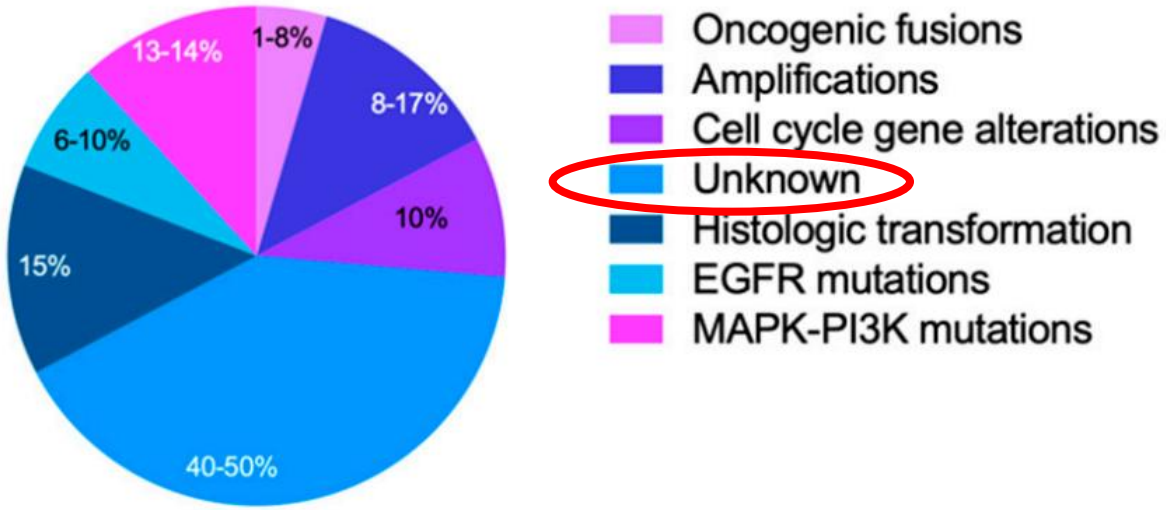
Resistance to Osimertinib

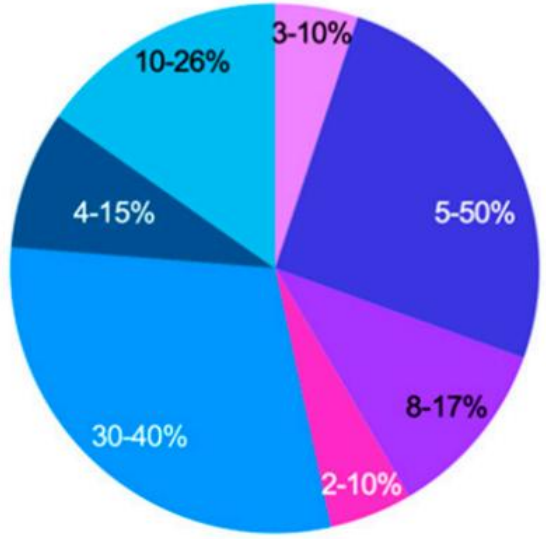
- Osimertinib: irreversible inhibitor of EGFR-sensitizing and T790M-resistant mutations
- Irreversibly binds to the cysteine-797 located in the adenosine triphosphate (ATP) binding site within the TK domain of the EGFR
- AURA & FLAURA studies

Ríos-Hoyo, A.; Moliner, L.; Arriola, E. Acquired Mechanisms of Resistance to Osimertinib—The Next Challenge. Cancers 2022, 14, 1931. https://doi.org/10.3390/ cancers14081931

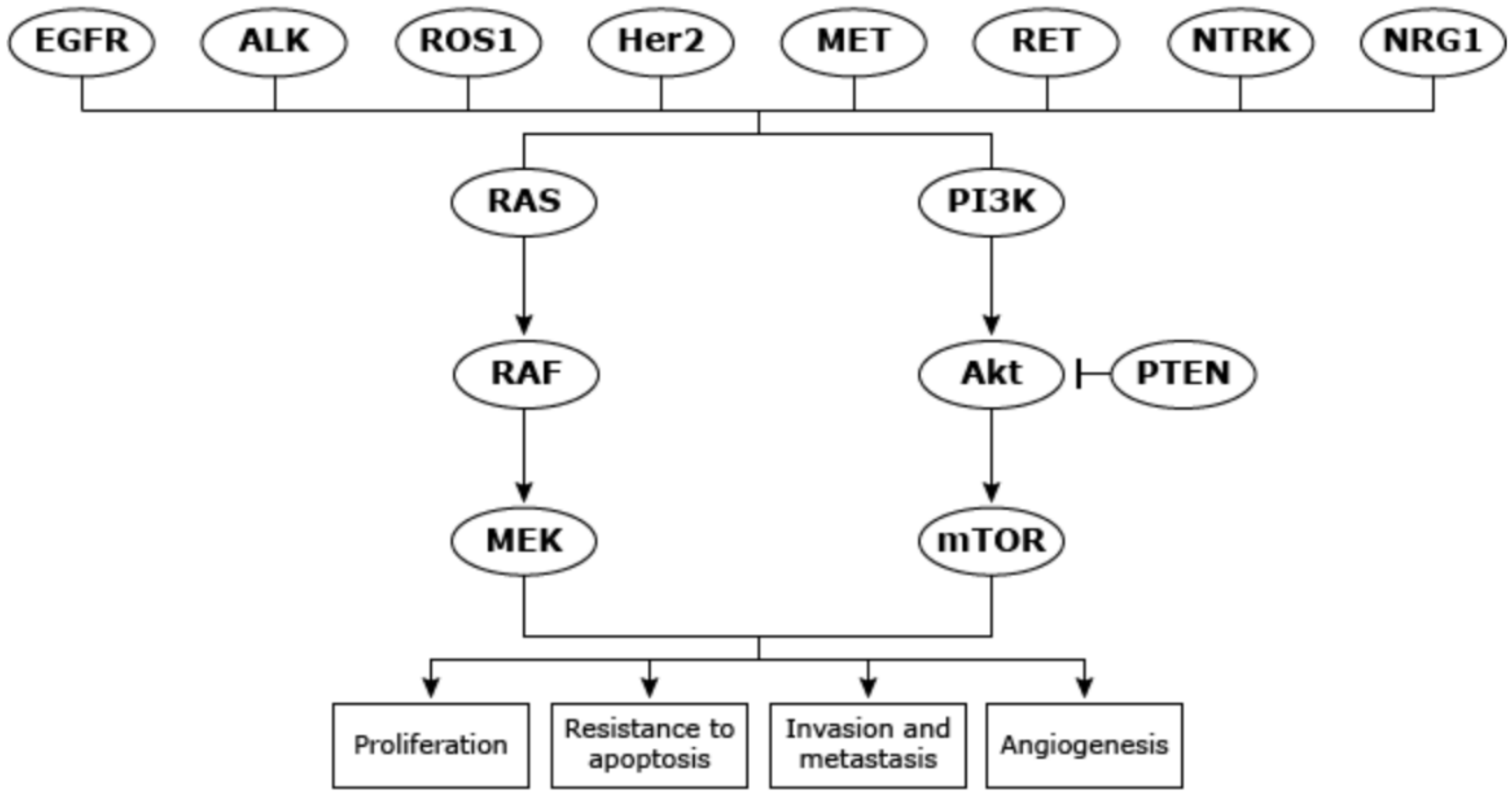
RESISTANCE MECHANISMS TO FIRST–LINE OSIMERTINIB



RESISTANCE MECHANISMS TO SECOND–LINE OSIMERTINIB



Leonetti, A.; Sharma, S.; Minari, R.; Perego, P.; Giovannetti, E.; Tiseo, M. Resistance mechanisms to osimertinib in EGFR-mutated non-small cell lung cancer. Br. J. Cancer 2019, 121, 725–737.


EGFR
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ROS1
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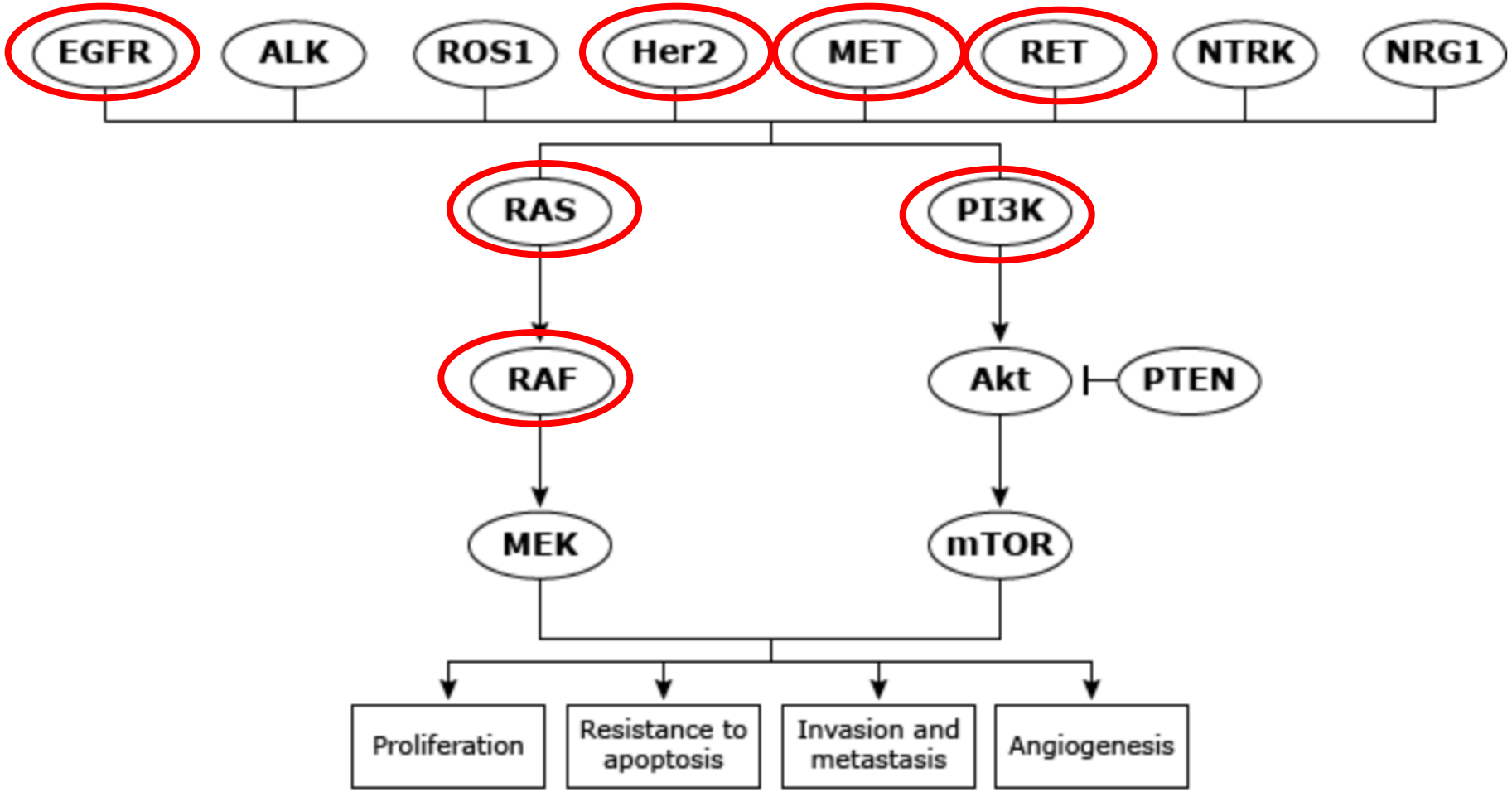
mTOR
Proliferation
Resistance to apoptosis
Invasion and metastasis
Angiogenesis


EGFR
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1. EGFR-Dependent Mechanisms of Resistance

C797X Mutations

- Most Common (C797S)
- Incidence in First line: 7%
- Incidence in second line: 15-22%
- cis vs trans C797X mutation

Strategy	Response
Osi + Erlo/Gef	Case Report, PFS 3 mths
Osi + Erlo/Gef + Bev	Case Report, PFS 8 mths
Brigatinib+ Cetuximab	ORR 60%, PFS: 14 mths
Patritumab Deruxtecan	ORR 39%, PFS 8.2 mths

Ramalingam, S.S.; et al. Mechanisms of acquired resistance to first-line osimertinib: Preliminary data from the phase III FLAURA study. Ann. Oncol. 2018, 29, viii740.

Preventive Strategies

- One phase I/II study is exploring whether the combination of osimertinib and gefitinib could delay the emergence of acquired resistance mechanisms.

- To date, the combination has shown an acceptable toxicity profile.

- Fourth Generation EGFR TKIs (in Phase I studies, preclinical studies)

Rotow, J.K.; et al. Concurrent osimertinib plus gefitinib for first-line treatment of EGFR-mutated non-small cell lung cancer (NSCLC). J. Clin. Oncol. 2020, 38, 9507.

1.2. Less Common EGFR-Dependent Alterations

- There is limited evidence regarding the functional effect of these less frequent mutations

- G796X (2%)
- L792X (12%)
- L718Q (8%)
- G719X (1%)

- Probably decrease the interaction between osimertinib and its binding residue.

1.3. EGFR Amplification

- Incidence of 33% and 31% in the first and later lines, respectively.
- In vitro studies have confirmed EGFR amplification as an independent resistance mechanism to osimertinib, even though, in most cases (up to 50%), it co-occurs with other EGFR resistance mutations.
- One branch of the ORCHARD trial is currently evaluating the combination of osimertinib and necitumumab (IgG1 Mab EGFR antagonist)

Phase 2 Platform Study in Patients with Advanced Non-Small Lung Cancer Who Progressed on First-Line Osimertinib Therapy (ORCHARD). NCT03944772.

2. Histologic and Phenotypic Transformation

- Small Cell transformation
- Squamous Cell transformation

2.1. Small Cell Transformation

- Incidence: 6% - 15% in the second-line setting, 4% in first-line setting
- Consistently, most of the transformed tumors maintain the original activating EGFR mutation. However, after transformation, EGFR expression levels drop.
- Common mutations identified: TP53 (91%), Rb1 (58%), and PIK3CA (27%)

Marcoux, N.; et al. EGFR-Mutant Adenocarcinomas That Transform to Small-Cell Lung Cancer and Other Neuroendocrine Carcinomas: Clinical Outcomes. J. Clin. Oncol. 2018, 37, 278–285.

- A retrospective analysis examined a cohort of 67 patients diagnosed with transformed SCLC, where up to 30% had received osimertinib. The most common treatment was platinum-etoposide, achieving an ORR of 54%, mPFS of 3.4 months, and mOS of 10.9 months since the transformation.

- No responses among the 17 patients who were treated with nivolumab in monotherapy or combination with ipilimumab.

- An ongoing clinical trial is testing the combination of osimertinib and platinum-etoposide chemotherapy

2.2. Squamous Cell Transformation

- Incidence was similar in first-line osimertinib, with 7%, and later-lines, with 9%.
- All squamous cell-transformed tumors maintained the original EGFR mutation.
- There was no clear molecular pattern after evolving into squamous tumors, with only one patient gaining a PIK3CA mutation.
- Currently, there are no clinical data regarding therapeutic strategies in these patients; however, a histology-based approach is recommended.

3. MET Amplification

- The MET (mesenchymal-epithelial transition factor) gene is located on chromosome 7q21-q31. (a transmembrane receptor tyrosine kinase)

- Incidence: between 9% and 24% in second or later lines; between 7% and 15% to first-line

Strategy	Response
Osi + Savolitinib (TATTON)	ORR 30%, PFS 5.4 mths
Lazertinib + Amivantamab (CHRYSALIS)	ORR 50%
Tepotinib +/- Osimertinib (INSIGHT 2)	Ongoing
Osimertinib + Crizotinib (Case reports)	PFS 2 – 7 mths

4. HER2 Alterations

- Incidence in first-line: amplification 2%, HER2 mutations in 1%
- Incidence in second-line: HER2 amplifications 5%, additionally 3% co-occurring with other alterations.
- The most common mutations: in-frame exon 20 insertions & exon 16 skipping HER2 deletion.

Strategy	Response
Patritumumab Deruxtecan	Ongoing
Osimertinib + Lapatinib/Neratinib	Ongoing
Osimertinib + Patritumumab Deruxtecan	Ongoing
Trastuzumab Deruxtecan	Ongoing

5. RET Alterations

- The RET proto-oncogene encodes a receptor tyrosine kinase (RTK). Its activation can develop as a consequence of gain of function amino acid substitutions and genomic rearrangements, leading to the formation of fusion proteins; RET fusions are usually generated by pericentric and paracentric inversions of chromosome 10.

- Incidence: 2%

- RET alterations frequently coexist with other genomic alterations such as TP53, cell cycle-associated genes, the PI3K pathway, and mitogen-activated protein (MAP) kinase effectors.

- Case Reports: Osimertinib with selpercatinib

Papadimitrakopoulou, V.A.; et al. Analysis of resistance mechanisms to osimertinib in patients with EGFR T790M advanced NSCLC from the AURA3 study. Ann. Oncol. 2018

6. BRAF Alterations

- Incidence: 3% BRAF V600E, 1-2% BRAF fusions

- Combination of osimertinib with BRAF and MEK inhibitors, dabrafenib, and trametinib has been used effectively in the case of BRAF V600E acquired mutation and EGFR exon 19del/T790M, which had progressed to osimertinib, with acceptable tolerance to the treatment.

- The use of osimertinib in combination with a single BRAF inhibitor, vemurafenib, has been reported as a successful strategy in overcoming BRAF V600E acquired resistance to osimertinib.

- The use of osimertinib in combination with the MEK inhibitor trametinib has been reported as a fifth line therapy in a patient with NSCLC and EGFR exon 19del and an AGK-BRAF fusion, observing a partial response to this treatment.

7. KRAS Mutations

- KRAS is a member of the membrane-bound family proteins RAS. It possesses inherent GTPase activity. RAS can activate different effector molecules, such as RAF and the MAP kinase pathway, as well as PI3K, ultimately activating mTOR.

- Incidence: 1% (KRAS G12D) and 3% (KRAS A1467T, KRAS G12C, and KRAS G12D, 1% each mutation) in the AURA3 and FLAURA trials

- Concomitant use of osimertinib with novel KRAS G12C inhibitors, such as sotorasib or adagrasib, could be an attractive alternative in this particular mutation

8. PI3K Alterations

- PI3K is activated through different upstream pathways involving tyrosine kinases, G coupled proteins, and RAS-related GTPases. Its activation can lead to diverging downstream pathways, such as Akt, TEC family tyrosine kinases, and mTOR.

- Incidence: In the FLAURA trial, PIK3CA mutations were detected in 6% of the cases (E545K 4%, E453K 1%, H1047R 1%).

- A study involving 605 patients with NSCLC detected up to 14.9% PIK3CA, PTEN, or Akt mutations in patients who had progressed to EGFR inhibitors.

- Subsequently, six patients were treated with EGFR TKIs and everolimus, an mTOR inhibitor. This combination resulted in a limited antitumoral activity with stable disease in five patients and a progressive disease in one.

9. Cell Cycle Gene Alterations

- Cyclin D-dependent kinases (CDK4 and CDK6) are major oncogenic drivers; their sustained activation leads to cancer cells entering the cell cycle repeatedly by producing G1-S phase transitions and reducing the duration of the G1 phase. Genes associated with CDKs include CCND, CCNE, and CDKN.

- Incidence: 12% in AURA 3 trial; 11% In the FLAURA study,

- These genomic alterations have been associated with poor outcomes regarding progression-free survival and overall survival in the context of osimertinib treatment.

- A clinical trial with osimertinib in combination with a CDK 4/6 inhibitor in a population with EGFR mutated NSCLC with or without T790M is evaluating the efficacy of this combination; however, this trial excludes patients who previously received osimertinib.

10. AXL Overexpression

- AXL is a receptor tyrosine kinase, which belongs to the tumor-associated macrophage family (TAM), including TYRO-3 and MER. AXL ligand is Gas6, which binds to the ectodomain of AXL; its activation leads to cellular growth, proliferation, motility, and invasion and EMT.

- AXL overexpression has been reported as an acquired mechanism of resistance to first- and second-generation EGFR TKIs, as well as to second-line Osimertinib.

- In NSCLC EGFR mutated patients, baseline AXL overexpression was associated with a decreased response to first-line osimertinib, compared to non-overexpressing tumors .

- Enapotamab vedotin and ADC specific against AXL has shown activity in an in vivo model of osimertinib resistant NSCLC [51] and is currently being tested in a Phase 1/2 clinical trial including different tumors.

11. Insulin-like Growth Factor (IGF)-1 Receptor Activation

- The IGF-1 receptor belongs to the insulin receptor family and has roles in cell growth and differentiation. It can be activated by IGF1, IGF2, and insulin.

- Activation of the IGF-1 receptor as an acquired resistance mechanism to osimertinib was described in in vitro studies

- Treatment with linsitinib (an IGF-1 receptor inhibitor) and osimertinib restored sensitivity to osimertinib in in vitro studies

12. Epithelial-Mesenchymal Transition (EMT)

- EMT is a cellular process, allowing cells to enhance invasive capacity, cancer stem-cell similar properties, as well as resistance to treatments.

- Epithelial cells lose their cell polarity and cell-to-cell adhesion, including a downregulation of epithelial proteins, such as E-cadherin, and acquire mesenchymal characteristics, including increased migration and invasion properties, and an upregulation of proteins, such as N-cadherin and vimentin.

- EMT develops through the involvement of different proteins and pathways, including TGF-β, SMAD and MAP kinase pathways, induction of IGF-1 receptor, and Notch signaling.

- In vitro studies using cell lines support EMT as an additional mechanism of resistance to Osimertinib.

Qin, Q.; Li, X.; et al. Targeting the EMT transcription factor Snail overcomes resistance to osimertinib in EGFR-mutant non-small cell lung cancer. Thorac. Cancer 2021

Conclusion

- Resistance to Osimertinib can be mediated by acquired *EGFR* kinase domain mutations.
- Off-target resistance to Osimertinib is more prevalent than on-target resistance and is mediated by various mechanisms, including the activation of bypass signalling or phenotypic transformation.
- Novel approaches designed to overcome resistance beyond fourth-generation TKIs, including combination therapies, antibody–drug conjugates, bispecific antibodies and immune-directed approaches, are at various stages of clinical investigation.

- It is therefore of paramount importance to assess these genomic and phenotypic changes through a rebiopsy when feasible and alternatively using liquid biopsies.

- One of the main challenges when multiple alterations are found is to elucidate which mechanism is driving the resistance to efficiently target that mechanism and improve the chances of therapeutic success.

Mechanisms of resistance	Pre-treated (%)	Naïve (%)
Loss of *T790M*	68	
Maintenance of *T790M*	32	
EGFR mutations (*C797S, G724S, L718Q*)	22	7
Bypass pathway activation		
MET amplification	10	15
HER2 amplification		2
KRAS mutation	2	3
BRAF mutation	5	3
PIK3CA mutation	10	7
RET fusion	2	
FGFR3 fusion	2	
BRAF fusion	2	
SCLC transformation	15	

Mechanism of Resistance	Therapeutic Strategies
C797X	Gefitinib, erlotinib Osimertinib + erlotinib Brigatinib + cetuximab Patritumab deruxtecan EAI045 JBJ-04-125-02 CH7233163 BLU-945
Small cell transformation	Platinum-etoposide
Squamous cell transformation	Histology based approach
MET amplification	Osimertinib + savolitinib Lazertinib + amivantamab Tepotinib + osimertinib Osimertinib + crizotinib Patritumab deruxtecan
HER2 alterations	Patritumab deruxtecan Osimertinib + lapatinib * Osimertinib + neratinib * Osimertinib + T-DXd *
RET alterations	Osimertinib + selpercatinib
BRAF alterations	Osimertinib + dabrafenib + trametinib Osimertinib + vemurafenib Osimertinib + selumetinib or trametinib
RAS	Osimertinib + selumetinib or Aurora kinase b inhibitor Osimertinib + sotorasib * Osimertinib + adagrasib *
PIK3	EGFR TKIs and everolimus Osimertinib + alpelisib
Cell cycle gene alterations	Osimertinib + palbociclib Osimertinib + abemaciclib
AXL overexpression	Enapotamab vedotin
IGF-1 receptor activation	Osimertinib + linsitinib
Non-specific alterations	Datopotamab deruxtecan

Thank You ☺