

Review

Stereotactic Body Radiotherapy Immunological Planning – Road to Abscopal Effect by Design

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Simple Summary: There are increasing studies on the abscopal effect of radiotherapy since the introduction of stereotactic body radiotherapy and immunotherapy. Existing literature primarily focuses on various factors of stereotactic body radiotherapy optimum dose-fractionation schedules, local control, tumor microenvironments, vascular effects, and immunological effects. The available clinical literature favoring the abscopal effect is limited to small numbers or inconsistent outcomes. This review paper attempts to consolidate complex and interdependent factors influencing stereotactic body radiotherapy and combinations with other therapies into one convergent process to develop predictable abscopal clinical results.

Abstract: This review highlights normal and tumor tissue vasculature, immunological changes, and phenotypic alterations (VIP model) as fundamental in abscopal interaction. In the stereotactic body radiotherapy (SBRT) and immunotherapy era, we are moving toward “immunological radiation planning,” i.e., radiation scheduling with abscopal effect as a vital endpoint as well. Towards this end, this manuscript presents specific diagrammatic tumor models to optimize the outcome of abscopal response in SBRT, based on the principle of the four R’s - Repair, Redistribution, Repopulation, and Reoxygenation of radiotherapy. The article highlights the importance of restricting the dose of SBRT to < 10 Gy per fraction, appropriate use of dose painting, and concomitant/delayed SBRT boost potential. Current literature indicates that immunotherapy should not precede but follow SBRT within seven days. Included is the review of integrating “cyclical” antiangiogenics, immune adjuvants/immune-metabolites as abscopal effect enhancers with SBRT. The importance of proton, carbon-ion SBRT is dealt with briefly. Proposed six fundamental requirements for augmentation of the abscopal cascade are listed. The existing exploratory results need to develop a definitive strategy amidst complex interactions in SBRT, immunotherapy, immune-adjuvants, & abscopal effects. We now have enough literature evidence to convert

“abscopal by chance” to “abscopal by design” by harmonized combinatorial approach.

Keywords: SBRT, SABR, Abscopal, vascular-normalization, immunotherapy, phenotypic, antiangiogenics, immunoadjuvants, VIP-model, High-LET

1. Introduction

Presently, several significant developments have changed radiotherapy's (RT) scope regarding its planning and outcomes. First is the advent of Stereotactic Body Radiotherapy (SBRT). Technological evolution in stereotactic localization of cancer and its movement has led to very high precision therapy, limiting the dose to the normal tissue. This precision has helped to deliver extreme hypofractionation to the macroscopic disease safely.

The second has been the evolution of the concept of oligometastases. In stage IV, a subset of patients has limited metastases termed oligometastases. These patients are still amenable to treatment by radical approach. A seminal review article by Timmerman et al. in 2009 emphasized the role of SBRT in the treatment of oligometastases. With SBRT, in as much as 25% of patients with oligometastases long-term control of the disease was achieved [1].

There has been a gradual evolution in selecting patients with oligometastases for SBRT over the years. Initially, it was a single metastasis of limited size in an organ, which then later included a few metastases in a single organ. Presently, in general, a few metastases in multiple organs also come within the ambit of the oligometastases status, as long as the total volume does not exceed a particular level depending on the site of metastases. Indications for oligometastases are still evolving in clinical situations, with a few metastases persisting after chemo-immunotherapy (oligo-persistence) or a few coming back (oligo-recurrence) or progressing in a limited number of metastases (oligo-progression). Recently, the interest is amplified in results of SBRT by increasing reports of outside the field and distant (abscopal) response effects.

The third development is the arrival of several newer immunotherapy agents to target cancer cells and enhance the immune response against cancer at the same time. There has been a potential breakthrough with the combined use of SBRT with these immunogenic drugs.

For a long time, RT has been deemed locally immune-suppressive with its cytotoxic effects on leukocytes. This immunosuppressive action is widely used in total body irradiation (TBI) as a bone marrow transplantation-conditioning regimen. However, in recent years, the RT-induced immune-stimulatory effect has been increasingly recognized,

especially with SBRT, including its ability to trigger the regression of metastatic tumors (abscopal response) distant from the irradiated field. The mechanisms of the abscopal effect encompass radiation-induced normalization of the vasculature, allowing more efficient infiltration of effector T cells; IFN- γ (type II IFN) induced up-regulation of VCAM-1 and MHC-I expression leading to generation of tumor neo-antigens. These specific neo-antigens induce the maturation of DCs and migration to draining lymph nodes with the presentation of tumor antigens endogenously triggering the priming and infiltration of antigen-specific effector T cells, completing the virtuous loop. Additionally, SBRT inactivates immunosuppressive cells such as tumor-associated macrophages (TAMs), regulatory T cells (Treg cells), CD11b⁺ cells, and myeloid-derived suppressor cells (MDSCs). The characteristic of triggering this process of immunogenicity is a unique functional type of cell apoptosis named immunogenic cell death (ICD). Overcoming tumor immune tolerance is encouraged by antigen-specific, adaptive immunity [2].

The process and mechanism of immune response with SBRT, immunotherapy, and abscopal effect has been dealt exhaustively by Tharmalingam & Hoskin [3], Bernstein et al.[4], and Buchwald et al., [5]. A very comprehensive review done recently by Marcus et al. covers conventional RT to High Linear Energy Transfer radiation, RT dose scheduling for optimum immunological response, choosing appropriate window of opportunity, and vaccination to adoptive transfer of immunity [6]. The present review paper aims to consolidate complex and interdependent factors influencing SBRT and the abscopal effect *into a convergent actionable strategy* to have improved and dependable outcomes.

Theoretically, to maximize and to have a consistent abscopal reaction, there are three major components. a) There should be an effective cell kill locally (including that of resistant cells), each dose of SBRT, leading to the generation of varied tumor-specific antigens (neo-antigens), dynamically with every mutation in vivo. b) Need to eliminate tumor-suppressive cells and improve the Tumor-Infiltrating Lymphocytes (TILs) in the tumor microenvironment (TME) for improved local effects. c) Use immune-adjuvant(s) to amplify the in-vivo therapeutic vaccination effect.

2. Review

A). Vascular-Immunological-Phenotypic (VIP) Model and SBRT

A1. Tumor Morphology & VIP profile at diagnosis:

Figure 1 shows components of the VIP model in the diagrammatic cross-section of cancer mass. The figure represents the spectrum of vascular distribution within the cancer mass from well-perfused to avascular areas,

TME immunological milieu, and various cancer cells with diverse phenotypic profiles. It is essential to understand the ever-changing dynamics of these three VIP components as cancer therapy progresses to maximize the abscopal effect in SBRT successfully.

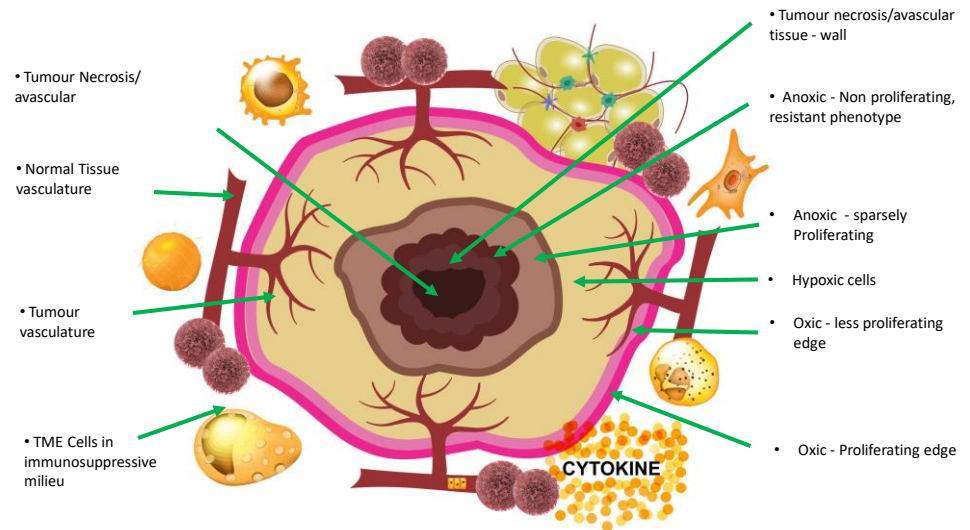


Figure 1: Model A – Morphological and pathophysiological aspects of vascular-immuno-phenotypic (VIP) tumour model for SBRT and vascular sparing dose painting technique in a range of 5 – 10 Gy per fraction (presently evidence favors intermediate dose) (*literature evidence available – see text*).

Radiation-induced cancer cell kill is most effective when well-oxygenated, and anoxic cells are the most resistant. The cancer tissue has nil to well-vascularised volumes, resulting in varied oxygenation, culminating in differential response to radiation. The hypoxic and necrotic area within the cancer tissue may be localized in the center, as the representative figure 1 shows *or could be eccentric or irregularly distributed* within the tumor. Additionally, due to mutations for survival, the cancer cells develop varied phenotypic profiles (Figure 2). Hence, differential targeting of the cells is required, based on this morphological and pathophysiological VIP model (Figure2).

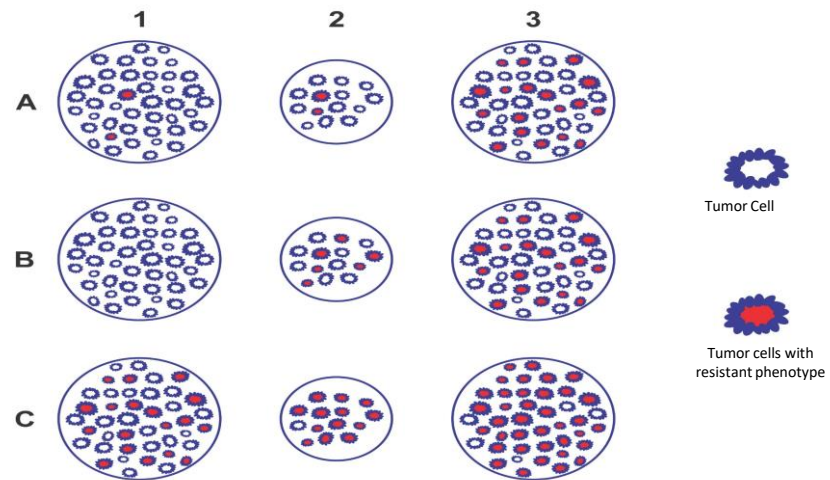


Figure 2: Model B - The figure depicts the phenotypic profile and criticality of appropriate timing of SBRT.

Example A: Initially few resistant phenotypes (A1) which survive during accelerated repopulation from day 21 to 30 of start of treatment or as residual lesion/oligo-persistence (A2) and these cells proliferate subsequently (A3).

Example B: Initially no resistant phenotypes (B1); appear during accelerated repopulation from day 21 to 30 of start of treatment or as residual lesion/oligo-persistence (B2) and these cells proliferate subsequently (B3).

Example C: Initially large number of resistant phenotypes is present (C1); survive during accelerated repopulation from day 21 to 30 of start of treatment or residual lesion/oligo-persistence (C2) and these cells proliferate to recur or metastasize (C3).

Situations A & B (receptor positive and differentiated tumors) indicates that SBRT can be reserved as “concomitant” or delayed boost. Situation C (e.g., receptor negative & aggressive tumors) indicates that SBRT to be considered upfront along with chemo/targeted/immuno-therapy followed by “concomitant” and/or “delayed” boosts subsequently (*preclinical trials required to establish the validity*).

In practical terms, three strategic aspects emerge. First, with improvements in functional imaging techniques, it would be possible to have three-dimensional models of oxygenation. The importance of figure 1 lies in the fact that with SBRT, we have the technology to titrate the dose accordingly by creating controlled hot spots (dose-painting). The dose-painting has dual benefits with SBRT technology. One, a higher required gradient dose could be delivered efficiently, if needed. Two, normal tissue (including the vasculature) sparing will be more effective. Tubin et al. demonstrated the feasibility of this concept by contouring and treating only

the hypoxic tumor segment. He delivered 10-12 Gy 1 to 3 fractions to a hypo-vascularized and hypo-metabolic junctional zone between the central necrotic and peripheral hyper-vascularized-hypermetabolic tumor segment as a palliative approach and observed abscopal effect in non-irradiated segments and nodes [7].

Second, some cancer cells are more sensitive and than others, requiring different strategies. Primarily, two strategies are target cells with some receptors/markers that are susceptible to cell kill or cells that have reached the level of undifferentiation requiring the aggressive approach. The strategy requires systematic combinations of treatment with *appropriate intensity and timing*, where SBRT would be invaluable.

Third, the genomic landscape of cancer is dynamic and ever-changing in response to the fluctuating tumor microenvironment and cancer-directed treatment. This change requires an adoptive personalized approach in the SBRT delivery. The major categories of cells with different phenotypic profiles are stem cells, especially in a vascular niche or growing edge; anoxic clonogenic cells in the wall of necrotic areas; hypoxic/anoxic clonogenic cells in G0 phase of cell cycle; cells with differential SUV uptake and cells with varying mutation burden. With improving imaging technology, it would be possible to get three-dimensional information facilitating matched approach and dose painting with SBRT.

A2. VIP profile of Responding and Residual lesions:

It is crucial to study the tumor profile during the treatment and later residual tissue. Figure 3 enumerate the changing profile of cancer with the treatment. During therapy with either fractionated RT or chemotherapy, decreased interstitial pressure happens due to tumor size reduction following initial cancer cell kill and improved vasculature, leading to additional cell kill of oxygenated cells. TME also evolves, resulting in improved immunological reaction (Figure 3). However, there could be accelerated repopulation (AR) of surviving relatively resistant cancer cells at this time.

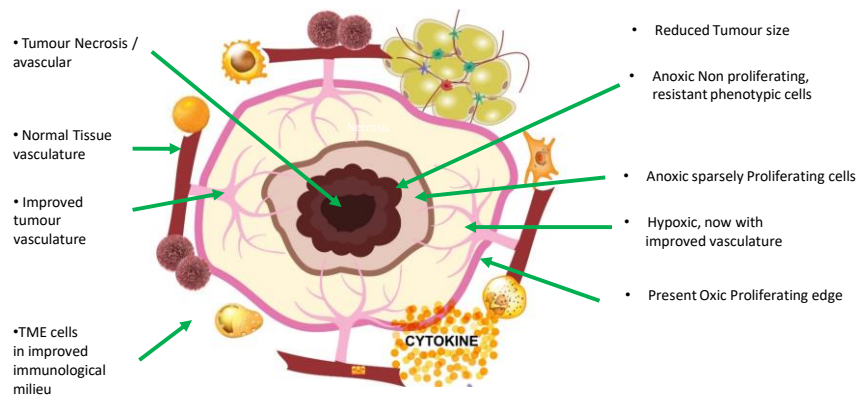


Figure 3: Model C – Morphological and pathophysiological aspects of VIP tumour model during accelerated repopulation from day 21 to 30 of start of treatment requires concomitant SBRT boost with or without dose painting (*preclinical trials required to establish the validity*).

Figure 4 represents the tumor profile at six weeks to three months after completing chemotherapy or conventional RT / SBRT. At this time, the residual disease, if present, is likely to have vascularised to the maximum possible extent due to shrinkage. Pathophysiologically, individual tumor cells are either hypoxic or oxidic with nil or with the least number of anoxic cells possible. Theoretically, this period could be the *window of least tumor burden* in a particular patient's timeline of cancer therapy. Also, left untreated or if on maintenance therapy, if it does not regress further, will start repopulating, developing resistance to the treatment, forming a potential source of recurrence and reseeding for metastases. The cells in this residual mass are most likely to be very resistant stem cells, if present, and require salvage surgery or SBRT boost in locally advanced disease in response or oligo-persistent / oligo-progression situation.

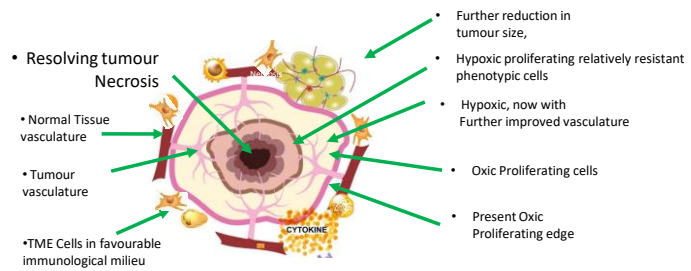


Figure 4: Model D – Morphological, pathophysiological aspects of VIP Tumour model, a) After completion of prescribed course of chemo/targeted/immunotherapy or 3 months after radiotherapy, residual/oligo-persistence/early recurrent or oligo-progression, if present, requires “delayed” SBRT boost with or without dose painting (*preclinical trials required to establish the validity*).

The dosage protocol should be such that there should, on the one hand, cause maximum possible tumor cell kill. On the other hand, it should retain the viability of the TME vasculature, ECM suppleness, and continued immune response. This balance is the way forward to convert “abscopal by chance” to “abscopal by design.” Hence, it is crucial to consider the interplay of the several factors enumerated below in the background of the fundamental principles of the VIP model discussed.

B). Radiobiology of SBRT

B1. Five R’s of Radiotherapy (1 more R added later after the initial proposal of 4 Rs):

To counter effectively both factors of anoxia and differential responding cells, the five R’s of radiotherapy - Reoxygenation, Repopulation, Radiosensitivity, Repair, and Redistribution, have been fundamental to the evolution of modern-day radiotherapy. Fractionated RT was the basic technique, which brought radiation therapy out of the dark era about 100 years back. It is, therefore, imperative to consider these four factors to use the SBRT-abscopal interaction optimally.

B1.1 Reoxygenation:

In SBRT, Kim et al. elucidated that oxygen consumption would drastically diminish after a massive death of tumor cells, and thus the surviving hypoxic cells may be reoxygenated [8]. Shibamoto et al. proposed the concept of 'reoxygenation utilization rate' (RUR) in SRT [9]. With two, four, six, eight and thirty fractions were 50%, 75%, 83%, 87.5%, 97% respectively. Therefore, the authors theorized that, unlike single fractions, six to eight fractions of SBRT treatments might be sufficient to utilize the reoxygenation phenomenon [9]. This study indicates that in fractionated SBRT, unlike single fraction, reoxygenation does contribute to increased cancer cell kill like in conventional RT.

B1.2 Repopulation and Radiosensitivity:

Withers et al. initially described the phenomenon of clonogenic repopulation in squamous cell carcinoma of the head and neck, accelerating after a lag period of four weeks (\pm one week) after the initiation of RT [10]. Although the onset of accelerated repopulation is not explicitly known for a particular type of cancer in the temporal timeline, one to two weeks of treatment with fractionated SBRT may be advantageous in reducing the acceleration in repopulation. This phenomenon particularly holds good for rapidly proliferating cells and may contribute to the improvement of local control [11] in aggressive disease. The delayed acceleration can happen in slow responding tumors like prostate cancer, as late as 69 days, depending on the stage. Since relatively radioresistant cells are in the proliferation phase (with apoptosis of sensitive cells), intensifying therapy with SBRT dose schedule, with planned SBRT boost at 3-4th week (concomitant boost) and 10-12th week (delayed boost) of initial SBRT, may be critical. The basis for this proposed plan is on the literature analysis by Garau MM et al. [11], about enhanced repopulation period varying from 19 days to 69 days, depending on the type & stage of cancer.

B1.3 Redistribution and Repair:

The less explored biological significance of Redistribution and Repair for SBRT is the limitation of these two factors. However, it is logical to presume that the more partial repairs & faster redistribution of cancer cells that are likely to occur with SBRT make them more susceptible to cell kill and dysregulated repair with a higher probability of double-strand deoxyribonucleic acid (DNA) breaks. This dysregulation of repair might be much more effective with the combined effect of SBRT and chemotherapeutic therapy.

B2. Radiobiology of Normalization versus Vasculature disruption:

Microvascular damage due to extensive endothelial apoptosis and consequent disruption of vasculature was the initially proposed SBRT

mechanism of action. Reports by Garcia-Barros et al. indicated that microvascular disorder and death of the tissue regulates tumor cell response to radiation in the clinically relevant dose range [12]. Fuks & Kolesnick showed that endothelial apoptosis becomes significant above a dose of 10 Gy [13] in addition to a direct effect on the cancer cells [14]. Additionally, the apoptosis switches from DNA double-strand breaks intrinsic pathway changes over to extrinsic or membrane-stress-ceramide pathway at a high dose [15]. Genetic data indicate an acute wave of ceramide-mediated endothelial apoptosis, initiated by acid sphingomyelinase (ASMase), which regulates tumor stem cell response to single-dose RT of >10 Gy [16]. With the present-day technology of SBRT, it is possible to deliver such vascular disruption doses of a high order, at least to resistant sub-volumes, and one could expect total tumor elimination. In addition, anti-vascular endothelial growth factor 2 (Anti-VEGFR2) induces ASMase activation and resets ceramide rheostat apoptosis if high dose RT is delivered immediately (within 24 hours), sensitizing the vasculature to SBRT further. In contrast, anti-VEGFR2 microvessel normalization requires at least 24 h to manifest [16]. Therefore, a concurrent combination of antiangiogenics and immediate single-dose SBRT as a combined ceramide pathway vascular disruption strategy should have been a standard operative therapy approach by now.

However, the work of Moding et al. is contrary to vascular disruption as an optimum strategy for the SBRT dose schedule. His and his colleague's studies have generated a fresh look at maximizing the endothelial apoptosis mechanism of cancer control. They utilized Flp recombinase to initiate primary sarcomas and Cre recombinase to delete Ataxia-telangiectasia mutated (Atm) or Bax nuclear-encoded protein selectively in the endothelial cells of mice vasculature. With this dual recombinase technology (DRT), the endothelial cells could be either sensitized or protected from the proposed membrane damage-triggered apoptosis. Deleting Bax from the vasculature did not affect radiation-induced endothelial cell death or tumor response to doses of radiation commonly used in SBRT. In contrast, deletion of Atm in endothelial cells successfully increased endothelial cell death 24 hours after radiation treatment. In most of this group, the tumor recurred despite extensive radiosensitizer effect on endothelial damage after a single dose of 50 Gy (where tumor cells were not radiosensitized), signifying endothelial cell death just prolonged the control rate, but did not contribute to sarcoma eradication. When Atm is deleted specifically within tumor cells, which substantially sensitized tumor cells, it increased tumor eradication through radiation therapy. These results in a primary cancer model system suggest that the increased long-term tumor control observed with SBRT for many tumors is not due to increased endothelial cell death.

Additionally, tumors can re-establish their vasculature. Finally, the authors do not exclude the vasculature as a possible target for radiosensitizers used in combination with SBRT [17, 18, 19]. However, their results clearly show that endothelial cells cannot be a critical target for cancer treatment with RT.

Drawing upon these findings of DRT, it is reasonable to propose that the ideal dose would be the one that causes maximum tumor cell lysis and preserves or normalizes and enhances the tumor and surrounding normal tissue vasculature (vascular normalization). Therefore, there is a robust case for using dual recombinase technology of Moding et al. in future *preclinical trials* to identify/escalate the optimum dose per fraction of SBRT (to eliminate the cancer cells selectively) with any of the combination therapies. To reiterate, genetic modulation protecting the endothelial cells might permit delivery higher dose per fraction with better cell lysis, and abscopal cure rate than that is possible now.

B3. Which is the best choice? Apoptosis or Ablation/Necrosis:

The teaching from the onset of modern radiotherapy is that the apoptosis approach is curative and necrosis of >3% is not acceptable in the curative treatment of cancer. The introduction of SBRT and the acceptance of inhomogeneous dose distribution within the tumor requires an update with these basic concepts. Based on this, although SBRT is used synonymously with Stereotactic Ablative Body Radiotherapy (SABR) presently, a distinction can be made where the predominant action of former is apoptosis (with ≤ 10 Gy per fraction), and that of latter is an intentional vascular disruption consequently accepting the impact of tumor necrosis (with >10 Gy per fraction).

B3.1. Radio Frequency Ablation (RFA) or Microwave Ablation (MWA):

RFA has several similarities with SABR/SBRT. Both induce immunological changes in the TME. A single amino-acid substituted macrophage inflammatory protein-1 alpha (MIP-1 α) enhanced tumor growth inhibition and the abscopal effect following local antitumor therapy such as radiation, radiofrequency ablation (RFA), or hyperthermia treatment [20]. Bäcklunda et al., in a case report, showed how a stereotactically navigated microwave ablation (MWA) of multiple lung metastases showed possible immunological benefits with pembrolizumab with a complete response in a recurrent setting [21]. Slovak et al. give a detailed review of immune-thermal radiation to boost anticancer immune response, especially in patients who have had a recurrence and underwent multiple therapies [22].

B3.2. Intratumoral Approach:

The other technique of differential dosing is the intratumoral, invasive approach. Confino et al. developed an innovative method of tumor ablation using diffusing intratumoral alpha-irradiation [23]. They used alpha-radiation wires in combination with a myeloid-derived suppressor cells (MDSC) inhibitor sildenafil, Treg inhibitor (low dose cyclophosphamide), and an immune-stimulant in mice bearing mammary adenocarcinoma with metastases. The combination of all four therapies led to a complete rejection of primary tumors in tumor-bearing mice along with the elimination of lung metastases. This study indicates the potential beneficial effect of localized ablative radiation with immune-adjuvants [23]. Ablative methods like alpha therapy cause intense localized necrosis.

In summary, abscopal events do happen with intense ablative approaches. Presently, results like these are available primarily in recurrent cases and patients who undergo multiple therapies sequentially. Compiling observational study outcomes in these recurrent patients who undergo a combination of SBRT/SABR, RFA/MWA, or alpha therapy in an opportunistic sequence with immunotherapy would help generate hypotheses for the optimization of SBRT abscopal ramifications with ablative procedures. Nonetheless, *according to components of the VIP model, these approaches, even with abscopal effect, at best could have prolonged palliative benefit in locally advanced/oligometastatic (>3 cm) malignancies.* Ablative therapies like radiofrequency ablation (RFA) or microwave ablation (MWA) require at least two centimeters of normal tissue around the tumor. Else, there were increased chances of local recurrence [24]. Therefore, *preclinical trials* are required to evaluate the cure rate rather than the local control rate with ablative versus multiple fractions incorporating the DRT principles. However, in animal trials, one caveat is that proper evaluation of long-term survival may not be possible with their limited life span.

C). Optimizing Tumor/stem-cell lysis and immunogenicity

SBRT has the potential to be a powerful clinical & immunological weapon. There are indications that sudden disintegration of a significant number of cells in SBRT (unlike conventional RT) will lead to a massive release of tumor antigens, stimulating antitumor immunity [8]. The ideal SBRT dose schedule strategy should have a high degree of spatial accuracy, maximum immunogenic cell death with minimum possible disruption of endothelial cells, and an appropriate dose per fraction to handle the varied phenotypes. A combination of therapies along with SBRT should have the ability to generate tumor-specific neo-antigens to prime DCs for in-vivo vaccination effect. The technique of SBRT should induce maximum bystander effects with the inactivation of immunosuppressive Tregs and MDSCs. The following literature shows the way for optimization of these factors.

C1. Dose per Fraction in SBRT - Single High Dose versus Multiple fractions:

C 1.1 Immunological effects:

a). *Dose per fraction >10 Gy per fraction as vasculature disruptive, immunogenic dose:* Single fraction 20–24 Gy causes the massive release of antigens, death-associated molecular patterns (DAMP) ligands, and Toll-like receptors (TLR) and stimulates antigen-presenting cells (APC) [25]. In an animal model, fractionated radiotherapy with 5 x 2 Gy or 5 x 5 Gy combined with the immunocytokine L19–IL2 resulted in control of all primaries and delayed the growth in distant tumors. When compared to the medium doses, a single dose of 15 Gy resulted in complete remission of 20% of the non-irradiated tumors in addition to local control in all tumors in both arms [6], indicating immediate immunogenicity is higher for disruptive doses. With the technique of increase in tolerance of endothelial cells selectively as described by Moding et al., [17] use of a higher dose per fraction may become practically applicable.

b). *Dose per fraction <= 10 Gy as balanced Immunogenic dose:* There are several critical advantages of choosing of <=10 Gy compared to >10 Gy dose per fraction, *presently*.

- High dose RT (15–20 Gy) may permanently reduce blood flow, limiting further infiltration of immune cells and aggravating hypoxic immunosuppressive microenvironment.
- When a dose of >10 Gy led to activated M-2 macrophages polarization through T helper type 2 (Th2) pathway, on the other hand, 1 to 10 Gy dose per fraction reprogrammed from macrophage type 2 (M-2) towards an M-1 like antitumor phenotype through T helper type 1 (Th1) pathway [26]. Doses of 5–10 Gy have increased nitric oxide synthetase, which repolarizes macrophages to the pro-immunogenic M1-phenotype [27].
- Along with an anti-CD40 agonistic antibody, 6 Gy showed equal or better abscopal responses than 10 Gy and 15 Gy.
- Dendritic cell activation: Cytosolic DNA has a crucial impact on the activation of antitumor immunity by enhancing DNA sensor cyclic GMP-AMP (cGAMP) synthase (cGAS) and its downstream effector, Stimulator of Interferon Genes (STING). This cascade results in interferon-beta secretion, which in turn causes dendritic cell recruitment-activation, an essential element for priming CD8 T cells antitumor immunity. The doses above 12–18 Gy per fraction cause activation of DNA exonuclease Trex-1 resulting in degradation of cytosolic DNA attenuating the immunologic response. These studies indicate that this delicate balance between cytosolic DNA and activated Trex1 is optimal at RT for 8 Gy x 3 fr for the emergence of the abscopal effect when combined with immunotherapy [26]. A Clear limit emerges for the induction of TREX1 upregulation is by the single radiation dose and *not determined by the total dose delivered* [28]. Although in vitro studies suggest that radiation compromises the stimulatory activities of DCs, in vivo models demonstrate that radiation at

intermediate radiation doses 5×8.5 Gy enhances the ability of DCs to capture tumor antigens and promotes DCs migration to lymph nodes in a toll-like receptor-dependent manner. In a murine melanoma study testing intratumoral DC vaccination, 5×8.5 Gy enhanced the ability of DCs to capture tumor antigens without inducing enhanced DC maturation but improving cross-priming of T-cells.

- In a glioma model, high-dose radiation 1×15 Gy induced more marked recruitment of immunosuppressive CD11b+ myeloid cells than lower doses 1×8 Gy [27].
- In another study 8 Gy three times enhanced the upregulation of IFN-I [28].
- In a mouse tumor model, fractionated radiotherapy and not single-dose RT induced an immune-mediated abscopal effect when combined with anti-CTLA-4 antibody [6].
- Results published by Schaeue et al. about maximizing tumor immunity with fractionated radiation in the murine melanoma mouse model showed that 7.5 Gy in two fractions and 5 Gy in 3 fractions affected regulatory T cells (Tregs) representation [29].
- Therefore, literature favors a window of 5 Gy to 10 Gy per fraction regarding immunological response [2], and a dose per fraction of >12 Gy appears to be counterproductive.

C 1.2 Dose per fraction effect on endothelial cells (ECs) and vascular permeability:

EC integrity is a surrogate of vascular normalization. With doses above 10 Gy per fraction, there will be extensive endothelial damage, causing reduced vascular flow, increased interstitial pressure, vascular collapse, hypoxia, and late extensive fibrosis [27]. Ten Gy in a single fraction is the threshold for induction of apoptosis in ECs, and doses of 4–10 Gy per fraction may induce tumor vessel normalization, with dilation, reduced leakage, and consequently increased tumor oxygenation. A single dose of 8 Gy post-RT 4 hours causes minimal damage to microvessels and the ECs, with a modest $<5\%$ reduction in perfusion. In another study, irradiation of bovine aortic ECs with gradient dose from 5 to 15 Gy, there were two-fold increases in flattened senescent-like cells at a higher dose of 15 Gy when compared to 10 Gy. At 15 Gy, massive endothelial cell death manifested at 2–5 weeks compared to transient morphological alterations with 5 Gy [27].

C1.3 The importance of extracellular matrix (ECM) and dose per fraction:

Generally, increased tissue stiffness and tensile strength happen due to augmented collagen deposition in solid tumors. This stiffness of ECM interferes with the motility of antitumor T-cells, antigen-antibody interaction, and delivery of immune-chemotherapeutic drugs. This feature of tumors dramatically weakens the immune surveillance and response to immunotherapy, as it is. Added to this 1×15 Gy increased collagen-I

staining in xenograft tumors preclinical study when excised 17 days post-RT, but not with doses 2 Gy and 5 Gy. Essentially master switch for the fibrotic program is TGF- β , which stimulates collagen production and facilitates its functions. In lung tissue in a mice study with a single dose of 12 Gy triggered TGF- β release, which peaked after 12 hours but had an insignificant rise with 6 Gy dose [27]. Therefore, given increasing stiffness of ECM along with disruption of the vasculature with high dose per fraction >10 Gy, it can induce sanctuary for the persisting resistant cells by debilitating immune-surveillance, immunological interactions, and hamper subsequent delivery of drugs.

C1.4. Dose per fraction and outcomes:

- *Preclinical studies:* Poleszczuk et al., with their mathematical models, show that to maximize the immune response, the dose per fraction needs to be between 10 Gy and 13 Gy [30]. In the mouse breast carcinoma model, Dewan et al. found that in the mouse tumor model, a dose of 20 Gy in 1 fraction did not significantly improve the response. The different schedules tested found that 8 Gy in three fractions was superior to 6 Gy in five fractions in inducing abscopal outcome and tumor-specific T cells [31]. These results suggest a specific therapeutic dose window between 6 Gy and 10 Gy for SBRT in combination with cytotoxic T-lymphocyte-associated protein (CTLA) blockade.
- *Clinical studies:* A trial by Videtic et al. indicated that fractionated SBRT might give better clinical results. In a randomized phase II study, they compared two schedules of SBRT for medically inoperable patients with early peripheral Non-Small Cell Lung Cancer. Compared 34 Gy in a single fraction to 48 Gy in four fractions showed better two-year overall survival (OS) and disease-free survival (DFS) 61.3% vs. 77.7% and 56.4% vs. 71.7% respectively, with lower and favorable \geq Grade III toxicity for 48 Gy arm. Although the trend of OS favored the 48 Gy arm, their study was not powered to address survival differences [32].

C2. VIP model and innovative SBRT schedule harmonization

C2.1. Possible role of concomitant SBRT Boost during accelerated repopulation at Day 21 of RT (Figure 2 & 3):

As discussed earlier, accelerated repopulation occurs around the third to fourth week of the first dose of RT. This period might be a suitable window period for SBRT to improve the cell kills in newly oxygenated hypoxic cells or evolving resistant phenotypes (Figure. 2 & 3). The study of the history of conventional RT indicates that of all the accelerated and/or hyperfractionated techniques tried, the accelerated hyper-fractionated concomitant boost technique, second

fraction of the day delivered after > 8 hours starting from day 21, was the one that encouraging results in the pre-cisplatin era (*cf.*- in concurrent technique additional dose/fraction starts from day 1). Overall, in a meta-analysis of six clinical trials, having 988 patients, Matuschek et al. concluded that accelerated RT techniques did not improve loco-regional control or overall survival in high-risk patients. Additionally, acute if not late radiation toxicity was more frequent [33].

Nevertheless, when looking at the results of Ang et al., in their multi-institutional, prospective, randomized trial, the comparison between conventional radiation and concomitant boost in high-risk post-operative patients showed that the concomitant arm had significantly better loco-regional control and overall survival without increasing the toxicities. This improvement in outcomes is after considering the post-operative period and radiation overall-time together [34]. Therefore, the institution of preclinical trials about the feasibility of giving SBRT boost to the gross tumor volume (GTV) anywhere between days 21 and 30 after the initial course of SBRT not only to increase the intensity of treatment but also to make use of the potential enhanced cell kill during proliferation phase is required.

C2.2. Possible role of delayed SBRT Boost with chemo-immunotherapy (Figure 4):

Paik et al. published the results of 23 patients with 29 oligo metastases, treated with a split course technique [35]. They delivered one to three sessions of SBRT course initially, and a second course at around four weeks with a range of 18-60 days, to reduce the dose to critical Organs based on the observation of faster rates of tumor regression with SBRT compared to that of conventional RT. Their data showed a partial response in 55% of the patients before the second course of SBRT [35]. Triple therapy of anti-PD-1, a checkpoint inhibitor, indoximod an immune-metabolic adjuvant, together with 2 x 12 Gy RT induced rapid tumor regression in mice bearing melanoma. In this trial, eventual tumor recurrence was associated with increased apoptosis of intratumoral T cells. Re-irradiation with 2 x 10 Gy at a late tumor regression phase or after relapse cured the majority, which correlated with more memory T cells in the tumor-draining lymph nodes and spleen. Also, re-irradiation effectively delayed the relapse in mice having poorly immunogenic mammary carcinoma [6]. This finding could signify that the delayed boost schedule fits in with synchronizing the SBRT boost during the phase of least tumor burden and possibly having the most resistant residual cells. In addition, treating selected patients who have residual lesions with reduced irradiation volume would be a plus point in reducing the overall side effects and is worth exploring.

C3. Optimal Sequencing of SBRT with chemo-immunotherapy

The optimum scheduling would be to deliver the maximum permissible dose per fraction and total dose of SBRT without vascular disruption during the

window period, which would enhance the uniform delivery of immune-chemotherapy drugs within the tumor and augment the immune stimulation.

C3.1 Evidence against immunotherapy before RT:

There is a theoretical concern that SBRT may come in the way of immune response if immunotherapy precedes SBRT. The mechanism presumed is the obliteration of the recently infiltrated and reinvigorated T-cell response in checkpoint inhibitor immunotherapy [5]. RT of 10 Gy single dose *before* starting immunotherapy with L19-IL2 was not beneficial in a murine F9 terato-carcinoma model, and anti-OX40 agonist antibody was optimal when given *a day* following radiation during the window period of amplified antigen presentation [6].

C3.2 Evidence for concurrent or ≤ 7 days of SBRT:

- *Vascular permeability* would be a surrogate indicator for improvement in the drug delivery to the cancer cell. In the skin of C3H-mice exposed to local irradiation 2, 15, or 50 Gy vascular permeability peaked **24 h** post-radiation, followed by a steady decline to baseline over 3–10 days. A colon adenocarcinoma xenograft study showed 1×4 Gy RT increased vascular permeability at **24 h** post-RT, but no difference at 72 h. In another study with a radiation dose 5 or 15 Gy to mammary adenocarcinoma xenografts, drug administration before and after RT showed 1.2- to the 3.3-fold enhancement of probe accumulation in tumors lasting the first *two days* post-RT. These results show that intermediate to high doses of radiation, even if not optimal to achieve tumor control, are sufficient to enhance drug delivery [27].
- *Immune cell infiltration & outcomes*: Several published clinical studies of radioimmunotherapy combinations report abscopal effects when used concurrently or immediately afterward, depending on the type of immunotherapy. Immune infiltration started within **2–4 days** after irradiation with 2×5 Gy, in a CT26 colorectal mouse model. RT of 2 Gy X 5 fraction increased OS when used in schedules with anti-PD-L1 day 1 to 5 and not in the schedule given on day 7. AB16 melanoma model demonstrated infiltration of CD8+ T cells *five days* after irradiation with 2×12 Gy [6]. In a mouse study, decreased PD-L1 expression and anergy of tumor-reactive T-cells were reported *seven days* after the last dose of RT by Dovedi *et al.* [36, 37]. Buchwald *et al.* propose that anti-PD-1/L1 and RT should be concurrent [5]. In PEMBRO-RT randomized study, patients in the SBRT arm received a dose of 24 Gy in 3 fractions along with standard pembrolizumab within *seven days* of the last dose of RT to a single site of metastatic NSCLC. In addition to the improved response rate from 20% to 50%, patients with SBRT arm had

improvement in both median PFS and OS, although non-significant. The finding of importance was that 22% and 4% of patients with 0% PD-L1 staining (immunologically cold tumor) had a response, respectively, in the SBRT group *vs.* pembrolizumab alone arm. Despite several limitations to the study, it was a well-designed randomized clinical trial and was the first of its kind [38].

C3.3 Evidence for SBRT with immunotherapy with or without chemotherapy beyond 7 days:

- In a preclinical study of colon cancer as a model, MHC-II positive DC recruitment into tumors was observed only between days after first radiation dose **5 and 10** [27].
- In the landmark PACIFIC trial, durvalumab delivered after chemoradiotherapy led to improved survival for patients with unresectable stage III lung cancer. Initiating durvalumab within **14 days** of completing fractionated RT experienced a better survival benefit than those who started on durvalumab from 14 days to 42 days [38].

C3.4 Evidence for any time after SBRT:

With 2-5 Gy, observation of upregulation of the immunosuppressive M2-gene signature to the pro-immunogenic M1-phenotype in vitro & in vivo in few days of irradiation lasts long for several weeks [27]. KEYNOTE-01 clinical trial of non-small cell lung cancer, the analysis determined that group who received immunotherapy even at a median of 9.5 months after RT had longer OS and PFS compared to those who did not receive RT. This study shows the lingering synergistic benefit of radiation given before, although this study has limitations being a retrospective review of a single-arm trial [38]. These studies indicate that immunological interaction will continue for a long time after the initial 2 – 7 days, even if it is not the optimal response.

C3.5. SBRT with multiple Combination Immunotherapies:

- RT in combination with dual immune checkpoint blockade by anti-CTLA-4 and anti-PD-L1 or anti-PD-1 resulted in the long-term survival of the mice. This improved action is due to triple action of broadening of the T cell repertoire by RT, depletion of intratumoral regulatory T cells by anti-CTLA-4, and reinvigoration of the exhausted T cells by anti-PD-L1.
- In another study, PD-L1 upregulation resulting from a concurrent blockade of TGF β along with 6 Gy X 5 fraction radiation when nullified by anti-PD-1 delayed the tumor recurrence and extended mice survival.

- Radiation of 5 x 5 Gy combined with a bifunctional fusion protein (M7824) blocking both TGF β & PD-L1 led to increased tumor-specific CD8 T cells, resulting in rejection of irradiated and abscopal tumors.
- Triple therapy-induced better tumor regression in mice bearing tumors following local radiation to one tumor along with an agonistic anti-CD137 (4-1BB) and a neutralizing PD-1 antibody.
- An oligonucleotide aptamer enhanced tumor response by simultaneously targeting vascular endothelial growth factor (VEGF) and 4-1BB ligand, up-regulated VEGF for tumor-targeted radiation dose of 12Gy X 1 fraction [28]. These studies indicate the critical place for trials of combination of immunotherapies with SBRT with overall <10 Gy per fraction.

C3.6 Synchronization with Treg cells targeting:

Immune tolerance associated with cancer is responsible for a poor prognosis. Increased Treg cells, a particular type of CD4+ T cells, play a crucial role in immune tolerance and tumor progression [2]. In a mouse model, the combined RT and anti-CD25/CTLA4 monoclonal antibody decreased Tregs, PD1+CD8+, and PD1+CD4+T cells resulted in suppression of locally irradiated and distal unirradiated tumor growth, improved OS, and reduced liver metastasis [39]. Minimum 5 Gy is required to set in motion the inflammatory response with immunotherapy, and in a study, 2 x 7.5 Gy schedule resulted in similar tumor growth inhibition as 15 Gy in a single dose. Additionally, lower Treg cell numbers were present in the spleens than in a single dose [6].

C4. Optimization of Dose Painting: *Biological target volume*

One technique of differential dose delivery is dose painting. The cancer cells in the infiltrating edge of the gross disease areoxic and proliferating. They are likely to be the most sensitive cells (except possibly resistant stem cells) in the entire tumor compared to those within. These cells are likely to respond initially and maximally. Varied hypoxia manifests in cancer cells well within this infiltrating edge, either as a concentric gradient (Figure 1) or eccentric /diffuse irregular fashion. Hypoxic and anoxic regions require relatively higher doses per fraction of RT for comparable cell kill. Since we have not yet found a clinically applicable effective hypoxic cell sensitizer, optimization of delivering differential doses to these varied areas by the technique of dose painting may be worth exploring diligently.

Even with the strategy of increasing the intra-tumoral dosage with dose painting, which is technically easy, there is a limit of vascular endothelial cell tolerance beyond a particular dose-per-fraction level, as discussed above. Within these limitations, one strategy worth exploring is to deliver controlled hot spots within the gross tumor volume to target the resistant cells, especially cells in the “necrotic wall.” The other strategy

would be to gradually increase the dose gradient in the hypoxic/necrotic area of the tumor from the periphery, assisted by 3D functional imaging dose ranging from 6 Gy to 10 Gy per fraction. Implementation of this dose gradient is easily achievable with modern-day SBRT technology.

Crane et al. adopted dose-painting techniques in large HCC tumors, with photon as well as proton therapy [40]. They safely used a very high dose (up to 140 Gy BED), simultaneous integrated protector volumes, longer fractionation, and boost to the sub-volume within gross tumor volume. Even though not conclusive, results were encouraging with local control rates of 85%-90% and without significant toxicity [40]. In a rat rhabdomyosarcoma model, by delivering a sub-volume boost of 40% and a 60% dose gradient to the high fluorodeoxyglucose (FDG) uptake area, Trani et al. did not find any improvement in tumor control, and in certain conditions, the tumor growth accelerated [41]. This study indicates critical optimization of boost uptake-based sub-volumes within positron emission tomography with computerized tomography (PETCT) scan delineated gross disease is open for SBRT biological targeting. Since PET CT scan reflects cell activity, maximization of PETCT scan information for biological planning can be rewarding.

C5. Improving the Tumor Vasculature

Following SBRT, the indirect effect of radiation and fixation damage by free radicals in the presence of oxygen persists for six to twelve weeks with continued cell death. It leads to the hypothesis that, during the post SBRT potential lethal damage fixation period, cells would continue to be susceptible to cell kill with the local immune response and abscopal response. For this local immune response to occur, at least a skeletal tumor vasculature needs to be present post-SBRT.

The first step is maintaining the vascular integrity with an appropriate SBRT dose schedule. As discussed above, >10 Gy per fraction likely to induce reduced perfusion, EC apoptosis-cell death & increase hypoxia with ensuing worsening of immunosuppression. Less than 10 Gy per fraction doses promotes the dilation, normalization, vascular integrity of existing vessels, pericyte recruitment, and maturation of surviving vessels [27], along with limited/recoverable damage in ECs.

The second step is the improvement of the vasculature with combination therapies. The exploitation of normalization action of antiangiogenics is by optimally in combining with SBRT. Usually, after commencement of antiangiogenics, starting in 1–2 days, normalization of tumor vasculature results in a reduction in tumor hypoxia, a drop in interstitial tumor pressure, improved tumor perfusion, decrease in the peritumoral edema, and the majority of evidence comes from preclinical studies, in mice subjected to continuous antiangiogenics therapy. These

vascular normalization features were eventually lost and replaced by pronounced vascular regression in mice subjected to continued antiangiogenics therapy. These temporal changes demonstrated the existence of a “normalization window.” Usually, this vascular normalization “time window” persisted for at least 28 days. There was “uncoupling” of the timing of different aspects of vessel normalization (i.e., vessel size and permeability) in clinical studies, not observed in preclinical studies due to shorter observation time. Clinical MRI studies also showed changes in patients on toxicity-related “drug holidays,” the normalization phenotype reversed while patients were off the drug. The normalization window opens in human patients with GBM as early as 24 h after cediranib therapy commences and lasts at least 28 days [42]. Antiangiogenics beyond the window period carries the risk of increased tumor hypoxia, in turn aggravating immunosuppression in a dose-dependent manner [43]. These findings have several implications.

- Typically, in clinical practice, antiangiogenics are administered on the same day as chemotherapy. A delay of chemotherapy drug delivery by a specified time after antiangiogenic administration allowing normalization to set in can enhance the response.
- Proper synchronization of antiangiogenics can enhance the efficacy of immunotherapy, independent of other effects of VEGF suppression [42].
- Need to investigate with preclinical studies cyclical administration of antiangiogenics in combination therapies, including SBRT, by “switching on” the “switched off” normalization window, giving planned antiangiogenics “drug holidays.”
- SBRT effects will improve if the vasculature becomes normal or enhanced due to a decrease in hypoxia. Utilize normalization effect by delaying SBRT for a minimum 2 days after the administration of antiangiogenics. Preclinical trials to find out the harmonized cyclical combination of antiangiogenics and SBRT, matching with normalization window “on,” with or without chemo-immunotherapy, likely to improve the abscopal response.

Other than antiangiogenics, several molecules presently used in cancer therapy have the component of vascular normalization. A review article by Karar and Maity innovatively illustrates that a specific class of drugs, human immunodeficiency virus protease inhibitors (HPIs) (nelfinavir, amprenavir, and saquinavir), blocks the PI3K-Akt signaling axis. Nelfinavir decreases hypoxia-inducible factors-1 α and vascular endothelial growth factor (VEGF) expression in vitro and in vivo [44]. Pore et al. noted that nelfinavir improves tumor oxygenation in A549 lung carcinoma xenografts [45]. Qayum et al. found that nelfinavir treatment

normalized the tumor vessels and observed that they were more regular with increased inter-branch length and reduced tortuosity [46]. Results with nelfinavir are very similar to the one with erlotinib. Erlotinib, followed by radiation, inhibited tumor regrowth to a greater degree than radiation alone [47]. These reports open up new avenues in improving the tumor vasculature, in turn possibly influencing the abscopal response, and can be used in SABR combination therapies.

C6. Immune Metabolism

The other important dimension in TME is immune-metabolism, which needs exploration in combination with SBRT. Activation of an interconnected complex series of processes involving inflammation, immunomodulation, revascularisation, cycling hypoxia (which directly affects radio-sensitivity), immune metabolites, and radiation-induced fibrosis is observed in TME [48]. The immune cells also have to compete with the cancer cells for nutrients, essential metabolites, and oxygen [49]. All immune cells need to adapt to navigate a punitive metabolic environment created by the cancer cells. Hypoxia results in the generation of adenosine, a metabolite that is highly suppressive of cytotoxicity by natural killer (NK) cells. mTOR is a critical driver of NK cell metabolic reprogramming [50]. Therefore, exploitation of this pathway can enhance natural killer cell activity. Vanherwegen et al. enunciated the importance of Vitamin D's ability to control the human dendritic cell activity to induce functional regulatory T cells by regulating glucose metabolism [51].

D). Immunoadjuvants and abscopal effect enhancers (AEEs)

Several constituents can enhance the primary abscopal interaction between SBRT and immunotherapy agents (abscopal effect enhancers). Communicable diseases are primarily under control due to several vaccines with adjuvants contributing to their efficacy. Like conventional vaccines, if we can incorporate an effective adjuvant that can enhance this immune reaction to SBRT in-vivo/in-situ, it would answer the therapeutic cancer vaccines need.

The objective of a combination of RT with different immunotherapeutic modalities is to induce action at independent levels using dendritic cells, natural killer cells, conjugated antibodies, and immune checkpoint inhibitors. Radiation of 2 x 8 Gy boosted immunogenicity of un-methylated cytosine-guanine with oligonucleotides even in poorly immunogenic mouse breast carcinoma [6].

Concept of patient-specific neo-antigens:

After the initial enthusiasm, SBRT has not shown abscopal effects to the expected level compared to molecularly defined vaccines. The most important reason could be that the flooding of non-mutant peptides will dilute the neoantigens released coming in the way of organized specific mutation-oriented antigen presentation. Recent technological innovations have made it possible to dissect the immune response to patient-specific neoantigens that arise because of tumor-specific mutations. Recognition of such neoantigens is now critical [52]. Augmentation of such specific neoantigen response or inactivation of non-mutant peptides along with SBRT would be a valuable area of trials.

Compared with traditional RT, a single dose of 20-24 Gy SABR generates more DNA double-strand breaks, less DNA damage repair, massive release of antigens. It releases more death-associated molecular patterns ligands and induces Toll-like receptors activating immune cell responses [25]. In contrast, multiple fractions of radiation can produce an increased number and diversity of tumor neo-antigens, unlike in a single fraction [38].

E). High Linear Energy Transfer (LET) SBRT

High LET radiation expected to have more significant immunogenic potential than photon radiotherapy due to Bragg peak effect, higher ionization density, RBE of 1.1 (proton) to 3 (carbon ion), higher unrepaired damage leading to more complex clustered DNA lesions with genomic instability ending up in micronuclei and neo-antigens with greater diversity, and less irradiated leukocytes. HIF-1 stabilization, photon radiotherapy (PRT) feature, contrarily, Carbon Ion Radiotherapy (CIRT) attenuated HIF-1 signaling. CIRT is more effective against cancer stem cells residing in the hypoxic niche than photon radiotherapy. Largely, high LET radiation will be expected to be more effective in combination with immunotherapies in hypoxic tumors. CIRT formed less distant metastases in the mouse osteosarcoma model than photons after exposure **to iso-effective dose** single dose of 10 Gy (5 GyE). With greater efficacy against the primary tumor, CIRT dose might be facilitating the development of the protective immunological memory [6].

In an osteosarcoma mouse model, CIRT alone reduced the number of lung metastases more efficiently than PRT, and in combination with IT, both radiation types suppressed metastasis outgrowth, but with greater efficiency for carbon ions. However, using the same physical dose of 10 Gy (not biological equivalent dose) in both groups might have introduced bias to the study. Results are awaited from the majority of the ongoing trials [6]. According to the present author, making use of better normal tissue sparing, higher immunogenic potential with high LET radiation appears to be encouraging; yet, preclinical studies are required to identify the optimum

dose with attention to the integrity of vasculature. The same principles hold good for FLASH radiotherapy, an additional advantage being its ability to spare the vasculature better.

F). Dose versus toxicities: Newer drugs

In the PEMBRO-RT trial, in the SBRT plus immunotherapy arm, 12/35 (34%) had grade 3+ toxicities. On another phase II trial, 4/29 (14%) patients with advanced lung cancer treated with SBRT followed by maintenance chemotherapy grade 3+ toxicities). SBRT with immunotherapy showed grade 3+ toxicity rates of 7–31% in any extracranial disease treatment, as shown by the review studies [38]. The effective use dose per fraction (presently <10 Gy per fraction), appropriate total dose, and technique of dose painting reduces the potential lethal toxicities with SBRT, keeping in mind unknown toxicities of newer drug combinations.

3. Discussion

Moving away from anatomical and biological planning, we may be approaching an era of immunological planning in the field of radiation oncology by decoding “abscopal by chance” to “abscopal by design,” resulting in a statistically predictable and consistent effect.

3.1 SBRT dose schedule:

The objective is to kill the cancer cells and optimally enable and enhance the immunity against the cancer cells in combination with chemo-immunotherapy. The key components in the stratagem of improving tumor lysis and immunogenicity are: choosing an appropriate dose per fraction in improving cancer cell kill while simultaneously reducing the EC damage by genetic modulation (e.g., DRT), improving the vessel permeability, reducing hypoxia, accelerating deactivation of cancer immune-suppressive pathways, proper synchronization of chemo-targeted-immunotherapy, and improving the presentation of specific neo-antigens. For a robust immunological response and to have an amplifying abscopal effect, along with tumor lysis, intact vasculature is required.

Even though an increasing dose of a single fraction has the accelerated potential to induce tumor lysis, in the absence of an effective vascular conduit, the subsequent antigen-antibody reaction may be mitigated, thus affecting the abscopal response. The number of fractions and total dosage would depend on the clinical situation and tolerance of surrounding tissues. Buchwald et al. concluded that optimal radiation dose appears to be somewhere between 8 Gy and 10 Gy per fraction (intermediate dose) in one to three fractions [5]. An intermediate dose range in multiple fractions seems optimal in terms of effective cell kill for the first and subsequent doses (with continued tumor cell kill and generation of tumor antigens) and local and abscopal response compared to a single high dose.

More balanced in stimulating pro-inflammatory and inhibiting anti-inflammatory signals appears to be happening with intermediate-dose [6].

3.2. *Optimal Sequencing of SBRT with immunotherapy:*

For treatment-naive patients, initiating immunotherapy within one week of completing SBRT may lead to improved responses until the availability of more data represents a potential standard practice [38]. Based on permeability and preclinical outcome studies enumerated above, the optimal time maybe the second day after SBRT. However, one has to consider the overall potential toxicity for the total dose planned.

3.3. *The way forward:*

- Present literature favors SBRT with a gradient dose of 6-10 Gy per fraction (10 Gy sub-volume boost), which may be the trade-off between maximum tumor lysis and minimal vascular disruption, followed by immediate immunotherapy. After the initial course of SBRT, 8-10 Gy per fraction concomitant boost dose SBRT at 3-4 weeks and a delayed SBRT boost dose of 8 -10 Gy per fraction at 6-12 weeks with “shrinking volume” dose painting technique is worth exploring. Studies are required to identify the optimum immunogenic dose between 6 to 10 Gy. The total dose of initial, concomitant, and delayed boost put together depends on the size of the lesion (treatment volume), surrounding critical structures (organs at risk), and response, respecting the permissible dose constraints. This strategy appears to satisfy the requirements of the VIP model, e.g., integrity of the vasculature, handling of accelerated repopulation at 3-4 weeks, and residual resistant phenotypic stem cells at 6-12 weeks resulting in the maximum generation of varied evolving immunogenic tumor antigens (Figures 1-4). Table 1 shows the optimum utilization of the VIP model for the SBRT harmonized combinatorial schedule. This approach also fulfills the requirement of delivering the maximum possible tolerable dose with acceptable side effects.
- Future trials should incorporate DRT technology enunciated by Moding et al. [18] to increase the cancer cell kill, simultaneously protecting the endothelial cells to rework the optimum dose of ICD.
- The other convergent action trials are the following. **a)** Cyclical SBRT before each dose/cycle of immunotherapy. **b)** Cyclical administration of antiangiogenics making use of the window of normalization “off and on” with the delivery of SBRT with each normalization “on” after planned antiangiogenic “drug holidays.” **c)** Enhancing the vasculature during cancer-directed therapy. **d)** Immunoadjuvants for in vivo vaccination effect, and immune-metabolites as abscopal effect enhancers. Consolidating all the above perspectives, concepts, and clinical results, the author proposes five important fundamental requirements optimization of SBRT (TABLE 2). The author has proposed and discussed the hypothetical foundation, principles, and analysis of the vascular-immuno-phenotypic (VIP)

model in general aspects of cancer therapy of locally advanced and oligometastases [53].

4. Conclusions:

These basic concepts enunciated above should be part of systematic clinical trials and artificial intelligence-based analysis. There is a need to collate and analyze varied multi-institutional SBRT data through observational studies to develop an effective model given AE response being multi-factorial. SBRT is a powerful immunological weapon that requires proper dose schedule, immaculate timing of window of opportunity, deactivating immunosuppressive factors in TME, and enhancing tumor vasculature and tumor-specific neoantigens to induce in-situ/in-vivo therapeutic vaccination in a strategy of harmonized combination therapies.

Table 1: SBRT dose schedules with or without harmonized combination therapies matching tumor profile of vascular-immuno-phenotypic (VIP) model

SBRT Dose Schedule / Strategy	Tumor Profile Targeting <i>Vis-a-vis</i> normal tissue effects
1. High dose, disruptive, Single fraction immunogenic dose (> 12 Gy dose) as per the present literature	Vascular disruption with subsequently increased hypoxia; Higher dose may not be adequate to kill resistant cells and hence "wasted radiation" component (?); a one-time flood of antigen generation and presentation; dose modification not possible for concurrent side effects; <i>DRT clinical trials required.</i>
2. Intermediate Immunogenic dose (< 10 Gy/fraction), multiple fractions with optimum cell kill vis-a-vis vascular disruption	One of the fundamental 5 R's of RT "Re-oxygenation" is accounted for to an extent resulting in increased levels of hypoxic cells lysis; vascular & ECM integrity maintained; multiple time, scalable neo-antigenic presentation; leeway for optimization of total tolerable dose of SBRT based on concurrent side effects; <i>DRT clinical trials required to identify optimum dose between 6 & 10 Gy.</i>
3. Intermediate Immunogenic dose (<10 Gy/fraction), Multiple fractions with Boost during AR (concomitant boost), and residual lesion (delayed boost)	Targets proliferating resistant cells and stem cells; vascular and ECM integrity maintained with better oxygenation & drug delivery; continued scalable neo-antigen presentation; optimization of volume and total tolerable dose of SBRT based on response with limitable "titratable" acute side effects; <i>clinical trials required.</i>
4. "Cyclical" SBRT: Immunogenic dose (<10 Gy/ fraction), Multiple fractions before each	SBRT as sensitizer secondary to primary therapy i.e., chemo-immunotherapy; Optimum reoxygenation; repeated scalable neo-antigenic presentation; vascular and ECM integrity

immunotherapy dose and/or cyclical antiangiogenics	maintained; optimization of total tolerable dose of SBRT with limitable and titratable acute side effects; <i>clinical trials required.</i>
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Table 2: Fundamental requirements to facilitate *augmented* abscopal effect

Fundamental Requirements	Effects
1. Minimal disruption of tumor bed & normal tissue vasculature	Enhances oxygenation, fixes potentially lethal damage, and maintains sensitivity to further doses of SBRT; enhances tumor hostile TME, e.g., normal Physio-biochemical response & immune-metabolism; permits continued delivery of subsequent doses of drugs; encourages cancer cell-TILs and NK cell interaction; carries tumor neo-antigens and primes cancer killer cells for abscopal action, reduces side effects.
2. Harmonization of a combination of therapies with SBRT/radio-sensitization of cancer cells	Additive/ synergistic (very rarely antagonistic) effects; augments Immune-stimulation; handles heterogeneous cancer cell population.
3. Enhancing tumor vasculature (under cover of anticancer treatment) or Increasing resistance of endothelial cells, e.g., DRT or both	Converts hypoxic and anoxic cells to oxic cells to sensitize them for subsequent doses of SBRT; clears degraded and dead necrotic cell products; continues to present neo-antigens; avoids endothelial senescence and long-term toxicities.
4. Immunoadjuvants and abscopal effect enhancers	Has multiplier effects of abscopal reaction; facilitates in-vivo / in-situ therapeutic vaccine induction.

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- | | |
|--|--|
| 5. Immunological RT planning: Appropriate Dose per fraction, dose painting, and concomitant SBRT boost | Optimizes SBRT for abscopal effect; improves cancer stem cell kill, improves immunological milieu, maintains supple ECM simultaneously reduces the side effects. |
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- | | |
|--------------------------|--|
| 6. SBRT as delayed boost | Targeting residual resistant population and stem cells to prevent recurrence & reseeded; reduced side effects. |
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Appendix A: Nil

Appendix B: Nil

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