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Review Article

Mechanisms of target therapy resistance in non-small cell lung cancer

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Abstract

In the past two decades, research on driver mutations has revolutionized lung cancer treatment with the emergence of targeted therapies as a new therapeutic strategy that significantly improves the prognosis of lung cancer. Targeted therapies are designed to recognize and disrupt specific proteins or pathways involved in the growth, spread and survival of cancer cells with high effectiveness. The use of targeted therapies has been shown to provide better progression-free survival and overall survival compared to traditional chemotherapy in NSCLC patients with targeted mutations. However, most patients eventually develop resistance regardless of the type and line of targeted therapy used. Resistance can occur in patients who initially respond targeted therapy. This is due to adaptive changes in tumor cells and the tumor microenvironment during drug exposure, through genetic and epigenetic processes forming secondary resistance. Understanding targeted therapies and their resistance mechanisms is essential to manage effective treatment for patients.

Keywords: *mutation, resistance, targeted therapy*

Abstrak

Dalam dua dekade terakhir, penelitian mengenai mutasi penggerak telah merevolusi pengobatan kanker paru dengan munculnya terapi target sebagai strategi terapeutik baru yang secara bermakna meningkatkan prognosis kanker paru. Terapi target dirancang untuk mengenali dan mengganggu protein atau jalur spesifik yang berperan dalam pertumbuhan, penyebaran dan kesintasan sel kanker dengan efektivitas yang tinggi. Penggunaan terapi target terbukti telah memberikan kesintasan bebas progresivitas dan kesintasan keseluruhan lebih baik dibandingkan kemoterapi tradisional pada pasien KPKBSK yang memiliki target mutasi. Namun, sebagian besar pasien akhirnya menunjukkan resistansi terlepas dari jenis dan lini pengobatan terapi target yang digunakan. Resistansi terjadi pada pasien yang awalnya merespons terapi target. Hal ini disebabkan oleh perubahan adaptif pada sel tumor dan lingkungan mikro tumor selama pajanan melalui proses genetik maupun epigenetik membentuk resistansi sekunder. Pengetahuan mengenai terapi target dan mekanisme resistansinya penting dipahami untuk dapat mengelola terapi efektif pada pasien.

Kata kunci: *mutasi, resistansi, terapi target*

Background

Cancer is the second leading cause of death in the United States and an unsolved global public health problem. In the United States, it is estimated that there will be 1,918,030 new cases of cancer and 609,360 cancer deaths by 2022 with lung cancer accounting for approximately 350 deaths each day and being the leading cause of cancer deaths. Statistically, lung cancer results in higher mortality than breast, prostate, colorectal and leukemia cancers combined. Lung cancer is one of the most life-threatening cancers worldwide with a five-year survival rate of only 19%, second only to pancreatic cancer.[1]

In general, lung cancer diagnoses are divided into two main groups: small cell carcinoma lung cancer (SCLC = KPKSK) and non-small cell carcinoma lung cancer (NSCLC= KPKBSK)). About 13% of cases are SCLC which is the more aggressive type with a lower five-year relative survival rate. others are NSCLC which accounts for 85% of all lung cancer diagnoses. NSCLC lung cancer can be further classified into adenocarcinoma with a five-year relative survival rate of 17%, large cell carcinoma has a five-year relative survival rate of 9% and squamous cell carcinoma a five-year relative survival rate of 14%.[2]

Current treatment options for lung cancer include surgical resection, chemotherapy, radiotherapy, targeted therapy and immunotherapy. In the last two decades, research into driver mutations has revolutionized the treatment of lung cancer with the emergence of targeted therapy as a new therapeutic strategy significantly improving the prognosis of lung cancer. In contrast to traditional chemotherapy that attacks rapidly dividing cells in general, targeted therapies are designed to recognize and disrupt specific proteins or pathways that play a role in cancer cell growth, spread and survival.[3,4]

In the last two decades, the identification of genetic alterations that regulate tumor growth and survival has significantly changed the therapeutic pathway of JPBSK based on its molecular characteristics. These genetic alterations become therapeutic targets. This is because tumor dependence on one or more oncogenic proteins or signaling pathways that result in tumor cell proliferation is usually regulated by driver mutations. Inhibition of these specific oncogenes can interfere with tumor growth so that the tumor stops growing or even shrinks and disappears. Drugs targeting these pathways have been developed and some of them have been clinically approved for NSCLC patients due to their high response rate and better specificity compared to standard chemotherapy.[5]

Targeted therapy first emerged with the discovery and development of tyrosine kinase inhibitors (TKIs) that target mutations in the epidermal growth factor receptor (EGFR). Successful therapy using first-generation TKIs, such as gefitinib and erlotinib as well as second-generation (afatinib, dacomitinib) has consistently shown high efficacy in the treatment of JPBSK with EGFR mutations. Furthermore, the identification of genetic rearrangements, such as anaplastic lymphoma kinase (ALK) and c-ros oncogene 1 (ROS1) has enabled the development of more specific and effective targeted therapies, such as crizotinib and alectinib.[6]

Despite extensive research, the prognosis of lung cancer remains disappointing with a five-year survival of around 15%. This is partly due to the development of drug resistance in the cancer tissue. Understanding the mechanisms of drug resistance provides an opportunity to develop more effective therapeutic strategies, including drug combinations, next-generation development and personalized approaches by adjusting the genetic profile of individual patients. Therefore, continuous research and multidisciplinary collaboration are necessary to translate scientific findings into effective interventions.[6,7]

Targeted Therapy Type

Targeted therapy is a treatment approach designed to target specific molecules or biological pathways involved in cancer growth and development. This approach is based on understanding the role of specific genetic mutations or molecular changes to be the main driver of tumor growth in contrast to chemotherapy which works by attacking all rapidly dividing cells including normal cells. Drugs are specifically designed to interfere with or inhibit the activity of such target molecules thereby stopping or slowing the growth of cancer cells with high effectiveness using targeted therapy.[3]

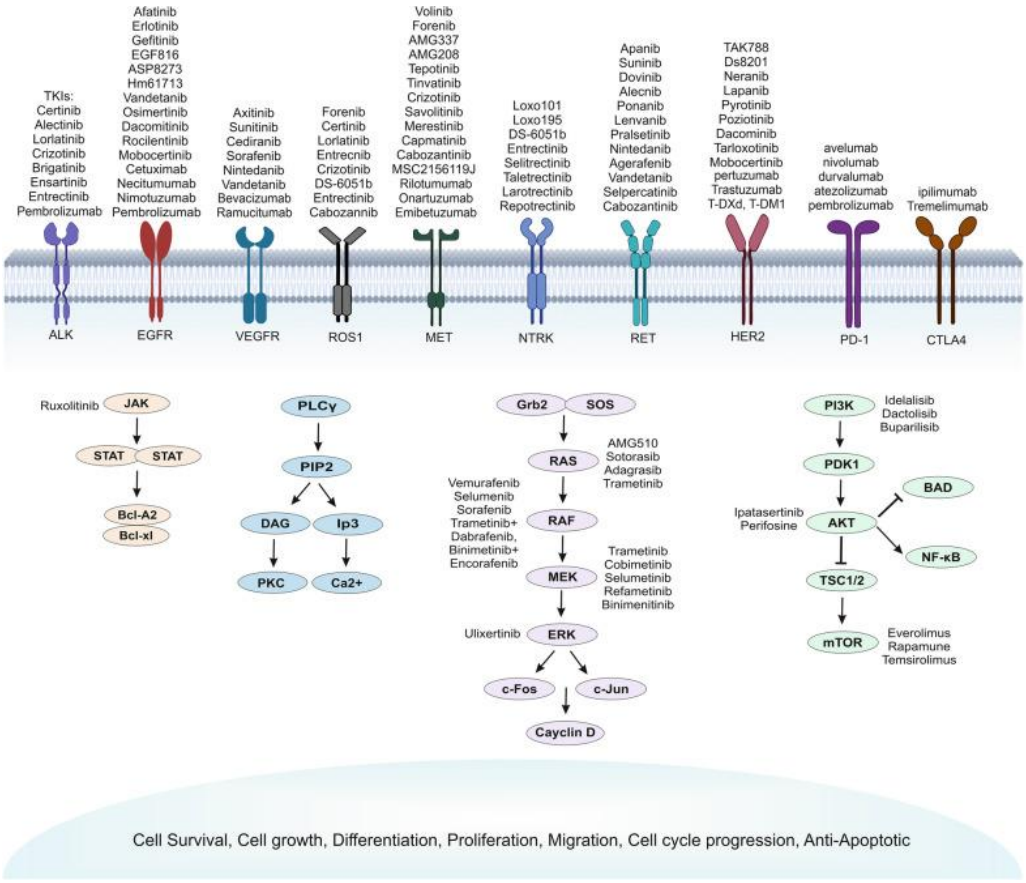

Figure 1. Target therapy[6]
Source: Araghi M, Mannani R, Heidarnejad maleki A, Hamidi A, Rostami S, Safa SH, et al. Recent advances in non-small cell lung cancer targeted therapy: an update review. Vol. 23, Cancer Cell International. BioMed Central Ltd; 2023.

By understanding and identifying specific genetic and molecular changes in cancer cells, these therapies can directly target pathways that are important for cancer growth or survival. This not only improves the effectiveness of treatment, but also reduces damage to normal cells, reduces side effects and improves the quality of life for patients. Targeted therapies have brought about revolutionary changes in the clinical management of lung cancer by providing more targeted treatment options and improving patient life expectancy. Some examples of targeted therapies in lung cancer include inhibition of EGFR, ALK, ROS1 and v-Raf murine sarcoma viral oncogene homolog B1 (BRAF).[3]

EGFR Inhibitors

EGFR mutation is the most common driver mutation in NSCLC. This mutation occurs in about 16% of adenocarcinoma patients. Currently, the use of targeted therapy in the form of TKIs has been widely used with a response of 50%-80%. The efficacy of TKIs varies based on the driver mutation. Mutations in exons 19 and 21 account for 85%-90% of all EGFR mutations and are considered to have a good response to TKIs. In contrast, patients with exon 20 mutations that occur in about 4% of EGFR mutations do not respond to TKIs.[8]

First-generation EGFR-TKI therapies work reversibly and compete with ATP for binding to the EGFR tyrosine kinase domain. In 2015, first-generation EGFR-TKIs, namely gefitinib, erlotinib and icotinib have been used as the first line of NSCLC therapy with EGFR mutations. The 2009 Iressa Pan-Asia study comparing gefitinib with chemotherapy using carboplatin and pacliataxel conducted in East Asia concluded that first-generation TKIs provide better progression-free survival (PFS) than chemotherapy.[9] Similar results were also obtained from the 2012 European Tarceva versus Chemotheraphy study conducted in Europe. In this study erlotinib was proven superior compared to platinum-based chemo-therapy.[10]

Second-generation EGFR TKIs, afatinib and dacomitinib, were then developed to combat first-generation resistance. Afatinib is an irreversible dual-specificity EGFR inhibitor designed to bind covalently to both EGFR and human epidermal growth factor receptor (HER2), while dacomitinib is an irreversible pan-HER inhibitor. The broad spectrum of activity allows second-generation EGFR-TKIs to improve tumor growth inhibition compared to first-generation EGFR-TKIs.[1]

In the 2017 ARCHER study, dacomitinib, a first-generation EGFR inhibitor, significantly prolonged PFS and overall survival time compared to gefitinib in CKD patients with EGFR activating mutations.11 However, afatinib and dacomitinib showed low maximum tolerated doses so that the second generation was reported to cause more frequent and severe side effects including skin and gastrointestinal toxicity. After receiving first- and second-generation EGFR-TKI therapy for 9-13 months, most patients develop resistance. The T790M mutation in exon 20 is the most common mechanism of resistance, accounting for 50%-60% of patients treated with first- and second-generation TKIs.[1,11,12]

A third-generation EGFR-TKI therapy was then developed to counter the previous generation of resistance, Osimertinib, which has good efficacy against secondary resistance. Osimertinib selectively targets the T790M mutation by covalently binding to residue C797 at the adenosine triphosphate (ATP) binding site on the EGFR receptor. The ATP molecule cannot bind and becomes inactive because it is irreversible. Besides being used for the T790M mutation, Osimertinib is also used for the L868R mutation. Osimertinib shows good tolerability with lower toxicity reports compared to previous generations. Osimertinib also shows about 200 times greater potency against EGFR L858R or T790M mutations compared to wild-type EGFR.[1]

Table 1. Target Therapy of EGFR TKIs in NSCLC

Class	Medicine	EGFR Sensitization Mutation	EGFR Binding
First Generation	Gefitinib	Deletion 19/L858R	Competitive Reversible
	Erlotinib	Deletion 19/L858R	
	Icotinib	Deletion 19/L858R	
Second Generation	Afatinib	Delesi 19/L858R/T790M	Covalent Irreversible
	Dacomitinib	Delesi 19/L858R/T790M	
Third Generation	Lazertinib	Delesi 19/L858R/T790M	Covalent Irreversible
	WZ4002		
	Rociletinib		
	Osimertinib		
	Olmutinib		
	Avitinib		
	Nazartinib		
	Maverletinib	Delesi 19/L858R/T790M	
	Naquotinib	Delesi 19/L858R/T790M	
	Almonertinib	Delesi 19/L858R/G719X/L861Q/T790M	
	Alflutinib	Delesi 19/L858R/G719X/L861Q/T790M	
Fourth Generation	EAI1001	L858R/T790M/C797S	Allosteric Reversible
	EAI1045	L858R/T790M/C797S JBJ-09-063	
	JBJ-09-063	L858R/T790M/C797S	
	BLU945	L858R/T790M/C797S	Unknown
	BBT176	L858R/T790M/C797S	

Source: Chhouri H, Alexandre D, Grumolato L. Mechanisms of acquired resistance and tolerance to EGFR targeted therapy in non-small cell lung cancer. Cancers. 2023;15:2- 18

In patients who progressed on first-generation EGFR-TKI treatment and harbor the T790M mutation, osimertinib significantly improved PFS and OS compared with chemotherapy. Osimetinib has shown better efficacy than gefitinib or erlotinib in NSCLC who have not received EGFR-TKIs. AZD9291 (Osimertinib) versus platinum-based doublet-chemotherapy in locally advanced or metastatic non-small cell lung cancer research (AURA3) compared the efficacy of osimertinib in patients receiving osimertinib. Patients given osimetinib had a lower incidence of serious adverse events than those treated with first- or second-generation EGFR- TKIs.[13] However, patients with EGFR activating mutations who were given

osimertinib as first-line treatment also eventually developed resistance. [1,13,14]

Despite the favorable outcomes of targeted therapies compared to conventional chemotherapy, their effectiveness is often limited by the emergence of secondary resistance. One of the most common mechanisms of resistance is the emergence of the T790M mutation in EGFR. This mutation occurs in approximately 50%-60% of patients who initially respond to first and second generation TKI therapy. The T790M mutation occurs when the amino acid threonine at position 790 in the EGFR tyrosine kinase domain changes to methionine. This mutation causes resistance to first- and second-generation EGFR by reducing the drug's affinity for EGFR.[8]

ALK Inhibitors

One of the molecular receptors targeted by targeted therapy is alteration of the anaplastic lymphoma kinase (ALK) gene encoding the fusion-driving oncoprotein. Mutations and rearrangements in the ALK gene that result in abnormal fusion proteins have been identified as the main driver in about 5% of NSCLC cases. ALK inhibitors were then developed to target and inhibit the abnormal activity of the ALK fusion protein. Crizotinib, a first generation ALK inhibitor showed good efficacy in inhibiting the proliferation of ALK positive tumor cells and improving FS compared to chemotherapy. However, resistance to crizotinib is often found through secondary mutation mechanisms in the ALK kinase domain or activation of alternative signaling pathways. To overcome this, second- and third-generation ALKs such as ceritinib, alectinib and lorlatinib were developed and are now widely used.[15,16]

There are currently five ALK tyrosine kinase inhibitors that have been widely used to treat ALK-positive NSCLC. These drugs include the first generation ALK-TKI crizotinib, the second generation ceritinib, alectinib and brigatinib and the third generation lorlatinib. Although the latest generation ALK-TKIs have better kinase selectivity and higher ability to overcome drug resistance, resistance is inevitable. Most of the resistance mechanisms to ALK-TKIs are dependent on ALK protein kinases. ALK-dependent resistance mechanisms including gene amplification and secondary mutations in ALK kinases account for half the incidence of resistance to crizotinib, including mutations in the L1196M, L1152R and G1202R genes. These mutations alter the ALK protein to become active without the need for stimulation, increasing binding affinity with ATP and leading to drug resistance.[3]

Table 2. Target Therapy of ALK TKI in NSCLC

Generation	Medicine	*Objective Response Rate* (ORR)	Median PFS (Months)
First	Crizotinib	74%	10.9
Second	Ceritinib	73%	16.6
Second	Alectinib	83%	25.7
Second	Brigatinib	74%	24
Third	Lorlatinib	76%	NR
Third	Ensartinib	75%	25.8

Source: Wu J, Lin Z. Non-small cell lung cancer targeted therapy: Drugs and mechanisms of drug resistance. Int J Mol Sci. 2022;23:1-18.

Secondary mutations in the ALK kinase domain are more common in patients with secondary resistance to newer generation ALK inhibitors, accounting for approximately 50%-70% of resistant cases compared to primary ALK resistance. One of the secondary mutations that often occurs after administration of first-generation ALK-TKIs is mutations in the G1202R gene. G1202R gene mutations account for about 42% of brigatinib-resistant cases, 21% of ceritinib-resistant cases and 29% of resistant cases in patients taking alectinib.

These mutations result in alterations in the adenosine triphosphate (ATP) binding domain of the ALK receptor, which reduces the affinity of the TKI to the target, thereby reducing the effectiveness of the drug.[1,17]

The third generation ALK inhibitor lorlatinib is a small macrocyclic molecule with good brain permeability. Lorlatinib can inhibit most of the first and second generation ALK inhibitor-resistant mutations especially the G1202R mutation. Clinical data showed lorlatinib produced an objective response of 45% including in ALK-positive patients who already had secondary resistance. Lorlatinib was approved by the Food and Drug Administration in 2018 as second-line therapy for ALK-positive NSCLC. However, lorlatinib drug resistance is also inevitable. Studies show that about 35% of the resistance mechanisms to lorlatinib are combined mutations of two or three gene sites, such as I1171N + L1198F, G1202R+ L1204V+ G1269A.[18]

C-ros Oncogene 1 (ROS1) Inhibitor

The ROS1 gene is a gene encoding one of the receptor tyrosine kinases located at 6q22 on the long arm of chromosome six. This gene belongs to the insulin receptor family that plays a role in cell growth

and proliferation. In NSCLC mutation or rearrangement of the ROS1 gene leads to constitutive activation of signaling pathways that promote cancer cell growth and survival. This mutation or rearrangement is in the form of a gene fusion, where the ROS1 gene joins with other genes produce a constitutively active fusion protein. One example of a gene fusion that often occurs is CD74-ROS1, which is a merger between the CD74 gene and ROS1.[19]

Expression of the ROS1 fusion gene results in autophosphorylation of the tyrosine kinase ROS-1 which initiates a signal cascade through the Mitogen-Activated Protein Kinase (MAPK) pathway and phosphorylation of RAS. Phosphorylation is the addition of phosphate groups into the structure of a protein. As a result, the receptor tyrosine kinase is constitutively active which means the protein will transmit a signal pathway for the cell to proliferate without the need for external ligands. Activated signaling pathways include Mitogen-Activated Protein Kinase/Extracellular signal- Regulated Kinase (MAPK/ERK) which is important for the regulation of cell growth, differentiation and cell survival. Phosphorylation of ERK proteins in this pathway promotes transcription of genes that support proliferation and inhibit apoptosis.[19-21]

There is also activation of the phosphoinositide 3-Kinase/ Protein kinase B (PI3K/AKT) signaling pathway. The PI3K/AKT pathway is dysregulated due to mutation or amplification of the gene encoding the receptor protein. As a result, the signal is continuously activated without a trigger. Activation of this pathway will result in rapid, uncontrolled cancer cell division and cancer cells are more resistant to apoptosis. Cancer cells will create metabolic conditions favoring cancer cell growth and survival and will also increase the ability of cancer cells to spread to other tissues.[19,21-23]

A similar mechanism is also obtained with the activation of the Janus Kinase/Signal Transducer and Activator of Transcription (JAK/STAT) signaling pathway, a biochemical reaction that starts with the activation of cytokine receptors on the cell surface and culminates in the activation of STAT transcription factors in the nucleus. This pathway plays an important role in regulating the expression of genes involved in various cellular processes. Proliferation of cancer cells will be increased, apoptosis inhibited and increase the ability of cancer cells to metastasize. The JAK/STAT pathway has a significant role in various processes of cancer development, so it can be an important target in the development of cancer therapy. JAK inhibitors are currently being developed and to inhibit this pathway and reduce cancer progression.[19-22]

ROS 1 inhibitors are targeted therapies designed to inhibit ROS1 protein kinase activity. ROS1 inhibitors work by binding to the kinase domain of the rearranged ROS1 protein, inhibiting its catalytic activity and preventing autophosphorylation. With the ROS1 receptor inhibited, ERK activation can be prevented thereby stopping the signal cascade of the MAPK/ERK signaling pathway so that the proliferation ability of cancer cells is reduced. addition, the PI3K/AKT pathway will also be disrupted due to the failure of the autophosphorylation results in binding sites for adaptor proteins, such as Growth Factor Receptor-Bound Protein 2 (GRB2) or GRB2-Associated Binding Protein 2 (GAB2) becoming inactive so that the mechanism of and proliferation of cancer cells is inhibited and cancer cells are more easily apoptotic.[19,21]

There are several approved tyrosine kinase inhibitors that inhibit both ROS1 and ALK, including crizotinib, ceritinib and lorlatinib. There is also entrectinib which inhibits both ROS and neurotrophic tyrosine receptor kinase (NRTK). Crizotinib was the first drug used in patients with ROS1 mutations. Duration of Response (DOR) was 72% (95% CI, 58%-84%) with a median duration of response of 17.6 months and median PFS of 19.2 months. Despite the high initial response rate, most crizotinib- treated patients eventually experienced disease progression due to inadequate central nervous system (CNS) penetration or the development of ROS1 resistance mutations.[1,19]
Another drug that has also been widely used is ceritinib, a second-generation TKI given to TKI-naive and crizotinib-resistant ALK-positive NSCLC patients. Ceritinib selectively inhibits ROS1 with 20 times more potency than crizotinib in preclinical studies. Clinical trials showed response in crizotinib-naive patients ORR 67% (95% CI, 48-81%) DOR for 21 months (95% CI, 17-25 months) and PFS 19.3 months (95% CI, 1-37 months). There was also lorlatinib with strong CNS penetration. The response of lorlatinib was significantly higher in TKI-naive patients with an ORR of 62% (95% CI, 38%-82%) PFS of 21 months (95% CI, 4.2-31.9) compared to those with crizotinib ORR of 35% (95% CI, 21%-52%) and PFS of 8.5 months (95% CI, 4.7-15.2). In patients with intracranial metastases, intracranial responses were achieved in 64% of naïve patients and 50% of patients who had received crizotinib.[19]

Resistance Mechanism

Targeted therapy has been shown to provide better PFS and OS in patients with CKD. However, most patients eventually show resistance regardless of the type and line of treatment used. The spatiotemporal heterogeneity of tumors facilitates the complexity and diversity of molecular resistance mechanisms. Resistance to targeted therapy is divided into two groups, namely innate or primary resistance and secondary resistance resulting from the adaptive capacity of the body and cancer cells to target therapy exposure. Each consists of tumor intrinsic and extrinsic mechanisms.[23,24]

Primary Resistance

Primary resistance is the innate resistance possessed by the tumor as well as by the individual. The development of resistance occurs before the tumor is exposed to the targeted therapy. Primary resistance is caused by mutations of both driver and passenger genes. This results in decreased treatment potency. Intrinsic primary resistance is where the tumor does not respond to the target therapy from the start of treatment. Primary resistance is mostly related to the lack of sensitive target receptor sites, or the cancer has the ability to activate alternative signaling pathways that can replace the function of the inhibited target protein. For example, EGFR exon 20 or KRAS mutations can provide enough signal to support tumor growth even though the EGFR receptor is inhibited with EGFR-TKIs.[25]

In primary resistance, tumor extrinsic factors may also play a role. Extrinsic factors refer to mechanisms originating from the tumor microenvironment or external factors that affect the effectiveness of the target therapy. Extrinsic factors include ineffective antigen presentation, T cell priming, activation, transport and migration or the presence of suppressive immune cells in the tumor microenvironment that are not targeted by immunotherapeutic agents. Tumor extrinsic mechanisms of primary resistance include mutations in genes that harbor immune regulatory function resulting in reduced neoantigen presentation and impaired antitumor immune responses.[25,26]

Secondary Resistance

Acquired resistance occurs when patients who initially respond to targeted therapy later relapse and develop resistance. This can be caused by adaptive changes in tumor cells and the tumor microenvironment during treatment exposure through epigenetic or translational events that establish secondary resistance. Secondary resistance may result from clonal evolution of tumor cells that acquire specific genetic changes that interfere with the antitumor immune response. Response rates and tumor control rates to EGFR-TKIs effectively do not reach 100% but range from 75%-80% regardless of drug generation; some patients respond only for a very short initial duration (<3 months). A special situation of secondary resistance is pharmacokinetic therapy failure.[28]

The main mechanisms of tumor cell intrinsic activity that led to secondary resistance to targeted therapy are resistance, activation of alternative pathways and tissue transformation. Target resistance and alternative pathway activation are acquired through genetic alterations. Both mechanisms depend on the core signaling pathways of the driving oncogene. Examples are secondary kinase mutations and amplification of driver genes. Tissue transformation or phenotypic change mechanisms are forms of phenotypic plasticity that involve cellular reprogramming. These mechanisms cover a wide spectrum, ranging from transient phenotypic changes to full transition into a new histologic subtype, such as the change of NSCLC to SCLC. [4,27]

Molecular Resistance Mechanism

One of the best known and genomically simplest mechanisms of resistance to targeted therapies is genetic alterations in driver oncogenes that allow the continuation of tumor cell signaling pathways despite exposure. This condition is often also described as therapeutic resistance. The two most common manifestations of secondary resistance in JPBSK are T790M kinase domain mutations and overexpression of target oncogenes in tumor cells. Resistance mechanisms in the form of genetic alterations are seen in response to almost every targeted therapy in NSCLC starting from first generation inhibitors.[4]

T790M Mutation

The T790M mutation is the main secondary resistance mechanism that occurs in patients with EGFR-TKIs. In cancers with EGFR mutations, the kinase domain of EGFR undergoes structural changes that result in the tumor being more active in sending signals for cancer cell growth and proliferation. Targeted therapy TKIs bind to the EGFR kinase domain at the ATP binding site, preventing ATP from binding and activating the signaling pathway. Without ATP, EGFR cannot transfer phosphate to intracellular proteins that transmit signals, thus inhibiting cancer cell growth. The T790M mutation replaces the amino acid threonine (T) with methionine (M) at position 790 in the EGFR kinase domain which is located close to the ATP receptor site.[27]

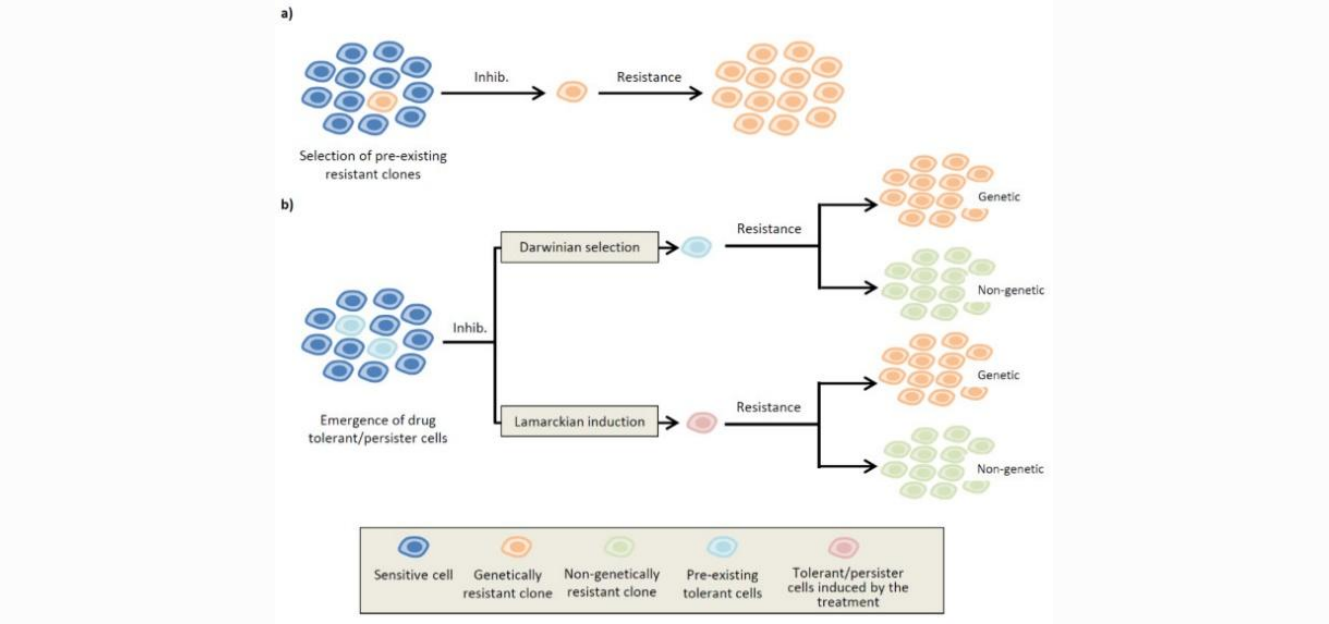

Figure 2. Clonal Selection Results in Resistance[28]
Source: Swayden M, Chhouri H, Anouar Y, Grumolato L. Tolerant/sister cancer cells and the path to resistance to targeted therapy. Cells. 2020;9:1-13

The T790M mutation causes structural changes around the ATP receptor site so that the affinity of EGFR for ATP increases. Although TKIs try to bind to the kinase domain, ATP competes more easily and binds to the receptor due to its higher affinity. As a result, EGFR remains active in sending growth signals leading therapy resistance. Tumor cells with the T790M mutation start because they are able to survive and continue to grow despite continued targeted therapy. The number of resistant cells increases and the tumor size increases again or stops shrinking.[27]

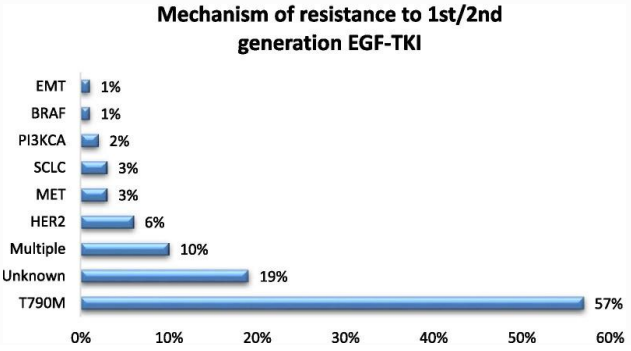

Figure 3. Secondary Resistance Caused By 1st and 2nd Generation EGFR-TKIs[21]
Source: Majeed U, Manochakian R, Zhao Y, Lou Y. Targeted therapy in advanced non-small cell lung cancer: Current advances and future trends. J Hematol Oncol. 2021;14:1-20

MET Amplification

MET amplification mechanism is one of the secondary resistance responses in EGFR- TKI administration. MET amplification is an important mechanism in resistance to targeted therapy in lung cancer. MET protein is a receptor tyrosine kinase involved in various biological processes including cell growth, differentiation and proliferation. Activation of MET by its ligand, hepatocyte growth factor (HGF) leads to the activation of intracellular signaling pathways that are important for the regulation of various cellular functions. Amplification of the MET gene leads to an increase in the number of MET receptors expressed on the cell surface which in turn can result in excessive signal activation. MET amplification occurs in approximately 10%-15% of patients with lung cancer who have received erlotinib, gefitinib or afatinib. MET amplification remains a major resistance mechanism in approximately 15% of patients with failure of first-line osimertinib therapy.[29]

MET amplification may occur as a mechanism of resistance to EGFR inhibitors, such as gefitinib or erlotinib. MET activation through gene amplification can trigger downstream signaling pathways independent of the EGFR pathway. MET amplification causes resistance by continuously activating signaling pathways, such as those regulated by MAPK, STAT and PI3K/AKT independent of EGFR activation or

signaling. These signals are relayed through the two adaptors HER3/ERBB3 when MET is triggered by genomic amplification or growth factor receptor associated binding protein 1 (GAB1) when MET is activated by HGF. Consequently, MET can trigger resistance by activating signaling pathways independent of EGFR through interactions with specific adaptors depending on its activation mechanism.[29]

The AURA3 study showed that MET amplification was the most common mechanism of resistance (19%), often co-occurring with the EGFR C797S mutation and possibly associated with CDK6 and BRAF amplification.[13] MET amplification can also occur as a mechanism of resistance to third-generation TKIs, either with or without loss of the T790M mutation. Osimertinib, although effective against the T790M mutation, is not designed to address MET amplifycation. Therefore, detection of MET amplification in patients who experience disease progression during therapy with osimertinib or other EGFR-TKIs is crucial.[13,29]

Activation Of Alternative Pathways

Activation of alternative signaling pathways refers to the condition that tumor cells develop mechanisms to avoid the inhibitory effects of targeted therapies by activating other cellular signaling pathways. This activation may occur as a mechanism of acquired resistance to EGFR-TKIs, ALK-TKIs, ROS1 inhibitors and other targeted therapies. When tumor cells are administered targeted therapies that target signaling pathways, cancer cells can adapt by activating other pathways that promote cell survival and proliferation independently. One common example is the activation of the MET signaling pathway known as hepatocyte growth factor receptor, which can compensate for the inhibited EGFR pathway and support tumor growth.[25]

Alternative pathway activation caused by ALK-TKI administration includes mutations in the G1269A, C1156Y, I1171T/N/S, S1206C, E1210K, L1152P/R, V11180L, G1128A, F1174V and L1196M genes. Patients with EGFR-TKI resistance, ROS-1 inhibitors and ALK-TKIs show similarities in that there can be recurrent mutations in RTK-KRAS (EGFR, KRAS, BRAF), TP53 and other genes in TKI- independent pathways. These genes will function as downstream mediators or alternative signaling pathways so that cancer cells can still metabolize, avoid apoptosis and proliferate even though the main pathway is inhibited by targeted therapy.[1]

Phenotypic Changes

One of the resistance mechanisms that can occur is phenotypic changes, especially through epithelial to mesenchimal transition (EMT) and transformation.

This change in phenotype allows cancer cells to avoid the inhibitory effects of targeted therapies and still proliferate and metastasize. The mechanism of EMT is the biological process of epithelial cells losing their epithelial characteristics, such as strong cell-cell adhesion and cell polarity and acquiring mesenchymal properties, including increased migration, invasion power, resistance to apoptosis as well as the ability to differentiate into various other cell types. These properties make EMT play a key role in tumor progression and metastasis.[28]

In the EMT mechanism, there are changes in the expression of genes that regulate cell properties, there is a decrease in the expression of epithelial cell proteins including E- cadherin which forms a complex with beta-catenin and other proteins to mediate the adhesion of epithelial cells. E-cadherin proteins also maintain epithelial cell polarity which is important for structural defense and cellular communication. The EMT process is also accompanied by increased expression of N-cadherin proteins. N- cadherin protein is a mesenchymal cell protein that functions for mesenchymal cell adhesion. The resulting bonds from N-cadherin proteins are looser so that cells can detach from the tissue and migrate in the extracellular matrix. The decreased intercellular density caused by the loss of E-cadherin which is replaced by N-cadherin allows the cells to become invasive and more therapy-resistant.[6]

There is one other protein that contributes to the EMT process, and that is vimentin. Vimetin is an intermediate filament protein that is a key marker of mesenchymal cells. Vimentin plays an important role in cell mobility, supporting cell shape changes necessary for migration and invasion. This protein interacts with the cytoskeleton and modifies the cytoskeleton so that cells have the structural flexibility needed for migration. Vimentin concentrations are very low early in tumor development, but increase as the tumor spreads, so elevated vimetin levels are also associated with poor prognosis in malignancies.[30,31]

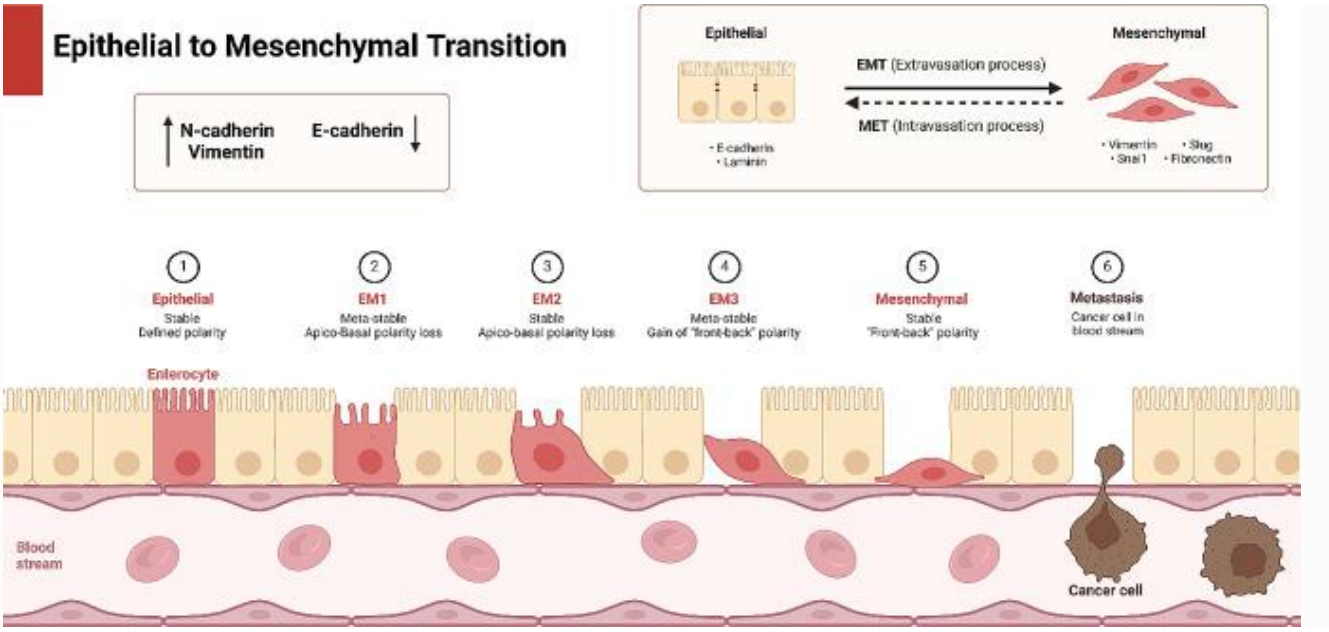

Figure 4. Epithelial to Mesenchymal Transition[32]
Source: Ashrafizadeh M, Dai J, Torabian P, Nabavi N, Aref AR, Aljabali AAA, et al. Circular RNAs in EMT-driven metastasis regulation: Modulation of cancer cell plasticity, tumorigenesis and therapy resistance. Cell Mol Life Sci. 2024;81:1-25.

In addition to EMT, cancer cells also have the ability to change into other histologic types, also known as histologic transformation, for example the transformation of NSCLC into SCLC. Histologic transformation into SCLC was first reported in America in 2006 in a woman diagnosed with adenocarcinoma. The patient was given erlotinib with partial response. At month 18, tumor progression occurred in the form of tumor metastasis in the brain. The second biopsy showed SCLC with positive synaptomycin. At autopsy, it was found that the SCLC had metastasized to several organs without any adeconarcinoma tissue.[33]

Histological changes caused by continuous exposure to the targeted therapy led to clonal selection. Cells that do not have the target therapy receptor will tend to survive and replicate to become dominant in the tumor cell population. Among these surviving cells are often cells with mutations of the tumor protein p53 (TP53) and retinoblastoma 1 (RB1) genes, which are part of the tumor suppressor gene family. The TP53 gene acts as a guardian of the genome by controlling DNA repair and apoptosis. Mutation or loss of TP53 function causes cancer cells to avoid apoptosis resulting in uncontrolled proliferation. RB1 gene expression functions as an inhibitor of cell proliferation by regulating G1 phase to S phase in the cell cycle by binding to and inhibiting transcription factors.[31]

There is also a role for the transcription factor Achaete-Scute Complex-Like 1 (ASCL1) in regulating the expression of genes involved in the differentiation of neuroendocrine cells. ASCL1 factor is highly expressed in neuroendocrine tumors, including SCLC. This process is driven by signaling pathways such as TGF- β/SMAD. Signals from TGF-β induce phosphorylation of Sma and Mad-related protein 2 (SMAD2) which then forms a complex with SMAD4. This complex moves to the nucleus and binds to the promoter of the ASCL1 gene thereby increasing its transcription. Increased ASCL1 expression leads to a change in phenotype from epithelial NSCLC cells to a neuroendocrine phenotype that is characteristic of NSCLC. These transformed cells exhibit more aggressive properties and are resistant to targeted therapies, such as EGFR-TKIs.[31,34]

Epigenetic Changes

Cancer has traditionally been viewed as a genetic disease, but recent studies have shown epigenetic changes play an important role in cancer development. Epigenetic changes are a major contributor to transcriptional heterogeneity, causing changes in the expression of key oncogenes and tumor suppressor genes, affecting various signaling pathways. Epigenetic changes are a major contributor to transcriptional heterogeneity, causing changes in the expression of

key oncogenes and tumor suppressor genes thereby affecting various signaling pathways. Major epigenetic regulation mechanisms include DNA methylation, histone modification, regulation by non-coding RNA and chromatin remodeling.[35]

DNA Methylation

DNA methylation involves the addition of methyl groups to cytosine residues in nucleotides that cause suppression of gene expression. In NSCLC, methylation of tumor suppressor gene promoters can result in loss of expression of the gene, allowing cancer cells to evade normal growth control. DNA methylation mainly occurs at CpG dinucleotides (concentrated in dense regions called CpG islands) that inhibit RNase binding to the gene strand thus leading to the deactivation of the corresponding gene. DNA methylation is a normal condition that occurs with age. However, DNA methylation is controlled by DNA methyltransferase (DNMT) and DNA demethylase (TET).[35]

In the mechanism of cancer progressivity, there is also a decrease in DNMT expression. Reduced expression of DNMT1 inhibits lung cancer cell growth in vitro and in vivo, while low expression of DNMT3A is associated with poor prognosis. The relationship between overall DNA methylation changes and EGFR-TKI response was illustrated in a study involving 79 patient subjects with adenocarcinoma before and after EGFR-TKI administration conducted by Fang Su et al in China in 2021. The researchers identified 216 CpG sites whose methylation differed in EGFR-TKI responding and non-responding patients. Most of the probes (203/216) showed higher DNA methylation in non-responders compared to responders. The findings suggest that DNA hypermethylation correlates with poor EGFR-TKI response.[35-37]

Histone Modification

Histone modifications are chemical changes that occur in histone tails, including acetylation, methylation and phosphorylation. These modifications affect chromatin structure and the regulation of gene expression, playing an important role in cancer development and resistance to therapy. Acetylation is the addition of an acetyl group (CH_3CO) to the lysine in the tail of a histone. Acetylation will reduce the positive charge on the histone resulting in a weakening of the histone's bond to negatively charged DNA. As a result, DNA is more accessible to transcription factors and gene expression increases, including oncogenes that play a role in tumor growth and resistance to targeted therapies.[35]

There is also the process of histone methylation, which is the addition of one or more methyl groups (CH_3) on lysine or arginine residues in the histone tail. This modification can change the architecture of the nucleosome, which consists of DNA wrapped around a histone. These modifications will affect how tightly or loosely the DNA is bound to the histone thus regulating the accessibility of those genes for transcription. Methylation occurs on lysine residues such as H3K4, H3K9, H3K27, H3K36 and H4K20, but also on arginine residues, such as H3R2, H3R8 and H4R3. These changes contribute to the complexity of genetic regulation and may influence the development of therapeutic resistance in cancer.[38]

In addition, histone modification in the form of phosphorylation also affects the replicative ability and resistance of cancer cells. Histone phosphorylation is a post- translational modification that involves the addition of phosphate groups to specific residues in histone proteins. Phosphorylation is carried out by protein kinases that add phosphate groups (PO_4^{3-}) on serine, threonine or tyrosine residues in the tail of histones. Phosphorylation often occurs on histone H3 at residues Ser10 and Ser28 and on H2AX at residue Ser139 known as γH2AX. These modifications can change the chromatin structure to be more open, allowing accessibility of transcription factors to the DNA and increasing transcription of genes especially progenitors.[38]

Non-coding RNA

Non-coding RNA (ncRNA) chains are RNAs that are not translated into proteins but have important functions in gene regulation including microRNA (miRNA), long non-coding RNA (lncRNA) and circular RNA (circRNA). Despite its very small size of about 22 nucleotides, miRNA can regulate the expression of oncogenes and tumor suppressor genes. MiRNAs can bind to messenger RNA (mRNA) that will be translated. MiRNAs that have bound to mRNA will be recognized by a protein complex called RNA- induced silencing complex (RISC). The recognized complex will then be cut by RICS so that there is no translation of gene. Some miRNAs, such as miR-21, miR-200c and miR-20a have been identified to suppress tumor suppressor genes and induce EMT.[39]

In addition to NSCLC, miR-214 upregulation in various tumor types, including ovarian cancer and esophageal squamous cell carcinoma promotes tumor progression and drug resistance. The miR-214

molecule may act as a biological courier that propagates resistance capabilities. Transfer of exosomal miR-214 from gefitinib- resistant cells to gefitinib-sensitive cells may confer a resistance phenotype that ultimately leads to resistance to gefitinib. However, the signaling pathway through which exosomal miR-214 confers resistance to gefitinib remains unidentified. Further research is needed to decipher the mechanism by which exosomal miR-214 mediates resistance to gefitinib.[39,40]

In addition to miRNAs, lncRNAs are also known to have longer nucleotides, namely 200-220 nucleotides, which have an important role in tumor development and resistance. Exosomes can carry lncRNAs in the exchange of information between cells, including in NSCLC patients. LncRNAs in exosomes in tumor patients can provide a picture of tumor progression and may be involved in changes in the tumor microenvironment. Studies show the expression of lncRNA in increased in osimertinib-resistant NSCLC patients compared to osimertinib-sensitive patients so lncRNA may be related to osimertinib resistance.[41]

One of the mechanisms of lncRNAs is by regulating the wingless untegration 1 (Wnt) signaling pathway that mediates the interaction between DNA, mRNA, miRNA and protein and thus directly plays a role in the initiation of tumor growth and its resistance to targeted therapies. In an in vitro study by, deletion of lncRNA urothelial carcinoma-associated 1 (UCA1) can reduce the likelihood of gefitinib resistance by inhibiting the signal transducer and activator of transcription 3 (STAT 3) signaling pathway in NSCLC. In addition, lncRNA UCA1 also interacts with the TGF-β signaling pathway which plays an important role in cancer cell migration and invasion. However, the mechanism of how Whether lncRNAs in exosomes contribute to target therapy resistance is not fully understood.[41]

Tumor Microenvironment

The tumor microenvironment is a complex environment around the tumor that consists of different cellular components, including tumor cells, T cells, B cells, dentritic cells, myeloid-derived suppressor cells (MDSCs), tumor-associated macrophages (TAMs), carcinoma-associated fibroblasts, tumor blood vessels, lymphatic tissue, fat cells, extracellular components, microvesicles, cytokines and chemokines. Under physiological conditions, these components are responsible for maintaining immunological homeostasis but can also trigger inflammation that leads to the formation of precancerous lung lesions and carcinogenesis. During the progression of SCI, changes occur in the TME that support inflammation and angiogenesis, modulation of the immune system which overall contributes to the progress of SCI, spread of metastasis and determination of prognosis.[42-44]

Under physiological conditions, the innate and adaptive immune systems function to detect and eliminate cancer cells. However, cancer cells can adapt and develop resistance to antitumor effects by disrupting the relationship between antagonistic effectors (CD8 cytotoxic) and regulatory T cells (CD4 Tregs). The resulting TME imbalance can aid cancer cell survival. In addition, tumor cells can excrete cytokines such as IL-4, IL-10, IL-13 and TGF-b resulting in TAM polarization. TAM polarization will encourage macrophages to become M2 which has anti-inflammatory and pro-tumor properties compared to M1 which has pro-inflammatory properties.[44]

M1 macrophages respond to infection and tissue damage. M1 macrophages produce proinflammatory cytokines such as TNF-α, IL-1β and IL-6 as well as reactive oxygen and nitrogen species (ROS and RNS) to eliminate pathogens and tumor cells. These cytokines stimulate the activity of T cells and natural killer (NK) cells that can attack and destroy tumor cells.

In addition, M1 macrophages have strong phagocytic ability and promote adaptive immune responses by enhancing antigen presentation to T cells. This ability makes patients with M1 polarization tend to have a better prognosis.[42,46,47]

M2 macrophages under physiological conditions play a role in wound healing, tissue regeneration and suppressing excessive immune responses. These macrophages secrete anti-inflammatory cytokines such as IL-10 and TGF-β as well as growth factors that support angiogenesis and tissue remodeling. TGF-β cytokines produced by M2 promote EMT making cancer cells resistant to targeted therapies with a high propensity for metastasis. In addition, M2 macrophages produce matrix metalloproteinases (MMPs) that remodel the extracellular matrix (ECM), allowing cancer cells to spread and increasing resistance to therapy. [42,46,47]

Polarization of TAMs to the M2 macrophage phenotype supports resistance to targeted therapies, tumor growth and metastasis by creating an immune-suppressive environment, supporting angiogenesis and reducing effector T cell activity so that TME becomes beneficial to cancer cells. Several studies have showed that a high number of TAMs and

polarization to the M2 phenotype were associated with poor prognosis and resistance to EGFR mutation in CKD patients. In addition to modulating TAMs, cancer cells can also modify ECM components and fibroblasts to provide favorable structural support for tumor cell growth. [42,46,47]

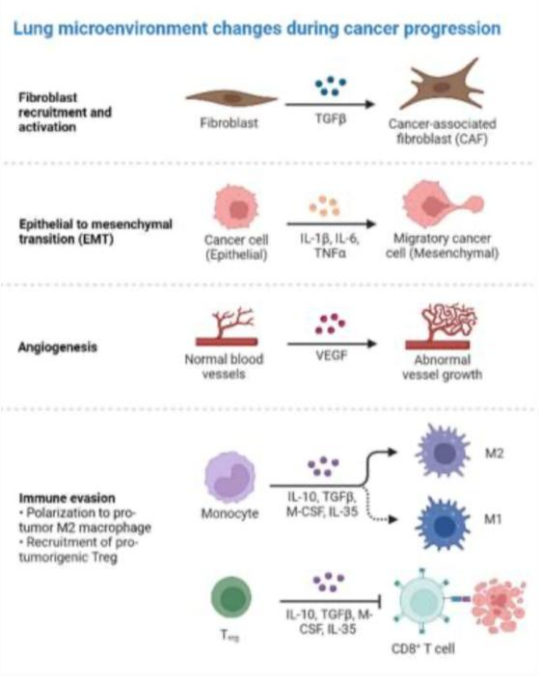

Figure 5. Changes in Tumor Microenvironment[42]
Source: Madeddu C, Donisi C, Liscia N, Lai E, Scartozzi M, Macciò A. EGFR-mutated non-small cell lung cancer and resistance to immunotherapy: Role of the tumor microenvironment. Int J Mol Sci. 2022;23:1-16.

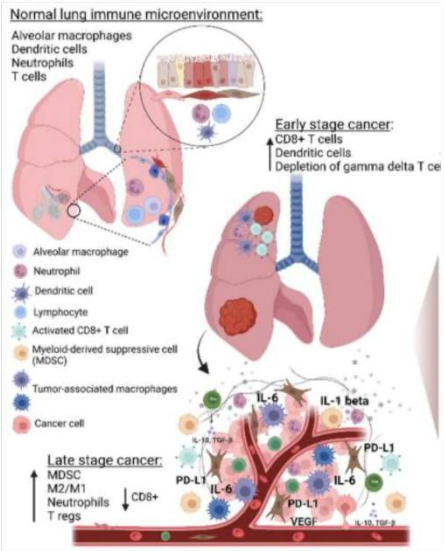

Figure 6. Changes in Tumor Microenvironment[42]
Source: Madeddu C, Donisi C, Liscia N, Lai E, Scartozzi M, Macciò A. EGFR-mutated non-small cell lung cancer and resistance to immunotherapy: Role of the tumor microenvironment. Int J Mol Sci. 2022;23:1-16.

Drug Efflux and Metabolism

Drug release mechanism is one of the important processes in the development of resistance to targeted therapy in patients with CKD. Drug expulsion is the mechanism by which cancer cells pump drugs out of cells, reducing the intracellular concentration of drugs and reducing the effectiveness of therapy. Previous studies have increasingly shown that multi drug resistant (MDR) genes in cancer mediate increased chemotherapy drug expenditure that reduces drug uptake by cancer cells. Increased drug release is accomplished by increases the regulation of protein transporters located in the cell membrane and regulates transporter expression so that the number of transporters increases.[22]

In cancer stem cells, there is an upregulation of four MDR family genes. The MDR1 gene, also called ATP Binding Cassette Subfamily B Member 1 (ABCB1) encodes the glycopotein-P (P-gp) protein, a Ca2+-dependent efflux pump. Due to the increase of P-gp, the target therapy that has entered the cell will be released back out of the cell quickly, thus reducing the intracellular accumulation of the drug and the desired effect of therapy cannot be achieved. This regulation does not only occur in targeted therapy, but also in alkaloid, paklitaxel and antibiotic chemotherapy. Up-regulation of MDR genes is usually followed by resistance to many types of drugs. Furthermore, using cRNA, MDR genes are transferred to other cells that are still sensitive to the target therapy.[22]

Target Therapy Resistance Implications

Evaluation of Treatment

Advances in targeted therapy have brought new hope for lung cancer patients, especially those with specific mutations that can be effectively intervened. Targeted therapies offer a more targeted approach in inhibiting the growth and spread of cancer cells. However, as these therapies advance, it is important to understand that patient response to therapy may vary. addition, cancer cells can develop resistance which must be monitored for cancer management navigation. Regular check-ups are crucial in the management of patients receiving targeted therapy.[24]

Periodic detection of resistance is required as one of the strategies to deliver targeted therapy. One of them is the examination of DNA strands released by tumor cells into the bloodstream identified as circulating tumor DNA (ctDNA). The ctDNA chain can provide

ctDNA analysis provides specific molecular information about tumor cell-derived DNA and the mutations that take place. ctDNA analysis from blood overcomes some of the obstacles faced by invasive tumor biopsies, allowing for easier, cheaper and periodic sampling of tumor DNA. Quantitative and qualitative analysis of ctDNA provides an immediate evaluation useful for diagnosis and prognosis. Therefore, ctDNA is a potential biomarker for detecting targetable mutations, monitoring therapy response and identifycation of novel drug resistance mechanisms in JPBSK.[45]

Another use of ctDNA analysis after administration of therapy is for quantitative assessment of drug effects by measuring ctDNA sequentially during treatment. A correlation between ctDNA changes and treatment effects has been demonstrated in several NSCLC studies. In addition, some studies have shown that ctDNA analysis can identify the emergence of drug resistance mutations several months before conventional radiographic imaging, providing a potential opportunity to improve or change therapy before clinical deterioration to achieve better clinical outcomes. However, to date there is no recommendation on what time interval molecular identification should be performed to detect resistance and the effectiveness of therapy.[45]

Strategy Against Resistance

The evolution of targeted therapies does not stop at the development of new drugs. Treatment approaches are now more focused on personalization by mapping the molecular profile of individual tumors to determine the most effective therapy. One of the resistance-fighting strategies being developed includes combining two or more targeted drugs that inhibit different signaling pathways to prevent cancer cells from developing resistance to a single pathway. Combination of targeted therapy with immunotherapy, combination of targeted therapy with chemotherapy and combination of targeted therapy with other targeted therapies are currently being vigorously conducted in clinical trials. [18,24,50]

Combination of EGFR TKI with Pyruvate Dehydrogenase Kinase

Glycolysis is the main way that cancers obtain energy from glucose, a process that greatly influences progression. Targeting one of the enzymes involved in the process, pyruvate dehydrogenase kinase (PDK) is one way to fight resistance that is currently being developed either given alone or in combination with other systemic therapies. The PDA inhibitor, dichloroacetate (DCA) has been developed to reduce cancer progressivity. Recent studies have shown that the combination of erlotinib and gefitinib with DCA provides a synergistic effect in the therapy of EGFR mutation- positive JPBSK.[47]

Combination of EGFR TKIs with Bcl-2 inhibitors

Overexpression of the antiapoptotic gene family Bcl-2 (consisting of Bcl-s, Bcl-XL and Mcl-1) accompanied by dysregulation of the proapoptotic gene family (including Bad, Bom, Bac and Bak) has been investigated to be one of the mechanisms of chemotherapy and radiotherapy resistance in lung cancer. This shows that Bcl-2 has the potential to be a therapeutic target. The Bcl-2 gene has four homologous domains, namely BH1, BH2, BH3 and BH4. Administration of BH3 mimic agents binds to the hydrophobic arm of Bcl-2 or Bcl-XL to be a competitive inhibitor. Gossypol (AT- 101) showed a pan-Bcl-2 inhibitor in in vitro and in vivo studies increasing gefitinib sensitivity in NSCLC with EGFR T790M mutation and increasing apoptosis in tumors.[47]

Immunotherapy

Immune checkpoint inhibitors are a class of drugs that inhibit immune control points (checkpoints) typically used by cancer cells to avoid detection and elimination by the immune system. The two most targeted checkpoints are programmed death 1 (PD- 1)/ Programmed death-ligand 1 (PD-L1) and Cytotoxic T-Lymphocyte Antigen (CTLA-4). In NSCLC this strategy has shown success in overcoming resistance to targeted therapies, such as EGFR- TKIs. Immunotherapy can currently be administered if a positive PD-1 or PD-L1 result is obtained on immunohistochemical examination. The combination of EGFR- TKIs with PD-1/PD-L1 inhibitors can improve treatment effectiveness by activating T cells to attack EGFR-TKI-resistant cancer cells and improve anti-tumor response by suppressing M2 TAMs activity. Studies show that the combination of pembrolizumab with osimertinib results in increased antitumor activity.[46]

Combination of EGFR TKIs and Chemotherapy

Combination of EGFR-TKIs with chemotherapy is one way to delay resistance. In the phase II NEJ005 trial, the combination of gefitinib with carboplatin and pemetreksed showed an overall improvement in PFS and OS in patients with EGFR mutations, especially with concurrent combination regimens with a median OS of 42 months.50 The phase III NEJ009 trial compared carboplatin and pemetreksed chemotherapy plus gefitinib with gefitinib alone. The combination group had

a significantly longer PFS of 20.9 months compared with 11.9 months for gefitinib alone. [47,48]

Combination of EGFR-TKIs with Specific Small Molecule Inhibitors

The combination of EGFR-TKIs with specific inhibitors selected based on the underlying secondary resistance mechanism has shown good improvement in therapeutic outcomes. EGFR-TKI resistance mechanisms involving activation of alternative signaling pathways are overcome through alternative pathway inhibition. The combination of EGFR-TKIs with specific small molecule inhibitors allows the targeting of multiple signaling pathways at once that support cancer cell proliferation and survival. Studies show that the addition of PI3K inhibitors to EGFR-TKIs can overcome resistance and the combination of PI3K inhibitors with EGFR-TKIs can reduce the risk of cancer cell proliferation and survival.

MEK and PI3K inhibitors have also been shown to be an effective therapeutic strategy to control JPBSK with secondary EGFR-TKI resistance. ROS1 rearrangements caused by EGFR-TKIs can be overcome by combining crizotinib.[47]

Developing A New Generation of inhibitors

The development of new drugs not only aims to obtain drugs with higher efficacy but also aims to overcome resistance caused by the previous generation. Currently, fourth-generation EGFR-TKI inhibitors are in the clinical trial phase with a primary focus on targeting the T790M/C797S drug-resistant mutation. The fourth-generation inhibitor EAI045 is the first selective small molecule variant inhibitor in studies to have shown efficacy in a mouse model of JPBSK when used together with cetuximab. Another targeted therapy, JBJ-04-125-02 has shown good ability in slowing down C797S resistance both as monotherapy and in combination with other agents. Another fourth-generation EGFR-TKI inhibitor under development, tQB3804 can overcome resistance due to secondary mutations and is currently in phase one clinical trials.[24]

Next-generation ROS1 inhibitors are also under development. PF-06463922 is currently undergoing clinical evaluation in NSCLC with positive ALK fusions and ROS1 mutations. This drug is a new generation small molecule ROS1/ALK inhibitor that has shown potent inhibition against various oncogenic ROS1 fusion variants and selective activity against various kinases. Repotrectinib (TPX-0005) shows effectiveness against secondary mutations occurring in the ROS1, NTRK1-3 and ALK genes. Repotrectinib is designed to have higher affinity and better brain penetration compared to first- and second-generation inhibitors. Repotrectinib binds simultaneously to the kinase domains of ROS1, ALK and NTRK preventing autophosphorylation and activation of downstream signaling pathways that support cancer cell growth and survival.[51-52]

Conclusion

1. Targeted therapy improves PFS and OS in patients with NSCLC who have target mutations better than chemotherapy.
2. Resistance to targeted therapy is classified into primary resistance, which is a mutation already present before exposure to targeted therapy, and secondary resistance acquired after targeted therapy administration.
3. The development of secondary resistance occurs due to phenotypic and genotypic changes that result in target immunity, activation of alternative pathways and tissue transformation.
4. Periodic evaluations are required in patients on targeted therapy to monitor resistance patterns developing in cancer cells and guide therapy delivery.
5. To combat resistance several strategies are used including the development of new drug generations and the combination of targeted therapy with targeted therapy, chemotherapy or immunotherapy.

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Conflict of Interests

The authors declare no conflict of interest..

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