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Radiotherapy and Remodelling of the Tumour Neoantigen Landscape: Mechanisms, Determinants of Neoantigen Quality, and Translational Prospects in Breast Cancer

Mustafa Nasrat Abdulraheem [*, **, ***♦], Laavanya Karunanithi [**], Norazmi M Nor [****], Hasmah Hussin [**, *****], Mohd Hafiz Mohd Zin [**, ******], Gokula Kumar Appalanaido [******], Rafeezul Mohamed [*, **], Kazumasa Minami [*******], Yutaka Takahashi [********], Nor Effa S Zulkafli [*, **]

[*]*Department of Biomedical Sciences, Pusat Kanser Tun Abdullah Ahmad Badawi, Universiti Sains Malaysia, Kepala Batas, Pulau Pinang, Malaysia*
[**]*Breast Cancer Translational Research Program (BCTRP), Pusat Kanser Tun Abdullah Ahmad Badawi, Universiti Sains Malaysia, Kepala Batas, Pulau Pinang, Malaysia*
[***]*College of Education, University of Anbar, AL Qaim, Iraq*
[****]*School of Health Sciences, Health Campus, Kubang Kerian, Universiti Sains Malaysia*
[*****]*Department of Clinical Sciences, Pusat Kanser Tun Abdullah Ahmad Badawi, Universiti Sains Malaysia, Kepala Batas, Pulau Pinang, Malaysia*
[******]*Department of Biomedical Imaging, Pusat Kanser Tun Abdullah Ahmad Badawi, Universiti Sains Malaysia, Kepala Batas, Pulau Pinang, Malaysia*
[*******]*School of Health Science, Graduate School of Medicine, University of Osaka, Yamadaoka, Suita, Osaka, Japan*
[********]*Department of Medical Physics and Engineering, Graduate School of Medicine, University of Osaka, Yamadaoka, Suita, Osaka, Japan*

♦Corresponding Author
Mustafa Nasrat Abdulraheem, PhD
Department of Biomedical Sciences,
Pusat Kanser Tun Abdullah Ahmad Badawi,
Universiti Sains Malaysia, Bertam,
Penang, Malaysia
Email: alrawimustafa335@gmail.com

Abstract
Radiotherapy has long been used to achieve local tumor control through deoxyribonucleic acid damage, while ionizing radiation alters tumor immunogenicity as well. This review investigates how radiotherapy reshapes the tumour neoantigen landscape in breast cancer, and identifies which effects are supported by direct evidence. PubMed, Scopus, and Web of Science were searched to August 2026 for studies of radiation-associated antigenic remodeling, neoantigen biology, and antigen presentation; peer-reviewed work from 2015 to 2026 was prioritized. In this narrative synthesis, no formal quality appraisal, statistical testing, or quantitative pooling was performed. Three frequently conflated processes were defined and used consistently in this study: radiation-generated neoantigens, encoded by new mutations; radiation-exposed neoantigens, pre-existing mutations revealed by increased transcription; and radiation-modulated presentation, the reshaping

of the major histocompatibility complex (MHC) class I immunopeptidome without new mutations. There is much stronger evidence that radiotherapy modulates antigen presentation in human breast cancer than that it creates de novo, naturally presented, immunogenic neoantigens.
Immunogenic cell death and cyclic guanosine monophosphate-adenosine monophosphate synthase-stimulator of interferon genes-dependent type I interferon signaling can upregulate MHC class I and the antigen-processing machinery, although both pathways are context dependent and may also reinforce immunosuppression. Therapeutic relevance depends on neoantigen quality rather than quantity, specifically stable human leukocyte antigen binding, efficient processing, clonal expression, dissimilarity from self, and productive TCR engagement. Tumor heterogeneity, antigen-presentation defects, an immunosuppressive microenvironment, and over-prediction by current computational tools limit translation, which remains to be tested in breast cancer.

Keywords: Breast neoplasms, Radiotherapy, Immunotherapy, Antigen presentation, Neoantigens

Introduction

Global burden and persistent clinical challenges

Despite advances in early detection and multidisciplinary treatment, breast cancer remains the most commonly diagnosed cancer in women and a leading cause of cancer-related death, with approximately 2.4 million new cases and nearly 700,000 deaths each year worldwide.[1] Outcomes are poorest in low- and middle-income settings, where delayed diagnosis and limited access to oncology services result in more advanced disease at presentation.[2-5]

Breast cancer is not a single disease but a group of biologically distinct malignancies. Molecular profiling established the intrinsic subtypes luminal A, luminal B, human epidermal growth factor receptor 2 (HER2)-enriched, and basal-like/triple-negative breast cancer (TNBC), each with distinct genomic alterations, therapeutic vulnerabilities, and clinical outcomes.[6-7] Subtypes differ in mutational load, chromosomal instability, copy-number profile, and oncogenic signaling.

This heterogeneity is compounded by clonal evolution under the selective pressure of treatment. Chemotherapy, endocrine therapy, HER2-directed agents, and immunotherapy enrich for resistant subclones with greater metastatic potential, altered antigen expression, and enhanced immune evasion.[8-10] Breast cancer therefore behaves as an evolving ecosystem in which malignant, stromal, and immune populations adapt to treatment.

Modern management combines surgery, radiotherapy, systemic chemotherapy, endocrine therapy, HER2-directed agents, antibody-drug conjugates, and cyclin-dependent kinase 4/6 (CDK4/6) inhibitors, which have reduced locoregional recurrence and improved disease-free and overall survival across subtypes.[11-13] Durable control is nonetheless not achieved in a substantial minority of patients, because eradicating the primary tumor does not eliminate occult micrometastatic disease, and recurrence, metastatic dissemination, and acquired resistance remain the principal causes of breast cancer death.

These limitations argue for treatments that extend local control and also modify tumor immunogenicity by acting on antigenicity, antigen processing and presentation, immune-cell infiltration, and the functional state of the tumor microenvironment. Radiotherapy has been proposed to act in this way and has been described as capable of reshaping the neoantigen landscape so that the irradiated tumor functions as an in situ vaccine. The strength of the evidence supporting each component of this proposal

differs substantially, and distinguishing those components is the central purpose of this review.

Rationale and novelty of the study

Several recent reviews have examined how radiotherapy can reprogram the tumor immune microenvironment (TIME) or whether neoantigens can be predicted and exploited in general.[14-17] The axis linking radiotherapy to the neoantigen itself remains comparatively unexplored. This axis includes mechanisms involved in the generation and radiation-induced exposure of neoantigens; mechanisms involved in the reconfiguration of the antigen-processing and presentation apparatus; factors that influence whether peptides are immunologically productive; and the application of these principles to breast cancer. As shown in Table 1, this review is positioned among recent reviews on the topic and identifies the gaps it fills.

This synthesis is warranted for three reasons. Firstly, only a fraction of breast cancers, primarily a subset of TNBC, is sensitive to checkpoint blockade, leaving a vast group of breast cancer patients for whom it is understood that there is a great unmet need to boost immunogenicity. Second, radiotherapy is delivered to most patients with breast cancer so that any capacity it has to modify neoantigens would be broadly rather than selectively applicable. Third, the field has converged on the view that neoantigen quality, rather than raw quantity, determines clinical benefit, which changes how radiation-associated antigenic change must be assessed. This review will frame and structure the evidence on neoantigen quality and outline the mechanistic steps from deoxyribonucleic acid (DNA) damage to neoantigen specification to facilitate the design of radiotherapy-immunotherapy combinations.

Search strategy and selection criteria

The present review article was conducted through a literature search on PubMed, Scopus, and Web of Science, with the last search done in August 2026. Search terms included breast cancer, radiotherapy, ionizing radiation, neoantigen, immunopeptidome, cyclic guanosine monophosphate-adenosine monophosphate synthase-stimulator of interferon genes (cGAS-STING), immunogenic cell death (ICD), and antigen presentation. Studies were eligible, if they addressed radiation-associated antigenic/immunological remodeling, neoantigen biology, and antigen presentation, with a focus on breast cancer and a translational aspect to solid tumors. Peer-reviewed primary experimental, genomic, and immunopeptidomic studies from 2015–2026 were prioritized, and foundational mechanistic papers were kept when the primary source for an established concept was provided, as were selected preprints for the newly emerging field of computational methods. Non-English records and conference abstracts with incomplete data, or where the study did not have a clear mechanistic or translational relevance, were excluded. If discrepancies still arose between studies, the one that directly analyzed immunopeptidomics or functionally validated T-cells was given priority over studies relying on predictions based on binding affinity. Each included study was additionally assigned one of the four evidence grades defined in the next paragraph (Table 2). A small number of pre-2013 citations were retained because they are the primary reports of foundational mechanisms whose priority cannot be reassigned to a later source, and each is accompanied by a contemporary reference; 76 of the 85 references cited are from 2013 onward. It is a recognized limitation that formal quality appraisal, statistical analysis, and quantitative meta-analysis were not performed, because the review is narrative rather than systematic.

Operational definitions and grading of evidence

Since the phrase radiotherapy-induced neoantigen remodeling has been applied to biologically distinct processes that are not interchangeable, the following operational definitions were adopted and used consistently throughout this review, including in the abstract, figures, tables, and conclusion.

Radiation-generated neoantigens: Peptides encoded by somatic mutations that arise as a consequence of irradiation and subsequent error-prone repair, and that were absent from the tumor before treatment.

Radiation-exposed neoantigens: Peptides encoded by mutations already present before irradiation but transcriptionally silent or poorly expressed, which become immunologically available because radiotherapy increases expression of the mutated gene. No new mutagenesis is required.

Radiation-modulated antigen presentation: Quantitative and qualitative changes in the MHC class I immunopeptidome that follow from altered antigen processing, peptide loading, and surface display, without any change in the underlying mutational sequence.

The evidence supporting these three processes is of unequal strength. Evidence that radiotherapy modulates antigen presentation and reshapes the immunopeptidome is substantially stronger than evidence that radiotherapy generates de novo, naturally presented, immunogenic neoantigens in human breast cancer, for which direct demonstration is currently lacking. To make this asymmetry explicit, every study summarized in Table 2 is assigned one of four evidence grades: directly demonstrated in breast cancer; demonstrated in another solid tumor; inferred from general radiation immunology; or hypothesis-generating and not yet validated. The same grading is applied in the text, so that the breast-cancer-specific evidence base can be distinguished from evidence extrapolated from other settings.

Radiotherapy as a driver of immune remodeling

For many years, radiotherapy was primarily seen as a treatment that targeted specific areas, relying on irreversible DNA damage. This damage led to processes such as mitotic catastrophe, apoptosis, senescence, and other forms of tumor cell death. This understanding made radiotherapy a crucial part of breast cancer treatment, enhancing local control and reducing the risk of recurrence following both breast-conserving surgery and mastectomy. However, over the last 20 years, growing evidence has transformed this perspective, revealing that the biological impacts of ionizing radiation extend far beyond direct cell killing.[18]

Radiotherapy is now recognized as an effective immunomodulatory intervention that modulates interactions among cancer cells, stromal elements, and innate and adaptive immune cells. Irradiation does not just kill tumor cells; it triggers a chain of events at the molecular and cellular levels, affecting the tumor-immune ecosystem.[19] These events involve the secretion of immunostimulatory danger signals, activation of innate immune sensing, processing and presentation of antigens, recruitment and activation of antigen-presenting cells, regulation of immune cell trafficking, and remodeling of the tumor immune environment. These processes may turn irradiated tumors from being targets for cytotoxic treatment alone to systemic antitumor immunizing target(s).

The immunological effects of radiotherapy depend on context, including radiation dose, fractionation regimen, tumor genotype, tumor microenvironment, and immune environment. Depending on their nature and intensity, these factors can favor either

productive antitumor immunity or counter-regulatory mechanisms that attenuate it. Therefore, knowledge of the mechanisms by which radiotherapy regulates immune system activation is crucial for making optimal use of radiotherapy in combination with modern immunotherapies. These effects now work together and should be considered as a coordinated process of immune remodeling (Figure 1). The main mechanisms are discussed in turn, i.e., ICD and molecular pathways that alter the tumor neoantigen landscape.

ICD

Radiotherapy is traditionally regarded as a local cytotoxic therapy that kills tumor cells by damaging their DNA; however, an important determinant of its therapeutic effectiveness is its ability to initiate anti-tumor immunity. Largely mediated by ICD, which involves the release of damage-associated molecular patterns (DAMPs), it transforms dying tumor cells into an endogenous source of immune stimulation rather than immunologically inert debris.[20-22] A defining feature of ICD is the surface exposure of calreticulin (CRT) on stressed tumor cells, where it acts as an eat-me signal that promotes dendritic cell-mediated phagocytosis and antigen uptake. Obeid et al. established this principle by showing that preventing CRT exposure abolished the immunogenicity of dying tumor cells despite comparable apoptosis, thereby identifying CRT as an essential determinant of effective antitumor priming.[23]

ICD also promotes the extracellular release of adenosine triphosphate (ATP), which recruits antigen-presenting cells to the irradiated microenvironment and activates inflammasome signaling to facilitate T-cell priming.[24] Ghiringhelli et al. demonstrated that inhibition of ATP-mediated purinergic signaling markedly impaired dendritic cell activation, interleukin-1 beta production, and tumor-specific CD8-positive T-cell responses, thereby identifying ATP as a critical mediator of treatment-induced antitumor immunity.[24] A third DAMP, high-mobility group box 1 (HMGB1), engages Toll-like receptor 4 (TLR4) on dendritic cells, and disruption of HMGB1-TLR4 signaling reduces the efficacy of anticancer therapy and impairs the generation of adaptive antitumor responses. Together, CRT exposure, ATP release, and HMGB1 signaling constitute the principal molecular determinants of radiation-induced ICD, functioning cooperatively to promote antigen uptake, dendritic cell maturation, and cross-presentation, and thereby establishing conditions permissive for T-cell activation. The presence of these signals is necessary but not sufficient. CRT exposure, ATP release, and HMGB1 release are markers of a cellular stress response rather than proof that cell death is functionally immunogenic, and irradiated tumors may display all three without eliciting a protective T-cell response. Establishing functional immunogenicity requires evidence such as protection on vaccination with irradiated tumor cells or measurable expansion of antigen-specific T cells; this evidence exists for a limited number of preclinical models and is absent for human breast cancer. The release of DAMPs should therefore be interpreted as creating permissive conditions for priming rather than as demonstrating that priming has occurred. Although ICD represents the initial immunological response to radiotherapy, durable antitumor immunity requires the activation of innate immune sensing that connects DNA damage to type I interferon production and systemic immune activation. This function is served chiefly by the cGAS-STING pathway.

The cGAS-STING pathway

The initiation of ICD alone is insufficient to generate durable antitumor immunity, and effective activation requires converting radiation-induced DNA damage into innate

immune signals that orchestrate dendritic cell activation and T-cell priming. Among the pathways activated after irradiation, cyclic GMP-AMP synthase-stimulator of interferon genes (cGAS-STING) has emerged as the principal molecular link between DNA damage and systemic antitumor responses.[25-26] Ionizing radiation induces extensive double-strand breaks that frequently produce chromosome fragmentation and micronuclei, and the rupture of these micronuclei releases double-stranded DNA into the cytoplasm, where cGAS recognizes it as a danger signal.[27] Deng et al. showed that STING-deficient mice failed to produce type I interferons after radiotherapy, with impaired dendritic cell activation and defective CD8-positive T-cell priming, thereby establishing STING signaling as an essential mediator of the immune effects of radiation rather than a secondary consequence of tumor cell death.[28] Upon binding cytosolic DNA, cGAS catalyzes the synthesis of cyclic GMP-AMP (cGAMP), which activates STING on the endoplasmic reticulum membrane. STING activation triggers phosphorylation of TANK-binding kinase 1 (TBK1) and interferon regulatory factor 3 (IRF3), culminating in the production of type I interferons and pro-inflammatory cytokines that coordinate innate and adaptive responses.[29, 30] The main effectors of this axis are the type I interferons, which mediate the expression of a series of genes involved in communication between irradiated tumor cells and immune cells in the microenvironment.[31] Burnette et al. found that inhibition of type I interferon signaling completely blocked the therapeutic effect of radiotherapy, even though the same number of tumor cells were killed, suggesting a role for interferon signaling that cannot be replaced by tumor killing alone.[32]

cGAS-STING has a dose- and fractionation-dependent immunological output. Vanpouille-Box et al. demonstrated that higher single doses (~12-18 Gy) induced the three-prime repair exonuclease 1 (TREX1), which degraded cytosolic DNA and consequently reduced type I interferon production, whereas fractionated doses preserved cytosolic DNA, maintained cGAS-STING activation, and increased the CD8-positive T-cell response.[33] Complementary work indicates that low-dose radiotherapy, delivered in single fractions of approximately 0.5 to 2.0 Gy, can also reprogram the tumor immune milieu.[14] These observations identify cGAS-STING as a principal mechanism translating radiation-induced DNA damage into innate immune activation and, beyond interferon induction, into dendritic cell maturation, enhanced cross-presentation, and an inflammatory context that can support recognition of both pre-existing and newly generated tumor antigens. The output of cGAS-STING signaling is not uniformly favorable, and the pathway should not be read as a linear route to antitumor immunity. Sustained or chronic STING activation can drive an immunosuppressive program that includes the recruitment of myeloid-derived suppressor cells, induction of indoleamine 2,3-dioxygenase, expansion of regulatory T cells (Tregs), and upregulation of programmed death-ligand 1 (PD-L1), and type I interferon itself exerts both stimulatory and inhibitory effects depending on the duration and cellular context of signaling. Dose and fractionation determine which output predominates, because high single fractions induce TREX1 and curtail interferon production. Cell-intrinsic silencing or loss of cGAS or STING is common in cancer and abolishes the response altogether, and the magnitude of pathway activation after clinically used breast radiotherapy schedules has not been measured in patients. The pathway is therefore better regarded as a context-dependent amplifier of antigen presentation

than as a reliable driver of neoantigen-specific T-cell immunity.

Remodeling of the TIME

The efficacy of radiotherapy extends beyond tumor cell death to the remodeling of the tumor TIME. In solid tumors such as breast cancer, the TIME is typically characterized by poor T-cell infiltration, deficient antigen presentation, and accumulation of immunosuppressive cells, all of which limit antitumor immunity. Radiotherapy can transform this suppressive milieu into one that supports immune activation and improves antigen recognition.[14-16,34] Irradiation increases the recruitment of dendritic cells into the tumor, which aids antigen uptake and the initiation of adaptive responses.[35] cGAS-STING-driven type I interferon further promotes the recruitment and activation of Batf3-dependent dendritic cells, the loss of which markedly impairs CD8-positive T-cell priming [33]. Radiotherapy also increases major histocompatibility complex (MHC) class I expression and antigen-processing capacity, improving the presentation of tumor-derived peptides to cytotoxic T lymphocytes.[36] Reits et al. demonstrated that ionizing radiation enhanced MHC class I surface expression and peptide generation, improving recognition and killing by CD8-positive T cells.[36] In parallel, radiation increases the levels of inflammatory chemokines such as CXCL9, CXCL10, and CCL5 that recruit effector T cells,[37] and Matsumura et al. showed that local irradiation enhanced CXCL16 expression in breast tumors, thereby increasing the infiltration of activated CD8-positive T cells.[38]

These immunostimulatory effects are counterbalanced by immunoregulatory programs. Irradiation can increase Tregs, myeloid-derived suppressor cells, and checkpoint molecules such as PD-L1, creating compensatory pathways that restrain T-cell function. These observations help to explain the limited efficacy of radiotherapy alone and provide a rationale for combining it with immune checkpoint blockade. Overall, radiotherapy reshapes the TIME by improving antigen presentation, activating dendritic cells, recruiting effector T cells, and modifying inflammatory signaling, which converts immunologically cold tumors into more inflamed, immune-responsive lesions that better recognize both pre-existing and radiation-associated neoantigens. This remodeling provides the basis for the generation, presentation, and immunological use of neoantigens, as discussed in the next section.

Radiotherapy and the tumor neoantigen landscape

Radiation-associated neoantigen remodeling involves more than the accumulation of DNA mutations, as it also alters the tumor immunopeptidome, the repertoire of peptides presented on MHC class I. This reshaping changes the antigenic landscape available for T-cell recognition and positions radiation as both a mutagen and a regulator of antigen processing and presentation, influencing the immunological visibility of tumor antigens.[39] The dynamic nature of tumor antigenicity is underscored by genomic studies of tumor evolution during treatment. Yates et al. showed that relapse- and metastasis-seeding clones in breast cancer continue to acquire mutations after dissemination, with many distant metastases acquiring driver mutations absent from the primary tumor.[40] Brady et al. found that therapeutic pressure selects pre-existing subclones with reduced antigen presentation and altered growth-factor signaling, phenotypes associated with immune evasion and drug resistance.[8] Tumor immunogenicity therefore evolves throughout treatment rather than remaining static, and therapeutic interventions reshape the neoantigen landscape by influencing both clonal evolution and antigen availability.

Radiotherapy affects this landscape not only through de novo mutations but also by altering the epitope (or peptide) presented by MHC class I, thereby changing the antigen recognized by cytotoxic T lymphocytes. A combination of integrated proteomic, immunopeptidomic, and exome analyses revealed that ionizing radiation upregulates the expression of critical antigen processing machinery, such as transporter associated with antigen processing 1 (TAP1), endoplasmic reticulum aminopeptidase 1 (ERAP1), beta-2-microglobulin, and MHC class I, with an expansion of the immunopeptidome and an increase in radiation-specific peptides, including a single radiation-specific tumor-specific neoantigen, Mtch1.[41] This expansion was both qualitative and quantitative; many of the peptides presented were novel, and they came from proteins that did not show an increase in total protein expression, suggesting that irradiation rearranged antigen processing, peptide loading, or both. The present study was performed in irradiated colorectal cells and therefore constitutes evidence demonstrated in another solid tumor rather than direct evidence in breast cancer.

Mechanisms of radiation-associated neoantigen generation and exposure

Radiation-induced double-strand breaks are repaired by several pathways, of which non-homologous end joining (NHEJ) is the most frequently used and the most error-prone. Misrepair by NHEJ principally yields small insertions and deletions (indels), larger deletions, and structural chromosomal rearrangements, and a radiation-associated deletion signature has been described across tumor types.[42, 43] SNVs should not be ascribed to NHEJ alone: after irradiation, they also arise through base and nucleotide excision repair of oxidative lesions, replication stress, mismatch repair, and translesion synthesis.

The mutational consequences of radiation are therefore context dependent and arise from several repair pathways acting together with chromosomal instability and the selection of pre-existing subclones. Such changes can alter protein-coding sequences and in principle yield novel peptides presented by MHC class I. Daino et al. reviewed experimental animal and human data, indicating that radiation leaves characteristic mutational signatures capable of generating mutation-derived neoantigens.[44] The chain of evidence from a radiation-induced DNA lesion to a clinically significant neoantigen-specific T-cell response is long and should not be compressed: the mutation must be fixed in a surviving and proliferating clone, transcribed, translated, processed, loaded onto an available human leukocyte antigen (HLA) allele, displayed at sufficient surface density, and recognized by a T-cell receptor (TCR) that has escaped tolerance. Each step is a potential point of failure, and in human breast cancer the available evidence extends only to the earliest of them. Frameshift indels are a particularly immunogenic subclass because they produce extended stretches of foreign sequence absent from the normal proteome, thereby increasing the likelihood of high-affinity HLA-binding epitopes and eliciting stronger CD8-positive T-cell responses than missense mutations. Roudko et al. demonstrated that frameshift mutations in microsatellite-unstable tumors generate shared poly-epitope peptides, in which single mutations encode multiple MHC-binding epitopes that elicited robust tumor-reactive CD8-positive responses. Ballhausen et al. mapped this shared frameshift landscape across microsatellite-unstable cancers and found evidence of ongoing immunoediting.[45, 46] In breast cancer, Turajlic et al. reported that TNBC, especially BRCA1-mutated disease, carries a higher burden of indels and mutant-specific neoantigens, a burden that correlates with response to checkpoint inhibitors.[47]

Hansen et al. confirmed that both point-mutation- and frameshift-derived neoepitopes are recognized by tumor-infiltrating T cells, with frameshift epitopes contributing disproportionately given their rarity,[48] and Jin et al. found that indel-derived neoepitopes generate a higher abundance of neoantigen-specific CD8-positive T cells than SNV-derived epitopes.[49]

Radiation-exposed neoantigens, as previously defined, do not require new mutagenesis. Radiotherapy can upregulate the expression of genes carrying pre-existing but poorly expressed mutations, thereby revealing immunogenic mutations rather than creating them. Using paired whole-exome and RNA sequencing with mass spectrometry-based immunopeptidomics, Lhuillier et al. showed that radiotherapy uncovers mutant CD8-positive and CD4-positive neoepitopes in a poorly immunogenic breast cancer model, thereby improving tumor control without new mutations, and subsequently detailed the pipeline for identifying such radiation-exposed neoantigens. This work was conducted in a preclinical breast cancer model, and equivalent direct evidence in human breast cancer is not yet available.[50-52]

Beyond generating and exposing candidate peptides, radiation reconfigures the machinery that processes and displays them, and Storozynsky and Hitt described how radiation-induced DNA damage activates cGAS-STING and type I interferon signaling to enhance MHC class I antigen-processing components, thereby facilitating efficient loading of newly available peptides.[25] Together, these three routes, namely radiation-generated neoantigens, radiation-exposed neoantigens, and radiation-modulated presentation as previously defined, may expand the antigenic landscape through parallel mechanisms (Figure 2, Tables 2 and 3) and provide a mechanistic rationale for combining radiotherapy with immunotherapy.

Beyond canonical MHC class I: Additional radiation-responsive antigen sources

Most work on radiation-associated neoantigens has centered on peptides presented by MHC class I to CD8-positive T cells, yet several additional antigen sources are relevant and may be disproportionately important in breast tumors with a low mutational burden. Radiation enhances dendritic cell cross-presentation and can increase tumor MHC class II expression, thereby enabling CD4-positive T-cell help and epitope spreading to contribute to durable responses. MHC class II neoantigens shape tumor immunity and the response to checkpoint blockade, which argues for extending radiation-neoantigen discovery to CD4 epitopes rather than restricting it to the class I ligandome.[53]

The presented repertoire also includes non-canonical species. A substantial fraction of HLA class I ligands are proteasome-generated spliced peptides,[54] and post-translationally modified peptides, for example phosphorylated or oxidized species, expand the antigenic space in ways that exome-based prediction does not capture.[55] Radiation-induced DNA-damage signaling can derepress endogenous retroviral elements, producing double-stranded ribonucleic acid (RNA) that drives an interferon response through viral mimicry and yields potentially antigenic products;[56] in parallel, alternative splicing and adenosine-to-inosine RNA editing, the latter governed by adenosine deaminase acting on RNA 1 (ADAR1), modulate both immunogenicity and sensitivity to immune attack.[57] These are especially relevant in classical low mutational burden hormone receptor positive breast cancer, where the neoantigen supply is limited. With the exception of MHC class II neoantigens, the radiation responsiveness of these antigen classes has not been

demonstrated directly in breast cancer, and their inclusion here is hypothesis-generating rather than established. Table 4 lists these antigen classes and their significance in relation to radiation.

Neoantigen quality determines therapeutic success

A central conclusion of the past decade is that raw neoantigen quantity, whether from baseline mutational burden or radiation-induced mutagenesis, is a poor predictor of antitumor immunity, and that neoantigen quality is more important.[58] This distinction is directly relevant to radiotherapy, which may generate or expose large numbers of candidate peptides yet yield only a small subset that T cells recognize or that translates into durable control.[51,59] Balachandran et al. proposed a quantitative framework for neoantigen quality in long-term pancreatic cancer survivors, defining high-quality neoantigens by their similarity to pathogen-derived epitopes and their differential MHC binding or T-cell activation relative to wild-type peptides.[60] In parallel, Luksza et al. developed a neoantigen fitness model integrating quality and clonality to predict responses to checkpoint blockade, and showed that a few high-quality neoantigens can be more clinically consequential than many low-quality ones.[61] A subsequent ten-year follow-up found that high-quality neoantigens declined over time in survivors, consistent with quality-based immunoediting.[62]

To make neoantigen quality operational rather than descriptive, its determinants can be grouped into three sequential layers, each of which must be satisfied before a candidate peptide is therapeutically useful. The presentation layer determines whether a peptide reaches the cell surface at all, and comprises proteasomal and aminopeptidase processing, TAP-dependent translocation, peptide-HLA binding affinity and complex stability, and the density and duration of surface display. The recognition layer determines whether a presented peptide is seen as foreign, and comprises dissimilarity from the wild-type sequence and the self-proteome, similarity to pathogen-derived epitopes, and the availability of a TCR repertoire that has escaped central and peripheral tolerance. The tumor-context layer determines whether recognition produces durable control, and comprises clonal or truncal expression across tumor regions, stability of expression under immune selection, and the permissiveness of the local microenvironment. These layers are sequential rather than additive, so a peptide that fails at any one of them cannot be rescued by strength at another, and candidates should be prioritized only when all three are satisfied (Table 5).

Independent of intrinsic foreignness, clonal status strongly influences therapeutic relevance. McGranahan et al. showed that in non-small cell lung cancer and melanoma, clonal neoantigen burden, and not total mutational burden, was associated with better survival and response to checkpoint blockade, and that T cells targeting clonal neoantigens were present in durable responders, whereas therapy-induced subclonal neoantigens predominated in poor responders.[63] Immunogenicity also depends on peptide-HLA binding affinity and stability, efficient processing, sufficient dissimilarity from self to overcome central tolerance, and productive TCR recognition (Figure 3, Table 5). Consequently, only a limited subset of radiation-associated neoantigens ultimately activates functional CD8-positive responses. Modern prediction increasingly integrates somatic mutation analysis with HLA typing, processing prediction, transcript expression, clonality, and TCR recognition probability to prioritize high-fitness candidates, which improves patient-specific selection for therapeutic vaccines and adoptive T-cell therapies. For

the evaluation of radiotherapy-induced remodeling, these findings indicate that a small number of highly immunogenic, clonally expressed neoantigens may be sufficient to drive systemic immunity, which supports the combination of radiotherapy with precision neoantigen discovery.

Antigen-presentation escape and other challenges

Radiotherapy may generate neoantigens, expose pre-existing ones, and modulate their presentation, but these changes do not reliably translate into durable antitumor immunity, and only a limited number of radiation-associated neoantigens prove immunologically relevant. Several biological and technical factors constrain therapeutic success (Table 6). Tumor heterogeneity and clonal evolution are foremost among them, and McGranahan and Swanton showed that intratumor heterogeneity enables Darwinian selection that shapes therapeutic outcomes.[64] Many radiation-associated neoantigens are expressed only in specific subclones. Although Lussier et al. found that subclonal radiation-induced mutations can still drive antitumor immunity when antigen is generated broadly across the tumor,[59] immune selection may eliminate highly immunogenic clones over time, allowing less immunogenic subclones to expand. Dijkstra et al. described how high heterogeneity hinders immunosurveillance by underdosing subclonal antigens, fostering competition and immunodominance, and driving unfavorable T-cell differentiation, which collectively push tumors toward less immunogenic states.[65]

A second limitation is the inefficient presentation of newly generated neoantigens. Even when radiotherapy increases antigen availability, recognition requires efficient processing, stable HLA presentation, and adequate TCR engagement. Gettinger et al. found that patient-derived xenografts from checkpoint-inhibitor-resistant tumors frequently showed beta-2-microglobulin downregulation and loss or near-absence of surface HLA class I, often without outright genomic loss, which implicates additional genomic and non-genomic mechanisms in immune escape.[66] Huber and Bassani-Sternberg similarly linked reduced antigenicity to defects in antigen processing and presentation, including HLA class I and II loss-of-heterozygosity and functional beta-2-microglobulin loss, which directly connect presentation defects to the failure of immunogenic neoantigens to elicit T-cell responses.[67]

These presentation defects are not uniform across breast cancer. Allele-specific HLA loss of heterozygosity is a recurrent route of immune escape in solid tumors and, together with beta-2-microglobulin loss and defects in the Janus kinase-signal transducer and activator of transcription and interferon pathways, can render radiation-exposed neoantigens invisible even when they are generated.[68] Triple-negative and BRCA1-associated tumors, which carry the highest indel and neoantigen burden and the strongest interferon signatures, are expected to show the most pronounced radiation-driven presentation remodeling, whereas hormone-receptor-positive tumors, with low mutational burden and frequently colder microenvironments, may depend more on the non-canonical antigen sources described above.[47] Reported magnitudes of radiation-induced change in MHC class I, TAP1, and ERAP1, and in the fraction of radiation-specific peptides, vary across models, and standardized quantitative reporting would aid cross-study comparison and patient stratification. Systematic, subtype-resolved data on HLA loss of heterozygosity, beta-2-microglobulin status, and interferon-pathway integrity in breast cancer remain limited, and generating them is a priority for patient selection.

Third, the immunosuppressive microenvironment constrains the benefit of neoantigen remodeling. Liu et al. reported that stereotactic body radiotherapy (6 to 10 Gy per fraction over 3 to 5 fractions) more than doubled Foxp3-positive Treg density and increased CD204-positive macrophages, while shifting myeloid populations toward an immunosuppressive phenotype, which indicates that radiotherapy can reinforce the suppression it aims to relieve.[15] Yazdimamaghani et al. showed that tumor-enriched Tregs secrete immunosuppressive mediators such as interleukin-10, interleukin-35, transforming growth factor beta, and prostaglandin E2 that blunt cytotoxic effector activity, and that depleting Tregs alongside antibodies against programmed cell death protein 1 (PD-1) and cytotoxic T-lymphocyte-associated protein 4 increased CD8-positive cytokine production, reduced tumor growth, and improved survival, although Treg-specific depletion remains technically difficult.[69] Suppressive myeloid populations can neutralize T-cell responses through redundant pathways even when immunogenic neoantigens are present.[70] Consistent with the need for combinations, Zhang et al. found that radiotherapy alone recruited Tregs and that Treg depletion controlled tumors without improving survival, whereas vaccination followed by radiotherapy increased effector T-cell influx and produced a markedly better antitumor effect, which underscores the importance of sequencing and combination.[71]

Because immunopeptidomics underpins any claim that radiotherapy reshapes the presented repertoire, what mass-spectrometry-based HLA ligandome analysis can and cannot establish should be stated precisely. It can establish that a given peptide was eluted from HLA molecules of a sample at a detectable abundance, can compare repertoires between irradiated and unirradiated conditions, and can identify presented species that exome-based prediction would never nominate, including proteasome-spliced and post-translationally modified peptides. It cannot establish that a detected peptide is immunogenic, because presentation and T-cell recognition are separate properties and only functional assays address the latter. It cannot establish absence, because detection depends on peptide abundance, ionization efficiency, the HLA alleles represented in the elution, the quantity of input material, and the completeness of the search database, so that a peptide not detected may nonetheless be presented. Mutation-derived peptides are especially vulnerable to this limitation, as they are typically of low abundance and are recovered in small numbers even from deeply profiled samples. Quantitative comparison between conditions further requires careful normalization, since an apparent expansion of the immunopeptidome may reflect increased HLA surface expression rather than a change in repertoire composition. Claims of radiation-specific neoantigens therefore require paired genomic, transcriptomic, and immunopeptidomic data together with functional T-cell validation, and it is relevant that the single radiation-specific neoantigen reported to date, Mtch1, was identified in a colorectal rather than a breast cancer model.

Lastly, there are technical restrictions to neoantigen identification. Most pipelines rely on genomic sequencing, peptides, and HLA-binding affinity prediction algorithms. While automated workflows like pVAC-Seq can combine expression and mutation information with tools such as sequence alignment, HLA typing, and NetMHC-based binding prediction, they often fail to consider TCR recognition or similarity to known immunogenic epitopes, and affinity alone is not enough.[17] Some correctly predicted neoantigens are never processed and presented, whereas other clinically relevant neoantigens remain undetected. Current

MHC class I and class II presentation models have positive predictive values of approximately 0.60 to 0.70, which means that false predictions are common,[55] and the Neo-intline framework has shown that not all peptides with predicted affinity for an MHC are presented.[72] Integrating genomics with transcriptomics and mass-spectrometry-based immunopeptidomics helps to overcome these limits by directly profiling presented peptides, and deep-learning models that exploit mass-spectrometry data further support multi-modal validation, although immunopeptidomic databases remain constrained and require pairing with functional T-cell assays to confirm clinical relevance.[17,55] Collectively, these limitations show that radiation-associated neoantigen remodeling is a first step rather than a complete solution, and that future strategies must improve neoantigen quality, presentation, immune recognition, and reversal of immunosuppression to realize its clinical potential.

Translational outlook and future directions

The evidence synthesized in this review is substantial but unevenly distributed across the three processes defined previously. The strongest evidence supports radiation-modulated antigen presentation, since irradiation reproducibly upregulates MHC class I, TAP1, ERAP1, and beta-2-microglobulin and expands the immunopeptidome, although the direct demonstration was made in colorectal rather than breast cells. The evidence for radiation-exposed neoantigens is moderate and rests principally on preclinical breast cancer models in which irradiation increased expression of pre-existing mutations and improved tumor control without new mutagenesis. The evidence for radiation-generated neoantigens is the weakest of the three, being largely inferential and derived from radiation mutational signatures rather than from demonstration of de novo, naturally presented, immunogenic peptides in human tumors. Across all three processes, therapeutic relevance was determined by neoantigen quality rather than quantity, and the principal obstacles identified were tumor heterogeneity and clonal evolution, antigen-presentation defects, a counter-regulatory immunosuppressive microenvironment, and over-prediction by current computational pipelines.

The mechanisms reviewed here support a defined translational approach. Because radiotherapy expands and reshapes the neoantigen landscape while also activating compensatory immunosuppression, its clinical benefit is more likely to be obtained in combination than as monotherapy. The leading candidates are the addition of immune checkpoint blockade, which counteracts the PD-L1 upregulation and regulatory T-cell-mediated immunosuppression induced by irradiation, and personalized neoantigen vaccines or adoptive T-cell therapies, which would focus the response on high-quality, clonally expressed targets.[59,69,71] In breast cancer specifically, in which checkpoint blockade benefits mainly a fraction of TNBC, radiotherapy offers a broadly available means of increasing tumor immunogenicity, and preclinical models already show that irradiation exposes actionable neoepitopes in otherwise poorly immunogenic disease.[47,51,59]

Dose and fractionation warrant deliberate optimization. The dependence of cGAS-STING signaling on cytosolic DNA implies that very high single fractions may be self-limiting through TREX1 induction, although this has been shown in preclinical models and not in irradiated human breast tumors, whereas fractionated or lower-dose schedules can sustain type I interferon output and immune priming.[14, 33] Selection of the radiation schedule according to the immunological objective, in addition to local

control, is likely to influence the quality and durability of the resulting response. In parallel, biomarkers that capture antigen-presentation integrity, clonal neoantigen burden, and the balance of stimulatory versus suppressive immune infiltrate could help to identify the patients most likely to benefit and guide the sequencing of combination therapy (Figure 4).

Several questions remain unresolved. It is not yet established how to prospectively quantify neoantigen quality in the clinical setting, how radiation dose influences both the quality and quantity of exposed peptides, or how immunosuppression following irradiation can be counteracted without impairing effective T-cell priming. Addressing these questions will require breast-cancer-focused translational studies that couple immunopeptidomic profiling of irradiated tumors with immune-monitored combination trials.

Neoantigen discovery tools and tissue analytics

Realizing this potential will require discovery that moves beyond binding-affinity prediction and is supported by interpretable, multi-criteria tools. Platforms such as pVACtools and its visualization companion pVACview operationalize quality-based triage by combining binding, processing, expression, and clonality into a reviewable ranking.[73, 74] Emerging models that jointly represent peptide-HLA and peptide-T-cell-receptor specificity, MHC class II presentation predictors, and structure-aware modeling of T-cell-receptor-peptide-MHC interactions extend prediction toward the recognition and processing determinants emphasized in this review, although processing is not yet fully modeled in all frameworks.[75-77] Applying these tools to radiation-exposed peptides, rather than to baseline sequencing alone, would align neoantigen prioritization with the antigenic changes that radiotherapy actually produces.

Quantifying radiation-induced remodeling in human tissue requires analytical methods matched to the question. Deconvolution of bulk transcriptomes and spatial transcriptomics can resolve the location and magnitude of antigen-presentation niches and antigen-presenting-cell and T-cell ingress after irradiation, thereby enabling multi-cohort, subtype-resolved comparisons in breast cancer.[78, 79] Paired pre- and post-radiotherapy sampling, analyzed with these methods, is the most direct route to human evidence for the mechanisms proposed here.

Radiobiology, human evidence, and safety

Beam quality may itself shape the antigenic output. Proton and heavy-ion beams, variation in linear energy transfer, and ultrahigh-dose-rate (FLASH) delivery alter the topology of DNA damage and normal-tissue responses and are therefore likely to influence mutational signatures, cytosolic DNA and TREX1 dynamics, and the balance between neoantigen generation and innate activation.[80-82] Whether FLASH preserves or attenuates immunogenic signaling relative to conventional dose rates is an open and clinically consequential question.

Human evidence is beginning to accrue. Trials that combine radiotherapy with immune modulation in breast cancer, such as the Neo-CheckRay study of preoperative stereotactic radiotherapy with immune-modulating agents, and individualized neoantigen-vaccine studies combined with radiotherapy in solid tumors, provide the clinical scaffolding for measuring pre- and post-treatment changes in presented antigens and T-cell-receptor clonality.[83, 84] Scheduling is likely to be decisive, and dynamical models of checkpoint therapy can help formalize whether radiotherapy is best delivered before, during, or after immunotherapy to align windows of neoantigen exposure with T-cell activation.[85]

Translation must also account for safety and feasibility. Because irradiation increases

antigen presentation in both normal and malignant tissue, bystander inflammation and the theoretical risk of autoimmunity warrant monitoring, particularly in adjuvant settings and when nodal basins are treated, while breast-specific constraints such as cardiac and lung dose remain paramount. The manufacturing time for personalized vaccines or adoptive T-cell products must be reconciled with the transient windows of radiation-induced antigen presentation. Table 7 outlines a trial-ready pipeline that links patient selection, sampling, assays, prioritization, and combination strategy for breast cancer.

Certain limitations of this review should be acknowledged. It is a narrative rather than a systematic synthesis, so no protocol was registered, no formal risk-of-bias or quality appraisal was applied, and no quantitative pooling or statistical analysis was performed; the selection of studies therefore reflects the authors' judgment and is susceptible to selection bias. The search was restricted to three databases and to English-language records, and conference abstracts and records lacking extractable mechanistic detail were excluded, so relevant work may have been missed. Much of the mechanistic evidence derives from cell lines, preclinical models, and tumor types other than breast cancer, and its extrapolation to human breast cancer is therefore provisional. Published reports of radiation-induced change in MHC class I, TAP1, and ERAP1 expression use heterogeneous models, doses, fractionation schedules, and sampling timepoints and are not quantitatively comparable, which precluded any pooled estimate of effect size. Finally, because the field is advancing quickly, a small number of preprints were retained for computational methods and have not undergone peer review.

Conclusion

Radiotherapy can no longer be regarded solely as a local cytotoxic treatment. Through ICD, cGAS-STING-dependent interferon signaling, and remodeling of the TIME, irradiation may modify the neoantigen landscape through the three distinct processes defined in this review: generation of new mutations; exposure of previously silent ones; and modulation of the machinery that presents them. These processes are supported by evidence of unequal strength, and the case for radiation-modulated presentation is considerably stronger than the case for de novo neoantigen generation in human breast cancer. The expansion of the candidate peptide pool is not in itself sufficient for therapeutic benefit. Whether radiation-associated neoantigens produce durable antitumor immunity depends on their quality, including HLA binding, clonality, foreignness, processing, and T-cell recognition, and on the extent to which tumor heterogeneity, antigen-presentation defects, an immunosuppressive microenvironment, and the limitations of current prediction tools can be overcome. In breast cancer, in which immunotherapy remains only selectively effective, a quality-based, multi-omic approach to radiation-associated neoantigens applied within rational combination regimens may allow radiotherapy to be developed as a broadly applicable means of enhancing systemic antitumor immunity. This remains a prospect to be tested in breast-cancer-specific translational studies rather than an established result.

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Authors' Contribution
MN Abdulraheem contributed to the literature search, data collection, interpretation of the literature, and drafting of the manuscript. ES Zulkafli contributed to the conception and design of the review, supervised the work, critically revised the manuscript, and provided final approval of the version to be published. R Mohamed contributed to the conception and design of the review. All authors have read and approved the final manuscript and agreed to be accountable for all aspects of the work, ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Conflict of Interest
None declared.

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Table 1. Positioning of the present review against recent related reviews of radiotherapy, the TIME, and neoantigen prediction, showing the primary focus of each review and the specific contribution of the present work

Recent review (year)	Primary focus	Contribution of the present review
Charpentier et al. (2022)	Radiation-induced remodeling of the TIME	Centers on stromal and immune-cell reprogramming; here the organizing axis is the neoantigen itself, including its generation, presentation, and quality.
Liu et al. (2023)	Radiotherapy remodels the microenvironment to enhance immunotherapy sensitivity	Emphasizes microenvironmental sensitization; the present review traces the molecular route from DNA repair to a presented neoantigen and its quality determinants.
Yi et al. (2026)	Central role of radiotherapy in immune-microenvironment remodeling	Provides a broad mechanistic overview; the present scope is narrower and more detailed on antigenic remodeling, with an explicit breast-cancer focus.
Zhang et al. (2025)	Advances and challenges in neoantigen prediction	Addresses computational prediction across tumor types; here prediction limits are related specifically to radiation-generated and radiation-exposed peptides.
Recent breast-cancer neoantigen and vaccine reviews (2024-2025)	Neoantigen discovery and vaccines in breast cancer	Do not treat radiotherapy as a neoantigen-remodeling modality; the present review adopts radiotherapy as the organizing mechanism.

DNA: Deoxyribonucleic acid; TIME: Tumor immune microenvironment

Table 2. Synthesis of key studies on radiation-associated immune and neoantigen remodeling published between 2015 and 2026, showing for each study the study type, the model or system examined, the principal finding, its relevance to radiotherapy-associated neoantigen remodeling, and the grade of evidence assigned in this review

Study (year)	Study type	Model/system	Key finding	Relevance to radiotherapy-neoantigen remodeling
Shapiro and Bassani-Sternberg (2026)	Review	Immunopeptidomics	Mass spectrometry-based immunopeptidomics advances neoantigen discovery; current presentation predictors show positive predictive values of about 0.60 to 0.70 (over-prediction)	Supports functional and mass-spectrometry validation of radiation-exposed neoantigens. Evidence grade: inferred from general tumor immunology.
Huber and Bassani-Sternberg (2026)	Review	Antigen processing and presentation	Defects such as HLA loss of heterozygosity and beta-2-microglobulin loss drive immune evasion	Presentation failure blunts the efficacy of radiation-generated neoantigens. Evidence grade: inferred from general tumor immunology.
Yi et al. (2026)	Review	Radiotherapy and TIME	Radiotherapy is central to immune-microenvironment remodeling; low-dose radiotherapy (0.50 to 2.00 Gy) can reprogram the immune milieu	Provides dose-dependent immune-remodeling context. Evidence grade: inferred from general radiation immunology.
Zhang et al. (2025)	Review	Neoantigen prediction	Binding-affinity-centered pipelines neglect TCR recognition and processing	Defines technical limits for prioritizing radiation-associated neoantigens. Evidence grade: inferred from general tumor immunology.
Daino et al. (2025)	Review	Radiation mutational signatures (animal and human)	Radiation leaves characteristic mutational signatures that generate neoantigens	Genomic basis of radiation-induced neoantigen generation. Evidence grade: inferred from general radiation immunology.
Liu et al. (2025)	Review	Tumor irradiation	DNA-damage induction and misrepair shape the immunogenic response	Links DNA repair to neoantigen generation. Evidence grade: inferred from general radiation immunology.
Zeng et al. (2025)	Experimental	Nasopharyngeal carcinoma	High-dose radiotherapy promotes dendritic cell maturation through ICD	Connects radiotherapy to antigen-presenting cell priming. Evidence grade: demonstrated in another solid tumor.
Yazdimamaghani et al. (2025)	Experimental	Nanoformulated TLR7/8 agonist and PI3K-delta inhibitor with radiotherapy	Microenvironment immunomodulation enhances radiotherapy benefit; Treg depletion with checkpoint blockade improves outcomes	Combination strategy to overcome immunosuppression. Evidence grade: demonstrated in another solid tumor.
Han et al. (2025)	Single-cell RNA sequencing	Metastatic breast cancer	Maps tumor evolution and therapeutic resistance	Clonal evolution reshapes the antigen landscape. Evidence grade: directly demonstrated in breast cancer.
Jin et al. (2025)	Genomic and immunologic	Acute myeloid leukemia	Indel-derived neoepitopes yield more neoantigen-specific CD8-positive T cells than SNVs	Mutation class governs immunogenicity. Evidence grade: demonstrated in another solid tumor.

Study (year)	Study type	Model/system	Key finding	Relevance to radiotherapy-neoantigen remodeling
Liu et al. (2023)	Experimental	SBRT models	SBRT (6.00 to 10.00 Gy per fraction over 3 to 5 fractions) increased Foxp3-positive Treg density (more than 2.00-fold) and CD204-positive macrophages (1.50-fold)	Radiotherapy can deepen immunosuppression, supporting combinations. Evidence grade: demonstrated in another solid tumor.
Wolf and Samuels (2023)	Perspective	Conceptual	Neoantigen quality outweighs quantity	Core organizing principle of this review. Evidence grade: hypothesis-generating and not yet validated.
Dijkstra et al. (2023)	Review	Clonal heterogeneity	Heterogeneity impairs immunosurveillance through antigen under-dosing and immunodominance	Limits the therapeutic value of subclonal radiation-associated neoantigens. Evidence grade: inferred from general tumor immunology.
Li et al. (2023)	Method (Neo-intline)	Bioinformatics	Integrated in-silico epitope presentation improves neoantigen design	Improves prioritization beyond binding affinity. Evidence grade: hypothesis-generating and not yet validated.
Tailor et al. (2022)	Multi-omics	Irradiated colorectal cells	Ionizing radiation expands the MHC class I immunopeptidome, upregulates TAP1/ERAP1/beta-2-microglobulin, and reveals a radiation-specific neoantigen (Mtch1)	Direct evidence that radiotherapy reshapes the immunopeptidome. Evidence grade: demonstrated in another solid tumor.
Charpentier et al. (2022)	Review	Radiotherapy and TIME	Radiotherapy remodels the TIME	Complementary microenvironmental perspective. Evidence grade: inferred from general radiation immunology.
Luksza et al. (2022)	Longitudinal (10-year)	Pancreatic cancer survivors	High-quality neoantigens decline over time through immunoediting	Immune selection operates on neoantigen quality. Evidence grade: demonstrated in another solid tumor.
Lhuillier et al. (2021)	Multi-omics preclinical	Poorly immunogenic breast cancer	Radiotherapy exposes mutant CD8-positive and CD4-positive neoepitopes and improves control without new mutations	Keystone breast-cancer evidence for radiation-exposed neoantigens. Evidence grade: directly demonstrated in breast cancer (preclinical model).
Lussier et al. (2021)	Preclinical	Low mutational-burden tumors	Radiation-induced neoantigens broaden the immunotherapeutic window	Radiotherapy expands the pool of actionable antigens. Evidence grade: demonstrated in another solid tumor.
Kocakavuk et al. (2021)	Genomic	Pan-cancer	Radiotherapy is associated with a deletion mutational signature affecting outcomes	Defines the mutagenic footprint of radiotherapy. Evidence grade: inferred from general radiation immunology.
Zhang et al. (2021)	Preclinical	Vaccine with radiotherapy	Vaccination followed by radiotherapy increases effector T-cell influx and antitumor effect	Sequencing and combination determine benefit. Evidence grade: demonstrated in another solid tumor.

Study (year)	Study type	Model/system	Key finding	Relevance to radiotherapy-neoantigen remodeling
Roudko / Ballhausen et al. (2020)	Genomic and immunologic	Microsatellite-unstable cancers	Shared frameshift poly-epitope neoantigens under ongoing immunoediting	Establishes frameshift immunogenicity. Evidence grade: demonstrated in another solid tumor.
Storozynsky and Hitt (2020)	Review	cGAS-STING	Radiation-induced DNA damage leads through type I interferon to MHC class I and processing upregulation	Mechanistic link to enhanced presentation. Evidence grade: inferred from general radiation immunology.
Vanpouille-Box et al. (2017)	Preclinical	Multiple tumor models	High single doses induce TREX1 and impair interferon; fractionation preserves cGAS-STING	Grounds dose and fractionation optimization. Evidence grade: demonstrated in another solid tumor.
Balachandran et al. (2017)	Computational and clinical	Pancreatic cancer	Neoantigen quality (pathogen similarity) predicts long-term survival	Provides a quality framework. Evidence grade: demonstrated in another solid tumor.
Turajlic et al. (2017)	Pan-cancer genomic	Indel neoantigens	TNBC and BRCA1 tumors carry high indel and neoantigen burden linked to checkpoint-blockade response	Breast-cancer neoantigen biology. Evidence grade: directly demonstrated in breast cancer.
Yates et al. (2017)	Genomic	Breast cancer relapse and metastasis	Relapse- and metastasis-seeding clones acquire new driver mutations	Antigen landscape evolves under therapy. Evidence grade: directly demonstrated in breast cancer.
McGranahan et al. (2016)	Genomic and clinical	Non-small cell lung cancer and melanoma	Clonal (not total) neoantigen burden predicts survival and checkpoint-blockade response	Clonality determines therapeutic relevance. Evidence grade: demonstrated in another solid tumor.
Demaria et al. (2016)	Review	Radiotherapy immunology	Reframes radiotherapy as a partner for immunotherapy	Conceptual foundation of the field. Evidence grade: inferred from general radiation immunology.
McGranahan and Swanton (2015)	Review	Intratumor heterogeneity	Darwinian selection shapes therapeutic outcomes	Frames the heterogeneity challenge. Evidence grade: inferred from general tumor immunology.

TIME: Tumor immune microenvironment; ICD: Immunogenic cell death; TNBC: Triple-negative breast cancer; SNV: Single-nucleotide variant; Indel: Insertion or deletion; SBRT: Stereotactic body radiotherapy; HLA: Human leukocyte antigen; Treg: Regulatory T cell; DNA: Deoxyribonucleic acid; RNA: Ribonucleic acid; MHC: Major histocompatibility complex; TAP1: Transporter associated with antigen processing 1; ERAP1: Endoplasmic reticulum aminopeptidase 1; B2M: Beta-2-microglobulin; cGAS-STING: Cyclic guanosine monophosphate-adenosine monophosphate synthase-stimulator of interferon genes; TREX1: Three-prime repair exonuclease 1; TCR: T-cell receptor; CD4: Cluster of differentiation 4; CD8: Cluster of differentiation 8; CD204: Cluster of differentiation 204; Foxp3: Forkhead box P3; BRCA1: Breast cancer susceptibility gene 1; TLR7/8: Toll-like receptor 7/8; PI3K-delta: Phosphoinositide 3-kinase delta; Mtch1: Mitochondrial carrier homolog 1; Gy: Gray

Table 3. Mechanistic routes of radiation-associated neoantigen remodeling, showing for each route the underlying molecular events and the representative published evidence supporting it

Route	Molecular events	Representative evidence
Genomic generation	Error-prone NHEJ repair of double-strand breaks produces SNVs, indels (notably immunogenic frameshifts), and structural variants, together with a radiation-associated deletion signature	Kocakavuk et al. (2021)[43]; Daino et al. (2025)[45]; Roudko/Ballhausen et al. (2020)[46, 47]; Turajlic et al. (2017)[48]
Transcriptional exposure	Upregulated expression of pre-existing, poorly expressed mutated genes reveals otherwise silent immunogenic mutations without new mutagenesis	Lhuillier et al. (2021, 2023)[52, 53]; Lussier et al. (2021)[54]
Enhanced presentation	cGAS-STING and type I interferon upregulate MHC class I, TAP1, ERAP1, and beta-2-microglobulin, expanding the immunopeptidome, including radiation-specific peptides such as Mtch1	Reits et al. (2006)[37]; Storozynsky and Hitt (2020)[25]; Tailor et al. (2022)[42]
Microenvironmental amplification	Dendritic cell maturation and chemokine-driven CD8-positive T-cell recruitment (CXCL9/10/16) increase the functional visibility of presented neoantigens	Matsumura et al. (2008)[39]; Zeng et al. (2025)[36]

NHEJ: Non-homologous end joining; SNV: Single-nucleotide variant; Indel: Insertion or deletion; MHC: Major histocompatibility complex; TAP1: Transporter associated with antigen processing 1; ERAP1: Endoplasmic reticulum aminopeptidase 1; cGAS-STING: Cyclic guanosine monophosphate-adenosine monophosphate synthase-stimulator of interferon genes; CXCL: C-X-C motif chemokine ligand; CD8: Cluster of differentiation 8; Mtch1: Mitochondrial carrier homolog 1

Table 4. Radiation-responsive antigen sources beyond canonical MHC class I neoantigens, showing for each source the proposed link to radiotherapy, its relevance to breast cancer, and the representative published evidence

Antigen source	Radiation link and breast-cancer relevance	Representative evidence
MHC class II / CD4 neoantigens	Radiation enhances cross-presentation and tumor MHC class II; CD4 help and epitope spreading sustain CD8 responses and may broaden targets in low-burden disease	Alspach et al. (2019)[55]
Proteasome-spliced peptides	Spliced ligands constitute a large fraction of the HLA class I immunopeptidome; radiation-altered processing may shift them, expanding targets beyond exome prediction	Liepe et al. (2016)[56]
Post-translationally modified peptides	Phosphorylated and oxidized species generated under stress add neoepitopes not captured by exome-based prediction, relevant where mutations are few	Shapiro and Bassani-Sternberg (2026)[57]
Endogenous retroviruses / viral mimicry	Radiation-induced DNA-damage and chromatin change can derepress ERVs, yielding double-stranded RNA that drives interferon signaling and potential antigens in cold tumors	Chiappinelli et al. (2015)[58]
Alternative splicing / A-to-I RNA editing (ADAR1)	Radiation modulates splicing and ADAR1 activity, altering neoepitopes and checkpoint-blockade sensitivity; ADAR1 is a candidate combination target	Ishizuka et al. (2019)[59]

MHC: Major histocompatibility complex; HLA: Human leukocyte antigen; CD4: Cluster of differentiation 4; CD8: Cluster of differentiation 8; DNA: Deoxyribonucleic acid; RNA: Ribonucleic acid; ERV: Endogenous retrovirus; A-to-I: Adenosine-to-inosine; ADAR1: Adenosine deaminase acting on RNA 1

Table 5. Determinants of neoantigen quality relevant to radiotherapy, grouped by the presentation, recognition, and tumor-context layers, with a description of each determinant and the representative published evidence

Determinant	Description	Representative evidence
Presentation layer: HLA binding affinity and stability	Stable, high-affinity peptide-HLA complexes are required for durable surface presentation and T-cell engagement	Zhang et al. (2025)[17]
Recognition layer: foreignness and dissimilarity to self	Similarity to pathogen epitopes and divergence from wild-type peptides predict immunogenicity and help to overcome central tolerance	Balachandran et al. (2017)[61]; Luksza et al. (2017, 2022)[62, 63]
Tumor-context layer: clonality (truncal expression)	Clonal neoantigens, rather than total mutational burden, predict survival and response to checkpoint blockade	McGranahan et al. (2016)[64]
Recognition layer: mutation class	Frameshift indels generate poly-epitopes and elicit stronger CD8-positive responses than SNVs	Roudko et al. (2020)[46]; Turajlic et al. (2017)[48]; Jin et al. (2025)[50]
Presentation and recognition layers: processing and TCR engagement	Beyond binding, efficient antigen processing and productive TCR engagement are required for a functional response	Huber and Bassani-Sternberg (2026)[65]; Shapiro and Bassani-Sternberg (2026)[57]

HLA: Human leukocyte antigen; TCR: T-cell receptor; SNV: Single-nucleotide variant; Indel: Insertion or deletion; CD8: Cluster of differentiation 8

Table 6. Barriers to the clinical translation of radiation-associated neoantigen remodeling, showing for each barrier its consequence for antitumor immunity and the candidate mitigation strategy proposed in this review

Barrier	Consequence	Candidate mitigation strategy
Tumor heterogeneity and clonal evolution	Immune selection favors less-immunogenic subclones, and subclonal antigens are under-dosed	Prioritize clonal and truncal neoantigens; use multi-region sampling and clonality-aware selection[66, 67]
Antigen-presentation defects (beta-2-microglobulin loss, HLA loss of heterozygosity)	Immunogenic neoantigens fail to be displayed	Screen antigen-processing integrity; pursue strategies to restore MHC class I expression[65, 68]
Immunosuppressive microenvironment (Treg, MDSC, TAM, PD-L1)	Effector T-cell function is blunted despite antigen availability	Combine with checkpoint blockade and Treg or myeloid targeting; optimize radiotherapy sequencing[15, 70, 72]
Dose- and fractionation-dependent STING attenuation	High single doses induce TREX1, degrade cytosolic DNA, and reduce type I interferon	Use fractionated or optimized dosing to sustain cGAS-STING signaling[14, 33]
Prediction-pipeline limitations	Binding affinity is an insufficient surrogate for immunogenicity, and over-prediction is common	Integrate immunopeptidomics and functional T-cell assays with genomic prediction[17, 57, 73]

DNA: Deoxyribonucleic acid; MHC: Major histocompatibility complex; HLA: Human leukocyte antigen; B2M: Beta-2-microglobulin; Treg: Regulatory T cell; MDSC: Myeloid-derived suppressor cell; TAM: Tumor-associated macrophage; PD-L1: Programmed death-ligand 1; cGAS-STING: Cyclic guanosine monophosphate-adenosine monophosphate synthase-stimulator of interferon genes; TREX1: Three-prime repair exonuclease 1

Table 7. A trial-ready pipeline for radiotherapy-neoantigen strategies in breast cancer, showing for each stage the required action, the assays or tools involved, and the decision point that governs progression to the next stage

Stage	Action	Assays / tools	Decision point
Patient selection	Stratify by likelihood of benefit	Subtype (TNBC, HER2-positive, HR-positive); BRCA/HRD and APOBEC/indel burden; PD-L1 combined positive score; baseline TILs and TCR clonality; HLA LOH and B2M status	Enrich for high-neoantigen, presentation-competent, immune-primed tumors
Baseline sampling	Characterize tumor and immune state	Whole-exome and RNA sequencing, HLA typing, immunopeptidomics, TCR-beta sequencing, spatial profiling	Establish the pre-radiotherapy antigen and immune baseline
Radiotherapy	Deliver an immunogenic schedule	Fractionated (e.g., 8.00 Gy × 3) or hypofractionated (6.00 to 10.00 Gy × 3 to 5) regimens; low-dose fields (0.50 to 2.00 Gy) to recondition suppressive niches	Sustain cGAS-STING; avoid TREX1-inducing high single doses
On-treatment sampling	Capture radiation-induced change	Early post-radiotherapy biopsy; repeat immunopeptidomics, RNA sequencing, and TCR sequencing	Identify radiation-exposed and radiation-specific peptides
Neoantigen prioritization	Rank high-quality clonal targets	Binding, processing, expression, clonality, and TCR-likeness (pVACtools/pVACview; joint peptide-HLA-TCR models)	Select truncal, presented, immunogenic candidates
Functional validation	Confirm immunogenicity	Minigene and antigen-presenting-cell assays; T-cell reactivity screening	Advance validated targets only
Combination therapy	Overcome suppression and focus the response	Checkpoint blockade (PD-1/PD-L1, CTLA-4); STING agonists; Treg or myeloid targeting (e.g., PI3K-delta inhibition); personalized vaccine or adoptive T-cell products	Sequence relative to radiation-induced windows of antigen presentation

TNBC: Triple-negative breast cancer; HER2: Human epidermal growth factor receptor 2; HR: Hormone receptor; BRCA: Breast cancer susceptibility gene; HRD: Homologous recombination deficiency; APOBEC: Apolipoprotein B messenger RNA editing enzyme, catalytic polypeptide-like; Indel: Insertion or deletion; PD-L1: Programmed death-ligand 1; TIL: Tumor-infiltrating lymphocyte; TCR: T-cell receptor; HLA: Human leukocyte antigen; LOH: Loss of heterozygosity; B2M: Beta-2-microglobulin; RNA: Ribonucleic acid; Gy: Gray; cGAS-STING: Cyclic guanosine monophosphate-adenosine monophosphate synthase-stimulator of interferon genes; TREX1: Three-prime repair exonuclease 1; PD-1: Programmed cell death protein 1; CTLA-4: Cytotoxic T-lymphocyte-associated protein 4; PI3K-delta: Phosphoinositide 3-kinase delta

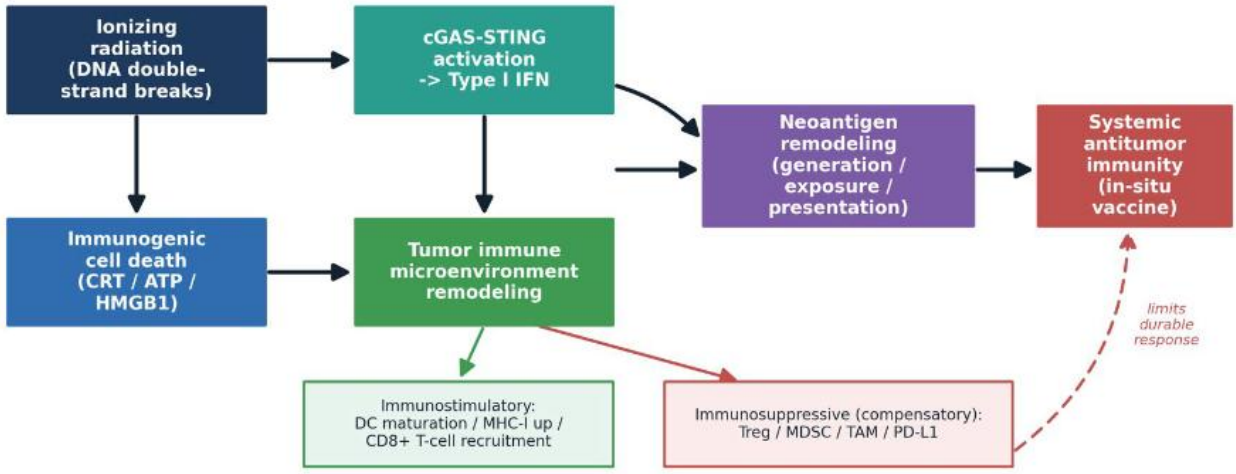

Figure 1. This figure shows the radiotherapy-associated immune remodeling proceeds as a coordinated cascade. Ionizing radiation causes DNA damage that triggers immunogenic cell death, with surface exposure of CRT and release of ATP and HMGB1. Cytosolic DNA released from ruptured micronuclei activates cGAS-STING signaling and type I IFN production, which promotes dendritic cell maturation and upregulation of MHC class I and the antigen-processing machinery. The figure shows these events as parallel inputs into antigen presentation and CD8-positive T-cell priming; it does not imply that every step is uniformly immunostimulatory, and the counter-regulatory outputs of each pathway are described in the text.

ATP: Adenosine triphosphate; CD8: Cluster of differentiation 8; cGAS-STING: Cyclic guanosine monophosphate-adenosine monophosphate synthase-stimulator of interferon genes; CRT: Calreticulin; DC: Dendritic cell; DNA: Deoxyribonucleic acid; HMGB1: High-mobility group box 1; IFN: Interferon; MDSC: Myeloid-derived suppressor cell; MHC-I: Major histocompatibility complex class I; PD-L1: Programmed death-ligand 1; TAM: Tumor-associated macrophage; Treg: Regulatory T cell

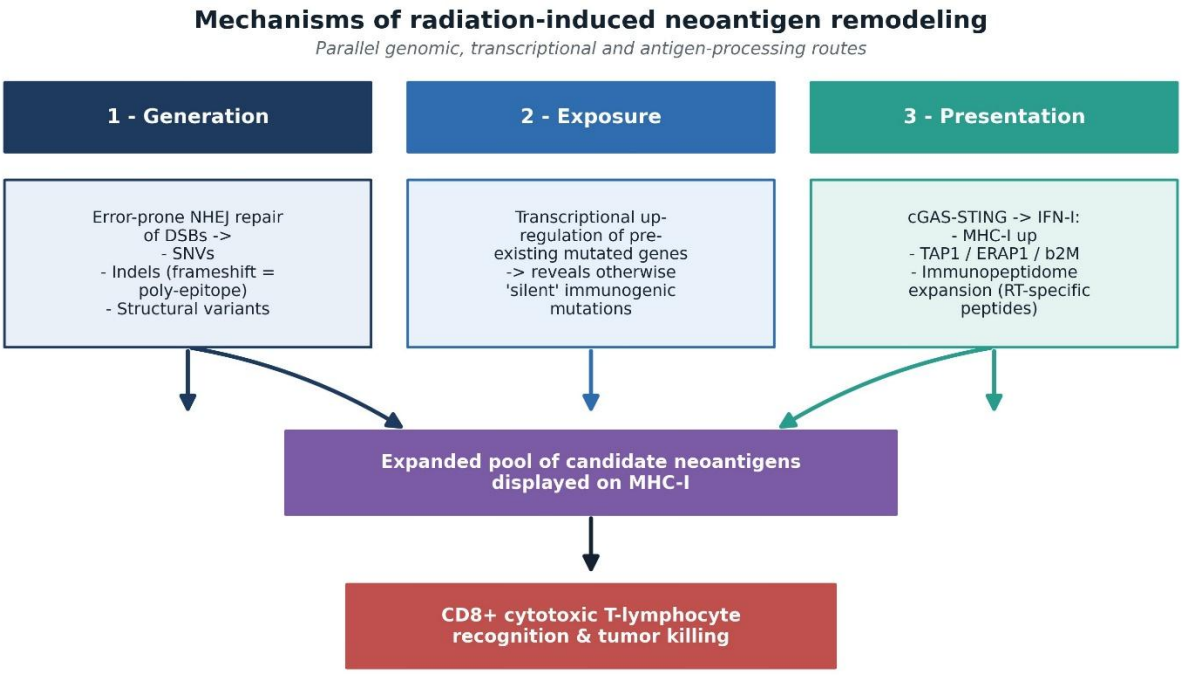

Figure 2. This figure shows the mechanisms of radiation-associated neoantigen remodeling. The figure shows the three processes by which radiation may expand the antigenic landscape. Radiation-generated neoantigens (route 1) arise from indels, larger deletions, and structural variants produced by error-prone NHEJ repair, together with SNVs arising from several additional repair pathways. Radiation-exposed neoantigens (route 2) arise from transcriptional upregulation of pre-existing but poorly expressed mutated genes, without new mutagenesis. Radiation-modulated presentation (route 3) results from cGAS-STING and type I interferon-driven upregulation of MHC class I and the processing components TAP1, ERAP1, and beta-2-microglobulin. Together these routes produce an expanded and partly radiation-specific immunopeptidome that can be recognized by CD8-positive T cells. The three routes are supported by evidence of unequal strength, with route 3 the best substantiated and route 1 the least.

b2M: Beta-2-microglobulin; CD8: Cluster of differentiation 8; cGAS-STING: Cyclic guanosine monophosphate-adenosine monophosphate synthase-stimulator of interferon genes; DSB: Double-strand break; ERAP1: Endoplasmic reticulum aminopeptidase 1; IFN-I: Type I interferon; Indel: Insertion or deletion; MHC-I: Major histocompatibility complex class I; NHEJ: Non-homologous end joining; RT: Radiotherapy; SNV: Single-nucleotide variant; TAP1: Transporter associated with antigen processing 1

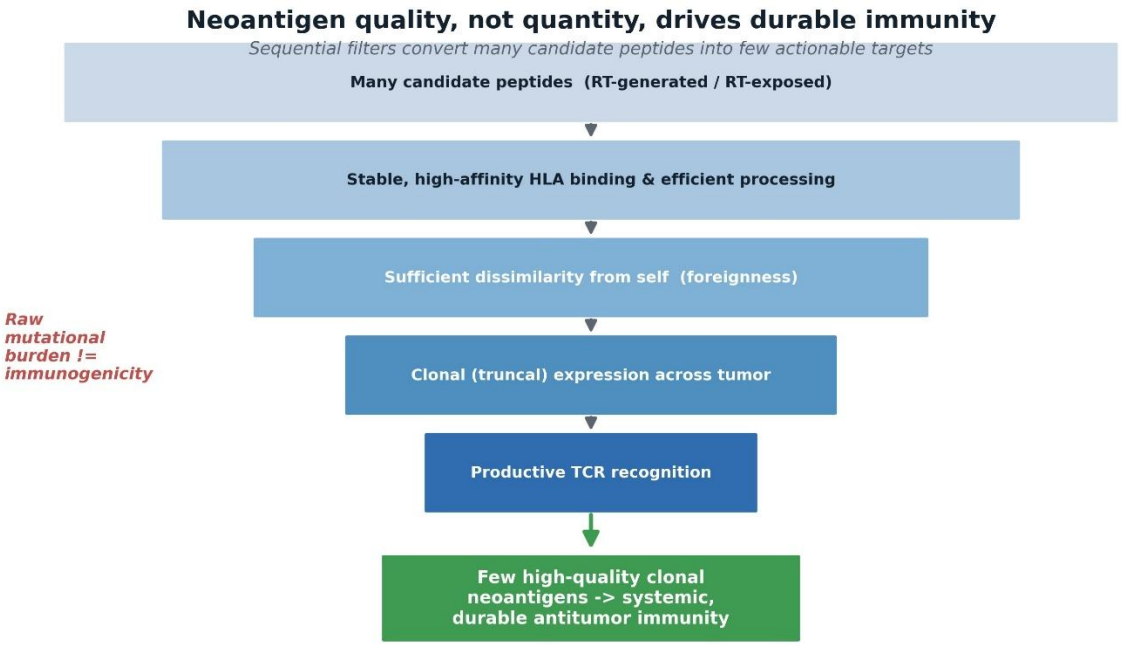

Figure 3. This figure shows the neoantigen quality, rather than quantity, drives durable immunity. Of the many candidate peptides that radiation generates or exposes, only those that combine stable high-affinity HLA binding, efficient processing, sufficient dissimilarity from self, clonal (truncal) expression, and productive T-cell receptor recognition become therapeutically actionable. Raw mutational burden is therefore a weak surrogate for immunogenicity.
HLA: Human leukocyte antigen; RT: Radiotherapy; TCR: T-cell receptor

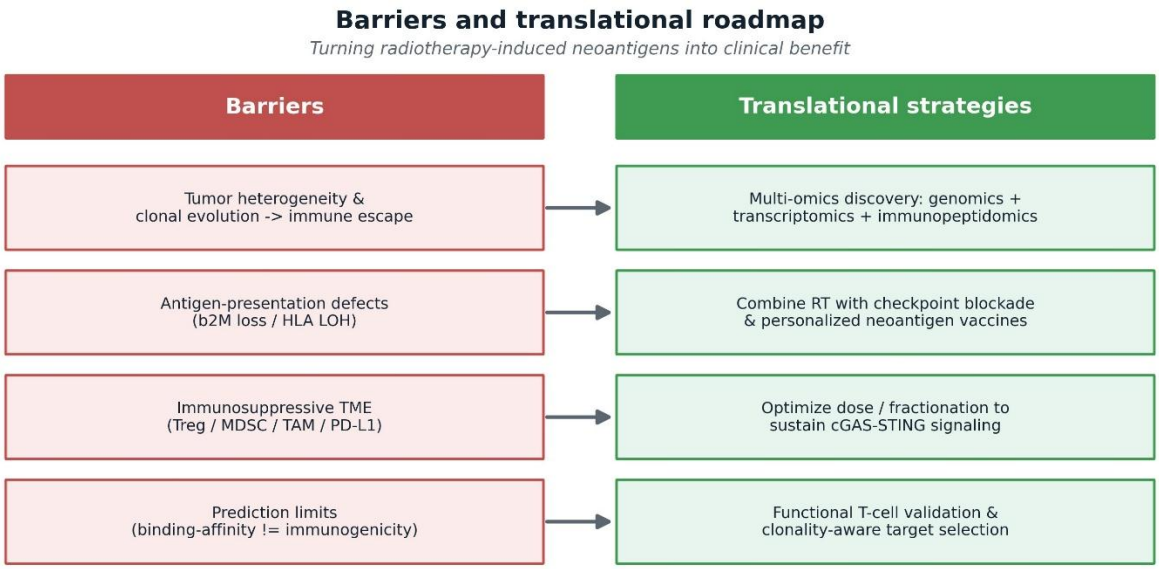

Figure 4. This figure shows the barriers and a translational roadmap for radiation-associated neoantigen remodeling. Each principal barrier (left) maps to a candidate mitigation strategy (right): multi-omic neoantigen discovery with functional validation, combination with checkpoint blockade and personalized vaccines, dose and fractionation optimization to sustain cGAS-STING signaling, and clonality-aware target selection.

b2M: Beta-2-microglobulin; cGAS-STING: Cyclic guanosine monophosphate-adenosine monophosphate synthase-stimulator of interferon genes; HLA: Human leukocyte antigen; LOH: Loss of heterozygosity; MDSC: Myeloid-derived suppressor cell; PD-L1: Programmed death-ligand 1; RT: Radiotherapy; TAM: Tumor-associated macrophage; TME: Tumor microenvironment; Treg: Regulatory T cell