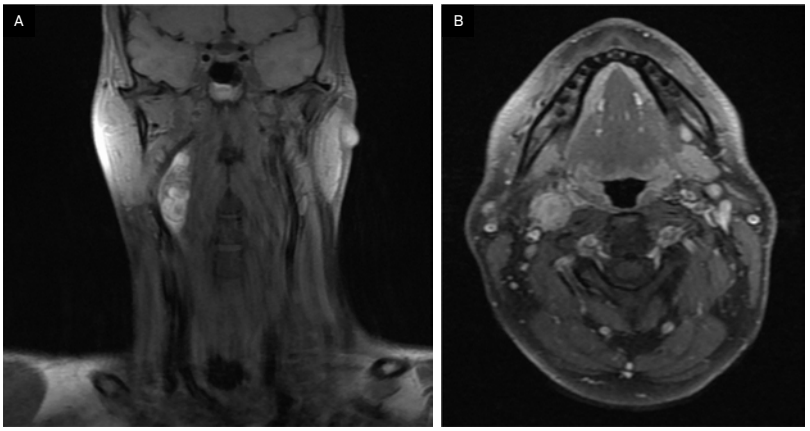
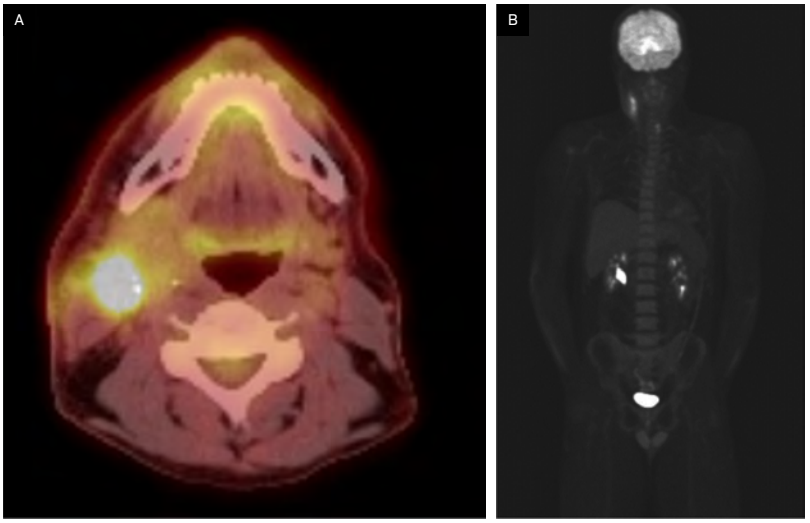


Figure 2. Preoperative F-18 fluorodeoxyglucose (FDG) PET-CT demonstrating FDG-avid lesions.



lesion in the right carotid space, splaying the internal and external carotid. The radiological differential included a carotid body tumor, with other etiologies like schwannoma or an enlarged lymph node less likely. An MRI demonstrated a 4 cm enhancing lesion in the right carotid space, similarly suspicious for carotid body tumor and, less likely, schwannoma or pathologic lymph node (**Figure 1**). A chest/abdominopelvic CT scan was negative for thoracic or abdominal paragangliomas and any metastatic disease. A hypermetabolic lesion in the right upper cervical neck was seen on preoperative PET-CT.

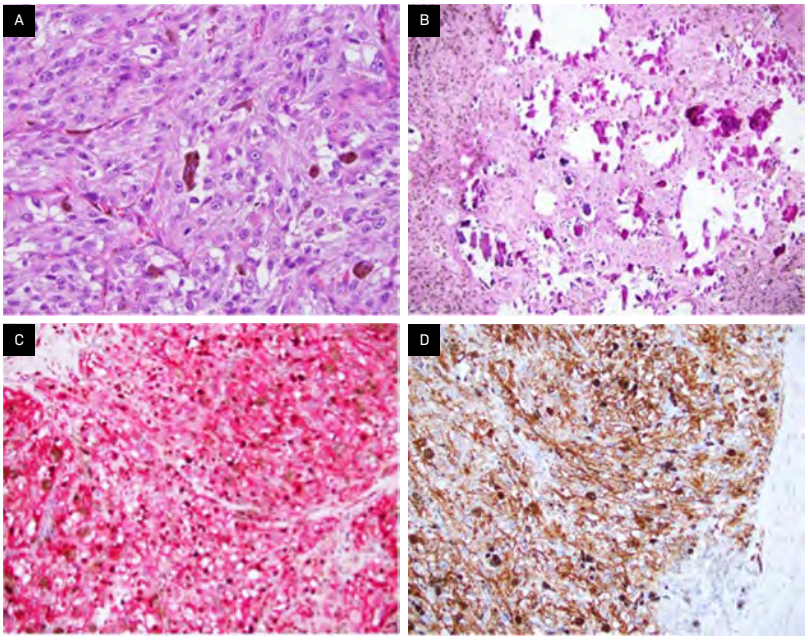
Postoperative PET-CT indicated postsurgical changes in the right upper cervical region with F-18 fluorodeoxyglucose (FDG)-avid, heterogeneous, asymmetric thickening of the anterior aspect of the right sternocleidomastoid muscle and was negative for hypermetabolic lymphadenopathy (**Figure 2**). There were no findings suspicious for distant metastatic disease.

Pathology

Gross examination of the right parapharyngeal space tumor specimen revealed a 4.0 × 2.0 × 1.4 cm dark tan nodule with a smooth, glistening surface. Serial sectioning demonstrated a well-circumscribed mass with grossly negative margins. Intraoperative frozen-section analysis revealed a pigmented epithelioid neoplasm, with the final diagnosis deferred to permanent sections. Microscopic examination of the permanent sections revealed a well-circumscribed, heavily pigmented neoplasm, arranged in nests and composed of epithelioid cells with round nuclei and prominent nucleoli, with admixed melanophages (**Figure 3A**). Psammoma bodies and areas composed predominantly of melanophages were present (**Figure 3B**). No necrosis, lymphovascular invasion, or perineural invasion was identified. The surgical margins were negative for tumor. A right neck dissection showed 3 lymph nodes negative for malignancy. A combined Melan A/Ki67 immunohistochemical stain of the psammoma bodies was positive for Melan A and showed a Ki67 proliferation index <10% (**Figure 3C**). Immunohistochemistry (IHC) also revealed that the tumor was positive for multiple additional melanocytic lineage markers, including SOX10, S100 protein, MITF, and HMB-45 (**Figure 3D**) but negative for CK8/18 and synaptophysin. IHC for *BRAF (VE1)* was negative in the tumor; thus, there was no indication of an underlying *BRAF* V600E mutation. PD-L1 expression by IHC was also negative.

Comprehensive genomic profiling using a hybrid capture-based DNA+RNA sequencing platform (Caris Life Sciences) revealed a *PRKAR1A* p.S321fs* pathogenic frameshift

Figure 3. Examination shows fascicles of large epithelioid cells with pigmented cytoplasm and prominent nucleoli, as well as admixed melanophages (A, H&E stain; original magnification: ×400). Psammoma bodies typical of this entity are also identified (B, H&E stain; ×200). Immunohistochemistry reveals the tumor is diffusely and strongly positive for melanocytic markers Melan A (C, red chromogen; × 200) and HMB45 (D, × 200), with Ki67 labeling fewer than 10% of tumor nuclei with brown chromogen (C, × 200).



mutation (RefSeq transcript NM_001276289.1; c.962delC), at 44% variant allele frequency. No genomic alterations in *BRAF*, *CDKN2A*, or the *TERT* promoter were identified. A review of the copy number plot demonstrated monosomies of chromosomes 1, 2, 4, 17, 21q, and 22q. Taken together with the findings of routine histopathologic examination and IHC, this genomic profile, with mutation in *PRKAR1A* only and monosomies of multiple chromosomes, including 1, 2, and 17, was diagnostic for MMNSTs.

Dedicated constitutional (germline) testing using a DNA and RNA-based platform (Invitae Multi-Cancer+RNA Panel) was performed on whole blood and was negative for all alterations, including in *PRKAR1A*. This finding supports that the above *PRKAR1A* mutation identified in tumor tissue is somatic in origin.

Discussion

MMNSTs, previously termed melanotic schwannomas, are uncommon tumors classified by the presence of melanin-producing Schwann cells.[3] These entities are known for their aggressive nature and rarity as they make up <1% of all nerve sheath tumors.[2] They typically originate in the spine or paraspinal soft tissue, although this particular case report presents one within the carotid space.[4] Psammomatous MMNSTs account for approximately half of all MMNSTs, and approximately half of these tumors are associated with the Carney complex, a disorder characterized by skin pigmentation, endocrine abnormalities, and increased risk of pituitary adenomas, testicular tumors, and cardiac myxomas.[5] This disorder is often associated with mutations in the *PRKAR1A* gene. However, in this case, the patient does not have a history of the Carney complex, and dedicated germline testing was negative for *PRKAR1A* mutation.

In the limited number of MMNST cases reported, patients commonly presented with neuropathic symptoms such as pain and paresthesia, similar to the findings in this case.[6]

Key imaging features include enhancement at CT and FDG-avidity at PET-CT. It has also been noted that MMNSTs typically grow along a spinal nerve root with a unique "dumbbell" configuration, though this is a nonspecific finding, as many types of tumors assume this configuration if they contain intradural and extradural components.[7-9] MRI is useful in distinguishing them from other tumors, as MMNSTs have intrinsic T1 hyperintensity (**Figure 1**), while schwannomas and neurofibromas tend to be hypointense on T1 and hyperintense on T2.[10]

MMNSTs are also characterized microscopically by spindle and epithelioid cells arranged in interlacing fascicles, with marked accumulation of melanin in neoplastic cells and admixed melanophages. Psammoma bodies are also typical.[3] Protein kinase A regulatory subunit-α (*PRKAR1A*) is genomically inactivated in MMNST. Monosomies of chromosomes 1, 2, and 17 are also characteristic.[11] This entity is distinguished from other heavily pigmented melanocytic neoplasms, including pigmented epithelioid melanocytoma and melanoma, by the presence of these specific monosomies and a lack of additional genomic alterations (*TERT* promoter, *CDKN2A* gene mutations, etc) encountered in melanoma.[12]

Complete resection is the primary curative treatment, with prior reports suggesting adjuvant radiation for larger or more aggressive

Table 1. Malignant Melanotic Nerve Sheath Tumor Case Reports and Treatment Outcomes

N	AGE/GENDER	LOCATION OF TUMOR	TYPE OF SURGERY (R0, R1, R2)	ADJUVANT TREATMENT	RADIATION DOSE IF RECEIVED	OUTCOME (30 MO NED, ETC)
1	52 y/male[17]	Parotid gland	R1	None	None	No recurrence
1	21 y/female[18]	Left foraminal L5/S1	R0	None	None	Recurred (i.e., locally at L5/S1) and metastasized (ie, leptomeningeal spread) throughout the neuroaxis within 4 postoperative months
1	45 y/female[19]	C6 nerve root, with both intradural and extradural components	R0	RT and then combination immunotherapy with nivolumab and ipilimumab	Not specified	Recurrence and widespread metastasis with death at 15 mo after diagnosis
1	59 y/female[20]	Left paraaortic area	R0	The tumor was resected en bloc by laparoscopic surgery and subsequent adjuvant RT	Not specified	No evidence of recurrent or metastatic disease 11 mo post- RT
1	18 y/female[21]	S1 nerve root	R0	Adjuvant stereotactic radiosurgery	40 Gy in 5 fractions, prescribed to 84% isodose line, with 25 Gy to bony spinal canal	No evidence of recurrent or metastatic disease after 2.5 y
2	58 y/female; 72 y/male[6]	T11-T12; T11	R0; R1	Case 1: complete resection with no adjuvant treatment Case 2: incomplete resection due to severe adhesions and bleeding, continued annual MRI, abdominal CT, and CXR without distant metastasis	None	None in both cases
2	35 y/male; 50 y/female[2]	Lumbar spinal L1/2 to L3/4 and cervical spinal at C6	R2, R1	Case 1: L2-L3 laminectomy and adjuvant RT Case 2: C5-7 laminectomy with partial excision of the lesion and adjuvant RT	Case 1: RT; 50.4 Gy in 28 fractions Case 2: 50.4 Gy followed by 5.4 Gy boost	Case 1: no recurrence; currently on follow-up 6 mo post RT Case 2: therapy ongoing

Abbreviation: R0, resection with cure or complete remission; R1, resection with microscopic residual tumor; R2, resection with macroscopic residual tumor; RT, radiation therapy.

lesions.[1,13] However, studies have found that 35% of patients experience local recurrences, suggesting that adjuvant radiation should be considered more broadly, particularly given the sensitive locations in which these neoplasms often arise that prohibit meaningful salvage operations. MMNSTs also have notable malignant potential; in one study, nearly half of the patients developed metastases.[1] Although MMNSTs are histologically and clinically distinct from malignant peripheral nerve sheath tumors (MPNSTs), treatment strategies are often extrapolated from the MPNST literature owing to the rarity of MMNSTs. Targeted therapies such as oncolytic herpes simplex virus have shown promise in preclinical models of MPNSTs.[14] Similarly, multimodal treatments such as preoperative chemotherapy, surgery, and postoperative radiation therapy (RT) have led to extended remission in some cases of MPNSTs.[15] Further research is needed to identify effective targeted therapies and treatment strategies for MMNSTs.[16]

As noted in **Table 1**, data documenting a strong association between adjuvant treatment and outcomes after surgical treatment of MMNSTs are lacking.

Adjuvant Treatment

After consultation with the patient, adjuvant RT was recommended owing to the locally aggressive nature of this tumor. The radiation dose required for optimal disease control remains uncertain; multiple experts recommend 60-70 Gy in standard and

Figure 4. Axial and coronal images of the final proton therapy plan. Axial slices (clockwise from top left) correspond to the superior field (A, B), mid-field (C, D), and inferior field (E). The patient was treated with 3 anterolateral proton beams using pencil-beam scattering. Isodose lines represent (from innermost to outermost): 63 Gy (orange), 54 Gy (yellow), 31.5 Gy (green), and 15.75 Gy (fuchsia).

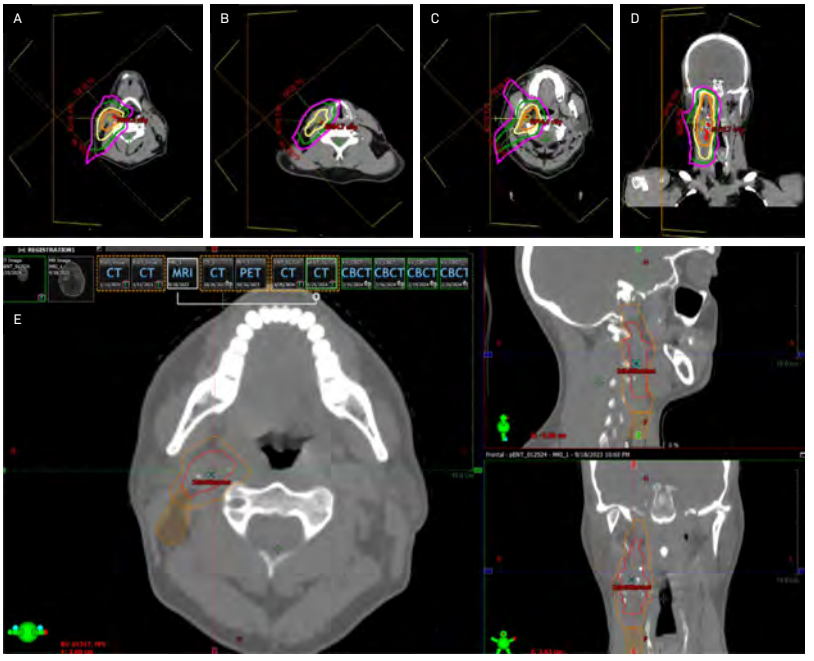
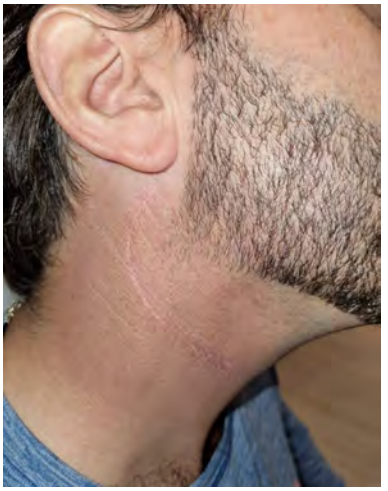


Figure 5. Clinical photograph of the right neck 6 mo post-treatment.



slightly hypofractionated regimens, consistent with that for aggressive soft tissue sarcomas or MPNSTs of the head and neck with negative surgical margins.[22,23] In this case, a compromise was reached among radiation oncologists to use an integrated boost where the postop bed/high-risk area was treated to 63 Gy in 2.1 Gy fractions; the intermediate-risk area was treated with 60 Gy in 2 Gy fractions to a radial margin of 0.5 cm on the postop bed, cropped back from bone, air, and 1.5 cm superior/inferior margin on the postop bed for concern about tracking along the vessels and nerves. The low-risk area was treated with 54 Gy in 1.8 Gy fractions to level 2b and the rest of level 3, to complete contouring of the involved LN basins surrounding the tumor bed and to add small margins on the postop bed. The regional lymph nodes were included in the low-dose area because of the possibility of aggressive local behavior and the propensity of head and neck cancers to metastasize to the regional neck nodes.

The patient was simulated supine with a long Aquaplast mask. Given their young age and planned ipsilateral treatment, intensity-modulated proton therapy delivered in Gy (RBE) was recommended to minimize radiation dose to nearby and contralateral healthy organs based on improved radiation dosimetry compared with IMRT. Image guidance with daily cone beam CT was utilized to minimize interfraction variability. No planning target volumes were added because the patient was treated with proton therapy. Slices from the primary RT plan are shown in **Figure 4**. The patient did not miss any treatments and tolerated the expected side effects of treatment, including grade 1 dermatitis of the right neck and right cheek, along with hair loss in the treatment field. However, the patient experienced no fatigue, changes in taste or saliva, or mucositis.

Post-Treatment Follow-Up

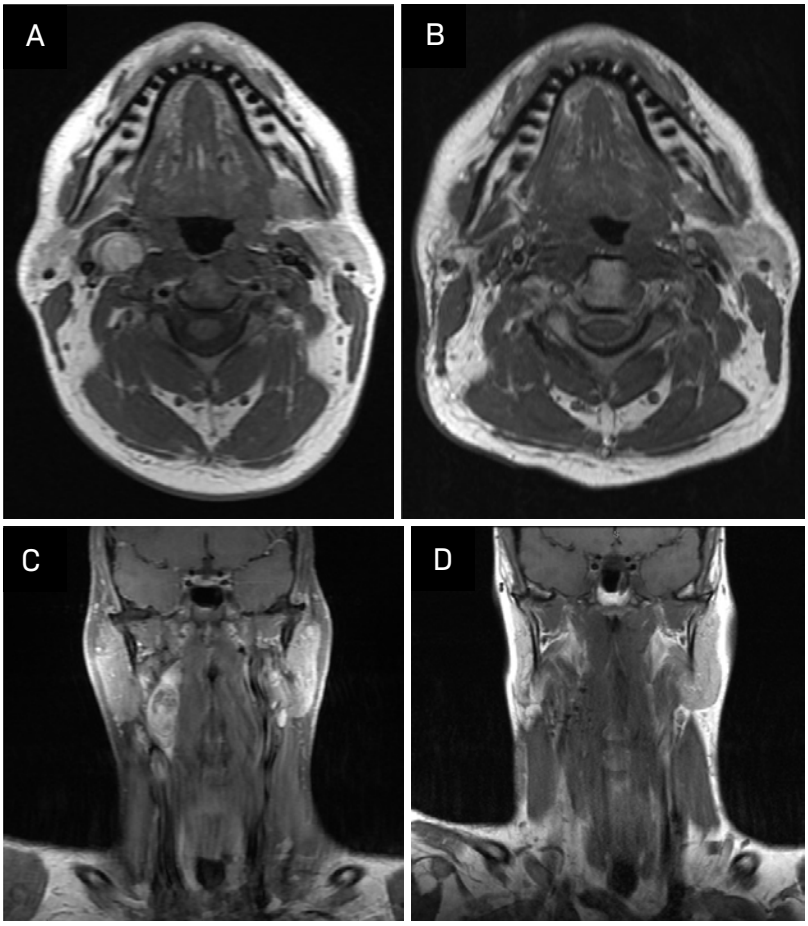
At evaluation 3 months postradiation, the patient's skin showed no residual reaction other than mild hyperpigmentation. Beard on the cheek, but not on the neck, grew back (**Figure 5**).

MRI 3 months post-treatment demonstrated no evidence of local or regional recurrence. Imaging showed increased signal on STIR and some enhancement in the right aspect of the neck, including the right aspect of the oropharynx and supraglottic larynx, presumably postradiation effects. There was also increased T2 signal and enhancement in the right submandibular gland, which was also compatible with postradiation effects (**Figure 6**). We plan to repeat MRI every 6 months for 2 years and then yearly for 5 years. Routine clinical follow-up will be scheduled after every imaging study.

Conclusion

Managing MMNSTs remains challenging owing to their rarity and aggressive nature, and presenting these cases to multidisciplinary

Figure 6. Comparison of pretreatment and post-treatment imaging. (A, C) Pretreatment: T1-weighted fast spin echo (FSE), noncontrast. (B, D) Post-treatment: T1-weighted turbo spin echo (TSE), noncontrast.



tumor boards is imperative. Data to guide treatment recommendations are lacking; our case highlights the need for further research to improve understanding of, develop data-driven treatment recommendations for, and improve long-term patient outcomes in this rare malignancy.

References

1) Torres-Mora J, Dry S, Li X, et al. Malignant melanotic schwannian tumor: a clinicopathologic, immunohistochemical, and gene expression profiling study of 40 cases, with a proposal for the reclassification of "melanotic schwannoma". *Am J Surg Pathol*. 2014;38(1):94-105. doi:10.1097/PAS.0b013e3182a0a150

2) Sahay A, Epari S, Gupta P, et al. Melanotic schwannoma, a deceptive misnomer for a tumor with relative aggressive behavior: a series of 7 cranial and spinal cases. *Int J Surg Pathol*. 2020;28(8):850-858. doi:10.1177/1066896920923146

3) Alexiev BA, Chou PM, Jennings LJ. Pathology of melanotic schwannoma. *Arch Pathol Lab Med*. 2018;142(12):1517-1523. doi:10.5858/arpa.2017-0162-RA

4) Meyer A, Billings SD. What's new in nerve sheath tumors. *Virchows Arch*. 2020;476(1):65-80. doi:10.1007/s00428-019-02671-0

5) Shields LBE, Glassman SD, Raque GH, Shields CB. Malignant psammomatous melanotic schwannoma of the spine: a component of carney complex. *Surg Neurol Int*. 2011;2:136. doi:10.4103/2152-7806.85609

6) Yeom JA, Song YS, Lee IS, Han IH, Choi KU. Malignant melanotic nerve sheath tumors in the spinal canal of psammomatous and non-psammomatous type: two case reports. *World J Clin Cases*. 2022;10(24):8735-8741. doi:10.12998/wjcc.v10.i24.8735

7) Liu E, Sun T, Liu C, Wang S, Wang S. Giant melanotic malignant peripheral nerve sheath tumor in the pelvis: contrast-enhanced CT and 18f-FDG PET/CT finding. *Clin Nucl Med*. 2019;44(11):895-897. doi:10.1097/RLU.0000000000002677

8) Benson JC, Marais MD, Flanigan PM, et al. Malignant melanotic nerve sheath tumor. *AJNR Am J Neuroradiol*. 2022;43(12):1696-1699. doi:10.3174/ajnr.A7691

9) Abul-Kasim K, Thurnher MM, McKeever P, Sundgren PC. Intradural spinal tumors: current classification and MRI features. *Neuroradiology*. 2008;50(4):301-314. doi:10.1007/s00234-007-0345-7

10) Koeller KK, Shih RY. Intradural extramedullary spinal neoplasms: radiologic-pathologic correlation. *Radiographics*. 2019;39(2):468-490. doi:10.1148/rg.2019180200

11) Wang L, Zehir A, Sadowska J, et al. Consistent copy number changes and recurrent PRKAR1A mutations distinguish melanotic schwannomas from melanomas: SNP-array and next generation sequencing analysis. *Genes Chromosomes Cancer*. 2015;54(8):463-471. doi:10.1002/gcc.22254

12) Williams EA, Shah N, Danziger N, et al. Clinical, histopathologic, and molecular profiles of PRKAR1A-inactivated melanocytic neoplasms. *J Am Acad Dermatol*. 2021;84(4):1069-1071. doi:10.1016/j.jaad.2020.07.050

13) Bradford D, Kim A. Current treatment options for malignant peripheral nerve sheath tumors. *Curr Treat Options Oncol*. 2015;16(3):328. doi:10.1007/s11864-015-0328-6

14) Antoszczyk S, Spyra M, Mautner VF, et al. Treatment of orthotopic malignant peripheral nerve sheath tumors with oncolytic herpes simplex virus. *Neuro Oncol*. 2014;16(8):1057-1066. doi:10.1093/neuonc/not317

15) Landy H, Feun L, Markoe A, et al. Extended remission of a recurrent median nerve malignant peripheral nerve sheath tumor after multimodal treatment: case report. *J Neurosurg*. 2005;103(4):760-763. doi:10.3171/jns.2005.103.4.0760

16) Natalie Wu LM, Lu QR. Therapeutic targets for malignant peripheral nerve sheath tumors. *Future Neurol*. 2019;14(1):FNL7. doi:10.2217/fnl-2018-0026

17) Li Z, Niu Y. Malignant melanotic nerve sheath tumor of the parotid gland: a case report and literature review. *Ear Nose Throat J*. 2025;104(1_suppl):339S-344S. doi:10.1177/01455613221145803

18) Shui C, Davey L, Scholsem M. Leptomeningeal dissemination of a malignant melanotic nerve sheath tumor: a case report and review of the literature. *Surg Neurol Int*. 2022;13:59. doi:10.25259/SNI_31_2022

19) Solomou G, Dulanka Silva AH, Wong A, Pohl U, Tzerakis N. Extramedullary malignant melanotic schwannoma of the spine: case report and an up to date systematic review of the literature. *Ann Med Surg (Lond)*. 2020;59:217-223. doi:10.1016/j.amsu.2020.10.003

20) Lin K-Y, Chen L, Hung S-W, et al. A para-aortic malignant melanotic nerve sheath tumor mimicking a gastrointestinal stromal tumor: a rare case report and review of literature. *BMC Surg*. 2022;22(1):293. doi:10.1186/s12893-022-01727-4

21) Hall JC, Chang SD, Wilson TJ, et al. Post-operative stereotactic radiosurgery of malignant melanotic schwannoma. *Cureus*. 2022;14(3):e22849. doi:10.7759/cureus.22849

22) Roohani S, Claßen NM, Ehret F, et al. The role of radiotherapy in the management of malignant peripheral nerve sheath tumors: a single-center retrospective cohort study. *J Cancer Res Clin Oncol*. 2023;149(20):17739-17747. doi:10.1007/s00432-023-05449-9

23) Hayes AJ, Nixon IF, Strauss DC, et al. UK guidelines for the management of soft tissue sarcomas. *Br J Cancer*. 2025;132(1):11-31. doi:10.1038/s41416-024-02674-y

Entrustable Professional Activities in Radiation Oncology: A Framework for Competency-Based Training

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As residents in radiation oncology (RO), we navigate a unique learning journey that encompasses both technical expertise and nuanced clinical judgment. Do all residents feel equally prepared for independent practice at the time of graduation? Are training gaps a function of institutional environment or individual variability? Do we have a reliable way to assess readiness beyond case logs and rotation completion?

In our recent survey of graduating RO residents,[1] over 90% of residents report high overall program satisfaction and ~80% felt they had appropriate autonomy; however, disparities exist in perceived confidence across specialized treatment modalities. For instance, while 96% of residents reported perceived confidence in lung stereotactic body radiation therapy, only 25% felt prepared for independent practice in prostate high dose rate brachytherapy.[1] Prior studies echo this trend; Marcrom and colleagues reported that only half of PGY-4/5 residents felt confident in starting a brachytherapy practice upon graduation.[2] Our data show that program size could play a role in training disparity; smaller programs may lack access to specialized modalities like proton therapy or adaptive radiation therapy, while larger programs may face barriers to hands-on procedural experience, particularly in brachytherapy. Besides case log requirements outlining a minimumnumber of cases to graduate, enforced by the Accreditation Council for Graduate Medical Education (ACGME), we currently do not have a reliable way to assess readiness. There is a growing interest in standardized, outcomes-focused education as exposure does not necessarily guarantee competence. Entrustable professional activities (EPAs) offer a practical, competency-based framework for assessing clinical readiness.

What Are EPAs, and Why Do They Matter to Us as Residents?

EPAs are discrete, observable tasks that represent the core work of a specialty. In 2023, the Project Leadership Committee within the Radiation Oncology Education Collaborative Study Group (ROECSG) published a consensus framework defining 52 EPAs across 4 developmental stages: Transition to Discipline, Foundations of Discipline, Core of Discipline, and Transition to Practice.[3] This framework seeks to shift assessment from time-

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Disclosures: The authors are members of ARRO's executive committee. MMB is the current ASTRO Government Relations fellow for the year 2024-2025. No other potential conflicts of interest to report. No outside funding was received for the production of this original manuscript, and no part of this article has been previously published elsewhere.
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Published: June 1, 2025. https://doi.org/10.37549/ARO-D-25-00017

based progression to demonstrable competence in real-world clinical tasks.

For residents, EPAs offer 4 key benefits:

1. **Clarity of Expectations**: EPAs demystify what "on track" looks like. For example, moving from "interpreting a radiation therapy treatment plan" in the Foundations phase to "providing feedback on a radiation therapy treatment plan to planning staff or peers" in the Transition to Practice phase offers clear milestones for skill acquisition.
2. **Individualized Learning Trajectories**: Time-based models assume uniform progression. In contrast, EPAs recognize that residents may advance at different rates across domains. This framework enables tailored support where needed and autonomy where earned.
3. **More Meaningful Feedback**: EPAs provide a blueprint for personalized mentorship and high-quality end-of-rotation feedback to move away from *"read more"* and other boilerplate language to more actionable feedback in evaluating a treatment plan to use the above example.
4. **Portability and Transparency**: Whether transferring institutions or entering independent practice, a resident's documented entrustment levels provide meaningful insight into their competencies beyond case logs.

Raising case minima could help mitigate deficiencies in some aspects of RO training. However, EPAs offer a structured approach to developing and assessing readiness regardless of program structure, one that focuses on teaching core skills and not maximizing volume or meeting an arbitrary cutoff. Future efforts should also be directed to developing a competency-based assessment framework specifically for procedural skills in brachytherapy and other specialized modalities as the current framework lacks granularity to properly assess procedural readiness.

EPAs in Practice: An Example

Consider EPA number 32: "Contouring complex target volumes and organs at risk using appropriate imaging modalities" in the Core of Discipline phase.[3] Rather than simply checking a box that a resident has completed a certain number of contouring cases, faculty would observe the resident's approach to a complex case, assessing their ability to integrate information from various imaging modalities, apply anatomical knowledge, and make appropriate clinical judgments about target delineation. The faculty may rate the resident on a 5-point entrustment scale from "observation only" to "teaching others." When sufficient observations consistently demonstrate competence, the resident would be entrusted to perform this activity with increasing level of independence. This process provides both specific feedback for improvement and documentation of progressive competence, aside from abstract feedback or lack thereof.

EPAs and Board Certification: Complementary or Competing?

How will EPAs align with existing assessment frameworks, such as ACGME milestones and board certification requirements? Ideally, EPAs would complement rather than compete with these systems. While milestones provide a somewhat abstract developmental framework across 6 core competencies, EPAs integrate these competencies into observable clinical activities that more closely mirror day-to-day practice. This integration could ultimately streamline assessment and provide more meaningful data for all stakeholders.

Implementation Considerations

Successful EPA implementation will require thoughtful design. Faculty development, streamlined assessment tools (e.g., use of a phone app), and integration into existing workflows are essential to minimize burden and maximize meaningful feedback. Experience from general surgery and radiology underscores these challenges as variability in faculty engagement and time constraints remain major barriers[4,5]; this is especially relevant when teaching comprises a small percentage of overall faculty responsibility and the promotions package. Moreover, the rise of artificial intelligence in operationalizing simple tasks may threaten knowledge acquisition in early training years. RO must anticipate similar issues.

Departments will need champions to guide cultural change. The onus is also on us, the residents, who will need to embrace a growth-oriented mindset that values demonstrated competence over passive rotation through services.

A Vision for the Future of Radiation Oncology Training

The Canadian RO training model has implemented an EPA-based curriculum with 15 comprehensive EPAs with structured assessment plans, milestones, and contextual requirements.[6] The US model, while with 52 distinct EPAs, remains in its infancy. Efforts are underway both at ARRO and ROECSG to further develop those EPAs. Brisson and colleagues at ROECSG are running a pilot survey study of 4 pairs of attending and resident physicians to assess the feasibility of using EPAs as a framework for actionable performance feedback.[7] ARRO's EPA working group, led by Sayeh Fattahi, MD, and Zohaib Sherwani, MD, is developing a guide describing key features of each of the EPAs and will be made available to stakeholders upon completion.

References

1) Basree MM, MacDuffie E, LeCompte M, et al. Trends in program satisfaction and perceived confidence levels among United States Radiation Oncology Residents: a 5-year aggregate analysis of the 2020-2024 association of residents in radiation oncology graduating resident survey. *Int J Radiat Oncol Biol Phys*. 2025. doi:10.1016/j.ijrobp.2025.03.035

2) Marcrom SR, Kahn JM, Colbert LE, et al. Brachytherapy training survey of radiation oncology residents. *Int J Radiat Oncol Biol Phys*. 2019;103(3):557-560. doi:10.1016/j.ijrobp.2018.10.023

3) Jeans EB, Brower JV, Burmeister JW, et al. Development of a United States radiation oncology curricular framework: a stakeholder Delphi consensus. *Int J Radiat Oncol Biol Phys*. 2023;115(5):1030-1040. doi:10.1016/j.ijrobp.2022.12.009

4) Hirvonen J, Becker M, Aronen HJ, European Society of Radiology (ESR). Resident education in radiology in europe including entrustable professional activities: results of an ESR survey. *Insights Imaging*. 2023;14(1):139. doi:10.1186/s13244-023-01489-4

5) Brasel KJ, Lindeman B, Jones A, et al. Implementation of entrustable professional activities in general surgery: results of a national pilot study. *Ann Surg*. 2023;278(4):578-586. doi:10.1097/SLA.0000000000005991

6) Royal College of Physicians and Surgeons of Canada. CBD Specialty Standards Document Suite. Accessed February 8, 2025. https://www.royalcollege.ca/en/standards-and-accreditation/information-by-discipline/ibd-cbd-documents.html

7) Brisson RJ, Holmes JA, Shiue KR, et al. Exploring the feasibility and utility of entrustable professional activities in resident assessment and feedback: a pilot survey study. *Int J Radiat Oncol Biol Phys*. 2024;119(4):e4. doi:10.1016/j.ijrobp.2024.04.030

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