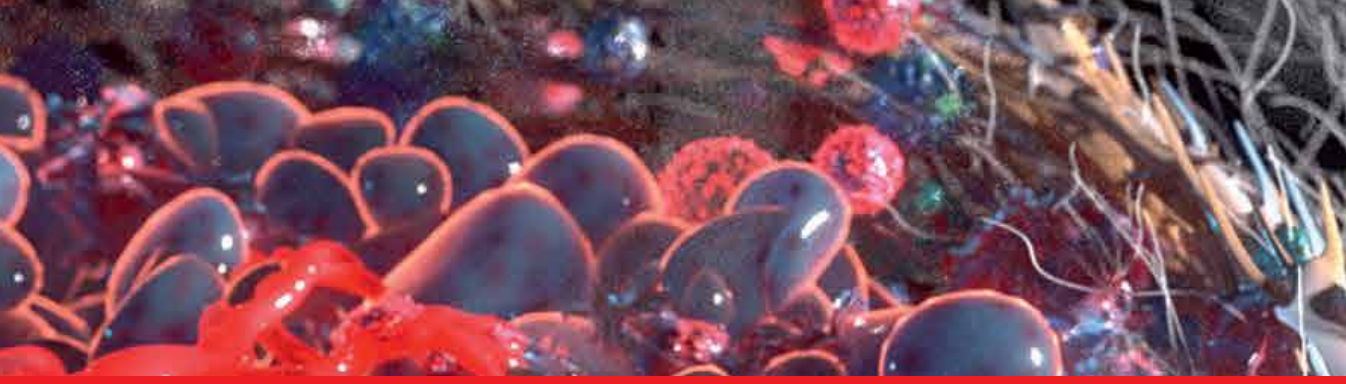

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# Tumor Microenvironment

## New Insights

*Edited by Ahmed Lasfar*

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# Meet the editor

Dr. Ahmed Lasfar is a faculty member at Rutgers University and a full member of the Rutgers Cancer Institute of New Jersey, USA. He is an editor and board member of several international journals and books. Dr. Lasfar is a scientific adviser and consultant to the pharmaceutical industry and a member of several medical and scientific societies. He graduated in Medical and Applied Science (BS/MD) and obtained a Ph.D. in Immunology from Paris Diderot University, France. Dr. Lasfar completed his postdoctoral training in cancer immunology at Robert Wood Johnson Medical School, New Jersey, USA.

# Contents

# Preface

The tumor microenvironment (TME) is a crucial aspect of all cancer types. It is well established now that TME plays an important role in both the control and the development of solid tumors. The histology of TME consists of a variety of normal resident and recruited cells, which are involved in concise and dynamic interactions with cancer cells. These interactions, which occur via released factors or cell-to-cell contact, are fundamental in tumor-induced suppression and metastatic dissemination of cancer cells, ultimately leading to morbidity and/or mortality for most cancer patients. Considerable progress has been made in understanding the mechanisms by which the TME contributes to the inhibition or promotion of cancer, enabling the emergence of a range of novel targeted therapies. In addition to stroma-targeted strategies, checkpoint inhibitor-based immunotherapy has emerged as a new treatment of choice for many advanced cancers. However, many cancer patients remain resistant to current therapies, necessitating the development of more innovative therapeutic strategies based on the identification of new targets and combining drugs that could counteract resistance. In this book, *Tumor Microenvironment – New Insights*, the authors highlight this aspect with chapters that describe and discuss innovative and impactful studies.

Prominent efforts and collaborations with leading experts in cancer were crucial in achieving this highly valuable book. We thank all the authors for their tremendous expertise and their exceptional quality in pointing out the crucial role of TME in cancer. Understanding and targeting TME constitutes a new hope for cancer patients, particularly those with advanced diseases.

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Section 1

# Novel Diagnosis and Treatment of Cancer

Chapter 1

# Liquid Biopsy: A New Strategy for Future Directions in Lung Cancer Treatment

*Maria Palmieri and Elisa Frullanti*

## Abstract

The gold standard for cancer diagnosis has always been based on radiological imaging followed by surgical tissue biopsies for molecular testing and pathological examination and surgical resection to remove the tumoral mass when possible. However, the resulting information is a limited snapshot in space and time, which poorly reflects clonal heterogeneity or tumor evolution and metastasis. Over a decade since its inception, the ability to use non-invasive methods such as a liquid biopsy to analyze tumor biomarkers has transformed the vision of future cancer care into a better patient experience thanks to real-time monitoring and early diagnosis. The liquid biopsy essay is an effective tool for detecting cancers at an early stage, when there are very few tumor-derived materials circulating in the bloodstream, being a very sensitive technique. For this reason, liquid biopsy is particularly suitable for early-stage diagnosis (stage I or II) of lung cancer whose diagnosis often occurs in the final stages of the disease as well as monitoring cancer progression and driving target therapies.

**Keywords:** liquid biopsy, lung cancer, CfDNA, new strategies, Circulating Tumor DNA (CtDNA)

## 1. Introduction

### 1.1 An early opportunity to catch cancer

A cancer patient's torturous journey begins with formulating the diagnosis. The detection of tumor mass, the stage, and the molecular profile are all clues that lead to the proper treatment. The advent of new technologies allows us to deepen our knowledge of molecular data useful to physicians to guide them toward specific therapies.

Cancer is the World's second biggest killer after heart disease, in which some of the body's cells grow uncontrollably and spread to other parts of the body. Currently, 90% of cancer patients do not die from the primary tumor but are killed by its distant metastases. Current treatment of patients with metastatic cancer is generally driven by the molecular characteristics of the primary tumor. Detection and monitoring of the disease are carried out with tissue sampling in a common and invasive difficult way for patients with solid tumors. Recently, sequential peripheral blood tests have been introduced as a non-invasive technique, resulting in the use of liquid

biopsy [1]. Liquid biopsy refers to a test, usually carried out from blood samples, to analyze tumor molecular biomarkers that can diagnose cancer and inform clinical decision-making [1].

Here, we will explore the possible molecular biomarkers that can be used:

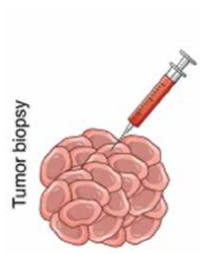
- *The circulating tumor cells* (CTCs) are released from both primary and metastatic tumor sites into the bloodstream. Tumor cells are recognized by the shape and/or physical elements such as size, density, electric charges and deformability and biological characteristics, cell surface protein expression and viability [1]. CTCs have a short half-life, between 1 and 2.5 hours and the process by which the CTCs are released into the bloodstream is not yet well understood. Although their role in metastasis remains poorly undigested, it is highly probable that CTCs are the precursors of the different metastatic populations [2] even if there are less than 10 CTCs per 7.5 ml of blood [3]. For this reason, CTCs require detection and enrichment processes being caught through positive (which relies on antibodies capturing the surface tumor antigen expressed on the CTCs) or negative (removing the other blood components using size filtration) selection. CTCs can be analyzed in a multidimensional characterization, at protein, DNA, and RNA level.

- *The Circulating Tumor DNA* (CtDNA) is the fragmented tumoral DNA that can be detected in the bloodstream, derived from apoptotic and necrotic tumor cells. During the normal apoptosis processes (programmed cell death) or necrosis (cell trauma—premature death), the cell undergoes a series of morphological changes, and the chromatin is condensed and degraded into small fragments, approximately 200 bp in length, and released circulating in the blood. These DNA fragments are also known as cell-free DNA (cf-DNA) and can be acquired by isolating DNA from plasma or serum. Nowadays, next generation sequencing (NGS)-based analyses and digital PCR (dPCR)-based methods are the most frequently used to detect ctDNA.

The analytes mentioned above are the most studied as they are detected more easily alone or in combination, but exosomes, RNA, extracellular vesicles and, last but not least, methylation must also be counted among others.

To date, the gold standard for cancer analysis in clinical practice is tissue biopsy. This implies that, despite the advantages of liquid biopsy that immediately appear very clear, it is necessary to demonstrate that this non-invasive approach is actually better than the current one. The greatest limitation of all tissue biopsies is the lack of representativeness of tumor heterogeneity and plasticity. Tumors are highly heterogeneous, even down to the single cell level, and their characteristics change over time and under treatment pressure. The recovery of the sample through tissue biopsy is a highly invasive practice for the patient and is not easily repeatable over time, thus making the information of the data obsolete over time. For its part, the liquid biopsy can be defined as minimally invasive and allows the monitoring of the evolution of the tumor characteristics over time thanks to the possibility of being able to repeat the practice of blood sampling and in a sequential way also during the course of treatment (**Figure 1**).

Even if the initial approach leads us to think of using the liquid biopsy rather than the tissue biopsy, we should start thinking of starting to collect different data deriving from both approaches to really have clear and comprehensive information that guides cancer treatment.



| | | At metastatic diagnosis | After subsequent lines of therapy |
|---|---|---|---|
| Tumor biopsy | Advantages | • Key pathological information<br>• Ability to assess non-DNA biomarkers (protein, RNA, etc) | • Important for research and discovery<br>• Critical if assessment of non-DNA biomarkers needed |
| | Disadvantages | • Longer turnaround time for sequencing limits first-line precision-therapy selection<br>• Limited tissue quantities can constrain breadth of testing or cause assay failure | • Requires repeat invasive procedure<br>• Longer turnaround time for sequencing results may hinder rapid selection of therapy |
| Liquid-biopsy cfDNA | Advantages | • High concordance with tissue biopsy<br>• Ready sample availability<br>• Rapid turnaround to facilitate first-line precision-oncology therapies<br>• Baseline for subsequent liquid biopsy | • Non-invasive, easy to obtain serial samples<br>• Captures heterogeneous resistance alterations<br>• Rapid turnaround can enhance clinical-trial enrollment |
| | Disadvantages | • Parallel assessment with tumor testing increases cost<br>• Cannot assess non-DNA biomarkers | • Cannot assess non-DNA biomarkers |

**Figure 1.**
*Liquid biopsy versus tumor biopsy for clinical-trial recruitment [4].*

## 1.2 The potential use of liquid biopsy through the patient's journey

Thanks to the sensitivity and specificity achievable with high-depth sequencing, liquid biopsy can be used for the early diagnosis of cancer, and in the next future also as a screening in healthy people. To justify this use, however, it is necessary to demonstrate that the tests are better than those currently in use. Indeed, some studies have shown that ctDNA analysis was able to diagnose lung cancer in the early stages (stage I or II) [5], as well as the detection of some mutations (i.e. *TP53* or *KRAS* genes) which is possible many years earlier, when the individual is still asymptomatic, compared to the time of the classic diagnosis [6].

Additionally, liquid biopsy can be extremely useful in detecting residual molecular disease (MRD) after treatment. In fact, it is very common that after specific medical treatments the current radiological imaging techniques are not able to detect the MRD [7] responsible for relapse.

However, before liquid biopsy enters the clinical routine, several clinical trials are needed for normalization and experimentation and would help answer several unsuspended questions. In fact, clinical trials allow facing challenges such as [8]:

- reproducibility and sensitivity.
- distinguish positive results from negative results.
- biomarker efficacy.

A recent study [9] shows that at the moment the analyses of cfDNA are mainly included in clinical trials for cancers that have a higher incidence and mortality such as lung, breast, and colon cancer. Additionally, the study found some main drawbacks that were common among several trials that risk demoralizing and/or confusing the patient:

- The lack of consensus on terminology
- The false-positive rate: normal cells may have tumor-related mutations
- Lack of a standardized protocol for evaluating cfDNA: hinders the routine use of liquid biopsy in laboratories as an ordinary test
- Lack of numerous cohorts that allow the standardization of the data

### 1.3 The liquid biopsy for non-small cell lung cancer

Lung cancer is the leading cause of cancer death in industrialized countries. In the USA it represents the leading cause of death in men and has now passed breast cancer in females leading to first place in mortality. For non-small cell lung cancer (NSCLC), treatment decisions follow the assessment of the staging of pathological node tumor metastases (pTNM). The more advanced the clinical stage, the more this is associated with the risk of death. However, it is estimated that only 40% of patients have stage 2–3 and have a minimal residual disease (MRD), and this means that the remaining 60% are likely to be over-treated with the possibility of giving rise to high toxicity risks. Somatic molecular alterations in NSCLC can lead to oncogene activation through multiple genetic mechanisms (point mutations, insertions, deletions, gene rearrangements, etc.) and the treatment of cancer has thus evolved from broad chemotherapeutic approaches to therapies targeted against specific molecular abnormalities that drive tumor growth. A robust and accurate assessment of molecular alterations within tumor cells is mandatory in routine clinical practice to determine which patients are suitable for these targeted therapies.

The TRACERx study (Tracking Cancer Evolution through Therapy) is a British national observational study for patients with NSCLC who have undergone surgery. Through this study, they try to evaluate the natural history of the evolution of the disease in order to understand the biology of MRD when it is impossible to access a tissue biopsy again. By monitoring 30 tumor variants, they were able to identify cases of disease recurrence by detecting MRD prior to clinical surveillance. They are currently looking to implement over 200 variants and limit of detection (LOD) of the technique in well over 1000 plasma samples. Therefore, thanks to this pioneering study it is possible to use two approaches:

- the first allows using the ctDNA as a biomarker after the surgery of a patient affected by NSCLC in two temporal points in order to detect the MRD and to enroll the patient in combined therapy;
- the second approach is MRD surveillance to identify those patients who already have a relapse in order to intervene immediately with the therapy in a time.

Historically, the first clinical application of liquid biopsy in advanced NSCLC was the detection of *EGFR* mutations (**Figure 2**). From these pioneering studies, the scientists moved on to the analysis of next-generation sequencing (NGS) which allowed expanding the investigation to other driver mutations as well, that could provide prognostic and predictive information [10].

The first methods used were those of RT-PCR (real-time PCR) capable of detecting *EGFR* mutations and at the moment the only one approved by the FDA for the

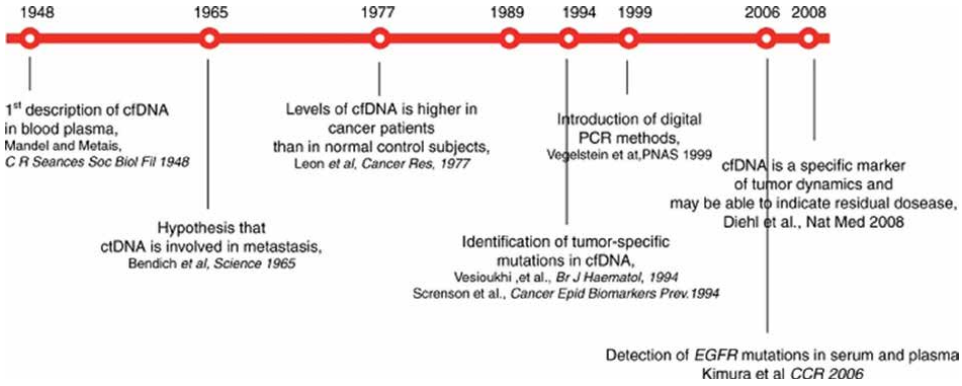



**Figure 2.**
*Timeline of the development of liquid biopsy [10].*

detection of the T790M resistance mutation of the *EGFR* gene [11]. Indeed, in 2016, the Food and Drug Administration (FDA) approved the liquid biopsy test for patients with NSCLC to verify EGFR-targeted therapy, while a European consortium from the European Liquid Biopsy Academy (ELBA) using biomarkers such as ctDNA, CTC, exosomes, and tumor educated platelets (TEP) [12].

Currently, ESMO guidelines recommend testing at least *EGFR* mutations, *BRAF* mutations, *ALK* fusions, *ROS-1* fusions, *MET* exon 14 skipping mutations, *RET* rearrangements and PD-L1 expression levels in non-squamous advanced NSCLC [13]. This panel could be further implemented considering *KRAS* mutations, *HER2* mutations, *MET* amplification, and *NTRK* rearrangements [13].

## 2. Conclusion

In conclusion, we can state that the liquid biopsy is significantly helping the management of patients with lung cancer by crossing the threshold of the use of off-label drugs in therapeutic pathways [14], but we must be aware that liquid biopsy cannot replace the PDL-1 expression assay for investigation of the tumor immune microenvironment, as well as cytological analysis of tissue biopsy. Therefore, in order to obtain the most complete treatment possible, we must consider liquid biopsy not as a competitive approach to the already existing ones, but as another valid mutation detection option.

## Declaration

The authors have no conflicts of interest.

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Chapter 2

# Minimally Invasive Surgery for the Management of Lung Cancer

*Gaetana Messina, Mary Bove, Giorgia Opromolla, Vincenzo Di Filippo, Mario Pirozzi, Marianna Caterino, Sergio Facchini, Alessia Zotta, Giovanni Vicidomini, Mario Santini, Alfonso Fiorelli, Fortunato Ciardiello and Morena Fasano*

## Abstract

Lung cancer is the leading cause of cancer-related death and the most diagnosed cancer. The treatment of Non-Small Cell Lung Cancer (NSCLC) depends on clinical staging. Surgical radical resection is recommended for patients with stage 1 or 2 of disease and represents the treatment of choice. In the last decades, the surgical approach for lung cancer changed moving from an open approach to a minimally invasive approach, represented by Video Assisted Thoracic Surgery (VATS) and Robot-Assisted Thoracic Surgery (RATS). In this chapter, we illustrate the characteristics of lung cancer, the diagnosis, the classification, the staging and the preoperative evaluation. Then we focus on the surgical treatment of lung cancer and on how it has changed during the years. We explain the open approach represented by the traditional posterolateral thoracotomy and by the muscle-sparing thoracotomy. We illustrate VATS approach and evolution: from the hybrid approach to the pure VATS that can be triportal, biportal or even uniportal. Then, we focus on RATS approach, characterized by the use of multiple ports in the same intercostal space and how it evolved toward the uniportal approach. The objective is to combine the advantage of uniportal VATS (lower postoperative pain, enhanced recovery) and RATS (better visualization, more degrees of movements).

**Keywords:** NSCLC, thoracic surgery, VATS, RATS, Uniportal, minimally invasive surgery

## 1. Introduction

### 1.1 Epidemiology

Lung cancer is one of the main causes of death in several countries. The incidence of lung cancer is 3% in men and 1% in women. 236.740 new cases of lung cancer and 130.180 deaths have been recorded in 2022 [1]. 5-years survival rate is 21.7%, in particular it is 15% for men and 19% for women [2]. It represents the first cause of death for tumor for men and the second for women.

Cigarette smoking is the main risk factor for lung cancer, because of its carcinogenic chemicals. Relative risk of lung cancer is related to number of cigarettes smoked per day, years of smoking and level of tar in cigarettes. Exposed non-smokers also have an increased relative risk of developing lung cancer.

Many agents, such as asbestos, beryllium, cadmium, chromium, diesel fumes nickel, are known as carcinogens. They increase the risk of lung cancer in exposed people, especially in smokers. About 80–90% of lung cancer is caused by smoking. The risk of lung cancer is increased in ex-smokers than in never smokers [3]. Some genetic factors, such as overexpression of Epidermal Growth Factor (EGFR), are related to the development of non-small cell lung cancer (NSCLC) [4].

### 1.2 Lung cancer screening

The National Lung Screening Trial (NLST) was the first trial to demonstrate that early diagnosis of lung cancer with annual low-dose CT scan in individuals with high-risk factors reduces the mortality rate related to this disease of 20% compared to chest radiographs. In this trial, individuals with high-risk factors were current or former smokers with a 30 or more pack-year smoking history, 55 to 74 years of age with no evidence of lung cancer [5]. Different organizations such as European Respiratory Society (ERS), European Society of Radiology (ESR), European Society of Thoracic Surgeons (ESTS), European Alliance for Personalized Medicine (EAPM), European Society of Medical Oncology (ESMO) e Swiss University Hospitals recommend lung cancer screening with low-dose CT scan. Anyway, low-dose CT screening and follow-up do not substitute smoking cessation.

### 1.3 Classification and prognostic factors

World Health Organization (WHO) divides lung cancer into two main categories non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC). The 80% of lung cancer is represented by NSCLC. It is divided into two groups: (1) non-squamous: adenocarcinoma, large-cell carcinoma and other subtypes; (2) squamous cell carcinoma [6]. **Table 1** summarizes WHO classification of lung cancer.

In the last decades, the histological definitions of NSCLS become critical for the development of new therapies based on the histotype. Diagnosis can be obtained with morphological criteria based on hematoxylin and eosin stain or specific stains, such as May-Grunwald-Giemsa, but immunohistochemistry is crucial for the definition of poorly differentiated NSCLC or Not Otherwise Specified (NOS).

Immunohistochemical investigation can be conducted both on histological or cytological samples.

The study of the molecular characteristics of lung cancer, the individuation of disease-associated mutations (EGFR mutations) or immune biomarkers (PD-L1) is crucial for target therapy, that is effective in patients with specific mutations [7].

### 1.4 Clinical manifestations

Lung cancer can manifest with symptoms like cough, dyspnea, pain, fatigue or hemoptysis. Symptoms related to advanced stages of disease are weight loss, pleural effusion, dysphagia, lymphadenopathy, paraneoplastic syndromes [8].

| |
|---|
| Epithelial tumors |
| Papillomas |
| Squamous cell papilloma, NOS Squamous cell papilloma, inverted Glandular papilloma<br>Mixed squamous cell and glandular papilloma |
| Adenomas |
| Sclerosing pneumocytoma Alveolar adenoma Papillary adenoma<br>Bronchiolar adenoma/ciliated muconodular papillary tumor Mucinous cystadenoma<br>Mucous gland adenoma |
| Precursor glandular lesion |
| Atypical adenomatous hyperplasia Adenocarcinoma in situ<br>Adenocarcinoma in situ, nonmucinous<br>Adenocarcinoma in situ, mucinous |
| Adenocarcinomas |
| Minimally invasive adenocarcinoma<br>Minimally invasive adenocarcinoma, nonmucinous Minimally invasive adenocarcinoma, mucinous<br>Invasive non-mucinous adenocarcinoma Lepidic adenocarcinoma<br>Acinar adenocarcinoma Papillary adenocarcinoma Micropapillary adenocarcinoma Solid adenocarcinoma<br>Invasive mucinous adenocarcinoma<br>Mixed invasive mucinous and nonmucinous adenocarcinoma |
| Colloid adenocarcinoma |
| Fetal adenocarcinoma |
| Adenocarcinoma, enteric type |
| Adenocarcinoma, NOS |
| Squamous precursor lesion Squamous cell carcinoma, NOS<br>Squamous cell carcinoma, keratinizing Squamous cell carcinoma, nonkeratizing Basaloid squamous cell carcinoma<br>Lymphoepithelial carcinoma |
| Large cell carcinoma |
| Adenosquamous carcinoma |
| Sarcomatoid carcinomas Pleomorphic carcinoma<br>Giant cell carcinoma Spindle cell carcinoma<br>Pulmonary blastoma<br>Carcinosarcoma |
| Other epithelial tumors NUT carcinoma<br>Thoracic SMARCA4-deficient undifferentiated tumor |
| Salivary gland-type tumors Pleomorphic adenoma Adenoid cystic carcinoma Mucoepidermoid carcinoma<br>Hyalinizing clear cell carcinoma Myoepithelioma<br>Myoepithelial carcinoma |
| Lung neuroendocrine neoplasms |
| Precursor lesion<br>Diffuse idiopathic neuroendocrine cell hyperplasia |
| Neuroendocrine tumors<br>Carcinoid tumor, NOS/neuroendocrine tumor, NOS Typical carcinoid/neuroendocrine tumor, grade 1 Atypical carcinoid/neuroendocrine tumor, grade 2 |
| Neuroendocrine carcinomas Small cell carcinoma<br>Combined small cell carcinoma Large cell neuroendocrine carcinoma<br>Combined large cell neuroendocrine carcinoma |

| |
|---|
| Tumors of ectopic tissues |
| Melanoma |
| Meningioma |
| Mesenchymal tumors specific to the lung |
| Pulmonary hamartoma |
| Chondroma |
| Diffuse lymphangiomatosis |
| Pleuropulmonary blastoma |
| Intimal sarcoma |
| Congenital peribronchial myofibroblastic tumor |
| Pulmonary myxoid sarcoma with EWSR1-CREB1 fusion |
| PEComatous tumors Lymphangioleiomyomatosi PEComa, benign<br>PEComa, malignant |
| Hematolymphoid tumors |
| MALT lymphoma |
| Diffuse large B-cell lymphoma, NOS |
| Lymphomatoid granulomatosis, NOS Lymphomatoid granulomatosis, grade 1 Lymphomatoid granulomatosis, grade 2<br>Lymphomatoid granulomatosis, grade 3 |
| Intravascular large B-cell lymphoma |
| Langerhans cell histiocytosis |
| Erdheim-Chester disease |

**Table 1.**
*WHO classification of lung cancer.*

## 2. Diagnosis

Clinical suspicion of lung cancer is based on clinical evaluation and history (smoking history, symptoms, age, previous cancer history, family history, other lung disease). Chest X-ray is generally the first investigation performed. Incidental radiological finding of a suspected lung cancer is frequent and it often presents as a solitary or peripheral nodule. Suspicion findings have to be investigated by CT with contrast (**Figure 1**). Radiological features of the pulmonary nodule that suggest the diagnosis of lung cancer are: size, shape and density. The size of the neoformation and especially its growth over time is closely related to the risk of malignancy. However, the doubling of the volume of the nodule in less than 7 days indicates benign lesion (inflammation/infection). Spiculation, irregular margins and pleural retraction are associated with an increased risk of malignancy. Density of the neoformation can be homogeneous or inhomogeneous and varies from solid lesions to "ground glass" or partially solid lesions [9, 10].

Positron Emission Tomography/Computed Tomography (PET/CT) is playing a significant role as a potential diagnosis and staging test in patients with non-small cell lung cancer (NSCLC) [11] and allows, moreover, to direct the biopsy on suspect areas with elevated glucidic metabolism, increasing the likelihood of reaching a diagnostic result.

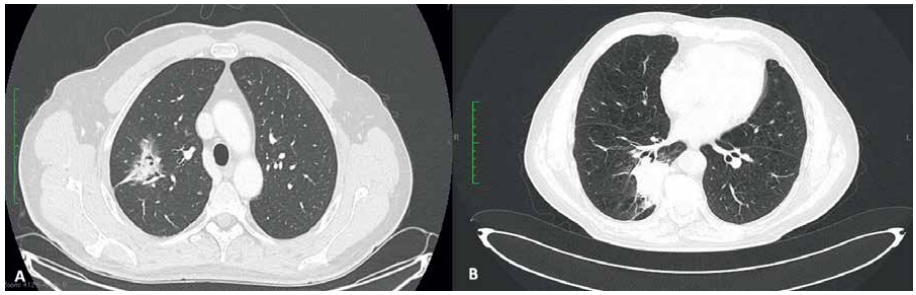


**Figure 1.**
*Examples of lung cancer at CT scan: (A) tumor located at right upper lobe; (B) tumor located at right lower lobe.*

Tissue diagnosis employs several techniques:

- Sputum cytology
- Bronchoalveolar Lavage (BAL)
- Image-guided transthoracic needle core biopsy or fine needle aspiration
- Bronchoscopy with biopsy or Transbronchial Needle Sspiration (TBNA)
- EBUS-guided biopsy
- EUS-guided biopsy

If a preoperative tissue diagnosis cannot be obtained, the alternative is intraoperative diagnosis (wedge resection or needle biopsy). The choice of diagnostic technique mainly depends on the location of the lesion (central or peripheral) but also on the size of the tumor and the clinical condition of the patient [12, 13].

In case of abnormal mediastinal and/or hilar lymph nodes at CT and/or PET, needle aspiration EBUS or EUS-guided is recommended. If malignant nodal involvement is not found by this techniques, surgical staging is recommended [12].

## 3. Staging and TNM classification

Lung cancer staging is necessary to establish the prognosis and the therapeutic program. Staging involves performing a contrast-enhanced CT scan of the chest and upper abdomen to determine local invasiveness, nodal involvement and distant metastasis, particularly in the liver and adrenal glands. The evaluation of these parameters defines the staging of the neoplastic disease according to the TNM system. TNM classification is universally accepted and routinely applied in clinical practice. The T category describes the size and the extension of the primary tumor; the N category defines regional lymph node involvement; the M category establishes presence of distance metastases. **Table 2** summarizes VIII edition of the TNM classification for lung cancer [13].

| T | Primary tumor |
|---|---|
| TX | Primary tumor cannot be assessed or tumor proven by presence of malignant cells in sputum or bronchial washings but not visualized by imaging or bronchoscopy |
| T0 | No evidence of primary tumor |
| Tis | Carcinoma *in situ* |
| T1 | Tumor ≤3 cm in greatest dimension surrounded by lung or visceral pleura without bronchoscopic evidence of invasion more proximal than the lobar bronchus (i.e., not in the main bronchus)[1] |
| T1mi | Minimally invasive adenocarcinoma[2] |
| T1a | Tumor ≤1 cm in greatest dimension[1] |
| T1b | Tumor >1 cm but ≤2 cm in greatest dimension[1] |
| T1c | Tumor >2 cm but ≤3 cm in greatest dimension[1] |
| T2 | Tumor >3 cm but ≤5 cm with any of the following features[3]:<br>• Involving main bronchus regardless of distance from the carina, but without involving the carina<br>• Invading visceral pleura<br>• Presence of atelectasis or obstructive pneumonitis that extends to hilar region (involving part or entire lung) |
| T2a | Tumor >3 cm but ≤4 cm in greatest dimension |
| T2b | Tumor >4 cm but ≤5 cm in greatest dimension |
| T3 | Tumor >5 cm but ≤7 cm in greatest dimension or direct invasion of chest wall (including superior sulcus tumor), phrenic nerve, parietal pericardium or separate tumor nodule(s) in the same lobe as the primary tumor |
| T4 | Tumor >7 cm in greatest dimension or invasion of diaphragm, mediastinum, heart, great vessels, trachea, recurrent laryngeal nerve, esophagus, vertebral body, carina or separate nodule(s) in a different ipsilateral lobe to that of the primary tumor |
| N | Regional lymph nodes |
| NX | Regional lymph nodes cannot be assessed |
| N0 | No regional lymph nodes metastasis |
| N1 | Metastasis in ipsilateral peribronchial and/or ipsilateral hilar lymph nodes and intrapulmonary nodes, including involvement by direct extension |
| N2 | Metastasis in ipsilateral mediastinal and7or subcarinal lymph node(s) |
| N3 | Metastasis in contralateral mediastinal and hilar, ipsilateral or contralateral scalene or supraclavicular node(s) |
| M | Distant metastasis |
| M0 | No distant metastasis |
| M1 | Presence of distant metastasis |
| M1a | Separate tumor nodule(s) in a contralateral lobe to that of the primary tumor or tumor with pleural or pericardial nodule(s) or malignant pleural or pericardial effusion[4] |
| M1b | Single extrathoracic metastasis[5] |
| M1c | Multiple extrathoracic metastases to one or more organs |

[1]*The uncommon superficial spreading tumor of any size with its invasive component limited to the bronchial wall, which may extend proximal to the main bronchus, is also classified as T1a.*

[2]*Solitary adenocarcinoma, ≤3 cm with a predominately lepidic pattern and ≤ 5 mm invasion in any 1 focus.*
[3]*T2 tumors with these features are classified as T2a if ≤4 cm in greatest dimension or if size cannot be determined, and T2b if >4 cm but ≤5 cm in greatest dimension.*
[4]*Most pleural/pericardial effusions with lung cancer are due to tumor. In a few patients, however, multiple microscopic examinations of pleural/pericardial fluid are negative for tumor. In these the effusion should be excluded as a staging descriptor.*
[5]*This includes involvement of a single distant (nonregional) lymph node.*

**Table 2.**
*TNM classification.*

In cases where CT does not show evidence of distant metastases, imaging staging should be completed with 18-FDG PET-CT, which has higher sensitivity and specificity than contrast-enhanced CT and higher sensitivity than (18)F-FDG PET in staging NSCLC in detecting extrathoracic and bone metastases. PET/TC has, however, low sensitivity in detecting brain metastases [14]. Staging brain MRI with contrast is used to evaluate the presence of cerebral metastases in patients with neurological symptoms or in the investigation of a suspected CT lesion [15].

Staging is divided into clinical staging (presurgical) and pathologic staging (after surgical resection of the tumor, lymph nodes or metastases) (**Table 3**).

## 4. Treatment of early stage lung cancer

Radical surgery allows to obtain a full recovery or to significantly improve the prognosis in patients with early stage disease and is not recommended for patients with advanced disease. Surgery needs to be taken in consideration in NSCLC stage I, II and in selected stage IIIA/IIIB (T1-T2, N2 single station, non-bulky). It should be performed in high-volume centers, by expert surgeons. It has been demonstrated that the outcome of patients undergoing lung resection for lung cancer is better for those treated in high-volume centers [16]. Before surgery, lung cancer patients need to be studied in order to define their operability. A tumor that can be completely resected with surgery is considered resectable. Even if a tumor is anatomically resectable, it is necessary to evaluate if the patient can tolerate surgery and is functionally operable according to his functional preoperative situation and, most importantly, his predicted postoperative status, especially with regard to respiratory and cardiovascular function. Cardiorespiratory evaluation is mandatory for patients that are candidate to a lung resection surgery, in order to predict the operatory risk and postoperative lung function. Lung function is evaluated mainly with: spirometry, Diffusion Lung CO (DLCO), hemogasanalysis, ergometric tests, lung perfusion scintigraphy. In case of lower values (FEV1 and DLCO <80%), Cardio Pulmonary Exercise Testing (CPET) is indicated and if the maximal oxygen consumption (VO2max) is less than 10 mL/kg/min the risk of serious postoperative complications is high. For cardiovascular assessment, the use of recalibrated thoracic Revised Cardiac Risk Index (RCRI) is recommended (**Table 4**).

An RCRI <2 has been reported to be associated with a low-cardiac risk, and no additional tests are needed. However, an RCRI >2 has been associated with an increased cardiac risk and a cardiac consultation with non-invasive testing is recommended [17, 18].

| STAGE | T | N | M |
|---|---|---|---|
| Occult carcinoma | TX | N0 | M0 |
| 0 | Tis | N0 | M0 |
| IA1 | T1mi | N0 | M0 |
| | T1a | N0 | M0 |
| IA2 | T1b | N0 | M0 |
| IA3 | T1c | N0 | M0 |
| IB | T2a | N0 | M0 |
| IIA | T2b | N0 | M0 |
| IIB | T1a | N1 | M0 |
| | T1b | N1 | M0 |
| | T1c | N1 | M0 |
| | T2a | N1 | M0 |
| | T2b | N1 | M0 |
| | T3 | N0 | M0 |
| IIIA | T1a | N2 | M0 |
| | T1b | N2 | M0 |
| | T1c | N2 | M0 |
| | T2a | N2 | M0 |
| | T2b | N2 | M0 |
| | T3 | N1 | M0 |
| | T4 | N0 | M0 |
| | T4 | N1 | M0 |
| IIIB | T1a | N3 | M0 |
| | T1b | N3 | M0 |
| | T1c | N3 | M0 |
| | T2a | N3 | M0 |
| | T2b | N3 | M0 |
| | T3 | N2 | M0 |
| | T4 | N2 | M0 |
| IIIC | T3 | N3 | M0 |
| | T4 | N3 | Mo |
| IVA | Any T Any T | Any N Any N | M1a M1b |
| IVB | Any T | Any N | M1c |

**Table 3.**
*Staging of NSCLC.*

Brunelli et al. [19] proposed a physiologic evaluation resection algorithm for major anatomic resection (lobectomy or greater).

For positive and low-risk or negative cardiac evaluation, we calculate postoperative FEV1 (ppoFEV1) and postoperative DLCO (ppoDLCO):

| Weighted factors | Points |
|---|---|
| Ischaemic heart disease | 1.5 |
| History of cerebrovascular disease | 1.5 |
| Serum creatinine >2 mg/dL | 1 |
| Pneumonectomy planned | 1.5 |
| Classes of risk | |
| A | 0 |
| B | 1–1.5 |
| C | 2–2.5 |
| D | >2.5 |

**Table 4.**
*Recalibrated thoracic revised cardiac risk index.*

- If ppoFEV1 or ppoDLCO <30%, cardiopulmonary exercise test (CPET) is recommended.
    - If VO2max is >20 ml/kg/min or > 75%, the patient is considered at low risk for major anatomic resection.
    - If VO2max is 10–20 ml/kg/min or 35–75%, the patient is at moderate risk for major anatomic resection.
    - If VO2max is <10 ml/kg/min or < 35%, the patient is at high risk for major anatomic resection.
- If ppoFEV1 or ppoDLCO <60% and both >30%, stair climb or shuttle walk is recommended.
    - If stair climb is >22 m or shuttle walk is >400 m, the patient is considered at low risk for major anatomic resection.
    - If stair climb is <22 m or shuttle walk is <400 m, cardiopulmonary exercise test (CPET) is recommended.
- If ppoFEV1 and ppoDLCO is >60%, the patient is considered at low risk for major anatomic resection.

For positive high-risk cardiac evaluation, cardiopulmonary exercise test (CPET) is mandatory:

- If VO2max is >20 ml/kg/min or > 75%, the patient is considered at low risk for major anatomic resection.
- If VO2max is 10–20 ml/kg/min or 35–75%, the patient is at moderate risk for major anatomic resection.

- If VO2max is <10 ml/kg/min or < 35%, the patient is at high risk for major anatomic resection.

A multidisciplinary evaluation is necessary to discuss the different therapeutic options and their potential results. The surgical procedure depends on the extent and the localization of the tumor and on the cardiopulmonary reserve of the patients. Preoperative or intraoperative cytohistologic diagnosis is recommended before anatomic lobectomy, bilobectomy or pneumonectomy. Anyway, when the diagnosis is technically difficult to obtain, or it is at high risk for the patient and the radiological and clinical probability of lung cancer is high, it is possible to perform an anatomic resection without tissue confirmation of lung cancer.

Anatomic lobectomy with mediastinal lymphadenectomy is the gold standard treatment for lung cancer.

When the lesion is not resectable through a lobectomy, for instance if it infiltrates the main bronchus or the main artery, or if it invades the fissure to the adjacent lobe, pneumonectomy is indicated.

If anatomically applicable and if negative margin can be achieved, sleeve lobectomy is preferred over pneumonectomy.

Anatomic segmentectomy is acceptable for Ground Glass Opacities (GGO) or for very early stage of disease (Tis or T1a) or in patients who are not eligible for lobectomy. It is possible because GGO more often are diagnosed as in situ adenocarcinoma or minimally invasive adenocarcinoma. When segmentectomy is performed, parenchymal resection margins should be 2 cm or more, or they should be the size of the nodule or larger. In these cases, segmentectomy is preferred over wedge resection [20].

## 5. Surgical techniques

### 5.1 Thoracotomy

The first pulmonary resection for lung cancer was performed in 1912. At the beginning, surgical resection for lung cancer was pneumonectomy. In 1960s, lobectomy was recognized as the gold standard treatment. Traditional surgical approach was a 15–20 cm posterolateral thoracotomy. This traditional approach implies the resection of multiple muscle layers (latissimus dorsi and serratus anterior) and ribs divarication with metal retractors. Ribs fractures are common during divarication and sometimes ribs segments are resected to avoid fractures and to improve surgical exposure. This kind of thoracotomy allows an optimal view of the hilum and the use of two hands by the surgeon. This incision can result in pain and shoulder and chest wall dysfunction. 44% of patients undergoing thoracotomy develop chronic pain for 1 year after the procedure and 29% of patients experience pain for more than 1 year after surgery [21].

Noirclerc et al. [22] were the first to describe the muscle-sparing thoracotomy. The objective of this approach is to preserve muscles, in particular the latissimus dorsi. This technique reduces postoperative complications and consents a better postoperative mobilization of the shoulder, compared to traditional posterolateral thoracotomy.

### 5.2 Video-assisted thoracic surgery (VATS)

During the last decades, Minimally Invasive Surgery (MIS) was applied for lung cancer surgery. The first Video-Assisted Thoracoscopic Surgery (VATS) for lung resection was performed in the early 1990s [23]. At the beginning, the term VATS indicated the use of a videothoracoscope during thoracic surgery procedures, performed through traditional thoracotomy. For example, Okada et al. [24] described a hybrid approach, with a mini-thoracotomy and a camera port, used to see areas not visible with direct vision. Substantially, hybrid approach integrated direct and thoracoscopic vision. Then, there was the development of "pure" VATS using only thoracoscopic vision.

Most centers use a 3–5 cm utility incision located anteriorly, one port for the optic and another port located posteriorly. Gossot et al. [25] described pure thoracoscopic lobectomy using three incisions with a mini-thoracotomy for the extraction of the lobe. McKenna Jr. et al. [26] use three or occasionally four ports.

Hansen et al. [27] perform a standardized anterior three-port approach, with the ports located always in the same place, independently of the lobe to resect: a utility incision of 4–5 cm is located anteriorly at the 4th intercostal space, the 1–1,5 cm camera port is located anteriorly at the level of the diaphragm (8th intercostal space) and a posterior 1,5 cm incision is done at the same intercostal space.

Burfein and D'Amico perform double-port VATS lobectomy [28]: a 2 cm camera port is located at the 7th or 8th intercostal space in the mid-axillary line and a utility incision of 4,5 cm is located anteriorly at the 5th or 6th intercostal space. The double-port technique is characterized by a different lung exposure and the camera has to be moved between the camera port and the utility incision during surgery.

In all cases, systematic lymph node dissection is performed.

Compared to open approach, VATS lung resection is associated with lower postoperative pain, lower incidence of postoperative complications (including atrial fibrillation, atelectasis, prolonged air leak), shorter length of hospitalization, lower postoperative mortality. The reduced hospitalization also consents a rapid access to adjuvant chemotherapy [29]. Different studies analyzed the oncological equivalence of VATS compared to open approach. Some studies aimed to analyze the effectiveness of nodal dissection in VATS compared to that obtained with thoracotomy. Medbery et al. [30] affirmed that there is no difference in staging if nodal dissection is performed in high-volume centers. According to Watanabe et al. [31] a complete lymphadenectomy is possible in VATS also in N2 stage intraoperatively diagnosed. A retrospective study of the National Cancer Data Base (NCDB) showed no difference in nodal staging and overall survival between patients operated in VATS or in open resections [26]. It is also demonstrated that there is no difference in long-term survival [32].

During the years, VATS surgery evolved to a uniportal approach, with only a single incision used for all instruments and for lobe extraction. Rocco et al. [33] were the first to describe the uniportal approach in 2004 for wedge resection, not performing lobectomies. Gonzales-Rivas et al. [34] did the first uniportal VATS lobectomy in 2010. The utility incision is done at the 5th intercostal space, its size is the same of the utility incision used for triple or double-port approach. The surgeon and the assistant are placed both in front of the patient in order to have the same vision and to coordinate movements. A 30 degrees camera is used and it follows the instruments, giving

a vision that closely resembles that of the open approach. Uniportal VATS lobectomy follows the same principles of all major pulmonary resection in VATS. Dissection of veins, arteries, bronchus and fissure is performed, with a complete mediastinal lymphadenectomy.

Different studies compared the outcome of uniportal and "multiportal" VATS, demonstrating a reduction of complications, length of hospitalization and duration of drain tube. Uniportal VATS also allows for a reduction in postoperative pain due to several factors. Firstly, it involves only one intercostal space, minimizing the overall surgical trauma. Secondly, the absence of trocars eliminates the potential compression on the intercostal nerve that may occur during the movements of the camera in traditional multiport VATS procedures [35]. An interesting evolution of the uniportal VATS was the development of the subxiphoid approach, in order to reduce the pain due to intercostal nerve damage [36]. It also can be used to treat bilateral disease, even if the visualization is limited, compared to transthoracic techniques [37]. More studies are necessary to compare subxiphoid to transthoracic approach.

General anesthesia with single lung ventilation is required for lung surgery and it is obtained through a double lumen endotracheal tube or through a bronchial blocker. The patient is positioned in lateral position. Both the surgeon and the assistant stay in front of the patient in order to have the same vision. In general, the port position is the same for every lobectomy. A 30° videothoracoscope and long and curved instruments are usually used. Hilum dissection is performed bluntly with instruments, suction device, peanuts or energy devices. Vascular and bronchial elements are isolated and resected through linear endo-staplers. Also the fissure is divided with endo-stapler device. The specimen is extracted using an endo-bag. The chest tube is inserted in the camera port at the end of surgery. Then, the lymphadenectomy is done mainly using energy device.

Lymphadenectomy is necessary for the correct staging of the disease. Systematic lymph node dissection is recommended. Anyway some authors recommend the lobe-specific mediastinal lymphadenectomy. According to them, nodal metastasis is related to the localization of the primary tumor: upper lobe tumors tend to metastasize upper lymph nodes and lower lobe tumors tend to spread to the inferior and subcarinal nodes [38]. Systematic mediastinal lymph node dissection allows the detection of more metastatic lymph nodes and a better oncologic outcome than lobe-specific nodal dissection [39]. Gooseman and Brunelli [40] recommend systematic lymphadenectomy and in particular, even for the selected cases in which lobe-specific nodal dissection could be accepted (peripheral T1 squamous cell carcinoma), they recommend always the dissection of subcarinal lymph nodes.

The surgeon has to be prepared to convert to thoracotomy in case of technical difficulties in dissection or in case of bleeding.

### *5.2.1 Right upper lobectomy*

The first step involves performing a mediastinal release maneuver. The lung is retracted anteriorly and the posterior pleura is dissected at the level of the bronchial bifurcation. It helps the dissection of the bronchus from the anterior approach. Then, the lung is retracted posteriorly and the dissection of the veins is performed. Once identified and dissected the upper lobe vein, it is resected with vascular endo-stapler. The resection of the vein exposes the pulmonary artery. The arterial branches to the upper lobe (truncus anterior and ascending arteries) are dissected and divided through

vascular endo-stapler. Then, the bronchus is divided through bronchial stapler and the fissure is completed with endo-stapler device. The specimen is extracted in an endo-bag through the utility incision.

### *5.2.2 Left upper lobectomy*

The lung is retracted anteriorly and the posterior pleura is dissected in order to identify the posterior artery and to facilitate the maneuvers with the anterior approach. The lung is retracted posteriorly and, after the identification of the veins, the upper lobe vein is dissected and divided through endo-stapler, exposing the pulmonary artery and the upper lobe bronchus. The arterial branches for the anterior, posterior and apical segments and the lingular branches are exposed and divided through vascular endo-staplers. Then, the upper bronchus and the major fissure are divided through endo-staplers. The specimen is extracted in an endo-bag through the utility incision.

### *5.2.3 Middle lobectomy*

The middle lobe vein is dissected and divided. Then the fissure to the lower lobe and the bronchus to the middle lobe are divided through endo-staplers. Then the artery branches are dissected and divided and at the end the fissure to the upper lobe is completed through endo-stapler. The specimen is extracted in an endo-bag through the utility incision.

### *5.2.4 Lower lobectomies*

The first step of lower lobectomies is represented by the division of the inferior pulmonary ligament. This confers mobility to the lobe and exposes the vein to the lower lobe. The lower lobe vein is dissected and divided with endo-stapler (**Figure 2**). Then the procedure can continue in two ways: the surgeon can dissect the arterial branches to the lower lobe (for the apical segment and for the basal pyramid) and the bronchus to the lower lobe within the fissure. The other option is represented by the fissureless technique: after the dissection and resection of the vein, the lower lobe is retracted cranially and the plane between the bronchus and the artery is dissected and

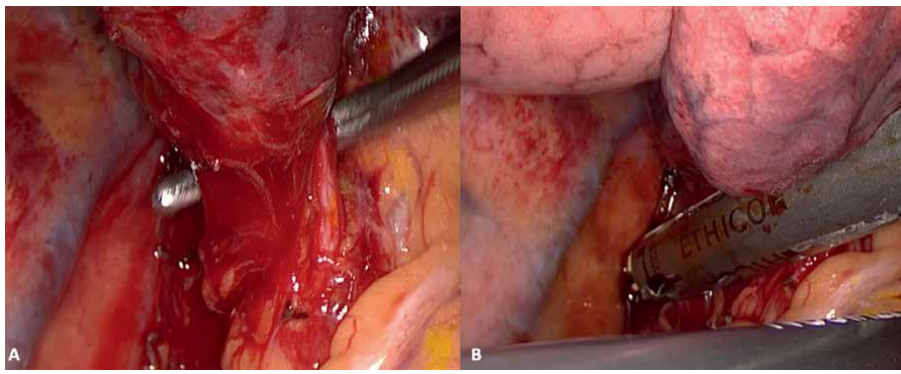


**Figure 2.**
*Right lower lobectomy: (A) right lower lobe vein dissection; (B) right lower lobe division through endo-stapler.*

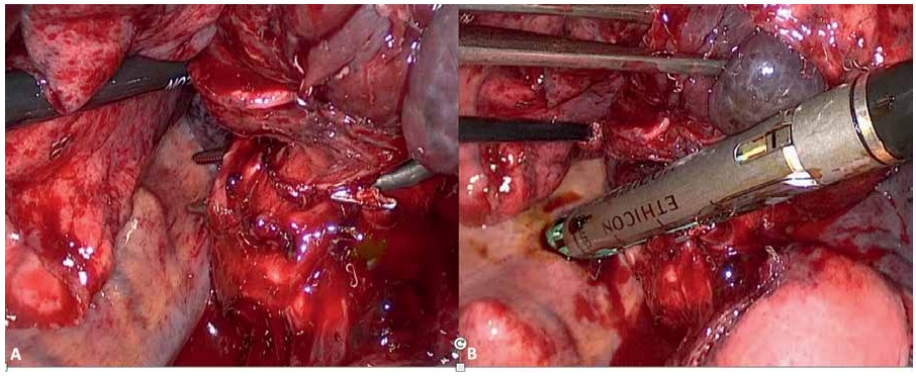


**Figure 3.**
*Right lower lobectomy: (A) dissection right lower lobe bronchus; (B) division of right of right lower lobe bronchus through endo-stapler.*

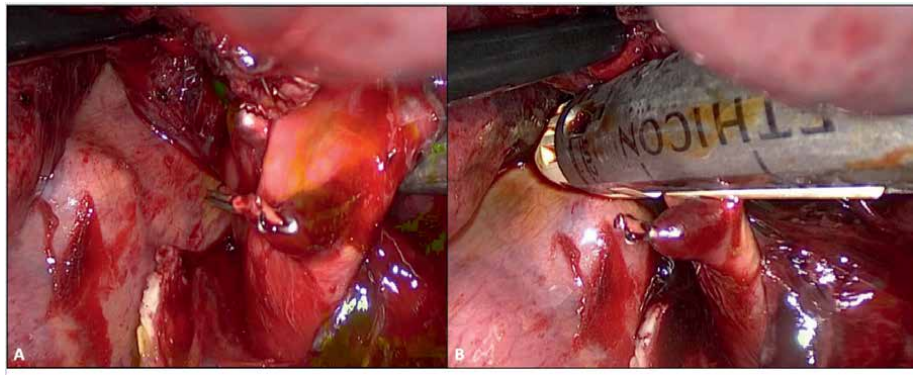


**Figure 4.**
*Right lower lobe artery: (A) dissection and (B) division of right lower lobe artery through endo-stapler.*

the bronchus is divided through endo-stapler (**Figure 3**). Then, the arterial branches are divided and the fissure is divided at last (**Figure 4**). The specimen is extracted in an endo-bag through the utility incision.

### 5.3 Robotic-assisted thoracic surgery (RATS)

The most recent minimally invasive technique applied to thoracic surgery is robotic approach. The progress in the field of robotic technology generated interest in thoracic surgeons, that started to perform Robot-Assisted Thoracic Surgery (RATS). The first robotic lobectomies were described by Morgan et al. [41] and by Ashton et al. [42] in 2003. Since then, robotic lobectomy started to be performed in different centers. Cerfolio et al. [43] described their initial results using a completely portal 4-arm robotic operation with insufflation of carbon dioxide. The four ports are located at the same intercostal space (7th intercostal space). They achieved a complete R0 resection, performing a median number of 5 mediastinal lymph node station dissections. They recorded a significant reduction in morbidity and hospital stay compared to thoracotomy. When compared to VATS, Kent et al. [44] reported less postoperative pain and a rapid return to normal activities.

In 2021, Yang et al. [45] were the first to describe a uniportal RATS lobectomy for a tumor located in the right upper lobe. A single 4 cm incision was made at the 4th intercostal space on the mid-axillary line. The 30° camera arm was placed on the upper end of the incision and the two instrument arms were placed intercrossed inside the chest. With this approach, they were able to perform a radical lobectomy and lymphadenectomy. The recovery was fast and the patient was discharged three days after surgery.

RATS consents a three-dimensional (3D) high-definition view, intuitive articulation of the robotic hands and more flexibility of instruments, with seven degrees of motion. Its superior instrumentations consent to perform accurate and safe dissection, in particular lymph node dissection that is crucial for the correct staging of lung cancer. It also can be used for difficult cases at high risk of conversion such as central tumors, sleeve lobectomy and pneumonectomy. To date, studies comparing VATS and RATS lobectomy do not show significant differences in terms of outcome. For this reason, a challenging question arises regarding the cost-benefit analysis [46, 47].

## 6. Conclusions and future perspectives

At the beginning of the era of minimally invasive thoracic surgery, a great limit of the technique was represented by the vision, because of the low definitions of cameras. Surgeons preferred the direct vision to have a better control of the procedure. The progress in the field of technologies consented to have high-definition cameras also with additional features, such as 3d vision or integration with augmented reality surgery navigation systems. Nowadays, cameras are largely used by surgeons even in thoracotomy approach to improve visualization and lightning. The progress made in the field of instrumentations has been appreciated by surgeons and instruments made for minimally invasive surgery such as endo-staplers are now used also for open approach, because of their thickness and flexibility.

Another challenge for VATS surgery is the use of rigid instruments that have to be moved through the rigid chest wall. Human hand consents to perform several traction and counter-traction movement and provides tactile feedback. With the development of minimally invasive surgery, that limits the tactile feedback, surgeons started to operate mainly relying on the vision. This happens, in particular, in RATS. Since the chance to palpate nodules or ground glass opacities is little in VATS and even null in RATS, surgeons has to rely only on visual signals. For this reason, they can use intraoperative ultrasound or they can mark nodules with coils in hybrid operating room [48]. Also artificial intelligence is developing in order to help surgeons during procedures.

The field of minimally invasive thoracic surgery is developing in two directions: reducing the number and the size of the surgical access (uniportal VATS) and increasing the use of RATS. The objective is to combine the advantage of uniportal VATS (lower postoperative pain, enhanced recovery) and RATS (better visualization, more degrees of movements).

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Chapter 3

# Trends of Pediatric Cancer in India

*Sajna Panolan, Srinivas Govindarajulu, S. Kalpana, Valarmathi Srinivasan and Joseph Maria Adaikalam*

## Abstract

Compared to developed countries, only a limited number of studies systematically engage with India's experience with the burden of childhood cancer and its implications for public healthcare in the country. This study aims to assess the long-term trend in the incidence of cancerous conditions, demographic factors, and the burden of the disease among children. The study has used the Madras Metropolitan Tumor Registry (MMTR), covering cancer cases reported among children (0–14 years) in Chennai for the last 34 years (1982–2016). The study analyses the incidence of the pediatric tumor for different age groups, gender, and type of cancer and the long-term trend over the years and compares the same with existing studies. The trend indicates that more cases are reported during 2007-11and the least number of cases are reported during 2012–2016 (respectively 16.7% and 11.9% of total cases reported).

**Keywords:** childhood cancer, pediatric cancer, tumor, trend, Chennai

## 1. Introduction

Globally, the incidence of childhood cancer has been increasing steadily and throws new challenges in public health management and policy making. Its nature, types and risk factors vary across the countries. As a developing country, India's experience with its given context is very important in understanding the role of epidemiological, demographic, socio-economic factors, and policy engagements in addressing public healthcare challenges. Several studies are looking into the experience of developed countries in addressing the cancer prevalence of cancer among children, their treatment, and attempts to connect them with countries' epidemiological transition. Compared to this, only a limited number of studies systematically engage with India's experience with the burden of childhood cancer and its implications for public healthcare in the country.

Available evidence indicates that India also experiences a steady increase in the number of children affected by different types of cancer. The details suggest that leukemia is the most common cancer affecting children followed by lymphoma and retinoblastoma. The profile of children affected by cancer shows variation across the age groups. The incidence of retinoblastoma, renal tumors, neuroblastoma, and hepatic tumors was found higher among children aged below five years whereas lymphoma, leukemia, bone tumors, and central nervous system tumors were found more among children aged above five years [1].

Globally, the annual number of new cases of childhood cancer exceeds 2, 00,000 and more than 80 percent of the reported cases are from the developing world [2]. Thirteen percent of the annual deaths worldwide are cancer-related and 70 percent of them are in the low- and middle-income countries [3]. Childhood cancer (age at diagnosis 0–14 years) is associated with a variety of malignancies and its incidence varies by age, sex, ethnicity, and geography, as reported by canceretiology [4, 5]. The incidence of childhood cancer across the countries ranges from 75 to 150 per million children per year. For instance, only 0.5 percent of all cancer cases reported in England occur in children less than 15 years of age whereas in India this proportion appears higher at 1.6–4.8 percent with variation by place of residence. This is related to the population structure (33% of the population in India is less than 15 years of age compared to 18% in England) [6, 7]. Though it remains less than the cases reported in the developed world, about 1.6 to 4.8 percent of all cancer reported in India are found in children below 15 years of age, and the overall incidence of 38 to 124 per million children, per year [8].

As 75 percent of the world population lives in these countries, developing countries bear more than half of the global cancer burden [9]. Because of population growth, aging and urbanization, changing dietary habits, better control of infections, and increasing tobacco consumption, developing countries are anticipated to bear a greater cancer burden, including that of greater lympho-hemopoietic malignancies [10]. India found to have 3 million persons is reported with cancer at any time, with 0.8 million new cases of cancer diagnosed each year [11]. There is a constant rise in cancer cases, but the trend and pattern vary according to the geographical region [12].

India's experience with a fast-growing economy and change in lifestyle-related behaviors can be connected to increasing cancer load [13, 14]. The relative differences in the incidence of lympho-hemopoietic malignancies in urban and rural populations can be connected with the differences in the environmental and socioeconomic factors affecting the dietary habits and lifestyle in rural and urban areas [15]. They tend to follow the larger trends noticed in terms of disease risk connected with the relative contributions of environment and genetics in the etiology of specific cancers. Studies consider their contribution to risk due to variation in exposure to carcinogens (in the external environment, or through lifestyle choices), or in genetic susceptibility to them [16].

This study broadly highlights the intensity of childhood cancer and its implications for child healthcare and health management in the global, national and local contexts. It aims to assess the long-term trend in the incidence of cancerous conditions, demographic factors, and the burden of the disease among children in Chennai from 1982 to 2016.

## 2. Materials and methods

This study has used the Madras Metropolitan Tumor Registry (MMTR), a population-based cancer registry (PBCR) based at the Cancer Institute (WIA), Chennai covering all cases reported among children (0–14 years) in Chennai for the last 34 years (1982–2016). All cases of childhood cancer from 0 to 14 years of age that were registered from 1st January 1982 to 31st December 2016 were included in this study. The study analyses the data on the incidence of the pediatric tumor for different age group, gender, and type of cancer and the long-term trend over the years and compare the same with existing studies. Childhood cancers (age at diagnosis

0–14 years) comprise a variety of malignancies, with incidence varied by age, sex, and ethnicity that provided insights into cancer etiology. The analysis looks into the types and incidence rate of cancer across the different age groups of children. The proposal was reviewed and approved by the ethical and scientific committees of the university.

## 3. Result and discussion

The analysis covers 34 years (1982 and 2016) and shows the trend of the cancerous condition of children of madras. The long-term trend indicates that more number of cases is reported during 2007–2011 (639cases) constitutes 16.7 percent of the total cases reported during this period. At the same time, the least number of cases are reported during 2012–2016 (458cases), constituting 11.9 percent of the total cases reported (**Figure 1**).

**Table 1** describes the Sex-wise distribution of pediatric cancer during this period and shows that more number of cases are reported among male children (2313 cases) constituting 60.3% of total cases reported (3834). Compared to this, only 1521 cases (39.7%) are reported among female children.

**Figure 2** describes the age group distribution of pediatric cancer reported from 1982 to 2016. When the children are classified into three agegroups, the data shows that more pediatric cancer is reported in 0–4 years of age (1417 cases) accounting for 37 percent of the total cases reported (3834 cases). The details show that the highest number of cases (370 cases, constituting 9.7%) was reported at three years of age.

**Table 2** shows the distribution of reported cases among the major religious groups. Compared to other religious groups, more pediatric cancer cases were reported in the Hindu community, (3172 cases) constituting 82.7 percent of the total 3834 cases. A large number of cases were reported among children from Muslim (392 cases, 10.2%), and Christian (247 cases 6.4%) communities.

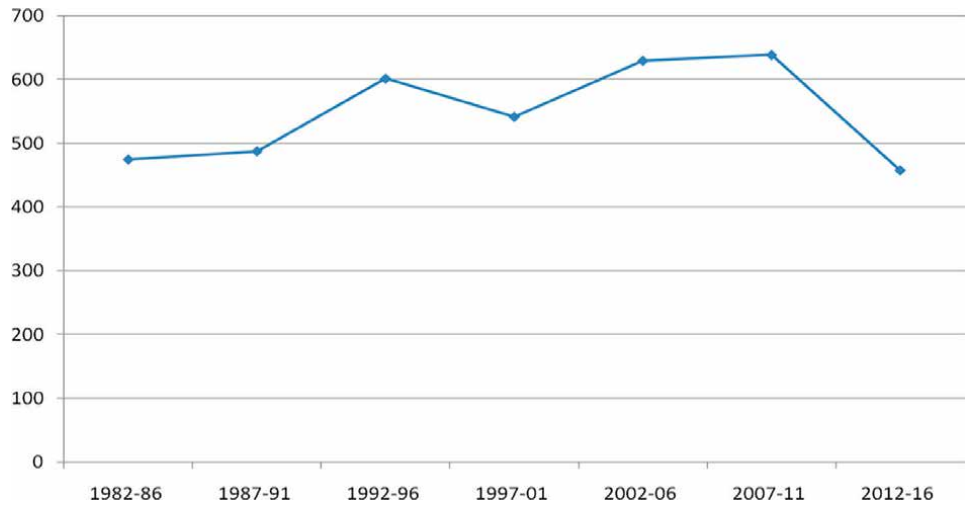


**Figure 1.**
*Number of cases reported: 1982–2016.*

| SEX | Frequency | Percent (%) |
|---|---|---|
| Male | 2313 | 60.3 |
| Female | 1521 | 39.7 |
| Total | 3834 | 100 |

**Table 1.**
*Sex-wise distribution of reported cases (1982–2016).*

| Religion | Frequency | Percent (%) |
|---|---|---|
| Hindu | 3172 | 82.7 |
| Muslim | 392 | 10.2 |
| Christian | 247 | 6.4 |
| Sikh | 1 | 0.0 |
| Jain | 22 | 0.6 |
| Total | 3834 | 100 |

**Table 2.**
*Religion-wise distribution of reported cases 1982–2016.*

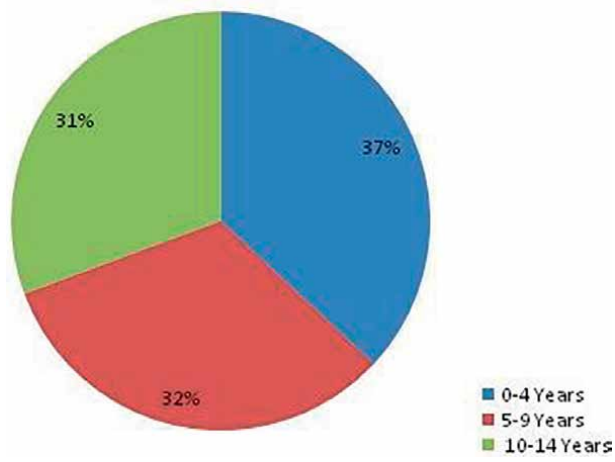


**Figure 2.**
*Distribution of reported cases across the age groups (share in %).*

**Table 3** describes the distribution of different types of pediatric cancer reported during this period. The trend indicates that lymphoid leukemia is the most common type of cancer reported (1002 cases, constituting 26.1% of 3834 cases). Non-Hodgkin's lymphoma, Myeloid Leukemia, Hodgkin's disease, Brain Tumor, Eye Cancer, and other type's cancers.

**Table 4** shows that the pattern of reported cases changes across the years. Types of major cancer reported between different periods show that more number of cases were reported during 2007–2011 (639 cases, 16.7%). Major types include Non-Hodgkin's lymphoma (39 cases, 6.1%), brain tumor (29 cases, 4.5%), rectum cancer

| Type of CA | Frequency | Percent (%) |
|---|---|---|
| Lymphoid Leukemia | 1002 | 26.1 |
| NHL | 297 | 7.7 |
| Myeloid Leukemia | 261 | 6.8 |
| Hodgkin's Disease | 204 | 5.3 |
| Brain Tumor | 139 | 3.6 |
| Eye Cancer | 120 | 3.1 |
| Other Cancer | 1124 | 29.3 |
| Bone Cancer | 95 | 2.5 |
| Rectum Cancer | 192 | 5.0 |
| Testis Cancer | 53 | 1.4 |
| Adrenal gland Cancer | 69 | 1.8 |
| Liver Cancer | 40 | 1.0 |
| Kidney Cancer | 140 | 3.7 |
| Leukemia unspecific | 46 | 1.2 |
| Multiple myeloma | 52 | 1.4 |
| Total | 3834 | 100 |

**Table 3.**
*Major types of cancer reported 1982–2016.*

(37 cases, 5.8%), kidney cancer (33cases, 5.2%), and other cancers (219cases, 34.3%). The other categories of cancer include cancers of the Nose, Pinna, fingers, nasopharyngeal cancers, etc.

**Figure 3** shows the distribution of reported cases with their types and gender. The trend indicates that most types of cancer reported remain high among the male children, except myeloid leukemia (7.1%), eye cancer (4%), bone cancer (2.7%), liver cancer (1.2%), kidney cancer (4.1%) and other types of cancers (33.9%).

**Table 5** shows the incidence of different types of pediatric cancer for different age groups. More pediatric cancers are reported in 0–4 years of age (1417 cases, 37%) out of 3834cases. Which include myeloid leukemia (14.7%), eye cancer (6.4%), adrenal gland cancer (3.8%), liver cancer (2.3%), and multiple myeloma (1.5%). Compared to this, more cases of lymphoid leukemia (29.1%), non-Hodgkin's lymphoma (9%), Hodgkin's disease (7%), brain tumor (5.2%), rectum cancer (5.1%), testis cancer (2.3%), kidney cancer (4.2%), and unspecific leukemia (1.3%) were reported in 5–9 years of age. The number of cases reported on Bone cancer (2.5%), and other cancer (29.3%) was found high among the children 10–14 years of age.

The overall incidence of pediatric cancer has gradually decreased in Chennai during the period 2012–2016, compared to the previous years. Leukemia emerges as the most common pediatric cancer as indicated by many studies (**Table 4**). The results broadly follow some of the existing studies like the highest incidence occurring between 0 and 4 years of age (**Table 2**) and non-Hodgkin's disease exceeds Hodgkin's disease (**Table 4**) as reported in India between 2012 and 2014 (Suman

| Types | 1982–1986 | 1987–1991 | 1992–1996 | 1997–2001 | 2002–2006 | 2007–2011 | 2012–2016 | Total |
|---|---|---|---|---|---|---|---|---|
| Lymphoid Leukemia | 92 (19.4) | 106 (21.7) | 173 (28.7) | 138 (25.5) | 209 (33.2) | 172 (26.9) | 112 (24.5) | 1002 (26.1) |
| NHL | 51 (10.7) | 43 (8.8) | 57 (9.5) | 48 (8.9) | 34 (5.4) | 39 (6.1) | 25 (5.5) | 297 (7.7) |
| Myeloid Leukemia | 44 (9.3) | 50 (10.2) | 34 (5.6) | 32 (5.9) | 41 (6.5) | 31 (4.9) | 29 (6.3) | 261 (6.8) |
| Hodgkin's D | 19 (4.0) | 38 (7.8) | 35 (5.8) | 35 (6.5) | 31 (4.9) | 26 (4.1) | 20 (4.4) | 204 (5.3) |
| Brain Tumor | 14 (2.9) | 30 (6.1) | 14 (2.3) | 13 (2.4) | 22 (3.5) | 29 (4.5) | 17 (3.7) | 139 (3.6) |
| Eye Cancer | 29 (6.1) | 18 (3.7) | 26 (4.3) | 39 (7.2) | 8 (1.3) | 0 (0.0) | 0 (0.0) | 120 (3.1) |
| Other CA | 147 (30.9) | 138 (28.3) | 165 (27.4) | 136 (25.1) | 164 (26.0) | 219 (34.3) | 155 (33.8) | 1124 (29.3) |
| Bone CA | 13 (2.7) | 16 (3.3) | 13 (2.2) | 13 (2.4) | 16 (2.5) | 10 (1.6) | 14 (3.1) | 95 (2.5) |
| Rectum CA | 24 (5.1) | 23 (4.7) | 31 (5.1) | 24 (4.4) | 28 (4.4) | 37 (5.8) | 25 (5.5) | 192 (5.0) |
| Testis CA | 11 (2.3) | 8 (1.6) | 12 (2.0) | 8 (1.5) | 9 (1.4) | 2 (0.3) | 3 (0.7) | 53 (1.4) |
| Adrenal gland CA | 3 (0.6) | 0 (0.0) | 0 (0.0) | 8 (1.5) | 18 (2.9) | 18 (2.8) | 22 (4.8) | 69 (1.8) |
| Liver CA | 3 (0.6) | 2 (0.4) | 11 (1.8) | 3 (0.6) | 8 (1.3) | 7 (1.1) | 6 (1.3) | 40 (1.0) |
| Kidney CA | 14 (2.9) | 10 (2.0) | 14 (2.3) | 22 (4.1) | 27 (4.3) | 33 (5.2) | 20 (4.4) | 140 (3.7) |
| Leukemia unspecific | 3 (0.6) | 4 (0.8) | 8 (1.3) | 14 (2.6) | 9 (1.4) | 5 (0.8) | 3 (0.7) | 46 (1.2) |
| Multiple myeloma | 8 (1.7) | 2 (0.4) | 9 (1.5) | 9 (1.7) | 6 (1.0) | 11 (1.7) | 7 (1.5) | 52 (1.4) |
| Total | 475 (100) | 488 (100) | 602 (100) | 542 (100) | 630 (100) | 639 (100) | 458 (100) | 3834 (100) |

**Table 4.**
*Major types of cancer: Trends across specific intervals (share in %).*

Das et. al) [17]. Similarly, overall cancer in children is more common among males than females (Stiller C 2007; [18] Gurney JG. et al. 2006) [19]. Existing studies report that both Hodgkin's and Non-Hodgkin's disease had the highest incidence among 10–14 years age group for both sexes (Suman Das.et al. 2017) whereas the present study finds that the Non-Hodgkin's disease and Hodgkin's disease had the highest incidence among 5–9 years of age group (**Table 5**). Our analysis also highlights that brain tumor had the highest incidence among 5–9 years of the age group for both sexes (**Table 5**). Eye and liver tumors had the highest incidence among the 0–4 years age group while bone and gastrointestinal tumors had the highest incidence among the 10–14 years age group for both sexes (**Table 5**).

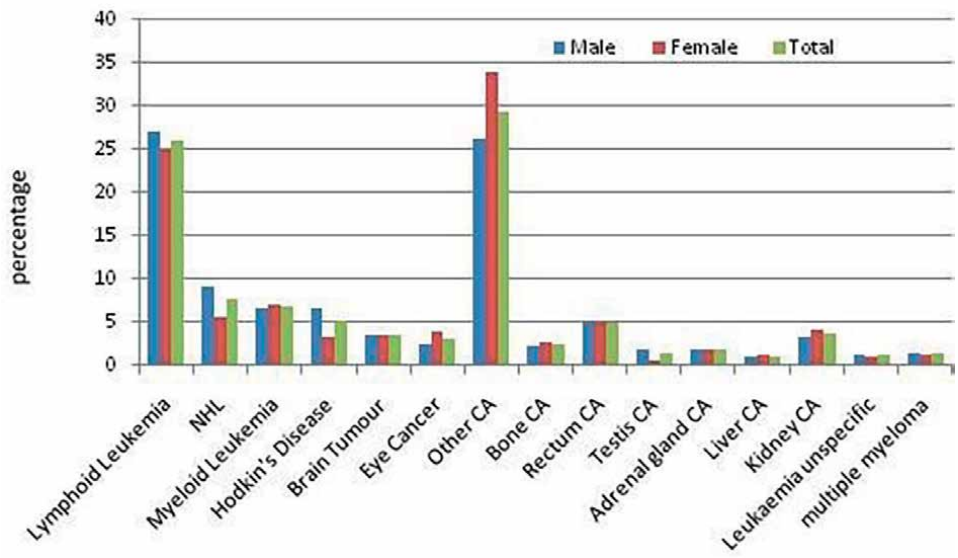


**Figure 3.**
*Sex-wise distributions of reported cases and types of cancer (share in %).*

| Type of CA | 00–04 Years | 05–09 Years | 10–14 Years |
|---|---|---|---|
| Lymphoid Leukemia | 28.9 | 29.1 | 26.1 |
| NHL | 4.7 | 9.0 | 7.7 |
| Myeloid Leukemia | 14.7 | 3.7 | 6.8 |
| Hodgkin's Disease | 1.7 | 7.0 | 5.3 |
| Brain Tumor | 2.5 | 5.2 | 3.6 |
| Eye Cancer | 6.4 | 2.0 | 3.1 |
| Other Cancer | 24.3 | 27.5 | 29.3 |
| Bone Cancer | .5 | 1.3 | 2.5 |
| Rectum Cancer | 3.9 | 5.1 | 5.0 |
| Testis Cancer | .6 | 2.3 | 1.4 |
| Adrenal gland Cancer | 3.8 | 0.8 | 1.8 |
| Liver Cancer | 2.3 | 0.5 | 1.0 |
| Kidney Cancer | 3.1 | 4.2 | 3.7 |
| Leukemia unspecific | 1.0 | 1.3 | 1.2 |
| Multiple myeloma | 1.5 | 1.0 | 1.4 |
| Total | 100 | 100 | 100 |

**Table 5.**
*Incidence of pediatric cancer across age group (share in %).*

## 4. Conclusion

The analysis covers three thousand eight hundred and thirty-four cases of pediatric cancer registered at Madras Metropolitan Tumor Registry from 1982 to 2016.

Overall, the results indicate a gradual decline in childhood cancer during this period and indicate that maximum cases are reported during 2007–2011. The results confirm some of the established patterns including a higher incidence of cancer among male children (60.3%), and a high incidence among the children in 0–4 yearsage group. Leukemia is the most common pediatric cancer and it constitutes 27 percent in males and 25 percent in females. Overall cancers are more reported in the Hindu community, while specific types like myeloid leukemia, NHL, brain tumor, and multiple myeloma are found high in the Jain community. Lymphoid leukemia and rectum ca are more common in the Muslim community.

The pediatric tumor showed wide variation concerning different age groups. The genetic and environmental factors played role in the etiology of pediatric cancer. Most pediatric cancer is curable if it has been detected early. Thus, the study offers some important insights and updates on the pediatric cancer trends in the city of Chennai and may serve as a reference source for clinicians and researchers on pediatric oncology and policymakers engaged in public health.

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Section 2

# Role of Immune Cells and Metabolism in Cancer

Chapter 4

# Perspective Chapter: Dendritic Cells in the Tumor Microenvironment

*Dan Jin, Laura Falceto Font and Catherine T. Flores*

## Abstract

Tumor infiltrating dendritic cells (DCs) play a critical role in initiating the process of anti-tumor immune responses. They can uptake tumor antigens either directly at the tumor site or from circulating antigens, and elicit T cell activation and adaptive immunity in secondary lymphoid organs. Subtypes of dendritic cells have various roles in immunity and tumor rejection. In this chapter, we will summarize the role of dendritic cell populations on mounting anti-tumor immunity. Conversely, we will discuss tumor-mediated dysfunction of dendritic cells that aid immune evasion including prevention of recruitment, impairment in antigen presenting and mediation of tolerance. At last, we briefly introduced the progress in DC vaccine applications in clinic.

**Keywords:** dendritic cell, tumor microenvironment, antigen presenting, T cell activation, DC tolerance, DC vaccine

## 1. Introduction

Dendritic cells (DC) are responsible for activating effector responses and mediating adaptive immunity. Immune responses are dependent on multiple factors including the DC type, maturation status, and immunogenicity of antigens. DCs have the capacity of inducing protective immunity as well as generating a tolerogenic immune environment. The complexity of how cancer impacts the spectrum of response varies depending on the cancer type, largely on the cancer immunophenotype. Here we discuss how different DC subtypes interact between cancer and adaptive immunity. We also touch on various cancer-mediated immune evasion strategies that alter DC function. Lastly we evaluate immunotherapeutic strategies that employ DCs to elicit anti-tumor T cell responses.

## 2. Anti-tumor roles of different dendritic cell sub-populations

DCs are composed of heterogenous sub-populations with each subtype possessing unique functions to compensate for each other. They cooperate to elicit both innate and adaptive immunity. In this section, we will generally review the roles of different DC sub-populations in anti-tumor immunity (**Figure 1**).

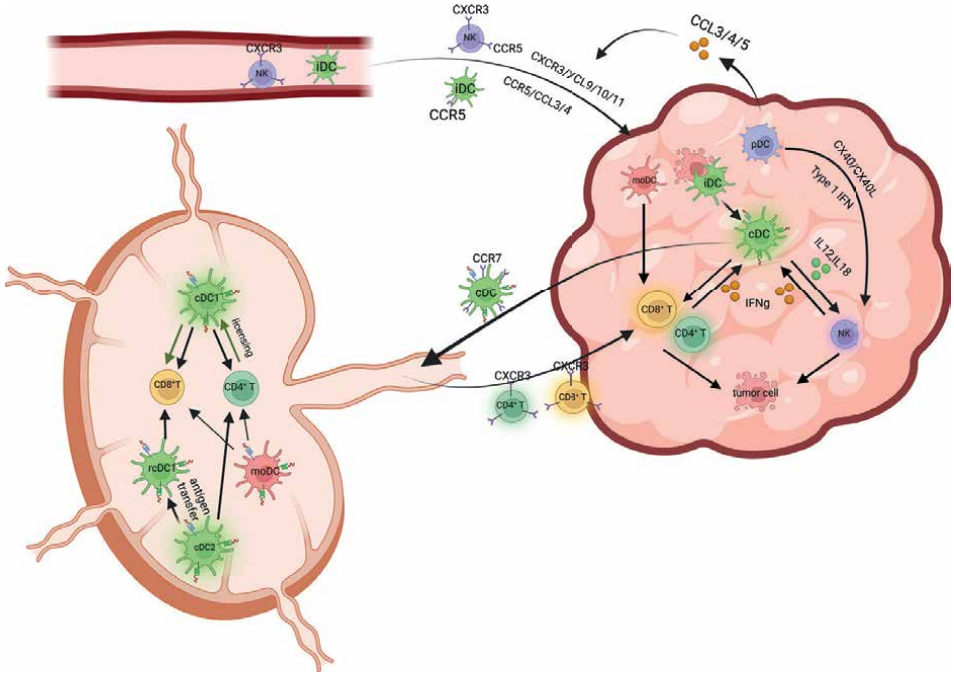


**Figure 1.**
*Roles of DC subpopulations in anti-tumor response. Immature DCs in peripheral tissue can be recruited into tumor site through CCR5/CCL3/4 chemoaxis. Both cDC1 and cDC2 uptake tumor antigens in situ and migrate to tdLN in a CCR7-dependent way, trafficking tumor antigens into tdLN. In tdLN, antigen-loaded cDC1 primes both naïve CD8 T cells and CD4 T cells. Primed CD4 T cells further boost CD8 T cell activation through licensing cDC1 in a CD40/CD40L dependent way. Antigen-loaded cDC2 predominantly primes naïve CD4 T cells. It can alternatively activate CD8 T cells through transferring antigens to LN resident cDC1(rcDC1), which primes naïve T cells in LN. Activated T cells migrate to tumor site in a CXCR3 dependent chemokine recruitment way. Effector T cells can be further stimulated by antigen activated cDCs and moDCs in tumor and exert cytotoxic tumor killing. moDCs exert CD4 and CD8 T priming function to compensate for cDCs when they are dysfunctional or depleted. Activated pDCs secrete CCL chemokines to recruit and activate NK cells through type I IFN and OX40-OX40L interaction. Activated NK cells promote cytotoxic CD8 T priming through activation of cDC in an IFNγ-dependent way, meanwhile, activated cDCs secrete cytokines like IL12, IL18 that enhance NK cell activation.*

## 2.1 Conventional DCs

Conventional DCs (cDCs) are derived from common DC precursors (CDP) in the bone marrow, which are comprised of two main subsets: cDC1 and cDC2. The infiltration of cDCs in the tumor has a positive correlation with patient survival in some solid cancers [1]. cDC1 development is specified by transcriptional factors BATF3, IRF8, and ID2, while cDC2 development depends on transcriptional factors RELB, IRF4, ZEB2 and KLF4 [2, 3]. BATF3 is required for maintaining IRF8 expression during cDC1 commitment in specified cDC1 progenitor [4]. BATF3 is also required for cDC1 cross-presentation function and cross-presentation independent anti-tumor immunity functions [5, 6]. BATF-dependent cDC1 is specified by its unique role to initiate naïve $CD8^+$ T cell activation in tumor-draining lymph nodes(tdLN), as well as enhance both T cell accumulation and local CD8 T cell cytotoxicity. The abundance of cDC1 in the tumor microenvironment positively correlates with cancer patient survival and response to immunotherapy across different cancer types [7, 8]. $CD103^+$ cDC1s sample tumor antigen in tumor mass and migrate to tumor-draining lymph nodes via CCR7, where they prime T cell responses [9]. Tumor-resident $BATF3^+$ cDC1s

secret CXCL9 and CXCL10 to recruit CXCR3 expressing effector T cells and NK cells [10, 11]. In turn, IFNγ produced by tumor effector T cells and NK cells induce CXCL9/10/11 production by myeloid cells, creating a feedback loop in this response [12]. cDCs also secrete IL-12, IL-18 and IL-2 provoking NK cells to produce IFNγ, TNFα, or GM-CSF, which further promotes DC activation [13].

Unlike cDC1s, cDC2s have limited capacity to cross-present tumor antigens to CD8 T cells. The function of cDC2s are largely restricted to priming of CD4 T cells in tdLN or in tumor [14–16]. cDC2s mediate cross-presentation of soluble antigens and is enhanced by TLR7 agonist [17, 18]. cDC2s complement the function of cDC1s by also activating CD8 T cells. Migratory cDC2 capture antigens in tumor and transfer antigens to LN resident cDC1s through antigen vesicles and synaptic transfer, which is capable of activating CD8 T cells [19]. In the absence of cDC1s, activating cDC2s by type I IFN can stimulate CD8 T activation in tumor [20]. In preclinical models where cDC1 function is impaired, deletion of cDC1 population improves cDC2 migration into tdLN and CD4 T activation [1].

### 2.2 Plasmacytoid dendritic cells

Plasmacytoid dendritic cells(pDC) are largely regarded as immunomodulating cells through secretion of massive amounts of type-I interferon during anti-virus immune responses. The role of pDC in anti-tumor immunity is controversial. pDC in tumors have been found to have impaired response to Toll-like receptor activation and decreased type-I IFN production. They recruit and expand immune regulatory T cells in the tumor microenvironment (TME) and are associated with poor prognosis [15, 21]. As an escape mechanism, tumor cells attract pDCs to induce an immunosuppressive environment through secreting chemokine CXCL12 [22].

On the contrary, in some solid tumors, pharmacological agents can be used to overcome immunosuppression. For example, imiquimod stimulation can induce pDC mediated tumor killing via secretion of TRAIL and granzyme B independent of adaptive immunity [23]. pDCs can also drive anti-tumor response by activating adaptive T cell immunity mediated by cDC activation dependent on type-I IFN [24]. Direct injection of TLR9 activated pDC into B16 melanoma tumor bearing mice induces robust cytotoxic T lymphocytes (CTL) cross-priming against tumor, leading to tumor regression. TLR9 activated pDCs produce large amounts of chemokines CCL3, CCL4, and CCL5 within the tumor, which recruits $CCR5^+$ NK cells. Recruited NK cells are activated by pDC through cell-to-cell interaction via OX40/OX40L and type I IFN secreted by pDC. Tumor cells lysated by NK cells cause tumor antigen release into cDCs and IFNg secreted by activated NK cells also help activate CTL in dLN [25]. Such an activated subset of pDC with higher levels of OX40 is also found in head and neck squamous cell carcinoma (HNSCC) tumor with distinct immunostimulatory and cytolytic function and can synergize with cDCs in generating tumor antigen-specific $CD8^+$ T cell responses [26].

### 2.3 Monocyte-derived dendritic cells

Monocyte-derived dendritic cells (moDCs) are differentiated from monocytes under inflammatory conditions. Activation of p53 in MDSCs and monocytic progenitors can induce moDC-like population differentiation in tumor, which potentiates the anti-tumor response [27]. Increase of moDC in tdLN can be a measurable indication of immune activation, particularly after treatment with pharmacological agents

such as TLR agonists [28]. moDCs in tumor are essential for CD8 T activation and antitumor response after local immunostimulatory agent treatment [29]. In mice with Zbtb46-DTR bone marrow chimeras, which are deficient in cDC production after diphtheria toxin (DT) treatment, moDC compensate for the loss of cDCs and account for intratumoral CTL expansion and function [30]. Compared with cDC, moDCs are less efficient at inducing CD4 T cell proliferation but more efficient at inducing Th1 and Th17 differentiation [31]. However, moDCs are also able to cross-present antigens through the vacuolar pathway and activate naïve CD4 T and CD8 T cells [32]. *In vitro* differentiated moDCs have been used in clinical trials as vaccines for cancer patients and encouraging responses have been shown when combined with other cancer therapies [33–37], which will be further discussed in the last section of this chapter.

## 3. Tumor-mediated immune evasion: impact on dendritic cells

### 3.1 DC tumor infiltration and migration to LN

cDCs in tumor are found to be sparse among tumor infiltrated immune populations [8, 38]. Increased cDC amount within tumor is associated with improved prognosis and response to check-point inhibitor immunotherapy [8, 39]. Tumor cells secret soluble factors that suppress DCs infiltrating to tumor site and migrating to LN (**Figure 2**).

Tumor cells suppress chemokine CCL4 production through activating beta-catenin signaling, and beta-catenin activation induces ATF3 expression. ATF3 binds the promoter of CCL4 gene and suppresses CCL4 expression. Decreased CCL4 leads to decreased intratumoral cDC recruitment by CCL4/CCR5 axis [40]. beta-catenin signaling suppresses CCL5 level in tumor loci. CCL5/CCR5 chemoaxis recruits cDC1 into tumor. Increased CCL5 expression in tumor recruits cDC tumoral infiltration and promotes anti-tumor immune response and promotes efficacy when combined with anti-PD1 [41]. Prostaglandin E2 (PGE2), a prostanoid lipid catalyzed by enzyme cyclooxygenase (COX), is highly produced in tumor [42–44]. cDC1s are absent from

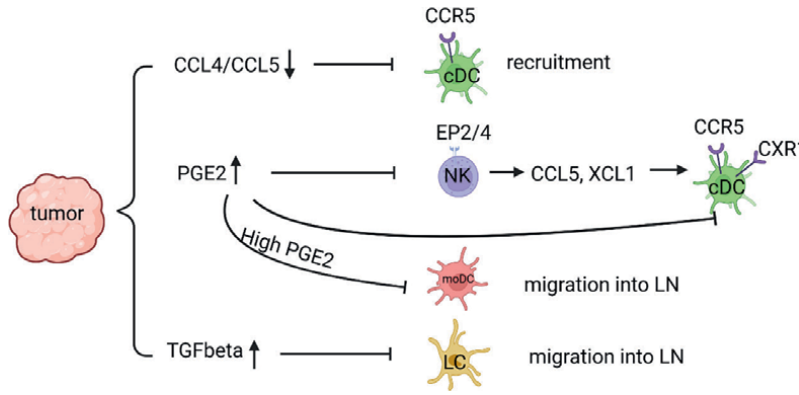


**Figure 2.**
*Impact of DC recruitment and migration by tumor. Tumor cells suppress CCL4/5 expression by inducing β-catenin signaling pathway, which inhibits cDC recruitment via CCL4/5/CCR5 chemoaxis. PGE2 produced from tumor directly acts on intratumoral NK cells through EP2/4 receptors. PGE2 inhibits CCL5 and XCL1 secretion by NK cells, which results in decreased cDC recruitment. PGE2 can also inhibit CCR5 and CXR1 expression on cDC to impair cDC recruitment. High concentration of PGE2 inhibits moDC migration into dLN. TGFβ from tumor inhibits LC migration into LN.*

PGE2 producing tumor. PGE2 suppresses NK cells mediated cDC1 recruitment in tumor. Intratumoral NK cells secret cDC1 chemoattractant CCL5, XCL1, which are inhibited by tumor derived PGE2 through PGE2 receptor EP2, EP4 on NK cells. However, expressing CCL5, or XCL1 in tumor is insufficient to reverse intratumoral DC exclusion in PGE2 producing tumor. Further study shows that PGE2 can also downregulate CXR1 and CCR5 expression in DC, which leads to impairment of response to chemokine even in the existence of chemoattractant [21, 44, 45].

DC cells uptake antigens in tumor site and then migrate to dLN for priming T cells. TGFβ secreted from tumor inhibits DC migration to dLN in both autocrine and paracrine way. Langerhans cells (LCs), skin-resident DCs, play critical role in eliciting immune response in skin disease. Besides of tumor cells, LCs are also active TGFβ producer. Knock-out of TGFβ or its receptor in LCs induces mass migration of LCs to regional LN in both steady and inflammation states [46]. In skin tumor model, TGFβ inhibits tumor infiltration and migration to skin-dLN by LCs [47]. Role of PGE2 in regulating DC migration relies on its concentration. High concentration of PGE2 suppresses DC migration while it has also been shown as a positive regulator of CCR7 expression and migration of moDCs [48, 49].

### 3.2 Antigen presentation

Tumor cells develop mechanisms to impair antigen capture and presenting by DCs (**Figure 3**). Molecules released or exposed from dying cancer cells can act as danger signals to circulating DCs. DCs recognize dying/dead tumor cells or tumor derived debris through danger-associated molecular patterns (DAMPs) mediated by pattern recognition receptors (PRRs) like TLRs, and phagocyze dying-tumor cells or tumor derived debris [50]. DAMPs include ATP, heat shock proteins (HSPs), HMGB1, calreticulin, annexin A1, dsDNA, but are not limited to these [50]. Antigen uptake will stimulate DC maturation and migration to dLN. Internalized antigens will be processed and presented on the DC surface by MHC-I and MHC-II molecules. MHC-I molecules used to be thought for intracellular peptide presenting, while MHC-II is for exogenous peptide. However, this is not always the case. Cross-presenting is termed for presenting exogenous antigens by MHC-I, which plays a critical role in eliciting anti-tumor immune response by DCs [51]. Proteins internalized by DCs are degraded in phagosomes into peptides [52]. Peptides are then translocated into endoplasmic reticulum (ER) by transporter associated with antigen presentation (TAP). The MHC-I heterodimer is assembled in ER from a polymorphic heavy chain and a light chain β2-microglobulin (β2m) and stabilized by chaperone proteins like calreticulin and tapasin when peptide is not loaded. Chaperones will be exchanged when peptide is loaded. Peptides fit into the MHC-I peptide binding groove which stabilizes the peptide-MHC-I complex. MHC-II comprises transmembrane α- and β-chains and an invariant chain. MHC-II will be transported to a MHC-II compartment, an endosomal compartment, for invariant chain digestion, resulting a class II-associated lipid peptide (CLIP). With the help of H2-DM/HLA-DM, CLIP is exchanged with antigen peptide [53]. In LN, DCs present tumor antigens to CD8 T cells and CD4 T cells dependent on MHC-I and MHC-II respectively. Tumor proteins will be processed into immunogenic peptides and loaded on MHC-I or MHC-II molecules on cell surface.

Tumor cells evolved multiple immune escape strategies to prevent recognition by DCs. For example, tumor-derived stanniocalcin 1(STC1) interacts with DAMP signal, calreticulin (CRT), to prevent CRT membrane from exposing to APCs, thereby abrogating membrane CRT-directed phagocytosis by DCs. High expression of STC1

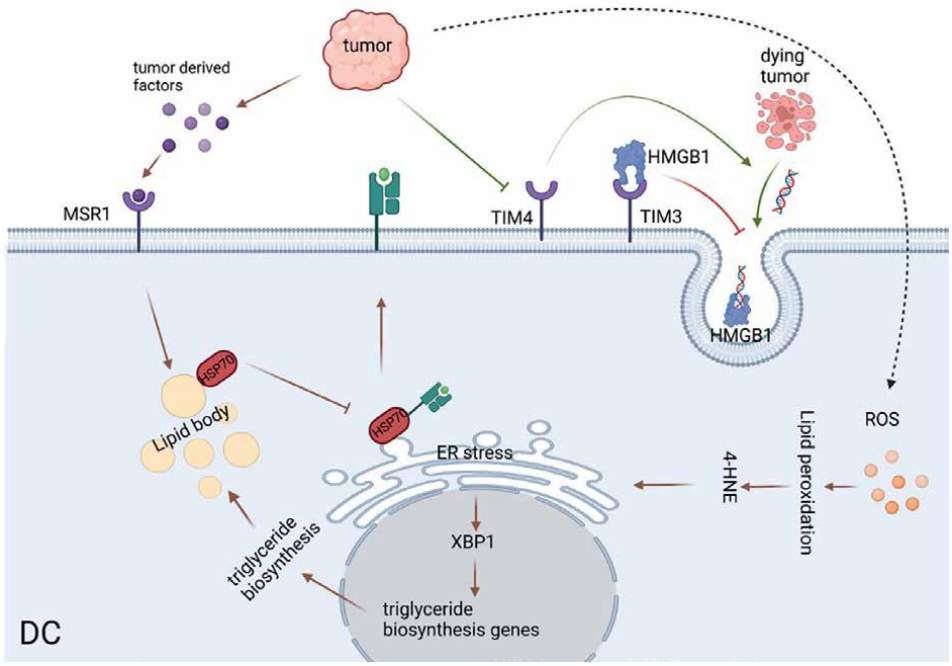


**Figure 3.**
*Tumor induced impairment of DC on tumor antigen capture and presentation. Tumor derived factors mediate lipid body accumulation in DC by inducing MSR1 expression. ROS, induced by tumor microenvironment, leads to increase lipid peroxidation in DC. Byproduct of lipid peroxidation, 4-HNE, induces ER stress, which activates XBP1 transcription factor. XBP1 then increases genes synthesizing triglyceride. Increased triglyceride leads to increased oxidized lipid bodies. The lipid bodies competitively bind HSP70 with pMHC, preventing pMHC been translocated onto cell surface. TIM3 competitively binds HMGB1 with dying tumor derive DNA, inhibiting DNA stimulated immune response via preventing DNA been internalized into DC. Tumor inhibits TIM4 expression on DC, thus inhibiting TIM4 mediated tumor associated antigen capture.*

in tumor is significantly correlated with poor responses to immunotherapy in patients [54]. In another immune escape mechanism, the mevalonate (MVA) pathway, which is highly activated in tumor cells, reduces F-actin exposure, a DAMP signal, on tumor cells, evading recognition mediated by Clec9A on cDC1. The MVA increases protein geranylgeranylation on Rac1, a small GTPase controlling actin cytoskeleton, resulting reduced F-actin in tumor [55–57]. The immune modulator TIM-3 is also highly expressed by DCs and has been shown to play an inhibitory role on DC activation. TIM-3 inhibits tumor derived DNA uptake by cDC1 through inhibiting endocytosis. HMGB1, a ligand of TIM-3, also acts as a DAMPS signal and binds tumor-derived DNA and is taken up by DCs. TIM-3 inhibits this process through sequestering HMGB1 bound DNA on cell surface [58]. TIM4, another T- cell immunoglobulin and mucin domain gene as TIM3, is also expressed on APCs like macrophages and dendritic cells. DAMP signal induces TIM4 expression on intratumoral macrophages and DCs [59]. Though TIM4 on tumor associated macrophage (TAM) has been shown impedes tumor antigen presentation through activating autophagy in TAM upon tumor antigen uptake in mouse melanoma model [59], TIM4 on lung resident cDCs in lung adenocarcinoma model shows a positive role in promoting anti-tumor immune activation [60]. TIM4 expression is downregulated in cDC1 from advanced lung tumor. Blocking TIM4 or knocking out of TIM4 abolishes tumor antigen uptake by lung resident cDC1 and impairs antigen presenting to CD8 T cells in vitro and in vivo.

DCs from tumor-bearing host have been found with accumulated lipids. Tumor derived factors induce oxidized lipid accumulation in cDCs from tumor bearing host. DCs have high oxidized lipids shows impaired cross-presentation while not affecting presenting endogenous antigens, and nor affecting the level of MHC-I. Scavenger receptor, MSR1, induced by tumor derived factors, accounts for the lipid accumulation in DC [61, 62]. ER stress signaling also involved in oxidized lipid accumulation in DC from tumor bearing host. 4-HNE is a byproduct from lipid peroxidation mediated by ROS and triggers ER stress and XBP1 activation. XBP1, a multitask transcription factor in response to ER stress, induces triglyceride biosynthesis. Elevated triglyceride biosynthesis leads to accumulated abnormal lipids and suppresses DC function [63]. HSP70 is a chaperon protein that binds with pMHC, and facilitates pMHC trafficking onto cell surface. Oxidative lipid bodies, not non-oxidized lipid bodies, competitively bind HSP70 covalently, preclude HSP70 interaction with pMHC, thus affect pMHC trafficking to cell surface [64].

### 3.3 Tolerance

Tumor cells have evolved different mechanisms to promote DC tolerance to facilitate immune escape (**Figure 4**). Tolerized DCs experience higher co-inhibitory markers, including PD-L1, PD-L2 and higher arginase activity, and lower MHC-II and co-stimulation markers, including CD80, CD86, CD40 [39, 65]. Tolerized DCs are not capable of activating T cells, while promote immune suppression through mechanisms like, for example, Treg upregulation.

#### *3.3.1 Secreted factors from tumor environment*

Secreted tumor-derived factors is one of the major ways of driving DC tolerance, these include PGE2 and TGFβ which lead to subsequent induction of other immune modifiers. Tumor derived PGE2 suppresses cDCs activation by suppressing co-stimulation, IL-12 production, and increasing PD-L1 and Arg1 [44, 66]. PGE2 is the main inducer of arginase-1 during tumor induced DC tolerization [67]. TGFβ from

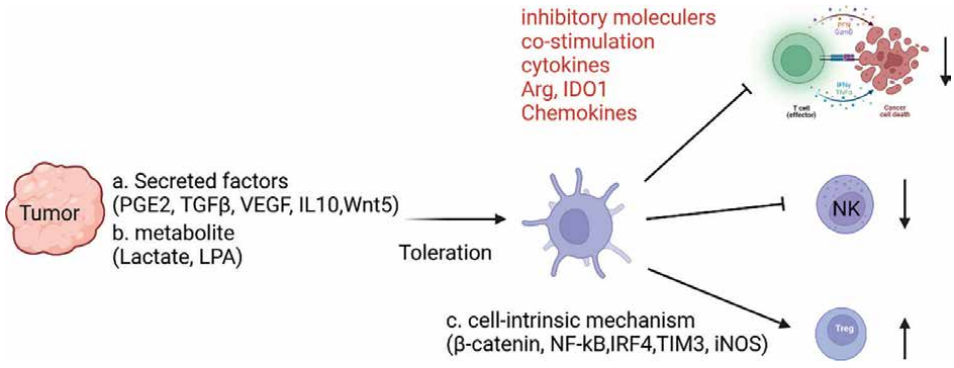


**Figure 4.**
*DC tolerance mechanism. Secreted factors or metabolites derived from tumor environment induce dendritic cell tolerance through activating or inhibiting cell-intrinsic signaling pathways in DC. DC tolerance leads to induced inhibitory molecular expression, Arg and IDO1 upregulation, anti-inflammation cytokine production and suppress co-stimulation and pro-inflammation cytokine production. And also result into dysregulated chemokine secretion. DC tolerance abolishes anti-tumor immune response through inhibiting T cell and NK recruitment and mediated tumor killing, and promoting immunosuppressive Treg differentiation and recruitment.*

tumor also induces DC tolerization [67, 68]. TGFβ induces IDO expression in pDC and enhances expression of CCL22 by myeloid DCs in tumor. IDO suppresses effector T cell activity and promotes Treg differentiation and activation. DC-derived CCL22 chemokine promotes CCR4-dependent recruitment of Tregs to the tumor microenvironment [69]. pDCs in tumor environments are associated with poor survival. Co-culture with TGFβ containing medium inhibits pDC activation and type I IFN secretion. Tolerized pDCs promote tumor growth through inhibiting NK cell infiltration and recruitment of Treg cells [70]. DCs with high TGFβ expression are poor at eliciting the activation of naive CD4 T cells and sustaining their proliferation and differentiation into Th1 effectors. Vascular endothelial growth factor (VEGF) inhibits LPS induced DC maturation via Nrp-1 receptor on DC. NRP1 interacted with LPS receptor TLR4 and suppressed downstream ERK and NF-κβ signaling, resulting in increased expression of MHC-II and costimulatory molecules (CD40, CD86) as well as proinflammatory cytokine production inhibition [71]. Infiltrating macrophages were the primary source of IL-10 within tumors, blocking IL-10 signaling increases intratumoral dendritic cell expression of IL-12 during chemotherapy in breast cancer [65].

LPA is a bioactive lipid produced by tumor cells. Blocking LPA-generating enzyme autotaxin in ovarian cancer cells elicits anti-tumor immune response driven by type-I IFN. LPA induces PGE2 synthesis by DCs, which suppressed type-I IFN production and response in DC via autocrine EP4 engagement [72]. Lactate is an oncometabolite resulted from metabolic adaption in cancer cells via Warburg effect. Lactate in tumor attenuates pDC activation in response to TLR9 ligand and consequent type I IFN induction. pDC tolerization by lactate is partially through activating GPR81, a cell surface G-protein coupled receptor of lactate. GPR81 activation induces intracellular $Ca2^{+}$ mobilization and activates calcineurin phosphatase (CALN) expression. Inhibition of CALN reverses the inhibitory effect by lactate. Extracellular lactate can also influence pDCs through intracellular import via the monocarboxylate transporters (MCT). Inhibition of MCT genes resulte in significant reversal of the lactate-mediated inhibition of IFNα. Thus, both GPR81 triggering and cytosolic import via the MCT transporters mechanism are involved in lactate induced pDC tolerization. Lactate treated pDCs have enhanced tryptonphan metabolism, leading to excessive production of kynurenines which in turn induces Treg cell differentiation [73].

### *3.3.2 Cell-intrinsic mechanism*

Tumor-derived Wnt5a induces β-catenin signaling activation-dependent IDO expression in DCs. DCs conditioned by wnt5a promote Treg development and suppresses effector T cell activation [74, 75]. β-Catenin complexed with PPAR-γ upon wnt5a stimulation and transcriptionally activates fatty acid oxidation (FAO) synthesis gene, CPT1a, inducing the synthesis of heme prosthetic group, protoporphyrin IX, which is required for IDO enzymatic activity [75]. Wnt1/β-catenin signaling in DC suppresses chemokine production, leading to T cell exclusion in tumor and decreased T cell activation [76]. β-catenin signaling in DC also impairs CD8 T priming through inducing IL-10 secretion via mTOR activation. Even though the negative regulation of initial CD8 T priming by β-catenin/mTOR/IL10 in DC, β-catenin–regulated IL-10 also shown has an opposite anti-tumor immunity role through maintenance of primed CD8 T cells after clonal expansion [77, 78]. β-catenin can also interact with TCF4 and activates gene expression of Aldh1, an enzyme to produce retinoic

acid (RA) from vitamin A, resulting increased RA in DC [79]. Aldh1 expression in mature DC significantly correlated with immunoregulatory module including genes like PD-L1, PD-L2, CD83, and CCL22 [80]. RA induces Treg generation in vitro and in vivo [81–83]. DC maturation suppression could be mediated by E-cadherin based DC-DC adhesion. Disrupting this contact activates DC maturation through activating β-catenin/TCF, leading to increase of co-stimulatory molecules, MHC-II and chemokine receptors. However, such DC maturation is not coupled with proinflammation cytokine secretion and failed to prime CD4 T cells, coupled with a distinct transcriptional profile from those induced by TLR activation. DC matured by E-cadherin disruption also leads to Treg production. The data suggests a DC function regulatory role of E-cadherin/β-catenin/TCF axis [84].

Nuclear factor-κB (NF-κB) is an important transcription factor that participating in cancer inflammation. There are two general types of NF-κB signaling pathways: canonical and non-canonical pathways [85]. Canonical and non-canonical NF-κB pathways play different roles in DC functional regulation. Lung cancer patient derived tumor sera induce canonical NF-κB pathway inhibition, while activates non-canonical NF-kB pathway in human mo-DC [86]. IFNγ has been shown important for myeloid activation [87]. Canonical NF-κb/IRF1 mediated IFNγ response pathway is required for intra-tumoral cDC1 activation. IFNγ knock out or IFNGR1 knock out in cDC1 abolished IL12 production [88]. Impaired NF-κB or IRF1 loses control of tumor growth and expression of maturation markers and chemokines (CXCL9/10) for recruiting T cells [89]. Inhibiting NF-κB in BMDC has no effect on MHC-II or co-stimulation molecules, while promotes Treg differentiation in vitro [80]. VEGF mediated inhibition on LPS stimulated BMDC activation is dependent on the inhibition of canonical NF-κB signaling pathway [66]. Noncanonical NF-kB signaling in dendritic cells is required for IDO induction in the late stage of DC activation by CD40 ligation [90].

Inhibitory molecular expression on DC suppresses T cell activation and induces Treg differentiation. PD-L1 upregulation in tolerized DC is not dependent on the presence of type I and type II IFN signaling, nor is dependent on inflammasome or TRIF/MyD88 signaling. Instead, PD-L1 upregulation is dependent on phagocytic cell-surface receptor AXL activation upon antigen uptake. IL-4 signaling negatively regulates IL-12 production on DC. Blocking IL-4 signaling can increase IL-12 production without upregulating PD-L1 [88]. IRF4 plays a dural role of upregulating antigen presenting capability and tolerization of BMDC. Depletion of IRF4 reduces Aldh1 and PD-L2 expression, coupled with elevated cytokine IL-12 an TNF expression. IRF4-deficient DC is impaired for Treg generation in vivo. TIM-3 is predominantly found expressed in cDC cells in tumor. TIM-3 expression on DC can be induced by IL-10 or VEGF [91]. Blocking TIM-3 improve survival when combined with chemotherapy. The regulatory effect by TIM-3 blocking is neither through affecting cDC infiltration nor through regulating cDC activation. However, TIM-3 blocking increases CXCL9 secretion by cDC1, which is a ligand for CXCR3. CXCL9/CXCR3 chemoaxis attracts T cells into tumor [92]. TIM-3 on DC impaires DC recognition and response to tumor derived nucleic acids. TIM-3 serves as a receptor for DNA sensor, HMGB1, completing with nucleic acids for binding to th A-box domain of HMGB1. The binding of TIM-3 on HMGB1 inhibits nucleic acids to be internalized into endosomes [87].

DC activation is accompanied by an increased glycolysis metabolic process, which is required by both survival and effector function of activated DC. Bioactive gas nitric oxide (NO) is synthesized and secreted by activated DC, playing an

immunomodulating role of DC. Cellular production of NO is catalyzed by NOS enzymes, which converts substrates L-arginine, NADPH and O2 to L-citrulline, NADP+, and NO [93]. Inducible NOS (iNOS) is the primary NO-synthesizing enzyme expressed by DC. iNOS expression in $CD103^{-}CD11b^{+}$ intratumoral DC is required for tumor suppressive Th17 T cell differentiation in PDA model [94]. Glucose could inhibit DC function through mTOR/HIF1a/iNOS signaling axis, inhibiting co-stimulation molecular expression and IL12 secretion and restricting T cell activation. When T cells encounter DCs, they compete for glucose availability, which suppress the glucose sensitive pathway resulting T cell activation [95]. Monocyte-derived tumor associated DCs are prominent in tumor antigen uptake, but lack of strong T-cell stimulatory capacity due to NO-mediated immunosuppression [96].

## 4. Application of DC vaccine in tumor immunotherapy

### 4.1 DC vaccination

DCs are the most efficient professional antigen-presenting cells that can initiate an adaptive immune response by presenting antigens to T cells [97, 98]. In the past 25 years, many groups have exploited this characteristic to create dendritic cell vaccines to direct the immune system to fight cancer. DC cell-based vaccine approaches have been proved safe for their minimal toxicity, and their low association rates with autoimmunity [99, 100]. The general process of DC vaccine preparation including DC generation, antigen loading and DC maturation. To date, different strategies have been developed to generate DC vaccine for clinical applications.

The most commonly used approach to generate DCs is through ex-vivo differentiation from peripheral blood. The advantage of this method is the easy generation of sufficient autologous DCs for vaccination. However, therapeutic outcomes still have a lot of room for improvement, with less than 15% the patients showing objective response [101]. Due to the artificial *in vitro* differentiation process, moDCs have compromised functionality compared with naturally-occurring DCs with different transcriptional profiles. The limitation of using naturally-occurring DCs is the low frequency of DCs in peripheral blood, resulting in a highly labor-intensive process in DC isolation for clinical use. To overcome this, a growing effort in the field has been exerted to facilitate the developing a feasible protocol, for example, an automatic system that can prepare DCs [102]. For DC vaccine production, DCs are then be loaded with total tumor lysate or RNAs and tumor associated antigens. The loading methods include pulsing by co-culturing, electroporation, viral transduction or DC-tumor fusion [103]. Maturation cocktails used in the clinic consist of TLR agonists and cytokines, often in combination with co-stimulatory proteins like CD40L. Introducing mRNAs coding constitutively-active TLR4, CD40L and CD70 via electroporation has shown clinical success [33, 104].

### 4.2 DC vaccination clinical trial in glioblastoma

DC vaccination in the context of glioblastoma has shown both positive and negative results in clinical trials. Even though a phase III clinical trial aiming to assess DC vaccine targeting the EGFR deletion mutation EGFRvIII in newly diagnosed EGFRvIII-expressing GBM patients failed [105], some other clinical trials have shown promising results. Another phase III clinical trial utilizing an autologous

tumor lysate-pulsed dendritic cell vaccine combined with standard therapy showed significant overall survival benefit from 15 to 17 months to 23.1 months [36]. In another phase II clinical trial, ICT-107 (autologous dendritic cells (DC) pulsed with six synthetic peptide epitopes targeting GBM tumor/stem cell-associated antigens MAGE-1, HER-2, AIM-2, TRP-2, gp100, and IL13Rα2 was given to newly diagnosed glioblastoma patients in addition to standard therapy. Results showed progression free survival (PFS) increased 2.2 months in ICT-107 cohort compared with matched DC control cohort. HLA-A2 subgroup patients achieved a meaningful therapeutic benefit with ICT-107, in both the MGMT methylated and unmethylated prespecified subgroups, whereas only HLA-A1 methylated patients had an OS benefit [106, 107]. Combination with other intervention methods could help increase DC vaccine efficacy. Pre-conditioning at vaccinated site can improve DC vaccination efficacy. Mitchell et al. (2015) showed that glioblastoma patients pre-exposed to tetanus/diphtheria (Td) toxoid in the vaccine site before vaccination with pp65 RNA-pulsed DCs had improved tumor-antigen-specific DC migration and improved survival compared to the ones that were not pre-exposed to the toxoid through increasing DC migration to dLN [108]. Three phase II clinical trials (ATTAC; ELEVATE; NCT00639639, NCT00639639, NCT02366728) aim to test pp65 DC with Td vaccine in newly diagnosed GBM patients. Results to date have shown that despite a small cohort, three successive trials demonstrate consistent survival outcomes, supporting the efficacy of *cytomegalovirus* DC vaccine therapy in GBM [109].

## 5. Conclusions

Dendritic cells, as the most professional APCs, play key roles in mediating the bridge between innate and adaptive immunity in anti-tumor immunity. DC subpopulations, through use of different action mechanisms in activating adaptive immunity, collaborate with each other to elicit anti-tumor immunity. In the battle with tumor, DC functions become regulated by tumor cells or other components in the tumor microenvironment, leading to DC dysfunction. These include impairments on antigen uptake, antigen presentation, migration to LN, and DC tolerance. Secreted factors from the tumor environment play a key role in mediating DC regulation. These suppressive signals act on DCs inducing DC dysfunction through different cellular intrinsic pathways. DC vaccine development for tumor treatment has made significant progress in the last decades, but still faces challenges in achieving a wide and significant therapeutic success. Deepening our understanding on DC function and regulation in the tumor environment will help the field in developing new and more powerful therapeutic intervention approaches.

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## Conflict of interest

CF is a founder of iOncologi.

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Chapter 5

# Perspective Chapter: Impact of Tumor Metabolism on Immune Cells in the Tumor Microenvironment

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## Abstract

Metabolism is essential for a cell to obtain energy for its growth and development. In tumors, the rapid rate of cell proliferation leads to an increased demand for energy. Because nutrients in the tumor microenvironment are scarce, there is great competition between tumor cells and healthy cells to obtain them. Because of this, tumor cells undergo adaptations to outcompete healthy cells for nutrients. These adaptations cause characteristic changes to the tumor microenvironment, which in turn, causes changes to immune cells in the tumor tissue. These changes help the tumor evade immune detection and cause tumor growth and metastasis. This review will analyze the changes that take place in the tumor microenvironment, the impact they have on immune cells, and how this contributes to cancer progression.

**Keywords:** metabolism, nutrients, tumor microenvironment, immune cells, immune detection, cancer progression

## 1. Introduction

Metabolic reactions are chemical reactions that take place within cells or organisms and are essential for their survival. Metabolic processes include the breakdown of compounds for energy, the synthesis of necessary biomolecules, etc. Changes to the metabolic processes of cancer cells are a key characteristic of tumorigenesis. In order to supply their rapid rates of cell proliferation, tumor cells are in constant need of nutrients from the tumor microenvironment (TME), which are very scarce. This puts tumor cells in fierce competition with neighboring cells for these resources. Tumor cells undergo various adaptations, such as utilizing anaerobic glycolysis in favor of aerobic respiration, a process that allows them to synthesize ATP at higher rates. Such adaptations allow tumor cells to outcompete neighboring cells and allow the tumor to grow. The adaptations that the tumor cells undergo have an influence on the TME. For example, the aforementioned use of anaerobic respiration causes the TME to become more hypoxic and acidic.

These changes to the characteristics of the TME cause phenotypic alterations of immune cells within the TME. The TME includes cells of both the adaptive and innate immune systems, and they undergo notable changes to their metabolic pathways in response to the conditions of the TME or other signals within it. The former includes T cells and B cells, while the latter consists of tumor-associated macrophages (TAMs), natural killer (NK) cells, dendritic cells, and neutrophils.

These alterations of immune cells in the TME provide numerous benefits to the tumor. Namely, various altered pathways allow for the tumor to evade detection by the immune system, which contributes to the growth of tumors and the progression of cancer. This paper will discuss how the metabolic reprogramming of tumor cells contributes to changes in the conditions of the TME, the impact these changes have on the functionality of immune cells, and how they relate to the spread of cancer.

## 2. Changes to conditions of the TME

Tumor growth relies on the rapid proliferation of cells, which is an energetically demanding process. However, nutrients within the TME are often very scarce, and as a result, tumor cells are in fierce competition with healthy cells in the TME for these nutrients. Tumor cells adapt to these increased energy demands by shifting their metabolic pathways [1]. One such adaptation that tumor cells undergo is reprogramming of their glucose metabolism to utilize anaerobic glycolysis in preference to the tricarboxylic (TCA) and oxidative phosphorylation (OXPHOS) pathways ([2], **Figure 1**). This pathway, known as the Warburg effect, is active even in the presence of abundant oxygen, and it is key to a tumor cell's ability to outcompete neighboring cells.

Though the process of anaerobic glycolysis generates lower quantities of net ATP from glucose than the OXPHOS pathway, it allows for the metabolism of glucose to occur much more rapidly in tumor cells, thus leading to tumor cells outcompeting neighboring ones for nutrients. Additionally, other adaptive mechanisms of tumor

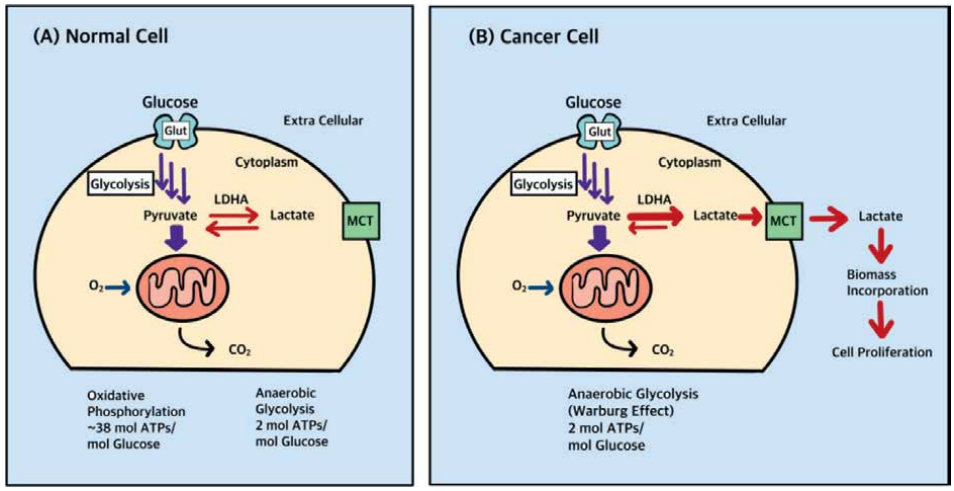


**Figure 1.**
*(Warburg effect): The Warburg effect is a major metabolic reprogramming that cancer cells undergo. Normal cells exhibit a usage of both glycolytic and OXPHOS pathways, while cancer cells rely on glycolysis and produce excess lactate as a by-product. This reliance on glycolysis and production of molecules, such as lactate, cause major changes to the conditions of the TME [3].*

cells allow them to overcome this inefficient method of obtaining energy. For example, many tumor cells can carry out autophagy, which allows them to recycle nutrients and prevents nutrient depletion [4]. Additionally, tumor cells can synthesize ATP using two ADP molecules, forming one ATP and one AMP [5, 6]. These adaptations make the Warburg effect a useful mechanism through which tumor cells can outcompete other cells within the TME for nutrients and proliferate. However, the process also causes drastic changes to the conditions of the TME.

The primary change caused by the Warburg effect is the acidification of the TME. These conditions are caused by the higher rates of anaerobic glycolysis and the production of lactic acid [7]. The acidic state of the TME confers numerous advantages for tumor growth, as it promotes the formation of new blood vessels, drug resistance, and suppression of the anticancer immune system [8]. The lactic acid that is produced can also act as a signaling molecule that regulates the migration of tumor cells: areas with a lower pH promote tumor cell invasion and metastasis [8].

Another important characteristic of the TME is its state of hypoxia. The delivery of oxygen and other nutrients to tissue occurs through blood vessels. Because tumors are undergoing constant growth, their receiving of blood flow is often irregular. In order to combat this, tumor tissues can form new blood vessels in a process referred to as angiogenesis [9]. This process allows tumors to continually receive the nutrients required to meet their metabolic demands. However, if angiogenesis fails, the aforementioned conditions of hypoxia and resource scarcity will arise in the TME. The tumor can still thrive under these conditions due to its reliance on anaerobic glycolysis [7]. Additionally, the hypoxic state acts as an additional stressor on the immune system and allows the tumor to evade immune attack.

The conditions in the TME also cause changes to the functionality of the immune system. For example, the hypoxic environment can negatively impact the immune detection of cancer cells and contributes to tumor immunity [10]. Signaling factors, called hypoxia-inducible factors (HIFs), are a key part of the regulation of tumor immunity genes. These factors can also inactivate lymphocytes in the TME, namely NK cells and CD8 T lymphocytes, thus preventing them from combating tumor growth. In this pathway, proinflammatory signals produced in hypoxic regions of the TME attract regulatory T cells (Tregs), which in turn suppress cytotoxic T cells from producing an immune response, thus promoting cancer growth [11]. The hypoxic conditions also act as a stressor on neutrophils and block them from attacking tumors. Finally, HIFs have negative impacts on the maturation of B cells, which they accomplish by increasing their rate of glycolysis. This metabolic change to B cells causes them to divide less rapidly (thus decreasing their immune response), prevents them from altering antibody production, and can even trigger cell death [10].

Additionally, the aerobic glycolysis pathway causes irregularities in the metabolite balance within tumor cells, a factor that causes changes to cell signaling and cell–cell interactions within the TME [12]. For example, the aforementioned acidic conditions of the TME created by the excessive lactate produced through glycolytic pathways interfere with the immune response of cytotoxic T cells. The lactic acid also interferes with the production of IFN-γ by NK cells, which inhibits phagocytic cells from attacking the tumor [13].

Amino acids, namely glutamine, arginine, and tryptophan, are also important metabolites that influence the function of immune cells within the TME. Glutamine is produced as a by-product of the catabolism of proteins in nutrient-scarce environments [13]. It is essential to the function of immune cells because it regulates immune cell activation and determination, namely that of T cells. When its availability is

limited, T-cell functionality is suppressed [13]. Similar to glutamine, arginine plays a role in the activation of T cells and NK cells. Additionally, it regulates the secretion of cytokines [13]. Tumor cells consume a significant amount of the exogenous arginine in the TME, thus inhibiting the effect it has on immune cells [13]. Tryptophan also plays a role in the regulation of T cells, namely its cell cycle. When tryptophan is unavailable, the rate of T-cell apoptosis increases drastically [13].

Finally, lipids play an important role in the regulation of immune cell signaling within the TME. Fatty acids are needed for macrophage maturation and proliferation [13]. Additionally, they are necessary for the synthesis of membranes for effector immune cells. However, the accumulation of fatty acids within the TME can cause metabolic alterations to immune cells and make them anti-inflammatory. [13]. Similar effects can be induced by the accumulation of cholesterol within the TME, which causes T cells to lose their antitumor functionality. This occurs because high cholesterol levels can cause the disruption of T-cell membranes, thus impeding their ability to attack tumors [13].

## 3. Immune cell subtypes in TME

The tumor microenvironment is comprised of tumor cells, resident host cells, extracellular matrix, cancer-associated fibroblasts, vascular cells, and tumor-infiltrating immune cells [14]. Although tumor-infiltrating immune cells of both innate and adaptive arms of the immune system are often present in the TME, specific subtypes of immune cells, their number, and function can vary significantly depending on the tumor type and on the different stages of progression [14]. Functionally, tumor-infiltrating immune cells have been shown to be responsible for both tumor-inhibitory (antitumor) and tumor-promoting properties [15]. Recruitment of immune cells into the TME is tightly regulated by chemotactic factors and the expression of chemokine receptors on immune cells which together define the recruitment of activator or suppressor type of immune cells into the TME [16]. Based on the extent of immune cell infiltration into tumor tissue, the TME can be classified as immune-infiltrated, immune-excluded, and immune-silent.

Immune cells of the adaptive response in the TME include T- and B- subsets of lymphocytes. Both subtypes of CD3+ T lymphocytes (CD4+ helper T cells and CD8+ cytotoxic T cells) can be observed within the TME, where CD8+ T cells are predominantly responsible for cytotoxicity response against the tumor cells and CD4+ T cells either support CD8+ cell cytotoxic activity or act as regulatory T cells (Tregs) that suppress the antitumor immune responses. The types of chemotactic factors in the TME and expression cytokine receptors therefore collectively determine which subtype of T cells predominate in the TME. For example, chemokines CXCR3, and CXCR4 aid in directing the migration of cytotoxic T cells and NK cells into the tumor, whereas CCR4 expression is linked to the recruitment of suppressor Tregs into the TME [16]. B cells, which are primarily responsible for antibody-mediated immune response, are also observed in the TME but in relatively small numbers when compared with T cells. Tumor-infiltrating B cells appear to mediate the formation of lymphoid-like structures within the TME where their interaction with T cells regulates tumor progression [16].

Immune cells of innate response that are constituents of the TME include NK cells, macrophages, neutrophils, and dendritic cells [14, 16]. Natural killer (NK cells) mediate antitumor activity either via direct cell-mediated killing of tumor cells or by

secretion of specific cytokines that indirectly contribute to the antitumor response. NK cells, although present in the TME, are less efficient at killing tumor cells within the tumor microenvironment, are highly effective against circulating tumor cells, and therefore more effective in preventing tumor metastasis [17]. Macrophages by far are the most common type of innate immune cells in TME and macrophage infiltration has been associated with poor prognosis of several solid tumors. Two distinct phenotypes of macrophages that mediate a pro-inflammatory response (M1 macrophages) and wound healing response (M2 macrophages) are commonly present in the tumor tissue [18]. However, the hypoxic state and presence of certain cytokines within the TME favor the M2 phenotype that supports tumor progression [18]. Neutrophils are the next variety of innate immune cells seen in the TME. Neutrophils are recruited into tumor tissue where they initially promote a local inflammatory response thereby promoting tumor cell apoptosis. As the tumor progresses, neutrophils can functionally support tumor growth through the modification of the extracellular matrix, and the release of growth factors that promote angiogenesis [19]. Dendritic cells (DCs), the most potent type of antigen-presenting cells; play an important role in cancer immunosurveillance and infiltration of DC into tumor tissue is associated with delayed tumor progression and metastasis [19].

## 4. Metabolism in lymphocytes: t cells

Metabolic pathways in T-lymphocyte vary depending on their differentiation status in their life cycle [20]. Naïve T lymphocytes mainly depend on TCA and OXPHOS to support basal metabolism. Continued signaling from cytokines, such as IL-7, is required to maintain glucose uptake by naïve T cells for sustaining the metabolism [20]. Following antigen recognition and activation, T cells undergo a metabolic change that is dependent on both glucose and amino acids as energy sources to support cell proliferation and to function as effector T cells [21]. Similar to the tumor cells, the effector T cells use Warburg metabolism to support energy demands associated with the secretion of cytotoxic cytokines and enzymes required for the removal of the tumor and virally infected cells. Therefore, within the TME, malignant cells compete with the effector T cells for energy sources and relatively nutrient deficiency in the TME can impair T-cell survival and proliferation [22]. The mechanisms underlying the regulation of T-cell effector functions by metabolic pathways also vary in different subsets of T cells. For example, in CD4 T cells, enzymes of the glycolytic pathway, such as GAPDH, can interact with mRNA of key cytokines, thereby preventing their translation [23]. Additionally, acetyl-CoA produced from citrate in cytosol due to the action of ATP citrate lyase (ACL) in both CD4 and CD8 T cells can directly modify histone acetylation status at the promoter regions of key cytokine genes involved in mediating effector functions [24]. Changes in mitochondrial structure and function are also implicated in the regulation of effector T-cell function as well as memory T-cell formation. Effector T cells, where mitochondria exhibit fragmentation, are poor in supporting electron transport machinery that leads to upregulation of anaerobic glycolysis, whereas in memory T cells, the mitochondrial fusion process allows proper function of ETC and facilitates lipid metabolism via fatty acid oxidation [25].

Due to similarities in the metabolic pathways utilized, within the TME, competition for nutrients exists between tumor cells and the effector T cells [26]. Tumor cells with functional mutations that confer survival advantage can therefore outcompete effector T cells leading to the reduced number and/or function of cytotoxic CD8

cells. Furthermore, lactate produced by tumor cells in the hypoxic regions creates an acidic environment that can inhibit T-cell activation by preventing glycolysis [27]. In contrast with cytotoxic T cells, Tregs, upon activation, induce fatty acid biosynthesis and oxidative phosphorylation, conferring them with a metabolic advantage to thrive within the TME [28]. Tumor cells evade the immune response by upregulation of inhibitory receptors, such as programmed cell death protein 1 (PD-1) and cytotoxic T-lymphocyte association protein 4 (CTLA4). These inhibitory receptors, known as immune checkpoints, are widely used as targets in cancer therapy as they also play a role in the metabolic regulation of T cells [29]. PD-1 expression downregulates glycolysis and increases fatty acid oxidation, which reduces their cytotoxic potential. PD-L1 expressed on tumor cells enhances glucose uptake and therefore blockade of PD-1/PD-L1 interaction can collectively potentiate antitumor activity of T cells [30]. CTLA-4 is a receptor expressed transiently on T cells following activation and plays an important role in regulating their activity. One of the mechanisms by which CTLA-4 suppresses T-cell activity is by down-regulating critical amino acid and nutrient transporters and inhibition of CTLA4 can restore the bioenergetic balance that favors the survival of T cells in the TME [29].

Another key area where understanding T-cell metabolism is critical is in cell-based therapies that utilize chimeric antigen receptor (CAR)-T and tumor-infiltrating lymphocytes (TIL). CAR-T treatment is an immunotherapeutic strategy in which samples of T cells taken from a patient's blood are genetically modified to produce receptors that target tumor cells [31]. In TIL therapy, T lymphocytes are taken from the tumor microenvironment and cultured *ex vivo*. The amplified TILs are then infused with the tumor in order to promote the targeting of cancer cells. These cells undergo metabolic reprogramming to inhibit glycolysis *in vivo*, which increases the

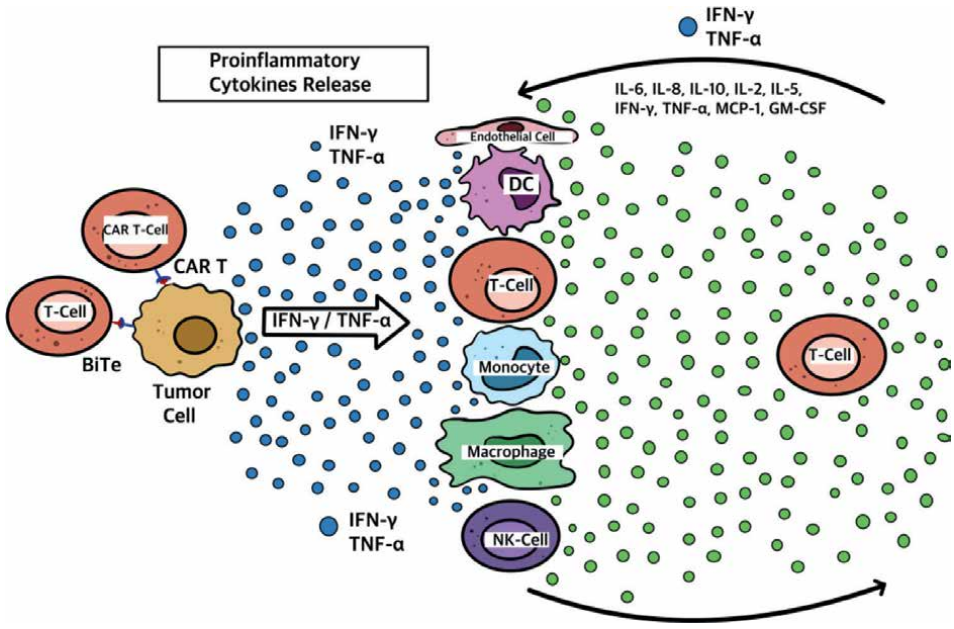


**Figure 2.**
*(cytokine release syndrome): CRS is an acute immune inflammatory response caused by the activation of the immune system, particularly T cells. This triggers the release of cytokines, which are molecules involved in directing immune function. These excess cytokines pose serious health risks, such as organ failure and potential death [34].*

proliferation of T cells and thus increases antitumor efficacy [32]. These methods have proven beneficial as alternative therapies when conventional therapies fail due to the acquisition of tumor resistance or where checkpoint inhibitors therapies are not a viable option due to lack of expression of those receptors as targets [33]. Both CAR-T and TIL therapies require isolation and *ex vivo* expansion of tumor-specific lymphocytes prior to administering to the patients. Despite having tumor-specific activity, engineered CAR-T-cell therapies are prone to adverse events in the form of cytokine release syndrome ([32], **Figure 2**). It is beginning to be understood that some of the mechanisms underlying CAR-T-cell properties, therapeutic efficacy, and potential adverse events are linked to metabolic pathways in the engineered cells. Conditions used for *ex vivo* expansion of TIL and CAR-T cells also may alter the metabolic state of these cells, thus impacting therapeutic effectiveness [35]. It is possible that redirecting the metabolic pathways during their expansion may result in cells with beneficial properties targeting tumors [35].

## 5. Metabolism in lymphocytes: nk cells

Resting NK cells predominately use glucose as fuel to carry out glycolysis and oxidative phosphorylation. Activation of NK cells via cytokine stimulation increases glucose uptake and the rate of glycolysis and oxidative phosphorylation, which support biosynthesis and secretion of IFN-Y and other key enzymes, such as granzyme, that are required for NK cell effector function [35]. In contrast with other lymphocytes, pyruvate generated from glycolysis in NK cells is preferentially converted to citrate via citrate-malate shuttle (CMS) rather than metabolism via the TCA cycle [36]. Two subsets of NK cells are recognized based on the expression level of phenotypic marker CD56 (CD56 dim and CD56 bright) that appear to be metabolically distinct. For example, CD56 bright NK cells involved in cytokine production express higher levels of glucose transporter proteins, thus rapidly taking up glucose upon activation [37]. In addition to glucose, glutamine is also important as a fuel source for the metabolism of activated NK cells. Glutamine can regulate the uptake of critical amino acids and the breakdown products of glutamine enter the TCA cycle for generating ATP [37]. Metabolic pathways in NK cells are tightly regulated both during development and activation. Specific signal transduction pathways and transcription factors are involved in regulating the metabolic pathways in NK cells. Transcription factor steroid regulatory element binding protein (SREBP) regulates the expression of the components of the CMS pathway and the mammalian target of the rapamycin (mTOR) pathway regulates NK cell proliferation and metabolism [36]. Consequently, reduced mTOR activity of mature NK cells is associated with diminished metabolic activity that results in impaired effector functions of NK cells. The multifunctional transcription factor c-Myc plays an important role by upregulating glucose transporters and critical enzymes of glycolysis in NK cells [36].

Although NK cells are highly effective in the targeted removal of tumor cells, the tumor microenvironment poses a challenge to the appropriate function of the NK cells. Firstly, changes in the metabolic properties of tumor cells create an environment that is low in critical nutrients (glucose and glutamine) and oxygen (hypoxic state) that are essential for the normal metabolism of NK cells [38]. Secondly, anaerobic glycolysis of tumor cells produces lactic acid that creates an unfavorable acidic environment, leading to reactive oxygen species (ROS) production in NK cells and induction of apoptosis [38]. Furthermore, transforming growth factor β

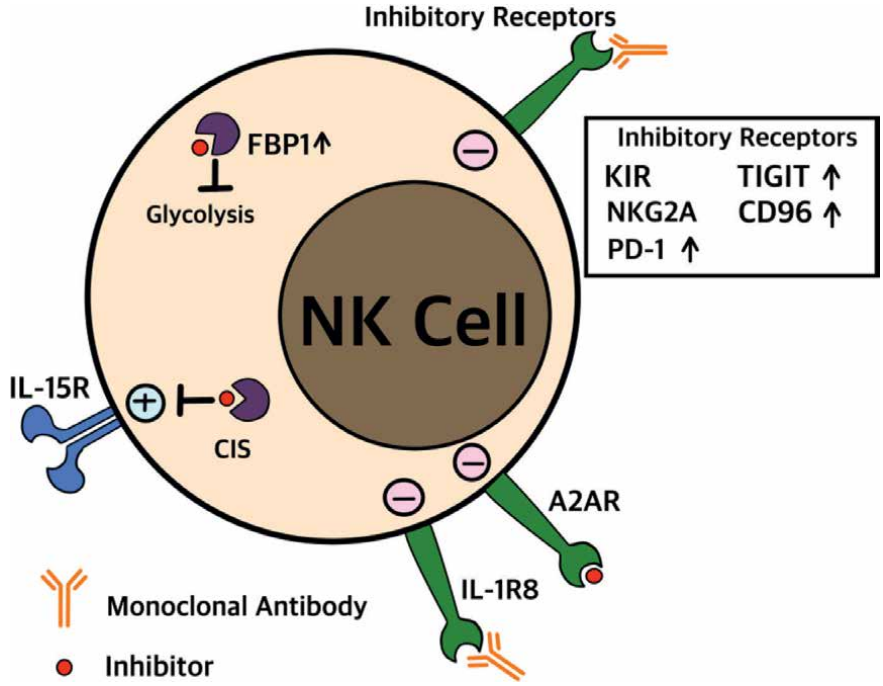


**Figure 3.**
*(targeting of NK-cell metabolic pathways): The targeting of major receptors and metabolites, namely FBP-1 in NK cells, holds great promise in restoring the antitumor efficacy of NK cells in the TME [40].*

(TGF-β), a cytokine that is commonly upregulated in several cancers can inhibit NK cell metabolism, presumably via the inhibition of mTor activity [39]. Metabolic adaptation of NK cells within the TME involves the activation of enzymes in the gluconeogenetic pathways, such as FBP-1, to generate glucose needed for NK cell metabolism. Therefore, dysregulated FBP-1 expression in NK cells further reduces their ability to survive in the TME and thus reduces immune function [40]. The hypoxic state of TME is associated with mitochondrial fragmentation in certain tumors, which perturb the survival and cytotoxic properties of NK cells [41]. In addition to the aforementioned factors, certain other metabolites that are elevated in the TME (adenosine, prostaglandin E2 (PGE2), and kynurenine) may also be responsible for reduced NK cell function via mechanisms that are yet to be understood [36]. Restoring normal metabolic function and survival of NK cells in the TME is one of the bases for pharmacological approaches to treat cancer where infiltrated NK cells have potent antitumor activity. Targeting TGF-β or its downstream signaling pathways and/or restoration of c-Myc protein levels via inhibition of enzymes (GSK3) are potential therapeutic approaches [39]. Additionally, culturing autologous NK cells *ex vivo* and inhibiting FBP-1 has proven to restore immune function, namely cytotoxicity ([40], **Figure 3**). Other cells in the TME (cytotoxic and Tregs, stromal fibroblasts, etc.) have also been shown to modulate the expression of various activating and inhibitory receptors on NK cells that in turn regulate the metabolic and antitumor properties of NK cells [38]. Therefore, targeting inhibitory NK cell receptors, such as NKG2A, is one of the strategies being evaluated as NK cell-mediated antitumor immunotherapy ([38], **Figure 3**).

## 6. Metabolism in the innate immune system: tumor-associated macrophages

Macrophages are specialized immune cells that develop from myeloid progenitor cells and are highly efficient in phagocytosis and the removal of pathogens [42]. Tumor-associated macrophages (TAMs) are macrophages that are specifically recruited into tumor tissue due to cytokines and growth factors secreted by cells within the tumor microenvironment [43]. TAMs are one of the most abundant leukocytes within the TME and have been implicated in tumor progression and metastasis [44]. Macrophages were further classified as inactive (M0), pro-inflammatory (M1), and anti-inflammatory (M2) subtypes based on specific immune responses elicited by these cells. Inactive macrophages (M0) are undifferentiated cells and can reprogram themselves into polarized M1 and M2 cells after exposure to stimuli [45]. These distinct subtypes of macrophages utilize different metabolic pathways to exert their functional effects and TAMs are further induced to undergo a metabolic switch to survive in the tumor microenvironment. The key features of M1 and M2 macrophages in the utilization of various metabolic pathways are as follows. Although both M1 and M2 macrophages metabolize glucose via glycolytic pathways, in M1 macrophages it is essential for pro-inflammatory properties, such as cytokine production, and in mediating phagocytosis [46]. Similarly, the pentose phosphate pathway, which produces NADPH, is also critical in M1 macrophages, where NADPH-oxidase-dependent generation of reactive oxygen species (ROS) and regeneration of glutathione [47]. Arginine is also metabolized differently in M1 and M2 macrophages by virtue of the

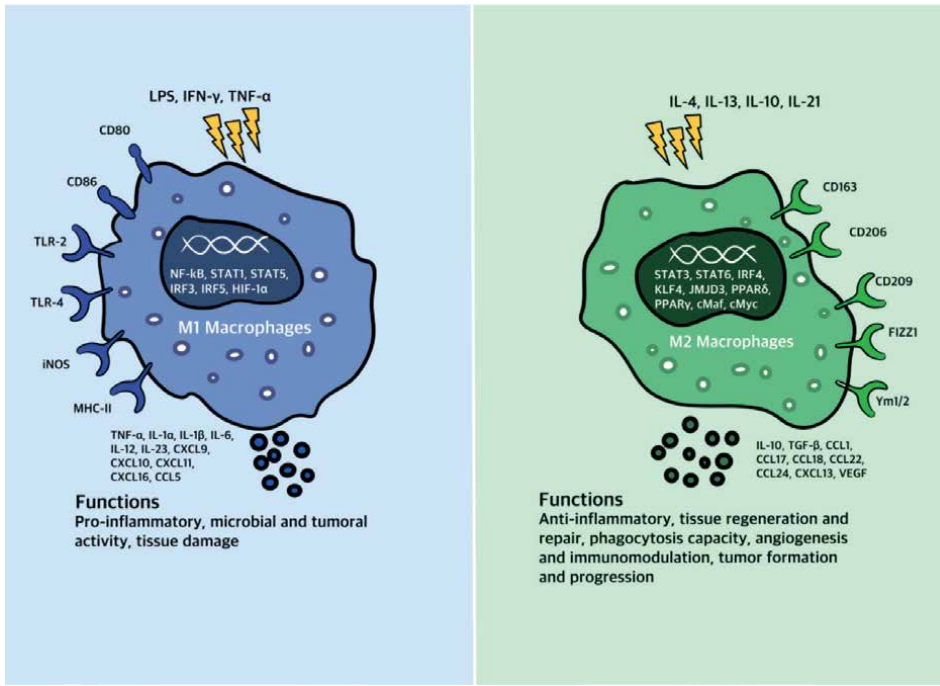


**Figure 4.**
*(TAM polarization): TAMs undergo metabolic changes that trigger polarization to the M2 phenotype, which has pro-tumorigenic properties and contributes to cancer progression [51].*

expression of distinct enzymes that break down arginine. Notably, M1 cells express inducible nitric oxide Synthase or iNOs that produces NO from arginine [46] and M2 macrophages express the enzyme arginase that metabolizes arginine to produce ornithine. NO has an important function in mediating pro-inflammatory response and ornithine serves as a precursor for polyamine synthesis that is critical in wound healing and repair processes that are mediated by M2 macrophages [48]. The TCA cycle in M2 macrophages is coupled to mitochondrial oxidative phosphorylation, whereas in M1 macrophages, intermediate metabolites of the TCA cycle, citrate and succinate, accumulate and are redirected toward the processes that lead to the production of inflammatory mediators, such as prostaglandin E2 (PGE2) [49].

The subtypes of macrophages within the TME vary with the progression of tumors. During the early stages of tumors, M1 macrophage polarization is favored, thus leading to the recruitment of cytotoxic CD8 cells and NK cells ([50], **Figure 4**) and the antitumor property of TAMs. However, as tumors progress, polarization to M2 macrophages is favored due to progressive changes in the TME ([50], **Figure 4**). Due to aerobic glycolysis of tumor cells, lactic acid in the TME induces M2-like TAM polarization of TAMs [46]. Additionally, TAMs have also been implicated in regulating tumor metastasis and angiogenesis further supporting the survival and spread of tumors. Metabolically, TAMs utilize glucose as the primary energy source with oxidative phosphorylation favoring their differentiation into pro-tumorogenic M2 macrophages [46]. As TAMs constitute the predominant cell population in the TME, potential therapies for cancer are based on metabolic targeting either to inhibit TAM polarization to an M2 phenotype or to selectively deplete M2 cells within the TME [52]. However, considering the complexity of concurrent metabolic processes occurring in other cells in the TME, these approaches have some limitations. Nevertheless, inhibition of OXPHOS pathways in TAMs has been shown to decrease tumor progression [53]. Future therapies directing metabolic processes via targeted drug delivery to TAMs may prove useful to overcome limitations associated with current strategies [52].

## 7. Conclusion

Cancer cells undergo key changes to their metabolic processes as an adaptation to outcompete other cells in the TME. This metabolic reprogramming causes the chemical conditions of the TME to change. The most notable of these changes is the development of hypoxic and acidic conditions due to a reliance on anaerobic glycolysis rather than OXPHOS pathways to produce ATP (Warburg effect), as well as the limited availability of nutrients. Additionally, the unique metabolism of cancer cells causes irregularities in the metabolite balance within the TME. Such changes have significant impacts on immune cells within the TME and their antitumor efficacy. All immune cell types in the TME of both the adaptive and innate immune systems undergo metabolic alterations in response to changes in the TME. These alterations greatly reduce immune function and contribute to tumor progression. The limited availability of nutrients in the TME downregulates the function of effector T cells and cytotoxic T cells and prevent their proliferation, and also prevents the formation of memory T cells. The antitumor efficacy of NK cells is reduced by the acidic and nutrient-scarce TME, which both triggers apoptosis, as well as the hypoxic state, which triggers mitochondrial fragmentation and reduces cytotoxic capabilities. The conditions of the TME cause TAMs to undergo polarization to the M2 subtype, which has pro-tumorigenic properties and can contribute to angiogenesis. Metabolism in

the TME has become a focus of cancer treatment. Common treatments are based on culturing autologous immune cell types *ex vivo* and modifying their metabolic properties. These immune cells are amplified in order to improve immune function and are then infused with the tumor. These treatments must be further explored, but the targeting of immune cell metabolism in the TME proves to be a promising strategy in the treatment of cancer.

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## Conflict of interest

The authors have declared that no conflict of interest exists.

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Section 3

# Cell Interactions and Signaling in Cancer

Chapter 6

# Perspective Chapter: Critical Role of Hedgehog in Tumor Microenvironment

*Xing-Guo Li and Jer-Yen Yang*

## Abstract

Hedgehog (Hh) signaling is a highly conserved pathway that plays a pivotal role during embryonic development. Mounting evidence has implicated Hh signaling in various types of cancer. Accordingly, inhibition of aberrant Hh signaling continues to be pursed across multiple cancer types -with some success in certain malignancies. In addition, with the renaissance of antitumor immunotherapy, an in-depth understanding of the molecular mechanisms underlying how the multifaceted functions of Hh signaling shape immunologically suppressive tumor microenvironment might be the key to unlocking a new era of oncological treatments associated with a reduced propensity for the development of drug resistance. Here, we focus on the latest advances regarding the immunological effects of misregulation of Hh signaling on tumor immunity. We also review the current status of clinically approved Hh inhibitors and dissect the mechanisms of drug resistance. Finally, we discuss the potential clinical applications that harness the immunomodulatory effects of Hh signaling not only to circumvent drug resistance, but also to achieve durable efficacy following immunotherapies, thus ultimately resulting in improved patient outcomes.

**Keywords:** hedgehog signaling, tumor microenvironment, immune cell, smoothened inhibitors, therapeutic targeting

## 1. Introduction

The Hedgehog (Hh) signaling pathway was discovered as a key regulator of organ development in *Drosophila melanogaster* by Christiane Nüsslein-Vollhard and Eric Wieschaus in the 1980s [1]. It was named after the gene locus associated with a spiky appearance of "hedgehog" phenotype in mutant *Drosophila* larve, findings based on which both investigators were awarded the Nobel Prize in Physiology or Medicine in 1995 "for their discoveries concerning the genetic control of early embryonic development," together with Edward B. Lewis [2]. Since then, the Hh signaling has been extensively studied as a highly conserved evolutionary pathway to orchestrate embryonic development, cell growth and differentiation, homeostasis [3]. Unlike other classical signaling cascades, Hh signaling is almost silent in the adult organisms but reactivated in a few tissues such as the skin, during tissue regeneration and wound

healing [3]. Not surprisingly, aberrant activation of this pathway has been demonstrated as a potent oncogenic driver to promote numerous hallmarks of cancer [4]. Therefore, the multifaceted role of Hh signaling may allow exploitation of this key pathway for novel and more effective cancer therapy [5].

Activation of Hh signaling is dependent on the primary cilium, a highly specialized organelle found on most vertebrate cells. Three Hh ligands, sonic hedgehog (Shh), desert hedgehog (Dhh), and Indian hedgehog (Ihh), are known to actuate the Hh pathway during embryonic and tissue development [6]. Whereas the expression patterns for Dhh and Ihh are tissue-specific, Shh has a broader expression pattern in various compartments and in multiple developmental stages [6]. In general, the Hh signaling is activated through either canonical or non-canonical mechanisms. In the canonical pathway, Hh ligands bind to the surface receptor Patched 1 (PTCH1), which alleviates the inhibitory effect of PTCH1 on a G-protein-coupled receptor (GPCR)-like protein, Smoothened (SMO), leading to migration of SMO to the tip of the cilium, which in turn signals suppressor of fused (SUFU) to release glioma-associated oncogene homolog proteins (GLIs). Finally, GLIs translocate into the nucleus, resulting in a signaling cascade through transcriptional regulation of Hh target genes [6]. Alternatively, GLI transcription factors can be activated through non-canonical mechanisms, which can be independently of PTCH1, SMO, or both [6]. Of note, mounting evidence has demonstrated that the signaling pathways that can induce non-canonical Hh signaling have been of known significance in oncogenesis, providing the mechanistic basis of the cross talk between Hh signaling and other signaling pathways to promote tumorigenesis, as well as the rationale for development of potential combination therapeutics [7–10].

The discovery of PTCH mutations in basal cell nevus syndrome (BCNS, or Gorlin syndrome, or nevoid basal cell carcinoma [BCC] syndrome), a hereditary form of BCC, provides the first link between the Hh signaling and tumorigenesis [11, 12]. Other than BCC, emerging evidence has involved abnormal activation of Hh signaling in a variety of cancer types, such as medulloblastoma, breast cancer, pancreatic cancer, and lung cancer [13].

So far, three models have been proposed to elucidate the role of Hh signaling in oncogenesis where Hh signaling is over-activated through ligand production, autocrine, juxtacrine, or paracrine reception of the ligand, as well as cross talk between Hh signaling and complex intracellular signaling cascades [13]. First, in BCC and medulloblastoma, activating mutations of Hh pathway have been identified, such as inactivating mutations in PTCH or SUFU, and activating mutations in SMO, as shown in 85% of sporadic BCC or 30% of medulloblastoma, respectively [11–16]. In this scenario, the autonomous activation of Hh signaling is independent of Hh ligands.

Second, Hh signaling is aberrantly activated through autocrine or juxtacrine ligand-dependent manner, where Hh is secreted and responded by the same or adjacent cells [13]. This category of cancers includes breast cancer, pancreatic cancer, lung cancer, prostate cancer, colorectal cancer, stomach and esophageal cancer, ovarian and endometrial cancer, melanomas, and gliomas [13]. Finally, in pancreatic cancer, prostate cancer, and colon cancer, Hh signaling is activated through a paracrine-dependent manner, where Hh ligands are secreted by tumor cells, whereas the PTCH receptor is expressed on stromal cells in the tumor microenvironment (TME). In this last model of Hh signaling activation, a feedback loop is generated, which allows the transmit of the growth-promoting signals from tumor cells to stromal cells and then back to tumor cells, leading to sustained tumor progression [17].

In the following sections, we will first highlight the key cellular components of TME involved in oncogenic Hh signaling to promote tumor progression. We will then

review the current status of the FDA-approved and non-approved inhibitors of Hh signaling, as well as the molecular mechanisms of drug resistance. Finally, we will provide a critical evaluation of recent studies on the treatments combining immunotherapeutic strategies with approved Hh inhibitors and will propose potential strategies that could be applied to harness existing knowledge to overcome the drug resistance.

## 2. The role of Hh signaling in the TME

Emerging evidence has suggested that TME is not just a silent bystander, but rather an active player of tumor progression [18, 19]. The composition of TME not only varies between tumor types, but also is continuously evolving in the different stages of tumorigenesis. Hh signaling has been intensively studied with respect to the classical hallmarks of cancer [3–6]. In contrast, its role in the modulation of TME has only become evident in recent studies [20–22].

### 2.1 Immune cells

The adaptive and innate immune systems cooperate to form a highly proficient immune surveillance machinery that can identify and eradicate genetically altered cells to prevent tumorigenesis. Tumor-infiltrating leukocytes (TILs), including T and B lymphocytes, monocytes and macrophages, myeloid-derived suppressor cells (MDSCs), dendritic cells (DCs), and natural killer (NK) cells, play diverse roles in tumor progression through interactions and production of cytokines, chemokines, and growth factors to support or suppress tumor growth and metastasis [20, 21]. There is increasing evidence from multiple experimental models that demonstrate an important and multifaceted role of Hh signaling in the modulation of immune cell functions. Aberrant Hh signaling induces a hostile, immunosuppressive microenvironment to dampen an effective antitumor immune response.

Regulatory T cells (Tregs) control the activity of effector immune cells such as granzyme B-expressing $CD8^+$ T cells and NK cells by secreting anti-inflammatory cytokines such as TGF-β and interleukin-10 (IL-10) [23]. The immune modulatory role of Hh signaling in T cells is evidenced by recent studies demonstrating that Hh signaling may directly regulate the expression and activity of TGF-β. Treg infiltration has been described for Hh-associated tumors, such as BCC [24], and medulloblastoma [25–28].

Intriguingly, elevated Treg infiltration is accompanied by an increase of TGF-β within intra- and peri-tumoral skin in a human UV-exposed facial BCC model [29]. In line with the putative immunosuppressive phenotype of Hh signaling, genetic abrogation of T-cell TGF-β signaling mitigated tumor progression in a transgenic medulloblastoma mouse model overexpressing smoothened A1 (SmoA1), an obligatory and conserved Hh signal transducer [25]. In this study, TGF-β signaling blockade led to nearly abolishment of Tregs and licensing of CD8 cytotoxic T lymphocytes for antitumor immunity [23].

Mechanistically, GLI2, an Hh effector, has been shown to directly activate the expression of TGF-β in human Tregs [30]. Thus, it has been proposed that Hh signaling may help generate a feed-forward loop where TGF-β induces the inversion of $CD4^+$ T cells to Tregs, which in turn secrete high levels of TGF-β, leading to

enforcement of the continued presence of immunosuppressive Tregs in the tumor microenvironment [31].

Myeloid cell infiltration has been described in multiple cancer entities where tumors may benefit from myeloid cells-mediated immunosuppression. The role of Hh signaling in the tumor-promoting function of myeloid cells has been postulated based on observations in multiple models of Hh-induced tumors. First, in a murine SMO-induced BCC model, tumor growth appears to be enhanced by the recruitment of immunosuppressive myeloid derived suppressor cells (MDSCs), accompanied by a reduction of effector lymphocytes in the tumor lesions [32]. This is mediated by the TGF-β-CCL2 (C-C motif chemokine ligand 2) axis secreted by oncogenic SMO-expressing keratinocytes and the CCL2 receptor expressed by MDSCs. *In vivo*, pharmacological suppression of the CCL2 receptor expression decreased infiltration of MDSCs and resulted in reduced tumor growth, indicating an immunosuppressive phenotype by the oncogenic Hh signaling [33]. Likewise, there is also strong evidence for immunosuppressive function of myeloid cells in Hh-associated medulloblastomas, which are characterized by high myeloid infiltration. For example, gene expression profiling of human Hh medulloblastoma tumors showed enrichment for an M2-like gene expression profile, consistent with immunosuppressive functions of myeloid cells [34]. Moreover, an inverse correlation has been observed between expression of M2-like markers (such as CD163) and survival of human Hh medulloblastoma patients [34].

Along these lines, the notion of an immunosuppressive function of Hh signaling was further affirmed by two recent studies in Hh-induced medulloblastomas. In a mouse model of Hh medulloblastoma (*Ptch1*$^{+/-}$; *Tp53*$^{-/-}$), Dang et al. showed decreased T-cell proliferation in a co-culture system of tumor-infiltrating myeloid cells and *ex vivo* stimulated T cells [35]. Mechanistically, the immunosuppressive phenotype appears to be mediated by CCL2. Genetic knockout of CCL2 receptor not only decreased infiltration of monocyte-derived macrophages but also increased levels of $CD8^+$ T cells in tumors [35]. Likewise, in another mouse model of Hh-induced medulloblastoma (Atoh1-SmoM2), pharmacological inhibition of colony stimulating factor 1 receptor (CSF1R) depleted macrophages and microglia, resulting in delayed tumor growth and prolonged mouse survival [36]. These recent studies support the notion of a tumor- promoting function of macrophages, which are consistent with an early study in another Hh-associated medulloblastoma tumor model, where the presence of MDSCs increases infiltration of Tregs and reduces the number of effector T cells [37]. Interestingly, infiltrating myeloid cells have been described as the predominant source of PD-L1 expression in a mouse model of Hh-induced medulloblastoma where the binding of PD- L1 to PD-1 on effector T cells resulted in T-cell exhaustion and immune escape of tumor cells [38]. Furthermore, an analysis of an immunocompetent breast cancer xenograft mouse model showed that inhibition of Hh signaling (SMO inhibitor vismodegib) led to reduced infiltration of immunosuppressive myeloid cells in the tumors, accompanied by an increase of effector $CD8^+$ T cells and M1 macrophages [39].

### 2.2 Tumor-associated astrocytes (TAAs)

Astrocytes, the most abundant type of glial cells in the brain, are integral partners with neurons in the regulation of neuronal development and brain function. Hh signaling has emerged as a critical player to support astrocyte-mediated modulation of neuronal activity [40–42]. A recent series of elegant work supports a key role of

tumor-associated astrocytes (TAAs) in promoting tumor growth and metastasis through distinct signaling, including Hh pathway [43–46]. First, TAAs were shown to secret the ligand Shh, which is required for maintaining cell proliferation of Hh-activated medulloblastoma through a Smo-dependent, but Gli1-independent manner, despite the absence of its primary receptor Ptch1. Of note, ablation of TAAs blocked tumor growth [43]. Furthermore, a recent study at single-cell resolution demonstrated that Hh-induced medulloblastoma cells can transdifferentiate into interleukin-4 (IL-4)-secreting TAAs, which in turn stimulates tumor-associated microglia to release insulin-like growth factor 1 (IGF1) to promote tumor progression [44]. Similarly, medulloblastoma-associated astrocytes have recently been shown to produce high levels of CCL2, a tumor-promoting cytokine shown to drive stemness maintenance and proliferation of disseminated tumor cells [45] and to promote metastasis [47]. Moreover, using single-cell RNA sequencing and lineage tracing analyses, Guo et al. investigated cellular origin of TAAs in a mouse model for relapsed Hh-activated medulloblastoma driven by *Ptch1* knockout [46]. This study has elegantly demonstrated that TAAs are predominantly derived from the transdifferentiation of tumor cells in relapsed MB, but not in primary MB, thus establishing the distinct cellular sources of astrocytes [46]. Interestingly, this study revealed that such transdifferentiation of medulloblastoma cells to TAAs depends on bone morphogenetic proteins (BMPs) and that pharmacological inhibition of BMP signaling repressed transdifferentiation and suppressed tumor relapse [46]. It remains to be determined what drives these transdifferentiation events and what intrinsic and extrinsic mechanisms, beyond Hh and BMP signaling, regulate the potential cooperation of TAAs and microglia in promoting the immunosuppressed state of medulloblastoma.

### 2.3 Cancer-associated fibroblasts (CAFs)

Cancer-associated fibroblasts (CAFs), the most abundant stromal cells in TME, have emerged as a central player in cancer progression and metastasis [48]. Through diverse phenotypes, origins, and functions, CAFs modulate the cross talk between inflammation and tumorigenesis and contribute to therapeutic resistance by producing various cytokines, chemokines, growth factors, and matrix-degrading enzymes [49].

There is increasing evidence indicating that CAF populations that support or suppress tumor growth and progression through stroma-specific Hh activation have been detected in multiple tumor types, including pancreatic cancer, colon cancer, and bladder cancer [50]. Recent advances in single-cell technologies have enabled detailed characterization of the heterogeneity and plasticity of differential CAF subsets, supporting a new therapeutic strategy in which tumor-supporting CAFs are reprogrammed into tumor-suppressing CAFs [50]. In pancreatic ductal adenocarcinoma (PDAC), Hh signaling pathway is activated in CAFs via a paracrine mechanism and has been associated with pancreatic tumorigenesis [49]. Initial studies indicated that inhibition of Hh pathway impaired tumor growth and sensitized tumors to chemotherapy in multiple PDAC models [51–56]. However, recent studies have challenged the concept of tumor-promoting CAFs. In the context of an oncogenic Kras-driven mouse PDAC model, conditional deletion of *Shh*, the predominant Hh ligand expressed in pancreas, led to cachexia and to poorly differentiated and highly vascularized tumors [57].

Moreover, by using three distinctly genetically engineered mouse PDAC models, another study showed that pharmacologic inhibition of Hh pathway activity

accelerated rather than delayed progression of oncogenic Kras-driven disease by affecting the balance between epithelial and stroma elements, leading to suppression of stromal desmoplasia but accelerated growth of pancreatic intraepithelial neoplasia [58]. These contradictory findings indicate that Hh signaling may play pleiotropic roles in PDAC progression. Interestingly, by using a combination of pharmacologic inhibition, gain- and loss-of-function genetic experiments, cytometry by time-of-flight, and single-cell RNA sequencing, a more recent study defines dosage-dependent effects of Hh signaling on the composition and function of CAFs in PDAC microenvironment [59]. Hh signaling is uniquely activated and differentially elevated in CAFs, with higher levels in myofibroblastic CAFs (myCAF) compared with inflammatory CAFs (iCAF) in both mouse and human PDAC. Driving high levels of Hh signaling promotes tumor growth, whereas Hh pathway inhibition alters the ratio of myCAF/iCAF populations, which is accompanied by a decrease in cytotoxic T cells and an expansion in regulatory T cells, thus altering the composition of CAFs, and shifting the inflammatory response toward a more immunosuppressive phenotype [59]. Given the differential functional implications for CAF subpopulations, changes in the ratio of CAF subtypes may lead to distinct antitumor outcomes. Consistent with, recent studies demonstrated a possible negative impact of current Hh pathway inhibitors on antitumor response in clinical trials, which were largely unsuccessful or even detrimental to patient health [60, 61]. Further understanding of the roles of Hh signaling in CAFs may open the possibility for more effective combination cancer therapies.

## 3. Therapeutic targeting Hh signaling in cancers

Given the multifaceted role of Hh signaling in cancer, inhibitors of Hh pathways have emerged as an important class of anticancer agents. These compounds fall into three main categories: Hh ligand inhibitors, SMO inhibitors, and GLI inhibitors [62]. Despite extensive efforts devoted to the discovery of Hh signaling inhibitors, so far only three drugs have been approved by the Food and Drug Administration (FDA), all targeting the upstream receptor of Hh signaling SMO, a membrane protein of the GPCR protein family [62].

### 3.1 FDA-approved inhibitors

To date, three SMO inhibitors, vismodegib, sonidegib, and glasdegib, have been FDA approved in 2012, 2015, and 2018, respectively, for cancer treatment. Cyclopamine, the first SMO antagonist, is a naturally occurring alkaloid found in the corn lily [63] later proved to bind to SMO and to inhibit activation of downstream Hh target genes [64].

Extensive efforts have been made to develop alkaloid derivatives to increase the bioavailability, sensitivity, and specificity of cyclopamine to target SMO [65]. Vismodegib (GDC-0449 or Erivedge), the first cyclopamine derivative and Hh pathway–targeting drug, is currently approved for the treatment of patients with locally advanced or metastatic BCC (US FDA). Compared to cyclopamine, vismodegib shows a higher potency and more favorable pharmacological properties [62]. The approval of vismodegib was based on results from the pivotal phase II ERIVANCE trial (Clinica lTrials.gov, NCT00833417) showing that vismodegib substantially shrank tumors or healed visible lesions (objective response rate, ORR) in 43% of patients with locally advanced BCC and 30% of patients with metastatic BCC, at 21 months, with a median

progression-free survival (PFS) duration of 9.5 months for both metastatic and locally advanced BCC patients [66, 67]. Up to the completion of this manuscript, there have been 86 clinical trials for vismodegib, both monotherapy and combination, in various cancer types (ClinicalTrials.gov).

Sonidegib (Erismodegib, NVP-LDE-225, LDE-225, Odomzo) is another cyclopamine-derived SMO antagonist discovered in 2010 through an *in vitro*, high-throughput screen, showing high tissue penetration and bioavailability, as well as the ability to cross the blood-brain barrier [68]. In 2015, sonidegib became the second SMO inhibitor approved for patients with locally advanced or recurrent BCC (US FDA). The approval of sonidegib was based on results from a multicenter, randomized, double-blind phase II BOLT trial (ClinicalTrials.gov, number NCT01327053), which showed the objective response rates of 38% and 43% in the 800 and 200 mg dosage groups, respectively after 30 months in patients with locally advanced BCC and the objective response rates of 17% and 15%, respectively in those with metastatic BCC [69]. Up to August 2022, there are 46 clinical trials for sonidegib in cancer treatment (ClinicalTrials.gov).

A third FDA-approved inhibitor of Hh signaling is glasdegib (PF-04449913, Daurismo), a benzamide derivative discovered in 2012 with high potency and oral bioavailability [70]. In 2018, glasdegib was approved for combination treatment with low-dose cytarabine arabinoside (LDAC) for patients with acute myeloid leukemia unsuitable for intensive chemotherapy. The approval of glasdegib was based on the results of the phase II BRIGHT 1003 trial (ClinicalTrials.gov, NCT01546038) showing the median overall survival of 8.8 months with glasdegib/LDAC as compared to 4.9 months with LDAC. Furthermore, 17.0% and 2.3% of patients in the glasdegib/LDAC and LDAC arms, respectively, achieved complete remission [71]. Up to this point, there have been 26 clinical trials for glasdegib in various cancer types (Clinica lTrials.gov).

### 3.2 Resistance mechanisms to FDA-approved inhibitors

The first retrospective study on drug resistance to SMO inhibitor therapy was reported in 2012 where 21% of BCC patients treated with vismodegib developed drug resistance, with a mean tumor recurrence time of 56.4 weeks in clinical examination [72]. Ever since, resistance to SMO antagonists has been observed in patients who never respond to SMO inhibitor therapy (primary resistance), as well as in those who initially respond but later develop resistance to SMO inhibitors (acquired resistance) [73]. Mechanistically, a number of models have been proposed to explain the basis of drug resistance to SMO inhibitor therapy. First, genetic analysis of resistant tumors has revealed mutations of SMO, loss of SUFU, and amplification of GLIs or Hh target genes, such as CCND1 and GLI1 [5, 10]. Second, accumulating evidence supports the notion that the resistance can be driven through the non-canonical Hh signaling, accompanied by the concurrent activation of other oncogenic signaling pathways, such as AP-1 and TGF-β signaling [74], RhoA signaling [75], and RAS-MAPK signaling [76]. Finally, a new mechanism has recently been uncovered to contribute to drug resistance through loss of primary cilia [77, 78]. This was supported by both preclinical and clinical evidence. In Hh-dependent medulloblastoma, recurrent mutations in oral facial digital syndrome 1 (OFD1), a culprit gene led to loss of cilia, and thereby caused resistance to SMO inhibitors [78]. Importantly, sequencing data analysis from resistant BCC patients showed recurrent mutations in ciliary genes, providing clinical relevance of this new mechanism [77]. Therefore, a better understanding of cilia-

regulating signaling pathways in resistant cancer may open up a new route to reintroduce cilia to sensitize resistant cancer cells to SMO inhibitors. Taken together, several strategies have been proposed to overcome the drug resistance through targeting the underlying mechanisms. These approaches include: (1) develop second-generation SMO inhibitors to retain anticancer activities that are not affected by the resistance-inducing mutations [5]; (2) target downstream components of SMO, such as GLIs (see below, non- approved inhibitors), or signaling molecules involved in the non-canonical Hh signaling pathway [8].

### 3.3 Non-FDA-approved inhibitors

Multiple novel inhibitors targeting SMO have been shown to be effective in preclinical models [5] and are now in active clinical trials, either monotherapy or combination for various cancer types. These compounds include saridegib (patidegib, IPI-926), taladegib (LY2940680), and BMS-833923 (XL139) (ClinicalTrials.gov). On the other hand, even though GLI1 antagonists are not as extensive as those targeting SMO, mounting evidence has shown that targeting the Hh signaling at the level of its final effector, GLI1, is a promising strategy to overcome resistance to currently available SMO inhibitors [79, 80]. In this regard, the promising pharmacological potential of direct and indirect GLI inhibitors, as well as GLI antagonists derived from natural products, has been in active investigation at the preclinical or clinical phase. It is anticipated that future study on these compounds will help develop new strategies tackling resistant mechanisms and tumor heterogeneity [81].

## 4. Hh signaling and antitumor immune response

In 2018, James P Allison and Tasuku Honjo were awarded the Nobel Prize in Physiology or Medicine "for their discovery of cancer therapy by inhibition of negative immune regulation" [82]. Although this breakthrough in cancer immunotherapy has revolutionized cancer treatment, only a subset of patients elicit favorable responses and most immunologically cold solid tumors are not responsive [83]. Given the immunosuppressive function of Hh signaling, inhibitors of Hh signaling pathway may hold promise in converting nonresponsive cold tumors into responsive hot ones, which may subsequently allow nonresponders to benefit from immunotherapies. Notably, clinically approved Hh inhibitors, as well as non-approved inhibitors, have been in active preclinical and clinical trials for combined therapies, including immunotherapies.

The first clinical trial with Hh inhibitors in combination with immune checkpoint inhibitors was conducted in 16 patients with advanced BCC (clincialtrial.gov, NCT02690948). This trial showed that pembrolizumab (PD-L1 inhibitor) is active against BCCs. Although the two groups of pembrolizumab with or without vismodegib were not directly compared, the response rate for the combination group was not superior to the monotherapy group [84]. Of note, most patients with advanced BCC progress on or are intolerant to Hh inhibitor therapy despite objective response rates of 30–60% [66–69, 85]. Until Feb 9, 2021, when cemiplimab, a PD-1 antibody, was approved by the US FDA fully for patients with locally advanced BCC, and accelerated for patients with metastatic BCC, after treatment with Hh inhibitors, or for whom Hh inhibitors are not appropriate [86], there was no standard second-line treatment option for these BCC patients [72]. A recent clinical trial study provides the

first report to show clinically meaningful antitumor activity of cemiplimab in patients with BCC after Hh inhibitor therapy ([87], clinicaltrials.gov, NTC03132636). In this trial, the efficacy and safety of cemiplimab were evaluated in patients with locally advanced BCC or metastatic BCC who had previously been treated with an Hh inhibitor. Among the efficacy population (n = 121), centrally reviewed objective response was observed in 31% of patients with estimated duration of response exceeding 1 year in 85% of responders [87].

Importantly, this study also showed that the safety profile was consistent with what is known for immune checkpoint class of drugs, even considering the advanced age of the patient population in the present study [87]. These findings demonstrate the efficacy of immune checkpoint blockade in treating BCC in patients who had previously received Hh inhibitor therapy, thus opening a new horizon for treatment of the many patients who discontinue Hh inhibitor therapy due to disease progression, toxicity, or drug resistance. Moreover, a recent case report demonstrated an impressive response to cemiplimab in a sonidegib-resistant giant basosquamous carcinoma, one form of BCC [88]. Finally, a dozen of clinical trials have been initiated to investigate the combination treatment of anti-PD-1, PD-L1, and CTLA-4 monoclonal antibody therapy with first-line Hh inhibitors in patients with a variety of cancer types (see **Table 1**). The outcome of these trials will not only inform about whether combinatorial treatments can increase the efficacy and duration of antitumor response, but also provide insights into the optimal customized regimen to circumvent resistance to Hh inhibitors.

Comparatively a few recent studies have indicated possible negative effects of the current Hh inhibitor therapy on antitumor immunity [89]. For instance, blockade of SMO signaling may inhibit formation of the immunological synapse [90]. Administration of SMO inhibitors caused the functional disruption of the immunological synapse, leading to the loss of T-cell effector activity [90]. Even though it remains unclear whether Hh inhibitor therapy may impede cytotoxic T-cell killing in cancer patients, a pilot clinical trial study of vismodegib in combination with pembrolizumab did not suggest additive clinical activity [84]. In the clinical context, there is an emerging paradigm that immunotherapy may show the greatest activity when administered early in the natural history of cancers. Further studies are warranted to evaluate the efficacy and duration of immune checkpoint blockade before Hh inhibitor therapy.

## 5. Conclusions

The Hh signaling pathway has attracted extensive research attention as a key player to contribute to the progression of a variety of human cancer types. With an in-depth understanding of the molecular mechanisms underlying the role of Hh signaling in tumorigenesis, enormous efforts have been made to develop specific inhibitors targeting molecular components of this pathway. Consequently, cancer therapy has undergone a paradigm shift from eradicating tumor cells to multidimensional targeting and normalizing tumor cells and TME. Herein, we reviewed the multifaceted function of Hh signaling in shaping immunologically suppressive TME to promote tumor progression, provided an up-to-date status of active clinical trials of FDA approved Hh inhibitors, and finally, highlighted possible therapeutic interventions that harness the immunomodulatory effects of Hh signaling not only to overcome drug resistance, but also to achieve durable efficacy following immunotherapies.

| SMO inhibitor | Combination | Cancer Type | Enrollment | Phase | Status | NCT # |
|---|---|---|---|---|---|---|
| Vismodegib (GDC-0449 or Erivedge) | + VEGF-A antibody and chemotherapy | Metastatic Colorectal Cancer | 199 | Phase 2 | Completed | NCT00636610 |
| | + Anti-hormone therapy | Prostate Cancer | 10 | Phase 1\|2 | Terminated | NCT01163084 |
| | + Chemotherapy | Pancreatic Cancer | 118 | Phase 1\|2 | Completed | NCT01064622 |
| | + VEGF-A antibody and chemotherapy | Ovarian Cancer\|Basal Cell Carcinoma\|Metastatic Colorectal Cancer | 19 | Phase 2 | Completed | NCT00959647 |
| | + Notch inhibitor | Breast Cancer | 13 | Phase 1 | Terminated | NCT01071564 |
| | + Chemotherapy | Gastric Cancer | 124 | Phase 2 | Completed | NCT00982592 |
| | + Chemotherapy | Pancreatic Cancer | 25 | Phase 2 | Completed | NCT01195415 |
| | + Chemotherapy | Myelodysplastic Syndromes, | 38 | Phase 2 | Terminated | NCT01880437 |
| | + Notch inhibitor | Sarcoma | 78 Phase1\|2 | Completed | NCT01154452 | |
| | + Photodynamic therapy | Basal Cell Nevus Syndrome | 24 | Phase 2 | Completed | NCT01556009 |
| | + IGF1R antibody and chemotherapy | Small Cell Lung Carcinoma | 168 | Phase 2 | Completed | NCT00887159 |
| | + Chemotherapy | Pancreatic Adenocarcinoma | 21 | Phase 1 | Unknown | NCT01713218 |
| | + Chemotherapy | Medulloblastoma | 24 | Phase 1\|2 | Terminated | NCT01601184 |
| | + Chemotherapy | Metastatic Pancreatic Cancer | 98 | Phase 2 | Completed | NCT01088815 |
| | + DNMT inhibitor | Acute Myeloid Leukemia | 40 | Phase 2 | Unknown | NCT02073838 |
| | + mTOR inhibitor | Pancreatic Cancer | 31 | Phase 1 | Completed | NCT01537107 |
| | + PD1 blockade | Skin Basal Cell Carcinoma | 16 | Phase 1\|2 | Completed | NCT02690948 |
| | + Chemotherapy | Breast Cancer | 40 | Phase 2 | Unknown | NCT02694224 |
| | + Radiation therapy | Basal Cell Carcinoma | 24 | Phase 2 | Completed | NCT01835626 |
| | + Radiation therapy | Carcinoma, Basal Cell | 14 | Phase 2 | Terminated | NCT02956889 |

| SMO inhibitor | Combination | Cancer Type | Enrollment | Phase | Status | NCT # |
|---|---|---|---|---|---|---|
| | + PD1/CTLA4 blockade | Basal Cell Nevus Syndrome | 0 | Phase 2 | Withdrawn | NCT03767439 |
| | + Tyrosine kinase inhibitor | Basal Cell Carcinoma | 84 | Phase 2 | Recruiting | NCT04416516 |
| | + Tyrosine kinase inhibitors and PARP inhibitors | Miscellaneous | 950 | Phase 2 | Recruiting | NCT02925234 |
| | + PDL1 blockade and Tyrosine kinase inhibitors | Miscellaneous | 676 | Phase 2 | Active, not recruiting | NCT02091141 |
| | + PDL1 blockade, Tyrosine kinase inhibitors and chemotherapy | Lymphoma, Non-Hodgkin | 720 | Phase 2 | Recruiting | NCT03297606 |
| | + PDL1 blockade, Tyrosine kinase inhibitors and chemotherapy | Cancer of Unknown Primary Site | 790 | Phase 2 | Recruiting | NCT03498521 |
| | + PDL1 blockade, Tyrosine kinase inhibitors and chemotherapy | Miscellaneous | 384 | Phase 2 | Recruiting | NCT04591431 |
| | + Targeted therapy and chemotherapy | Glioblastoma, Adult | 350 | Phase 1\|2 | Recruiting | NCT03158389 |
| | + Targeted therapy and chemotherapy | Meningioma | 124 | Phase 2 | Recruiting | NCT02523014 |
| | + PDL1 blockade, Tyrosine kinase inhibitors and chemotherapy | Miscellaneous | 300 | Phase 2 | Recruiting | NCT04341181 |
| | + PDL1 blockade, Tyrosine kinase inhibitors and chemotherapy | Miscellaneous | 6452 | Phase 2 | Recruiting | NCT02465060 |
| | + PDL1 blockade, Tyrosine kinase inhibitors and chemotherapy | Miscellaneous 40 | Phase 1 | Recruiting | NCT03878524 | |
| | + PDL1 blockade, Tyrosine kinase inhibitors and chemotherapy | Miscellaneous | 131 | Phase 2 | Not yet recruiting | NCT05238831 |
| | + PDL1 blockade, Tyrosine kinase inhibitors and chemotherapy | Advanced Cancer\|Solid Tumor | 250 | Phase 2 | Recruiting | NCT05159245 |

| SMO inhibitor | Combination | Cancer Type | Enrollment | Phase | Status | NCT # |
|---|---|---|---|---|---|---|
| | + Radiation therapy and chemotherapy | Medulloblastoma | 660 | Phase 2 | Recruiting | NCT01878617 |
| | + PDL1 blockade | Cancer Metastatic | 1000 | Phase 2 | Recruiting | NCT04817956 |
| | + Chemotherapy | Pancreatic Cancer | 55 | Phase 1 | Active, not recruiting | NCT00878163 |
| Sonidegib (Erismodegib, NVP-LDE-225, LDE-225, Odomzo) | + Radiation therapy and chemotherapy | Medulloblastoma | 205 | Phase 2 | Not yet recruiting NCT04402073 | |
| | + Chemotherapy | Lung Cancer | 19 | Phase 1 | Completed | NCT01579929 |
| | + JAK inhibitor | Miscellaneous | 50 | Phase 1\|2 | Completed | NCT01787552 |
| | + Chemotherapy | Pancreatic Ductal Adenocarcinoma | 23 | Phase 1\|2 | Terminated | NCT01431794 |
| | + Chemotherapy | Myelodysplastic Syndrome | 63 | Phase 1 | Completed | NCT02129101 |
| | + mTOR kinase inhibitor | Esophageal Cancer | 25 | Phase 1 | Completed | NCT02138929 |
| | + Chemotherapy | Plasma Cell Myeloma | 28 | Phase 2 | Completed | NCT02086552 |
| | + Chemotherapy | Pancreatic Cancer | 78 | Phase 1\|2 | Completed | NCT02358161 |
| | + Tyrosine kinase inhibitor and chemotherapy | Chronic Myelogenous Leukemia | 11 | Phase 1 | Completed | NCT01456676 |
| | + Tyrosine kinase inhibitor and chemotherapy | Miscellaneous | 108 | Phase 1 | Recruiting | NCT03434262 |
| | + Tyrosine kinase inhibitor | Miscellaneous | 120 | Phase 1 | Completed | NCT01576666 |
| | + Chemotherapy | Advanced Breast Cancer | 12 | Phase 1 | Completed | NCT02027376 |
| | + Tyrosine kinase inhibitor | Carcinoma, Basal Cell | 10 | Phase 2 | Terminated | NCT02303041 |
| | + Chemotherapy | Pancreatic Cancer | 18 | Phase 1 | Completed | NCT01487785 |
| | + PD1 blockade | Miscellaneous | 45 | Phase 1 | Recruiting | NCT04007744 |
| | Neoadjuvant + Surgery | Basal Cell Carcinoma | 10 | Phase 2 | Recruiting | NCT03534947 |
| | + Chemotherapy | Multiple Myeloma | 7 | Phase 2 | Terminated | NCT02254551 |

| SMO inhibitor | Combination | Cancer Type | Enrollment | Phase | Status | NCT # |
|---|---|---|---|---|---|---|
| | + Chemotherapy | Solid Tumor\|Ovarian Cancer | 30 | Phase 1 | Completed | NCT01954355 |
| | + Chemotherapy | Pancreatic Cancer | 39 | Phase 1 | Completed | NCT01485744 |
| | + Chemotherapy | Prostate Cancer | 0 | Phase 1 | Withdrawn | NCT02182622 |
| | + PD1 blockade | Basal Cell Carcinoma | 20 | Phase 2 | Recruiting | NCT04679480 |
| Glasdegib (PF-04449913, Daurismo) | + Chemotherapy | Glioblastoma | 75 | Phase 1\|2 | Active, not recruiting | NCT03466450 |
| | + Chemotherapy | Acute Myelogenous Leukemia | 30 | Phase 2 | Recruiting | NCT04231851 |
| | + Antibody-drug conjugate | Acute Myeloid Leukemia | 414 | Phase 3 | Recruiting | NCT04168502 |
| | + Chemotherapy | ACUTE MYELOID LEUKEMIA | 1 | Phase 2 | Terminated | NCT04051996 |
| | + Chemotherapy | Acute Myeloid Leukemia | 15 | Phase 3 | Active, not recruiting | NCT04842604 |
| | + Chemotherapy | Myelodysplastic Syndrome | 73 | Phase 1 | Completed | NCT02367456 |
| | + Chemotherapy | Leukemia, Myeloid, Acute | 730 | Phase 3 | Completed | NCT03416179 |
| | + Chemotherapy | Acute Myeloid Leukemia | 0 | Phase 1 | Withdrawn | NCT04655391 |
| | + Antibody-drug conjugate | Acute Myeloid Leukemia | 28 | Phase 3 | Terminated | NCT04093505 |
| | + PD1 blockade, antibody-drug conjugate and chemotherapy | Acute Myeloid Leukemia | 138 | Phase 1\|2 | Active, not recruiting | NCT03390296 |
| | + Chemotherapy | Acute Myeloid Leukemia | 48 | Phase 1 | Active, not recruiting | NCT02038777 |
| | + Chemotherapy | Leukemia, Myeloid, Acute | 0 | | Withdrawn | NCT04230564 |
| | + Chemotherapy | Acute Myeloid Leukemia | 255 | Phase 2 | Completed | NCT01546038 |
| | + Chemotherapy | Adult Acute Myeloid Leukemia | 75 | Phase 2 | Recruiting | NCT03226418 |
| | + Chemotherapy | Soft Tissue Sarcoma | 960 | Phase 3 | Recruiting | NCT03784014 |
| | + Chemotherapy and radiation therapy | Glioblastoma | 30 | Phase 1\|2 | Not yet recruiting | NCT03529448 |

| SMO inhibitor | Combination | Cancer Type | Enrollment | Phase | Status | NCT # |
|---|---|---|---|---|---|---|
| Saridegib (patidegib, IPI-926) | + Chemotherapy | Metastatic Pancreatic Cancer | 122 | Phase 1\|2 | Completed | NCT01130142 |
| | + Chemotherapy | Pancreatic Cancer | 15 | Phase 1 | Completed | NCT01383538 |
| | + Tyrosine kinase inhibitor | Head and Neck Cancer | 9 | Phase 1 | Completed | NCT01255800 |
| Taladegib (LY2940680) | + Chemotherapy and radiation therapy | Esophageal Adenocarcinoma | 7 | Phase 1\|2 | Completed | NCT02530437 |
| | + Chemotherapy | Small Cell Lung Carcinoma | 26 | Phase 1\|2 | Terminated | NCT01722292 |
| | + Chemotherapy and CDK inhibitors | Breast Cancer\|Colon Cancer\|Cholangiocarcinoma\|Soft Tissue Sarcoma | 94 | Phase 1 | Completed | NCT02784795 |
| BMS-833923 (XL139) | + Tyrosine kinase inhibitor | Leukemia | 33 | Phase 1\|2 | Completed | NCT01218477 |
| | + Tyrosine kinase inhibitor | Leukemia | 70 | Phase 2 | Terminated | NCT01357655 |
| | + Chemotherapy | Small Cell Lung Carcinoma | 5 | Phase 1 | Completed | NCT00927875 |
| | + Chemotherapy | Stomach Neoplasms\|Esophageal Neoplasms 39 | Phase 1 | Completed | NCT00909402 | |
| | + Proteasome inhibitors | Advanced Cancer, Various, NOS | 27 | Phase 1 | Completed | NCT00884546 |

*Data from clinicaltrials.gov (accessed on 2022/8/22).*

**Table 1.**
*Combination therapy of SMO inhibitors under clinical trials.*

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Chapter 7

# Perspective Chapter: Role of Cancer-Associated Fibroblasts in Oncogenesis

*Anyu Gu, Chikezie O. Madu and Yi Lu*

## Abstract

The tumor microenvironment consists of multiple types of cells, including endothelial cells, pericytes, neutrophil macrophage mast cells, lymphatic cells, basement membrane extracellular matrix, as well as fibroblasts. Fibroblasts populations found in cancers, also known as cancer-associated fibroblasts, have been implicated in the initiation, progression, and metastasis of tumors. This chapter will focus on the roles of cancer-associated fibroblasts in the progression of cancer and the studies of use of cancer-associated fibroblasts as a therapeutic target for cancer intervention.

**Keywords:** tumor microenvironment, cancer-associated fibroblasts, fibroblasts, cancer intervention, cancer

## 1. Introduction

The tumor microenvironment (TME) is the environment in which tumor cells or cancer stem cells exist [1]. The TME consists of multiple types of cells, including endothelial cells, immune cells, and fibroblasts [1–3]. The TME also consists of components such as the extracellular matrix (ECM), soluble factors such as cytokines and growth factors, and physical properties such as pH and oxygen content [2]. The TME and the interactions between its components help to promote tumor growth and cancer progression (**Figure 1**) [3].

Fibroblasts are the most common type of cell in connective tissue, commonly defined as structural cells that specialize in depositing and remodeling the ECM [4]. Fibroblast populations found in primary and metastatic cancers, known as cancer-associated fibroblasts (CAFs), are implicated in tumor initiation, progression, and metastasis [5]. CAFs have wide varieties of cells-of-origin, heterogeneous phenotypes, and diverse functions, all of which are shared by other cells found in the TME [6]. This chapter will focus on CAFs and their potential use in cancer intervention.

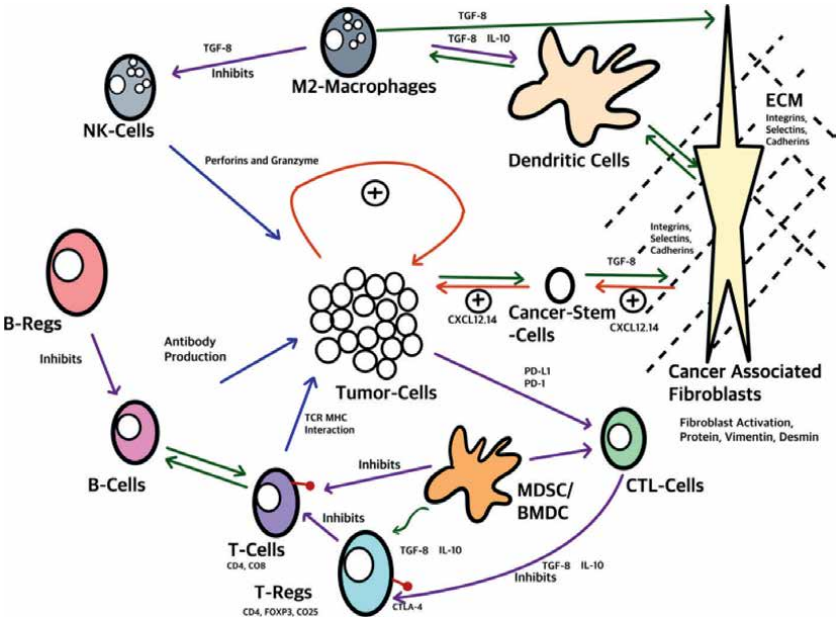


**Figure 1.**
*Important interactions and mechanisms of the TME [1].*

## 2. Fibroblasts and cancer-associated fibroblasts

The precursors of CAFs are generally considered to be dormant tissue-resident fibroblasts and pancreatic and hepatic stellate cells, though different studies have also identified bone marrow-derived mesenchymal stem cells, endothelial cells, and adipocytes [5]. Fibroblasts play a prominent role in coordinating the wound repair response in skin; therefore, it is likely that key CAF traits correspond to the normal physiological role normal fibroblasts play [7]. Fibroblasts transform into CAFs through tumor-derived stimuli, including soluble factors secreted by the tumor, immune infiltrate, lysophosphatidic acid, fibroblast growth factor, interleukin-1 (IL-1), IL-6, and granulin [8]. Transforming growth factor β (TGFβ) and lysophosphatidic acid are well-established activating signals for fibroblasts, which promote the activity of SMAD transcription factors and serum response factors, respectively [7]. These fibroblast activating signals converge to drive expression of the fibroblast marker αSMA, as well as increase the activity of the contractile cytoskeleton [7]. Fibroblasts may become activated through Notch signaling when in direct contact with tumor cells [7, 8]. Other mechanisms that can activate normal fibroblasts to become CAFs are shown in **Figure 2**. In the TME, tumor cells secrete factors such as TGFβ, platelet-derived growth factor (PGDF), and fibroblast growth factor (FGF) to convert fibroblasts to CAFs [3]. A build-up of CAFs is often associated with poor prognosis in many cancer types [3].

As shown in **Figure 3** [10], TGFβ is a common factor in the conversion of many different cell types into fibroblasts and CAFs. There are many types of TGFβ. TGFβ-1 is one that is secreted by stromal and tumor cells and is the main factor in promoting the mobilization of residential fibroblasts and their activation into CAFs [10]. Through SMAD-dependent and SMAD-independent pathways, TGFβ-1 activates fibroblasts into CAFs, expressing alpha-smooth muscle actin, periostin, α-fibroblast

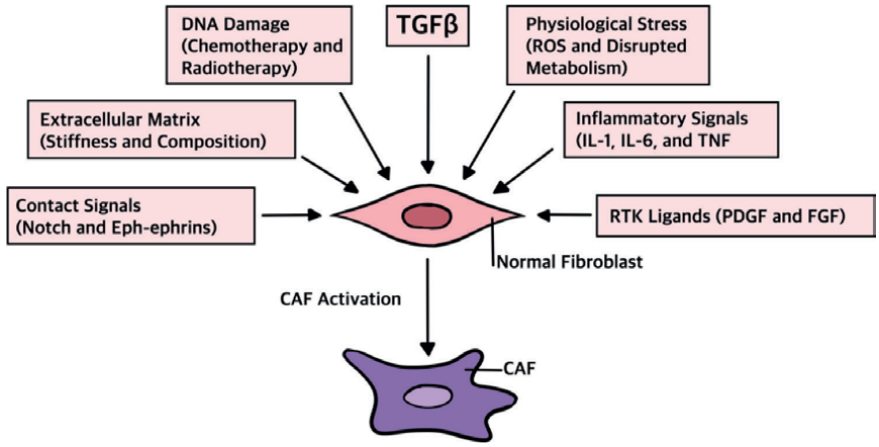


**Figure 2.**
*Mechanisms that activate normal fibroblasts to become CAFs. FGF, fibroblast growth factor; PDGF, platelet-derived growth factor; ROS, reactive oxygen species; RTK, receptor tyrosine kinase; TGFβ, transforming growth factor-β; TNF, tumor necrosis factor [7].*

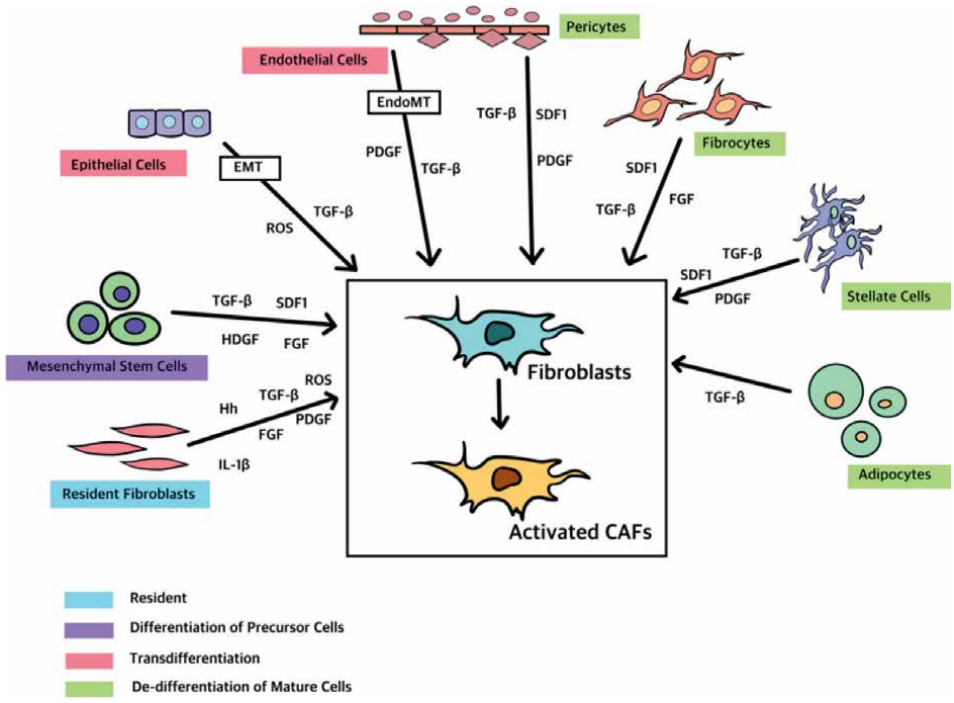


**Figure 3.**
*Different origins of CAFs [9, 10].*

activation protein, and fibroblast-specific protein-1 [10]. In addition, activated fibroblasts secrete TGFβ-1 [10], which could create a positive feedback loop, increasing fibroblast activation. TGFβ binds to the type 2 of TGFβ receptor (TGFBR2) on the surface of fibroblasts [11].

### 2.1 CAFs in tumors

CAFs are a type of myofibroblast that enhance the malignancy and progression of cancer [12]. The presence of CAFs is identified in almost all solid tumors [13]. This suggests that CAFs are important to the formation of solid tumors. In an established tumor, the TME represents a changed part of the original normal tissue of the host [13]. Tumor cells mostly contribute to the change in their favor. [13]. The stromal transformation of TME is primarily dominated and maintained by CAFs [13]. The CAF component of the TME is the most critical in influencing most of the functions of the TME in real time [13]. CAFs alter the TME by directly interacting with cancer cells and regulatory paracrine signaling, control the immune response to neoplasia, deposit ECM components, stimulate angiogenesis, and provide a scaffold for tumor invasion and metastasis [14]. Additionally, CAFs can produce many growth factors and pro-inflammatory cytokines to promote angiogenesis and recruit immunosuppressive cells to the TME to evade the immune system [9].

While CAFs have historically been considered to be cancer-promoting components, recent studies have shown that CAFs could have tumor-restraining functions in certain circumstances [15]. The tumor-restraining actions of CAFs are likely dependent on stimulation of anticancer immunity, pro-inflammatory secretome, tumor inhibitory signaling, and the synthesis of ECM components as barriers to tumor cell invasion and dissemination [5]. A study in mice has shown that myofibroblast depletion leads to increased tumor invasion, which is associated with decreased survival [16]. This study suggests that CAFs have functions in restraining tumors. This paradoxical nature of CAFs can potentially be explained by the heterogeneity of CAFs [15].

### 2.2 Heterogeneity of CAFs

There is mounting evidence that CAFs are a heterogeneous population of cells [9]. This likely depends on the numerous precursors of CAFs [9]. CAFs can be recruited to the tumor from a distant source, such as bone marrow [14], or transdifferentiate from non-fibroblastic lineages, such as epithelial cells, blood vessels, adipocytes, pericytes, and smooth muscle cells [9]. Numerous precursors of CAFs are shown in **Figure 3**. The study of genetically modified mouse models (GEMMs) designed to limit the accumulation of CAFs in growing pancreatic tumors or to conditionally delete the vascular endothelial growth factors in breast CAFs revealed that there are distinct functional subtypes of CAFs [17].

### 2.3 Functions of CAFs

CAFs have both pro-tumor and antitumor tendencies [17]. Pro-tumorigenic functions of CAFs are generally driven by their altered secretive [17]. Paracrine signaling between cancer cells and CAFs leads to tumor progression by enhancing the survival, proliferation, stemness, and metastasis-initiating capacity of cancer cells, promoting cancer progression and enhancing resistance to therapy [17]. CAFs also have an indirect influence in promoting tumor growth due to their ability to remodel the ECM [17]. The stiffness of the tissue, which plays a critical role in tumorigenesis, is influenced by modifications in the ECM's composition and cross-linking [18, 19]. CAFs express lysyl oxidase (LOX), an enzyme that cross-links and stiffens collagen fibers, promoting their stability [18]. CAFs also regulate the degradation of the ECM

[18]. CAFs secrete cytokines and chemokines that regulate tumor immunity and the intratumoral vascular program [17]. Several studies have indicated that CAFs play an important role in chemoresistance *via* different mechanisms, including but not limited to increasing stem cancer cells, secreting cytokines, and secreting miRNAs [10]. miRNAs have been shown to inhibit tumor-repressor genes, thus promoting cell growth and invasion, metastasis, and tumorigenesis [20].

CAFs trigger tumor initiation and progression [18]. *In vitro* coculture and *in vivo* transplantation experiments have shown that human prostatic CAFs induced the proliferation and the ability to form tumors from immortalized nontumorigenic human prostatic epithelial cells [18]. This effect was not exhibited by normal fibroblasts. It is thought that CAFs' secreted factors are what cause this tumor-initiating potential [18].

Numerous studies have shown that CAFs confer resistance to chemotherapy [6]. Some CAF-mediated resistant mechanisms include delivery of exosomes stimulating cancer cell survival, promoting cancer cell epithelial-mesenchymal transition, and thus decreasing expression of transporters responsible for drug uptake and scavenging chemo drug to reduce the amount of intratumoral chemotherapy drug [6]. CAFs also contribute to the resistance to targeted therapy [6]. Additionally, evidence suggests that CAFs contribute to immune evasion and immunotherapy resistance [6].

Antitumor functions of CAFs are predominantly associated with their functions as regulators of antitumor immunity [17]. Studies in mice have shown that fibroblast depletion leads to increased tumor invasion [16]. The use of defined gene promoter-driven expression of viral thymidine kinase proteins in GEMMs to study CAFs has allowed researchers to deplete populations of CAFs using ganciclovir, a substance that is toxic only to cells that express viral thymidine kinase [17]. A similar approach to deplete CAFs expressing αSMA suggested that αSMA+ stromal cells were predominantly acting to restrain cancer progression [17]. The depletion of these αSMA expressing CAFs yielded a more invasive tumor with enhanced intratumoral hypoxia [17]. A reduction in CAFs in GEMMs of pancreatic tumors with a deletion of sonic hedgehog (SHH) in the cancer cells also resulted in more aggressive tumors with increased cancer proliferation [17].

## 3. Targeting CAFs for cancer intervention

Numerous studies have proven CAFs' significant role in cancer progression and subsequently the potential of CAFs as targets for effective cancer intervention. Traditionally, therapies involved targeting cancer cells directly [21]. Recent complementary efforts aim to disrupt the networks that promote cancer cell activity and behavior [21]. The depletion of CAFs and targeting of CAF-dependent pathways can indirectly result in malignant cell death through both immune-dependent and immune-independent mechanisms [21]. Most conventional cancer therapies, such as radiotherapy and chemotherapy, are likely to affect CAFs as well by preventing cellular division by inducing DNA damage, impeding DNA and RNA synthesis, and blocking the cytoskeleton remodel required for cell division [17]. However, the unintended impact of these therapeutic methods on the function and accumulation of CAFs is largely unknown [17].

As a result, research is being conducted to help target CAFs through alternative methods [21]. One approach involves targeting the regulatory pathways leading to fibroblast differentiation and activation [9, 17, 21]. For example, TGFβ is a common

| Drugs | Target and mechanism | Cancer types | National Clinical Trial number | Status | Ref. |
|---|---|---|---|---|---|
| Sibrotuzumab | 131I-labeled anti-FAP mAb | Colorectal, non-small cell lung, breast, or head and neck cancers | NCT02198274 NCT02209727 | Phase I | [24] |
| Calcipotriol Paricalcitol | Vitamin D analogue | Early-stage skin cancer, breast cancer, pancreatic cancer | NCT03596073 NCT04617067 NCT02030860 NCT03138720 NCT04054362 | Phase I/II | [25] |
| Pamrevlumab (FG-3019) | Anti-CTGF mAb | Pancreatic cancer | NCT03941093 | Phase III | [26] |
| Plerixafor (AMD3100) BL-8040 (motixafortide) | CXCR4 receptor antagonist | Pancreatic cancer | NCT04177810 NCT02179970 NCT02826486 NCT03193190 | Phase I/II | [27, 28] |
| IPI-926 | Smoothened inhibitor | Pancreatic cancer | NCT01130142 | Phase I | [29] |
| S-3304 | MMP inhibitor | Advanced solid tumors | NCT00078390 NCT00033215 | Phase I | [30] |
| 131I-m81C6 | 131I-labeled anti-tenascin mAb | Brain tumors | NCT00002752 NCT00003461 | Phase II | [31] |
| Imatinib | PDGFR inhibitor | Advanced solid tumors | NCT00161213 NCT00281996 NCT01048320 NCT00485485 | Phase I/II | [32] |
| GS-6624 (simtuzumab) | LOXL2 mAb | Pancreatic cancer | NCT01472198 NCT01479465 | Phase II | [33] |
| Tetrathiomolybdate | Copper chelator, target LOX | Breast cancer, prostate cancer | NCT00195091 NCT00150995 NCT00405574 | Phase II | [34] |
| Pegvorhyaluronidase alfa (PEGPH20; PVHA) | Recombinant human hyaluronidase | Lung cancer, pancreatic cancer | NCT01453153 NCT02563548 NCT01839487 NCT02715804 | Phase I/ II/III | |

**Table 1.**
*Summary of various drugs' efficacy against CAF-induced cancer progression in clinical and pre-clinical studies [6].*

factor in the conversion of different cell types into CAFs. In a study, Mariathasan et al. found the two top scoring TGFβ pathway genes represent a ligand, TGFβ1, and receptor TGFβR2 [22]. In murine tumor models, blocking the TGFβ signaling by using the SM16 TGFβ receptor inhibitor or anti-TGFβ antibodies resulted in the recession of tumor growth [23]. By targeting these regulatory pathways, the activation of fibroblasts could be prevented, preventing CAFs from activating and functioning.

Another approach for targeting CAFs for cancer intervention is targeting CAF-secreted factors [11]. Numerous mitogens, chemokines, and matricellular proteins that CAFs release aid in the evolution of tumor progression and the development of drug resistance [11]. Targeting these CAF-secreted factors should prevent the promotion of tumor progression and drug resistance, making the tumor more susceptible to drugs. The heterogeneity of CAFs also proves as an advantage for cancer intervention *via* shifting the influence of pro- vs. antitumorigenic populations [21].

Currently, there are many drugs under trial as shown in **Table 1**. Of the potential targets identified in CAFs, fibroblast-activation protein (FAP) is the most studied. FAP has been neither detected in benign tumors nor in most normal quiescent adult stromal cells [35]. FAP is a type II integral membrane of the prolyl oligopeptidase family, or S9 family [36]. FAP is further classified into the dipeptidyl peptidase (DPP) subfamily (S9B) [36]. This class of enzymes is characterized by its capacity to cleave the pro-Xaa peptide link, where Xaa can be any amino acid. It has been demonstrated that this enzymatic activity contributes to the development of cancer by altering bioactive signaling peptides [36]. *In vivo,* FAP+ CAFs were successfully depleted by the FAP-depleting immunotoxin, and tumor models demonstrated strong tumor inhibitory effects. [6]. Other approaches in targeting FAP include DNA vaccine and chimeric antigen receptor (CAR) T cells [6].

## 4. Conclusions

Heterogeneous populations of CAFs exist in the TME. The heterogeneous nature of CAFs likely comes from their different origins, and this heterogeneity is likely the cause of the paradoxical nature of CAFs having both pro-tumorigenic and antitumorigenic functions. CAFs have many functions in the TME. CAFs alter the TME. They produce growth factors and pro-inflammatory cytokines.

CAFs are a promising target for cancer intervention. They have many pro-tumorigenic functions. CAFs can be targeted through their activation pathway by blocking a step in the pathway. One method is by preventing FAP from being produced by introducing siRNAs that are complementary to the FAP mRNA.

Over time, our understanding of CAFs and their contribution to cancer progression has expanded greatly. We now have a better understanding of their heterogeneity and their functions in the TME. While the antitumorigenic functions may act as a roadblock to targeting CAFs for cancer intervention, it may be possible to develop a treatment that targets the pro-tumorigenic functions of CAFs without targeting the antitumorigenic functions of CAFs by targeting subpopulations of CAFs that express pro-tumorigenic genes.

While CAFs are a promising target for cancer intervention due to their pro-tumorigenic functions, their antitumorigenic functions may act as a roadblock. Further research would be required before targeting CAFs as a conventional method of cancer intervention.

However, there are issues with targeting CAFs for cancer intervention. **Figure 4** shows the effects of targeting only tumor cells vs. targeting only the TME on a tumor. In both, there is a possibility that the tumor can grow back. In addition, studies in mice have shown that fibroblast depletion leads to increased tumor invasion [16]. In mouse models, deletion of SHH accelerated the progression of pancreatic ductal adenocarcinoma [37].

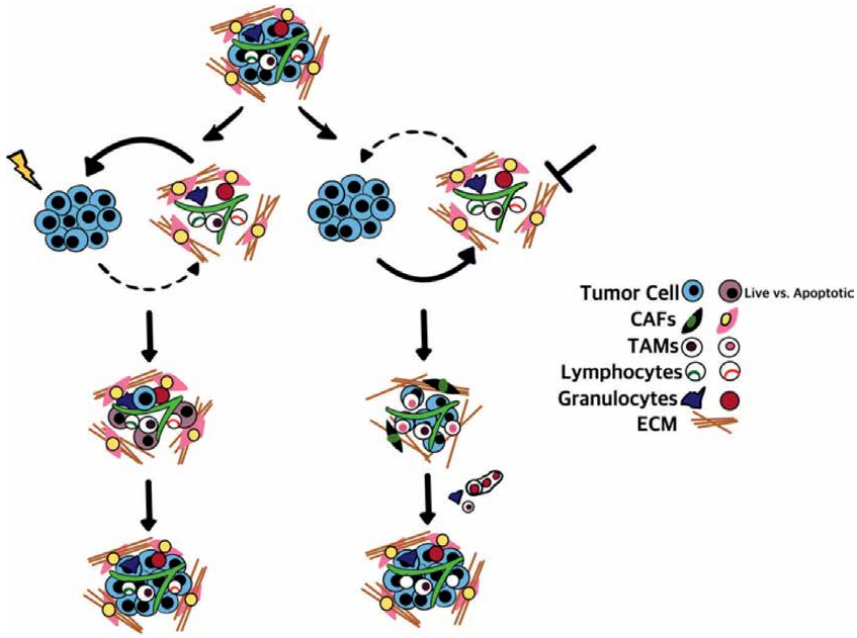


**Figure 4.**
*A schematic diagram of targeting tumor cell or TME only and their potential resistance mechanisms. Left: Targeting tumor cells only (such as chemotherapies) kills majority tumor cells. However, the residue tumor cells may survive due to the TME, leading to tumor relapse. Right: Targeting the TME can inhibit the recruitment and activation of pro-tumor cells and enhance antitumor responses. However, the TME will be reconstituted by tumor cells via recruitment and programming of bone marrow derived cells or local resident stromal or immune cells [6].*

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## Conflict of interest

The authors have declared that no conflict of interest exists.

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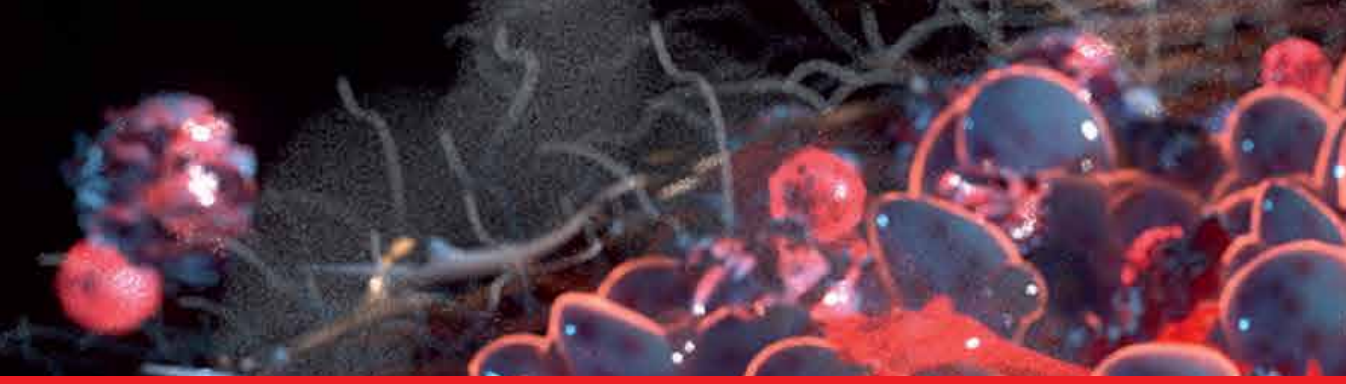

*Edited by Ahmed Lasfar*

This book offers outstanding approaches to understanding the role of the tumor microenvironment (TME) in cancer development and metastasis. The TME, with its multifaced role, is fundamental in the control and exacerbation of almost all cancer types. The outcome of many solid tumors is dependent on the modulation of the TME. Local tumor immunity, which is crucial in the control of cancer promotion, is one of the leading compounds of the TME. This book presents new insights and provides detailed and updated descriptions of the role of the TME in the control and the development of almost all cancer types. This book is an authentic source of knowledge, useful for researchers, medical doctors, students, and all individuals interested in understanding the mechanisms of cancer control and development.

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