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## THE RADIOTHERAPY MIGHT BE A VACCINE FOR IMMUNE RESPONSE

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**Abstract:** *Radiotherapy (RT) is one of the most important treatment options of cancer and also may activate the immune response. Because of this, we must discuss the question of "is the radiotherapy vaccine". If RT increases the immune response we must learn some more about the RT effects on the cells, cell surface antigens, immune checkpoints and their inhibition (ICI; CTLA4, PD1, PDL1 inhibition). It will be discussed the role of the radiotherapy and biologic effects of the radiotherapy to activate the immune response, radiotherapy dose, radiotherapy timing and the side effects of immune checkpoints inhibitors. Hypofractionated stereotactic body radiotherapy is widely used in clinical practice to achieve immune response, but with high conformal hypofractionated stereotactic radiotherapy the microenvironment around the cancer tissue such as fibroblast may survive and then may help cancer stem cells progression, and this is a very important subject should be taken into consideration in order to be better understood in future studies.*

**Keywords:** *Radiotherapy, Immune Response, Immune Checkpoints*

### 1.Introduction

In the multimodally approach we are fighting against cancer with Radiotherapy (RT), Chemotherapy (CT), Surgery (S) and Immunotherapy (IT). There is new information about to use IT and RT. In this paper will be discussed the role of radiotherapy for immune response. The immune system inhibits cancer cell growth and spread. As seen on Figure 1, also over the basal membrane in the stage 0 (in-situ) epithelial cancer, the Langerhans Cell (L) acts as Antigen Presenting Cells (APC) for an immune response [1].



**Figure 1.** The Langerhans Cell (L) over the basal membrane in the epithelial tissue.

In tumor microenvironment there are many cells; such as normal epithelial cells, mesenchymal cells, endothelial cells, macrophage (M), tumor-associated macrophage (TAM), fibroblast (F), cancer-associated fibroblast (CAF), dendritic cell (DC), antigen presenting cell (APC), T-B lymphocytes, cancer cells and many dead cells of cancer cells and normal cells. There are many dead tissues and necrotic secretions in the carcinoma microenvironment. These increase with treatments and may protect cancer. Inflammatory response increases pressure and may protect cancer. M2, TAM, CAF, peristicle, collagen tissue may protect cancer. (Figure 2).



**Figure 2.** Cancer microenvironment has many living and dead cells (Tc: T cytotoxic, T: T lymphocyte, DC: Dendritic Cell, F: Fibroblast, CAF: Cancer-Associated Fibroblast, M: Macrophage, TAM: Tumor-Associated Macrophage, C I: Dead Cells)

During RT planning usually, we include in the treatment volume primary gross tumor volume + clinical target volume + lymph nodes (Figure 3) [2]. But if we want a good immune response we need cancer antigens and lymphatics. Because of this, we must discuss to save regional lymphatics and to give RT before surgery.

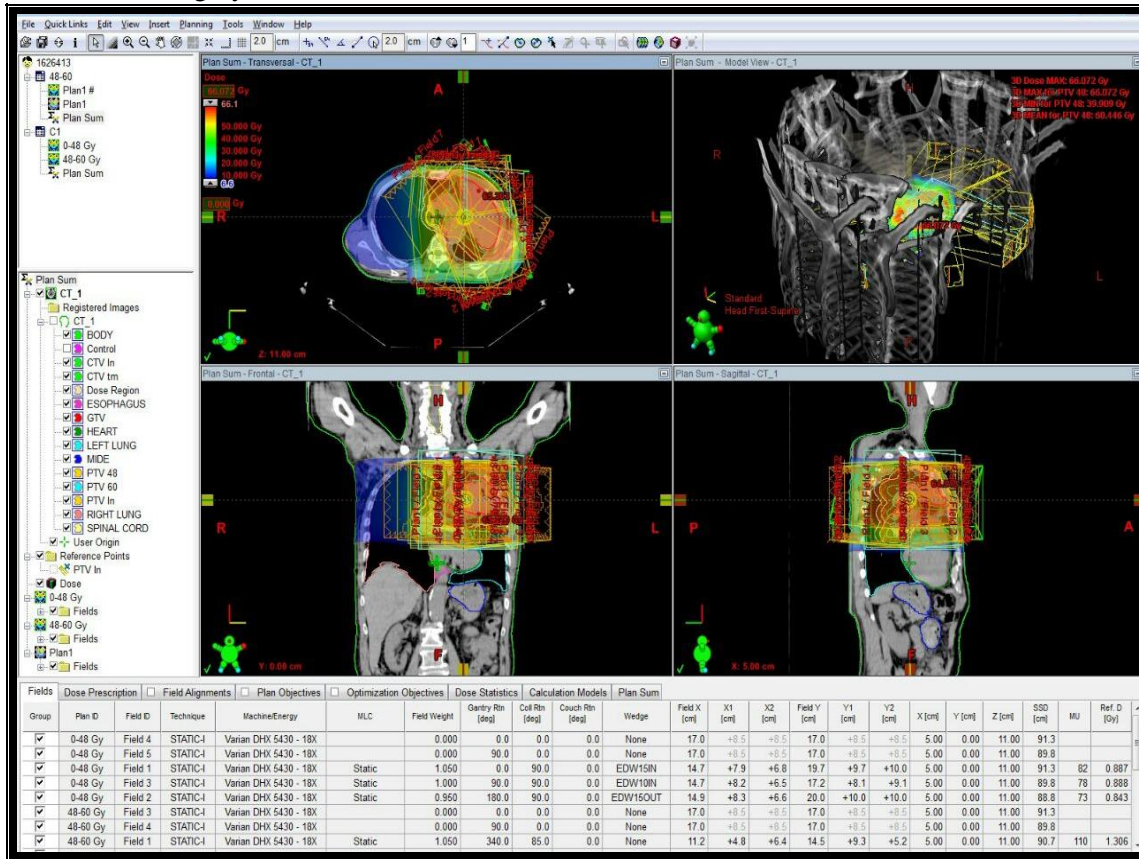
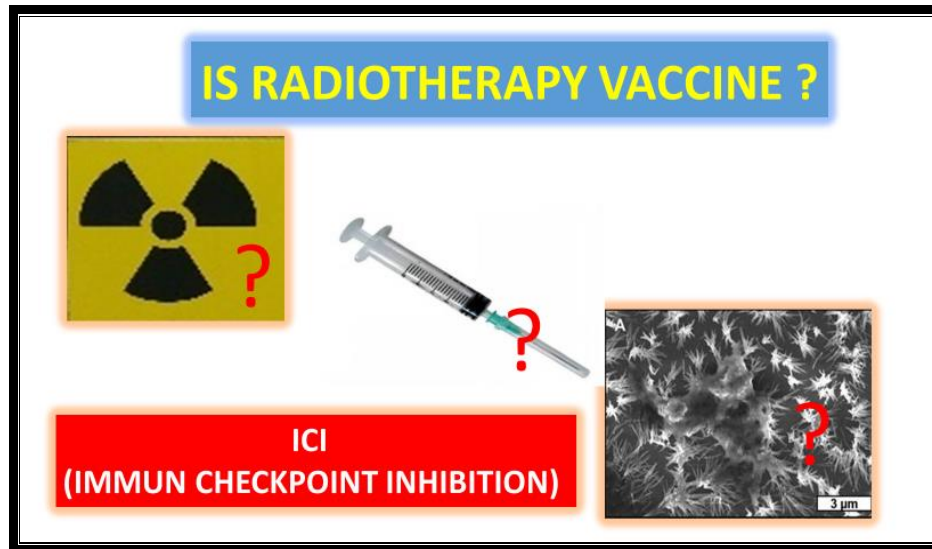


Figure 3. Radiotherapy treatment fields and isodoses on the left lung cancer case.

In the past and in classic radiation biology had 4R [3]; Repair, Redistribution, Reoxygenation (but RT may cause hypoxia, this may bad news and needs Anti VEGF), Repopulation (This may bad news because RT may stimulate cancer growth) and 5R; Radioresistant/Radiosensitive tissue or cancer. Now we have 6R; IMMUN REJECTION, this may be good news? Because Radiotherapy activates immune response (Figure 4). Also, we have the 7R; Remodelling means cancer lives in a microenvironment and crosstalk with cancer and microenvironment such as CAF, etc [4, 5].

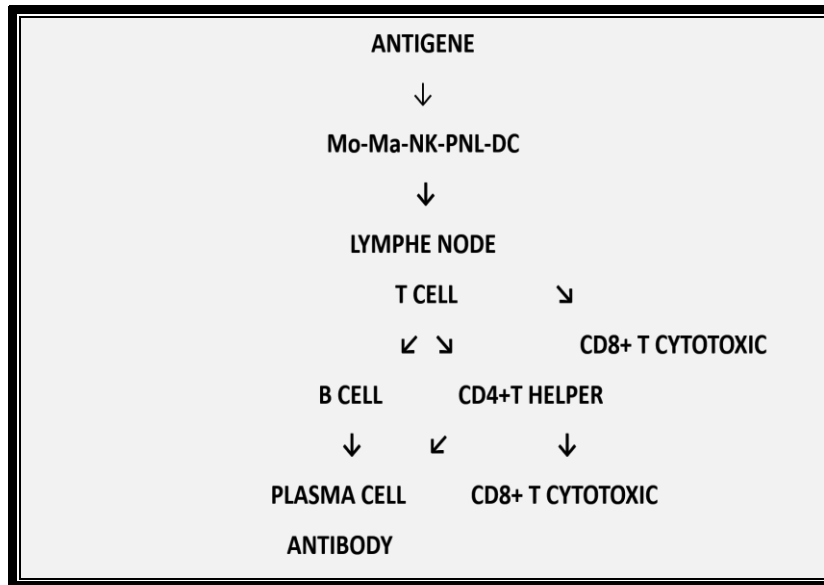


**Figure 4.** Is the radiotherapy vaccine [1, 6]?

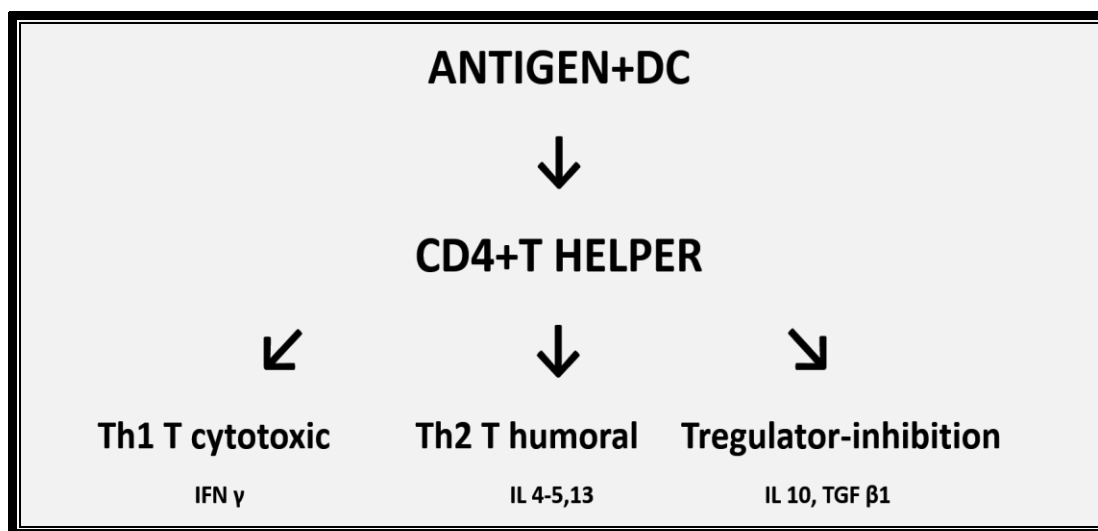
In oncology practice, there are some vaccines, such as; Oncolytic virus, BCG [7]. Also; Sipuleucel-T is a DC-based vaccine containing GM-CSF. White cells (DS, Mo-Ma, B cell) are taken from the patient's with apheresis and transferred to the fabrication, where meeting with recombinant antigen-Sipuleucel-T / Provenge, Dendritic cells mature with GM-CSF and the T cell is matured and activated, then is given back from the vein the patient. This is active cell immunotherapy in castration-resistant prostate cancer, and

Cimavax-EGF (CUBA): If meningitis bacteria inoculated to patient body, produces antibodies against EGF. The EGF amount is reduced [8, 9]. Cancer can not progress and can not metastasis. Another non-vaccine treatment also against the EGF receptor as TKI. Including to above; Is the radiotherapy vaccine? If RT increases the immune response we must learn some more about immune checkpoint inhibition (ICI; CTLA4, PD1, PDL1 inhibition) [10].

There are some molecules called Major Histocompatibility Antigene (MHC) or Human leukocyte Antigene (HLA) for immune activation after RT; MHC 1 (HLA- A, B, C) cell surface molecules is to deliver intracytoplasmic antigens such of viruses and tumor antigens presented to CD8 + cytotoxic T cells by the cell. MHC 2 (HLA-DP, DR, DQ) are the cell surface molecules, which has been taken by bacterial endocytosis to presented to CD4 + helper T (Figure 5-6) [11].



**Figure 5.** Immun activation after RT



**Figure 6.** CD4 + T helper

Th1 cells provide the cellular immunity of the person with interferon gamma (IFN- $\gamma$ ), interleukin-2 (IL-2) and tumor necrosis factor-alpha (TNF-alpha). IL-2 specifically proliferates cytotoxic T-cells and activates IL-2 NK-cells. Also, the TNF-alpha stimulates T-cells and NK-cells to attack the tumor. INF- $\gamma$  helps HLA-expression and enhances antigen presentation to effector T-cells [10-12]. Th2-cells secrete more IL-4, IL-5 and IL-10 than Th1-cells and Th2-cells support the immune response with antibody production. IL-4 stimulates B-cells through IgE. IL-10 contributes to the formation of B-cells, monocytes, and granulocytes [10-12].

## 2. Radiotherapy response

When we give RT to cancer microenvironment we may see many reactions [13-18];

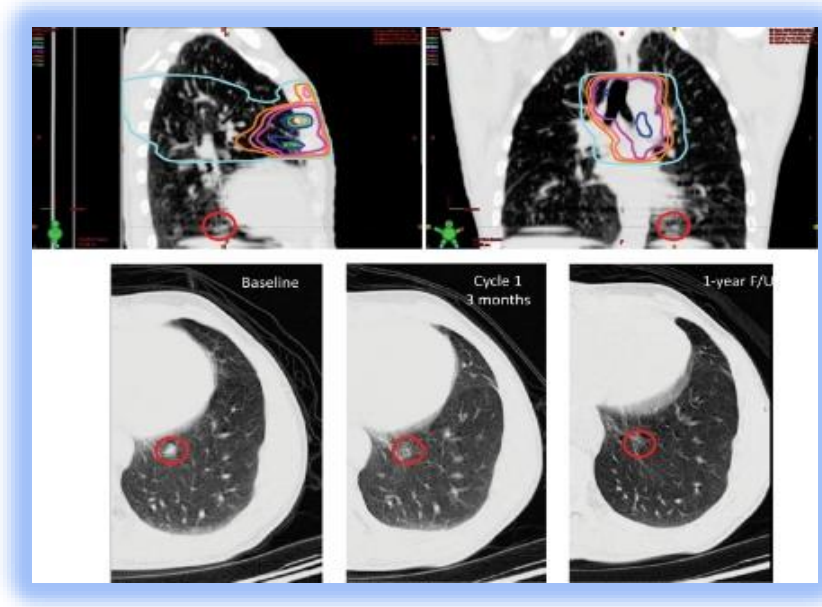
1. Tumor antigens (MHC1/2) excrete to the extracellular matrix. Macrophages and dendritic cells (Dendritic-Cell DC) take them, go to the lymph glands, and these antigens present to the effector T cells and activate T cytotoxic cells (Tc) (CD8 + T lymphocytes).
2. DNA binding protein HMGB1 (High Mobility protein Group Box 1) Increases with radiation. The HMGB1 protein release to extracellular space. HMGB1 is TLR4-associated dendritic cell activator.
3. ATP (Adenosine triphosphate) appears after RT damage, activate the immune response.
4. When DNA is damaged, ATM (Anti telengectase mutated protein) comes out.
5. NKG2D (Natural Killer group 2D) ligands are increased. NK and active CD8 + T cells attack these ligands on cancer. Also the activated Tc attacks to metaplasia and metastasis.
6. From the endoplasmic reticulum, Calreticulin appears on the tumor surface. Calreticulin pre-apoptotic is released and it is the message to "eat me" in cell translocation.
7. Uric acid and Nitric oxide increases are released into the tumor space and cancer is eliminated by macrophages
8. HSP (heat shock protein) increases. They stimulate the Dendritic Cell (DC). DC stimulates CD8 + Tc.
9. Ceramide is present in the vascular endothelial membrane and also in all membranes. Ceramide is a member of the sphingolipid family, leads to apoptosis. It also helps in immunotherapy (IT) by providing Dendritic Cell maturation with MHC1 increase.
10. ICAM1 (CD54): Intra Cellular Adhesion Molecule/Inter-Cellular Adhesion Molecule. After RT lymphatic endothelial cell adhesion increased to keep the immune cells for immune response
11. Fas (CD95): First Apoptosis Signal (Apo1 or tumor necrosis factor receptor superfamily member 6) binds to the protein-ligand (FasL), resulting in apoptosis signaling from the nucleus leading to cancer cell death.
12. The formation of ROS (reactive oxygen substrate) also kills cancer.
13. Radiation kills cancer directly but also activate immune responses through the Stimulator of Interferon Genes (STING)-mediated DNA-sensing pathway.
14. After RT, IFN gamma released against cancer to increases immune cell migration to the tumor site, has antiproliferative and antiangiogenic effects, the cytotoxic effect on cancer. IFN causes apoptosis in cancer. Also, IFN gamma / IL-12 are secreted by tumor-infiltrating monocytes/macrophages/ DCs and these activate the Natural Killer (NK) cells and this help to open holes in the cell with perforin granule enzyme and por forming the cytolytic protein. Por caspase or Fas-L caspase stimulus kill cancer.

## 3. Abscopal effect

(Ab: remote, away from. Scopes: target, purpose, aim, scope); Divided into two; The near abscopal effect (bystander effect, audience effect) and the remote abscopal effect [19-21].

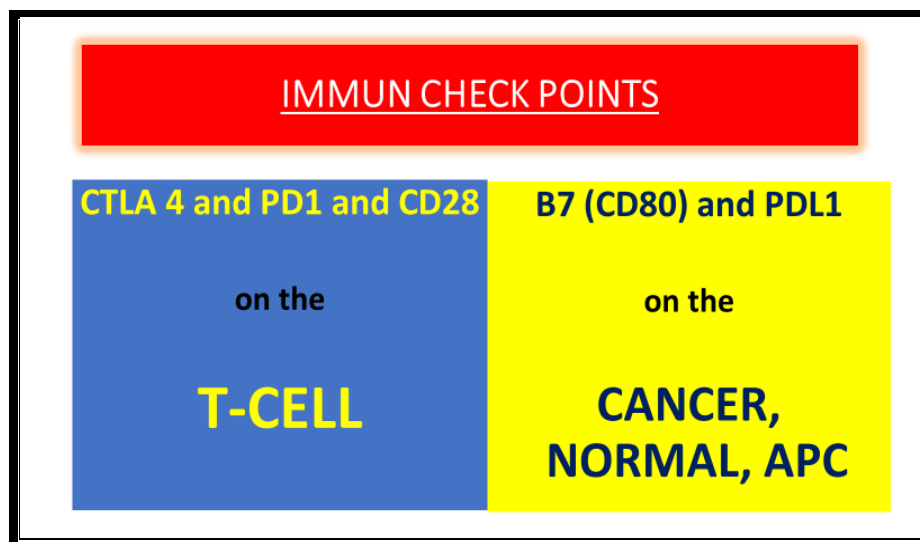
The abscopal effect cannot be obtained in non-T cells rats. After RT, close and distant cancer cells may shrink and disappear. Because the antigens released by RT-receiving cells are taken up by Macrophages and DCs, and in the lymph node these antigens recognized by the effector T-cell and the activated immune system kills close and distant cancer cells. CD8 + Tc and Type1 Interferon increase. As seen in

Figure 7, the left lower metastatic lesion has disappeared after RT to only the left hiler primary and mediastinal lymph nodes because of the abscopal effect of RT [22].



**Figure 7.** Abscopal effect [22]

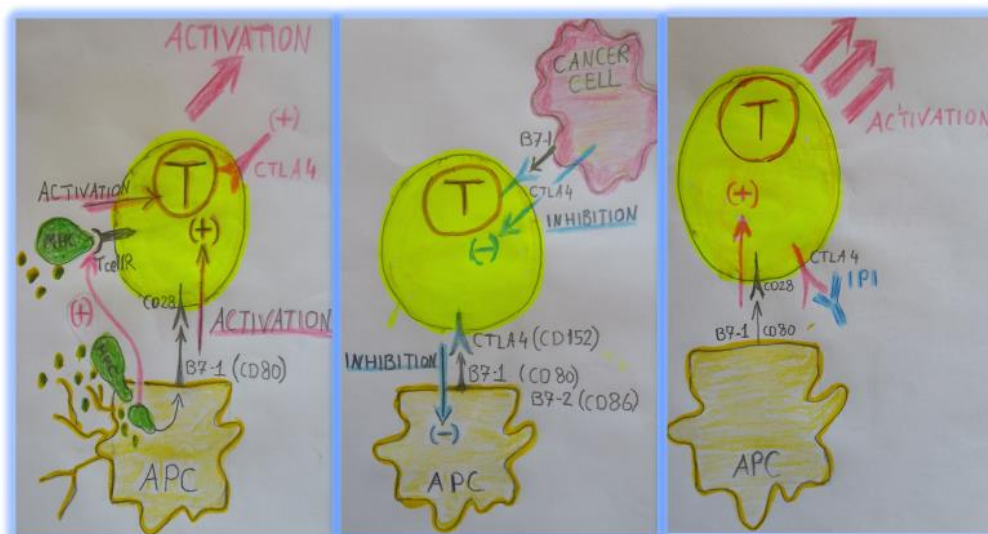
BUT, if proliferating cancers and metastases mutate, it may be a new antigenic construct and we can not see the abscopal effect. Cancer cells are heterogeneous and each cancer cell has similar and dissimilar antigens. For this reason, the immune response may not be against every cell. Also, it is very important that a normal person has immune checkpoints (ICP) to protect its self from over immune attack to from autoimmune diseases. If ICP works in cancer patients our immune system can not attack to kill the cancer cells. Because of these, we must discuss how the ICP works and we need ICP inhibition to fight against cancer (Figure 8).



**Figure 8.** Immun Check Points (ICP)

There are natural immunocompromised checkpoints against autoimmunization (CTLA4, PD1, PDL1) and block the immune response, otherwise, the body will accept its normal cells as foreign. CTLA4 (Cytotoxic T Lymphocyte Associated antigen 4) immunity checkpoint prevents self-attack in normal human and normal tissues, protects us and prevents autoimmune disease. CTLA4 is on T lymphocyte, and if B7 (B; Bursa of Fabricius was used to identify unknown B cell antigens and ligands in studies previously performed with monoclonal antibodies. Now, this terminology is also used in APC and DC. B7 peripheral membrane protein; T cell surface costimulatory molecule) are on the normal and APC or cancer cells. If CTLA4 and B7 binds; T lymphocyte cannot attacks.

The MHC antigen stimulates APC and DC receptors, also at the same time directly stimulates on T cell receptors. CD28 (Cluster of Differentiation 28) in the T cell communicates with B7 in APC provides immune activation. When this activation reaches a certain level, CTLA4 which is on the active T cell binds to the B7 ligands on DS or APC in order to avoid the autoimmune attack and immuno-suppression. If anti-CTLA4 is given, B7 continuously stimulates the T cell and increases the immune response. B7 and CTLA4 receptor interaction between the tumor cell and APC then inactivates T cell. If Anti CTLA4 is given, T cell becomes active. Anti CTLA4 T blocks CTLA4 on the cell. The APC stimulus continues at T and T attacks cancer. If anti-CTLA4 (Ipilimumab) is given, B7 cannot bind to CTLA4 in dendritic cells or in APC, and the attack continues (Figure 9). Also, APC (antigen presenting cell) stimulation continues on T-lymphocytes, the attack is exacerbated, but it is also necessary to pay careful attention to the attacking of normal tissues which cause hazards and side effects.



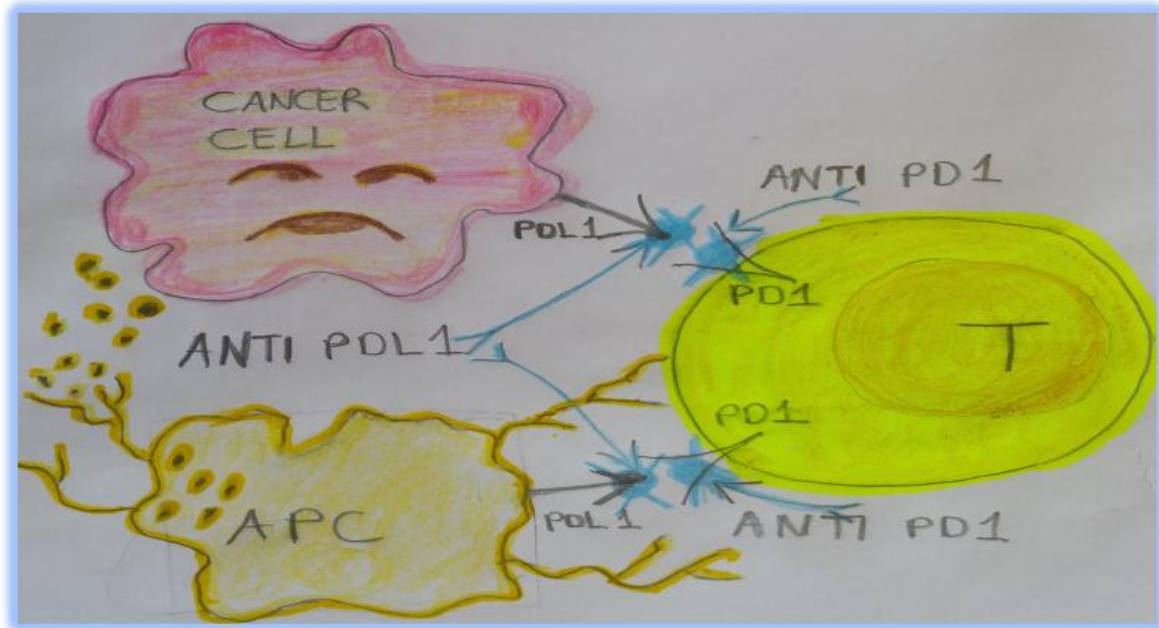
**Figure 9.** Activation and inhibition with B7, CD28 and CTLA4 interactions

The PD1 is on T CELL and PDL1 is on NORMAL and APC and cancer cells. When RT is given, PD1 and PDL1 are overexpressed. If a contact on T cell PD1+PDL1 on the tumor cell/normal/APC cell deactivates the immune T cell. ICI antibodies (anti-PD, anti-PDL1) continues to activate the immune response against cancer (Figure 10).

PD1 is present in activated T cells and must bind to PDL1 to suppress T cell immunity. PDL1-2 is present not only in antigen presenting cells but also in cancer cells, and T cell inhibition occurs when the PDL1 ligand of APC or tumor cell meets with PD1. Therefore, if anti PDL1 is given, PDL1 and PD1



will not meet, APC will not be suppressed, and it will continuously stimulate APC and the killer cell will attack cancer in the active state. Cancer can not prevent T cell attacks.



**Figure 10.** PD1/PDL1 interactions and anti PD1/anti PDL1

Anti PDL1 closes the PDL in cancer and in APC. The PD1 in cancer and in the APC cannot contact with PDL and the T attack continues. if we give an ICI, auto-immunity is exacerbated and side effect increases. If B7 is blocked, the CD28 stimulus is turned off and immune stimulation is reduced [23, 24].

#### 4. Side effects of ICI+RT [25, 26]:

The most common autoimmune side effect is on the skin, like vitiligo, but G3-4 is less. In addition, gastroenteritis, nausea, vomiting, reduced appetite, fatigue can be seen. Also, colitis, pneumonia, hepatitis, nephritis may occur. Attacks on the endocrine system can lead to hypothyroidism, thyroiditis, hypophysitis, colitis, pneumonia, these are threatening the life.

CTLA4 is the first and early control point and early side effects can be expected (dermatitis, vitiligo, gastroenteritis, hepatitis, endocrinopathy, thyroid dysfunction). PD1 / PDL1 is the second control point and late side effects can be expected. (Lung pneumonia in the late period).

#### 5. RT Dose and timing [10-14, 27]:

Low-dose RT is insufficient to activate the immune system. Very high doses completely eliminate the anti-tumor effect of the immunizing system. In addition, excessive antigen presentation may also exert a stifling effect on the immune response. Because of this, the dose must be stimulating cytotoxic cells while decreasing T regulatory cells.

Low doses below 2Gy can not kill cells. It is more likely to induce macrophage stimulation, where the macrophages (M2/ TAM) provide anti-inflammatory effects and thus impair the immune response. At doses above 2 Gy, cancer cell surface antigens (MHC1, ICAM, Fas) are exaggerated from the cell.

In X-Ray RT, the input and output doses act on normal tissues and also suppress the immunological response at the around and within cancer. Proton is better in this respect [28].

The hypofractionated RT with stereotactic body RT (SBRT) with 1x20Gy, 3x8Gy, 5x6Gy is recommended (3 or 5 fractions are preferred to 1 fraction. The best is 3x8Gy) such as 3x8Gy or 5x6Gy + anti-CTLA4 (3 mg/kg every 1-3 weeks for 4 doses) instead of a single dose of 1x20Gy or 1x15Gy, 5x3Gy, 3x5Gy, 2x7.5Gy (The best is 2x7.5Gy).

If ICI is given too long before RT, the RT given later will destroy existing cytotoxic T lymphocytes and the response will diminish. If immunotherapy is given just prior to RT, there is an immunologically prepared environment and RT effect is increased. If RT is given first, the cells that are found to recognize and to kill the tumor cells die and the immune response can only be achieved by introducing immunogenic cells from the outside. The best is given; just before RT, or during RT, or immediately after RT. But, in the near future, we must discuss for the use of SBRT because the microenvironment is very important and with hypofractionated stereotactic radiotherapy, the microenvironment around the cancer tissue such as fibroblast may survive and then may help cancer stem cells progression [4, 5].

## 6. Conclusion

It is clear that the RT activates the immune response [6, 29-33]. It is popular to use hypofractionated SBRT, but we have risk because of the protected fibroblasts around the irradiated volume may survive and then help cancer stem cell progression [4, 5, 31]. After RT there are many changes in the cancer cell or around the tumor microenvironment and these activate the immune response. But the cells have some immune checkpoints to protect autoimmune disease after a certain level of immune activation. If we use the ICI with RT we may see the better immune response but we may pay attention to the side effects of these applications. Hypofractionated stereotactic body radiotherapy is widely used in clinical practice to achieve an immune response, but in the microenvironment around the cancer tissue such as fibroblast may survive and may help cancer stem cells survive and progression, and this is very important subject should be taken into consideration in order to be better understood in future studies.

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