EGAS MONIZ SCHOOL of HEALTH & SCIENCE | INSTITUTO UNIVERSITÁRIO EGAS MONIZ

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MESTRADO INTEGRADO EM MEDICINA DENTÁRIA

BIOLOGICAL THERAPY FOR ORAL CANCER: IMMUNOTHERAPY

Trabalho submetido por

OUSSAMA AISSAOUI

para a obtenção do grau de Mestre em Medicina Dentária

Julho de 2024

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Prof.Doutor Carlos Zagalo

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DEDICATION:

This thesis is dedicated to my Family for their unwavering support and love, to my future wife, for her constant encouragement and patience, and to my friends for their steadfast camaraderie and belief in me. Lastly, I dedicate this work to myself, in recognition of the perseverance and dedication that have made this achievement possible.

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ABSTRACT:

Malignant tumors treatment, particularly in the case of oral cancer, presents a significant challenge within the realm of medical science. Current treatment modalities, ranging from surgical interventions to chemotherapy and radiation therapy, often yield mixed results. These approaches have their limitations, including the potential for tumor recurrence, non-resectable lesions, and the toxic side effects of radiotherapy and chemotherapy. As a result, there is a pressing need for innovative strategies to improve patient outcomes by reducing morbidity and mortality while enhancing their quality of life.

Immunotherapy, a relatively new avenue in cancer treatment, offers promise as a complementary approach. It has the potential to mitigate the shortcomings of traditional treatments when used in conjunction with them. By harnessing the patient's immune system to enhance the specific immune response to cancer cells, immunotherapy holds significant potential. This promising approach gained widespread recognition when it was deemed the most significant scientific breakthrough by Science, due to its remarkable efficacy and innovation.

Immunotherapy's role in the treatment of various malignant tumors, including oral cancer, is the central focus of this review. The review systematically explores the key elements involved, including the fundamental definition of immunotherapy. It delves into the intricate dynamics of the tumor microenvironment and the unique challenges associated with oral cancer, offering insights into the various techniques and strategies employed in immunotherapy. Furthermore, it highlights the challenges that researchers and clinicians face in this dynamic field, as well as the anticipated future developments.

In essence, this review strives to illuminate the potential of immunotherapy as an invaluable addition to the arsenal of treatment options for oral malignant tumors. It emphasizes the importance of considering immunotherapy in the context of this particular malignancy, highlighting the ongoing challenges and the promising prospects that could substantially benefit patients confronting this debilitating disease.

KEYWORDS: Immunotherapy, Oral cancer, Tumor microenvironment, Treatment modalities

RESUMO:

O tratamento de tumores malignos, particularmente no caso do cancro oral, representa um desafio significativo no domínio da ciência médica. As modalidades de tratamento actuais são essencialmente a cirurgia, a Radioterapia e a quimioterapia. Estes tratamentos produzem frequentemente resultados variáveis e têm limitações, incluindo o potencial de recorrência do tumor a existência de lesões não ressecáveis e os efeitos secundários tóxicos da radioterapia e da quimioterapia. Consequentemente, existe uma necessidade premente de estratégias inovadoras para melhorar os resultados dos doentes, reduzindo a morbilidade e a mortalidade e melhorando a sua qualidade de vida.

A imunoterapia, uma via relativamente nova no tratamento do cancro, é promissora como abordagem complementar. Tem o potencial de atenuar as limitações dos tratamentos tradicionais quando utilizada em conjunto ou em complemento destes. Ao utilizar o sistema imunitário do doente para melhorar a resposta imunitária específica às células cancerígenas, a imunoterapia tem um potencial significativo. Esta abordagem promissora ganhou amplo reconhecimento quando foi considerada a descoberta científica mais significativa pela Revista Science em 2013, devido à sua notável eficácia e inovação.

O papel da imunoterapia no tratamento de vários tumores malignos com incidencia particular no cancro oral, é o foco central desta revisão. A análise explora sistematicamente os elementos-chave envolvidos, incluindo a definição fundamental de imunoterapia. Aprofunda a intrincada dinâmica do microambiente tumoral e os desafios únicos associados à imunoterapia oral.

Essencialmente, esta revisão procura iluminar o potencial da imunoterapia como uma adição inestimável ao arsenal de opções de tratamento para tumores malignos orais. Salienta a importância de considerar a imunoterapia no contexto desta doença maligna específica, destacando os desafios actuais e as perspectivas promissoras que podem beneficiar substancialmente os doentes que enfrentam esta doença debilitante.

PALAVRAS-CHAVE: Imunoterapia, Cancro oral, Microambiente tumoral, Modalidades de tratamento.

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LIST OF ACRONYMS

ABZI: Amidobenzimidazole

AC: Actinic cheilitis

ADCC: Antibody-Dependent Cellular Cytotoxicity

AI: Artificial intelligence

AIDS: Acquired immunodeficiency syndrome

AKI: Acute kidney injury

AKT: Protein Kinase B

ALK: Anaplastic lymphoma kinase

AMP: Adenosine monophosphate

APCs: Antigen-presenting cells

ASCO: American Society of Clinical Oncology

ATP: Adenosine triphosphate

AZB: Azoximer bromide

BCR-ABL: Breakpoint Cluster Region-Abelson tyrosine kinase.

BTLA: B and T lymphocyte attenuator

CAF: Cancer-associated fibroblast

CAR: Chimeric antigen receptor

CDA: Cyclic dimeric adenosine

CDG: Cyclic dimeric guanosine

CDN: Cyclic dinucleotides

CEA: Carcinoembryonic antigen

COVID: CoronaVirus Disease

COX: Cyclooxygenase

CRS: Cytokine Release Syndrome

CSF: Colony-stimulating factor

CTL: Cytotoxic T lymphocyte

CTLA : Cytotoxic T lymphocyte antigen

DC: Dendritic cell

DDR: DNA damage response

DOI: Depth of invasion

ECM: Extracellular matrix

EMT: Epithelial–mesenchymal transition

ENDS: Electronic Nicotine Delivery Systems

ERK: Extracellular Signal-Regulated Kinase

ESMO: European Society of Clinical Oncology

FAD: Flavin Adenine Dinucleotide

FAP: Fibroblast activation protein

FDA: Food and Drug Administration

FU: Fluorouracil

GBD: Global Burden of Diseases

GM-CSF: Granulocyte–macrophage colony-stimulating factor

GMP: Guanosine monophosphate

GVHD: Graft-versus-Host Disease

HA: Hyaluronic acid

HBGF: Heparin-binding growth factor

HDI: Human Development Index

HIF: Hypoxia-inducible factor

HIV: Human Immunodeficiency Virus

HLA: Human Leukocyte Antigen

HNC: Head and neck cancer

HNSCC: Head and neck squamous cell carcinoma

HPV: Human papillomavirus

HVEM Herpes: Virus Entry Mediator

IARC: The International Agency for Research on Cancer

ICB: Immune checkpoint blockade

ICD: Immunogenic cell death

ICI: Immune checkpoint inhibitor

IDO : Indoleamine 2,3-dioxygenase

IFN : Interferon

IL : Interleukin

IO: Integrative oncology

LAG: Lymphocyte-activation gene

LFA-1: Lymphocyte function-associated antigen 1

LOC: Lip and oral cancer

MDSCs: Myeloid-Derived Suppressor Cells

MDT: Multidisciplinary Team

MHC: Major histocompatibility complex

MHP-1 : Malignant Hyperthermia Protein 1

MMPs: Matrix metalloproteinases

MSA-2: Methylsulfonylmethane-2

NADH: Nicotinamide adenine dinucleotide

NIR-PIT: Near-infrared photoimmunotherapy

NK: Natural killer

NPC: Nasopharyngeal carcinoma

NSCLC: Non-small cell lung cancer

OEPL: Oral epithelial precursor lesions

OLP: Oral lichen planus

OM: Oral mucositis

OPC: Oro-pharyngeal cancer

OPSCC: Oropharyngeal squamous cell carcinoma

ORN: Osteoradionecrosis

ORR: The overall response rate

OSCC: Oral squamous cell carcinoma

PDGF: Platelet-Derived Growth Factor

PEG: Polyethylene glycol

PFS: Progression-free survival

PLGA: Poly (lactic-co-glycolic acid)

PPI: Proton pump inhibitor

PSA: Prostate-Specific Antigen

RANKL: Receptor Activator of Nuclear Factor Kappa-B Ligand

RCNN: Region-Based Convolutional Neural Network

RNA: Ribonucleic Acid

ROS: Reactive oxygen species

SDF: Stromal cell-derived factor 1

SDI: Socio-demographic Index

STING: Stimulator of Interferon Genes

SVM: Support vector machine

TAA: Tumor-associated antigens

TAM: Tumor-Associated Macrophage

TCR: T cell receptor

TGF: Transforming growth factor

TIM: T-cell immunoglobulin and mucin domain

TLR: Toll-Like Receptor

TMB: Tumor mutational burden

TME: Tumor microenvironment

TNF : Tumor necrosis factor

TNM : Tumour, Node, Metastasis

TPF: Docetaxel, Cisplatin, and Fluorouracil

TREM2: Triggering receptor expressed on myeloid cells-2

TSNA: Tobacco-specific nitrosamines

VAE: Viscum album extract

VEGF: Vascular endothelial growth factor

VEGFR: Vascular endothelial growth factor receptor

I. Intoduction

Oral cancer is one of the most common malignant tumors in the head and neck region. Squamous cell carcinoma (OSCC), often linked with mucosal variations, is the most predominant type. It can occur in different parts of the mouth, including the lips, tongue, gums, inside of the cheek, and base of the mouth. It can also affect the soft or hard palate, oropharynx, jawbone, salivary glands, facial skin mucosa, oral base, and maxillary sinus. Several factors lead to the development of oral cancer, such as prolonged alcoholism, tabaco, poor oral hygiene, excessive sunlight exposure, usage of betel nut, malnutrition, leukoplakia or erythema of the mucosa, and oral ulcers.

The impact of oral cancer extends beyond aesthetics, affecting essential functions like eating and posing a severe threat to life. Treatment options for oral cancer are diverse. Commonly employed methods include surgery, chemotherapy, and radiotherapy. Surgical intervention is tailored to the tumor's size, with considerations for the appropriate extent of resection. However, post-surgery, individuals, particularly those with tongue cancer, may experience challenges in eating and speaking.

Radiotherapy, classified as a local treatment, may induce damage to the local skin and mucous membrane, resulting in conditions like radioactive skin and mucous membrane injuries and radioactive stomatitis. On the other hand, chemotherapy may lead to adverse effects such as pancytopenia and severe nausea with gastrointestinal reactions (Liu et al., 2022).

Head and Neck Cancer (HNC) presents distinctive challenges, constituting 3% of annual cancer cases in the UK. Unfortunately, early detection remains challenging, often leading to late stage diagnoses due to ambiguous symptoms. General Dental Practitioners (GDPs) are instrumental, requiring extensive training for early detection beyond the oral cavity.

Efficient referral systems are crucial, with GDPs considering various head and neck sites during routine examinations. HNC management operates within a Multidisciplinary Team (MDT) framework, tailoring treatment options from surgery to non-surgical approaches based on tumor stage and patient health.

Post-treatment evaluations, including pathology assessments, guide evidence-based follow-up in line with national guidelines. The emphasis on shared decision-making involves patients in treatment planning, ensuring a patient-centric approach.

The human immune system functions as a vigilant guardian, distinguishing between self and non-self to safeguard the body from external and internal diseases. This intricate

defense mechanism comprising white blood cells and crucial lymphatic organs orchestrates a symphony of responses to identify and eliminate threats, maintaining the delicate balance of homeostasis. Unlike cytotoxic chemotherapy, immunotherapy exploits the host immune system, revolutionizing cancer treatment.

Achieving victory against tumors requires a steadfast comprehension of the constantly evolving dynamic between cancer cells and the immune system. A historical exploration of immunotherapy's milestones becomes imperative, highlighting diverse types such as monoclonal antibodies and adoptive cell therapies. This narrative review navigates complexities, shedding light on the mechanisms governing immunotherapeutic interventions (Abbott & Ustoyev, 2019; Owens et al., 2022).

II. Development

1. Oral cancer

1.1. Word wide epidemiology

Lip, oral, and pharyngeal cancers pose significant challenges in the global cancer landscape. Therefore, there is a need for a comprehensive assessment of their impact worldwide. A study that used data from the 2019 Global Burden of Diseases, Injuries, and Risk Factors (GBD) Study aims to analyze the overall and risk-attributable burden of lip and oral cancer (LOC) and oro-pharyngeal cancer (OPC) across 204 countries. This study specifically focuses on distinctions in the Socio-demographic Index (SDI). It estimates the mortality, incidence, and disability-adjusted life years (DALYs) associated with LOC and OPC from 1990 to 2019(Da Cunha et al., 2023).

In 2019, the global health challenge posed by lip and oral cavity (LOC) and oropharyngeal (OPC) cancers manifested with approximately 370,000 cases, 199,000 deaths for LOC, 167,000 cases, and 114,000 deaths for OPC. This impact extended to DALYs, reaching 5.5 million and 3.2 million for LOC and OPC, respectively (Da Cunha et al., 2023).

Significant global disparities were observed, with consistently higher age-standardized mortality rates in low-middle and low Human Development Index (HDI) regions for LOC. Conversely, age-standardized incidence rates for OPC increased in high HDI strata. The incidence of lip, oral, and pharyngeal cancers differed among regions and socio-demographic indices. South Asia and high-income regions exhibited the highest incidence rates. Over time, high SDI regions experienced decreasing incidence and mortality rates, while low SDI regions consistently recorded the highest mortality rates (Barsouk et al., 2023; Da Cunha et al., 2023).

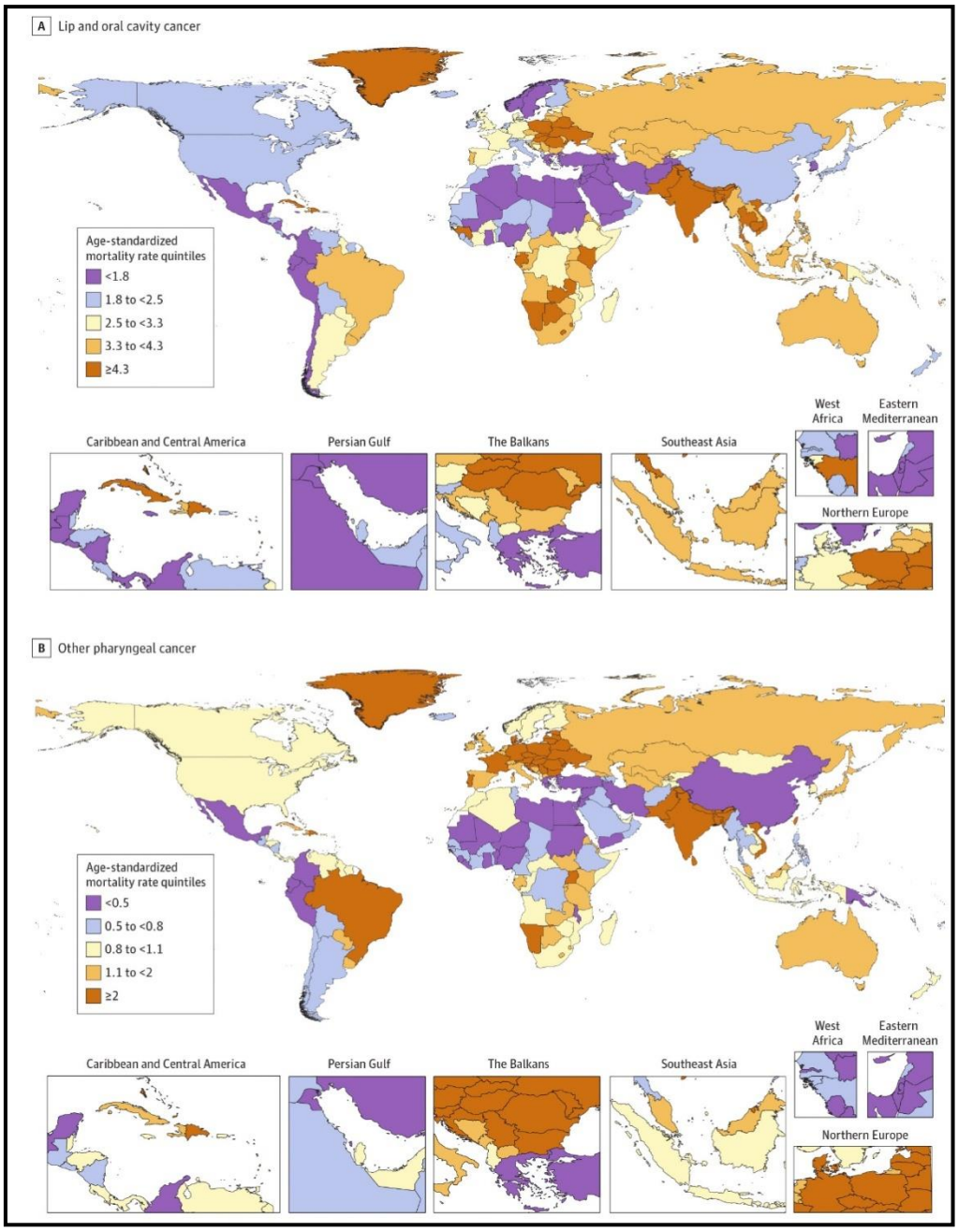

Figure 1: Age-standardized mortality rates for LOC and OPC in 2019. Adapted from (Da Cunha et al., 2023).

1.2. Risk factors

Alcohol and tobacco are identified as the principal risk factors for Head and Neck Squamous Cell Carcinoma (HNSCC). The International Agency for Research on Cancer (IARC) has recognized the presence of other carcinogens that show varying levels of evidence across different subsites of HNSCC. Since 1985, tobacco has been classified as a Group 1 carcinogen and has been strongly linked to several subsites of HNSCC. The risk of developing cancer is higher with an increase in daily cigarette consumption and usage duration, which resulted in a 71.4% tobacco-attributable fraction for oral

cavity and pharynx cancers in 2015 (Nokovitch et al., 2023).

Alcohol consumption results in the production of acetaldehyde, which is a carcinogenic substance. Acetaldehyde is produced during the absorption of alcohol and is classified as a Group 1 carcinogen by the International Agency for Research on Cancer (IARC). A study conducted in the United States found that alcohol use is linked to an increased risk of Oral Squamous Cell Carcinoma (OSCC) (Nokovitch et al., 2023).

The synergistic effect of alcohol and tobacco is noteworthy, as these habits are closely linked, and their combined effects are synergistic. Betel, especially when chewed, has been recognized as carcinogenic by the IARC since 2003, particularly in regions like India and Southeast Asia. A case–control study in 2003 revealed a high risk of oral cancer with combined tobacco and betel use (Nokovitch et al., 2023).

HPV16, which belongs to Group 1 carcinogen since 1995, has become the main cause of HNSCC but is responsible for less than 4% of OSCCs (Rahman et al., 2023). Other potential risk factors for OSCC include cannabis consumption and opium addiction, which may have links to HNSCC. Additionally, mouthwash usage, air pollution, and endocrine factors are suggested as potential risk factors. Despite advancements in understanding these risk factors, uncertainties persist, especially concerning other HNSCC subsites (Nokovitch et al., 2023).

Table 1: IARC-recognized carcinogenic agents for HNSCC with adequate human evidence (Nokovitch et al., 2023).

	Carcinogenic agents with high evidence in humans	Carcinogenic agents with low evidence
Oral cavity	Alcoholic drinks HPV-16 Betel Active and passive smoking	Occupational exposure to oxidized and hard bitumen and its emissions during roofing and mastic asphalt work. HPV-18
Oropharynx	HPV-16	
Pharynx	HPV-16 Alcoholic drinks Smoking Betel	Opium Asbestosis Passive smoking Printing processes
Larynx	Alcoholic drinks Smoking Acid smokes Strong inorganics Opium	HPV-16 Passive smoking Mustard gas Rubber production

Electronic Nicotine Delivery Systems (ENDS), like e-cigarettes, raise concerns regarding their potential carcinogenic effects. The heated propylene glycol in ENDS is identified as a carcinogen by the World Health Organization. The vapors produced by ENDS contain carcinogens similar to those found in traditional cigarette smoke, contributing to cytotoxicity, genotoxicity, and inflammation. Long-term exposure to these aerosols may lead to adverse cellular effects, including altered cell activity, morphological changes, apoptosis, a high concentration of pro-inflammatory cytokines, and DNA damage, heightening the risk of carcinogenesis (Mohamed et al., n.d.).

While ENDS exhibit lower levels of tobacco-specific nitrosamines (TSNA) compared to combustible cigarettes, they still contain these harmful compounds. Additionally, the aerosols generated by ENDS, varying in temperature, may play a role in their carcinogenic potential. The high temperatures can release aldehydes and contribute to conditions like nicotine stomatitis, identified as a premalignant lesion. Acrolein, a byproduct in ENDS, has been linked to oxidative stress and inflammation, potentially damaging endothelial cells (Gallagher et al., 2024).

Assessing Electronic Nicotine Delivery Systems (ENDS) as an isolated risk factor for oral cancer is a complex task because there are not enough long-term studies available. Additionally, ENDS users frequently have other established risk factors, such as smoking and alcohol consumption, which makes it challenging to determine the exact role of ENDS in the development of oral cancer (Gallagher et al., 2024; Mohamed et al., n.d.).

1.3. Biomarkers for potentially malignant oral tumors

To effectively prevent and detect oral tumors, it is crucial to identify potentially harmful lesions in the oral mucosa that may lead to cancer. This can be achieved through regular screening and early diagnosis. Additionally, it is important to address any local factors that may contribute to chronic inflammation, such as poor oral hygiene or tobacco use.

Timely recognition and appropriate treatment of such lesions are crucial. Clinical evaluation of oral mucosal lesions has demonstrated a high detection rate for oral cancers/premalignancies. According to the World Health Organization, any suspicious lesion persisting beyond two weeks after removing local irritants should undergo biopsy, which remains the gold standard for oral tumor diagnosis. It is essential to highlight that it comes with several drawbacks that may deter patients. These disadvantages include stress, fear, discomfort, pain, potential healthy tissue damage,

risk of infection, and aesthetic concerns. Clinical examination and biopsy are the most essential and effective diagnostic tools for oral tumors and premalignant lesions (Abati et al., 2020).

Various adjunctive diagnostic methods have been developed and studied to aid clinicians in this process, including:

- Toluidine blue vital staining, a straightforward, cost-effective, and minimally invasive technique utilized as an adjunct in diagnosing both malignant and premalignant lesions in the oral cavity. This metachromatic dye, known as toluidine blue, exhibits a cationic property and selectively imparts a royal blue color to areas of dysplastic epithelium (Lau et al., 2024; Romano et al., 2021).
- Autofluorescence imaging: Autofluorescence imaging relies on illuminating tissues with specific light wavelengths, prompting the production of autofluorescence from inherent fluorophores like elastin, keratin, collagen, and nicotinamide adenine dinucleotide (NADH), flavin adenine dinucleotide (FAD). The technique operates on the premise that dysplasia and cancer induce alterations in mucosal fluorescence, leading to a loss of green fluorescence in pathological conditions. Although it offers high sensitivity at an estimated 91% and is non-invasive, its main drawback is relatively low specificity, estimated at 58%. This limitation stems from benign conditions, including inflammatory diseases, being capable of causing changes in tissue autofluorescence similar to those observed in malignant and premalignant conditions (Lyu et al., 2021).
- Salivary markers of progression: In recent years, scientific research has been focused on identifying biomarkers present in biological fluids, especially saliva, which can help in the early detection of premalignant and malignant lesions that may have gone unnoticed. Saliva contains over 100 different biomarkers, including a combination of organic and inorganic molecules, peptides, proteins, and electrolytes, which can indicate various pathological processes and the onset of cancer. Some of these biomarkers include viruses, cytokines (such as IL-8, IL-1b, and TNF-α), protein receptors (CD44), as well as DNA and RNA markers that are overexpressed during carcinogenic processes (Abati et al., 2020; Bast et al., 2024; Umapathy et al., 2023).

Furthermore, research on saliva biomarkers for early recognition of head and neck squamous cell carcinoma (HNSCC) and oral squamous cell carcinoma (OSCC) shows promise. However, it requires further investigation to enhance sensitivity and

specificity, ultimately advancing their clinical utility in diagnosing malignant transformation of the oral mucosa (Khijmatgar et al., 2024).

1.4. Current challenges in oral cancer treatment

Treatment of head and neck cancer (HNC) typically involves a multidisciplinary approach incorporating surgery, radiotherapy, and chemotherapy, with the selection of modalities tailored to the specific stage of the tumor. Notably, the inclusion of oral cavity radiotherapy, a standard component of treatment, introduces a spectrum of acute adverse effects, mainly when administered concomitantly with chemotherapy. These effects encompass xerostomia, dysphagia, mucositis, erythema, pain, dysgeusia, and the potential for long-term complications impacting muscles, teeth, and bones (Corrao et al., 2023).

A substantial concern in the aftermath of head and neck radiotherapy is the heightened risk of osteoradionecrosis (ORN) after oral surgery. ORN manifests as exposed irradiated jawbone that fails to heal within a three-month timeframe, giving rise to symptoms ranging from pain and suppuration to mucosal ulceration and bone necrosis. The incidence of ORN exhibits variability, ranging from 2% to 22%, with a predilection for affecting predominantly elderly individuals, smokers, and habitual alcohol users exhibiting suboptimal oral health (Corrao et al., 2023).

Dysphagia constitutes a complication that emerges as a complex and multifaceted challenge, particularly prevalent among individuals undergoing therapeutic interventions for head and neck cancers. The intricate interplay of surgical procedures, radiation therapy, chemotherapy, and the resultant side effects significantly contributes to the development of dysphagia in this patient population (Vester et al., 2023).

Multiple factors contribute to dysphagia in these cases, including structural changes induced by surgery and radiation therapy, inflammation and mucositis caused by radiation and chemotherapy, the formation of scar tissue during the healing process, and neuromuscular impairment resulting from the adverse effects of cancer treatments on nerves and muscles coordinating swallowing. Additionally, dry mouth (xerostomia), a consequence of radiation-induced damage to salivary glands, further exacerbates the challenges associated with swallowing (Vester et al., 2023).

Moreover, the prevalence of oral mucositis (OM) compounds the challenges faced by individuals undergoing oncological treatments, such as radiotherapy, chemotherapy, and particle radiation for head and neck cancer. OM emerges as a dose-limiting toxic side effect, significantly impacting the prognosis of cancer patients. It is diagnosed in a

substantial percentage of individuals undergoing chemotherapy or radiotherapy, highlighting the urgency for effective, well-tolerated, and easily applicable strategies to overcome this debilitating condition. The ongoing research and advancements in supportive care play a vital role in navigating the intricate challenges linked to OM in cancer treatment, aiming to enhance patient outcomes and mitigate the negative impact on their quality of life (Wen et al., 2023).

1.5. Artificial intelligence for oral cancer

The differentiation between oral potentially malignant disorders (OPMDs) and malignant or benign lesions poses a significant challenge due to their diverse and often similar appearances. OPMDs, such as oral lichen planus, erythroplakia, proliferative verrucous leukoplakia, leukoplakia, and others, can manifest in various colors, including white, red, or white-red (Lorini et al., 2021).

Histopathological biopsy examination is the gold standard for detecting oral lesions, particularly premalignant and malignant lesions. However, this method necessitates a tissue biopsy, leading to potential discomfort, pain, and swelling for patients. Additionally, the process is time-consuming and expensive, especially when referrals to specialists are involved. A more streamlined clinical assessment that identifies only suspicious lesions requiring excision or referral could minimize unwarranted actions, difficulties, and expenses (Alajaji et al., 2024).

The integration of artificial intelligence (AI) in the medical field, particularly in skin cancer diagnosis, has become increasingly prominent. Machine learning, a type of AI, enables machines to recognize patterns in complex datasets without requiring explicit programming. Deep learning, a computational technique within machine learning, involves constructing nonlinear processing units with multiple hidden layers to comprehend and correlate inputs and outputs. Convolutional neural networks (CNNs), a specific type of deep learning, have gained popularity for image analysis, including image classification, object detection, and image segmentation (Dixit et al., 2023).

AI, deep learning, and machine learning have also been utilized to identify oral mucosa lesions in photographs. This AI diagnostic tool involves non-deep learning techniques such as support vector machine (SVM), random forests, and Naïve Bayes, as well as deep learning with various architectures such as Google Net, Alex-Net, ResNet, VGGNet, or Faster RCNN. The objective is to aid clinicians in screening for oral mucosa lesions, distinguishing between potentially cancerous and benign lesions, and subclassifying different OPMDs. The potential advantages of using AI include quicker

detection, targeted referrals, and increased survival rates(de Chauveron et al., 2024; Rokhshad et al., 2024; Tiwari et al., 2023).

AI algorithms have shown impressive capabilities in predicting treatment outcomes, evaluating clinical endpoints, and forecasting metastasis and recurrence in head and neck cancer patients, particularly in nodal lymph nodes. Although additional studies and standardized protocols are necessary, these AI algorithms demonstrate greater accuracy than commonly used prognostic models like staging or depth of invasion (DOI). Machine learning and deep learning algorithms in prognostic assessment have allowed the stratification of cancer patients with nodal involvement. The potential of AI in nodal lymph node assessment holds promise for personalized treatment strategies, which aim to optimize therapeutic interventions while minimizing unnecessary side effects. However, it is essential to conduct prospective clinical trials that directly compare AI to standard prognostic algorithms, particularly in nodal involvement, to establish its role as a valuable tool for disease management in oncology. Integrating AI into the management of oncology patients is crucial in influencing the future landscape of medical practices (Michelutti et al., 2023).

2. Biological treatment

2.1. The immune response

The human immune system can be divided into two primary branches, namely innate and adaptive immunity, working collaboratively to conduct immune surveillance and differentiate between self and non-self entities. This distinction operates at a biochemical level, involving factors such as DNA composition and glycoprotein structure. Although these two facets are simplified concepts, they frequently exhibit overlapping functions. The innate immunity is an initial line of defense present since birth and activates a non-specific immune response when encountering foreign materials. It contains physical barriers like mucous membranes and skin, physiological barriers like temperature and pH, and non-specific elements like dendritic cells, neutrophils, mast cells, and macrophages. This defense mechanism is quick and doesn't rely on antigens. It uses cytokines to mediate immune functions and generate a general response. If the innate response proves insufficient, the acquired or adaptive immune system comes into play. Unlike innate immunity, adaptive immunity is specific and time-dependent, evolving after recognizing foreign materials. The process entails the production of B-cell antibodies and the involvement of antigen-presenting cells, helper T cells, and cytotoxic T cells. Cytotoxic T cells specifically target non-self cells, and adaptive immunity culminates in immune memory formation, enabling a quicker and more robust response upon re-exposure (Abbott & Ustoyev, 2019).

Table 2: The Innate and Adaptive immune system (Abbott & Ustoyev, 2019).

	Innate	Adaptive
Specificity	- Non specific - Present all times - Reacts against all foreign pathogens	- Specific - Needs to be activated - A prompt reaction to a harmful microorganism.
Response time	- Immediate response - General defense	- Delayed response - Specific defense
Memory	- Absent	- Present - Antibody development
Cell components	- Dendritic cells - Macrophages - Neutrophils - Phagocytes - Natural Killer cells	- B Lymphocytes - T Lymphocytes

The adaptive immune response is a complex defense mechanism that depends on specialized lymphocytes and their products, including immunoglobulins and cytokines, to combat harmful microbes. Three crucial features distinguish this response. Firstly, it is highly specific, meaning that it can target a particular pathogen, immunogen, or antigen. Secondly, it is incredibly diverse, producing millions of distinct effectors (antibodies) to combat a wide range of intruders. Finally, it possesses a remarkable memory, enabling the immune system to identify a pathogen upon future encounters and launch a more rapid and robust response (Vaillant et al., n.d.).

While innate immunity provides immediate defense, adaptive immunity delivers a focused and memorized response. Both types jointly defend against foreign substances like bacteria. However, a question arises about the immune system's ability to recognize and fight cancer cells, which are considered "self.". Cancer cells, distinguished by their unique biochemical and antigenic features, can evade immune detection and proliferate unchecked, presenting a significant challenge to health (Abbott & Ustoyev, 2019).

2.2. Cancer immunity cycle

The cancer-immunity cycle outlines a dynamic sequence of events orchestrated by the immune system to identify and destroy malignant cells. This intricate process unfolds through seven key steps (Mellman et al., 2023):

1. Generation of Neoantigens: As cancer cells proliferate, they accumulate DNA mutations, forming neoantigens - novel proteins absent in healthy cells. The immune system identifies neoantigens as foreign substances.
2. Capture of Antigens by Dendritic Cells: Specialized immune cells called dendritic cells identify and process these neoantigens. They present the antigens to T cells, initiating an immune response.
3. Activation and Proliferation of T cells: When dendritic cells present neoantigens, T cells get activated and start multiplying. This process generates effector T cells, which directly target cancer cells, and stimulate memory T cells, which retain information for future responses.
4. Migration of effector T cells to malignant cells: Activated effector T cells reach the tumor site, aiming to eliminate cancer cells. However, the tumor microenvironment can hinder their movement.
5. Infiltration of the Tumor by Effector T cells: Despite challenges, some effector T cells successfully infiltrate the malignant site and initiate the process of cancer cell recognition and elimination.

6. Release of Neoantigens from Cancer Cell Death: As cancer cells die, they release their contents, including neoantigens, into the tumor environment. These neoantigens stimulate further immune responses by activating dendritic cells and T cells.
7. Long-Term Control of Cancer: Successful immune-mediated elimination of cancer cells can lead to long-term disease control. However, cancer cells may evolve mechanisms to evade immune detection, posing challenges to sustained remission.

The cancer-immunity cycle showcases the intricate interplay between cancer cells and the immune system, emphasizing the complexity of anti-tumor immune responses (Mellman et al., 2023).

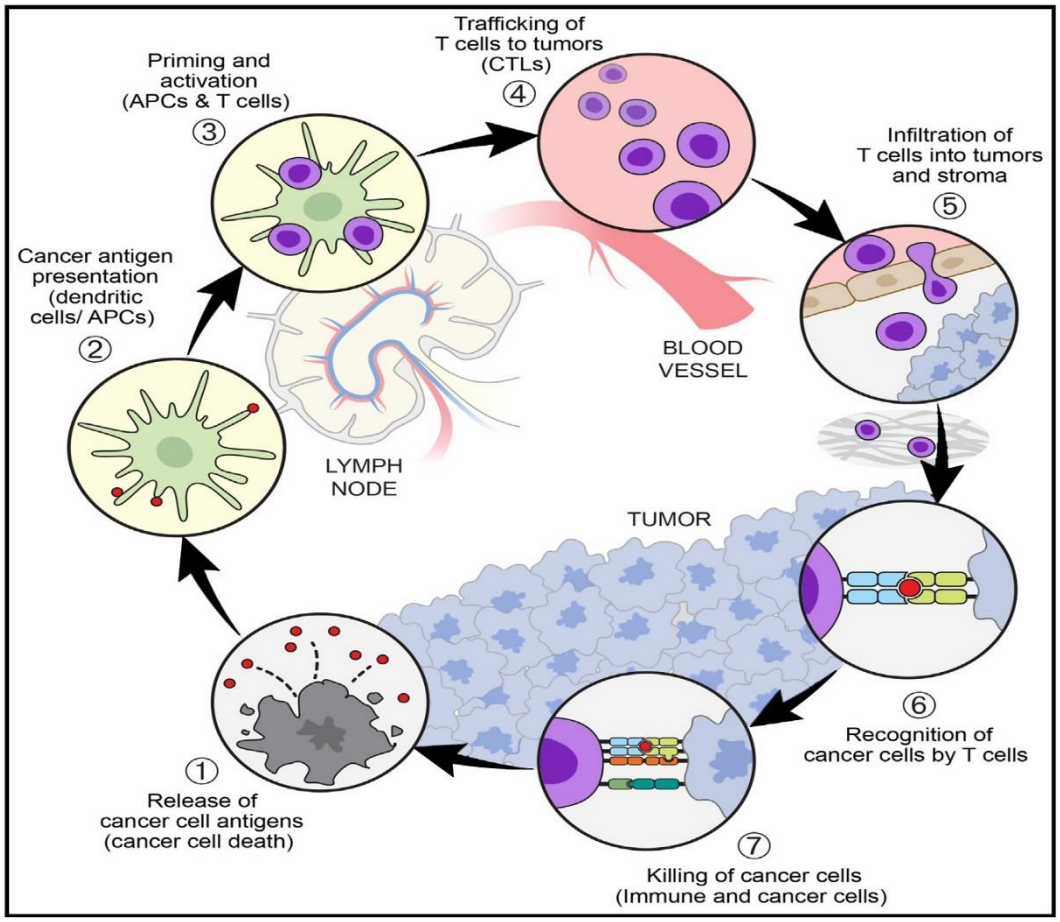

Figure 2: The cancer-immunity cycle delineates seven essential stages associated with immunity against cancer. Adapted from (Mellman et al., 2023).

The cancer-immunity cycle is a series of steps necessary for optimal immunity against cancer. Successful anti-cancer immunity can reinforce itself during this response.

However, various factors can disrupt this cycle, making it challenging for the immune system to control cancer effectively. Below are the factors that are causing disruption:

1. Tumor heterogeneity: characterized by the diverse morphological and phenotypic traits of various cancer cells, is a critical obstacle in developing effective cancer treatments. This diversity encompasses differences in appearance, genetic behavior, metabolism, motility, proliferation rates, and metastatic potential among tumor cells. Many cells with distinct molecular profiles can exist within a single tumor, resulting in varied responses to treatments (Li et al., 2024).

   This heterogeneity becomes particularly challenging when designing immunotherapies. For instance, immune checkpoint inhibitors may face resistance to these therapies due to subpopulations of unresponsive tumor cells. The effectiveness of CAR-T cell therapy addressed to the CD19 protein in B cell tumors is compromised because not all tumors uniformly express CD19. This variability can lead to resistance scenarios, where subclones of tumor cells initially lacking the CD19 protein may emerge, contributing to relapses post-CAR-T cell therapy. Understanding and overcoming this tumor heterogeneity is crucial for developing more precise and effective cancer treatment strategies (Li et al., 2024).
2. Immunosuppressive tumor microenvironment: Tumors create a hostile environment that undermines the immune system's effectiveness. This environment is created by the tumor cells themselves and influenced by cancer-associated fibroblasts and various immune cells. The tumor's ability to attract immunosuppressive cells, like Tumor-Associated Macrophages (TAMs), Myeloid-Derived Suppressor Cells (MDSCs), and Regulatory T cells (Tregs), actively suppresses the function of immune effector cells, contributing to the establishment of an immunosuppressive environment. Moreover, tumor cells can alter the extracellular matrix and surrounding stromal components, building physical barriers that impede immune cell infiltration and compromise their functionality within the tumor microenvironment (Li et al., 2024).
3. Immunoediting: Genes have a significant role in regulating normal body cells' growth, maturation, and programmed death. Daily, each cell experiences roughly 20,000 instances of DNA damage, which are usually repaired by DNA repair pathways. When cells are no longer necessary or become a threat,

> apoptosis, or programmed cell death, is triggered to prevent uncontrolled proliferation. Cancer cells are characterized by their unchecked growth, which involves mutated or abnormal cells that spread and infiltrate healthy tissue. Cancer progression entails eight processes: continuous proliferation, evasion of growth suppressors, resistance to cell death, angiogenesis, metastasis, replicative immortality, reprogrammed metabolism, and evasion of immune destruction (Li et al., 2024).

The study of evasion of immune destruction has a long history. In 1909, Paul Ehrlich proposed that the immune system could control tumors. Back in 1957, the theory of cancer immunosurveillance was put forth by Thomas and Burnet. This theory posits that lymphocytes play a crucial role in identifying and eradicating mutated cells. Despite this proposal, there was a lack of evidence to support the claim until more recent studies on cancer incidence in individuals with immune suppression (e.g., HIV/AIDS or post-allogeneic transplant patients) shed light on the matter. "Immunoediting" encompasses all phases of cancer and immune system interaction that go beyond immunosurveillance (Abbott & Ustoyev, 2019).

The immunoediting hypothesis can be divided into three stages. The first stage, known as the elimination phase, involves the active process of immunosurveillance, where the immune system detects and eliminates cells that have become cancerous by escaping the body's natural DNA repair mechanisms. This process is facilitated by the innate immune system, which presents tumor antigens to specific CD4+ and CD8+ T cells, resulting in the destruction of cancer cells (Abbott & Ustoyev, 2019).

The equilibrium phase follows, during which surviving tumor cells remain in balance with the immune system for extended periods of time, sometimes even for years. Finally, in the escape or evasion stage, cancer cells grow and metastasize as they become increasingly resistant to immune system attacks. This happens due to various mechanisms that enable malignant cells to evade elimination, including immune suppression by the tumor cell or genetic mutations that help the tumor cells suppress the immune system (Abbott & Ustoyev, 2019).

One such mechanism is the expression of immune checkpoint molecules on the surface of cancer cells. These molecules suppress T cells at immune checkpoints, making it difficult for the immune system to attack the cancer cells. This ability to evade the immune system is a significant characteristic of cancer pathogenesis (Abbott & Ustoyev, 2019).

The Cancer-Immunity Cycle framework has remained largely unchanged since its introduction, with only one modification being made to emphasize that blood-derived T cells often have to cross a stromal barrier before they can reach the tumor. However, recent advancements have brought several new concepts to light. One of these is the idea that within specific cancer types, tumors can be classified into distinct immunological phenotypes or "immunotypes" (Mellman et al., 2023).

These immunotypes, namely "immune inflamed," "immune excluded," and "immune desert," delineate different immune phenotypes observed within tumors based on the distribution and immune cell activity in the tumor microenvironment. Immune-inflamed tumors exhibit high infiltration of immune cells, such as cytotoxic T cells, dendritic cells, and natural killer cells, actively promoting an antitumor immune response. Such tumors typically have a favorable prognosis and are more responsive to immunotherapy. On the flip side, immune-excluded tumors display a lower concentration of immune cell infiltration, with immune cells often located in the tumor stroma, suppressing the antitumor immune response. These tumors generally have a less favorable prognosis than immune-inflamed tumors. Meanwhile, immune desert tumors exhibit minimal immune cell infiltration, lacking evidence of an active antitumor immune response. These tumors tend to be more aggressive with a poorer prognosis (Mellman et al., 2023).

Table 3: Characteristics of different immunotypes.

Phenotype	**Immune cell infiltration**	**Location of immune cells**	**Antitumor immune response**	**Prognosis**
Immune inflamed	High	Intratumoral	Active	Good
Immune excluded	Low	Stromal	Suppressed	Poor
Immune desert	Very low	None	Absent	Very poor

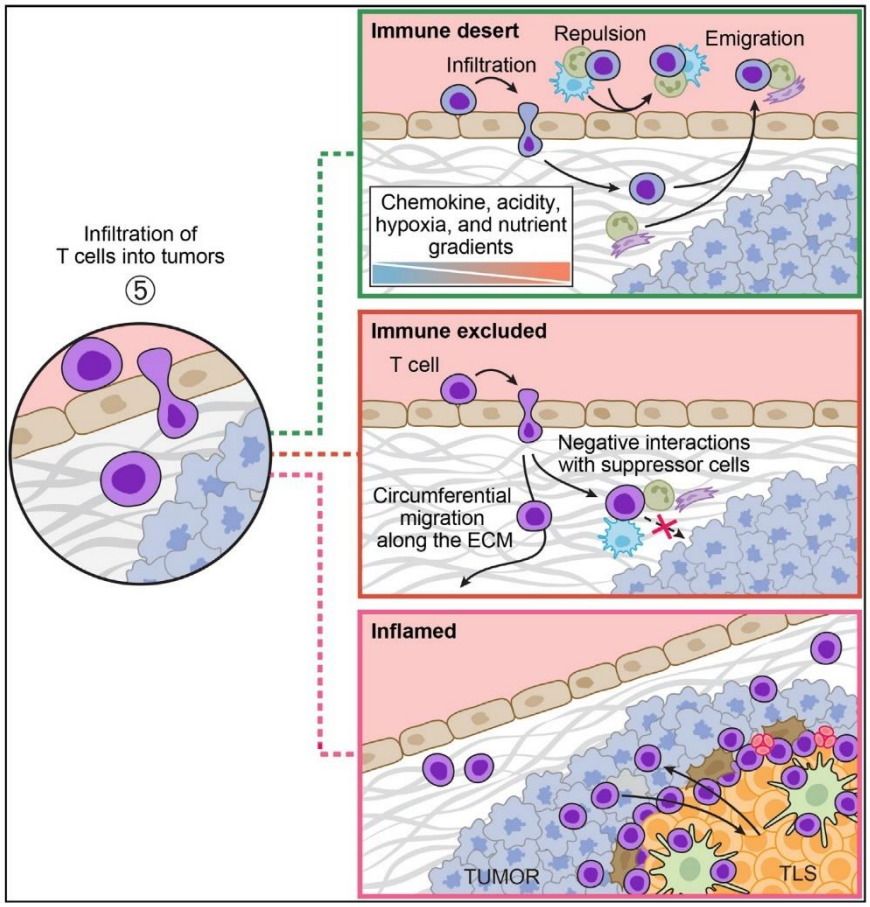

Figure 3: Immunotypes: immune desert (green), immune excluded (red), and inflamed (purple). Adapted from (Mellman et al., 2023).

Understanding a tumor's immune phenotype is a crucial prognostic factor for cancer patients, influencing the likelihood of responding to immunotherapy. Patients with immune inflamed tumors are more responsive, while those with immune excluded or immune desert tumors are less likely to benefit. Categorizing tumors as "hot" or "cold" over-simplifies their immunotypes (Mellman et al., 2023).

The tumor microenvironment (TME), including the tumor stroma, plays a crucial role in determining tumors' immunotype and immune trajectory. Apart from T cells, cells of the innate immune system like monocytes, natural killer (NK) cells, granulocytes, and non-immune cells such as cancer-associated fibroblasts (CAFs) are also of great importance. These cell types work together to form a collagen-rich fibrotic stroma, which restricts T cell immunity by suppressing their function and physically blocking their migration into tumor nests. Understanding these dynamics is very important for developing more effective immunotherapies (Mellman et al., 2023).

2.3. TME: Tumor microenvironment

In the current perspective, it is widely acknowledged that the progression and migration of cancers are heavily influenced by the tumor microenvironment (TME). Within the TME and tumor stroma, elements like the extracellular matrix regulate the proliferation and function of cancer cells. This regulation extends to controlling tumor metabolism, response to hypoxia, and releasing various anti-inflammatory and pro-inflammatory cytokines, chemokines, and other molecular factors (Qin & Wu, n.d.).

Precisely, the release of pro-angiogenesis molecules within this complex environment activates the formation of new blood vessels and microvessels, contributing to the dynamic and multifaceted nature of cancer progression (Qin & Wu, n.d.).

The intricate interplay among the tumor microenvironment (TME) components underscores the complexity of its network relationships. A profound comprehension of the composition and attributes of the TME serves as the foundation for advancing immunotherapy strategies based on this milieu.

In oral cancer, the development, advancement, and spread of tumors are closely associated with the surrounding environment. This environment encompasses various factors such as the function, structure, and metabolism of the tumor tissue as well as the internal conditions of cancer cells. It is important to note that the relationship between tumors and their microenvironment is characterized by a dynamic interdependence marked by both antagonistic and cooperative interactions (Liu et al., 2022).

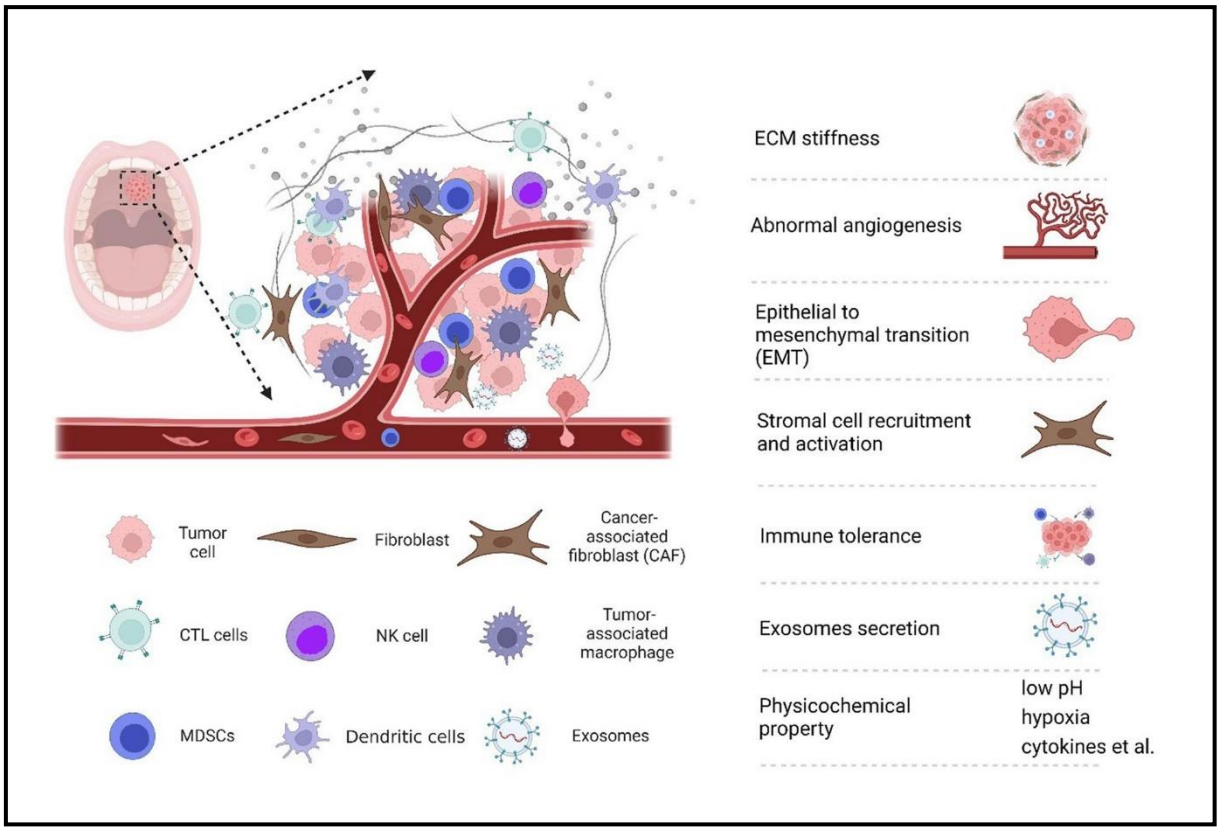

Figure 4: Schematic diagram of TME .Adapted from (Liu et al., 2022).

2.3.1. Non cellular elements: Extracellular matrix and their component

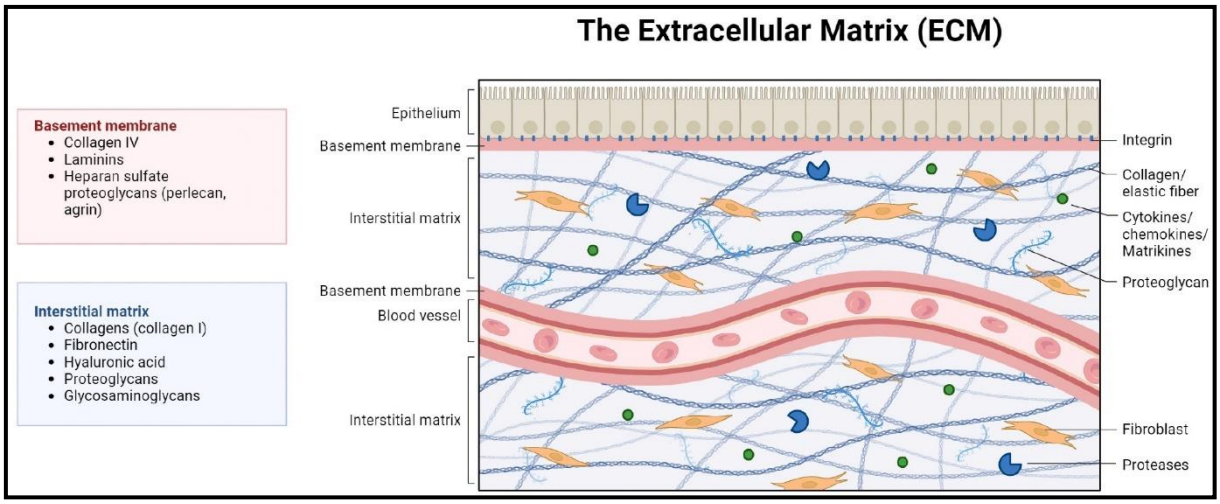

Figure 5: Visualizing the composition and organization of the extracellular matrix: A schematic overview of structural components. Adapted from (Baghy et al., 2023).

2.3.1.1. Collagens

Collagen, a predominant component comprising 30% of total proteins and 90% of the human extracellular matrix, plays a vital role in tissue strength and toughness through

cross-linking, forming various supramolecular structures. During tumor development, its degradation by matrix metalloproteinases (MMPs) releases signal molecules, impacting mechanical properties and signal transduction in the tumor microenvironment (TME) (Liu et al., 2022).

Collagen I and collagen III, primary structural proteins in the ECM, contribute to tumor matrix rigidity. An increased quantity of collagen is associated with unfavorable prognoses due to ECM stiffness during remodeling. MMPs, notably MMP-1, -8, -13, and -14, catalyze the replacement of the original ECM in tumor-associated stroma with new collagens, influencing the DNA damage response (DDR) (Baghy et al., 2023).

2.3.1.2. Fibronectin

In the extracellular matrix, fibronectin represents the most important adhesive glycoprotein; via integrins, it forms a crucial connection with epithelial cells. Despite a delayed recognition of its oncogenic potential and lingering contradictory data, fibronectin is consistently produced by fibroblasts and tumor-derived stromal cells, becoming a constant fixture in the fibrous ECM. There is increasing evidence that fibronectin plays a crucial role in the biological behavior of tumors, in addition to its many physiological functions. Fibroblasts and macrophages are the leading producers of fibronectin in cancer, and this production influences important aspects like tumor cell migration, invasion, and angiogenesis (Baghy et al., 2023).

2.3.1.3. Elastin

Elastin, an integral element within elastic fibers, is predominantly present in ligaments and vascular walls. Teaming with collagen, elastin is crucial in upholding tissue strength and resilience against deformation or breakage. Its remarkable elasticity distinguishes it from collagen due to its unique dynamic three-dimensional structure and amino composition. Moreover, working in tandem with collagen, laminin proteins construct the membrane's basement and actively participate in vascularization during vascular maturation. Laminin's upregulation during epithelialization is particularly notable, serving as a platform for epithelial cell adhesion. This interaction allows cells to adhere and stretch, pivotal in signaling pathways that can influence tumor metastasis (Baghy et al., 2023).

2.3.1.4. Hyaluronic acid

HA is a crucial component of the extracellular matrix (ECM) and plays a significant role in facilitating wound healing, regulating vascular wall permeability, and allowing for material transportation and diffusion. In addition to its structural functions, HA also

acts as a reservoir for water, playing an active role in buffered ion exchange and maintaining osmotic balance and water within the extracellular matrix. The selective permeability of substances and biomacromolecules, dictated by charged surfaces and specific domains, further underscores HA's versatile role (Liu et al., 2022).

HA serves as a crucial point of recognition for tumor cells by means of intracellular signal transduction molecules, especially the membrane receptor CD44. This specific interaction plays an essential role in facilitating the migration and invasion of tumor cells (Liu et al., 2022).

Moreover, the ECM is a catalytic entity, instrumental in realizing various growth factors. It acts as a reservoir for signaling molecules and growth factors, which is crucial for malignant transformation. Notably, the Platelet-Derived Growth Factor (PDGF) effectively accumulates in the ECM after binding with collagen, exemplifying its role as a storage site (Liu et al., 2022).

Associated with angiogenesis, heparin-binding growth factor 1 (HBGF-1) forms critical associations by binding to type I and IV collagen. This intricate interplay within the ECM showcases its dynamic involvement in shaping the microenvironment for tumor progression (Liu et al., 2022).

2.3.1.5. Growth Factors, Cytokines, Chemokines, and Matrikines

In the complex tumor microenvironment (TME), signaling molecules orchestrate critical processes such as inflammation, extracellular matrix (ECM) remodeling, and cancer progression. They intricately shape the behavior of, stromal cells, immune cells, and the extracellular matrix (Baghy et al., 2023).

For instance, these molecules drive the trans-differentiation of stromal progenitors into cancer-associated fibroblasts (CAFs), facilitate immune cell recruitment, and promote tumorigenesis and metastasis. Tumor-associated fibroblasts (TAFs) within the TME add another layer by supplying inflammatory mediators, growth factors, and ECM components, molding the microenvironment to support tumor growth (Baghy et al., 2023).

The interplay between these signaling molecules and the cellular components of the TME holds profound implications for cancer development. Understanding these roles is crucial for developing targeted therapies to disrupt pro-tumorigenic processes (Baghy et al., 2023).

In response to a tumor lesion, immune cells are recruited to the site, releasing transforming growth factor β (TGFβ). Initially a tumor inhibitor, TGFβ transforms into

a metastasis promoter at advanced carcinogenesis, reshaping the TME to sustain tumor growth (Baghy et al., 2023; Marozzi et al., 2021).

Stimulated by TGFβ, activated fibroblasts (CAFs) release cytokines and chemokines that foster an inflammatory response, attracting immune cells. Additionally, CAFs secrete pro-tumorigenic cytokines such as IL-6 and TNFα, which impact tumor growth and progression (Marozzi et al., 2021).

Cytokines and chemokines impact tumor growth through mechanisms such as controlling cell migration and promoting the survival of tumor cells. These molecules also play crucial roles in tumor angiogenesis, invasion, metastasis, and microenvironment modulation through immune cell recruitment and regulation of inflammatory responses. Cytokines, including interleukins, interferons, and tumor necrosis factors, contribute to chronic inflammation, angiogenesis, and immune evasion, facilitating tumor expansion (Chow & Luster, 2014).

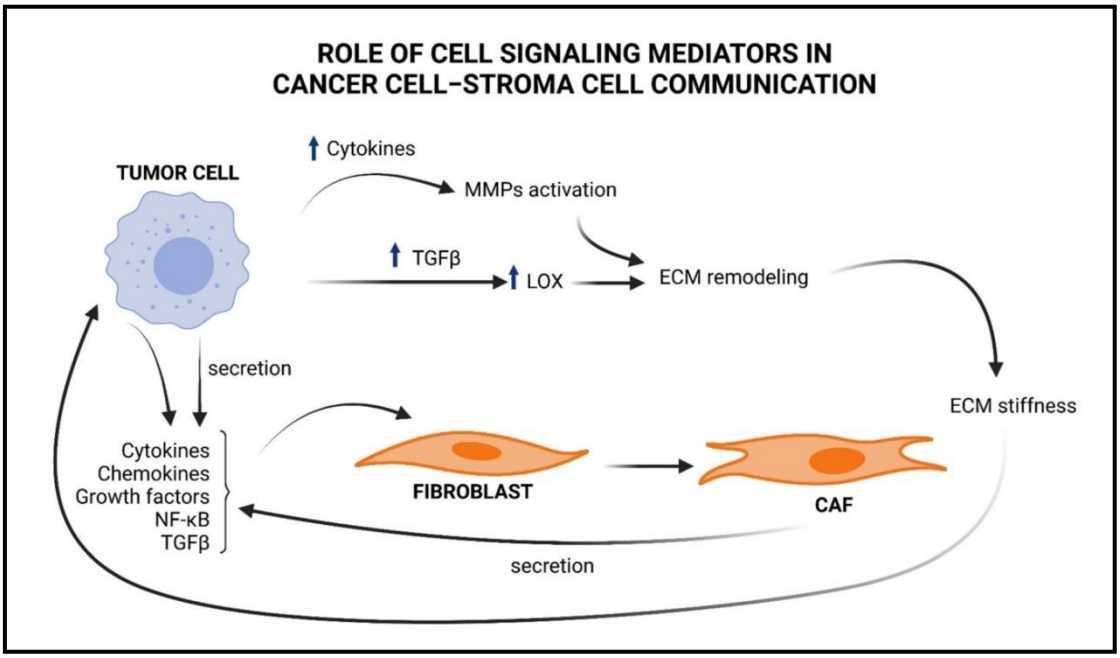

Figure 6: An overview of how cancer cells communicate with stromal cells and remodel the extracellular matrix through cell signaling mediators. Adapted from (Marozzi et al., 2021).

2.3.2. Vascular microenvironment

The tumor microenvironment (TME) intricately links with the initiation of tumor angiogenesis, a process vital for sustained tumor growth. Typically, tumors remain within a diameter of around 2 mm without angiogenesis. Beyond this threshold, as the solid tumor surpasses 2 mm, acquiring sufficient O2 and nutrients from the surroundings becomes challenging. Malignant cells respond by releasing angiogenic

factors into the tumor microenvironment, instigating the development of new vascular structures (Liu et al., 2022).

Malignant cells play a direct or indirect role in this process, particularly in hypoxic environments where hypoxia-inducible factor (HIF) orchestrates an upregulation of gene expression, including vascular endothelial growth factor (VEGF). The oxygen concentration drops from 21% to 0.3% significantly boosts VEGF mRNA expression, particularly evident in tumor tissue's necrotic and vascular-deficient zones. Tumor cells recruit and polarize neighboring cells to form tumor-associated stromal cells, amplifying the representation of vascular growth factors and implicitly stimulating tumor angiogenesis (Liu et al., 2022).

VEGF, crucial in this context, binds to vascular endothelial growth factor receptor (VEGFR) within malignant tissues, activating downstream signaling pathways. This activation guides changes in vascular permeability, ultimately fueling tumor angiogenesis. The complex interconnection among tumor growth, angiogenesis, and metastasis suggests that targeting the inhibition or disruption of the tumor angiogenic microenvironment could be a promising approach in potential tumor treatments (Liu et al., 2022).

2.3.3. Cellular elements of TME

The TME comprises a dynamic and intricate network of cells engaging in complex interactions mutually and with extracellular matrix components. Key components of the TME include malignant cells, fibroblasts, diverse immune cell types, and vascular system cells.This multifaceted milieu is pivotal in tumor development and progression, shaping therapeutic responses and influencing disease outcomes (Baghy et al., 2023).

2.3.3.1. Stromal cells

2.3.3.1.1. Cancer-associated fibroblasts

Cancer-associated fibroblasts (CAFs) exhibit distinct characteristics from normal fibroblasts (NFs), particularly in their activated state. The metamorphosis of NFs to CAFs is primarily stimulated by cytokines secreted by immune or malignant cells and involves significant epigenetic modifications. Quiescent CAFs share similarities with NFs, while activated CAFs demonstrate heightened proliferative and migratory properties (Gao et al., 2023).

Fibroblasts within tumors co-evolve with cancer cells, often hired and transformed into primitive CAFs that promote malignancy through the secretion of growth factors,

cytokines, and chemokines. Bone marrow-derived stem cells (BMDSCs) are a significant cellular source of recruited CAFs. In vitro studies illustrate that conditioned medium from various malignant cell lines induces a chemotactic effect on mesenchymal stem cells (MSCs), culminating in the acquisition of a CAF phenotype (Gao et al., 2023).

The tumor microenvironment activates CAFs, facilitating partial epithelial–mesenchymal transition (EMT). Beyond fibroblast cells, CAFs arise from various cell types, including adipocytes, epithelial cells, smooth muscle cells, pericytes, and endothelial cells, through processes like endothelial-mesenchymal transformation (Baghy et al., 2023).

Multiple signaling pathways, like platelet-derived growth factor (PDGF), TGF-β, and essential fibroblast growth factor (bFGF), contribute to the engagement and activation of NFs. PDGF, obtained from cancer cells, significantly alters the stromal microenvironment in favor of malignant cells. It initiates the recruitment of CAFs, thereby promoting tumorigenesis and angiogenesis through the generation of vascular endothelial growth factor (VEGF) by the recruited host stromal fibroblasts (Baghy et al., 2023; Gao et al., 2023).

In summary, the progression from NFs to CAFs involves a complex interplay of cytokines, epigenetic modifications, and various cell types within the TME. The activated CAFs play an important role in supporting malignancy through their enhanced proliferative and migratory properties and their ability to promote ECM remodeling and facilitate cancer cell invasion (Baghy et al., 2023; Liu et al., 2022).

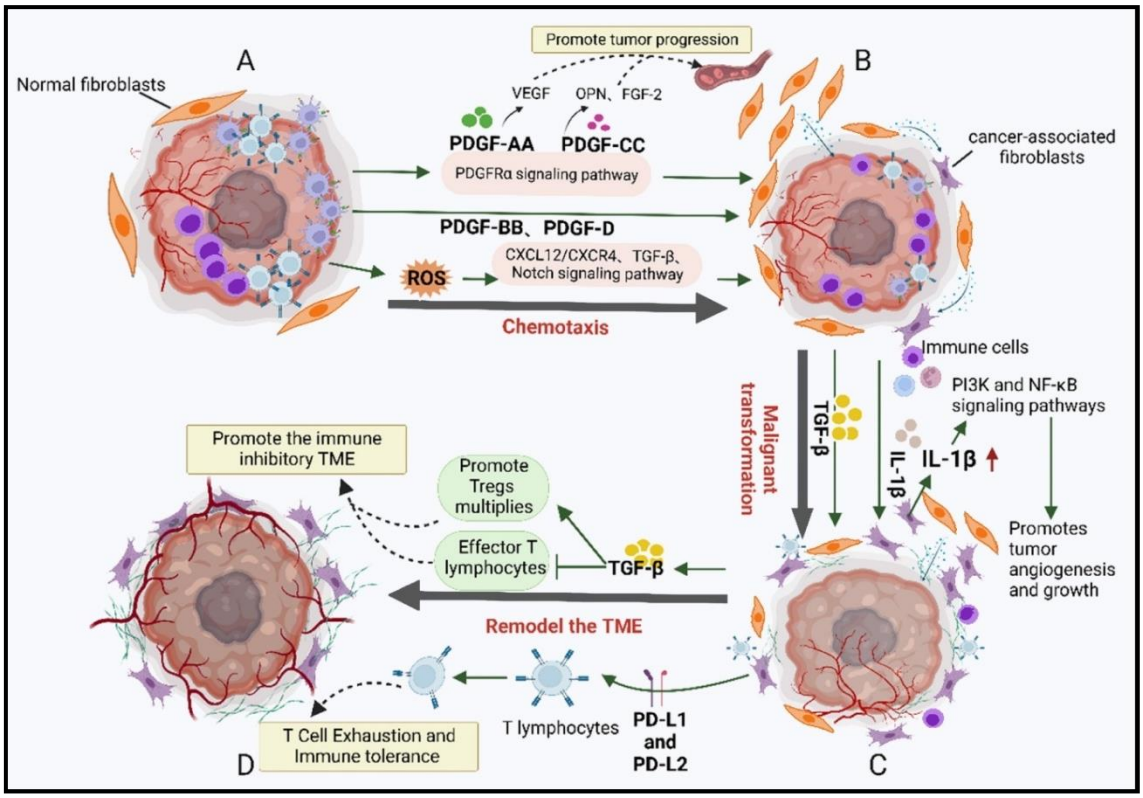

Figure 7: Dynamic Remodeling of TME: Tumor-Induced Recruitment and Transformation of Fibroblasts. Adapted from (Gao et al., 2023).

2.3.3.1.2. Macrophages

The stromal cells within tumor tissues exhibit significant diversity, with macrophages playing a crucial role. Macrophages have the ability to change their functions and characteristics according to the environment they are in. Broadly categorized into M1 and M2 subtypes, M1 macrophages are pro-inflammatory with bactericidal and anti-tumor properties, while M2 macrophages exhibit anti-inflammatory and immunosuppressive functions without cytotoxicity against tumor cells (Liu et al., 2022). Tumor-associated macrophages (TAMs) are influenced by cytokines and growth factors present in the TME, induced by factors like CCL2, granulocyte–macrophage colony-stimulating factor (GM-CSF), VEGF, IL-8, and IL-6. TAMs are drawn to hypoxic/necrotic regions of tumors through chemotactic molecules, surviving by shifting their metabolism to glycolysis. Additionally, TAMs produce elevated rates of TGF-β, hindering cytotoxic T cells' proliferation and killing effects while activating immunosuppressive Treg cells (Gao et al., 2023; Liu et al., 2022).

The interplay between M2-type TAMs and cancer-associated fibroblasts (CAFs) is reciprocal, creating a positive feedback loop supporting malignant cell development and immunosuppression. M2 macrophages stimulate CAFs by releasing molecules like

SDF-1, IL-6, and TGF-β, enhancing cancer cell invasiveness. CAFs influence monocyte recruitment and differentiation into M2 TAMs via various regulatory molecules, actively polarizing macrophages towards a pro-tumor phenotype, thereby promoting tumor growth (Gao et al., 2023; Liu et al., 2022).

Studies have also shown that stimulated bone marrow-derived mesenchymal stem cells (MSCs), acting as CAFs at the malignant site, increase macrophage invasiveness, supporting CAF proliferation and invasion in specific cancers. This intricate interaction between M2 macrophages, CAFs, and MSCs forms a complex network that contributes significantly to tumor progression (Gao et al., 2023; Liu et al., 2022).

2.3.3.1.3. Adipocytes

Adipocytes within the tumor microenvironment (TME) play a significant role by secreting a diverse range of tumor-related adipocytokines, influencing tumor initiation and progression. The TME, characterized by hypoxia and elevated pressure, induces pathological metabolic changes in adipocytes. This alteration in adipocyte metabolism leads to shifts in the secretion of lipid metabolites and adipokines surrounding tumor cells. Consequently, this prompts the activation of crucial signaling pathways, which include signal transducer and activator of transcription 3 (STAT3) and other pathways associated with tumorigenesis. These changes contribute to the acceleration of tumor development and deterioration (Liu et al., 2022).

2.3.3.2. Cells of the immune system

2.3.3.2.1. Natural Killer

Natural Killer cells, originating from bone marrow lymphoid stem cells, are pivotal elements of the immune system with the ability to target and destroy malignant cells independently of major histocompatibility complex (MHC). However, tumor cells deploy tactics such as releasing TGF-β and altering antigenic expression to resist NK cell activity. Regulatory T cells (Tregs) also hinder NK cells by competing for interleukin-2 (IL-2) (Gao et al., 2023; Liu et al., 2022).

The tumor microenvironment involves the interplay of NK cells with cancer-associated fibroblasts (CAFs). CAFs secrete cytokines, chemokines, and MMPs, inhibiting NK cell receptors and compromising their tumor-killing capabilities. Elevated PGE2 levels from CAFs, especially in the presence of NK cells, establish a feedback loop, further inhibiting NK cell function (Gao et al., 2023; Liu et al., 2022).

Additionally, CAFs decrease the expression of MHC I-like proteins, impairing NK cell

cytotoxicity. The intricate crosstalk between NK cells and CAFs involves downregulating activation receptors, granzyme B and perforin on NK cells, as well as hindrance in cytokine secretion (IFN-γ and TNF-α). TGF-β secreted by CAFs further diminishes IFN-γ production by NK cells, crucial for activating effector CD4+ TH1 cells indispensable for tumor clearance (Baghy et al., 2023; Gao et al., 2023; Liu et al., 2022).

2.3.3.2.2. T lymphocytes

The intricate interplay between T cells and TME forms the crux of anti-tumor immunity. A key player in this defense mechanism is the recognition of tumor-specific antigens (TSAs), which activate CD4+ T cells, CD8+ T cells, and specific antibodies. The dynamic nature of T cell functionality is underscored by their capability to inhibit or stimulate tumor progression, dependent on a delicate balance of signaling pathways within the TME (Wang et al., 2023).

CD8+ T cells, armed with the capacity to impede tumor proliferation through direct lysis or the release of critical cytokines like IFN-γ and TNF-α, embody a frontline defense against malignancy. However, a cautionary note arises with prolonged exposure to tumor-specific T cell antigens, leading to a state of exhaustion marked by the expression of immune markers like TIM-3 and PD-1 (Wang et al., 2023).

The TME further regulates the fate of T cells by orchestrating a delicate equilibrium between co-stimulatory and co-inhibitory signals. This balance dictates whether CD8+ T cells will be in a state of immune activation, actively combating the tumor, or succumb to immunosuppression.

Meanwhile, CD4+ T cells display a diversity of roles within this immunological landscape. T1, for instance, amplifies the tumor-destruction prowess of CD8+ T cells via the secretion of cytokines like IFN-γ, IL-2, and TGF-β. T2 provides support to eosinophils, contributing to their efficacy in eliminating tumor cells. T17 cells, depending on the cytokine milieu, can switch between anti-tumor and pro-tumor properties, showcasing the intricate dance within the TME (Wang et al., 2023).

In contrast, regulatory T cells (Tregs), a specific variant of CD4+ T cells, are recruited to the TME to suppress the anti-tumor immune response. Stimulated by recognizing tumor-associated antigens (TAAs), Tregs release various cytokines that effectively hinder the engagement of tumor-specific effector T cells, creating a formidable barrier against immune-mediated tumor killing (Wang et al., 2023).

The delicate balance of T cell subsets is pivotal in assessing tumor incidence and development. Ratios such as CD8+/CD4+ and CD8+/FoxP3+ serve as critical indicators, reflecting the vigor of the anti-tumor immune activity. Disturbingly, tumor cells deploy strategies to manipulate this balance, inhibiting the action of tumor-specific T cells and inducing the engagement of immunosuppressive Tregs. The outcome is a reduction in the CD8+ T cells/Tregs ratio, providing a window for tumor cells to evade immune attacks and progress unchecked (Liu et al., 2022).

2.3.3.2.3. B lymphocytes

The involvement of B cells is a topic marked by controversy and relative understatement compared to the well-discussed role of T cells. B cells, however, present a dual functionality in this context, extending beyond their conventional role in antibody secretion (Liu et al., 2022). They exert influence not only over T cells but also over responses from innate immune cells. The crucial processes of antigen processing and presentation by B cells, coupled with the intricate balance of B-cell subtypes and their functions, take a significant part in shaping both anti- and pro-tumorigenic responses (Gupta et al., 2022).

Within the cancer tumor microenvironment, B cells exhibit a multifaceted nature, offering both opportunities and challenges for immunotherapy. On the one hand, these cells showcase anti-tumor characteristics by recognizing tumor-specific "neoantigens" and catalyzing the production of antibodies, thereby effectively targeting and eliminating oncogenic cells. However, the pro-tumorigenic effects of B cells, influenced by factors like circulating immune complexes (CICs) and specific B cell subtypes such as CD19+, CD24+, and CD38+, present formidable obstacles (Gupta et al., 2022).

A noteworthy subtype of B cells, known as Breg cells, is influenced by inflammatory signals and contributes to immune tolerance. Breg cells can enhance Foxp3 expression in Treg cells, thus aiding immune regulation. Nevertheless, in specific scenarios, such as hepatocellular carcinomas, the expression of PD1/PD-L1 may counteract Bregs' anti-tumor activity (Gupta et al., 2022).

Overall, while B cells hold promise in bolstering immunotherapy goals through their anti-tumor capabilities, their pro-tumorigenic potential underscores the need for a nuanced understanding of their role within the tumor microenvironment. Balancing these dual aspects of B cell function is crucial for effectively harnessing their potential in cancer immunotherapy (Gupta et al., 2022).

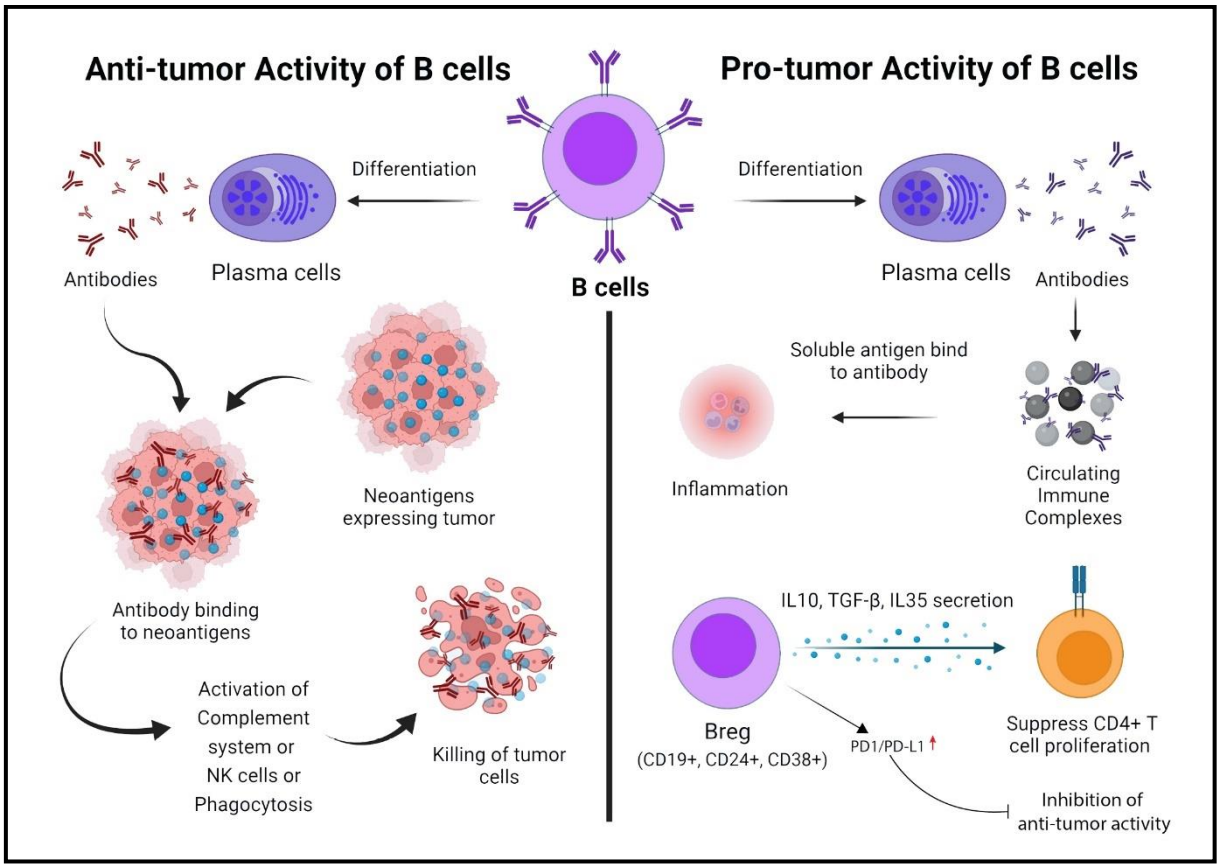

Figure 8: B cells in the microenvironment of cancer tumors: Maneuvering through their intricate dual characteristics. Adapted from (Gupta et al., 2022).

2.3.3.2.4. Dentritic Cells

Dendritic cells are pivotal as principal antigen-presenting cells within the human body. Their capacity to process and present antigens on MHC enables the initiation of antigen-specific immune responses by T cells. Three primary categories of DCs include conventional DCs (cDCs), monocyte-derived DCs (moDCs), and plasmacytoid DCs (pDCs). cDCs are also divided into cDC1 and cDC2, each characterized by distinct surface molecules and transcription factors (Peng et al., 2021).

Originating from bone marrow-derived precursors, cDCs are crucial in inducing T-cell-dependent adaptive immunity. Plasmacytoid DCs, on the other hand, serve as producers of type I interferons and are also derived from bone marrow. MoDCs, often generated in response to inflammation, differentiate from monocytes and are present in specific tissues under steady-state conditions (Liu et al., 2022).

While immature DCs lack costimulatory molecules and cytokine secretion, they can potently capture antigens. Activated DCs undergo maturation, characterized by

increased MHC II and CCR7 expression. Simultaneously, they downregulate antigen-capturing capacity and acquire the ability to migrate to lymph nodes. DC maturation markers incorporate the expression of MHC-peptide complexes, enhanced costimulatory molecules, cytokine secretion, and the capability to stimulate corresponding T cells (Peng et al., 2021).

In the tumor immune microenvironment (TME) context, dendritic cells play an essential role in activating and regulating both immune responses. Cancer-associated fibroblasts (CAFs) influence DCs by controlling their maturation and antigen presentation, ultimately contributing to immune escape by tumor cells. In certain studies, the fusion of DCs with CAFs has demonstrated the ability to stimulate T lymphocytes and generate a robust cytotoxic T lymphocyte (CTL) response. Additionally, CAFs, a significant source of TGF-β, mediate the co-stimulation of MHC II and downregulate key surface molecules on DCs, hindering antigen presentation and CTL activation. This intricate interplay highlights the sophisticated regulatory mechanisms inside TME, impacting DCs functional dynamics DCs and influencing adaptive immune responses (Gao et al., 2023).

2.4. Immunotherapy: Basics and Types

Immunotherapy represents a pivotal frontier in contemporary medical paradigms, influencing disease trajectories. Positioned as a therapeutic approach, it intricately engages the host's immune system to impede or propel disease progression. Its primary objective is to orchestrate a concerted response from the innate and acquired immune systems, aiming for the enduring obliteration of diseased cells (Fasano et al., 2022).

Broadly categorized into passive and active approaches, immunotherapy illuminates distinct avenues of intervention. In passive immunotherapy, clinicians administer ex vivo-generated immune elements like antibodies and immune cells directly to patients. This method sidesteps the stimulation of the host immune response, embodying a precise yet externally driven assault on the disease. Passive immunotherapies generally require routine and frequent administration due to their short-lived effects (Monk et al., 2022).

On the flip side, active immunotherapy beckons the immune system into action, fostering the development of specific immune effectors. The duration of active immunotherapy can vary depending on the individual's reaction and the treatment protocol. Here, the goal is not merely treatment but the cultivation of sustained immune memory, a formidable ally in the ongoing battle against diseases (Ritu et al., 2023).

Navigating the delicate balance between fortifying the immune system and avoiding unchecked inflammatory responses, immunotherapy holds promise in cancer treatment. While innate immunity contributes to cytokine releases, the adaptive immune system emerges as the protagonist, armed with the capability to target non-self antigens precisely. This nuanced understanding has given rise to various immune therapies, spanning monoclonal antibodies, vaccinations, and checkpoint inhibitors, each strategically designed to augment immune function through diverse mechanisms of action (Abbott & Ustoyev, 2019; Naran et al., 2018).

2.4.1. Passive immunotherapy

2.4.1.1. Cytokine-mediated immunotherapy

Treatment involving cytokines, involving interleukins, interferons, and chemokines, has emerged as a prominent strategy in tumor treatment. The FDA's approval of recombinant interferon-α (IFN-α) in 1986 marked a milestone in cancer immunotherapy, followed by the authorization of recombinant interleukin-2 (IL-2) in 1992 for treating metastatic melanoma and advanced renal cell carcinoma. Pro-inflammatory cytokines like interferon-γ (IFN-γ), interleukin-12 (IL-12), and interleukin-15 (IL-15) play pivotal roles in enhancing effector T cell activity, promoting T helper cell and cytotoxic T lymphocyte proliferation (Shang et al., 2022).

Despite its promising outcomes, systemic cytokine administration faces challenges such as high doses, short in vivo half-life, off-target toxicities, and low bioavailability. Local delivery methods have been explored to concentrate cytokines at the tumor site to address these limitations while minimizing exposure to normal tissues. Local scaffolds, particularly hydrogels, are appealing for site-specific cytokine delivery (Rahman et al., 2023).

Interleukins, these signaling molecules govern immune responses, with IL-10 exhibiting anti-inflammatory effects, while IL-2 and IL-15 stimulate immunostimulatory responses. Like thermosensitive hydrogels, local scaffold-assisted delivery has improved anti-tumor effects, sustaining cytokine release and reshaping the tumor microenvironment (Shang et al., 2022).

Interferons, known for their pleiotropic effects, are crucial in immune response regulation. However, their systemic use is hindered by dose-limiting toxicities. Innovative approaches, such as interferon-a2a (IFN-a2a)-incorporated hyaluronic acid-tyramine hydrogel, have shown promise in liver cancer treatment, enhancing drug concentration at the tumor site and inhibiting cancer cell proliferation (Shang et al.,

2022).

Chemokines, vital for immune cell migration to tumor sites, offer a potential avenue for cancer immunotherapy. Local delivery of chemokines, like CCL17-encapsulated scaffolds, demonstrates the ability to recruit immune-activating cells, contributing to immune-stimulation effects and suppressing tumor growth (Kane et al., 2024; Shang et al., 2022).

In summary, local scaffold-assisted delivery of cytokines, whether interleukins, interferons, or chemokines, presents an innovative approach to enhance cancer immunotherapy. These strategies address challenges associated with systemic cytokine administration, offering a more targeted and controlled means to improve therapeutic efficacy. Combining cytokine therapy with immune checkpoint blockade (ICB) may enhance anti-tumor immunotherapy outcomes by mitigating adverse immune effects and promoting sustained therapeutic responses (Kondoh & Mizuno-kamiya, 2022; T. Rahman et al., 2023).

2.4.1.2. Antibodies directed against tumor-associated antigen

Pioneering an exhaustive exploration through cutting-edge molecular and cellular methods, our intensive efforts to dissect immune responses in patients with neoplastic conditions have yielded groundbreaking insights. The outcome has been the identification of diverse molecules recognized by human T cells and antibodies in the context of tumors. These discoveries have delineated two primary categories of molecules with immense implications for cancer immunotherapy (Jacqueline & Finn, 2020).

At first, we discuss the concept of tumor-specific antigens, which include mutated neoantigens that are unique to individual tumors, as well as products resulting from oncogene mutations such as K-ras and N-ras, and gene translocations like BCR-ABL. Additionally, oncofetal antigens, common among many tumors but not present in normal adult tissues, have also been identified as important factors. It is worth noting that immune responses against shared oncogene mutations and oncofetal antigens can be targeted by both humoral and cellular immunity in patients, providing potential candidates for therapeutic cancer vaccines. Their consistent expression in certain tumor types makes them promising candidates for preventative cancer vaccines (Jacqueline & Finn, 2020).

In the second category, we explore tumor-associated antigens (TAA), including

differentiation antigens and those overexpressed or post-translationally modified. Differentiation antigens like CEA and PSA exhibit tissue-specific expression and are present in certain tumor types and their corresponding normal tissues. Overexpressed antigens, including MUC1, cyclin B1, HER2/neu, and others, are identified as promising targets due to their increased expression in epithelial tumors (Jacqueline & Finn, 2020).

Transitioning to the targeted therapy landscape, the focus shifts to developing biological therapies, prominently featuring monoclonal antibodies and T cells targeting particular tumor antigens. This avenue offers promising prospects for improving survival across various cancers. Notably, the success of antibodies like trastuzumab, targeting HER2/neu in breast cancer, exemplifies the potential of such targeted therapies (Alrhmoun & Sennikov, 2022).

In antibody-based therapies, passive immunotherapy with HER2-directed monoclonal antibodies, such as trastuzumab, pertuzumab, and margetuximab, has significantly improved clinical outcomes. The advent of antibody–drug conjugates (ADCs) provide a promising avenue, circumventing the toxicity concerns associated with conventional chemotherapy. Trastuzumab Deruxtecan (T-DXd), Trastuzumab Emtansine (T-DM1), and SYD985 represent noteworthy players in this domain (Maecker et al., 2023).

As we traverse the landscape of cancer therapy, particularly in the context of HER2-positive cancers, the evolving arsenal of treatments signifies a paradigm shift in improving patient outcomes. The approval of several HER2-targeted therapies by the FDA, with multiple new drugs entering the scene, underscores the dynamic nature of advancements in cancer therapeutics. The amalgamation of targeted therapies, immunotherapies, and preventative vaccines marks a transformative era in the quest for effective cancer treatments (Alrhmoun & Sennikov, 2022).

2.4.1.3. Engineering T cells

T cell-based immunotherapy has emerged as a groundbreaking methodology in tumor treatment, with notable successes in developing tumor-infiltrating lymphocytes, chimeric antigen receptor (CAR) T cells, and (TCR) T cells. CAR-T cells have succeeded in treating leukemia and lymphoma, gaining FDA approval and participating in over 480 reported clinical trials as of September 8, 2022 (June et al., n.d.).

One key advantage of CAR-T cell therapy lies in its independence from antigenic peptide-bound major histocompatibility complex (MHC), addressing a significant immunoevasion mechanism in cancer. However, the treatment comes with challenges,

such as cytokine release syndrome and neurotoxicity, necessitating careful management for potential immunotherapies (Hyun et al., 2023).

T cells, essential players in the immune system, navigate various tissues throughout their lifespan, encountering diverse mechanical environments in the thymus, bone marrow, blood vessels, and lymphoid tissues. Their journey involves:

- Flowing through the lymphatic or bloodstream.
- Extravasating vessels.
- Migrating through the stromal extracellular matrix (ECM).

T cells sense mechanical forces in these processes, adapt their shape, and interplay with antigen-presenting cells (APCs). These forces are essential in T cell processes, incorporating antigen recognition through the T cell receptor (TCR) binding to MHC (Hyun et al., 2023).

T-cell mechanosensing is a complex process that involves the identification of antigens presented by APCs. Antigen recognition is further reinforced by the formation of TCR clusters that help create an immunological synapse. Co-receptors such as CD28 and LFA-1 also provide supplementary stimulatory signals to the T-cells. Forces provided by the cytoskeletons facilitate these signals (Wang et al., 2022).

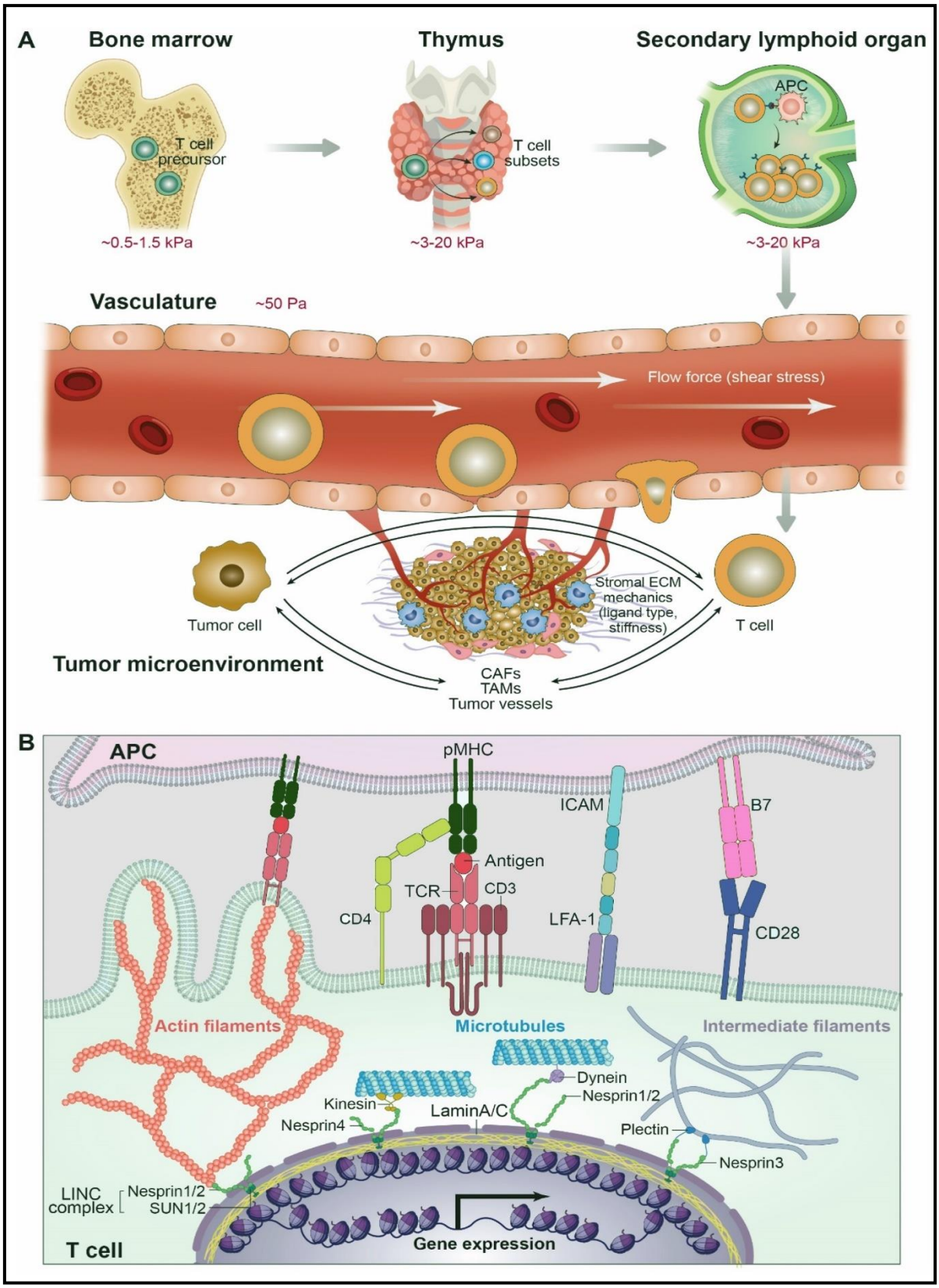

Figure 9: The movement of T cells within living organisms, the signaling processes that occur inside the cells, and their ability to detect mechanical stimuli. Adapted from (Hyun et al., 2023).

CAR-T cell therapy, currently limited to young adults under 25 and children dealing with lymphoma, leukemia, and melanoma subtypes, presents challenges due to potential serious side effects on healthy tissues. Overcoming immune escape and resistance is crucial for expanding cancer immunotherapy applications (Hyun et al., 2023).

Biophysical cues from ECM and neighboring cells, like stiffness and topography, impact T cell functions. Ex vivo culture on traditional plates diminishes primary T cells' therapeutic potential, prompting the development of engineered biomaterial substrates like polymer fibers and silica rods to enhance T cell signaling (Wang et al., 2022).

Immunotherapy faces challenges in solid tumors with heterogeneous cells and stiff ECM, inhibiting immune cell recruitment. Targeting stiff ECM for increased T cell infiltration involves strategies like a pharmacological intervention to soften ECM, demanding novel tumor-specific drug delivery systems. Mechano-sensitive CAR-T cells, designed for self-penetration into solid tumor ECM, show promise by manipulating mechano-sensitive machinery (Hyun et al., 2023).

Genome engineering allows spatiotemporal control of CAR-T cell stimulation via noninvasive methods like heat, light, and ultrasound. ECM and cell mechano-modulation, along with engineered T cell responses to mechanical forces, is a burgeoning research area with potential in immunotherapy (Hyun et al., 2023).

Engineering approaches for mechano-modulating T cells include biomaterials mimicking microenvironment mechanics, microfluidic devices providing controlled cues, and mechano-genomic engineered T cells sensitive to physical alterations. External forces on mechano-genomic modified CAR-T cells enhance cytotoxicity and activation while reducing off-tumor toxicity (Hyun et al., 2023).

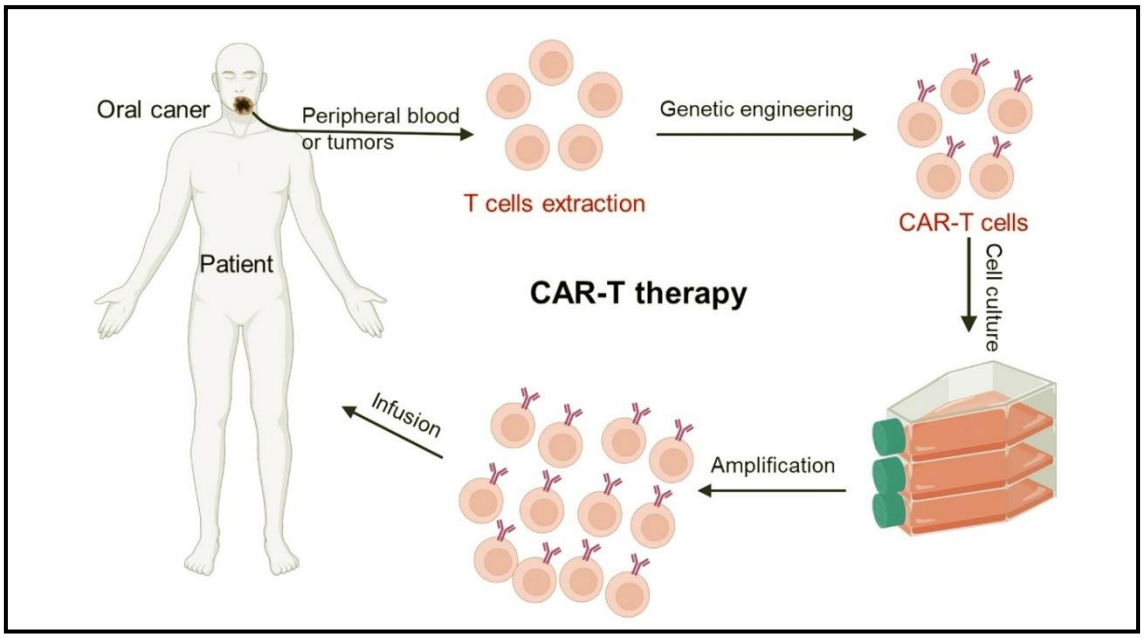

Figure 10: Illustrative representation of CAR-T therapy. Adapted from (Liu et al., 2022).

This transformative approach, lauded as a significant breakthrough by science in 2013, entails the ex vivo expansion of modified CAR T cells followed by their infusion back into the patient. The engineered T cells demonstrate increased effectiveness in detecting and eradicating tumors. While the therapeutic impact of CAR T cell therapy has been remarkable in hematological malignancies, its effectiveness against solid tumors remains limited owing to challenges posed by TME, low infiltration rates, and restricted survival duration of CAR T cells (Li et al., 2024).

Recent advancements, such as the fibrin-based gel developed by Dotti et al. for glioblastoma treatment, underscore efforts to overcome these barriers. The gel ensures the stability and biocompatibility of CAR T cells and facilitates extended contact with tumor cells within the resection cavity. This controlled release mechanism enhances antitumor activity compared to direct implantation of CAR T cells (Shang et al., 2022).

Despite these strides, CAR T cell therapy confronts impediments associated with T cell dysfunction within the immunosuppressive environment of solid tumors characterized by immune checkpoint molecules. Innovative strategies have been proposed to surmount these challenges, including developing multi-functional hydrogels. Hu et al. designed a hyaluronic acid hydrogel to improve CAR T cell therapy against tumor growth and recurrence by integrating Immune Checkpoint Blockade and cytokine therapy (Shang et al., 2022).

The hydrogel is a reservoir for CAR T cells, IL-15 and aPD-L1, providing targeted and sustained delivery. By addressing immune-related impediments, such as the immunosuppressive tumor microenvironment, this approach exhibited superior efficacy in curbing post-surgery tumor relapse and fostering durable immune memory (Shang et al., 2022).

Despite advancements, inadequate antigen presentation in CAR T cell therapy remains a challenge that can compromise clinical success rates. Overcoming these obstacles necessitates the activation of innate immune responses to augment the effectiveness of CAR T cells against phenotypically diverse solid tumors, preventing the evasion of CAR T cell identification due to the loss of targeting antigens. This comprehensive approach underscores the evolving landscape of cancer immunotherapy, combining genetic engineering, biomaterials, and multi-functional strategies to address the complexities associated with solid tumors (Shang et al., 2022).

2.4.1.4. Immune checkpoint inhibitors

Immune inhibitory molecules, like CTLA-4 and PD-1, play crucial roles in regulating T cell stimulation, guiding the release of anti-inflammatory cytokines, and establishing an immunosuppressive microenvironment. This orchestrated manipulation of immune cells directs the immune response towards suppression. Despite efforts in immunotherapy to counteract this suppression, challenges persist in achieving robust responses. Therefore, understanding the dynamic immunosuppressive network inside the TME is imperative. To achieve this, gaining deeper insights into the roles of T cells,antigen-presenting cells (APC), and natural killer cells (NK) is essential for a comprehensive understanding of immune modulation in this context (Mestiri et al., 2024).

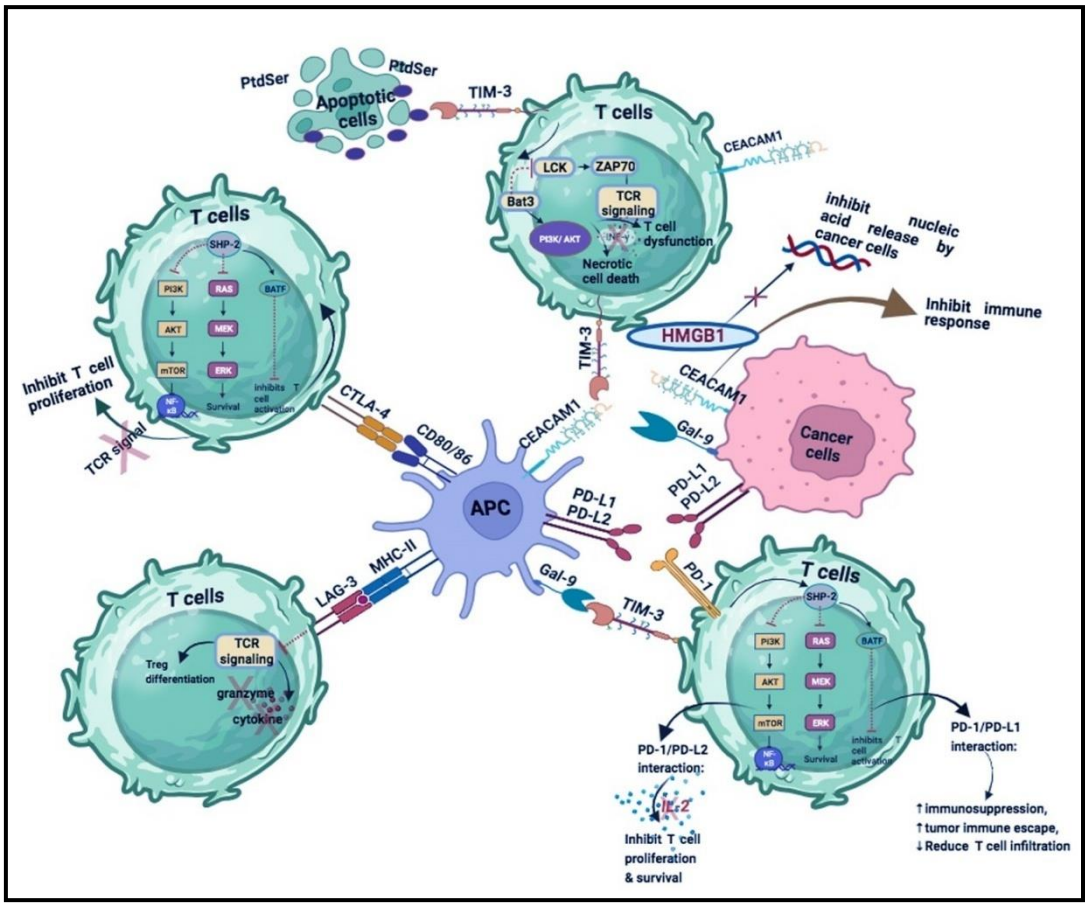

Figure 11: Immune inhibitory biomarkers. Adapted from (Mestiri et al., 2024).

French researchers' pivotal discovery of cytotoxic T lymphocyte antigen 4 (CTLA-4) in the 1980s triggered a revolutionary shift in cancer immunotherapy, setting the stage for the development of immune checkpoint inhibitors (ICIs). Dr. James Allison's groundbreaking work illuminated CTLA-4's role as an immune system brake, prompting the hypothesis that inhibiting this molecule could unleash T cells to combat cancer effectively. In 2011, the FDA approval of ipilimumab, a CTLA-4 checkpoint inhibitor, marked a significant milestone for metastatic melanoma treatment (Abbott & Ustoyev, 2019).

Simultaneously, Dr. Drew Pardoll's exploration of programmed death-1 (PD-1) and its ligand PD-L1, identified in Japan, uncovered another critical immune system regulator. The subsequent approval of PD-1 and PD-L1 therapies by the FDA, particularly as a front-line treatment for lung cancer patients with increased PD-L1 expression, highlighted the transformative potential of these immune checkpoint inhibitors (Abbott & Ustoyev, 2019).

The intricate interplay between PD-1 and its counterpart, PD-L1, on tumor cells instigates the PD-1/PD-L1 signaling cascade. This molecular dialogue is a roadblock to

further immune activation, allowing tumors to evade antigen-specific T-cell responses cleverly. Emerging as a powerful force in this arena, immune checkpoint inhibitors (ICIs) disrupt these conversations, offering a beacon of hope with their enduring responses and improved patient survival (Davoudi et al., 2024).

The success of ICI therapy in advanced-stage cancer has sparked curiosity about its potential in earlier disease stages. Within the dynamic theater of the tumor microenvironment (TME), immune cells play a Janus-faced role, either suppressing or promoting tumors, contingent upon context and tumor type. The presence of immunosuppressive cells (Tregs, myeloid-derived suppressor cells, tumor-associated macrophages, tumor-associated neutrophils, and tumor-associated dendritic cells), coupled with co-inhibitory receptor activation, becomes a critical determinant in the efficacy of ICI therapy (Davoudi et al., 2024).

To better design clinical trials and identify predictive biomarkers for cancer therapies, it is crucial to have a deep understanding of the mechanisms that drive tumor resistance. This understanding can be achieved by integrating multi-omic measurements. Such knowledge can also help us discover new drug targets and combinations, leading to more effective cancer treatments (Davoudi et al., 2024).

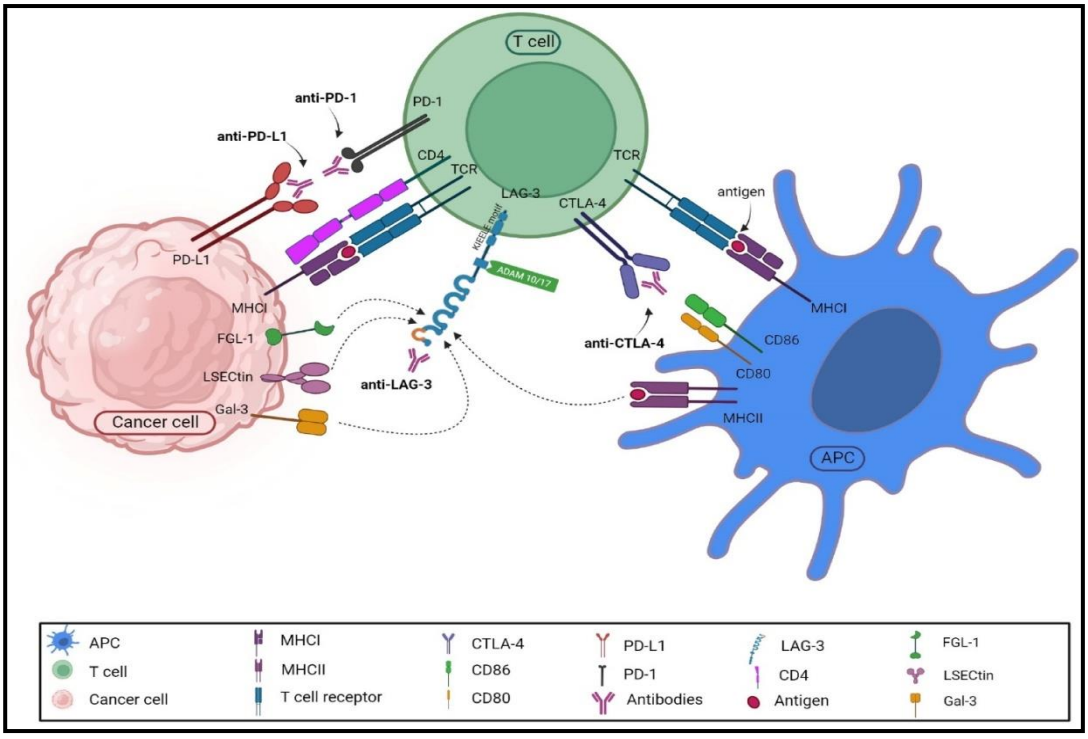

Figure 12: Immune checkpoint inhibitors that have been approved along with their target molecules. Adapted from (Baghy et al., 2023).

2.4.1.4.1. Cytotoxic T lymphocyte antigen 4

Cytotoxic T lymphocyte antigen 4 (CTLA-4), a crucial component of the immunomodulatory CD28-B7 superfamily, has garnered attention in melanoma and head and neck cancer (HNC) research. In melanoma, the CTLA-4 inhibitor ipilimumab has shown remarkable success, enhancing survival rates compared to standard therapy. However, its efficacy comes with a higher occurence of immune-related adverse events (Davoudi et al., 2024).

In HNC, CTLA-4 expression becomes a diagnostic and prognostic marker. Studies reveal elevated CTLA-4 levels in laryngeal and oral squamous cell carcinoma, with lower expression correlating with better outcomes. Nasopharyngeal carcinoma patients exhibiting lower CTLA-4 expression also display improved survival rates. The CD8+/CTLA-4 ratio emerges as a potential prognostic indicator for HNSCC (Mestiri et al., 2024).

In the clinical and research realm, preclinical studies demonstrate CTLA-4 monoclonal antibodies triggering reduction in tumor size and long-lasting immunity against the tumor. Although no approved immunotherapies target CTLA-4 in HNC, ongoing clinical trials explore merging anti-CTLA-4 antibodies with other treatments. This approach, flourishing in different cancer types, holds promise for improved clinical outcomes (Mestiri et al., 2024).

In summary, CTLA-4 is a significant player in cancer therapy, with ipilimumab's success in melanoma inspiring exploration in HNC. The dual perspective highlights the potential of CTLA-4 as a therapeutic target, encouraging continued research for enhanced cancer treatment strategies (Mestiri et al., 2024).

2.4.1.4.2. Programmed cell-death protein 1 (PD-1)

Programmed cell death protein 1 (PD-1) is a transmembrane protein belonging to the CD28/B7 immunoglobulins superfamily. It is typically found on the surface of activated T cells, B cells, and certain tumor-infiltrating lymphocytes (TILs). The primary role of PD-1 is to regulate immune responses by sending inhibitory signals when it binds to its ligands, programmed death ligand 1 (PD-L1) and programmed death ligand 2 (PD-L2), which helps maintain peripheral self-tolerance. The approval of anti-PD-1 antibodies such as nivolumab, pembrolizumab, and cemiplimab, alongside anti-PD-L1 antibodies including avelumab,atezolizumab, and durvalumab by the US Food and Drug Administration (FDA), underscores the clinical significance of targeting the PD-1/PD-L1 axis in immunotherapy (Davoudi et al., 2024).

In the context of HNSCC (Head and Neck Squamous Cell Carcinoma), distinguishing molecular profiles become apparent when classifying based on the infection status of human papillomavirus (HPV). Studies by Chen et al. have demonstrated a direct correlation between p16 protein expression and PD-1/PD-L1 levels in HNSCC samples, suggesting a potential prognostic value. Notably, positive PD-1/PD-L1 expression in HNSCC patients is associated with improved prognosis and reduced recurrence rates, indicative of favorable responses to anti-PD-1/PD-L1 therapeutic interventions. Additionally, investigations have revealed associations between PD-L1 expression and HPV positivity, further highlighting the intricate interplay between viral infection status and immune checkpoint regulation in HNSCC pathogenesis (Mestiri et al., 2024).

Further characterization of the tumor microenvironment in HNSCC has identified the role of tumor-associated macrophages (TAMs) and myeloid-derived suppressor cells (MDSCs) in modulating PD-1/PD-L1 expression through the CD47/SIRPα process. In addition, interferon-gamma (IFN-γ) has been implicated in the upregulation of PD-L1 expression via various signaling cascades, including the protein kinase D isoform 2 (PKD2) pathway and the IFNAR1/STAT1 axis. Notably, PD-1 expression on Natural Killer within the TME represents an activation phenotype, with PD-L1-mediated inhibition attenuating NK cell-mediated cytotoxicity against HNSCC cells. Additionally, the detection of soluble PD-L1 (sPD-L1) in patient plasma and PD-L1 positive exosomes further underscores the potential utility of these biomarkers in prognostic assessment and treatment stratification for HNSCC (Mestiri et al., 2024).

In clinical practice, ICIs targeting PD-1, such as nivolumab and pembrolizumab, have demonstrated efficacy in recurrent/metastatic HNSCC by disrupting suppressive signals mediated by the PD-1/PD-L1 pathway, thereby enhancing anti-tumor immune responses. (Mestiri et al., 2024)Notably, PD-L1 expression levels have emerged as a crucial determinant for patient selection in immunotherapy, with the PD-L1 combined positive score serving as a validated biomarker in recurrent/metastatic HNSCC. Despite ongoing investigations into the prognostic and diagnostic implications of PD-1 expression in HNSCC, its precise role remains to be fully elucidated (Mestiri et al., 2024).

In conclusion, the multifaceted roles of PD-1, PD-L1, and PD-L2 in HNSCC underscore their significance as therapeutic targets and prognostic biomarkers. Future research endeavors to unravel the intricate mechanisms governing immune checkpoint regulation in HNSCC are warranted to optimize treatments and improve patient

outcomes in this challenging malignancy (Mestiri et al., 2024).

2.4.1.4.3. Lymphocyte-activation gene 3 (LAG-3)

Lymphocyte-activation gene 3 (LAG-3) is a molecule found on the surface of various immune cells such as CD4/CD8+ T cells, plasmacytoid dendritic cells, B cells, and natural killer cells. When LAG-3 communicates with MHC II, it inhibits its interaction with T-cell receptors, leading to the downregulation of T-cell proliferation and activation. This downregulation is associated with resistance to treatment in multiple malignancies, such as melanoma, head and neck squamous cell carcinoma (HNSCC), non-small cell lung cancer (NSCLC), colorectal cancer, pancreatic cancer, colorectal cancer, and breast cancer (Davoudi et al., 2024).

The coexpression pattern of LAG-3 suggests its potential as a target for immune checkpoint inhibitor (ICI) therapy. High expression of LAG-3 was observed in different malignancies, with 22.6% of patients exhibiting elevated LAG-3 gene expression. Particulary, neuroendocrine, uterine, colorectal, and melanoma tumors showed exceptionally high LAG-3 expression (Davoudi et al., 2024).

A substantial correlation was found between high expression levels of LAG-3 and other immune checkpoints, including PD-L1, PD-1, CTLA-4, and a high tumor mutational burden, in a study that examined 397 genes in 514 cancer cases. The FDA recently approved Bristol Myers Squibb's medication Opdualag, a fixed-dose combination of relatlimab and nivolumab, for the first-line treatment of advanced or metastatic melanoma. Targeting both PD-1 and LAG-3 at the same time, this combination therapy has shown to be safe and effective, offering advantages above PD-1 blockage alone in terms of progression-free survival (Davoudi et al., 2024).

2.4.1.4.4. T-cell immunoglobulin and mucin domain 3 (TIM-3)

The T-cell immunoglobulin and mucin domain 3 (TIM-3) protein functions as a negative regulator of anti-tumor immunity and is linked to advanced malignancy and a bad prognosis. As a potential treatment for solid tumors and hematological malignancies, targeting TIM-3 has gained traction. It was discovered that TIM-3 is a negative regulator of T helper 1 (Th1) activity, and that Th1 cell death and T cell fatigue are caused by TIM-3's ligand Gal-9 (Mestiri et al., 2024).

T cell fatigue and immunological tolerance in cancer are linked to the relationship between TIM-3 and CEACAM1 on active T cells. Co-expression of TIM-3 and CEACAM1 in colorectal cancer is associated with T cell depletion; inhibiting both

pathways can reactivate the immune response against the tumor. TIM-3 also plays a role in suppressing the immune response by binding to HMGB1 in the tumor microenvironment, preventing the stimulation of nucleic acids released by tumor cells (Mestiri et al., 2024).

Intratumoral regulatory T cells (Tregs) in HNSCC express increased TIM-3, indicating a more suppressive phenotype. TIM-3 expression correlates with CD8+ T cells, myeloid-derived suppressor cells (MDSCs), recurrent disease, and upregulation after radiotherapy or chemotherapy (Mestiri et al., 2024).

2.4.1.4.5. B and T lymphocyte attenuator (BTLA)

B and T lymphocyte attenuator (BTLA) is a crucial co-inhibitory receptor that is widely expressed on a variety of immune cells, such as type 1 conventional dendritic cells (cDC1), resting Vγ9Vδ2 cells, and naïve CD4+ and CD8+ T cells. Its ligand, HVEM, is expressed in diverse cell types, and HVEM overexpression is observed in different malignancies. BTLA signaling inhibits T cell proliferation and function, and its depletion enhances TCR-induced proliferation. In cancer, BTLA-HVEM interaction acts as a defense mechanism for immune evasion. Variants in the BTLA gene may serve as biomarkers for early detection of oral squamous cell carcinoma (OSCC) and the effectiveness of treatment. Studies HNSCC reveal varied methylation patterns and mRNA levels of BTLA, with correlations to survival and PD-L1 expression. Circulating soluble BTLA is suggested as a non-invasive biomarker for immune checkpoint inhibitor therapy outcome prediction. BTLA inhibitors are being developed as prospective therapeutics for HNSCC, with ongoing clinical trials exploring a combination therapy using monoclonal antibodies against BTLA and PD-1 in patients with nasopharyngeal carcinoma (NPC) and OSCC (Mestiri et al., 2024).

A study of mouse tumors found that utilizing a PD-1 blocking antibody alone was ineffective for flank tumors but caused regression in 54% of orthotopic tongue tumors. Combining immunotherapies targeting PD-1 and CTLA-4 resulted in an impressive 93.3% survival rate in mice with tongue tumors and a significant reduction in immunosuppressive cells within the tumor microenvironment. Elevated interferon signaling and increased PD-1/PD-L1 levels in tongue tumors correlated with their enhanced response to PD-1 blockade. Notably, in a metastatic model involving both tongue and flank tumors, combining a STING agonist with systemic treatment led to sustained tumor regression in 71% of mice. This positive outcome was connected with

increased ratios of cytotoxic CD8+ T cells (CTL) to regulatory T cells (Treg) and functional myeloid-derived suppressor cells (MDSC), indicating a productive abscopal anti-tumor immunity (Dorta-Estremera et al., 2019).

In conclusion, immune checkpoint inhibitor (ICI) therapy has undeniably achieved remarkable clinical success in solid tumor treatment. However, the inherent variability in treatment response, associated expenses, and the impact of immune-related adverse events (irAEs) underscore the need for innovative, cost-effective strategies in integrative oncology (IO). This novel approach aims to improve treatment outcomes while minimizing adverse events (Fuller-Shavel & Krell, 2024).

As ongoing research in this field progresses, a pivotal shift toward pre-treatment assessments, encompassing both tumor and patient-associated biomarkers, is emerging. Personalized multimodal prehabilitation care plans and on-treatment support techniques are proposed. This includes targeted nutrition, physical exercise, and supplementation regimens that address systemic inflammation and gut microbiota modulation (Fuller-Shavel & Krell, 2024).

Integrative oncology (IO) emerges as a promising avenue to tailor responses to ICI therapy without undue toxicity. Key IO approaches, encompass a Mediterranean-style diet rich in fiber (over 20g), regular exercise, and the utilization of live biotherapeutics. Other integral elements involve ensuring vitamin D repletion while minimizing proton pump inhibitor (PPI) and antibiotic use. Simultaneously, various underexplored IO approaches await further investigation, spanning dietary interventions (polyphenols, prebiotics, omega-3) to diverse gut microbiome and immune modulation strategies (Chinese herbal medicine, mycotherapy) (Fuller-Shavel & Krell, 2024).

Cautionary considerations apply, notably in the case of medical cannabis usage in ICI-treated patients, due to known adverse effects on overall survival. In contrast, investigations using Viscum album extract (VAE) therapy have found no safety problems.

The understanding of chronic stress's impact on ICIs treatment outcomes calls for prudent discussions on stress management with patients. Exploring the use of mind–body modalities in ICI support is crucial for further investigation, promising holistic advancements in cancer care (Fuller-Shavel & Krell, 2024).

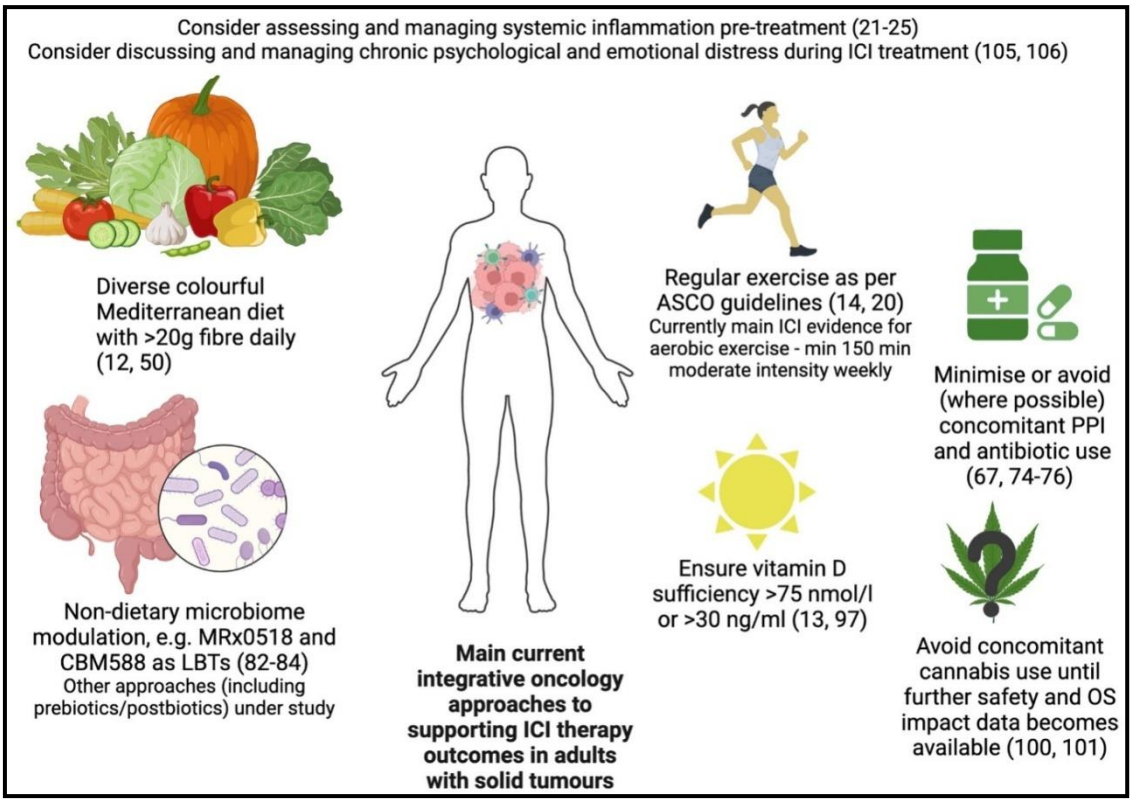

Figure 13: The primary modern approaches in integrative oncology aimed at enhancing the effectiveness of immune checkpoint inhibitor (ICI) therapy for solid tumor treatment in adults. Adapted from (Fuller-Shavel & Krell, 2024).

2.4.1.5. Targeting CAFs to modulate TME

The complex interaction between cancer-associated fibroblasts (CAFs) and the tumor microenvironment makes CAFs interesting targets for cancer therapy, owing to their high connection with unfavorable cancer prognoses. A pivotal study by Hayward et al. revealed that inhibiting epithelial CXCR4 expression effectively mitigated the tumorigenic response mediated by CAFs. Furthermore, Yuan et al. demonstrated the significant inhibition of breast cancer metastasis through MHP-1, down-regulated TGF-β signaling and the epithelial-mesenchymal transition (EMT) program (Lin et al., 2016). The current landscape of therapeutic strategies directed at CAFs encompasses a dual approach: enhancing the anti-tumor impacts of CAFs and mitigating their pro-tumor roles. Strategies to augment the anti-tumor potential of CAFs involve manipulating the extracellular matrix (ECM), modulating immune responses, and promoting anti-tumor metabolic processes. On the other hand, strategies to reduce the pro-tumor functions of CAFs focus on alleviating stromal solidification, eliminating ECM, reversing CAF activation, inhibiting pro-oncogenic cytokine secretion, and suppressing vascularization and inflammatory processes (Gao et al., 2023).

Crucially, the pursuit of effective interventions against CAFs can be approached through two distinct pathways: the clinical pathway and the natural pathway. Whether targeting specific clinical markers or harnessing the inherent mechanisms within the body, these dual strategies open avenues for innovative and tailored therapeutic interventions in cancer treatment (Gao et al., 2023).

In clinical therapeutics targeting cancer-associated fibroblasts (CAFs), two strategies with promising potential have emerged.

The first technique calls for the direct or indirect decrease of CAFs. Notably, the cell surface serine protease FAP (fibroblast activation protein) has emerged as a promising option for CAF targeting. FAP expression, which is lacking in mature somatic tissues save for wound repair and tumor mesenchyme, becomes a target for intervention. The use of relay chimeric antigen receptor (CAR)-T cell treatment directly targets CAFs by using FAP-specific CAR-T cells. However, problems like as myelotoxicity and cachexia have been found in various studies, motivating the investigation of alternative approaches (Gao et al., 2023).

One such alternative is the application of near-infrared photoimmunotherapy (NIR-PIT), showcased in vivo mice xenograft models. This technique effectively targets CAFs, inhibiting tumor growth without negative effects. The combination of NIR-PIT and 5-fluorouracil (5-FU) further demonstrates potential by sensitizing CAF-rich tumors to 5-FU, offering a promising approach to overcoming drug resistance through CAF elimination (Gao et al., 2023).

The second technique focuses on functionally modifying or reprogramming CAFs to a resting state. Preclinical studies advise against non-specific targeting or elimination of mesenchymal fibroblasts, highlighting the importance of targeting specific CAF subtypes or inducing an anticancer CAF phenotype. Notable instances include targeting vitamin D receptors in pancreatic cancer and changing active stellate cells into a calmer state, thus lowering disease aggressiveness (Gao et al., 2023).

Nidanib, a PDGF receptor β signaling pathway blocker, can inhibit the activation and development of CAFs. This inhibition considerably lowers IL-6 release by CAFs, affecting various signaling pathways, including ERK (Extracellular Signal-Regulated Kinase) and AKT (Protein Kinase B). As a result, the cytotoxic actions mediated by NK cells are eliminated. In CAF/tumor spheroid or xenograft models, co-administration of nidanib improves the tumor-killing capacity of chimeric antigen receptor NK cells, suggesting a synergistic impact with increased NK cell activity through blocking of IL-6

trans-signaling (Gao et al., 2023).

Natural molecules offer an intriguing approach to targeting CAFs, demonstrating anti-fibrotic and immunomodulatory activities within the tumor microenvironment (TME). These chemicals regulate carcinogenesis, progression, and regression by regulating the quantity, subtype, and cytokine secretion of CAFs, potentially increasing patient lifespan and quality of life. Specifically, the impact of natural small molecules on CAF regulation brings up new prospects for clinical cancer treatment, giving a theoretical basis for pharmacological investigations. Noteworthy strategies involve targeting the amount of CAFs, where curcumin exhibits dose-dependent inhibition of fibroblast proliferation and induces apoptosis in myCAFs, and inhibiting the pro-tumor activity of CAFs, with ginsenoside Rg3 effectively preventing tumor cell-induced activation of CAFs (Xia et al., 2022).

Additionally, functional modification and reprogramming of CAFs to a resting phenotype can be achieved through curcumin, which inhibits collagen expression and transforms active mesenchymal fibroblasts into normal fibroblasts (Gao et al., 2023).

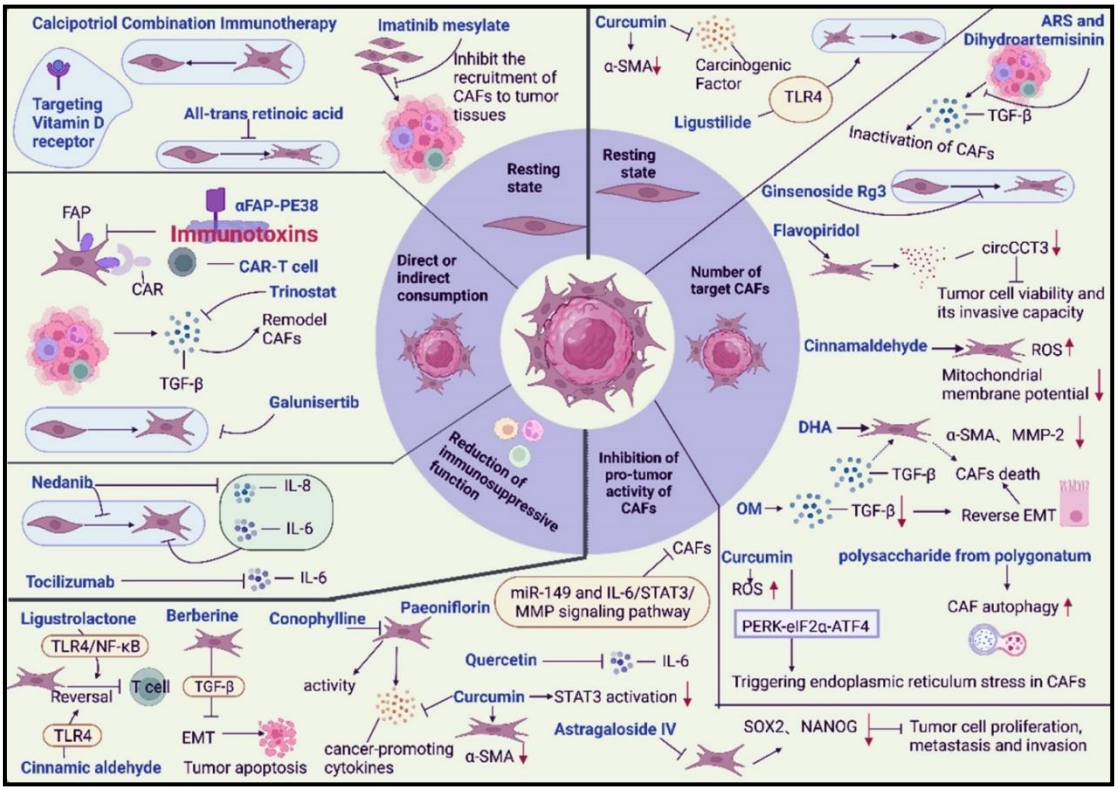

Figure 14: Targeted regulation of CAFs using clinical medicinal drugs and naturally occurring small molecules. Adapted from (Gao et al., 2023).

2.4.1.6. Modulation of macrophage metabolism

Exploring the many metabolic pathways within tumor-associated macrophages (TAMs) holds great promise for cancer immunotherapy. Over the last decade, significant progress has been made in the study of TAM-targeted immunotherapies, revealing five major categories of interventions: inhibiting monocyte recruitment, eliminating immunosuppressive macrophages, using Chimeric Antigen Receptor (CAR) macrophages, inducing macrophage reprogramming, and harnessing TAMs' intrinsic antitumor function (Dussold et al., 2024).

Strategies to impede monocyte recruitment, such as utilizing triggering receptor expressed on myeloid cells-2 (TREM2) inhibitors, aim to prevent the transformation of monocytes into TAMs at malignant sites. Tools like TREM2 inhibitors selectively eliminate immunosuppressive macrophages, promoting regular immune responses and suppressing tumor growth. Genetically modified CAR-Mac enhances the precision of antitumor immune responses by specifically targeting tumor cells. Techniques like inducing macrophage reprogramming with CLEVER-1 inhibitors shift TAMs from a pro-tumor to an antitumor phenotype. Additionally, approaches like SIRP1α–CD47 inhibitors tap into the antitumor functionalities of specific TAM subsets to hinder tumor growth (Dussold et al., 2024).

However, the heterogeneity of TAMs, revealed by high-dimensional data, presents obstacles and opportunities for TAM targeting. While focusing attention on certain phenotypic and functional subgroups can improve therapy outcomes, TAM heterogeneity complicates identification and targeting. Recent advances, such as blocking different axes like as CCL2-CCR2, CSF1-CSF1R, ANG2-TIE2, and CXCL12-CXCR4, help to eliminate specific TAM subsets that are important for tumor angiogenesis (Qian et al., 2024).

Simultaneously, ongoing clinical investigations focus on TAM reprogramming methodologies, such as using creatine and PI3Kγ inhibitors, which manipulate cytokine release and modulate TAM activation. Studies reveal that manipulating TAMs through removal (anti-CSF1R therapy) and reprogramming (agonistic anti-CD40 monoclonal antibodies) yields different impacts on the antitumor activity of infused T cells, emphasizing the need to consider the differential effects of TAM modulation (Qian et al., 2024).

Targeting specific functional subgroups is critical in the development of TAM-centric therapies, considering their heterogeneity's substantial impact on the tumor immune

milieu. For instance, targeting CX3CR1+TAMs shows potential in inhibiting metastasis in tumors abundant with this subset, paving the way for tailored therapies that address aggressive or resistant malignancies (Qian et al., 2024).

Given the significant metabolic reprogramming induced by the tumor microenvironment (TME) on TAMs, repolarizing macrophages represents an attractive therapeutic possibility. Strategies that focus on metabolic components of TAM activity, such as intracellular ATP levels and redox state, aim to change TAMs into an M1-like pro-inflammatory phenotype, hence influencing their capacities and decreasing M2-like anti-inflammatory polarization. The subsequent sections will delve into modern therapeutic techniques targeting TAM metabolism for effective TAM reprogramming (Dussold et al., 2024; Qian et al., 2024).

2.4.1.7. cGAS-STING pathways

The cGAS-STING pathways have gained prominence in the last decade, emerging as a significant area of research in immune system studies. Activation of STING signaling, triggered by cytosolic DNA sensing, has shown potential in inducing innate immune responses, especially interferon and pro-inflammatory cytokine expression. This pathway is critical for activating antigen-presenting cells and boosting adaptive immunity, especially in cancer therapy. New findings highlight the prospect of pharmaceutical manipulation, which makes STING a potential target for immune modulation, particularly in cancer immunotherapy (X. Chen et al., 2024).

The cGAS-STING pathway responds to various stimuli, including pathogen detection and cellular stress, accumulating cytosolic double-stranded DNA. Cyclic GMP-AMP (cGAMP) is produced by cGAS upon binding to dsDNA, and it interacts with STING, initiating a series of events involving oligomerization, translocation, and activation of downstream molecules. This ultimately results in the production of pro-inflammatory genes and type I interferons (Chin et al., 2023).

Activation of the cGAS-STING pathways has important consequences for antitumor immunity, as it influences dendritic cell maturation, tumor antigen cross-presentation to CD8+ T cells and increases the abundance and activity of innate immune cells in the tumor microenvironment. Additionally, STING activation transforms immunosuppressive cells, contributing to an increased antitumor immune response. Overall, STING-mediated immune responses reshape the immunogenicity of the tumor microenvironment, presenting potential avenues for therapeutic interventions (Chin et al., 2023).

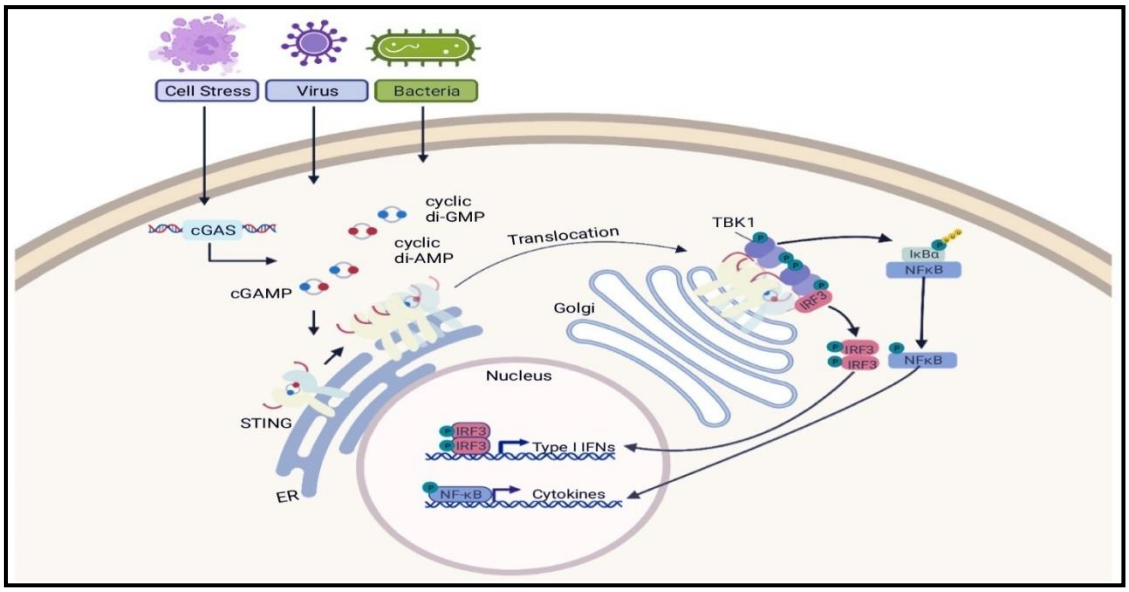

Figure 15: The initiation of cGAS-STING pathways, which facilitate the detection of cytosolic nucleic acids and innate immune responses. Adapted from (Chin et al., 2023).

In recent years, researchers have focused on exploring a diverse array of STING agonists, continuously assessing their potential in antitumor therapy. Among these agents are small-molecule compounds, notably cyclic dinucleotides (CDNs), which directly target the STING protein. CDNs, essential components of STING agonists, operate as secondary messengers, activating innate immune responses through IRF3-dependent type I IFN production. Natural CDNs derived from bacteria include cyclic dimeric guanosine monophosphate (c-di-GMP, CDG), 3′3′-cyclic AMP-GMP (3′3′-cGAMP), cyclic dimeric adenosine monophosphate (c-di-AMP, CDA), and endogenous 2′3′-cGAMP synthesized in mammalian cells. These CDNs have improved immunogenicity and tumor suppression in mouse melanoma, breast, and colorectal cancer models. However, their inherent hydrophilic and electronegative characteristics make them sensitive to rapid enzymatic breakdown, limiting their efficacy and bioavailability in tumor treatment. Efforts are being made to address these obstacles, including the production of synthetic CDNs and delivery vehicles or particles optimized for targeted delivery to tumor tissues (Chen et al., 2024).

In addition to CDNs, non-CDN substances have received attention for their pharmacological capabilities in activating cGAS-STING. DMXAA, a classic model medication that targets mouse STING, has successfully disrupted tumor vasculature. However, it has failed to succeed in clinical applications due to its inability to bind to human STING. Similar to the structure of DMXAA, its derivatives have inspired the possible development of new anticancer STING agonists. Furthermore, Ramanjulu et al.

developed diABZI, a new non-CDN derived from symmetry-related amidobenzimidazole (ABZI). This new agonist has a high binding affinity for human STING and has demonstrated encouraging results in promoting systemic tumor regression in colorectal cancer mice models. Subsequently, new stable non-CDN STING agonists, such as SR-717 and MSA-2, were identified to have high STING affinities and excellent anticancer properties. Despite the advances made using these techniques, the limitations of small-molecule STING agonists, such as random distribution, quick clearance, and low accumulation at tumor locations, continue to limit the efficacy of existing immunotherapies (Chen et al., 2024).

Nanoparticles (NPs) are increasingly being used to package small-molecule STING agonists due to their capacity to provide enhanced cellular uptake and tumor accumulation. This technique shows potential in addressing the limitations of free STING agonists. Nanotechnology has advanced rapidly and is frequently used in the biomedical field. Numerous nanoparticles are used in cancer treatment to encapsulate and efficiently distribute active medicinal components to tumor locations. The US Food and Drug Administration (FDA) has approved the use of various nanotechnology platforms, such as polymersomes, liposomes, and metal-based nanomedicines, to load anticancer drugs such as DaunoXome, Doxil, and Onivyde (Chen et al., 2024).

A new paradigm has arisen, centered on nanoplatforms incorporating STING agonists with organic and inorganic nanomaterials. These therapeutic NP platforms are intended to increase tumor penetration and accumulation, improve cellular absorption, and slow the degradation of STING agonists. This new approach provides a viable strategy to improve the efficacy of STING agonists in cancer therapy (Chen et al., 2024).

2.4.1.8. Natural Killer

Three fundamental approaches are now driving the use of natural killer (NK) cells in cancer treatment:

- NK Cell ADCC (Antibody-Dependent Cellular Cytotoxicity): This method harnesses the power of monoclonal antibodies (mAbs) to activate NK cells via the Fc receptor CD16a. The interaction induces CD16a activation signaling, ultimately resulting in the lysis of malignant cells coated with mAbs. Notably, several mAbs, including Pertuzumab, Trastuzumab, and Margetuximab for HER2+ cancers, Cetuximab for EGFR+ colorectal cancer, and Rituximab for non-Hodgkin's lymphoma, are commercially available. However, challenges arise from the CD16a variant 158F, which is present in most of the population and may diminish the

binding affinity for therapeutic mAbs. Strategies like fucosylation or editing of the Fc region of mAbs are explored to address this concern (Zhang et al., 2024; M. Zhang et al., 2023).

- CAR-NK Cell Therapy: This novel technique involves genetically altering NK cells to express Chimeric Antigen Receptors (CARs) that target specific tumor antigens. The modified CAR-NK cells detect and activate NK cells via their cytoplasmic tails, which include signaling modules. Compared to CAR-T cell therapies, CAR-NK cells produce more efficiently from allogenic sources and have a safer profile with lower risks of Cytokine Release Syndrome (CRS) and Graft-versus-Host Disease (GVHD).Despite these advantages, challenges persist, such as the short lifespan of CAR-NK cells, necessitating frequent infusions that escalate treatment costs. Genetic manipulation complexities, including lower transduction efficiency and the need for more significant NK cell numbers, also contribute to increased production expenses and time (Zhang et al., 2024; M. Zhang et al., 2023).
- NK Cell Engagers (NKCEs): Recombinant proteins such as Bispecific Killer Cell Engagers (BiKEs) and Trispecific Killer Cell Engagers (TriKEs) offer a novel approach by bringing tumor and NK cells together to activate NK cells. Notably, NKCEs offer several advantages over traditional approaches. In contrast to NK cell ADCC, CD16a-targeted NKCEs are less influenced by the CD16a polymorphism because they can detect regions unaffected by this genetic variation. Moreover, NKCEs have the potential to activate various immune cells, not solely limited to NK cells. Their manufacturing process is more straightforward and more cost-effective than CAR-NK cell therapy. Furthermore, NKCEs, when paired with adoptive NK cell transfer, as proven in studies using CYNK-101, Daratumumab, Trastuzumab, and Pembrolizumab, offer potential in increasing their efficacy for cancer treatment (Zhang et al., 2024; Zhang et al., 2023).

Despite the numerous advantages, NKCEs, as an immunotherapeutic modality, are still in the optimization phase. Careful consideration of critical factors during their development ensures their potency and effectiveness in clinical applications. Ongoing clinical studies exploring the co-administration of adoptive NK cell transfer with various NKCEs underscore the dynamic nature of this field, with continuous advancements on the horizon (Zhang et al., 2023).

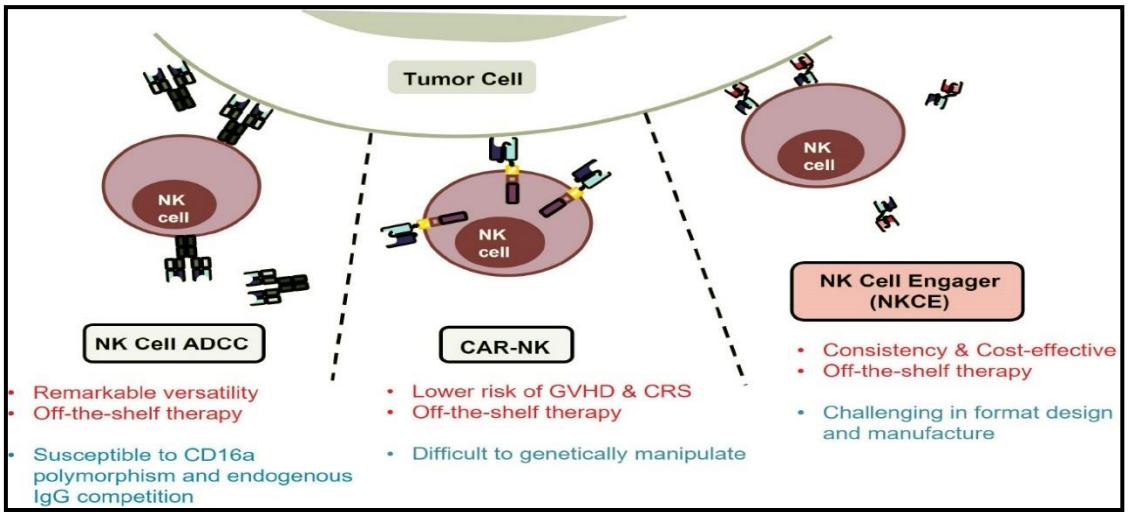

Figure 16: Strategies for NK immunotherapy. Adapted from (Zhang et al., 2023).

2.4.2. Active immunotherapy

2.4.2.1. Cellular immunotherapy DCs

Dendritic cells (DCs), discovered in 1973 by Ralph Steinman and Zanvil Cohn, play a critical role in both innate and adaptive immune responses. They trigger naive and memory immune responses, particularly when tumor antigens are present. The DC vaccine strategy, falling under cellular vaccines, utilizes ex vivo loaded and matured DCs infused back into cancer patients to circumvent immunosuppression in the tumor microenvironment (TME) and induce tumor-specific cytotoxic T lymphocytes (CTLs), aiming for long-term immunological memory (Lee & Kim, 2023).

Despite clinical trials confirming the safety and feasibility of DC vaccines, patient responses have been inconsistent. Variables such as DC cell type source, maturation stimuli, vaccine administration route, frequency, adjuvants, and overall immune system competence contribute to variable outcomes. Early DC vaccine efforts focused on advanced cancers faced challenges due to the TME's active immunosuppression. Limited DC source material further complicated vaccine development, with Sipuleucel-T being a notable success against prostate cancer, demonstrating increased median overall survival (Elwakeel et al., 2023; Makker et al., 2023).

Second-generation DC vaccine designs involve creating monocyte-derived DCs (MoDCs) ex vivo with maturation signals to provide a more reliable source of APCs.Various sources, including CD34+ hematopoietic stem progenitor cells (HSPCs), enhance DC generation. Maturation strategies have evolved to ensure full activation status, crucial for effective antigen presentation and T-cell stimulation. Current research focuses on next-generation DC vaccines, which may involve the cDC1 fraction for

optimum TAA-specific T cell responses, while obstacles remain in ex vivo generation (Elwakeel et al., 2023; Makker et al., 2023).

Despite evolving strategies, DC vaccines exhibit continued benefits in clinical settings, as demonstrated in recent phase III trials for glioblastoma. Adding autologous tumor lysate-loaded dendritic cell vaccine (DCVax-L) to standard care resulted in a statistically significant extension of survival, underlining the potential of DC vaccines as a therapeutic approach, especially in malignancies with historically poor survival rates (Elwakeel et al., 2023; Makker et al., 2023).

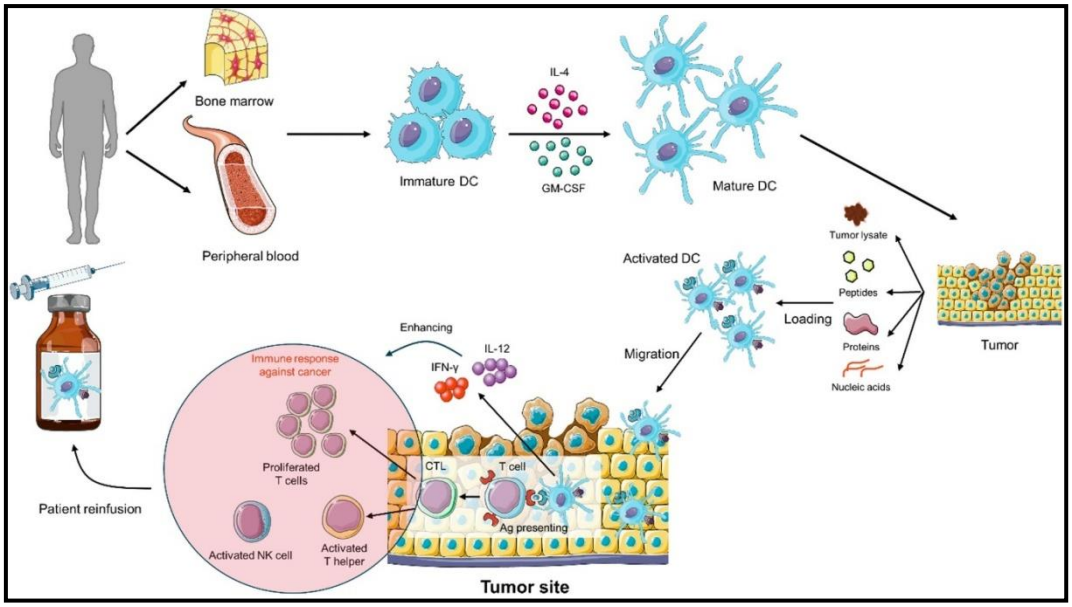

Figure 17: Depiction of the anticancer vaccination strategy utilizing dendritic cells (DCs). Adapted from (Shbeer, 2024).

2.4.2.2. Molecular immunotherapy

2.4.2.2.1. Tumor antigen-vaccine

The basic concept of cancer vaccination is to transfer antigens to dendritic cells (DCs), either directly or indirectly, so that they can be presented to T cells. Antigen selection is critical for therapeutic cancer vaccines; it should be tumor-specific and different enough to elicit a functional T cell response from non-tolerized T cells. Tumor-associated antigens (TAAs) such as gp100 or MUC1, which were initially appealing due to their widespread therapeutic use, confront hurdles since central tolerance restricts their efficacy. Cancer-testis antigens such as NY-ESO-1, which are expressed in malignant cells, are classified as tumor-specific antigens (TSAs) and have been extensively exploited in vaccine development. Mutant self-antigens have succeeded in certain

malignancies, such as KRASG12D and mutant anaplastic lymphoma kinase (ALK) (Makker et al., 2023).

Advancements in sequencing technologies have led to the identification of neo-antigens arising from somatic mutations, gene fusions, and other genomic events. These unique neo-antigens, specific to each patient, allow for personalized vaccines. The process involves sequencing non-malignant and tumor biopsies, predicting epitopes, and encoding selected neo-antigens in a vaccine vector. Early exome sequencing and mass spectrometry experiments demonstrated the feasibility of identifying neo-antigens and generating therapeutic vaccines in mouse models (Makker et al., 2023; Richard et al., 2024).

Clinical trials using exome sequencing to identify altered peptides for vaccination have shown an increase in neo-antigen-specific T cells and a broader T cell response. However, the clinical impact of vaccination, especially in combination with immune checkpoint inhibitors (ICIs), is still being determined. Various algorithms are employed for epitope prediction, focusing on detecting neo-antigens with strong binding affinities (Lee & Kim, 2023).

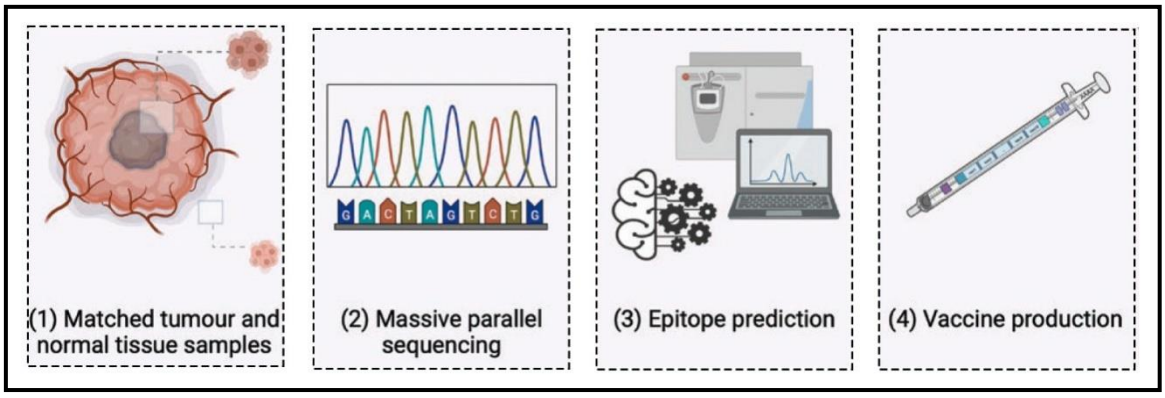

Figure 18: The neo-antigen vaccine development procedure. Adapted from(Makker et al., 2023).

The link between tumor mutational burden (TMB) and responsiveness to ICIs has sparked interest in employing neoantigens in cancer vaccine development. However, new studies indicate that TMB alone may not be a reliable predictor of ICI response and survival across all malignancies. The immunogenicity of neo-antigens and clonal heterogeneity within tumors are crucial factors influencing immunotherapy targets' success. Understanding the evolution of TMB is essential for identifying beneficial neo-antigens for immunotherapy (Janes et al., 2023; Makker et al., 2023).

2.4.2.2.2. mRNA tumor vaccine

Substantial progress has been made in the past few years in the creation of tumor vaccines for cancer immunotherapy. These vaccines immunize the body with a variety of tumor antigens, including inactivated tumor cells, tumor-related proteins or peptides, and genes that express tumor antigens. The goal is to elicit adaptive immune responses that selectively eradicate antigen-overexpressing malignant cells, resulting in long-term therapeutic responses and eventual elimination via immunological memory (Qu et al., 2023).

Despite these efforts, Dendreon's Provenge (Sipuleucel-T) is the only therapeutic tumor vaccine licensed by the United States Food and Drug Administration (FDA) in the last two decades, slightly increasing patients' average survival time. This limited effectiveness is related to tumor antigen variety and immunogenicity, which render standard recombinant protein or peptide-based vaccines ineffective for eliciting robust tumor-specific immune responses. As a result, there is an urgent need for more effective and adaptable vaccine platforms (Qu et al., 2023).

The success of messenger ribonucleic acid (mRNA) vaccines during the COVID-19 pandemic has garnered significant attention. mRNA vaccines, such as BNT162b2 for Pfizer, Spikevax for Moderna, and SYS6006 for CSPC Pharma, have demonstrated high safety, rapid production, and effectiveness. This success has sparked interest and confidence in applying mRNA technology to tumor vaccines (Makker et al., 2023; Qu et al., 2023).

Compared to existing vaccine platforms such as dendritic cells (DCs), peptides, viral vectors, and DNA, mRNA tumor vaccines provide numerous advantages. These include a short preparation cycle, the ability to express hydrophobic antigen peptides with high HLA affinity, the capacity to simultaneously deliver multiple tumor antigen fragments, cytoplasmic expression without nuclear entry, and inherent adjuvant effects, activating immune cells to release cytokines (Makker et al., 2023).

However, delivering mRNA intact into cells and ensuring effective translation poses challenges. mRNA faces barriers crossing non-polar cellular and tissue structures and is susceptible to degradation by nucleases. Protecting mRNA with proper delivery vectors, such as lipid nanoparticles (LNPs), is critical for successful in vivo expression. Despite the mature validation of LNPs during the COVID-19 pandemic, there are still limitations, including in vivo stability, specific targeting to the immune system, lysosomal escape, and the need to enhance antigen expression for a robust immune

response (Qu et al., 2023).

In conclusion, while mRNA vaccines hold promise for tumor immunotherapy due to their unique advantages, overcoming delivery challenges remains essential for their successful application in the complex in vivo environment. Addressing these limitations could further advance the development of mRNA-based drugs for tumor vaccines, with the potential for potent and specific immune responses against cancer (Qu et al., 2023).

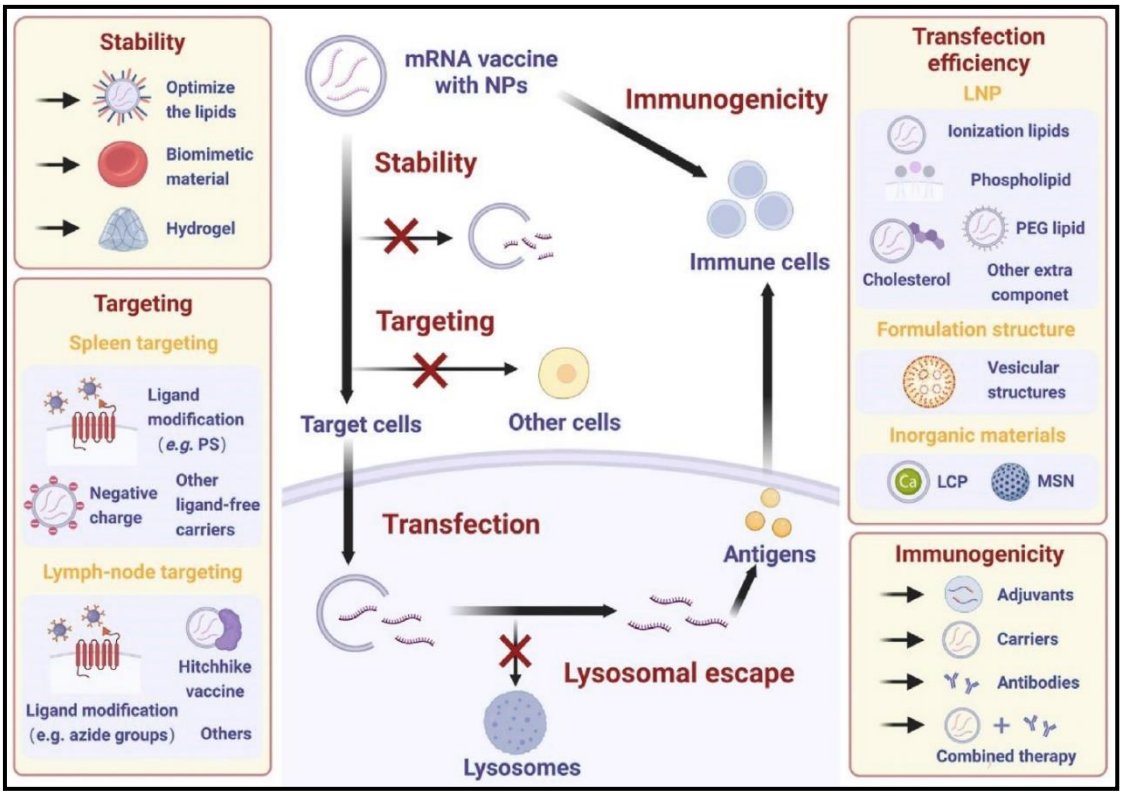

Figure 19: In vivo functionality of mRNA vaccines and nanoparticle-based engineering techniques for improving vaccination properties. Adapted from (Qu et al., 2023).

2.4.3. Biomembrane nanostructure

Nanoparticles offer a promising avenue for treating oral cancers by targeting the tumor microenvironment (TME), malignant cells, and immune cells. From 1 to 100 nanometers, these structures can comprise diverse materials, including organic polymers or inorganic compounds. Polymeric nanoparticles, specifically those derived from polymers like polyethylene glycol (PEG) or poly(lactic-co-glycolic acid) (PLGA), exhibit attributes conducive to drug delivery, featuring degradability and compatibility with biological systems (Qin & Wu, n.d.).

Various nanoparticle classes, including micelles, liposomes, nanogels, lipid

nanoparticles, and sponge nanoparticles, contribute to the repertoire of drug delivery options. Inorganic nanoparticles, like gold, iron oxide, and silica nanoparticles, possess unique properties beneficial for cancer-targeting (Qin & Wu, n.d.).

Nanoparticles loaded with cytokines, like interleukin-2 (IL-2), demonstrate potential in augmenting the activity of CD8+ T cells and natural killer (NK). These nanoparticles can specifically target immune cells, mitigating systemic side effects. Targeting immune checkpoints through nanoparticles, with natural products and monoclonal antibodies, shows promise for suppressing programmed cell death ligand-1/programmed cell death-1 (PD-L1/PD-1) in oral cancers (Zhang et al., 2024).

Furthermore, nanoparticles can be customized to improve anticancer immunity, targeting specific molecules in oral tumors. Cyclooxygenase-2 (COX-2), identified as a promising target, can be modulated by nanoparticles, increasing water solubility and therapeutic potency of anti-COX-2 drugs. Innovative approaches, such as celecoxib-loaded cubosomal sponges and chitosan-fucoidan-loaded celecoxib, exhibit effective tumor suppression in in vitro and in vivo models (Zhang et al., 2024).

Nanoparticles may also influence cellular mechanisms, impacting immune responses. For instance, cobalt-ferrocene nanoparticle-loaded hydroxychloroquine prevents autophagy and induces apoptosis in oral cancer cells, potentially averting immune escape. With their unique properties, metal nanoparticles, including gold, iron, and silver, hold promise in enhancing ionizing radiation toxicity, potentially inducing immunogenic cell death (Zhang et al., 2024).

While hypotheses surrounding reactive oxygen species (ROS)-generating products and metal nanoparticles for inducing anticancer immunity are promising, rigorous experimental studies are imperative to discern their consequences within the context of oral cancers (Qin & Wu, n.d.).

3. Immune-related adverse events (IRAES)

Immune-related adverse events (irAEs) arise from immune-checkpoint signaling disruption with Immune Checkpoint Inhibitors (ICIs), impacting various organ systems with variable severity. While most irAEs are transient and can be resolved with glucocorticoid use, some persist despite treatment adjustments involving immunosuppressive and immune-modulating agents. Current guidelines from ASCO (American Society of Clinical Oncology) and ESMO (European Society of Clinical Oncology) focus on acute irAEs, leaving a gap in guidance for chronic irAE management, potentially leading to prolonged morbidity and additional side effects from extended immunosuppression. As the use of ICIs grows, notably in neoadjuvant and adjuvant settings, a better understanding of chronic irAEs is required to advise patients about potential risks and benefits. In the event of these toxicities, ICI treatment may be delayed or discontinued, and immunosuppression may begin, with unknown long-term consequences (Barron et al., 2023; Yip et al., 2024).

Pneumonitis has been found as the most lethal complication linked with PD(L)1 monotherapy, particularly for thoracic malignancies due to the compromised organ system.It manifests as inflammation of the lung parenchyma, often presenting with symptoms like cough, shortness of breath, chest pain, and fever. Diagnosis is challenging, with high-resolution computed tomography imaging revealing new pulmonary infiltrates or, occasionally, no radiographic abnormalities. In non-small cell lung cancer (NSCLC), PD-1 monotherapy, like pembrolizumab or nivolumab, leads to a 3% to 7% incidence of pneumonitis (Ghanbar & Suresh, 2024).

Myocarditis is the predominant cardiac immune-related adverse event (irAE), although other cardiac issues such as cardiomyopathy, pericarditis, arrhythmias, heart failure, and pericardial effusion have been documented. Despite its low overall incidence (less than 1%), myocarditis stands out as the most fatal irAE associated with combined PD(L)1/CTLA-4 therapy, exhibiting a mortality rate of up to 50%. The risk of myocarditis is notably higher in patients undergoing PD(L)1/CTLA-4 combination treatment compared to PD(L)1 alone (O'Leary et al., 2023).

Endocrinopathies emerge as the most prevalent toxicities associated with PD(L)1 monotherapy, affecting both endocrine and exocrine systems induced by immune checkpoint inhibitors (ICIs). PD(L)1 monotherapy frequently results in thyroid dysfunction, with hypothyroidism being the most common endocrinopathy, whereas CTLA-4 inhibitors primarily cause hypophysitis. Hyperthyroidism or hypothyroidism

may arise independently or as part of ICI-mediated thyroiditis. Patients with thyroiditis experience a fast developing thyrotoxic condition, followed by a rapid reduction in free thyroxine (T4), leading to hypothyroidism (O'Leary et al., 2023).

Cutaneous toxicities are the first side effects of PD(L)1 monotherapy and combination therapies, occurring in up to 50% to 70% of patients receiving combination immune checkpoint inhibitor (ICI) regimens. Skin-related immune-related adverse events (irAEs) range in severity from minor symptoms like pruritus, rash/morbilliform exanthems, vitiligo-like depigmentation, and lichenoid dermatitis to more severe but uncommon reactions like Stevens-Johnson syndrome, toxic epidermal necrolysis, and bullous pemphigoid. Specific ICI classes tend to induce specific skin toxicities. CTLA-4 inhibitors typically cause pruritus and rash, while anti-PD(L)1 causes pruritus or vitiligo (O'Leary et al., 2023).

Nephritis emerges as a joint adverse event in the context of chemo-immunotherapy (Chemo-IO), with acute kidney injury (AKI) being an infrequent occurrence, although recent evidence suggests a higher prevalence when combined with chemotherapy and immune checkpoint inhibitors (ICIs). The estimated overall incidence of AKI and nephritis ranges from 2% to 7%, with some reports indicating up to 29%, particularly in patients receiving a combination of CTLA-4 and PD(L)1 inhibitors. However, ICI-induced AKI can manifest in up to 20% of ICI-treated patients, and only 2% to 5% progress to develop ICI-induced nephritis. Various causes, such as minimal change disease, acute interstitial nephritis, immunoglobulin A nephropathy, and podocytopathies, contribute to ICI-induced nephritis (O'Leary et al., 2023).

Table 4 : Most Common Immune-Related Adverse Events (O'Leary et al., 2023).

System	Toxicities
Pulmonary	Pneumonitis
Endocrine	Thyrotoxicosis Hypothyroidism Diabetes Hypophysis Primary adrenal insufficiency
Cardiac	Myocarditis Pericarditis Arrythmias Vasculitis Thromboembolism
Cutaneous	Bullous dermatoses Inflammatory dermatoses Severe cutaneous adverse reactions
Renal	Nephritis Immune-related acute kidney injury
Gastrointestinal	Hepatitis Colitis Gastritis Enterocolitis
Neurologic	Myasthenia Aseptic meningitis Encephalitis

4. Adjuvant and neoadjuvant immunotherapy

Immunotherapy has shown promise in treating recurrent/metastatic head and neck squamous cell carcinoma (R/M HNSCC), offering potential clinical benefits. However, its efficacy as a neoadjuvant treatment for HNSCC has yet to be thoroughly investigated. Recent studies indicate that combining chemotherapy with PD-1 inhibitors in neoadjuvant therapy significantly improves the overall response rate (ORR) compared to immunotherapy alone (61% vs. 22%) (Chen et al., 2023).

Various neoadjuvant therapies are available, with induction chemotherapy widely utilized, employing standard treatments like docetaxel plus cisplatin and fluorouracil (TPF) and cisplatin and fluorouracil (PF). Both TPF and PF effectively inhibit tumor growth, with ORRs of 68% and 54%, respectively. It is essential to exercise caution when interpreting these results with PD-1 inhibitors, as the assessment of ORR in immunotherapy may be influenced by the occurrence of inflammatory pseudotumors, a benign process with imaging findings resembling tumor responses (Chen et al., 2023).

Immunotherapy exhibits favorable 1-year overall survival (OS) and 1-year progression-free survival (PFS) rates, reaching 84% and 82%, respectively, in contrast to 72% and 52% for TPF and PF. This difference may be influenced by an increased incidence or potential biases, particularly the predominant representation of oral cavity cases. Furthermore, the link between human papillomavirus (HPV) infection and prognosis in oropharyngeal squamous cell carcinoma (OPSCC) could influence the outcomes (Chen et al., 2023).

Radiotherapy, a primary treatment for HNSCC, when combined with immunotherapy, induces antitumor effects. However, the success of this combination relies on the timing and sequence, with optimal results observed when immunotherapy is administered immediately following radiotherapy. Considering neoadjuvant immune therapy may be beneficial in creating an immune-active tumor microenvironment for subsequent radiotherapy (Chen et al., 2023).

Cancer immunotherapy, a promising approach to cancer treatment, has focused on cancer vaccines that activate and increase tumor-reactive T cells. Despite this, most cancer vaccines have had minimal therapeutic efficacy when taken alone. The challenges arise from the antigen targets being "self" proteins, inducing tolerance, and the suppressive tumor microenvironment. Adjuvants become essential for cancer vaccines to overcome immune tolerance and foster effective immune responses. Toll-Like Receptor (TLR) agonists are artificial substances that stimulate TLRs. TLRs, the

most common family of pattern recognition receptors (PRRs), detect infections or cellular damage and initiate innate immune responses. Incorporating TLR agonists into vaccines activates TLRs, triggering an innate damage response that contributes to developing a robust adaptive immune response to the antigen. TLR agonists' unique capacity to modulate innate immune responses makes them a valuable adjuvant for vaccines targeting infectious illnesses and cancer (Jeon et al., 2024).

Initially crafted for infectious vaccinations, immune adjuvants are now gaining increased attention in the context of advancements in cancer immunotherapy. These adjuvants operate by triggering and enhancing the inflammatory response, initiating innate immune reactions, and subsequently activating adaptive immune responses. Their primary role revolves around supporting antigen presentation and the maturation of dendritic cells. While commonly associated with vaccines, particularly the newer mRNA vaccines, only a limited number of immune adjuvants find use as drugs. Hydroxyapatite (HA) ceramics and azoximer bromide (AZB) emerge as underappreciated agents, previously employed in early clinical trials, showcasing clinical efficacy and favorable tolerance profiles. Initially developed for veterinary purposes, HA and an autologous vaccine targeted spontaneous lymphomas in canines.

On the other hand, AZB, an innovative immune modulator derived from heterochain aliphatic polyamines, holds licenses in the Commonwealth of Independent States, Russia, and Slovakia for infectious and inflammatory diseases. Presently, it is under exploration for its potential in cancer treatment, displaying promising results. The synergistic combination of these two immune adjuvants presents various possibilities in immunotherapy strategies (Guven et al., 2023; Rossi et al., 2024).

5. Relationship between oral diseases and immunotherapy

Amid the COVID-19 crisis, global health concerns have shifted towards maintaining oral health due to the heightened risk of increased viral transmission. Oral diseases pose a significant public health threat, with periodontitis being the primary cause of permanent adult tooth loss. Additionally, lip and oral cancer, ranking 15th among the most prevalent malignancies worldwide, directly impacts human life quality. The oral cavity, representing the entire body system, is susceptible to a diverse array of diseases and disorders, including chronic infectious conditions like apical periodontitis and periodontitis, autoimmune diseases such as Pemphigus Vulgaris (PV), and potentially malignant oral disorders (Zhang et al., 2021).

Once thought to be a simple bacterial infection, periodontitis is now recognized as a chronic inflammatory disease affecting the structures that support teeth. Progress in knowledge has brought to light a more intricate pathogenic mechanism, wherein a positive feedback loop exists between the host's inflammatory response to resident microorganisms and the oral microbiome. The localized immune response triggered by the formation of bacterial biofilm (plaque) on teeth can be managed with proper oral hygiene to address gingivitis. However, the accumulation of substantial plaque deposits in the dentogingival pocket results in biofilm enrichment, persistent local inflammation, and the permanent loss of supporting tissues, known as periodontal disease.

In the beginning, the biofilm consists predominantly of Gram-positive bacteria like Streptococcus spp., Staphylococcus spp., and Rothia spp., followed by Gram-positive bacilli such as Actinomyces spp. and Corynebacterium spp., along with a few Gram-negative cocci. An intricate three-dimensional extracellular matrix forms with bacterial division, providing a protective environment for Gram-negative anaerobic bacteria to thrive deep within the oral biofilm, away from the oxygen-rich oral cavity. Systemic consequences may arise from periodontitis due to elevated proinflammatory cytokines (e.g., IL-6), complement factors, and activation of signaling pathways by various microorganisms. Porphyromonas gingivalis, an uncommon pathogenic Gram-negative anaerobe in the oral cavity, expresses virulence factors that support its proliferation, overgrowth, and survival. Additionally, it contributes to biofilm maturation, adhesion with other bacteria, polymicrobial colonization, and aggregation of essential nutrients (e.g., nitrogen, carbon, and iron) necessary for bacterial growth and function (Guastaldi et al., n.d.).

The virulence factors of P. gingivalis, including lipopolysaccharides, gingipains, outer fimbriae, and membrane vesicles, play a crucial role in evading host immune clearance and recognition. They achieve this by cleaving antibodies involved in opsonization, degrading or downregulating specific proinflammatory chemokines, and cytokines, thereby hindering the effectiveness of immune cells recruited to eliminate the bacteria. Consequently, P. gingivalis can decouple bacterial clearance from inflammation, promoting dysbiotic conditions through various mechanisms, like enhancing selectins, complement activation, and the PD-1/PD-L1 pathways (Guastaldi et al., n.d.).

The presence of gingipains in P. gingivalis has a major impact on the complement pathway, an essential signaling cascade that assists the immune system in quickly eliminating infections by inducing inflammation and recruiting phagocytic cells.P. gingivalis uses gingipains to disrupt the complement system, preventing bacterial death while maintaining inflammation. This technique involves the production of C5a, which activates C5a receptors in various immune cells. P. gingivalis degrades MYD88 via interaction with TLR2 and C5a receptors, allowing the PI3K pathway to be activated and causing inflammation (Guastaldi et al., n.d.).

According to research, people with periodontitis had greater serum complement C3 levels than healthy controls. The pathways controlled by the C3a receptor and C5ar1 have been demonstrated to reduce effector T-cell function, signaling that untreated periodontitis may impede immunotherapy response (Guastaldi et al., n.d.).

Magrini and colleagues investigated how complement C3 or C3ar expression influences the efficacy of anti-PD-1 therapies. In a sarcoma model, an anti-PD-1 monoclonal antibody had little anticancer activity, while tumor formation was considerably reduced in homozygous C3-deficient animals. Anti-PD-1 treatment also reduced lung metastases in the C3-deficient metastatic sarcoma model. Similarly, Zha and colleagues discovered that C5a lowered the efficacy of PD-1 inhibition in a preclinical colon cancer model. This effect was confirmed in another preclinical model of non-small-cell lung carcinoma, in which simultaneous suppression of PD-1, C5a, and the C5a receptor inhibited tumor growth and metastasis. These findings suggest that overexpression of C3 or C5 can impair the efficacy of immune checkpoint blockade therapy (Guastaldi et al., n.d.).

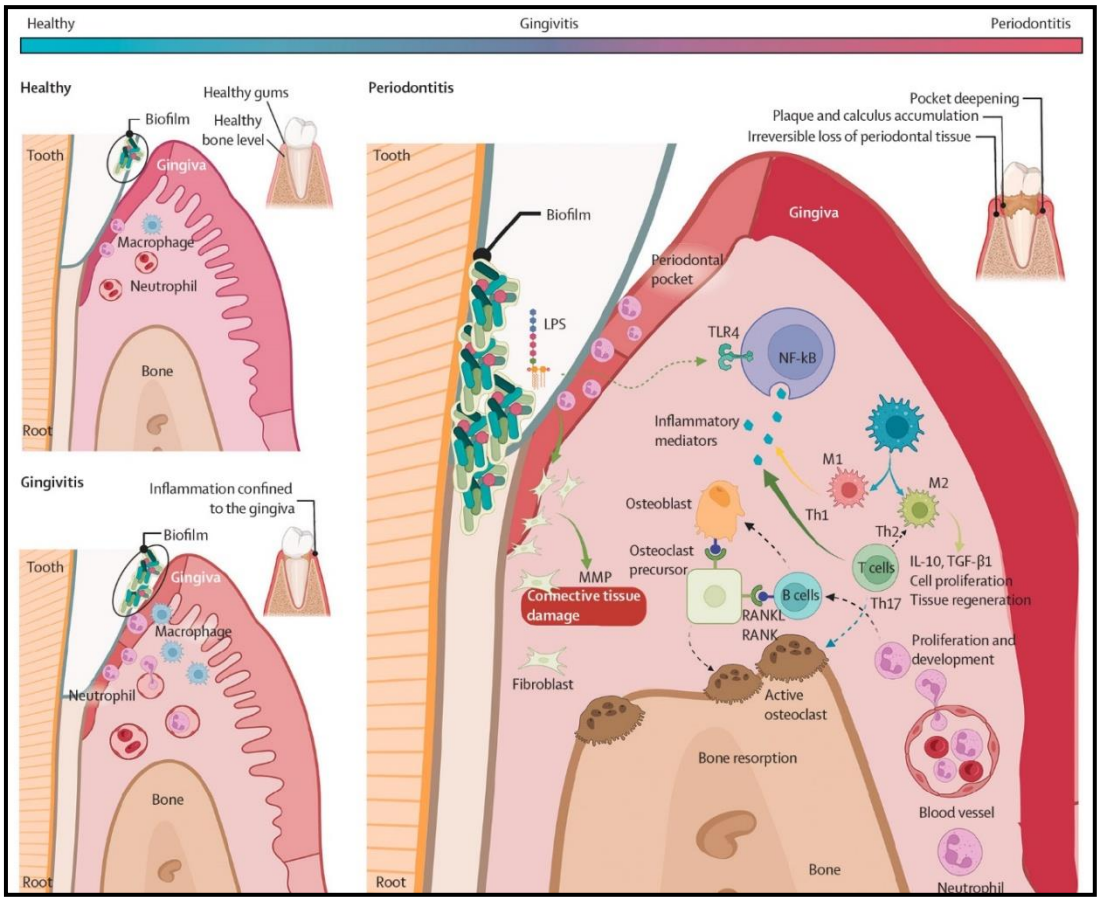

Figure 20: The clinical and pathological development of plaque-induced periodontal disease. Adapted from (Guastaldi et al., n.d.).

The immune system regulates T cell stimulation and proliferation by a balance of co-stimulatory and co-inhibitory receptors, which includes the PD-1/PD-L1 pathway. Proper communication via these receptors is required to start, amplify, and resolve adaptive immune responses. Persistent T-cell activation without appropriate inhibitory signals may result in autoimmunity and tissue damage at the infection site. Immune checkpoints such as PD-1/PD-L1 can impair the formation and maintenance of efficient immune responses in infections with selective antigen expression, such as bacterial or viral pathogens, making infection clearance more difficult (Guastaldi et al., n.d.).

Studies indicate increased PD-1/PD-L1 expression in $CD4^+$ and $CD8^+$ T cells associated with oropharyngeal malignancies caused by human papillomavirus and periodontitis. The bacteria P. gingivalis in periodontal tissue expresses the fimA protein, which increases PD-1 and PD-L1 expression in T cells and $CD11b^+$ cells. This promotes bacterial survival in the periodontal milieu. Other immune checkpoint molecules, like CTLA4 and TIM3, have also been overexpressed in periodontitis. Periodontitis' worldwide activation of several immune checkpoint pathways may contribute to the

development and progression of associated systemic illnesses (Guastaldi et al., n.d.).

Periodontitis, characterized by collagen degradation facilitated by P. gingivalis, can lead to bacterial translocation from periodontal pockets to distant sites like the gastrointestinal tract. This translocation influences the gut microbiome composition, with P. gingivalis causing a microbial shift similar to that observed in the oral cavity. A study of the gut microbiota in periodontal disease patients found lower diversity and a high prevalence of oral taxa in stool samples. Certain bacteria linked with periodontitis, such as Prevotella spp., Comamonadaceae spp., and Lactobacillales, were numerous in gingivitis patients, enabling the identification of those with periodontal disease. These oral pathogens can disturb the commensal microbial community, affecting responses to cancer immunotherapy (Guastaldi et al., n.d.).

Studies on clinical responders and non-responders to immunotherapy identified specific gut bacterial species that may modulate response to treatment. Notably, Prevotella spp and Ruminococcus spp, common in periodontitis patients and non-responders to immunotherapy, may influence treatment outcomes. Preclinical studies in mice administered P. gingivalis orally showed increased proliferation of bacteria associated with poor response to immunotherapy, such as Bacteroidales and Prevotella spp. Conversely, bacteria linked to positive responses to immunotherapy, like Actinobacteria and Proteobacteria, were reduced in mice with periodontitis. This shows that P. gingivalis may alter the gut microbiota towards a composition associated with poor immunotherapy responses, necessitating additional research into the link between periodontitis-related microbiome and immunotherapy responsiveness (Guastaldi et al., n.d.).

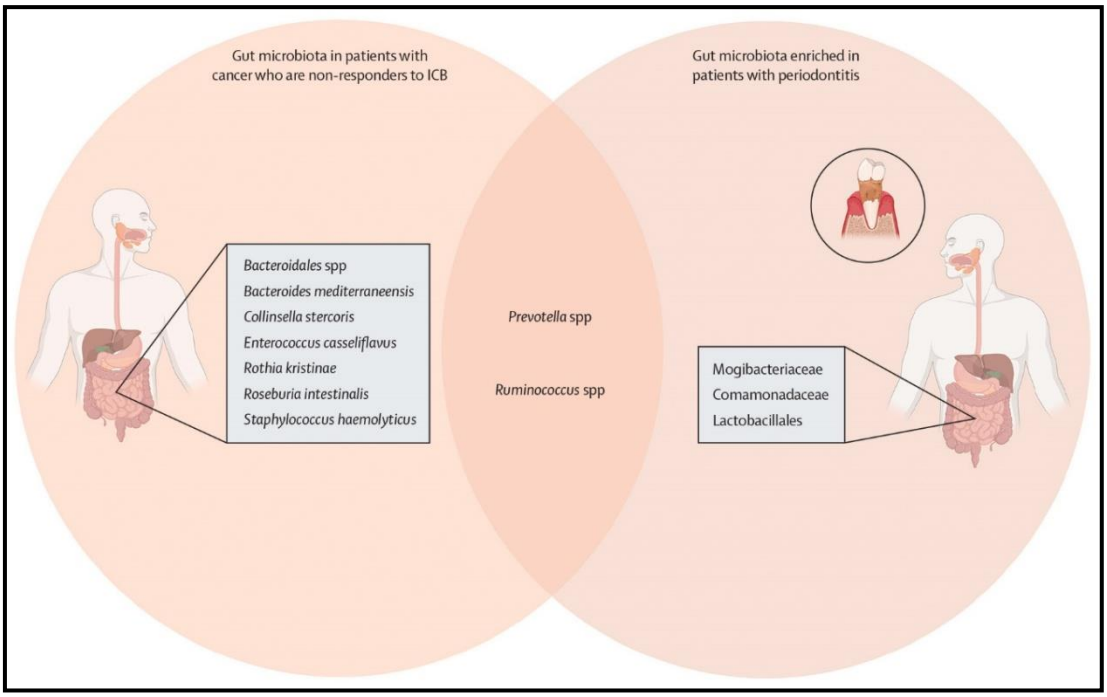

Figure 21: Comparisons and contrasts in the composition of gut microbiota between individuals with periodontitis and cancer patients who show resistance to immunotherapy. Adapted from (Guastaldi et al., n.d.).

Periodontitis is linked to more than 50 systemic inflammatory diseases and comorbidities, many of which are immune-related side effects associated with immunotherapy. The rate of severe adverse events varies with immunotherapy drug, ranging from 10-15% for anti-PD-1 to 55% for combined anti-CTLA4 and anti-PD-1 therapy. Periodontitis, which disrupts immunological homeostasis, may increase cancer patients' vulnerability to immune-related adverse events during immunotherapy (Baima et al., 2023; Guastaldi et al., n.d.).

P. gingivalis, a major cause of periodontitis, has been related to a variety of complications. This pathogen causes autoimmune reactions in myocarditis, resulting in oxidative stress, inflammatory infiltrates, and cardiac dysfunction. The transfer of P. gingivalis to the myocardium raises concerns about an increased risk of myocarditis, especially in individuals with a proinflammatory baseline who are undergoing immunotherapy (Baima et al., 2023).

The bidirectional link between periodontitis and inflammatory bowel diseases, such as colitis, is noteworthy. Oral pathogens like P. gingivalis may lead to gut dysbiosis by changing the gut microbiota and increasing inflammatory cytokine expression in the colon. The lower abundance of bacteroidetes in individuals with periodontitis may contribute to the development of colitis in cancer patients undergoing immunotherapy.

Immunotherapy-induced diabetes, affecting up to 40% of cancer patients, may also involve P. gingivalis translocation to pancreatic β cells. The intricate interplay between PD-1/PD-L1 blockade, periodontitis, and β cells could exacerbate the severity of ICB-associated diabetes, creating a potential feedback loop (Guastaldi et al., n.d.).

A connection between periodontitis and rheumatoid arthritis is observed, with P. gingivalis expressing a peptidylarginine deiminase linked to the development of arthritis. The interaction between PD-1/PD-L1 blockade, periodontitis, and arthritis warrants further exploration (Guastaldi et al., n.d.).

Furthermore, periodontal disease raises the risk of getting Sjögren's syndrome, an autoimmune disease that affects salivary glands and the lacrimal. Immunotherapy-treated cancer patients have described Sjögren's syndrome as an immune-related adverse event, implying a link between ICB exposure and the syndrome (Guastaldi et al., n.d.).

When the immune system malfunctions, either due to inherent issues or acquired conditions, it frequently leads to necrotizing ulcerative periodontitis, oral candidiasis, and a notable rise in tumor susceptibility. Treg cells are pivotal in preserving the balance and tolerance of the immune system within oral tissues. While they help prevent inflammatory conditions like apical periodontitis and periodontitis, they can also hasten the progression of premalignant lesions in oral mucosa (Zhang et al., 2021).

Periodontitis, characterized by tissue damage due to the host's immune response to microbial infection, poses a therapeutic challenge. Treg cells play an important role in modulating this immune response, with studies showing their accumulation in infected tissues, particularly in chronic periodontitis. Chemokines like CCL17 and CCL22 may attract Treg cells to inflammatory sites. However, functional variations, such as IL-17A+ FOXP3+ cells, suggest Treg plasticity, transforming into pro-inflammatory Th17 cells in the periodontitis environment. Treg cell dysfunction, observed in animal models, contributes to periodontitis progression. Positive regulation of Treg cells achieved through IL-35, nanofibrous spongy microspheres, and exosomes show promising results in mitigating bone loss. Imbalances in the Th17/Treg ratio are implicated in periodontitis, with Treg cells suppressing osteoclast differentiation and inhibiting RANKL expression, contrasting with Th17's role in promoting bone resorption. Understanding Treg subsets' diversity and plasticity is crucial for developing advanced and safer drug delivery systems for periodontitis immunotherapy, potentially impacting systemic diseases associated with periodontitis (Baima et al., 2023; Zhang et

al., 2021).

In apical periodontitis, a local inflammatory response to bacterial infection in root canals causes tissue damage and bone loss. The equilibrium of pro-inflammatory and anti-inflammatory responses, which is controlled by different CD4+ T helper cells, promotes disease progression. Treg cells, a protective subset of CD4+ T cells, are beneficial in limiting periapical inflammation. Studies show that Treg cell levels initially remain low but increase as lesions transition from acute to chronic phases. The presence of Treg cells correlates negatively with IL17+/Foxp3+ ratio and osteoclast count (Zhang et al., 2021).

Chronic periapical lesions, including periapical granulomas, residual radicular cysts, and radicular cysts, exhibit increased FOXP3 expression. FOXP3+ Treg cells are higher in advanced inflammatory infiltrate grades. In experiments, inhibiting Treg function exacerbates periapical lesions, while Treg cell expansion attenuates progression. Chemoattractant application for Treg cells is considered a promising treatment option. Trials in dogs undergoing regenerative endodontic treatment show enriched Treg cells around regenerating tissues, suggesting a role in tissue repair. Treg cells' dynamic modulation of harmful T cell phenotypes may provide an approach for treating periapical lesions and osteolytic disorders, underlining the necessity of boosting endogenous Treg recruitment-based treatment (Zhang et al., 2021).

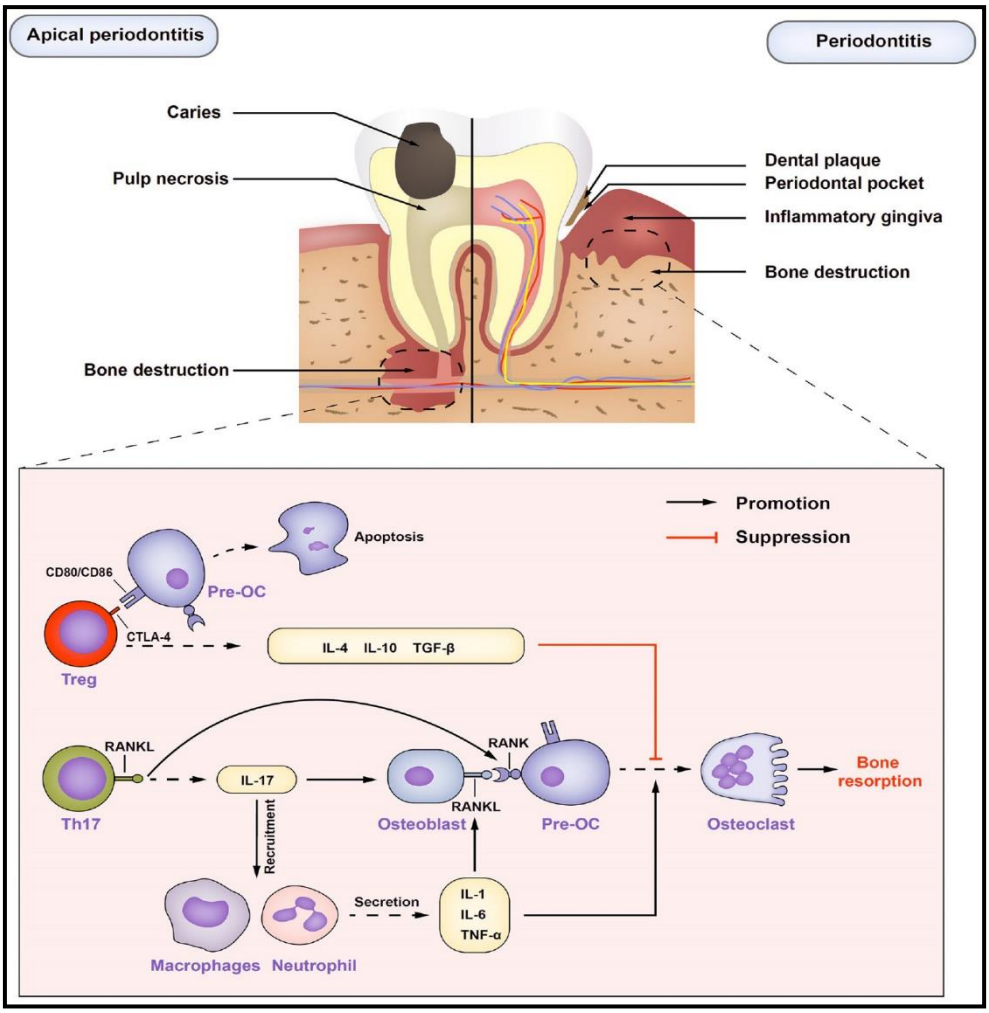

Figure 22: The immunoregulatory roles of Treg cells in both apical periodontitis and periodontitis. Adapted from (Zhang et al., 2021).

Treg cells are essential for oral mucosal health, serving as a critical barrier against pathogen invasion. Interestingly, FOXP3+ Treg cells expressing CTLA-4 and CD103 are significantly more prevalent in the oral mucosa than in secondary lymphoid organs and other mucosal barriers. This shows that the oral mucosa requires a large number of highly active Treg cells to maintain immunological quiescence. Notably, Treg cells in the oral mucosa rely mostly on recruiting and migrating exogenous Treg cells rather than local induction (Zhang et al., 2021).

Imbalances in Treg cell numbers contribute to various oral mucosal diseases. For instance, Pemphigus Vulgaris is associated with decreased Treg cell frequency, increased Th17 cells, and reduced CCL22 expression. In chronic inflammatory illnesses, such as aphthous ulcers, peripheral blood Treg cell frequency and inhibitory function decrease, possibly linked to decreased IDO expression. Conversely, Treg cell numbers increase in precancerous lesions of oral mucosal tissues (Zhang et al., 2021).

The severity of oral epithelial lesions is positively correlated with CD25 and FOXP3 expression, as observed in comparative studies of oral epithelial precursor lesions (OEPL) and oral squamous cell carcinoma (OSCC). In conditions like oral lichen planus (OLP) and actinic cheilitis (AC), there is an increase in FOXP3+ cell infiltration and a simultaneous decrease in the CD8+/FOXP3+ cell ratio, indicating the promotional role of regulatory T cells (Treg cells) in cancer progression. Furthermore, the transition from premalignant lesions to cancer witnesses a gradual shift from Th1 and Th17 phenotypes to a Treg phenotype in the spleen and lymph nodes (Zhang et al., 2021).

Regulatory T cells (Treg cells) are essential in preserving immune balance within the oral mucosal barrier. Utilizing anti-Treg immunotherapy could decelerate the advancement of precancerous lesions. Additionally, prompting the conversion of Treg cells to a Th17-like phenotype holds promise for intervening in the progression of precancerous lesions (Zhang et al., 2021).

Finally, the found link between periodontal disease and an increased risk of oral cancer, particularly given the link to chronic infection, highlights the possible role of oral health in cancer development. The identified aspects of dental hygiene and tooth loss add to the intricacy of this interaction. These findings underline the importance of ongoing research, including more multicentric trials and individual studies that focus on standardized reporting, larger sample sizes, increased event rates, and reduced bias.

As we navigate these issues, the conclusion emphasizes the crucial importance of improved oral healthcare practices. Improving oral hygiene practices and proactively preventing oral illnesses have emerged as critical strategy for reducing the risk factors associated with oral cancer (Mahuli et al., 2023).

6. Future perspective

Tumor eradication chances have significantly increased with recent advancements in cancer therapy, including the introduction of sophisticated radiation instruments and the approval of novel medications. Even with these developments, there is still an opportunity to improve the effectiveness of cancer treatment. New medications intended for targeted therapy and immunotherapy are being investigated in ongoing research, and pre-clinical investigations have shown great promise for combination therapy methods including radioimmunotherapy.

One particularly noteworthy area of exploration is adoptive cell therapy, which presents a personalized approach to cancer therapy. Adoptive cell therapy involves the extraction of immune cells, like NK cells or T cells, from a patient. Subsequently, these cells are expanded and engineered in a laboratory setting before being reintroduced into the patient's body. This method, known for its specificity and efficacy, employs T cells obtained directly from the patient's malignant tissue or via leukapheresis, where T cells are separated and expanded outside the body. This tailored method has the advantage of targeting specific antigens expressed by the patient's tumor, which improves therapeutic precision. Additionally, adoptive cell therapy, including chimeric antigen receptor (CAR) T-cell therapy, has shown promise in establishing long-lasting immune memory, potentially preventing cancer recurrence and offering prolonged protection against the disease (Zhang et al., 2024).

Tumor vaccine therapy represents another compelling avenue in cancer treatment. This approach harnesses the patient's immune system by stimulating it with specific antigens, awakening an anticancer immune response. Though challenges persist in identifying optimal tumor antigens and overcoming immune evasion, early clinical trials of tumor vaccine therapy have demonstrated encouraging results. Combining tumor vaccination with adjuvants has emerged as a strategy to boost efficacy, as seen in studies examining the synergistic effects of vaccine therapy and substances like metformin for oral cancer cells. These combinations have shown promise in enhancing the infiltration of immune cells and suppressing tumors more effectively (Igarashi & Sasada, 2020).

In the realm of innovative approaches, pH-sensitive nanoparticles targeting the acidic tumor microenvironment have garnered attention. Solid tumors, including those in oral cancers, often exhibit a low pH environment due to increased glycolysis and lactate production. This acidic environment stimulates tumor growth and contributes to resistance against conventional therapies. pH-sensitive nanoparticles offer a solution by

selectively releasing encapsulated anticancer drugs in response to the acidic tumor microenvironment. These nanoparticles, often composed of pH-responsive materials like polymers or lipids, protect the drugs during circulation, ensuring targeted release inside the tumor site. While experiments using pH-sensitive nanoparticles, such as those loaded with doxorubicin or graphene oxide, have shown promise in selectively sensitizing oral squamous cell carcinoma (OSCC) to photothermal therapy and chemotherapy, challenges persist (Murciano-Goroff et al., 2020).

The challenges encompass tumor heterogeneity, affecting pH-sensitive nanoparticles' efficacy in targeting tumors. Tumors exhibit pH levels, oxygenation, and perfusion variations, requiring future research to develop nanoparticles capable of responding to a broader range of pH levels and adapting to diverse microenvironments inside tumors. Furthermore, the unusual vasculature, dense extracellular matrix, and high interstitial fluid pressure within tumors present obstacles to the uniform distribution of nanoparticles. Addressing these challenges is crucial to optimizing pH-sensitive nanoparticles' safety and therapeutic potential (Wada et al., 2022).

Despite these obstacles, pH-sensitive nanoparticles have indisputable potential, as they can be used with other therapeutic modalities, including immunotherapy or targeted therapy, to improve patient outcomes. The distinctions between oral cancers and normal tissues can be used for the targeted administration of nanoparticles into tumors, significantly increasing their potential impact. While obstacles related to manufacturing consistency, scalability, cost-effectiveness, and regulatory approval exist for the clinical translation of pH-sensitive nanoparticles, future efforts should focus on overcoming these challenges to unlock their full potential in cancer therapy. In essence, a comprehensive strategy integrating adoptive cell therapy, tumor vaccination, and pH-sensitive nanoparticle drug delivery holds tremendous promise for advancing the treatment landscape of oral cancers (Murciano-Goroff et al., 2020; Qin & Wu, n.d.; Wada et al., 2022).

III. Conclusion

Immunotherapy has emerged as a promising treatment method for oral cancer. It focuses on targeting immune cells and elements in the tumor microenvironment (TME) to improve the immune system's ability to fight cancer cells. CAFs, TAMs, Tregs, MDSCs, NK cells, and CD8+ T lymphocytes are among the cells in the oral TME that contribute to or prevent tumor formation.

Studies have explored targeting anti-apoptosis mediators, chemokines, co-inhibitory molecules, and growth factors to modulate immune responses in oral cancer therapy. Additionally, natural products like retinoic acid, curcumin, and resveratrol have shown the potential to stimulate immune attacks on oral cancer cells. However, their low bioavailability can be addressed by delivering them through nanoparticles for better efficacy.

Furthermore, immunotherapy with immune checkpoint inhibitors (ICIs) has shown promise in allowing the immune system to target diverse tumors, including oral malignancies.

Combining radiotherapy with hypo fractionated techniques and certain chemotherapy drugs can induce immunogenic cell death (ICD) and subsequent anticancer immune responses.

The future of immunotherapy for oral cancer involves exploring innovative approaches such as dendritic cell-based immunotherapy, CAR-T cell therapy, and adoptive cell therapy to enhance immune responses against cancer cells. Nanoparticles are being investigated for targeted delivery of agents into tumors to improve drug accumulation while reducing side effects in normal tissues.

In conclusion, ongoing research focuses on developing novel immunotherapeutic strategies to modify the tumor immune microenvironment effectively. These advancements provide hope for improving outcomes in patients with oral cancer by optimizing existing therapies and exploring new targets.

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