Iranian Journal of Blood & Cancer

Journal Home Page: www.ijbc.ir

Review

Remodeling of Tumor Microenvironment for Enhanced Immunotherapy against Non-Melanoma Skin Cancer

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ARTICLE INFO

Article History:
Received: 20/04/2023
Accepted: 08/06/2023

Keywords:
Skin cancer
Tumor microenvironment
Immunotherapy
Oncolytic viruses
Radiotherapy

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Abstract

Non-melanoma skin cancer is a serious malignancy and white skinned people are highly susceptible to this cancer. About 918 deaths occurred due to NMSC in UK following the year 2018-2019. The incidence of NMSC is 18-20 times higher as compared to melanoma skin cancer. The tumor immune microenvironment (TME) of NMSC possess diversity of immune cells which exert pro-tumor and anti-tumor effects on the TIME. So by recognizing the tumor promoting entities, the TIME can be remodeled. Immunotherapy provides such a treatment that activates the person's immune system to fight against tumorigenic cells. Radiotherapy also cause the modulation of the immune system and increase the anti-tumor responses in patients. The use of immune checkpoint inhibitors (ICIs) after radiotherapy has produced significant survival rates in patients. Oncolytic virus therapy is a subtype of immunotherapy with positive response in the treatment of cancer. The synthetic viral promoter highly specific to tumor and introduction of transgenes help them to inhibit the tumor promoting cells and make the tumor susceptible to anti-tumor cells, thus helping the tumor to eliminate from the body. This characteristic of oncolytic virus convert the "cold TIME" to "hot TIME" which exert a highly positive response when used ICIs. In this article, a literature review is conduced to study the role of TIME in the progression of cancer and various methods that remodel the TIME such as immunotherapy, radiotherapy and oncolytic viruses that might help to treat NMSC.

Please cite this article as: Farman A. Remodeling of Tumor Microenvironment for Enhanced Immunotherapy against Non-Melanoma Skin Cancer. Iranian Journal of Blood and Cancer. 2023; 15(2):128-145.

1. Introduction

Skin cancer is affecting the population globally being more common among Caucasian population. There are two major types of skin cancer including malignant melanoma (MM) and non-melanoma skin cancer (NMSC) (1).As far as NMSC is concerned, there are three major types squamous cell carcinoma (SCC), Merkel cell carcinoma (MCC), basal cell carcinoma (BCC). According to WHO, the number of non-melanoma skin cancer worldwide recorded in 2020 are one million, one hundred ninety-eight thousand and seventy-three. The people of North america and Europe are mostly affected comprising over a combined percentage of 78 percent of all cases reported worldwide. In Asia total 87040 NMSC cases reported out of which 4352 cases are seen in Pakistan. Males are more susceptible to this cancer than women (2).

Till date the non-surgical treatments for NMSC are available such as photodynamic therapy and laser. Initially, NMSC was treated by "Photodynamic Therapy". In this method, the cancerous cells are made photosensitized by a drug that is stimulated by light and destroys them after applying a prescribed medicine levulan which is aminolevulinic acid. This is

a painful process because the skin is very sensitive due to levulan. The patient has to be completely stayed away from the sunlight and proper prevention is needed. Moreover, the patient feels itching after the treatment on the skin. Laser can also be used as treatment option at early stages of NMSC and actinic keratosis. Cryotherapy uses liquid nitrogen to kill the precancerous cells. A blister is formed on the skin when cryotherapy is applied. For Basal Cell Carcinoma, where the large skin area is affected Mohs' Surgery is the best option. This is called "Complete Margin Assessment Surgery". The objective of this surgery is to maximum eradicate the tumor. The affected part is removed by surgery and diagnosed in the lab under microscope time to time until there is no tumor left. Chemotherapy is the use of chemicals to inhibit the growth and proliferation of cancer. The "topical treatment" continues on the daily basis which include the use of medications such as diclofenac (Solaraze), ingenol mebutate (Picato) and fluorouracil on the skin in case of actinic keratosis. Chemotherapy is especially important in dealing with SCC in which fluorouracil cream (5FU) improved its effectiveness. This is somehow palliative type of treatment which temporarily slow down the growth of cancer. The topical medication such as imiquimod is used for BCC that activates the immune system to kill the tumor (3). After surgery and chemotherapy, radiotherapy (RT) is best option to treat cancer (4). About two third of all cancer patients receive radiotherapy during the course of their disease. The ionizing radiation target the DNA of tumor cells thereby inhibiting the proliferation of tumor (5).Radiation directly or indirectly cause cell death of the cancerous cells. The direct effect of radiation results in breaking the double strands of DNA while reactive oxygen species generate due to the indirect effect of radiation. As a result radiotherapy inhibit the proliferation of cancer cells (6).Radiotherapy (RT) uses highly energetic beam of particles to invade the cancerous cells. Patients usually prefer RT if they want to avoid the surgical removal of tumor. But in MCC, RT is applied after the patient has undergone surgery. Such a type of treatment is called "adjuvant therapy". It is to be noted that if the patient is suffering from nevoid BCC syndrome then RT is not a good option for him. Targeted therapy specifically inhibits the metastasis by targeting the genes, proteins or tumor microenvironment. Targeted therapy is applied in case of advanced metastatic BCC when there is no benefit with surgery and chemotherapy. Immunotherapy is a medication-based treatment which activates the patient's immune system to fight against infections. There are 3 FDA approved drugs for NMSC which are Cemiplimab (Libtayo), Pembrolizumab (Keytruda) and Avelumab (Bavencio). These are immune checkpoint inhibitors (ICIs) that interfere with PD-1 or PDL-1 pathway and help in the treatment of NMSC (3).

The term TIME refers to tumor immune microenvironment that facilitates tumor growth and cancer metastasis. TIME includes various immune cells, natural killer cells (7), dendritic cells (8), B cells (9) and T cells of adaptive immune system (10), along with fibroblasts (11),vascular endothelial cells (12) , neutrophils , stellate cells, adipocytes, extracellular matrix ,blood vessels along with stromal cells (13). TIME can be remodeled by various methods such as immunotherapy and radiotherapy or sometimes both are combined in order to increase the overall survival of the patients and increase the efficiency to kill the tumors (14). The oncolytic viruses (15) are another way to treat cancer which help in the regression of the tumors. The OVs either halt the growth of tumor by interfering with the protein synthesis or they directly invade unhealthy cells by releasing cytokines and tumor antigens (16).

Herein, the treatment of NMSC by the help of combinational therapies such as immunotherapy, radiotherapy, and oncolytic viruses a subtype of immunotherapy will be discussed. Tumor immune microenvironment (TIME) is important factor that plays a key role in the development of tumor and metastasis. The oncolytic viruses convert the cold tumor to the hot ones which respond better with ICIs. Similarly, radiotherapy remodel the TIME which give appreciable results when treated with ICIs. The idea of combining RT and immunotherapy allowed successful results in different cancer such as pancreatic cancer (17), head and neck cancer and metastatic melanoma (18).

2. Methodology

A literature search carried out on the sources such as Google Scholar and PubMed/Medline database, National Cancer Institute, Cancer.Net in order to mention the studies that is highly relevant to the review published till January 2023. The searched keywords were tumor immune microenvironment; immunotherapy; immune checkpoint inhibitors for the treatment of NMSC; radiotherapy for NMSC;

oncolytic viruses and variety of combinations of the aforementioned keywords. Moreover, the search was limited to English language articles.

3. Non-Melanoma Skin Cancer

The most prevalent neoplasm is the skin cancer that is very common among the European population (19). It comprises 1/3 of all cancers recorded in the world yearly (20). There are two types of skin cancer; malignant melanoma (MM) and non-melanoma skin cancer (NMSC). Malignant melanoma comprises the minimum percentage of skin cancer about 5 percent while the incidence of non-melanoma skin cancer NMSC is far greater than MM because this is associated with aging and recent statistics proved more than one million cases per year (21). The significant difference between Malignant Melanoma (MM) and NMSC lies in their metastasis which showed that MM is the major cause of death owing to greater rate of metastasis (22). Among NMSCs, the two major types are basal cell carcinoma (BCC) and squamous cell carcinoma (SCC). BCC represents about 80 to 85 percent cases resulting in morbidity but rarely cause death while SCC accounts for 15 to 20 percent cases (23). BCC and SCC are collectively called "keratinocyte carcinoma (KC)" previously known as NMSC. The morbidity rate is higher in BCC not causing significant deaths as compared to SCC which has higher rate of mortality especially the immunocompromised patients or the recipients of organ transplantation (24). Another type of non-melanoma skin cancer is Merkel Cell Carcinoma which is rare however can become dangerous if it affect lymph nodes and other distant organs (25). The data collected from the last five years indicate the mortality rate due to this skin cancer is 37% but if the lymph nodes affected the percentage increases up to 47% (26).

4. Environmental Factors

According to the joint methodology adopted by World Health Organization (WHO) and International Labor Organization (ILO), the ultraviolent radiation (UV) is a potential risk factor of both cutaneous melanoma and non-melanoma skin cancer. UV is categorized into different types depending upon the wavelength; ultraviolet A (UVA) ranges in wavelength from 340nm to 400nm. Likewise ultraviolet B (UVB) has a wavelength of 280nm to 320nm and ultraviolet C (UVC) comprises 200nm to 280nm wavelength of the electromagnetic spectrum (27). It has been noticed that "UVA" and "UVB" is the major cause of skin cancer. Since UVA has the longest wavelength, it is capable of approaching the dermis thereby generating reactive oxygen species (ROS) much likely to cause DNA damage. Contrary to that, a large portion of UVB is absorbed in the epidermis layer, resulting in the direct damage to DNA which produces pyrimidine dimers compromising the integrity and DNA repair mechanisms. Since UVC is almost absorbed by the ozone layer, so it is not potentially damaging to skin. It has been noticed that incidence of squamous cell carcinoma (SCC) is 50 to 70 percent while chances of occurrence basal cell carcinoma (BCC) is 50 to 90 percent among fair skinned people. Latest meta analytic studies showed that workers exposed to UVR during professional activities are more likely to have chances of SCC and actinic keratosis by 77 percent while 43 percent chances are there to have BCC as compared to the other non-workers. Not only environmental factors play a vital role in NMSC but there are certain genetic factors involved (28, 29).

5. Genetics of NMSC

The mutations in the genetic makeup of living organisms has always been under observation for the studies of the various types of tumors. These mutations usually alter the expression of many genes specifically tumor suppressor genes and oncogenes (30). Vechtomova et al. 2021 observed that UVR is the main cause of damage to DNA. Moreover, the recent studies showed that constant exposure upto one hour to the UVR causes 1000 to 2000 DNA lesions, interfering the replication and transcription mechanisms (31). Consistent with it, the mutations resulting from the NMSC lesions give rise to such phenotypic alterations that result in more complications. This plasticity of cancer cells increases the tendency to escape from cell regulation systems (32).The different cells such as fibroblasts, melanocytes and keratinocytes are isolated from human skin and these are exposed to UVB radiation. It was observed that a significant number of keratinocytes were affected, though there is DNA repair mechanisms in these cells was active but not efficient enough to maintain the stability of genome leading to the inability of these cells to undergo apoptosis (33).`Similarly, exome sequencing on the high risk SKH-1 hairless mice was carried out. These animal models were exposed to chronic and intermittent doses of solar stimulated UV radiations (SSUV), that lead to the development of SCC which has resemblance when compared to the histopathological spectra of the humans. Moreover, a number of signaling pathways and cellular

processes are affected by the mutations in the tumor suppressor genes (34). The enzymes such as "DNA photolyase" has prominent role in DNA repair processes which repair the DNA lesions caused by the photoproducts such as cyclobutane pyrimidine dimers and pyrimidine pyrimidone photoproducts when the skin is exposed to UVR (35). The genes that respond to UVR is identified. SerpinB2 is identified for a well-known role in the regulation of inflammation, apoptosis and metastasis but studies found that several cell lines of this gene is upregulated when activated by UVR. SerpinB2 removes nucleotide excision repair (NER) from DNA that can lead to tumor development (36). The study of further genes that respond to UV might be helpful in understanding the genetics of NMSC.

6.Overview of Tumor Immune Microenvironment (TIME)

Tumor immune microenvironment is such a cellular microenvironment that facilitate tumor growth and development. The key components of TIME are blood vessels, some cells of immune system, fibroblasts, bone marrow derived inflammatory cells, and signaling molecules. Within TIME, the interplay between tumor cells and normal cells determine the cancer metastasis. The tumor cells have the ability to promote tumorigenesis causing cell division rapidly. The tumor cells kill other normal cells and spread throughout the body via lymph and blood. Endothelial cells have a significant role in tumor progression and shield the tumor cells from the immune cells. The other cells found in TIME are granulocytes, lymphocytes, macrophages. Out of these, macrophages M2 have well known role in immunosuppression that lead to tumor progression. Tumor associated macrophages (TAM) can either enhance (M2) or suppress the immune response (MI). TAM are well known for their ability to make the tumors more susceptible to immunotherapeutic treatment (37). Fibroblasts are also an important component of TIME. Fibroblasts promote metastasis by permitting cancer cells to move from primary location into the bloodstream. Moreover, fibroblasts enhance angiogenesis in the tumor by the migration and proliferation of endothelial cells (38). The extracellular matrix (ECM) contribution in the development of tumor immune microenvironment has been confirmed by many previous studies. The ECM comprises of complex network of various glycoproteins, enzymes, and collagens that enhance biomechanical functions occurring in the body. The significant tissue components present in ECM help the cells to communicate, divide and adhesion with other cells. The integrins are important growth factors that help in the communication of cells with the TIME. Hypoxia and angiogenesis are also important characteristics of TIME. Cancerous cells need oxygen and nutrients to grow and proper blood supply which is a process called angiogenesis (39). Hypoxia which is essential for the progression of tumor and its aggressiveness, maintain metabolism, angiogenesis of the TIME and cause poor prognosis (40).

7. Tumor Promoting Cells

7.1. Regulatory T cells (Tregs)

The TIME recruit various immune cells and by suppressing the anti-tumor properties enhance the progression of tumor (41). Regulatory T cells (Tregs) and myeloid derived suppressor cells (MDSCs) are among the tumor promoting cells that enhance the tumor progression. Tregs belong to the family of T cells that are involved in maintaining the peripheral tolerance and homeostasis of the immune system (42, 43). For the differentiation of T cell line lineage and suppression of certain regulatory signals, Foxp3+ is essential marker expressed on the cell surface of Tregs together with the expression of CD4 markers (44). The activity of Foxp3+ on the Tregs is like a double-edged sword. The one end can suppress the autoimmune response and inhibit the hyper-active immune response. In contrast to that, within TIME the Tregs inhibit the activity cytotoxic T lymphocytes on the cancerous cells, thus enhancing the metastasis (45). Moreover, the single-cell sequencing of Tregs proved their heterogeneous nature (46) and rapid expansion of Tregs is an important feature in the prognosis of the patients (47).

7.2. Myeloid Derived Suppressor Cells (MDSCs)

In the previous years, a research carried out by Yang et al.2006 revealed that there is another kind of immune cells that play a significant role in the progression of cancer that are named as myeloid derived suppressor cells (48). There are two types of MDSCs; the one which are polymorphonuclear or granulocytic (PMN-MDSCs) behaves and functions much like neutrophils MDSCs and the other is monocytic MDSCs resembling and functioning much like monocytes. The significant population of MDSCs is composed of "PMN-MDSCs" accounting for 80% of the total MDSCs in most cancerous cells (49). The interaction between MDSCs and PMN-MDSCs is important for the development of tumor (50). A recent studies showed that MDSCs carry out metastasis by producing MMP9 (51), prokineticin 2 (52) and vascular endothelial cells (53). MDSCs

can produce arginase (54), nitric oxide synthase (55), transforming growth factor B (56) and interleukin-10 (57). Anderson et.al 2020 explained that MDSCs enhance the tumor suppression by inhibiting the activity of T cells through the release of various cytokines, reactive oxygen species, and production of Tregs, alteration in T cell receptor (58). Recent research is being done to enhance immunotherapy by inhibiting MDSCs trafficking that boost up the function of T cells. So a lot of work is under consideration related to find out different ways based on T cell immunotherapy (59).

7.3. Cancer Associated Fibroblasts (CAF)

Fibroblasts are known for their extracellular matrix remodeling properties (60).Fibroblasts are activated during wound repair, fibrosis. L.W. Chung et al. was the 1st who reported the role of fibroblasts in tumor progression so named as cancer associated fibroblasts (61). CAF may have pro and anti-tumor functions (62). The fibroblasts present within the tumor sites act as main source for the production of cancer associated fibroblasts. Moreover, TGF-B1 (63), platelet-derived growth factor (PDGF) (64), sonic hedgehog (SHH) and interleukin (IL) 1-β, fibroblast growth factor (FGF), released by the tumors stimulates the conversion of fibroblasts to CAF (65). Hypoxia inducible factor (HIF)-1α-mediated signaling pathway responsible for the formation of hypoxia in TIME also act as a factor that activates resident fibroblasts to CAF. These CAFs present a major resistance to PD-1 protein as seen in melanoma but human studies are needed to prove it (66).

8. Tumor Antagonizing cells

8.1. Effector T cells

There are three types of effector T cells; helper T cells, regulatory T cells and cytotoxic T cells. MHC class1 present intracellular peptides on the cell surface of nearly all the nucleated cells of the body that are recognized by cytotoxic T cells having CD8 receptors. In comparison to that, MHC class 2 present extracellular peptides on the cell surface of immune system; lymphocytes and macrophages recognized by helper T cells having CD4 receptors (67). CD8+T cells are converted to effector CD8+T cells which have cytotoxic role in the TIME when activated by Dendritic cells (DCs) (68).DCs are professional antigen presenters that can activate T cells and other immune cells such as natural killer cells and B cells. The activated CTL travel towards the TIME due to chemokines such as CXCL9 and CXCL10 which are released by the DCs (69). One of the main step in the recruitment of CD8+T cells includes the binding of ligand CD70 and CD80–CD86 present on DCs to the receptors such as CD27 and CD28 that are present on the CD8+T cells (68). CD4 helper T cells can also activate CD8+T cells both directly and indirectly. The IL-2 produced from CD4+T cells enhancing the expansion of CD8+T cells and also through the interaction by CD40–CD40L are the direct methods of activation of CD8+T cells (70, 71). CD4+T cells causes the DCs to activate them by presenting tumor antigens to CD8 T cells (72). CD4+T cells can also form memory CTL (73). In the TIME the different methods that cause the cytotoxicity by CD8+T cells include by exocytosis, Fas ligand (FasL) induced apoptosis, interferon-γ (IFN-γ) and tumor necrosis factor TNFα (68). Cancer cells can resist the CTLs if PDL-1 (74) bind to inhibitory checkpoints such as CTLA-4 (73) and PD-1 which can stop the activation of T cells. In the TIME, the CTLs can attain "exhausted state" which lead to dysfunctioning of T cells and help in the growth of tumor (75, 76).

8.2. Natural Killer Cells (NK Cell)

Natural killer cells from the blood span over the extracellular matrix and tumor stromal cells towards the tumor bed. Several chemokines such as CCL5-CCR5, CCL27-CCR10, and CX3CL1-CX3CR1 recruit NK cells to the tumor bed where they perform their tumor antagonizing activities (77). The tumor suppressing activities of NK cells are degranulation, or causes apoptosis via ADCC, FasL or Trail by producing granzymes and perforins (78).The activated NK cells activate the adaptive immune system that recruit T cells against PD-1 antibodies in the tumor, By the adoptive transfer of iPSC-NK cells. Activated T cells has the ability to direct other immune cells by releasing various cytokines such as IFN-γ, GM-CSF, G-CSF, M-CSF, TNF, IL-5, IL-10, IL-13, TGF-α, XCL, CCL3/4/5 (79).Among these IFN γ represent significant antitumor immune response that causes a number of immune cells to perform anti tumoral activities such as macrophages, DCs, T cells, B cells, and even NK cells (80).

8.3. M1 Macrophages

Macrophages are innate cells of the immune system

and are originated from monocytes. These are present in all tissues and have different names such as microglial and Kupffur cells. Macrophages are classified into two types depending upon the polarized state that can be pro-inflammatory M1 and anti-inflammatory M2 (81). M1 type causes the killing of tumor cells in two ways; via reactive oxygen specie (ROS) or nitric oxide (NO) that induce cytotoxicity of cancerous cells but this takes time usually 1 to 3 days (82). M1 macrophages also use anti-tumor antibodies which causes the invasion of tumor cells through antibody dependent cell mediated cytotoxicity (ADCC) (83). This is a quick response that occurs within few hours as compared to the cytotoxicity via NO or ROS. So these are some tumor antagonistic activities of pro-inflammatory M1 macrophages. The M2 macrophages are also known as tumor associated macrophages (TAM) due to their role in tumorigenesis. Therefore targeting TAM to stop the metastasis can be a promising therapy for the treatment of cancer in the future (84).

8.4. N 1 Polarized Neutrophils

Like macrophages, neutrophils are also N1 and N2 polarized. The research conducted on human and animal models have shown that neutrophils are bifaced that is they can suppress or promote tumor development. So neutrophils are classified as tumor antagonistic N1 and N2 are tumor associated neutrophils (TAM) that help in tumor development and progression (85). The granules of N1 neutrophils are enriched with antimicrobial and cytotoxic compounds that invade microbes and cancerous cells and also recruit other immune cells by secreting various cytokines and chemokines (86). Neutrophils also use ADCC pathway to kill cancerous cells which are antibody opsonized (87). Cancer cells resist antibody-mediated destruction by neutrophils through activation of the exocyst complex opsonized. A clinical trial showed that if the the interaction between CD47 receptor and its ligand signal regulatory protein SiRP is interrupted, the function of ADCC can be enhanced which ultimately invade the cancerous cells because neutrophils express Fc receptors through which recognition and phagocytosis occurs. So immune checkpoint that inhibits CD47\SiRP can give a new gateway to cancer immunotherapy (88). Tumorigenesis can be postponed by neutrophils as they present tumor antigens to CD8+T cells they also activate immune system and interferon gamma from CD4 CD8 unusual alpha-beta T cells by releasing interleukin 12 (IL-12) (89).

9. Immunotherapy

Immunotherapy helps to enhance the body's natural immune system to fight against cancer. This treatment is also called biological therapy, biological response therapy (BRM) or more commonly named as biotherapy because it relies on the substances derived from living organisms and prepared in the lab (90). It is one of types of the systemic treatment that target cancer cells throughout the body (91). Immunotherapy is now regarded as the 5th pillar of cancer therapy which connects the areas of surgery, chemotherapy, radiation, and targeted therapy (92).

9.1. The Importance of Immunotherapy to Treat NMSC

Various immunotherapeutic drugs also called immune checkpoint inhibitors are designed to treat non-melanoma skin cancer which is a prevalent form of cancer in these days. Immune system is regulated by checkpoints that are found on the surface of T cells and prevent T cells from killing non-cancerous cells. The binding of checkpoint to their respective partners on the tumor cells act as off signal for the T cells that inhibit the activity of T cells. In this way cancerous cells escape the immune system. In order to destroy the tumors scientists have synthesized immune check point inhibitors (ICI) that block the binding of immune checkpoints with their partners and T cells remain active and ultimately tumors are killed. One such drug known as anti-PD 1 or anti-PD L1 is synthesized and useful to treat a number of cancers. Another ICI is prepared that target CTL-4 on the surface T cells (93). Recently, another immunotherapeutic drug named as Opdualag is synthesized by combining two other drugs relatimab and nivolumab which is being used in the case of advanced melanoma approved by FDA (94).To treat the different stages of non-melonoma skin cancer various FDA approved drugs such as Cemiplimab (Libtayo), Pembrolizumab (Keytruda), Avelumab (Bavencio) are used today. Immunotherapy sometimes can cause side effects in different people how their body respond to the immunotherapeutic treatment.

9.2. Clinical Trials on Skin Cancer

SCC: In phase 1 clinical trial Pembrolizumab is approved For Squamous Cell Carcinoma (SCC) and Basal Cell Carcinoma (BCC) surgery remained the probable option to treat the affected area of the skin. Particularly, Mohs surgery is used to precisely excise

the skin cancer when there are chances that skin cancer may reappear. If the cancer is seen on larger area of the skin then radiotherapy may be employed. Radiotherapy kill the tumorigenic cells or cause a halt in further development of skin cancer. But for advanced squamous cell carcinoma surgery or radiotherapy may not be an effective treatment option. In this case, radiotherapy can only give appreciable results when used along with` immunotherapeutic treatments as concomitant therapy. For metastatic, advanced squamous cell carcinoma, the two clinical trials NCT02383212 (dose amplification study phase 1) and NCT02760498 (open-label EMPOWER SCC phase 2 study) were conducted in order to assess the safety, efficacy of the Cemiplimab for the patients that were not amenable to treatment by surgery or radiotherapy. The patients received Cemiplimab after every 2nd or 3rd week. These patients received either radiotherapy or surgery before injected Cemiplimab. They were kept under examination for probably 8.9 months after injecting Cemiplimab intravenously. The patients reported a significant tumor suppression or even successfully eliminated tumor. Based upon these results, Cemiplimab was approved by FDA in 2018 for metastatic and locally advanced squamous cell carcinoma (95). Moreover, another anti PD-1 drug Pembrolizumab approved by FDA based on the results of the open-label KEYNOTE-629 trial in 2020 (96).

BCC: Certain environmental factors such as UVR (97) or mutated hegdehog pathway (98) can lead to Basal cell carcinoma. The FDA approved targeted drugs for BCC is Hedgehog Signaling Inhibitors vismodegib (Erivedge) and sonidegib (Odomzo). These interfere with protein patched homologue 1 (PTCH1) mutations which is responsible for the development of BCC. The patients experienced a shrinkage of the tumor (99). Moreover, for advanced BCC FDA approved anti-PD-1 Cemiplimab in 2021 based on the results of the phase 2 open-label NCT03132636 study. This trial included 112 patients out of which 84 have locally advanced BCC and 28 have metastatic state. All these patients may or may not have undergone Hedgehog pathway inhibitor therapy. There was either a complete removal of the tumor or significantly reduced tumor in patients of locally advanced or metastatic BCC. These patients received a 350mg dose of Cemiplimab every 3rd week and this process continued for 2 years. The response rate recorded in the patients of locally advanced BCC was 31% and 21% recorded in the patients of metastatic BCC (95).

MCC: FDA approved Avelumab for the treatment of MCC based on the results of the open-label JEVELIN Merkel 200 phase 2 study. About 204 patients undertaken in this study were given 10mg/kg Avelumab intravenously every two weeks. Maximum responded effectively to this treatment either whole tumor terminated or significantly shrink tumor. However, a few patients seem to have side effects such as dermatitis psoriasiform. These side effects are often termed as treatment related adverse effects or immune related adverse effects. Pembrolizumab anti PD-1 drug is approved by FDA on the basis of the KEYNOTE-O17 study that included 50 patients suffering from recurrent or metastatic MCC. These patients were injected with a dose of 2mg/kg intravenously every 3rd week. The overall response rate recorded in these patients was 58%, which indicates that maximum patients had either whole tumor terminated or significantly shrink tumor While a few patients have some adverse effects (95). A trial carried out by Ryu et.al pointed out that major reason of MCC is a virus known as Merkel Cell Polyomavirus (MCPyV) while a few cases of MCC caused by mutations induced by UVR. Such patients were given anti PD-1 drug Pembrolizumab. The immune profiling of the patients that responded well to this drug revealed their blood enriched with MCPyV specific CD8+T cells, in addition with CD39+cells expressing skin associated markers such as CLA and tissue recirculating marker e.g. CD103. The high concentration of T cells can be used as biomarkers for immunotherapy for MCC (100). The different immunotherapeutic drugs approved by FDA for NMSC is shown in the **Table 1**.

9.3. Limitations of Immunotherapy

These possible side effects of immunotherapy can be nausea, vomiting, loss of appetite, itching, rash, swelling and constipation, pain, fever, cough. In some cases, dealt with immunotherapy, the healthy cells can be affected. Due to these aspects of this treatment, the patient should go for a personalized

Table 1. **The immunotherapeutic drugs approved by FDA for NMSC.**

Immunotherapeutic Drug	Target	SCC	BCC	MCC	Reference
Cemiplimb (Libtayo)	PD-1	●	●		(101), (102)
Pembrolizumab (Keytruda)	PD-1	●		●	(96), (95)
Avelumb (Bavencio)	PDL-1			●	(95)

immunotherapeutic treatment (3). Some other negative effects hypothyroidism, colitis, and pneumonia. These are often named as immune related adverse effects (103). A study performed including 123 patients suffering from tumors and some autoimmune disease when treated with ICIs responded such that 41% experienced severity of their autoimmune disorder, 25% patients reported immune related adverse effects while 9% patients reported both conditions (104).

9.4. Remodeling of TIME by Immunotherapy

The presence of immunosuppressive cells such as Tregs and MDSCs, M2 type, and N2 type all contribute to the immunosuppressive nature of TIME. Due to this reason, the function of immune checkpoint inhibitors (ICI) is affected. TAM has been a major reason of poor prognosis in the cancer patients (105) while CD68 enhance the immunotherapeutic resistance (106). Studies shows that the patients of pancreatic cancer and glioblastoma did not show any response even after the treatment with ICIs, PDL1 and CTL4.In such type of cancers, the immunosuppression of TIME is very high. So further research is needed to minimize the immunosuppression and convert the pro tumorigenic cells into anti-tumor cells that can be promising in the treatment of these cancers. A small percentage of patients e.g. 20-40% respond to immunotherapy. This is all due to the heterogenous TIME which is used by the cancer cells to proliferate further and can escape the immune attack due to which patient usually do not respond to ICIs or develop adaptive or acquired resistance (107). So, there is a need of clinical trials that may remodel the TIME and make cancerous cells more susceptible to the immunotherapeutic treatment because each patient's TIME respond differently to the treatment.

9.5. Clinical Trials to Remodel the TIME

CD40 is a cell surface marker belonging to the tumor necrosis factor receptor (TNFR) which has a noticeable ability to redirect the macrophages and monocytes to anti-inflammatory phenotypes in addition to increasing the activity of antigen presenting cells. So researchers have synthesized the antibodies of CD40 agonists. These antibodies along with ICIs enhance the activity of effector T cells and tumor infiltrating T cells (108) .The use of CD40 agonist antibodies are under the process of approval by FDA (109).Colony stimulating factor (CSF-1) and its respective receptor CSF1R have a significant role in the immunosuppression of TIME.CSF-1 offers the resistance to the adaptive immune system by activating Tregs, MDSCs and TAM polarization thereby enhancing M2 phenotype which is pro tumorigenic (110).The CD8+ cells causes the expression of CSF1 after the treatment with immunotherapy and this is also related to the enhanced infiltration of MDSCs and poor overall survival (OS) (111). The sensitization and reprogramming of TAM can be achieved by CD40 agonists that causes the inhibition of CSFIR when used in along with neoantigen vaccines and chemotherapy depending upon the patient's TIME. This step may be promising towards maximizing antitumor immune response in the patient (112).

The immunosuppression of Tregs can be minimized by targeting different expression markers other than CTL-4 for example CCR4 with mogamulizumab and agonist antibodies against GITR. This can result in enhancing the tendency of newly formed T cells towards effector T cells than Tregs. This type of immunotherapeutic treatment shows promising results along with the use of PD-1 in early trials (113).In some cases after PD-1 therapy, the level of indoleamine 2,3 deoxygenase 1 (IDO1) is increased which offers resistance to the immunotherapy.IDO1 is an enzyme that is expressed along with PDL-1 in the TIME. The function of IDO1 is to inhibit the activity of T cells and the production of tumorigenic M2 macrophages and DCs. IDO1 also enhance the activity of other tumor promoting cells such as Tregs (114).Different modulators that can interrupt IDO1 pathway such as indoximod, navoximod, and epacadostat show appreciable response rates in patients when applied along with anti PD-1 or anti CTL4 in phase 2 trials without the addition of toxicity when used in combination with anti PD-1 (115). But the results of phase 3 trial did

not give any benefit with epacadostat (116). However, the expression of IDO1 in this trial was not stratified that may demonstrate that patients in which enhanced expression of IDO1 in the tumor may be benefitted with this type of immunotherapeutic treatment and also highlights the importance of the group of patients on the basis of drug target.

Various trials regarding the use of antibodies against other molecules which has the ability to suppress the inhibitor signal from the innate immune are continuous. This includes CCR2 which recruits M2 macrophages, CXCR4 that decreases the infiltration of MDSCs in the TIME and increases the recruitment and activation of T cells (117), P13K gamma (118) which alters the M1 phenotype toward the M2 macrophages and TREM2 which is responsible for the activation of TAM (119).

The various types of cancer such as pancreatic cancer, glioblastoma which represent solid tumors show very poor response with ICIs. These cancers did not give efficient results when treated alone with PD-1 due to highly developed immunosuppressive TIME with high infiltration of Tregs, TAM and MDSCs which inhibit the adaptive immune response in the patients. Thus the combination of immunotherapies in addition that target such pro tumorigenic cells might be promising in enhancing the immune system to fight against such malignancies (120).

10. Remodeling of TIME by Oncolytic Viruses

10.1. Mechanism of Action

Oncolytic viruses (OV) are naturally occurring or modified genetically that has the ability to destroy cancerous cells. The genome of these viruses is modified by introducing tumor specific viral promotor or in deletions in the specific regions of the viral genome that ultimately kill tumor cells when entered in the patient. The other genetic modifications include many transgenes that are responsible for encoding interferon alpha, granulocyte macrophage colony stimulating factor (GM-CSF) and various cytokines that collectively have immunomodulatory function. Oncolytic viruses are helpful in awakening the immune system and produce immune response against cancerous cells. When oncolytic virus selectively infects a tumor cell, it makes multiple copies of itself until the cancerous cell destroys. These invading cells release tumor antigens and sends signals that can remodel the tumor immune microenvironment and ultimately converts the cold tumors to the hot ones. Cold tumors usually show a weak immune response and contain significant population of tumor suppressing entities. So by using viral therapy cold tumors can be converted to hot tumors which quickly respond to ICIs (121). In this way, oncolytic viral therapy by remodeling of TIME can be beneficial in immunotherapy (122).

10.2. Clinical Trials of Oncolytic Viruses to Treat Cancer

Many oncolytic viruses have been approved by FDA. Nowadays adenoviruses, polioviruses, reoviruses, poxviruses, and Newcastle disease viruses, herpes viruses, measles viruses, coxsackie viruses can be used as oncolytic viruses. Oncolytic viruses act in two major ways: either these can replicate directly within cancerous cells and result in the lysis of these cells or may activate the systemic immune system (123).Till date, three FDA approved OV drugs are available in market. The first one is "Rigvir" that is OVT used in the treatment of melanoma. It was approved in 2004 non-pathogenic ECHO-7 picornavirus (124). The 2nd OV came in market in 2005 which is used in treatment of head and neck cancer. It is "adenovirus" that has the ability to infect and replicate specifically in P53 tumors. But this OV is not effective in the metastatic state (125). The third OV is talimogene laherparepvec "(T-VEC)" which is approved in 2015 for the treatment of metastatic melanoma and cutaneous melanoma. It is recombinant herpes simplex type 1 virus and has two copies of GM-CSF by CMV promotors. This OV is used in combination with other immunotherapeutic drugs such as pembrolizumab (PD1 inhibitor) and ipilimumab CTL-4 inhibitor for better results (126).

10.3. The Importance of Oncolytic Viruses in Remodeling of TIME

The immunosuppression induced by MDSCs and Tregs can be modified by oncolytic viruses. The oncolytic virus can convert the MDSCs to tumor killing cells that causes the production of nitric oxide from MDSCs shifting them to antitumor phenotype can remodel the tumor microenvironment and help in removal of tumors. For example a research conducted by Eisenstein et al.2013 showed that oncolytic rhabdovirus that is a genetically modified strain of vesicular stomatitis virus (VSV) has strong affinity for MDSCs and causes them their transformation to antitumor phenotype that ultimately destroys the tumor cells (127).

The other significant pro-tumor cells are Tregs. OV can remodel the TIME by decreasing the concentration of tumor infiltrating Tregs. The oncolytic vaccinia virus (VV) demonstrate the reduced population of Tregs in murine models that have head and neck cancer. The OV induced Treg infection lower the levels of IL-2 that ultimately affect the pro-tumor activity of Tregs (128). Measles virus (MV) proved to inactivate Tregs having α-CTLA4 or α-PD-1 in the TIME after treatment (129). Some OV require additional agents to enhance the therapeutic efficacy such as vesicular stomatitis virus (VSV) need a CD25 antibody which is PC61 that significantly causes loss of Tregs in TIME and suppress Tregs mediated inhibition of NK cells (130).

Some oncolytic viruses enhance the activity of natural killer such as reovirus (131), maraba virus (132) and adenovirus (133).Dendritic cells when come in contact with oncolytic viruses release various chemokines which are CCL2, 3, 4, 5, 7, 8, 11 and CXCL10 that recruit and activate natural killer cells. Adenovirus induce cytotoxicity by contact dependent NK activation therefore act as potent anti-cancer agent. The activity of OVs can be affected by NK cells such as reported by HSV oncolytic virus in glioblastoma models (134) and VSV in hepatocellular carcinoma rat models (135). Chesney et al. reported that oncolytic virus e.g. adenovirus Delta24- RGD can convert protumor M2 macrophage phenotype to anti-tumor M1 phenotype in the TIME of glioblastoma patients (114).

11. Comparison of Different Therapies to Treat Non-Melanoma Skin Cancer

Table 2 summarizes different options available to treat NMSC depending upon the depth of tumor and patient response to therapy including the efficacy of the treatment and some the risks associated with it.

11.1. Radiotherapy

Ionizing radiation (IR) produce potent series of biological effects which appeared to be systemic and immune-induced anti-tumor response (145, 146). Park et al. 2014 proved the anti-tumor effects of IR acts in a series of steps rather than a single step of in the cycle of cancer immunity with ICIs (147). Some common effects induced by IR are release and presentation of tumor antigens (148, 149), activation of a number of immune cells (150), enhancing the population of tumor infiltrating lymphocytes (150), enhancing activity of T cells to recognize the tumor antigens thereby exerting anti-tumor responses (151). IR plays important role in remodeling of TIME and convert the "cold TIME" to hot TIME" because of pro-inflammatory signals and cytokines and required for response to ICIs (152, 153).

11.1.1 Radiotherapy Mediated Immuno-activation

Ionizing radiation (IR) can act directly on cancer cells and damage the DNA which is a key immunomodulatory function of RT and the production of ROS species (154) .The immunogenic cell death (155) enhanced by IR and damaged associated molecular pattern (DAMP) secreted from the damaging cancer cells also impact the immunomodulatory function of radiotherapy (RT) and affect the behavioral pattern of various immune cells (156).The release of DAMPs causes dendritic cell antigen presentation, and differentiation of naïve T cells to effector T cells. These T cells induce cytotoxicity in the local tumor sites. At non-irradiated sites of tumor, T cells may respond due to activation by dendritic cells (DCs) which have cytotoxicity against tumor antigens. The TIME of the patients now populated by T cells after radiotherapy quickly respond to ICIs due to presence of large number of PDL-1 as compared to the TIME of the patients enrich with MDSCs. In this case, patients are required to give immune-oncological agents along with radiotherapy which can reprogram MDSCs and enhance T cells infiltration which have anti-tumor effects. It is to be noted that single site radiotherapy has less anti-tumor effects as compared to multiple site radiotherapy due to less infiltrating T cells. The IFN-B and TNF after IR treatment enhance the N1 neutrophils antitumor phenotype which has cytotoxic effects and also activates CD 4+T cells and M1 macrophages which is important in abscopal effect and destroys the tumor by phagocytosis (157). A study recently performed by Kottler D et al. in Caen Hospital, France proved that combinational therapy such as radiotherapy along with immunotherapy pose durable benefit in the patients of squamous cell carcinoma. The study suggested that there was a significant higher response rate in patients treated with combinational therapy (radiotherapy along with Cemiplimab) than those that were treated with Cemiplimab only (158). Thus the antitumor activity is significantly enhanced by radiotherapy along with immunotherapy as dictated by this research.

Table 2. **Current therapeutic options to treat NMSC**

Treatment	Mode of Action	Efficacy	Side effects	Reference
Surgery	Surgical removal of the cancerous cells	Highly efficient at removing cancer	May leave a scar. Often followed by radiotherapy or chemotherapy if there is a chance of recurrence.	(136)
Moh's Surgery	Particular type of surgical treatment in which thin layers of the infected tissues removed successively, till the cancer removed completely	Useful for the tumors with undefined edges, priority if there is recurring BCC, MCC (if the tumor is seen at a delicate area of the body)	Time requiring, Lengthy procedure	(137)
Radiotherapy	High energy radiation that destroy cancerous cells	High success rate for localized tumors	Hypopigmentation, Lengthy time period 1-6 weeks	(138)
Topical Chemotherapy (5-Fluorouracil, Diclofenac)	Anti-cancer drug usually a cream or gel applied to the affected area of the skin	Appropriate for Actinic Keratosis, used to treat sub-clinical lesions efficiently	Prolonged time period 4-16 weeks, reduced persistence that cause low efficacy due to localized skin reactions.	(139)
Cryotherapy	Liquid nitrogen used to kill the abnormal cells of the skin	High success rate, very less recurrence.	Edema, scarring, necrosis of healthy cells may occur, increased ulceration if patient has diabetes mellitus	(140), (141)
Curettage and Electrodessication	Use of Curette and electric needle to destroy the cancerous cells	Cost effective therapy, suitable for primary BCC and SCC	Leave a scar, inflammation of the treated area of the skin	(141)
Photodynamic Therapy	A photosensitizer Compound e.g. ALA penetrate in the tumors when illuminated by light selectively causes destruction of cancerous cells.	High success rate, less recurrence, no scar is appeared	Increased sun sensitivity after therapy often results in inflammation	(142), (143)
Immunotherapy (Immune Checkpoint Inhibitors)	Immunotherapeutic drugs that activate the immune system to invade cancerous cells by blocking CTL-4 and PD-1/PDL-1	Efficacious for patients with advanced NMSC	Immune related adverse effects ….	(95)
Oncolytic viral therapy	Modified viruses that selectively replicate in tumor cells causing their lysis and activation of immune system	FDA approved for melanoma; more trials are needed to prove its efficacy for NMSC	Fatigue, fever or flue may occur as side-effects of this treatment, not suitable for immunocompromised or pregnant patients	(144)

11.1.2. Radiotherapy Mediated Immunosuppression

RT sometimes increases immunosuppression by enhancing MDSCs and Tregs in the TIME. The level of immunosuppressive cytokines are significantly enhanced after radiotherapy. Irradiation causes CD4+T cells to convert to Tregs through IL-10R STAT 3 pathway. The radiation activates ROS and p50 homodimer that favors the polarization towards M2 phenotype and secretion of TGFB and IL-10 prevent DCs function. After this, M2 macrophages secrete CCL2 which leads to the recruitment of Tregs infiltration at the tumor sites. The STING activation of CCL2/CCR2 and CSF1/CSF1R pathways also recruit Tregs. MDSCs can also be recruited by the CSF1/CSF1R

pathway. This can limit the effectiveness of RT (159).

11.1.3. The Therapeutic Approach to Overcome the Immunosuppression

The reappearance of T cells to the TIME accompanied by RT can be solution to this problem. Moreover, the inhibition of CCL2/CCR2 signaling pathway could prevent the recruitment of immunosuppressive MDSCs in the TIME. So efficiency of RT can be improved by the use of dual antagonists CCL2/CCL5 that target these monocytes have reported to decrease the metastatic rate in breast and pancreatic models indicating the importance of MDSCs (160, 161). Buhtoiarov et al. 2005 reported that CD40 agonists can be employed to reprogram macrophages towards M1 phenotype which can further lead to the priming of effector T cells (162). The activation of T cells can also restore by toll like receptors (TLR) agonists. The TLR agonists can also modulate macrophages towards M1 phenotype and help in the conversion of MDSCs to antigen presenting cells (APCs) that initiate T cell activation (163). So RT along with TLR agonists induce anti-tumor immune response and help in regression of tumors in many diseases (164).The function of NK cells may be inhibited by TGFB and of MHC class 1 on the surface of tumor cells. The In 2005, the foundation of radioimmunotherapy was laid by Demaria and colleagues as a way to treat cancer (165). Golden et al. 2015 reported that radiotherapy along with IO;GM-CSF produced significant abscopal effect (166) in a patient suffering from metastatic solid tumors with remarkable results (167). It has been noticed that the use of radiotherapy along with immuno-oncology (RT-IO) combinations may not be as beneficial as compared to multi-site irradiation. Therefore it is proposed that multi-site irradiation might decrease the disease burden and also enhance the chances of RT dependent-immune stimulation (168). During RT the relative dose of IO agent is also important. Moreover, RT-IO combination also depend upon various fact as type of the disease, and site of occurrence of tumor, and the combination of RT-IO (169).

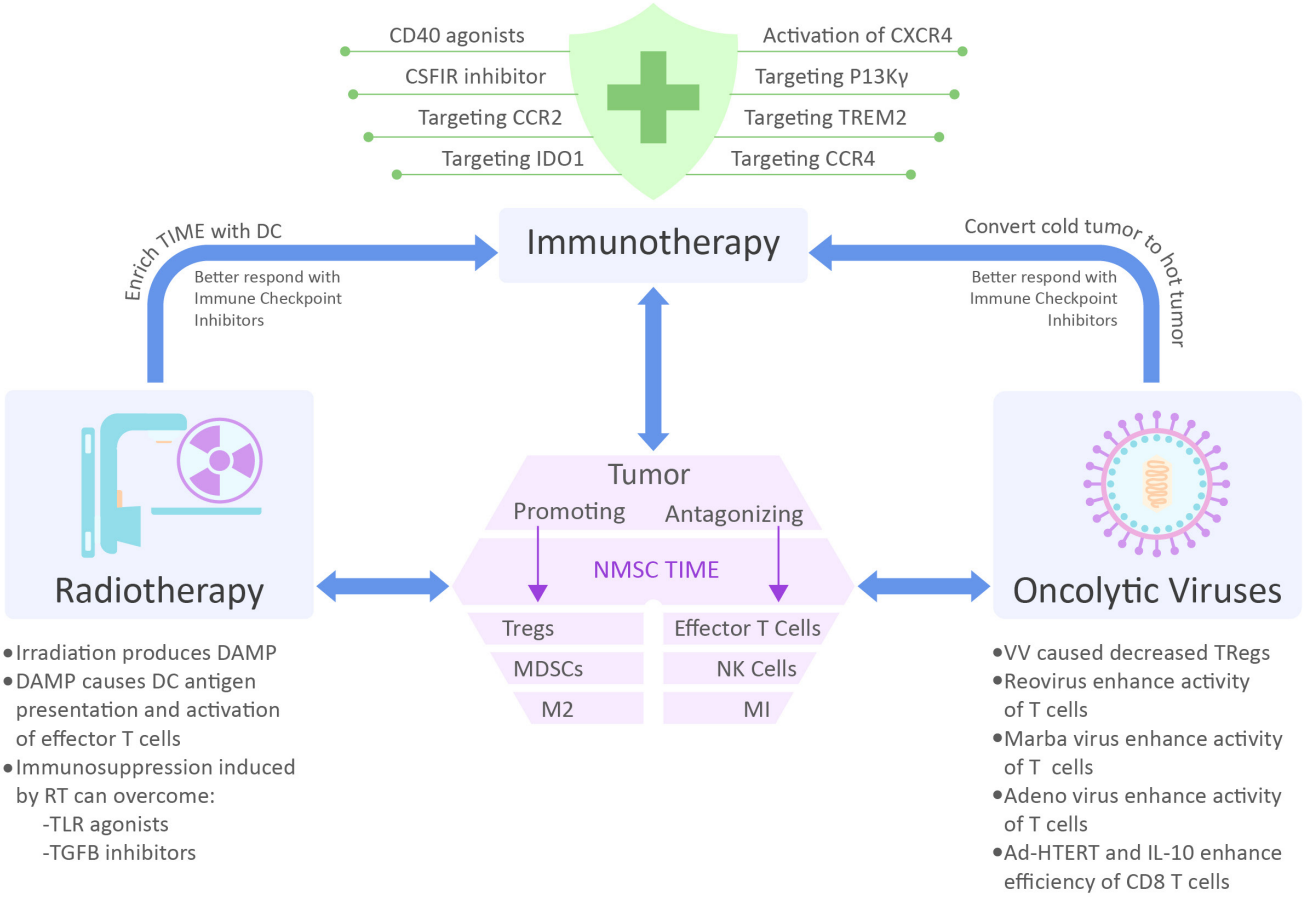

Figure 1. Interrelation of TIME, Immunotherapy, radiotherapy and oncolytic viruses.

12. Conclusion

TIME is important feature of any malignancy that determines the metastatic rate and overall survival of the patients. All the methods that has the ability to remodel the TIME and convert the pro-tumorigenic phenotypes to the anti-tumorigenic phenotypes can be promising in the treatment of that malignancy. Those patients whose TIME is not very complex respond to immune checkpoint inhibitors ICIs. Radiotherapy along with immunotherapy can be beneficial in the treatment against NMSC but sometimes it causes immunosuppression as observed in some malignancies. Viral therapy have also side-effects, so there is need to minimize these side effects. Various methods have devised to reprogram TIME after immunotherapy which increase the infiltration of anti-tumor entities but there is still a research needed to completely understood the mechanism of TIME and control the barrier of immunosuppression against NMSC. The development of dynamic markers is needed to demonstrate the immune-score at any stage of the treatment. There is also a need of personalized therapy because each patient's TIME respond differently. Immunotherapy and radiotherapy are individual treatments for NMSC at present but a lot of work is required in order to achieve the benefit regarding the "combinational therapy" as a solution for NMSC.

Conflict of Interest

The authors declare that they have no conflicts of interest.

Acknowledgement

I would like to appreciate Multiomics, Muhammad Junaid Iqbal from COMSATS University Islamabad and Aghna Maryam from University of Urbino for their invaluable support. I want to extend my heartfelt acknowledgement to my esteemed professor of immunology Dr. Shahbaz Aslam whose exceptional teaching skills ignite the passion for this intricate field of study I'm also thankful to the researchers and authors whose work has been cited in this study and the various databases that laid a solid foundation and provide the valuable research for this study.

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