Conformal Radiation with Immunotherapy: An Introduction to future of Radiation

Basis And Key Evidences

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INDEX

1. Relevant Basics of Cancer Immune System
   Mechanisms by which Immune Systems recognize tumors
   Effector mechanism against tumors
   Understand tumor escape mechanisms
   Immunotherapy -- manipulate immune system to kill tumors

2. Rationale of using Radiotherapy (RT) and Immunotherapy (IT)
3. Mechanism of Interaction between RT and IT
4. Challenges and Future of the Interaction
5. Conclusions
Cancer and the Immune System—To REMEMBER

• All Cells in The Body Can Communicate with the Immune System

• Immune Cells patrol the body and are continuously exposed to the environmental insults

• Something goes wrong—Immune system can Detect it

• Cancer Cells acquire aberrant features which can be detected by many immune cells that then react.

Janeway's immunobiology
Evidences For Tumour Immunity

Spontaneous regression: melanoma, lymphoma

Regression of metastases after removal of primary tumor: pulmonary metastases from renal carcinoma

Infiltration of tumors by lymphocytes and macrophages: melanoma and breast cancer

Lymphocyte proliferation in draining lymph nodes

Higher incidence of cancer after immunosuppression, immunodeficiency (AIDS)

Higher incidence in aged and neonates (less immunity)
Background

• **Cancer immune surveillance:**
  Immune system can recognize and destroy nascent transformed cells

• **Cancer Immunoediting:**
  Cancer cells acquire the ability to escape immune control before they develop into clinical cancer
BACKGROUND

• Similar immune escape ALSO contributes to resistance to immunotherapy

• HENCE---A Tumor-specific immune response , both local and systemic in patients with cancer, may overcome this immunosuppressive scenario

• Radiotherapy--A new role emerging in overcoming immunosuppression in the tumor microenvironment.

Relationship between Tumor and The Host Immune system --- Evolution of Cancer

[Cancer Immunoediting]
- Transformed
- Normal
- Elimination (Cancer Immunosurveillance)
- Equilibrium
- Escape

[Cancer Immunoediting]
- Innate & Adaptive Immunity
- Tumor antigens
- MICA/B, ULBP (Human), Rae-1, H60 (Mouse)
- Danger signals (urate acid, ECM products)
- Carcinogens, Radiation, Chronic inflammation, Inherited, Viruses

[Immune Evasion]
- CD8^+ T cells
- CD4^+CD25^+FoxP3^+ regulatory T cells (Treg)
- Genetic instability
- IFNγ response
- NK cells

[CANCER---Ability to escape the immune response]
The Immune System
Our Ultimate Line of Defence

Immune System

External
(Skin, mucous membrane, nasal hair)
Barriers

Internal
(Immunological cells)
Barriers

1st Line Defense

Phagocytes

Natural Killer Cells

2nd Line Defense

T Cells

B Cells

Innate Immunity

Adaptive Immunity

https://www.stemcellimmuneregenerative.com/what-is-the-immune-system
**T and NK Cells**

- Great cytotoxic capacity
- Probe the target cells to detect signs of abnormality
- Healthy Cells are Spared by T and NK cells
- T and NK cells Specifically kill dangerous Cells— Tumour Cells

- But Sometimes they Don’t Kill Tumour Cells--- ?????
T and NK Cells

• Cytotoxic T cells (CTLs) CD8+ Cells---Attach to Class I MHC- Peptide and destroy cancer cells by perforating the membrane with enzymes or through apoptosis.

• Helper T cells----CD4+ cells—React to Class II MHC, secrete Cytokines
T and NK Cells

- NK Cells—Lymphocytes, that destroy tumour cells without prior sensitization

- Tumours that do not express MHC Class I Ag, cannot be recognized by T cells

- Such Tumours can Trigger NK cells since NK cells are inhibited by MHC Class I molecules
Macrophages, T and NK Cells

• Macrophages are activated by factors like Lymphokines (Produced by T Cells) and Interferons

• Activated Macrophages kill tumour by production of reactive O2 metabolites or by secretion of TNF (Tumour necrosis Factor)

• TNF has potent anti-tumour activity

• T cells, NK cells and Macrophages collaborate in anti-tumour activity because interferons-y (a cytokine secreted by T cells and NK cells) is a potent activator of macrophages

• Dendritic Cells are Ag presenting cells present in skin, LNs etc

Nature reviews cancer 2012 Apr, 12(4): 265
SUMMARY OF TUMOUR AND IMMUNE RESPONSE

Tumor antigen or tumor cell

APC

T helper cell 1

Interferon

MHC I

Perforins, apoptotic signals

T cytotoxic cell

T cytotoxic memory cells

T cytotoxic effector cells

IL-1

IL-2

Cancer Cell

Endogenous antigen

Generally ineffective tumor surveillance, but some ADCC

B Cell

Eosinophil
Traditional Cancer Treatments Activate Immune System
Cancer has ways to hide from Immune System

<table>
<thead>
<tr>
<th>Low immunogenicity</th>
<th>Tumor treated as self antigen</th>
<th>Antigenic modulation</th>
<th>Tumor-induced immune suppression</th>
<th>Tumor-induced privileged site</th>
</tr>
</thead>
<tbody>
<tr>
<td>No peptide:MHC ligand</td>
<td>Tumor antigens taken up and presented by APCs in absence of co-stimulation</td>
<td>T cells may eliminate tumors expressing immunogenic antigens, but not tumors that have lost such antigens</td>
<td>Factors (e.g., TGF-β, IL-10, IDO) secreted by tumor cells inhibit T cells directly. Expression of PD-L1 by tumors</td>
<td>Factors secreted by tumor cells create a physical barrier to the immune system</td>
</tr>
</tbody>
</table>

- No adhesion molecules
- No co-stimulatory molecules
- No peptide:MHC ligand

Janet’s immunobiology
• Activation of Immune cells including T cells is regulated by **POSITIVE** and **NEGATIVE** Signals

• **BALANCE** between them is important for auto immunity

• **Tumors** may exploit the **negative** signals provided by the **INHIBITORY CHECK POINT RECEPTORS** to escape the Immune Response

**Inhibitory Receptors Regulate Immune Cell Activation**
Inhibitory receptors regulate immune cell activation

- T cells and NK cells Kill Tumour Cells
- These are Inhibited by Check point Receptors
- HENCE Check Point Blockade removes Breaks from The immune system
  
  and reactivate Immune cells against Cancer

The 2018 Nobel Prize for Physiology was awarded to James Allison and Tasuku Honjo for this discovery that there was a negative immune regulatory system that could itself be inhibited through PD-1 and PD-L1 modulation
Cancer Evades the Immune Response by

- Low immugenicity
- Lack of recognition
- Antigenic modulation
- Tumour induced suppression

Each of these evasions are targeted by:

- Adoptive cell transfer
- Antibodies
- Vaccination
- Oncolytic viruses
Adoptive cellular therapy is based on the idea that T cells are capable of fighting tumors. T cells with different specificity are expanded to be specific for the tumor and then re-infused into the patient.
CAR T cells: engineering T cells to fight cancer

Generating super-soldiers
the production of CAR-T cells

T-cell

CAR-T cell

CARs MAKE ALL T CELLS REACTIVE AGAINST TUMOR CELLS

facebook.com/pedromics

June et al.
Science 2018
Antibodies

Antibodies are Y Shaped molecules Produced by B Cells

AB Bind with high specificity to their Ag

AB Can be produced in the labs to bind to molecules present in the body

Eg– Ab Can activate immune cells against Cancer by **TAKing OFF THE IMMUNOLOGICAL BREAKS**
Rationale For Combination of RT and IT

Radiation affects both tumor cells and surrounding stromal cells.

• **LOCAL EFFECT**---Radiation-induced cancer cell damage makes tumor-specific antigens visible to immune surveillance and promotes the priming and activation of cytotoxic T cells.

• **SYSTEMIC EFFECTS** --- Radiation-induced modulation of the TME also facilitate the recruitment and infiltration of immune cells.

MECHANISTIC RATIONALE OF RADIATION

• DNA DAMAGE MEDIATED
  Radiation Increases Antigen Visibility

  Radiation Activates the cGAS-STING Pathway

• Radiation Modifies Tumor Stromal Microenvironments (TME)
Radiation Increases Antigen Visibility

- Activates the downstream immune responses and priming of T cells
- Upregulate expression of MHC-I on the tumor surface
- Radiation-induced DNA damage induces a systemic increase in antigen recognition
- Radiation induce the T cell-mediated inhibition of untreated distant tumors

APC engulf the tumor cells and present their antigens to naïve T cells through phagocytosis & enhance their clearing

Enable better presentation of tumor-specific peptides, & enhance visibility of the tumor to cytotoxic T cells

Generate neoantigen and trigger the immune surveillance. (known as the ABSCOPAL EFFECT)

(Reits et al., 2006)
(Demaria et al., 2004).
Radiation Activates the cGAS-STING

- The cyclic GMP–AMP (cGAMP) synthase (cGAS)—stimulator of interferon genes (STING) pathway plays a crucial role in the DNA damage-induced immune response

- RT activate immune responses through the Stimulator of Interferon Genes (STING) -mediated DNA- sensing pathway.

- STING is essential to protect hosts from DNA pathogens

- IFN-I generated by cGAS/STING pathway induces dendritic cell migration to the tumor and cross-priming of T cells,--- required for the antitumor effect of radiotherapy

(Sharma et al., 2011; Watson et al., 2012). Deng et al., 2014b).
Radiation Modifies Tumor Stromal Microenvironments (TME)

**TME**-- Stromal cells and their secreted signals (cytokines, chemokines, and growth factors)

RT Has both positive and negative effects on antitumor immunity and the TME

- Induce immunogenic tumor cell death and release of tumor-specific antigens
- TGF-β signaling is upregulated momentarily after radiation and triggers an immune-suppressive microenvironment
- Surviving TUMOUR CELLS undergo phenotypic changes in the expression of immune susceptibility markers
- Immuno-therapies can augment the efficacy of radiation therapy by targeting their detrimental immunologic effects.

(Klopp et al., 2007)
(Vanpouille-Box et al., 2015),

Immune responses induced by radiotherapy include those caused by DNA damage and those that occur in the tumor...
Interaction between Radiotherapy (RT) & Immunotherapy (IT).

**Red arrows** -- RT help IT to greater tumor control;
**Blue arrows** -- IT help RT.

TME, tumor microenvironment.

**SYNERGY IS BIDIRECTIONAL**

Clin Cancer Res; 26(12) June 15, 2020
Rationale For Combination of RT and IT

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Interaction between Radiotherapy (RT) & Immunotherapy (IT).

- Increased release of neoantigens -- presented to the immune system
- Radiation damaged DNA increases production of mutated non tumour specific antigens.
- Non-tumor specific antigens help in the upregulation of immune surveillance.
- Cytokines (type I interferons) release by damaged DNA which has escaped into the cytosol, upregulated through the (STING) pathway
- IFN-γ is also increased as a result of an increase in CD8+ T cells.
- MHC-1 molecules are more prevalent on the cell surface
Together with immunostimulatory responses, RT can also trigger immunosuppression.

RT upregulates the expression of the immune checkpoint PD-L1 limiting the activation of tumour T cells.

RT enhance release of immunosuppressive cytokines such as transforming growth factor beta (TGF-b) in the tumour environment.

TGF-b can repress the proliferation, activation and effector function of T cells and can also impact the maturation and function of tumour NK cells and macrophages (Dahmani and Delisle, 2018).

M. Mondini et al.
Combination of radiotherapy and immunotherapy
Interaction between Radiotherapy (RT) & Immunotherapy (IT). —

- Occurrence of tumour responses distant from the irradiated volume
- The abscopal effect is a rare event,
- Results from the induction of a systemic immune response triggered by the combined immunostimulatory effect of RT with immunotherapy.
Interaction between Radiotherapy (RT) & Immunotherapy (IT)--- SYNERGY IS BIDIRECTIONAL

• Immunotherapy – A Radiosensitizer?
• Increase T cell activation and may increase tumor response to radiation
• Immunotherapy can normalize the dysfunctional tumor vasculature, increasing the effectiveness of subsequent radiotherapy
• Radiation increases susceptibility of tumor cells to immune-mediated killing.
Interaction between Radiotherapy (RT) & Immunotherapy (IT)--- SYNERGY IS BIDIRECTIONAL

• Radiated tumor cells upregulate negative feedback elements (eg, checkpoint proteins), which can dampen the immune response.

• Immunotherapy blocks this negative feedback & reinvigorate an immune response primed by radiation

• Responses to immunotherapy often are delayed and may follow a transient increase in tumor burden

• Radiation can reduce the growth of lesions, allowing a greater window of opportunity for response to immunotherapy.

Nat Commun 2017;8:15618.
Influence of Dose, fractionation, and volume of radiation on immunologic effects

• FRACTIONATION induces expansion of unique immune populations, --
  Standard fractionation INCREASES  a Myeloid response

• Hypofractionation increase a Lymphoid response –
  (more favorable to adaptive antitumor immunity)

• Extreme HYPOFRACTIONATION (20–30 Gy in 1 fraction) ----
  Sabotage tumor immunogenicity by inducing DNA exonuclease Trex1 to block
cGAS-STING pathway activation

  (Vanpouille-Box et al., 2017; Ye and Formenti, 2017).

Influence of Dose, fractionation, and volume of radiation on immunologic effects

- Immunogenic tumor cell death increases as a function of increasing dose.
- At low doses (2-5 Gy), radiation release of cytokines that influence immune cell trafficking and activation.
- At low doses (1-3 Gy), radiation also ablates radiation-sensitive immune populations, -- suppressive and effector lymphocytes
- This is an opportunity for reconstitution with a more favorable infiltrate using immunotherapies
<table>
<thead>
<tr>
<th>Study</th>
<th>Type</th>
<th>RT</th>
<th>IT</th>
<th>Sequence</th>
<th>Results</th>
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</thead>
<tbody>
<tr>
<td>Allomari et al., 2016</td>
<td>Case report: brain metastases</td>
<td>SRS 22 Gy</td>
<td>ipilimumab, pembrolizumab</td>
<td>IT, RT, IT</td>
<td>Status improvement</td>
</tr>
<tr>
<td></td>
<td>Case report: brain metastases</td>
<td>SRS 20 Gy</td>
<td>nivolumab, ipilimumab</td>
<td>RT, IT</td>
<td>Remain asymptomatic neurologically 6 weeks after surgery</td>
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<td>Antoni et al., 2017</td>
<td>Stage III trial: lung cancer</td>
<td>Definitive RT</td>
<td>durvalumab</td>
<td>RT, IT</td>
<td>PFS improvement with durvalumab</td>
</tr>
<tr>
<td>Aryankalayit et al., 2014</td>
<td>Preclinical: human prostate cancer cells</td>
<td>1 Gy x 10 vs. 10 Gy</td>
<td>NA</td>
<td>NA</td>
<td>Multifraction radiation induced more DAMP release</td>
</tr>
<tr>
<td>Baird et al., 2016</td>
<td>Preclinical: murine pancreatic</td>
<td>10 Gy</td>
<td>Cyclic dinucleotides</td>
<td>Concurrent</td>
<td>STING activator and RT synergistically controlled local and distant tumors</td>
</tr>
<tr>
<td>Camphausen et al., 2003</td>
<td>Preclinical: murine lung (LLO)</td>
<td>10 Gy x 5 vs. 2 Gy x 12</td>
<td>NA</td>
<td>NA</td>
<td>Five fractions of 10 Gy induced more robust abscopal effects</td>
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<tr>
<td>Deng et al., 2014a</td>
<td>Preclinical: murine breast and colon</td>
<td>12 Gy</td>
<td>anti-PD-L1</td>
<td>RT, IT</td>
<td>Combination of radiation and immunotherapy could be more potent than either treatment alone</td>
</tr>
<tr>
<td>Dewan et al., 2009</td>
<td>Preclinical: murine breast</td>
<td>20 Gy x 1 vs. 8 Gy x 3 vs. 6 Gy x 5</td>
<td>anti-CTLA4</td>
<td>Concurrent</td>
<td>Abscopal effect was conducted only by fractionated radiation</td>
</tr>
<tr>
<td>Doveci et al., 2014</td>
<td>Preclinical: murine melanoma, colorectal and TNRB</td>
<td>10 Gy in 5 fractions</td>
<td>anti-PD-1 or anti-PD-L1</td>
<td>Concurrent, sequential</td>
<td>PD1/PD-L1 inhibition was effective only when given either concurrently or with or at the end of radiation</td>
</tr>
<tr>
<td>Haymaker et al., 2017</td>
<td>Case report: metastatic melanoma</td>
<td>WBRT 30 Gy in 10 fractions</td>
<td>ipilimumab, pembrolizumab</td>
<td>IT, RT, IT</td>
<td>Status improvement, long-term survival</td>
</tr>
<tr>
<td>Lee et al., 2009</td>
<td>Preclinical: murine melanoma (B16)</td>
<td>20 Gy vs. 20 Gy in 4 fractions</td>
<td>NA</td>
<td>NA</td>
<td>Immune response triggered by ablative radiation doses</td>
</tr>
<tr>
<td>Lugade et al., 2005</td>
<td>Preclinical: murine melanoma (B16)</td>
<td>3 fractions</td>
<td>15 Gy vs. 15 Gy in 3 fractions</td>
<td>IT, RT</td>
<td>15 Gy single-dose generated more tumor-infiltrating T cells</td>
</tr>
<tr>
<td>Nagasaka et al., 2016</td>
<td>Case report: head and neck</td>
<td>Palliative 30 Gy</td>
<td>anti-CTLA4, anti-PD-1</td>
<td>IT, RT</td>
<td>Significant radiographic response</td>
</tr>
<tr>
<td>Qian et al., 2016</td>
<td>Clinical: melanoma brain metastasis</td>
<td>SRS 12–24 Gy</td>
<td>pembrolizumab</td>
<td>IT, RT</td>
<td>IT given within 4 weeks of stereotactic radiosurgery led to improved response</td>
</tr>
<tr>
<td>Reits et al., 2006</td>
<td>Preclinical: murine colon</td>
<td>10 Gy</td>
<td>anti-CTLA4, anti-PD-1</td>
<td>Concurrent</td>
<td>Combination better inhibited tumor growth</td>
</tr>
<tr>
<td>Sørensen et al., 2017</td>
<td>Preclinical: colon Clinical</td>
<td>Various doses</td>
<td>T cell adoptive transfer</td>
<td>IT</td>
<td>Induction immunotherapy began more than 30 days before radiation resulted in longer OS</td>
</tr>
<tr>
<td>Schoenhals et al., 2016</td>
<td>Case report: lung cancer</td>
<td>Fractionated RT to primary and metastasis</td>
<td>nivolumab</td>
<td>RT, IT, RT</td>
<td>Abscopal effect</td>
</tr>
<tr>
<td>Shaver et al., 2017</td>
<td>Stage III trial: lung cancer</td>
<td>Various doses</td>
<td>pembrolizumab</td>
<td>RT, IT, IT</td>
<td>Patients who previously received any radiotherapy had better overall survival when treated with pembrolizumab</td>
</tr>
<tr>
<td>Shi et al., 2017</td>
<td>Case report: pancreatic cancer</td>
<td>45 Gy in 16 fractions</td>
<td>GM-CSF</td>
<td>Concurrent</td>
<td>When combined with radiation, anti-CTLA4 and anti-PD-L1 promotes response through different mechanisms</td>
</tr>
<tr>
<td>Teyman-Saint Victor et al., 2015</td>
<td>Preclinical: murine melanoma and pancreatic</td>
<td>20 Gy, 8 Gy</td>
<td>anti-CTLA4, anti-PD-L1</td>
<td>IT, RT</td>
<td>Anti-PD-1 prolonged survival of mice treated with RT and TGF-beta blockade</td>
</tr>
<tr>
<td>Vanpouille-Box et al., 2015</td>
<td>Preclinical: murine breast</td>
<td>6 Gy x 5</td>
<td>anti-TGF-beta, anti-PD-1</td>
<td>IT, RT</td>
<td>Anti-CTLA4 therapy was not able to synergize with high dose radiation to induce an abscopal effect</td>
</tr>
<tr>
<td>Therapie-Box et al., 2017</td>
<td>Preclinical: murine breast and colon</td>
<td>8 Gy x 3 vs. 20 Gy</td>
<td>anti-CTLA4</td>
<td>IT, RT</td>
<td>Anti-CTLA4 was most effective when given before the radiation</td>
</tr>
<tr>
<td>Young et al., 2016</td>
<td>Preclinical: murine colon</td>
<td>20 Gy</td>
<td>anti-CTLA4</td>
<td>IT, RT</td>
<td>Anti-OX40 was more effective when given 1 day after the radiation</td>
</tr>
<tr>
<td></td>
<td>Preclinical: murine colon</td>
<td>20 Gy</td>
<td>anti-OX40</td>
<td>IT, RT</td>
<td>Anti-OX40 was more effective when given 1 day after the radiation</td>
</tr>
</tbody>
</table>

SRS, Stereotactic Radiosurgery; WBRT, Whole Brain Radiation Therapy.
FUTURE DIRECTIONS

• Optimizing the Timing of Radiotherapy and Immunotherapy
• Optimizing the Dose of Radiotherapy: Conventional or Hypofractionation
• Minimizing the Direct Effects of Radiation on T Cells
• Because most immunotherapies depend on functioning T cells, lymphopenia is likely to undermine immunotherapy efficacy.
• Identifying Biomarkers to Predict Responders to Combination Therapy
• COULD IMMUNOTHERAPY BE A RADIATION SENSITIZER?
Conclusion

• RT acts by cytotoxic DNA damage with Tumour cell kill
• RT induces ‘immunogenic cell death’ (ICD), a type of cell death that promotes a T-cell-mediated immune response against antigens derived from dying cells
• Radiation synergize with immunotherapy via several mechanisms, such as increasing the visibility of tumor antigens, activating the cGAS-STING pathway, and modulating the tumor microenvironment.
• RT can assist IT by enhancing immune activation both systemically and locally
• IT can enhance the immune response induced by local RT.
• Future Directions--Optimizing the Timing and dose of Radiotherapy and Immunotherapy
REFERENCES

• Janeway’s Immunobiology 9th Edition
• Ainhoa Arina1,2, Stanley I. Radiotherapy and Immunotherapy for Cancer: From “Systemic” to “Multisite” Clin Cancer Res; 26(12) June 15, 2020
• Michele Mondini1,2, Antonin Levy, Radiotherapy–immunotherapy combinations – perspectives and challenges. Molecular Oncology 14 (2020) 1529–1537
• Hiro Sato 1,2,* , Sandra Demaria .The role of radiotherapy in the age of immunotherapy. Japanese Journal of Clinical Oncology, 2021, 51(4)513–522
• Nature reviews cancer 2012 Apr,12(4):265