Conformal Radiation with Immunotherapy: An Introduction to future of Radiation

Basis And Key Evidences

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ICRO CLASSES, 18th February 2022

INDEX

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.

Janeways 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

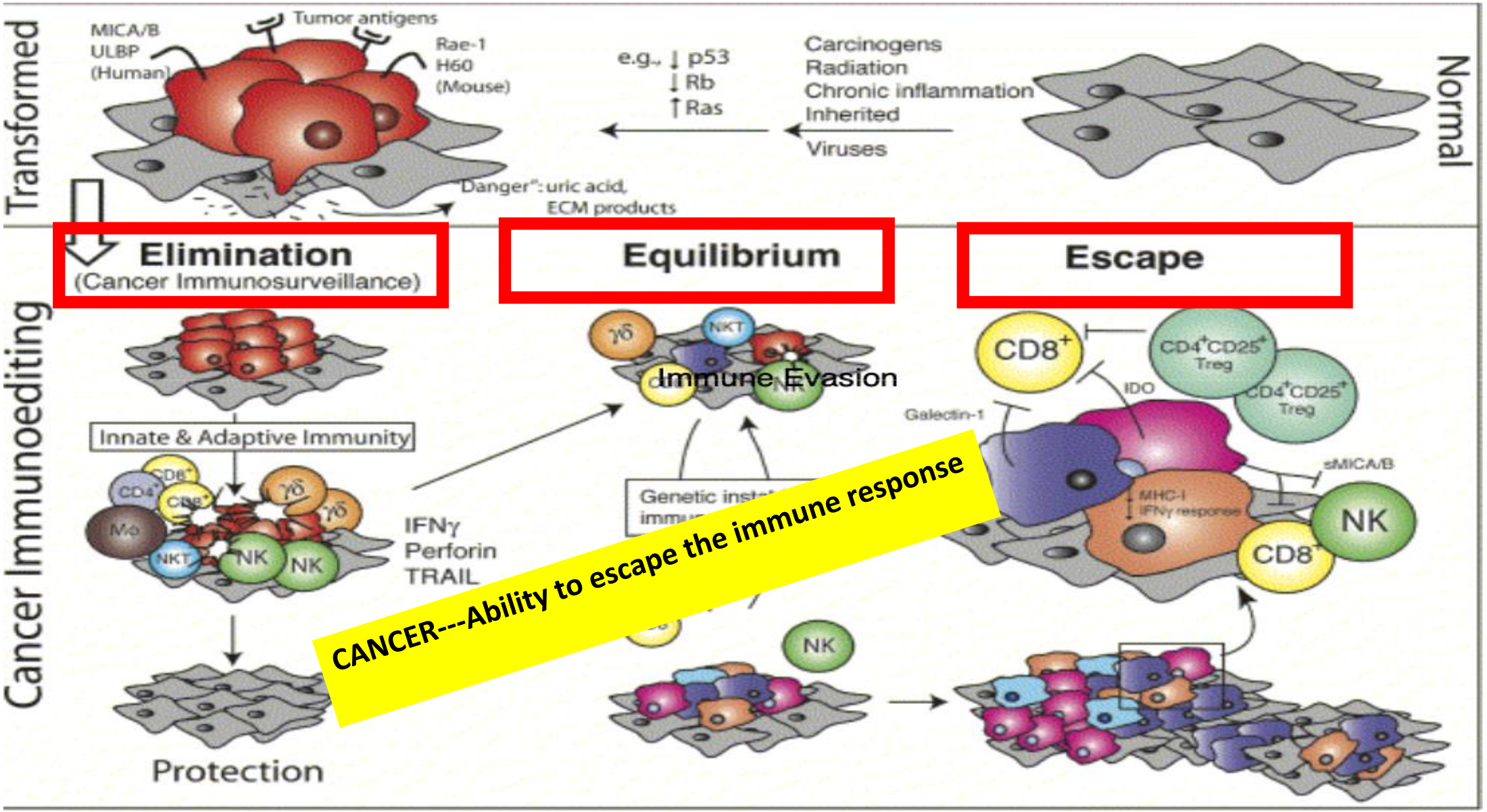
- **Cancer Immunoediting**:
  Cancer cells acquire the ability to escape immune control before they develop into clinical cancer

BACKGROUND

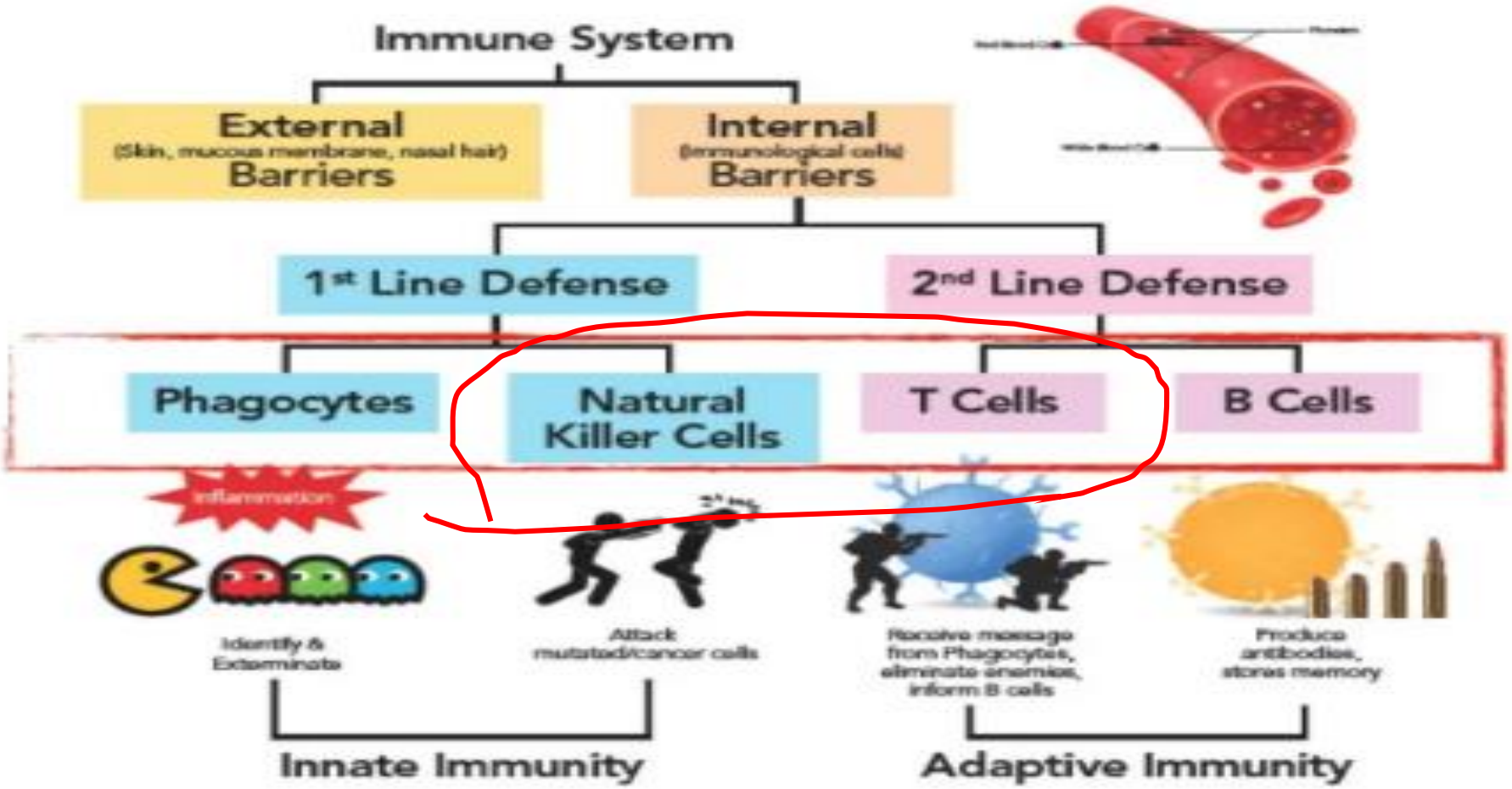
- Similar immune escape ALSO contributes to resistance to immunotherapy
- HENCE---A Tumor-specific immune response , both local and systemic in patients with cancer, may overcome this immunosuppressive scenario
- Radiotherapy--A new role emerging in overcoming immunosuppression in the tumor microenvironment.

Nat Rev Clin Oncol. 2019;16:151–67.

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



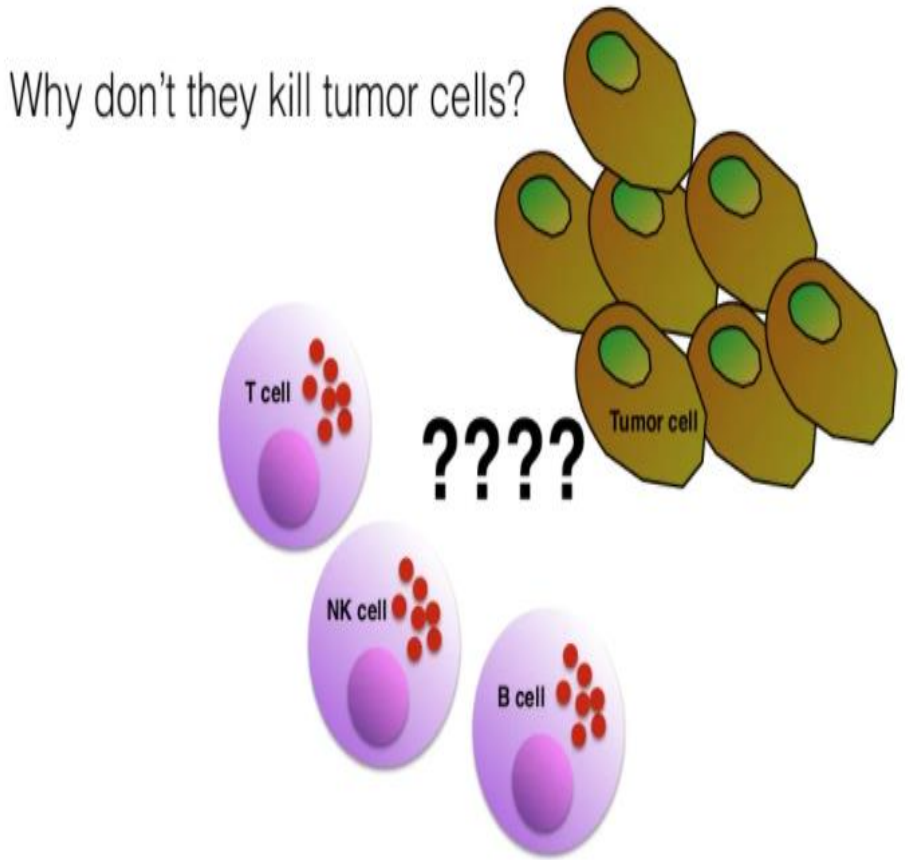
The Immune System
Our Ultimate Line of Defence



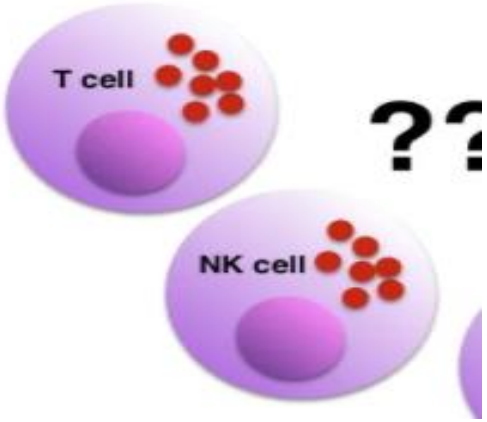
https://www.stemcellimmuneregenerative.com/what-is-the-immune-system

T and NK Cells

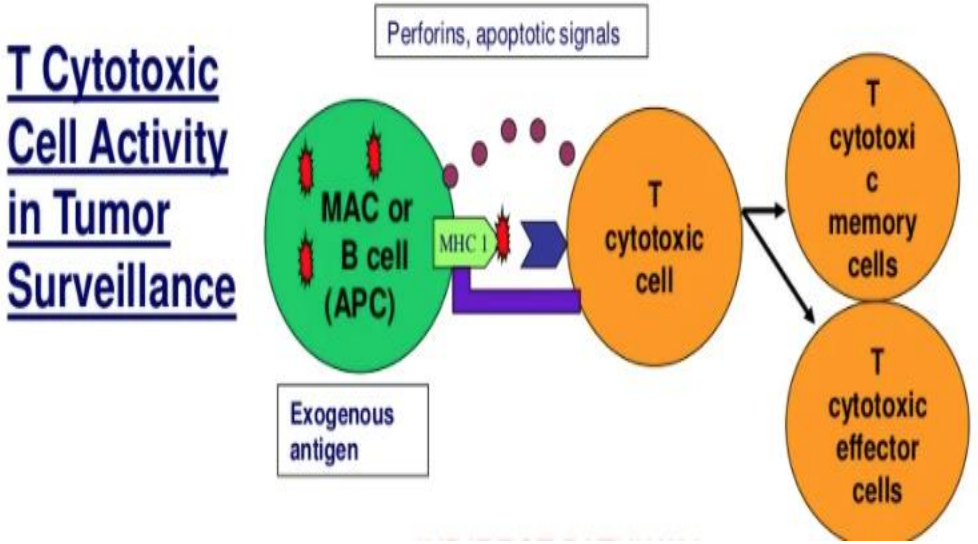
- Great cytotoxic capacity
- Probe the target cells to detect signs of abnormality
- Healthy Cells are Spared by T and NK cells
- T and NK cells Specifically kill dangerous Cells– Tumour Cells
- But Sometimes they Don’t Kill Tumour Cells--- ????????



T and NK Cells

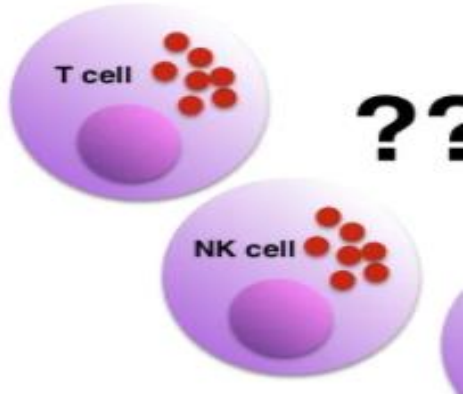


- Cytotoxic T cells (CTLs) CD8+ Cells--- Attach to Class I MHC- Peptide and destroy cancer cells by perfora
  or through apoptosis.



- 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

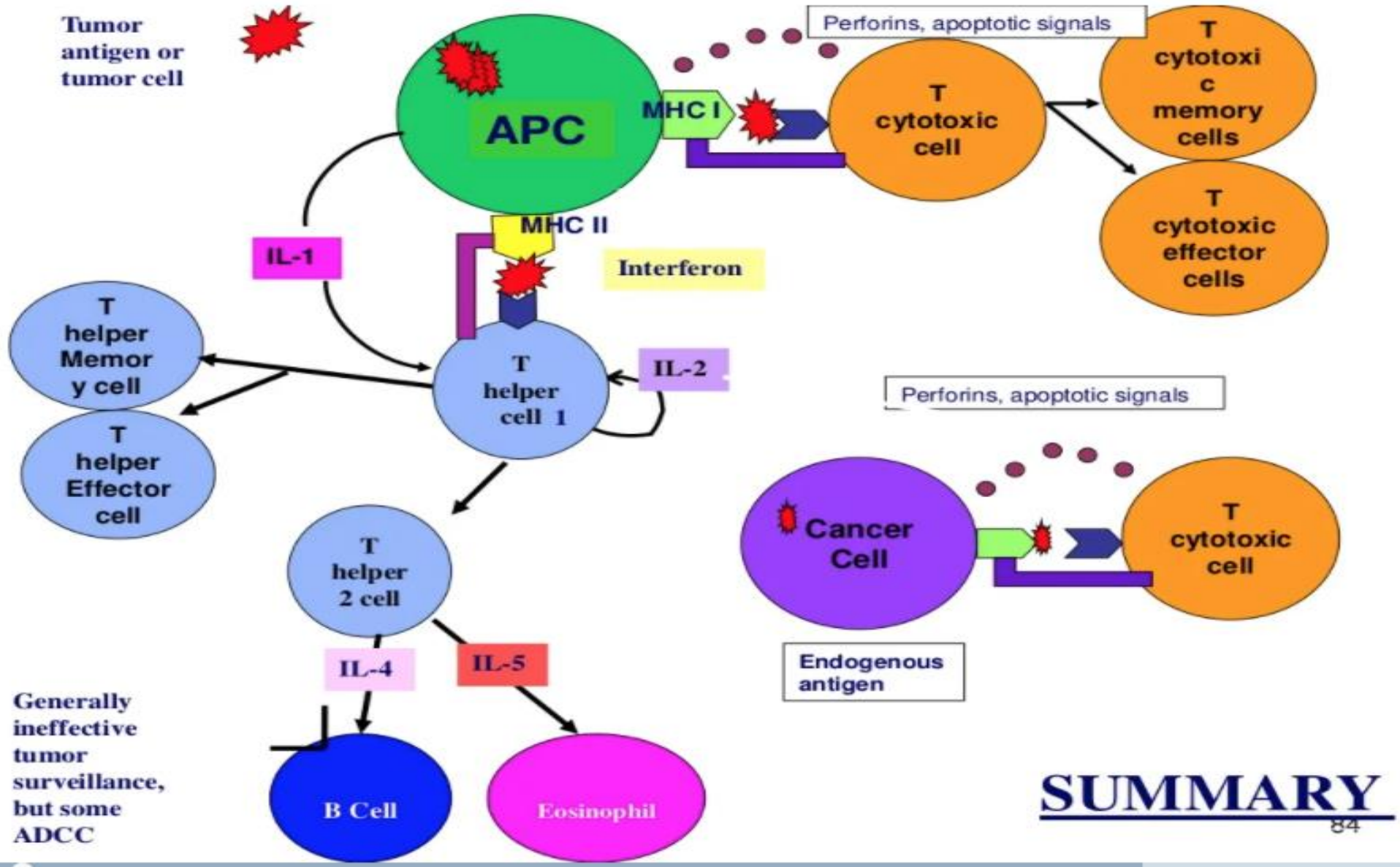
- Such Tumours can Trigger NK cells since NK cells are inhibited by MHC Class I molecules

Macrophages, T and NK Cells

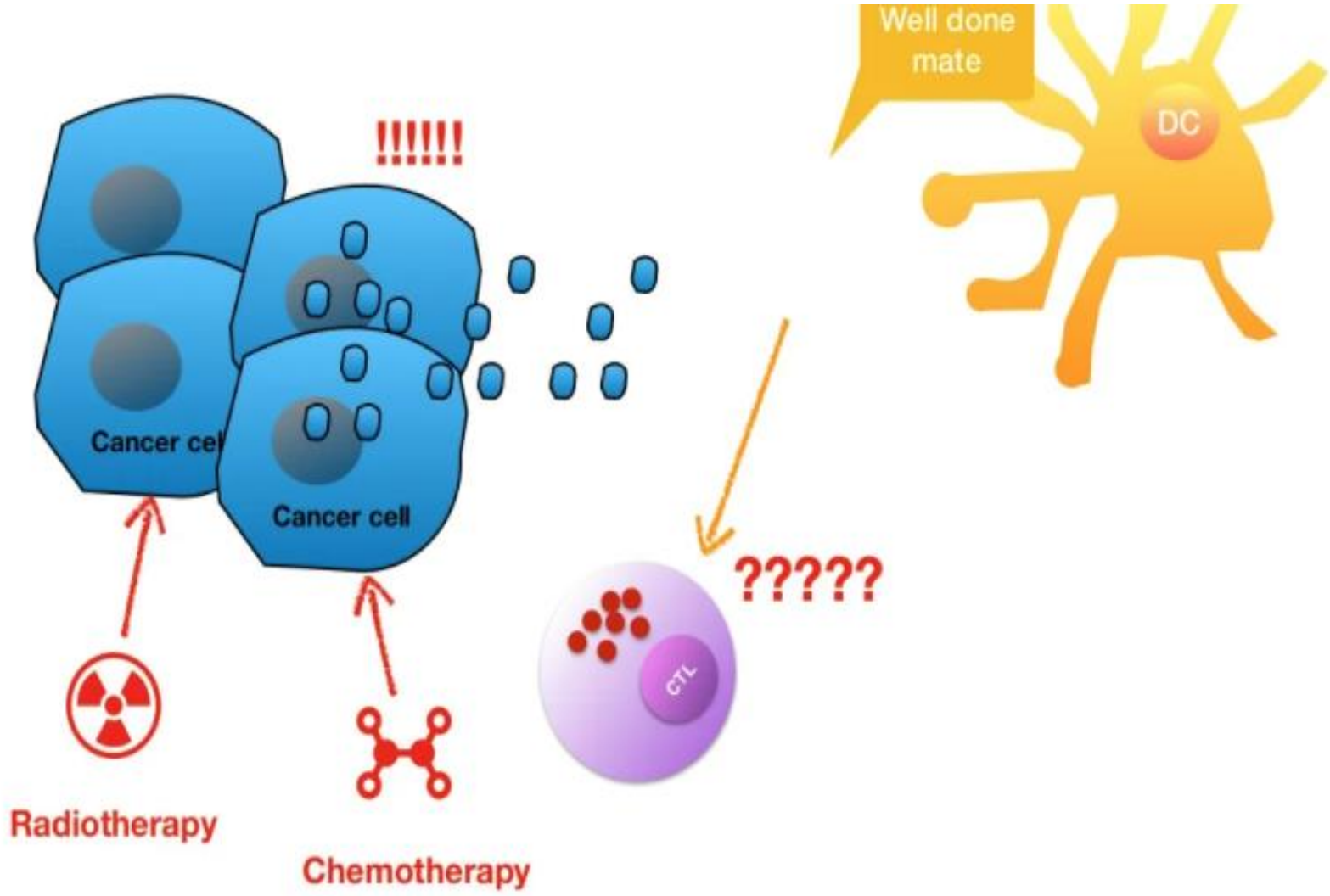
- Macrophages are activated by factors like Lymphokines (Produced by T Cells) and Interferons
- Activated Macrophages kill tumour by production of reactive O2 metabolites or by secretion of TNF (Tumour necrosis Factor)
- TNF has potent anti tumour activity
- T cells, NK cells and Macrophages collaborate 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

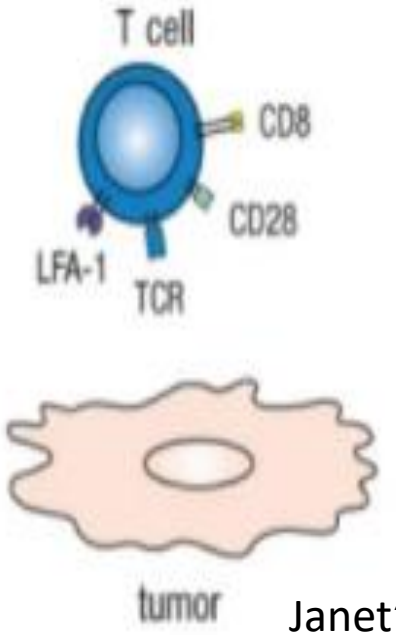
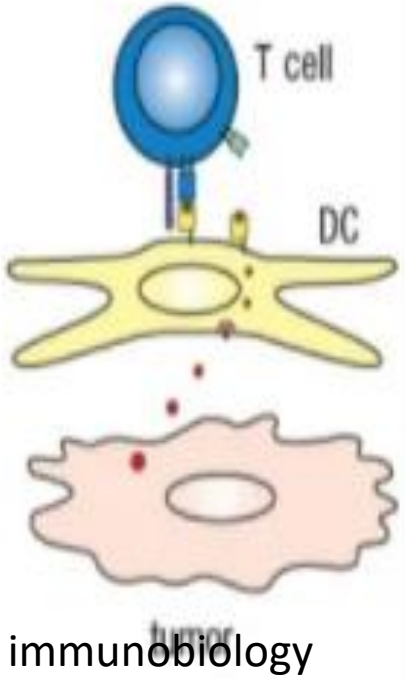
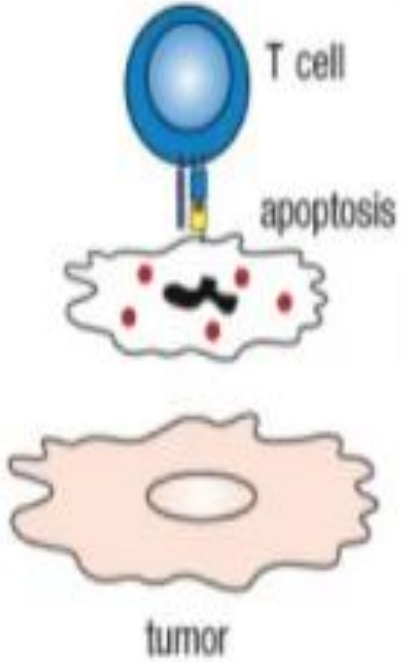
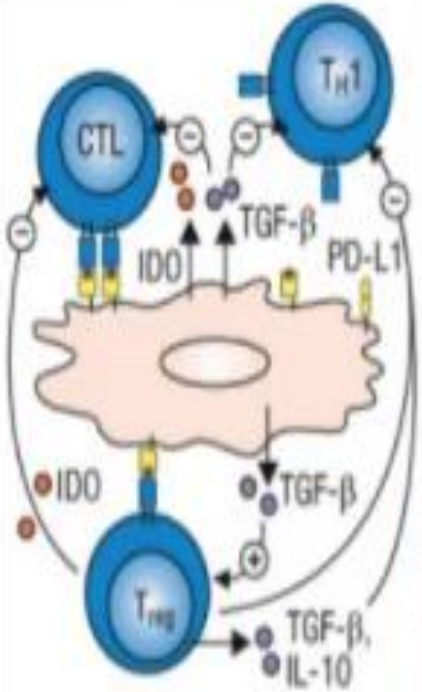
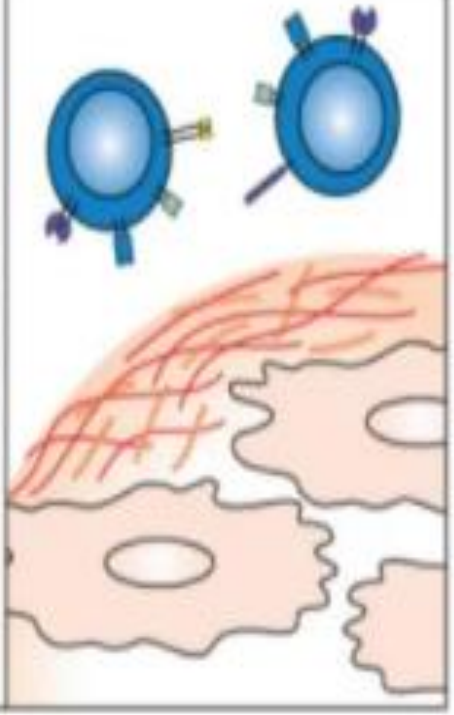
SUMMERY OF TUMOUR AND IMMUNE RESPONSE



Traditional Cancer Treatments Activate Immune System

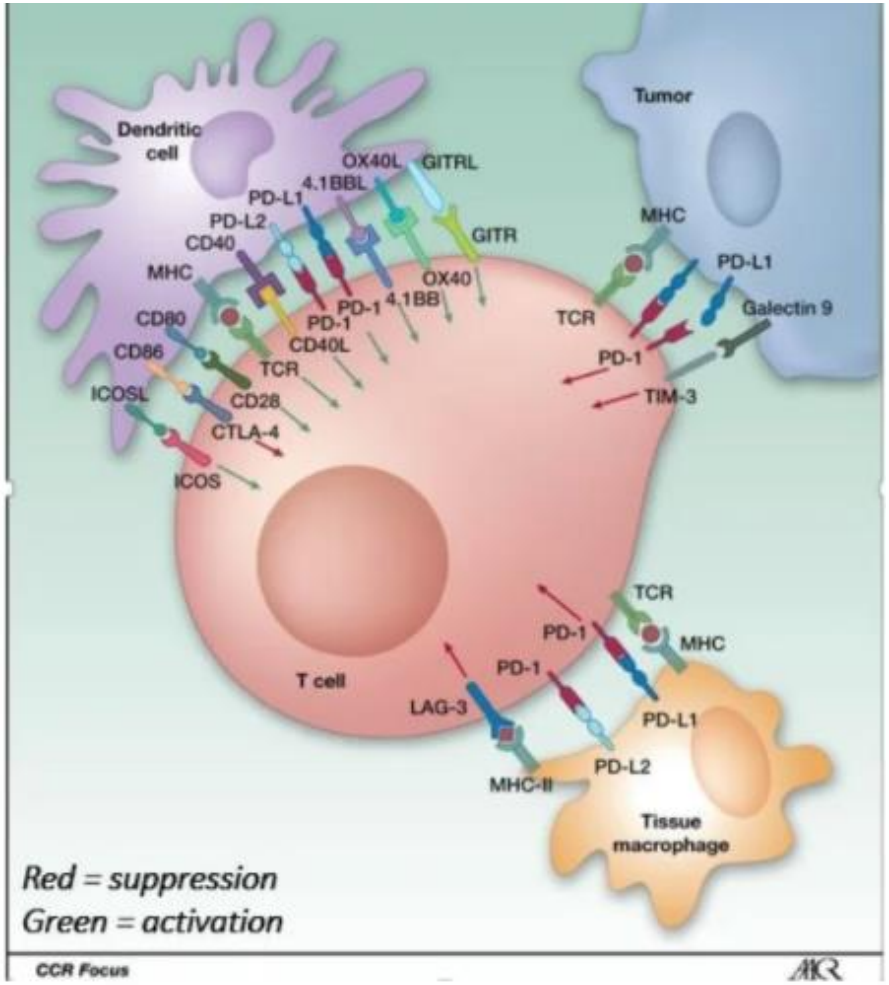


Cancer has ways to hide from Immune System

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

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 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

IMMUNOEDITING---Cancer Evades the Immune Response by

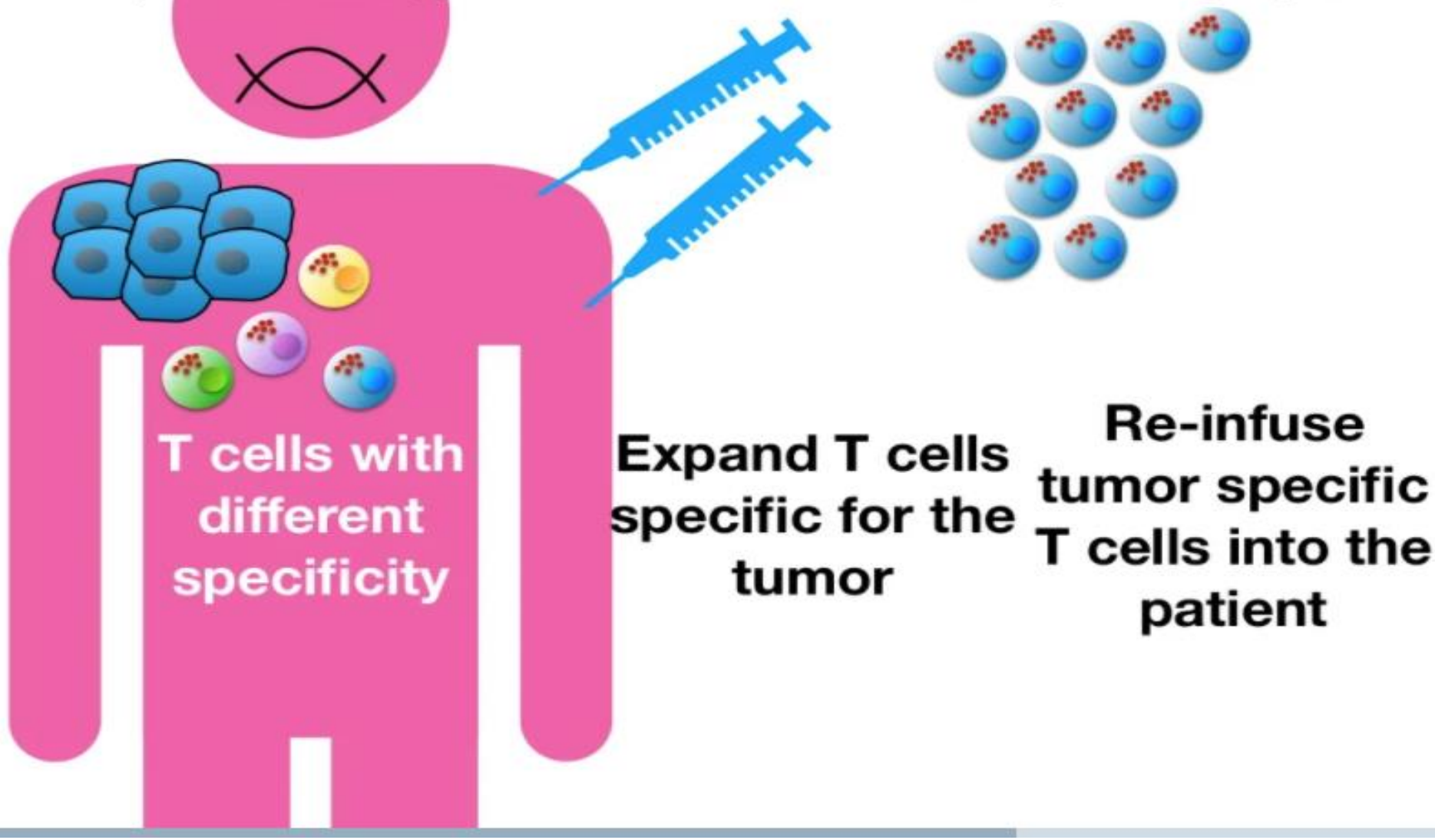
- LOW IMMUGENICITY
- LACK OF RECOGNITION ANTIGENIC MODULATION
- TUMOUR INDUCED SUPPRESSION

EACH OF THESE EVASIONS ARE TARGETED BY

- ADOPTIVE CELL TRANSFER
- ANTIBODIES
- VACCINATION
- ONCOLYTIC VIRUSES

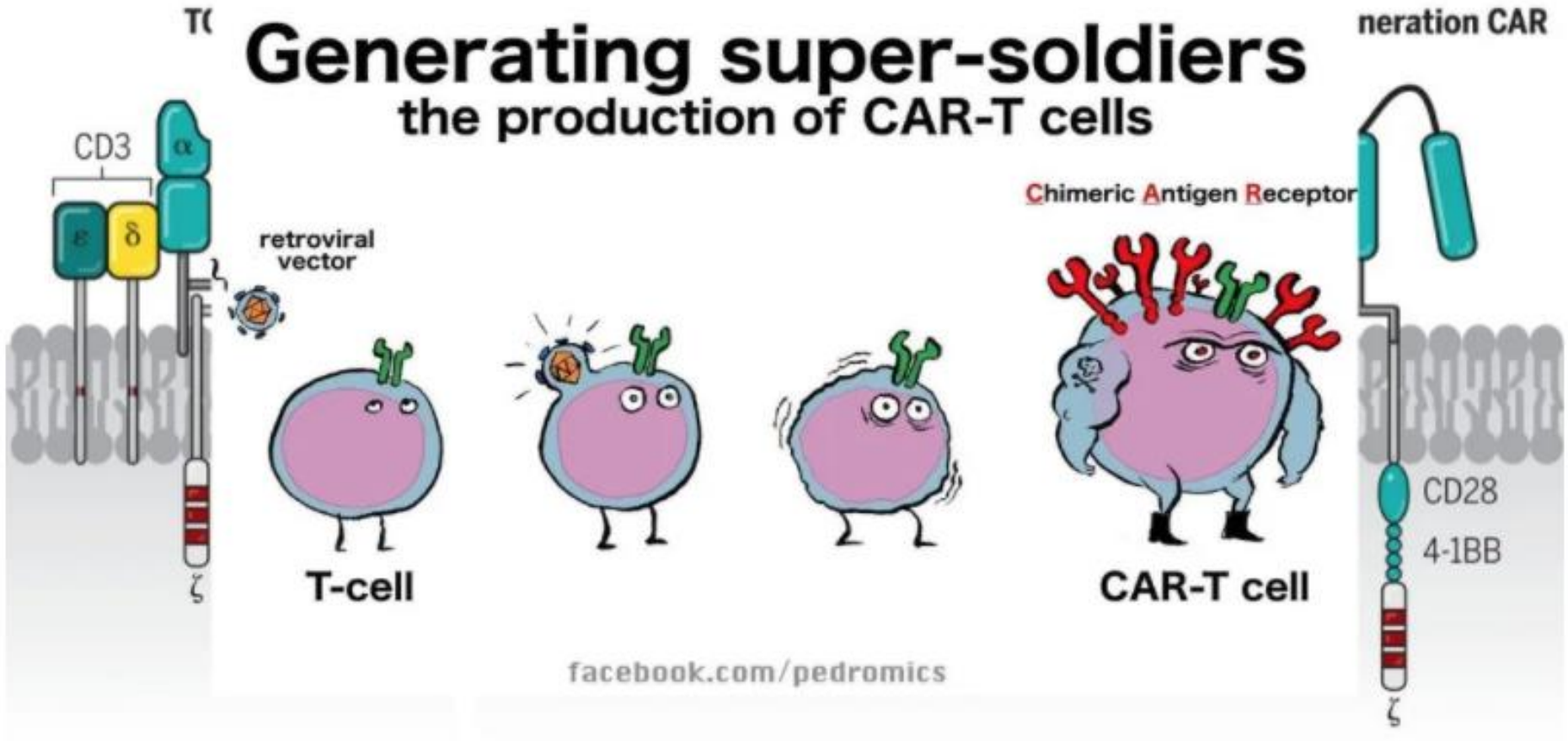
Cellular Therapy

Adoptive cellular therapy is based on the idea that T cells are capable of fighting tumors



Cellular Therapy

CAR T cells: engineering T cells to fight cancer



CARs MAKE ALL T CELLS REACTIVE AGAINST TUMOR CELLS

June et al.
Science 2018

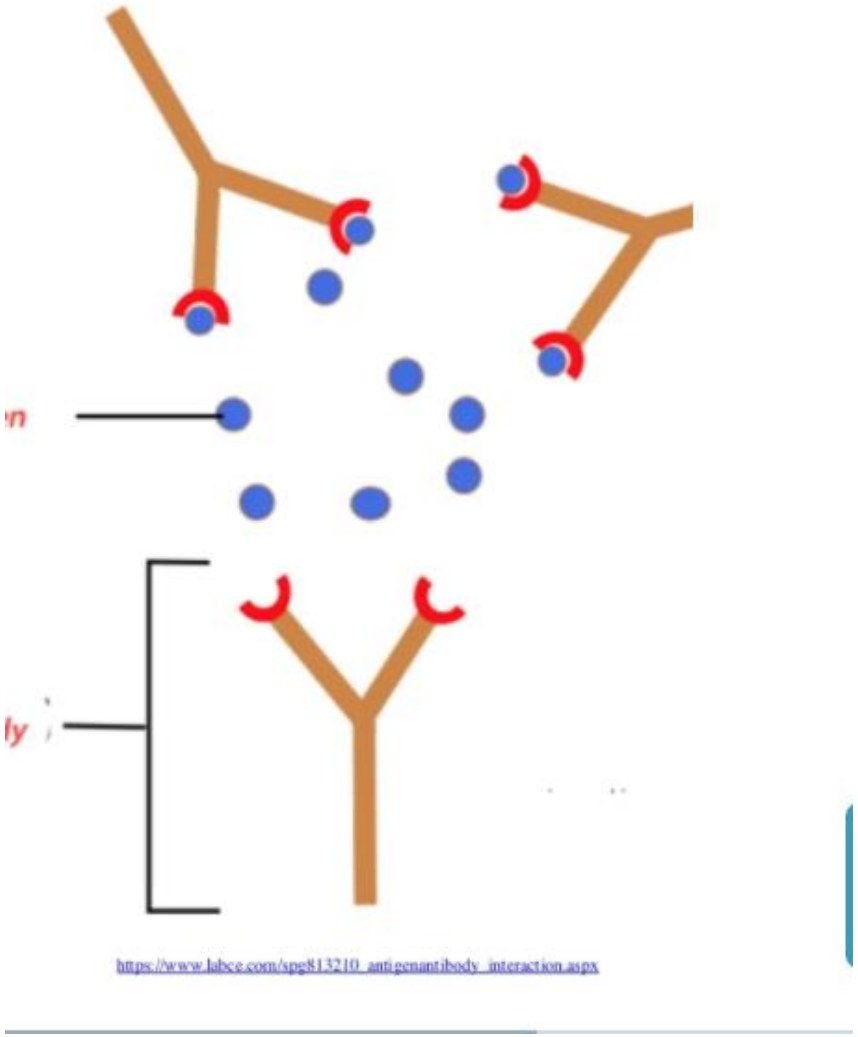
Antibodies

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**

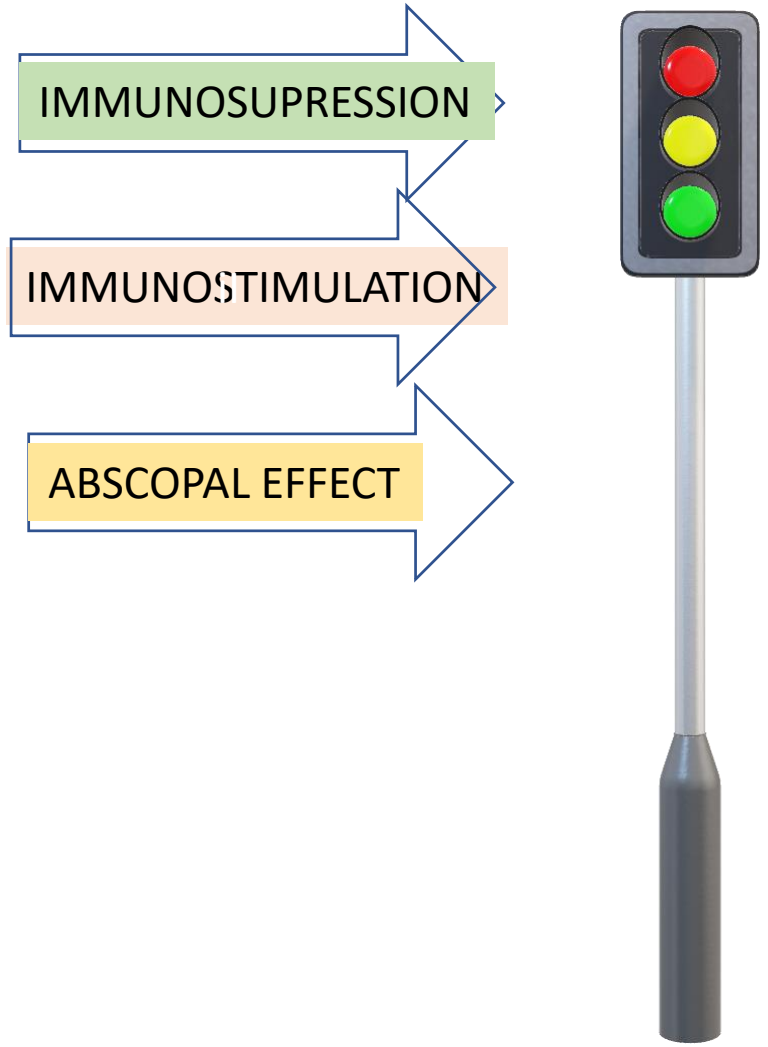


Rationale For Combination of RT and IT

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.



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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 → APC engulf the tumor cells and present their antigens to naïve T cells through phagocytosis & enhance their clearing
- Upregulate expression of MHC-I on the tumor surface → Enable better presentation of tumor-specific peptides, & enhance visibility of the tumor to cytotoxic T cells
- Radiation- induced DNA damage Induces a systemic increase in antigen recognition, → Generate neoantigen and trigger the immune surveillance.
- Radiation induce the T cell- mediated inhibition of untreated distant tumors → (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)

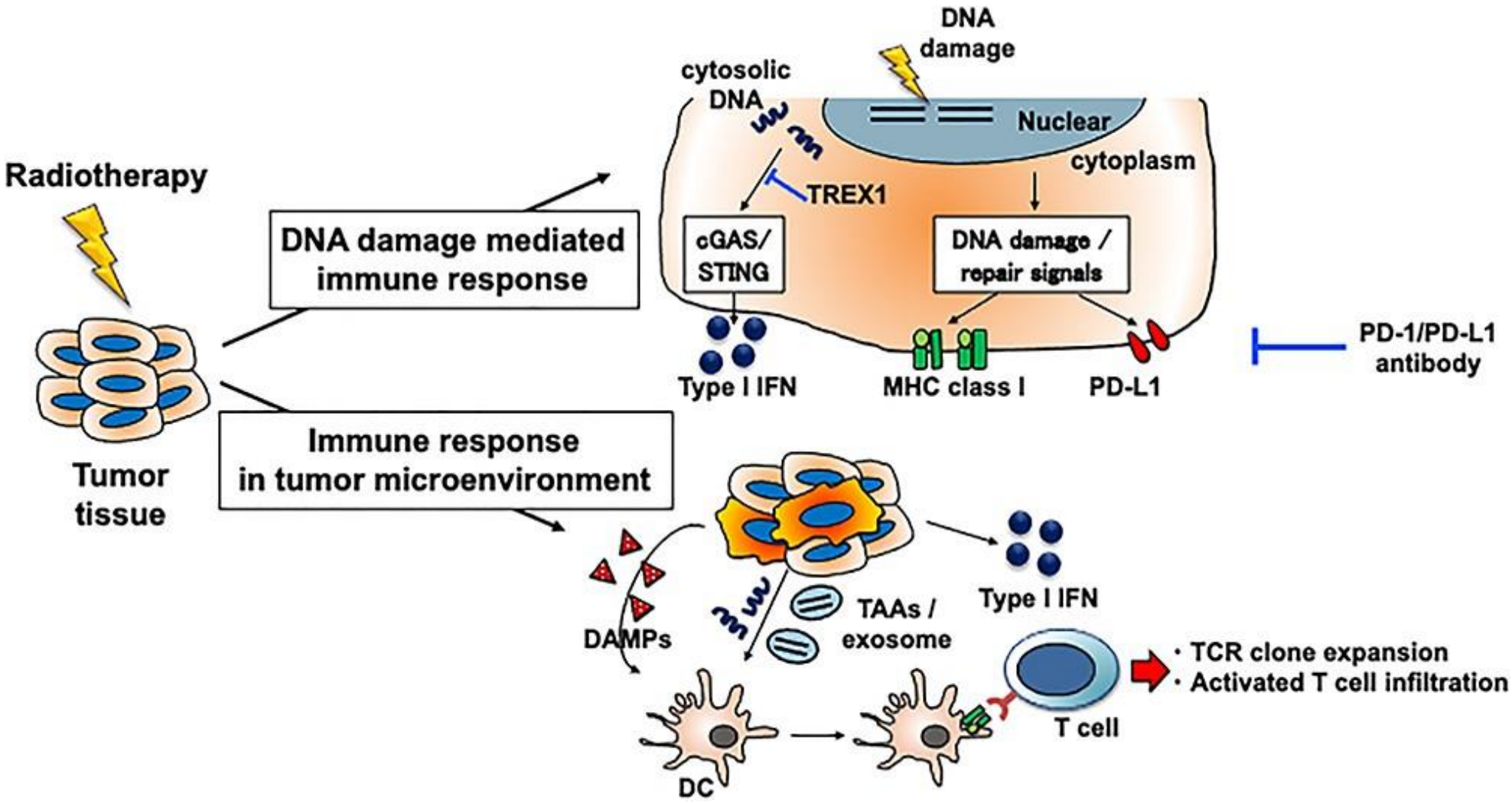
RT Has both positive and negative effects on antitumor immunity and the TME

- 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),

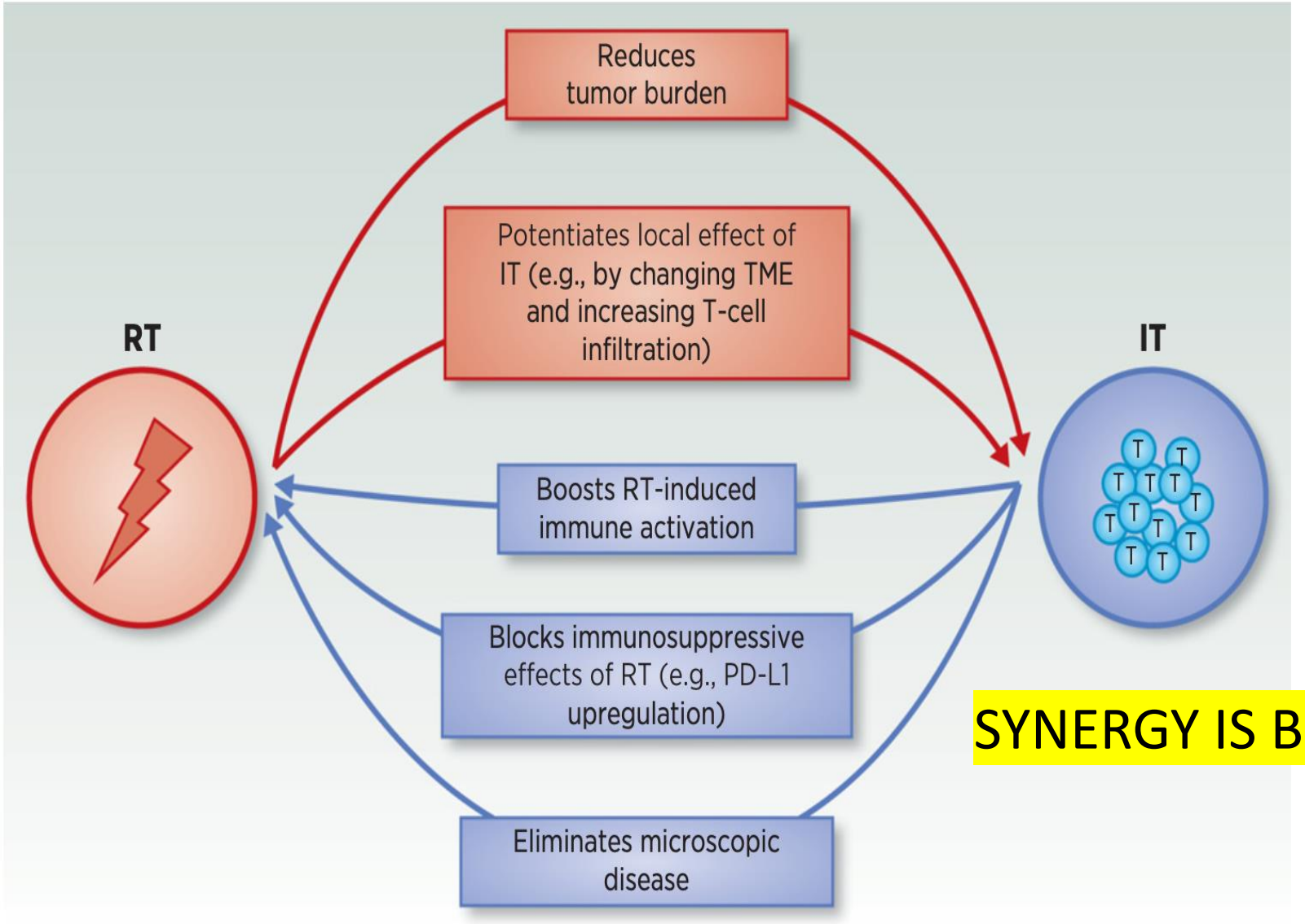
Annu Rev Immunol 2013;31:51-72.

Immune responses induced by radiotherapy include those caused by DNA damage and those that occur in the tumor ...



Jpn J Clin Oncol, Volume 51, Issue 4, April 2021, Pages 513–522, https://doi.org/10.1093/jjco/hyaa268

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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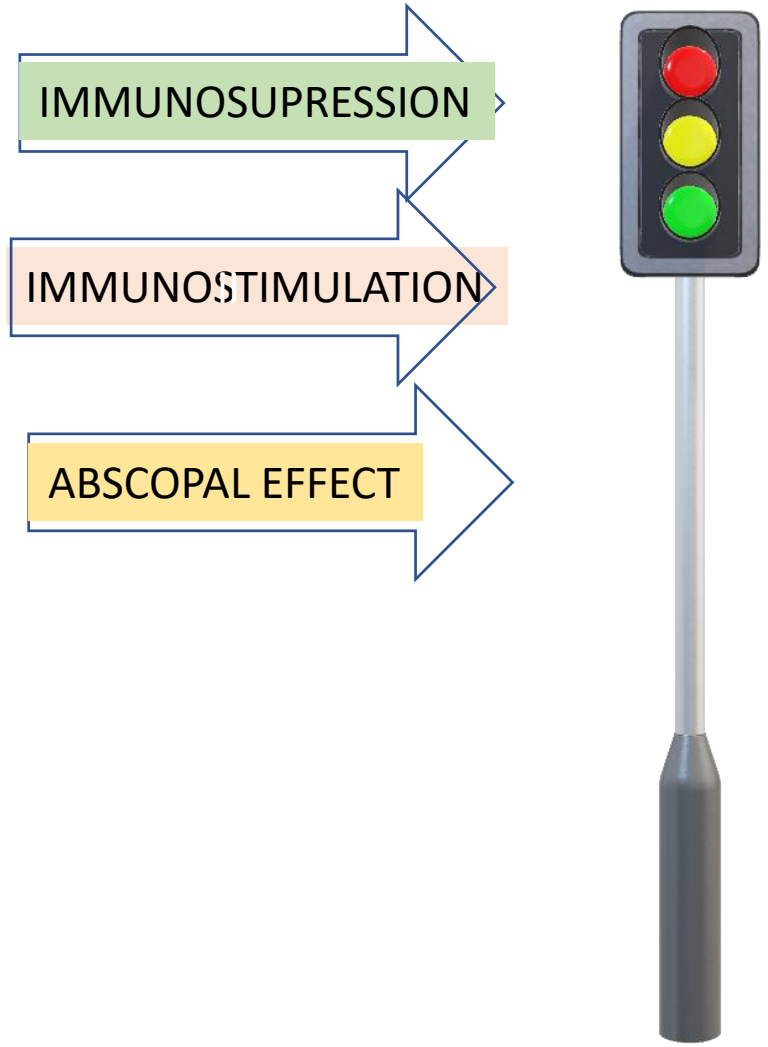

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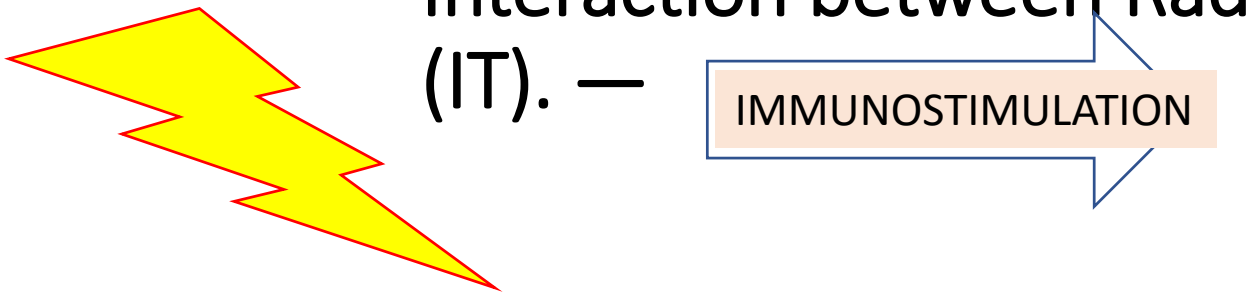
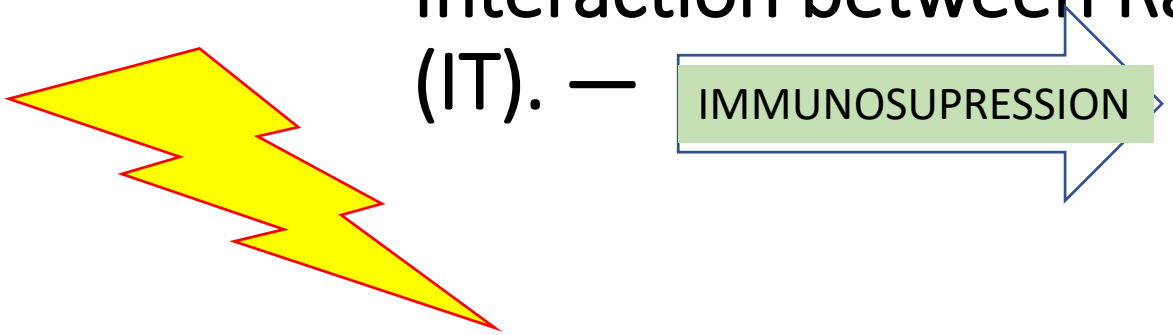


- Increased release of neoantigens -- presented to the immune system
- Radiation damaged DNA increases production of mutated non tumour specific antigens.
- Non-tumor specific antigens help in the upregulation of immune surveillance.
- Cytokines (type I interferons) release by damaged DNA which has escaped 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

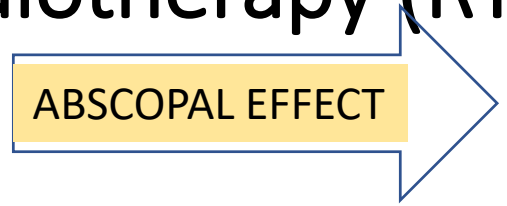
Interaction between Radiotherapy (RT) & Immunotherapy (IT). —



- 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 trigger 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.

Bioconjugate Chem. 2020, 31, 12, 2668–2678

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).

A promising new combination. J Immunother Cancer 2019;7:160.

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

STUDIES COMBINING RADIOTHERAPY &IMMUNOTHERAPY

Study	Type	RT	IT	Sequence	Results
Alomari et al., 2016	Case report: brain metastases	SRS 22 Gy	ipilimumab, pembrolizumab	IT, RT, IT	Status improvement
	Case report: brain metastases	SRS 20 Gy	nivolumab, ipilimumab	RT, IT	Remaind asymptomatic neurologically 6 weeks after surgery
Antonia et al., 2017	Stage III trial: lung cancer	Definitive RT (54 to 66 Gy)	durvalumab	RT, IT	PFS improvement with durvalumab
Aryankalayil et al., 2014	Preclinical: human prostate cancer cells	1 Gy × 10 vs. 10 Gy	NA	NA	Multifraction radiation induced more DAMP release
Baird et al., 2016	Preclinical: murine pancreatic	10 Gy	Cyclic dinucleotides	Concurrent	STING activator and RT synergistically controlled local and distant tumors
Camphausen et al., 2003	Preclinical: murine lung (LLC)	10 Gy × 5 vs. 2 Gy × 12	NA	NA	Five fractions of 10 Gy induced more robust abscopal effects
Deng et al., 2014a	Preclinical: murine breast and colon	12 Gy	anti-PD-L1	RT, IT	Combination of radiation and immunotherapy could be more potent than either treatment alone
Dewan et al., 2009	Preclinical: murine breast	20 Gy × 1 vs. 8 Gy × 3 vs. 6 Gy × 5	anti-CTLA4	Concurrent	Abscopal effect was induced only by fractionated radiation
Dovedi et al., 2014	Preclinical: murine melanoma, colorectal and TNBC	10 Gy in 5 fractions	anti-PD-1 or anti-PD-L1	Concurrent, sequential	PD1/PDL1 inhibition was effective only when given either concomitantly with or at the end of radiation
Haymaker et al., 2017	Case report: metastatic melanoma	WBRT 30 Gy in 10 fractions	ipilimumab, pembrolizumab	IT, RT, IT	Status improvement, long-term survival
Lee et al., 2009	Preclinical: murine melanoma (B16)	20 Gy vs. 20 Gy in 4 fractions	NA	NA	Immune response triggered by ablative radiation doses
Lugade et al., 2005	Preclinical: murine melanoma (B16)	15 Gy vs. 15 Gy in 3 fractions	NA	NA	15 Gy single-dose generated more tumor-infiltrating T cells
Nagasaka et al., 2016	Case report: head and neck	Palliative 30 Gy	pembrolizumab	IT, RT	Significant radiographic response
Qian et al., 2016	Clinical: melanoma brain metastasis	SRS 12–24 Gy	anti-CTLA4, anti-PD-1	Concurrent vs. non-concurrent	IT given within 4 weeks of stereotactic radiosurgery led to improved response
Reits et al., 2006	Preclinical: murine colon	10 Gy	T cell adoptive transfer	RT, IT	Combination better inhibited tumor growth
Samstein et al., 2017	Clinical	Various doses	anti-CTLA4, anti-PD-1/PD-L1	Concurrent, non-concurrent	Induction immunotherapy begun more than 30 days before radiation resulted in longer OS
Schoenhals et al., 2016	Case report: lung cancer	Fractionationed RT to primary and metastasis	nivolumab	RT, IT, RT	Abscopal effect
Shaverdian et al., 2017	Stage III trial: lung cancer	Various doses	pembrolizumab	RT, IT vs. IT	Patients who previously received any radiotherapy had better overall survival when treated with pembrolizumab
Shi et al., 2017	Case report: pancreatic cancer	45 Gy in 15 fractions	GM-CSF	Concurrent	Abscopal effect, survival benefit
Twyman-Saint Victor et al., 2015	Preclinical: murine melanoma and pancreatic	20 Gy, 8 Gy	anti-CTLA4, anti-PD-L1	Concurrent, sequential	When combined with radiation, anti-CTLA4 and anti-PD-L1 promotes response through different mechanisms
Vanpouille-Box et al., 2015	Preclinical: murine breast	6 Gy × 5	anti-TGF-beta, anti-PD-1	RT, IT	Anti-PD-1 prolonged survival of mice treated with RT and TGF-beta blockade
Vanpouille-Box et al., 2017	Preclinical: murine breast and colon	8 Gy × 3 vs. 20 Gy	anti-CTLA4	RT, IT	Anti-CTLA4 therapy was not able to synergize with high dose radiation to induce an abscopal effect
Young et al., 2016	Preclinical: murine colon	20 Gy	anti-CTLA4	IT, RT vs. RT, IT	Anti-CTLA4 was most effective when given before the radiation
	Preclinical: murine colon	20 Gy	anti-OX40	IT, RT vs. RT, IT	Anti-OX40 was more effective when given 1 day after the radiation

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

THANK YOU

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