*Basic and Translational Oncology*
*Italian-French Erasmus Intensive Course in Oncology*

# *Drug resistance in solid tumors*

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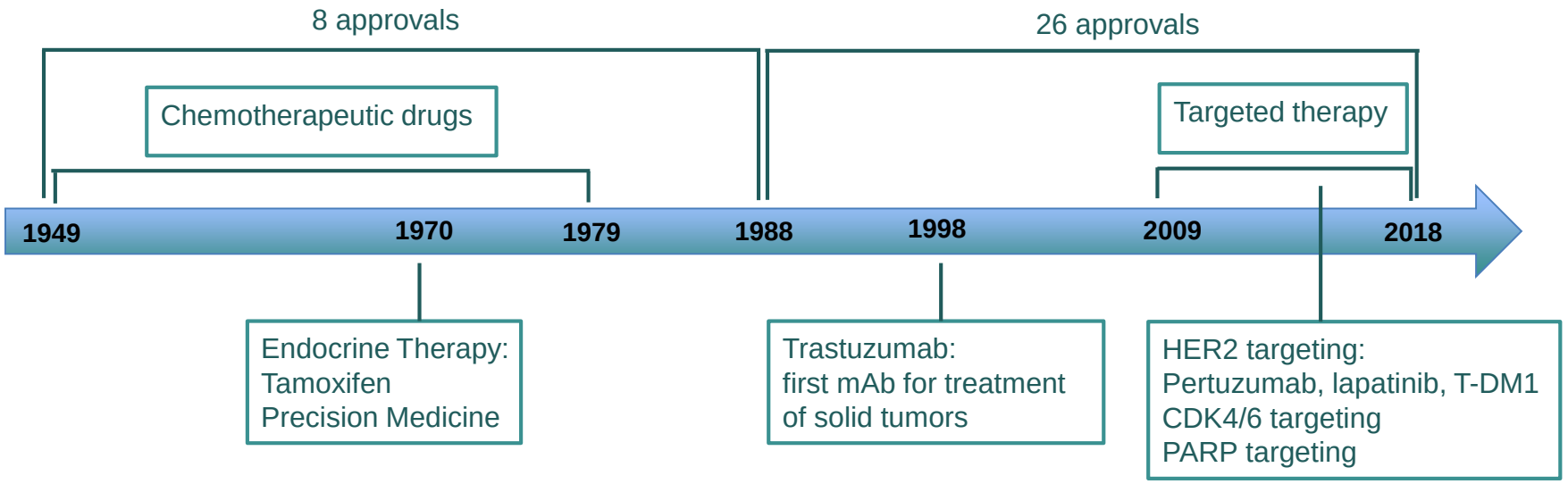
# The targeted therapies

In 2002, I.B. Weinstein defined the phenomenon of '**oncogene addiction**,' whereby cancer cells become excessively dependent on a particular 'driver' alteration for their survival

Cancers with these dependencies exhibit exquisite vulnerability to drugs that inhibit the drivers, so-called **targeted therapies**.

## Timeline of last 70-years FDA approvals for breat cancer

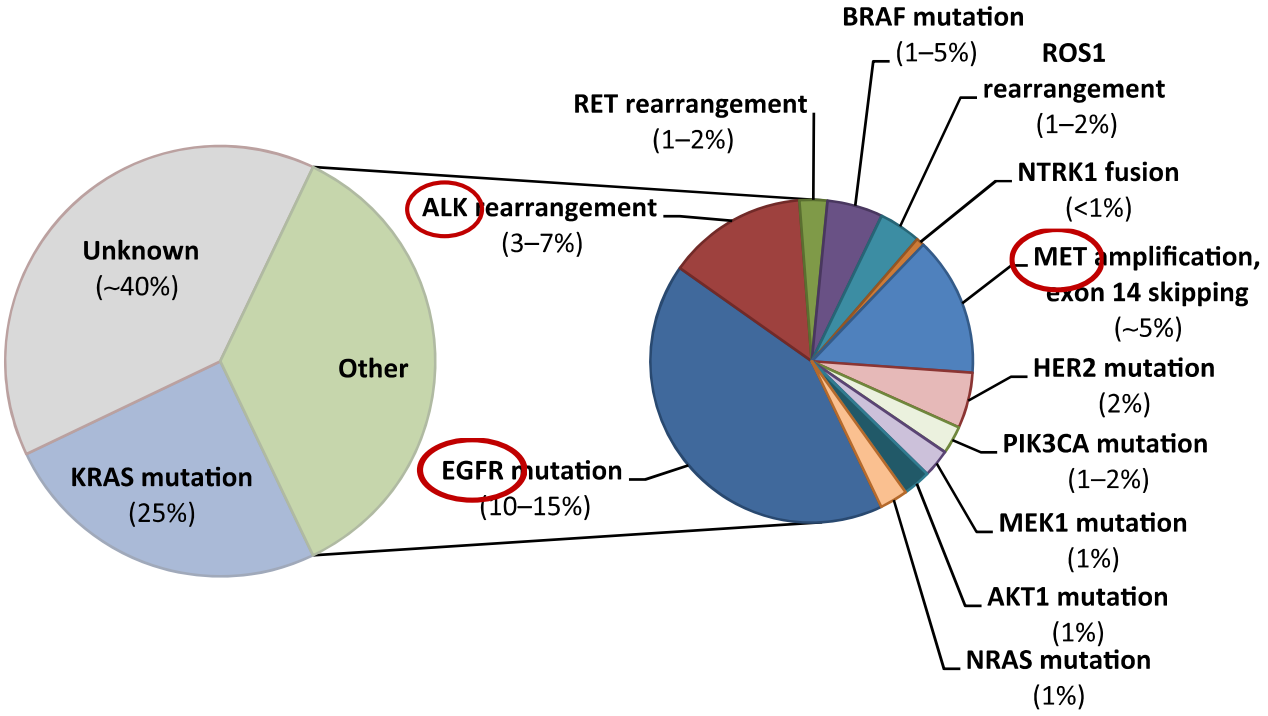


# Oncogenic Drivers in Lung Adenocarcinoma.

The past decade has witnessed numerous successes in targeting specific oncogenic drivers in different human malignancies; most notably non-small-cell-lung-cancer

85% cases of lung cancer are NSCLC

Somatic activating mutations in EGFR were the first driver alterations characterized in NSCLC. These mutations confer sensitivity to TKIs of EGFR, resulting in high response rates and prolonged PFS



# Current molecularly-targeted therapeutics, their associated targets, and acquired mutations conferring resistance

| Inhibitor | Target | Acquired Mutations Conferring Resistance | References |
|---|---|---|---|
| Erlotinib | EGFR | T790M, D761Y, T854A, L747S | [9–11] |
| Gefitinib | EGFR | T790M, D761Y, T854A, L747S | [9–11] |
| Afatinib | EGFR, HER2 | T790M | [12,13] |
| Osimertinib (AZD9291) | EGFR | C797, G796D | [14–20] |
| Rociletinib | EGFR | C797 | [21,22] |
| EAI045 | EGFR | Under Investigation | [20,23] |
| Crizotinib | ALK, MET, ROS1 | L1196M, C1156Y, F1174L, F1174V T1151K | [24–30] |
| TAE684 | ALK | G1123S, G1123SD | [31,32] |
| Ceritinib | ALK | G1202R, F1174C/V, G1123S, T1151K | [33–37] |
| Alectinib | ALK | G1202R, I1171T/N/S, V1180L | [34,38–42] |
| Brigatinib (AP26113) | ALK, ROS1 | Under Investigation | [34,43] |
| Lorlatinib (PF-06463922) | ALK, ROS1 | L1198F | [44,45] |
| Entrectinib (RxDx-101) | ALK, ROS1, NTRK1–3 | NTRK1, NTRK2, NTRK3 | [46] |
| Ensartinib (X-398) | ALK, ROS1, MET, SLK | Under Investigation | [47] |
| Dabrafenib | BRAF | G12D KRAS | [48,49] |
| Vemurafenib | BRAF | Alternate isoforms of RAF proteins | [50–52] |
| Trametinib | MEK | Under Investigation | [52,53] |

# The issue of resistance is the inevitable barrier that limits the effectiveness of targeted therapy

**Primary Resistance**: occurs in 4-10% of newly diagnosed patients, lack of response to targeted therapy

**Responsible mechanisms:**
- Non-sensitizing alterations within the target
- Genetic alterations outside the target kinase (MET amplification, NFkB pathway activation; alterations in genes leading to the induction of EMT)

**Acquired Resistance**: disease progression following initial clinical benefit

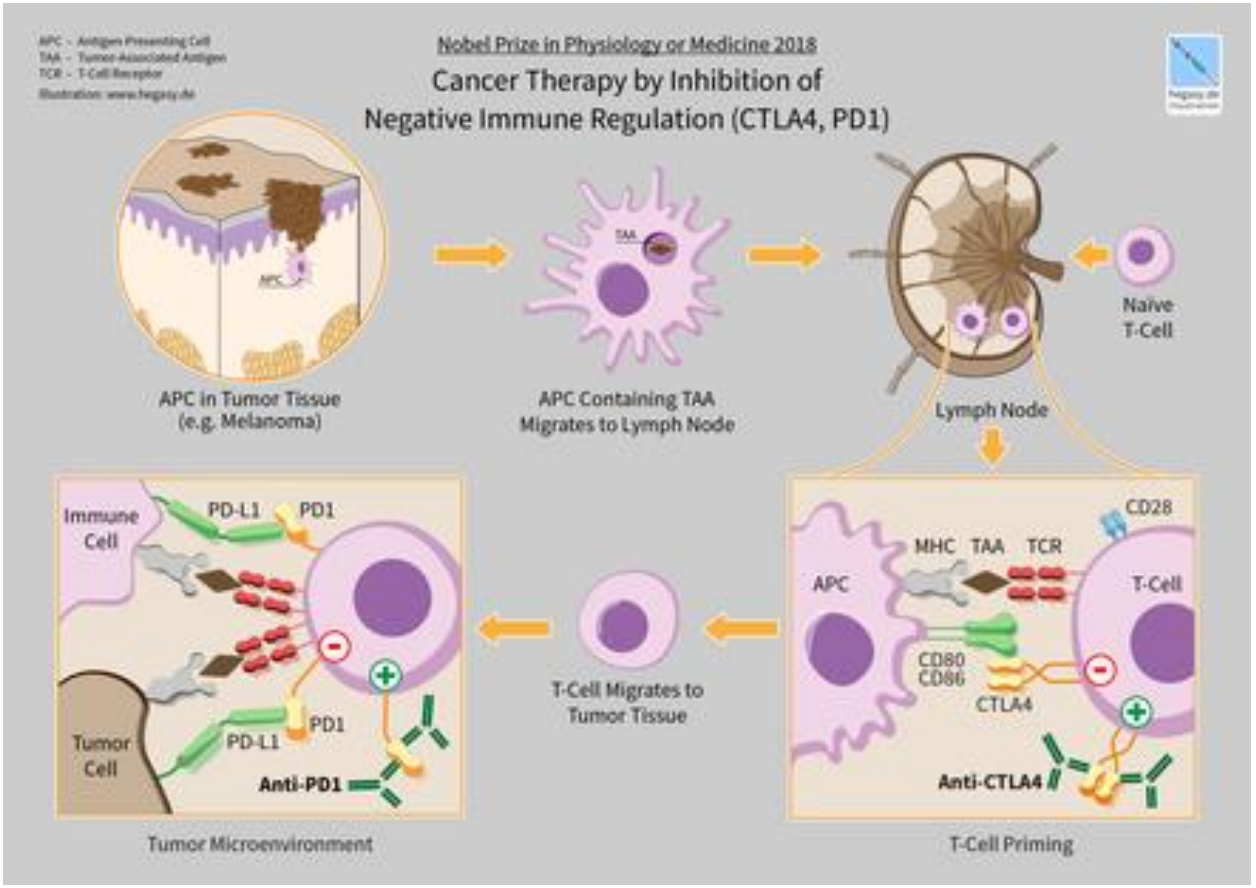
**Responsible mechanisms:**
- Acquisition of secondary target alterations
- Epithelial-mesenchimal transition (EMT)
- Activation of an alternative signaling pathway or downstream effector(s)

Cancer survival is driven by genetic diversity and accumulation of mutations, influenced by the selective pressures of TKI therapy

# Mechanisms of Acquired Resistance in EGFR- and ALK-Positive NSCLC Treated with TKIs

| Category | Alteration | Estimated frequency (%) | Refs |
|---|---|---|---|
| Resistance to EGFR-TKI | **EGFR *target alteration*** | ***~60*** | |
| | T790M | 50–60 (for 1st-generation EGFR-TKI) | [33–35] |
| | D761Y, T854A, L747S | 1-2 | [34–39] |
| | C797S | ~20 (for 3rd-generation EGFR-TKI) | [40–43] |
| | *EGFR* amplification | 8–10 | [33,39] |
| | ***Bypass signaling tracks*** | ***~20*** | |
| | *MET* amplification | 5–22 | [33,34,59] |
| | HGF overexpression | 1 of 2 cases reported | [60] |
| | *HER2* amplification | 12 | [33,34] |
| | FGFR3 activation | 1 case reported | [63] |
| | *BRAF* mutations | 1 | [64] |
| | *CRKL* amplification | 1 of 11 cases reported | [65] |
| | *NF1* reduced expression | 4 of 10 cases reported | [66] |
| | ***Phenotypic changes*** | ***3–10*** | |
| | Transformation to SCLC | 3–10 | [33–35] |
| | ***Unknown mechanism*** | ***10–20*** | |
| Resistance to ALK-TKI | **ALK *target alteration*** | ***~28–46*** | |
| | Secondary mutations in *ALK* | 22–36 | [44–47] |
| | *ALK* amplification | 7–18 | [46,47] |
| | ***Bypass signaling tracks*** | ***~40–50*** | |
| | EGFR activation | Up to 44 | [45–47] |
| | *c-KIT* amplification and SCF overexpression | 15 | [46] |
| | IGF-1R activation | 4 of 5 cases | [62] |
| | *MEK1* mutations | 1 case reported | [63] |
| | *PIK3CA* mutations | 1 case reported | [63] |
| | *MET* amplification | 1 case reported (for alectinib) | [61] |
| | SRC activation | Unknown % | [63] |
| | ***Phenotypic changes*** | ***<5*** | |
| | Transformation to SCLC | < 5 (case reports) | [72–74] |
| | ***Unknown mechanism*** | ***~15–30*** | |

# Advances in oncological therapy by Immunological approaches




Anti-CTLA4 and anti-PD1/PD-L1 monoclonal antobodies that disable negative regulators, or checkpoint, of the adaptive immune system have resulted in remarkable anti-tumor activity in multiple tumor types

Yet, eventual resistance remains the norm

Review

# A view on drug resistance in cancer

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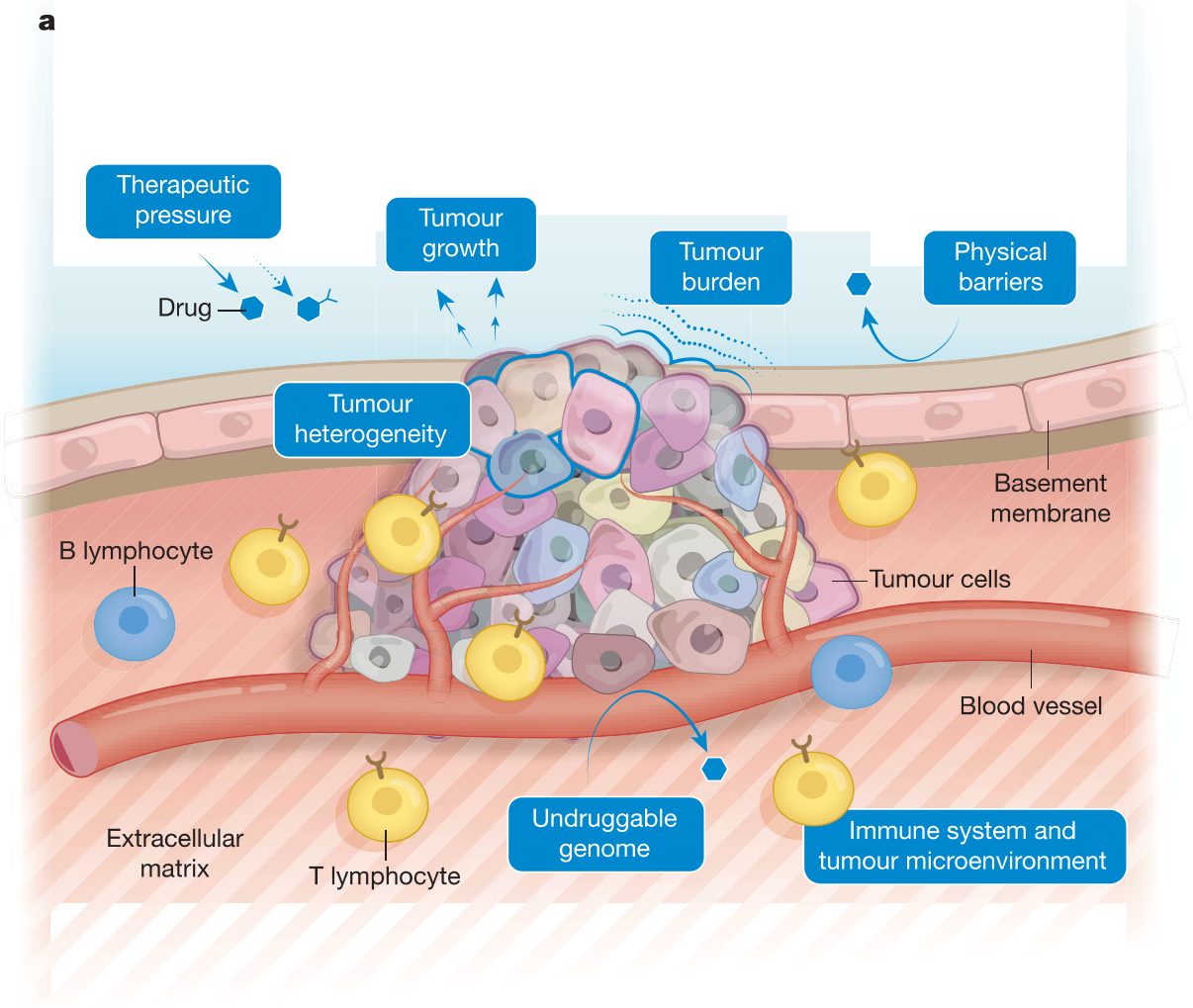
The problem of resistance to therapy in cancer is multifaceted. Here we take a reductionist approach to define and separate the key determinants of drug resistance, which include tumour burden and growth kinetics; tumour heterogeneity; physical barriers; the immune system and the microenvironment; undruggable cancer drivers; and the many consequences of applying therapeutic pressures. We propose four general solutions to drug resistance that are based on earlier detection of tumours permitting cancer interception; adaptive monitoring during therapy; the addition of novel drugs and improved pharmacological principles that result in deeper responses; and the identification of cancer cell dependencies by high-throughput synthetic lethality screens, integration of clinico-genomic data and computational modelling. These different approaches could eventually be synthesized for each tumour at any decision point and used to inform the choice of therapy.

# Biological determinants of resistance




**b**

**Tumour growth**
- Dynamic monitoring
- Functional-imaging studies
- Dose density and more complete killing of cells

**Tumour burden**
- Radiotherapy
- Surgery
- Neoadjuvant chemotherapy

**Tumour heterogeneity**
- Early detection
- Combination therapy
- Targeting tumour neoantigens

**Physical barriers**
- Local therapies
- Functional-imaging studies
- Small molecules engineered to penetrate sanctuary sites

**Immune system and tumour microenvironment**
- Enhancing tumour recognition by the immune system
- Anti-angiogenic therapy
- Cellular therapies

**Undruggable genome**
- Transcription-factor inhibitors
- Allele-specific inhibitors
- Restoring the function of tumour suppressors

**Therapeutic pressure**
- Next-generation TKIs that overcome resistance mutations
- Allosteric inhibitors
- Antibody–drug conjugates

# Biological determinants of resistance

-Tumour burden and growth kinetics. There is an almost universal correlation between tumour burden and curability. The rate of tumour growth and the changes in growth kinetics that are induced by therapy also have a critical role in responses to therapy and resistance

Physical barriers. Cancer cells can create spatial gradients within tumours that prevent adequate blood flow, thereby creating a pro-tumorigenic hypoxic environment and decreasing the effective exposure of a tumour to drugs

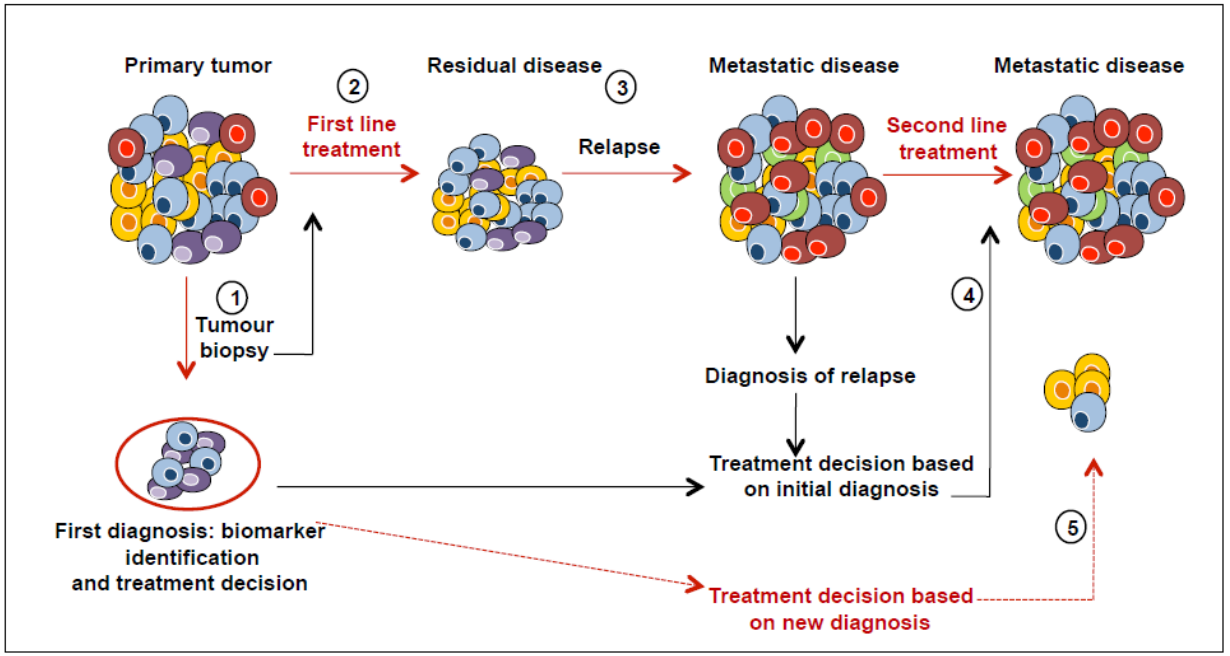
Immune system and tumour microenvironment. The tumour microenvironment may mediate resistance by several mechanisms - preventing immune clearance of tumour cells, hindering drug absorption, stimulating paracrine growth factors to signal cancer cell growth. Immunosuppressive cancer microenvironment (immune deserts) are now recognized as a major impediment to checkpoint inhibitors

Undruggable genomic drivers. Some of the most formidable oncogenes and tumour suppressor genes remain undruggable, including MYC, RAS, and TP53

Selective therapeutic pressure. Under targeted therapies, changes may be divided into *early adaptive responses* or - after prolonged exposure - *acquired resistance*. Phenotypic changes may occur that result in the evolution of treated tumours into new histological types.

Tumour heterogeneity.

# Heterogeneity and Policlonal Resistance

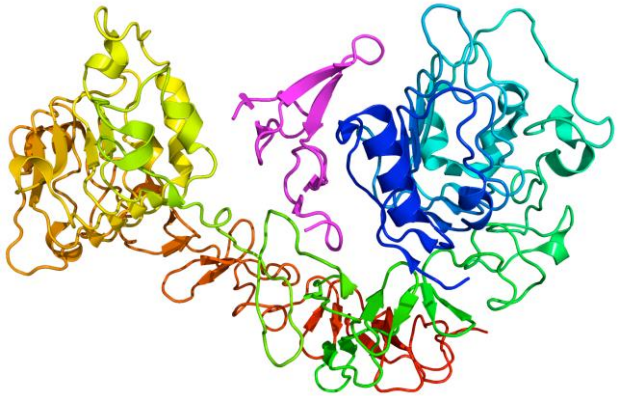



Cancer diagnosis is commonly based on a biopsy (**1**) that contains only a small fraction of tumour and may thus not be representative of all the subclones. The first line treatment can be successful in eliminating dominant clones (2), but resistant clones are selected and drive disease progression (**3**). Metastases can develop from primary tumour cells, or from clones that survive the initial therapy. Therefore, the clonal composition of metastatic lesions may be completely different from that of the primary tumour sample, and treatments based on the initial diagnostic sample may be suboptimal for the treatment of metastatic disease

Currently, heterogeneity is evaluated by genomic sequencing of either archived tumour samples at diagnosis or a subsequent biopsied tumour sample at recurrence.

This approach has serious limitations, as subclonal driver mutations are missed, nevertheless are sufficient to drive resistance to targeted therapies

# EPIDERMAL-GROWTH FACTOR RECEPTOR (EGFR)



EGFR is a transmembrane growth factor receptor for epidermal growth factor (EGF) soluble factor

It is a member of the ErbB family of receptors, which includes four closely related receptor tyrosine kinases: EGFR (ErbB-1), HER2/neu (ErbB-2), HER3 (ErbB-3) and HER4 (ErbB-4). Mutations in EGFR that result in its constitutive activation are believed to be an important contributor to the tumorigenesis of many cancer types

EGF ligand and its receptor was discovered by Stanley Cohen of Vanderbilt University. Cohen shared the 1986 Nobel Prize in Medicine with Rita Levi-Montalcini for their discovery of growth factors

# EGFR Activating Mutations in Cancer

Sensitizing mutations in the EGFR tyrosine kinase (TK) domain occur in about 15-18% of NSCLC patients

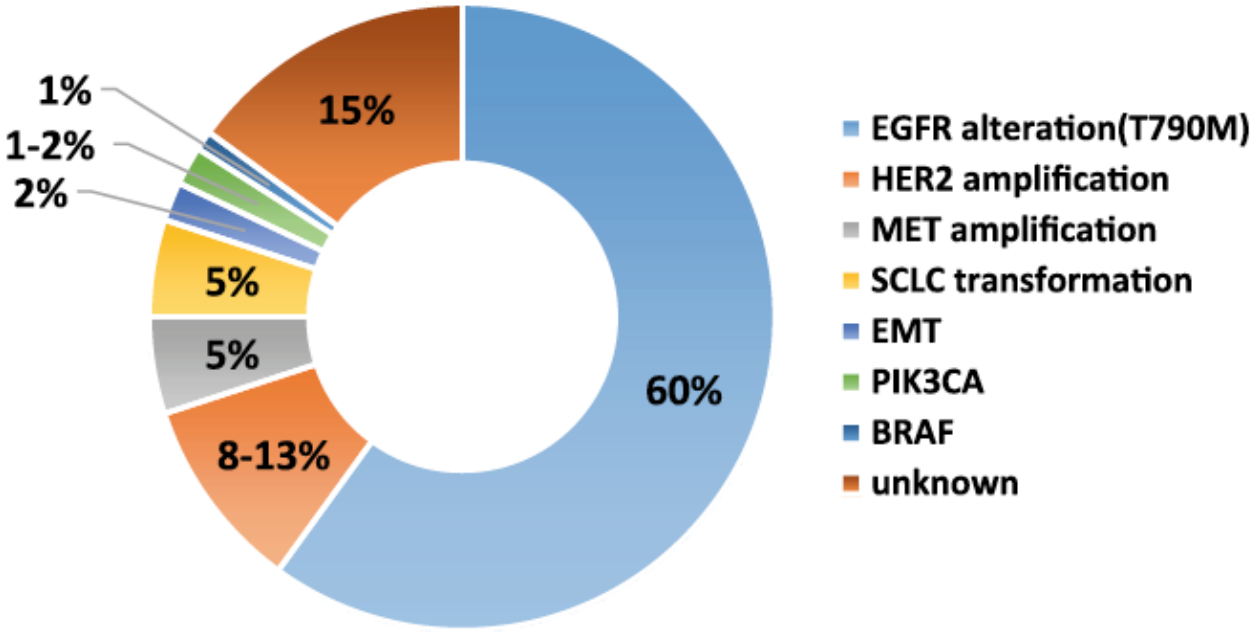
The most common activating mutations are:
- in frame deletion of exon 19
- single-point mutation  of exon 21 (Leu858Arg)

Together these mutations account for more than 80% of known activating mutations of the receptor

Patients receiving EGFR TKIs (according to ASCO, ESMO, and NCCN guidelines) have longer progressive free survival (PFS) than those receiving platinum-based chemotherapy as first-line of treatment

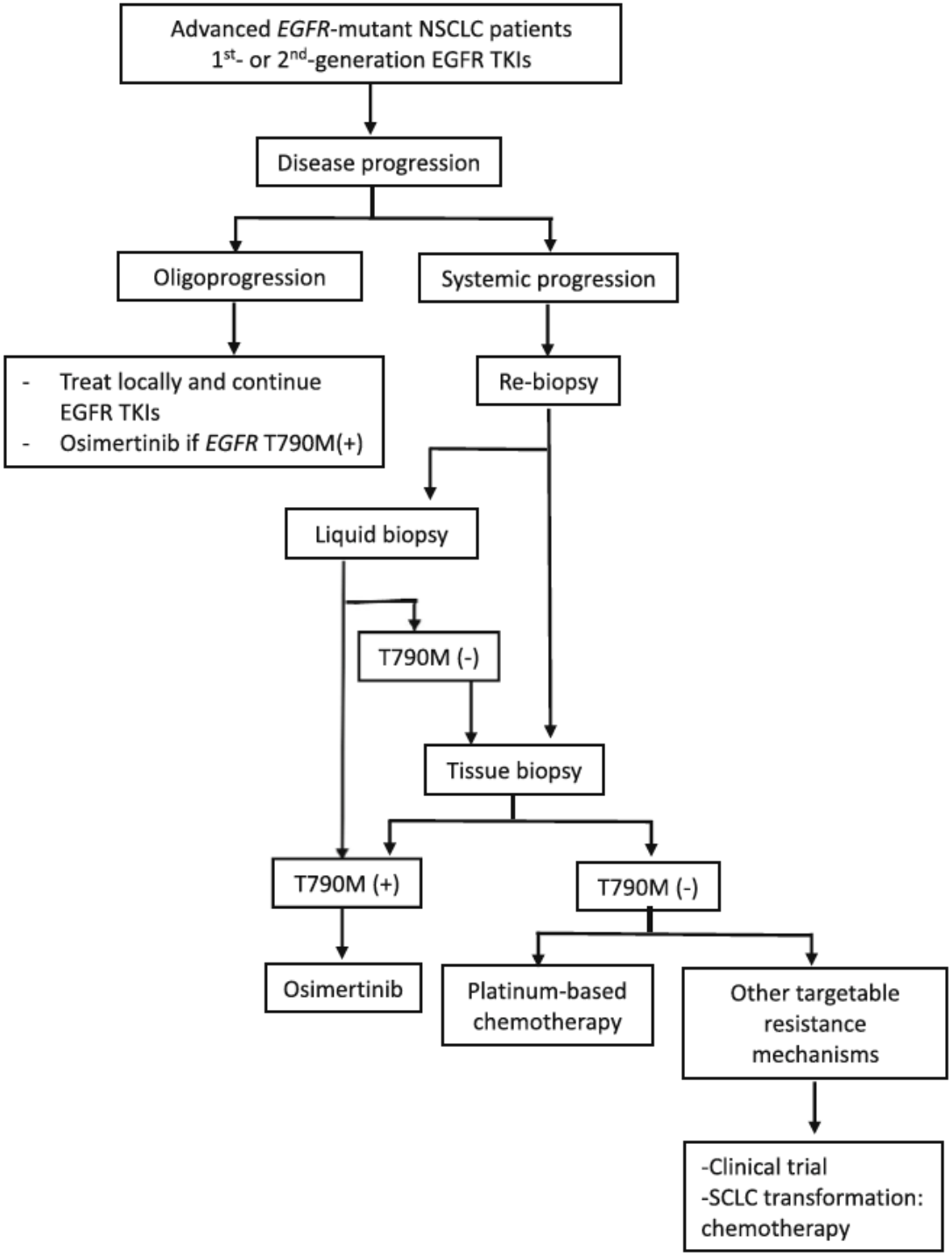
Most patients develop progressive disease within 1 year of treatment

# Mechanisms of acquired resistance to EGFR TKIs in NSCLC




Distribution of acquired resistance

*Molecular Cancer (2018) 17:38*


Advanced *EGFR*-mutant NSCLC patients
1st- or 2nd-generation EGFR TKIs
Disease progression
Oligoprogression
Systemic progression
- Treat locally and continue EGFR TKIs
- Osimertinib if *EGFR* T790M(+)
Re-biopsy
Liquid biopsy
T790M (-)
Tissue biopsy
T790M (+)
T790M (-)
Osimertinib
Platinum-based chemotherapy
Other targetable resistance mechanisms
-Clinical trial
-SCLC transformation: chemotherapy

# Different Generations of EGFR TKIs

| Generation | EGFR inhibition | Drug | Molecular Targets[a] | Adverse effect | Status |
|---|---|---|---|---|---|
| 1st-generation | Reversible; | Gefitinib | *EGFR del19, L858R* | Skin rash/acne, abnormal LFT | FDA approved |
| | competitive | Erlotinib | *EGFR del19, L858R* | | FDA approved |
| 2nd-generation | Irreversible; covalent | Afatinib | *EGFR del19, L858R*, uncommon mutations, HER2, HER4 | Diarrhea, paronychia. Skin rash | FDA approved |
| | | Dacomitinib | *EGFR del19, L858R*, HER2, HER4 | Diarrhea, skin rash/acne | Phase III |
| | | Neratinib | *EGFR G719X*, HER2, HER4 | Diarrhea, dyspnea, N/V | Phase II |
| 3rd-generation | Irreversible; | Osimertinib | *EGFR* mutations and *T790M* | Diarrhea, skin rash | FDA approved |
| | covalent | Rociletinib | *EGFR T790M* mutation, IGF-1R | Hyperglycemia, QTc prolong | Withdrawn |
| | | Olmutinib | *EGFR T790M* mutation | Diarrhea, skin exfoliation, nausea | Approved in South Korea |
| | | ASP8273 | *EGFR L858R, del19, T790M,* | Diarrhea, N/V, thrombocytopenia | Phase III Discontinued |
| | | Nazartinib | *EGFR L858R, del19, T790M,* | Rash, diarrhea, pruritus | Phase I/II |
| | | Avitinib (AC0010) | *EGFR L858R, del19, T790M,* | Diarrhea, skin rash, abnormal LFT | Phase I/II |
| | | HS-10296 | *EGFR* sensitive mutations *(G719X, del19, L858R, L861Q)* +/– T790M | None reported | Phase I/II |
| | | PF-06747775 | *EGFR L858R, del19, T790M,* | None reported | Phase I/II |

***N/V*** **nausea and/or vomiting,** ***LFT*** **liver function test,** ***del19*** **deletion in exon19,** ***EGFR*** **epidermal growth factor receptor,** ***FDA*** **Food and Drug Administration**
[a]**The targets included FDA approved or associated targets**

# Efficacy of Third-Generation EGFR TKIs in EGFR T790M-Positive NSCLC Patients

| Drug | Trial | Patients (N) | Dose | ORR T790M | PFS (mo.) |
|---|---|---|---|---|---|
| Osimertinib | AURA phase I [92] | Total: 253 T790 M(+): 138 | 20-240 mg QD | T790M(+): 61% T790M(–): 21% | T790M(+): 9.6 T790M(–): 2.8 |
| | AURA phase I T790M(+) | 63 | 80 mg QD | 71% | 9.7 |
| | AURA phase II | 210 | 80 mg QD | 70% | 9.9 |
| | AURA phase II extension [132] | 411 | 80 mg QD | 62% | 12.3 |
| | AURA phase III [84] | 416 -Osimertinib arm: 279 -Chemotherapy arm: 140 | | 71% 31% Odds ratio:5.39 (95% CI: 3.47–8.48) | 10.1 4.4 HR: 0.30 (95% CI: 0.23–0.41) |
| Rociletinib | TIGER-X phase I/II [98] | Total: 69 T790M(+): 51 | 500, 625 or 750 mg bid | 45% | T790M(+): 9.6 T790M(–): 2.8 |
| Olmutinib | HM-EMSI-101 phase I/II T790M(+) [133] | 76 | 800 mg QD | 62% | 6.9 |
| ASP8273 | NCT02113813 phase I/II [134] | Total: 63 T790M(+): 58 | 300 mg QD | 29% | 6.8 |
| Nazartinib | NCT02108964 phase I/II [105] | 152 | 75-350 mg QD | 46.9% | 9.7 |
| Avitinib (AC0010) | NCT02330367 phase I/II [106] | 136 | 50-350 mg QD | 44% | |

Third generation TKIs have been designed to target both EGFR with activating mutations and T790M resistance mutation in NSCLC patients

# Main mechanisms involved in acquired resistance to EGFR-TKIs and the associated targetable drugs

| Molecular alteration | Pathway | Targetable drug |
|---|---|---|
| HER2 amplification | | Afatinib, Trastuzumab, ado-trastuzumab emtansine (TDM1) |
| MET overexpression/genetic alteration | | • Anti-HGF antibody: Rilotumumab, Ficlatuzumab<br>• Anti-c-MET antibody: MET Mab, Emibetuzumab (LY2875358)<br>• Selective c-MET inhibitor: Tivantinib (ARQ197), Capmatinib (INC280), Savolitinib (AZD6094), Tepotinib (EMD 1214063), SGX523, SAR125844,<br>• Multikinase inhibitors: Crizotinib, Cabozantinib (XL184), Glesatinib (MGCD265), Merestinib (LY2801653), S49076 |
| PIK3CA | PI3K-AKT-mTOR | • PI3K inhibitor: Pilaralisib (XL147), Dactolisib (BEZ235) and Pictilisib (GDC-0941), Buparlisib (BKM120)<br>• AKT inhibitor: MK-2206<br>• mTOR inhibitor: Everolimus, Temsirolimus, Ridaforolimus |
| BRAF | Ras-Raf-MEK-ERK | Vemurafenib (PLX4032), Dabrafenib (GSK2118436), Selumetinib, LY3009120 |
| AXL overexpression | GAS6-AXL | • Tyrosine kinase inhibitor: Cabozantinib (XL 184)<br>• AXL antibody: E8, D9, Mab173<br>• AXL decoy receptor: AXL-Fc, MYDI |

# Combination therapy to overcome resistance

Vertical pathway:

Cetuximab (a chimeric MAb to EGFR) in combination with Afatinib for patients who have progressed after receiving EGFR TKI therapy and Chemotherapy

Horizontal pathway:

Bypass signaling pathway activation is an important acquired resistance mechanism of EGFR TKIs; thus the combination of EGFR inhibitor and inhibitors for the bypass signaling pathway has been investigated as a new strategy.
Unfortunately current results are preliminary and immature.

On October 30, 2018, the FDA approved pembrolizumab in combination with chemotherapy for first-line treatment of metastatic squamous NSCLC

# CONCLUSIONS

EGFR TKIs are currently the standard first-line treatment of patients with advanced NSCLC harboring EGFR activating mutations

After acquiring resistance to first-line EGFR TKI therapy, based on the mechanism of resistance, subsequent treatment can be chosen

Continuation of EGFR TKI therapy is suitable for selected patients with asymptomatic progression and/or oligoprogression

Repeat tumor biopsy to detect the EGFR T790M mutation is the current standard of care, and osimertinib has been approved for patients with acquired EGFR T790M-mutant disease

Liquid biopsy is an alternative method to detect plasma EGFR T790M mutation and to identify patients suitable for osimertinib therapy

More recently, the management of NSCLC patients has advanced by the approval of immunological approach

# Targeting ALK Receptor Tyrosin Kinase
# Precision medicine in lung cancer: where we are

The discovery of anaplastic lymphoma kinase (ALK) dates back to 1994 when a chromosomal rearrangement was described in anaplastic large-cell lymphoma (Morris SW et al., Science 1994)

Subsequent work over the next two decades identified ALK fusion proteins as the oncogenic driver in numerous different malignancies (salient example of the paradigm of “oncogene addiction”)

ALK encode a highly conserved receptor tyrosine kinase within the insulin receptor superfamily

In adult human ALK expression is limited to the nervous system, testis, and small intestines. It is activated by the ligand Augmentor a and b (FAM150)

With the advent of next-generation sequencing (NGS)-based diagnostics, more than 20 different ALK fusion partner genes have been reported

*Cancer Discovery 2017; 7(2):1-19*

# ALK rearrangements in cancer

| Cancer type | Frequency of *ALK* rearrangements | *ALK* fusion partner gene | Location of fusion partner | References |
|---|---|---|---|---|
| NSCLC | 3–7% | *TPR* | 1q31.1 | (2, 40–45) |
| | | *CRIM1* | 2p22.2 | |
| | | *EML4*[a] | 2p21 | |
| | | *STRN* | 2p22.2 | |
| | | *TFG* | 3q12.2 | |
| | | *HIP1* | 7q11.23 | |
| | | *PTPN3* | 9q31 | |
| | | *KIF5B* | 10p11.22 | |
| | | *KLC1* | 14q32.3 | |
| | | *CLTC* | 17q23.1 | |
| Anaplastic Large Cell Lymphoma ALCL | ~55% (in adults) | *TPM3* | 1q21.3 | (1, 21–29) |
| | | *ATIC* | 2q35 | |
| | | *TFG* | 3q12.2 | |
| | | *NPM1*[a] | 5q35.1 | |
| | | *TRAF1* | 9q33.2 | |
| | | *CLTC* | 17q23.1 | |
| | | *RNF213* | 17q25.3 | |
| | | *TPM4* | 19p13.1 | |
| | | *MYH9* | 22q12.3 | |
| | | *MSN* | Xq12 | |
| Inflammatory Myofibroblastic Tumor IMT | Up to 50% | *TPM3* | 1q21.3 | (30–37) |
| | | *RANBP2* | 2q13 | |
| | | *ATIC* | 2q35 | |
| | | *SEC31A* | 4q21.22 | |
| | | *CARS* | 11p15.4 | |
| | | *PPFIBP1* | 12p11 | |
| | | *CLTC* | 17q23.1 | |
| | | *TPM4* | 19p13.1 | |
| Diffuse Large B-cell Lymphoma DLBCL | <1% | *RANBP2* | 2q13 | (164–171) |
| | | *EML4* | 2p21 | |
| | | *SEC31A* | 4q21.22 | |
| | | *SQSTM1* | 5q35 | |
| | | *NPM1* | 5q35.1 | |
| | | *CLTC* | 17q23.1 | |
| Colorectal cancer | <1% | *EML4* | 2p21 | (172–175) |
| | | *WDCP* | 2p23.3 | |
| Breast cancer | N.D. | *EML4* | 2p21 | (173) |
| Renal Cell Carcinoma RCC | <1% | *TPM3* | 1q21.2 | (176–179) |
| | | *EML4* | 2p21 | |
| | | *STRN* | 2p22.2 | |
| | | *VCL* | 10q22.2 | |
| Renal Medullary Carcinoma RMC | N.D. | *VCL* | 10q22.2 | (180) |
| Esophageal cancer | N.D. | *TPM4* | 19p13.1 | (181, 182) |
| Ovarian cancer | N.D. | *FN1* | 2q35 | (183) |

# ALK Inhibitors

Preclinical studies have established that EML4-ALK is an oncogenic unit

Transgenic mice expressing EML4-ALK develop several adenocarcinoma nodules in their lung soon after birth

Although originally developed as a potent MET inhibitor, crizotinib was the first ALK-directed TKI to enter the clinic

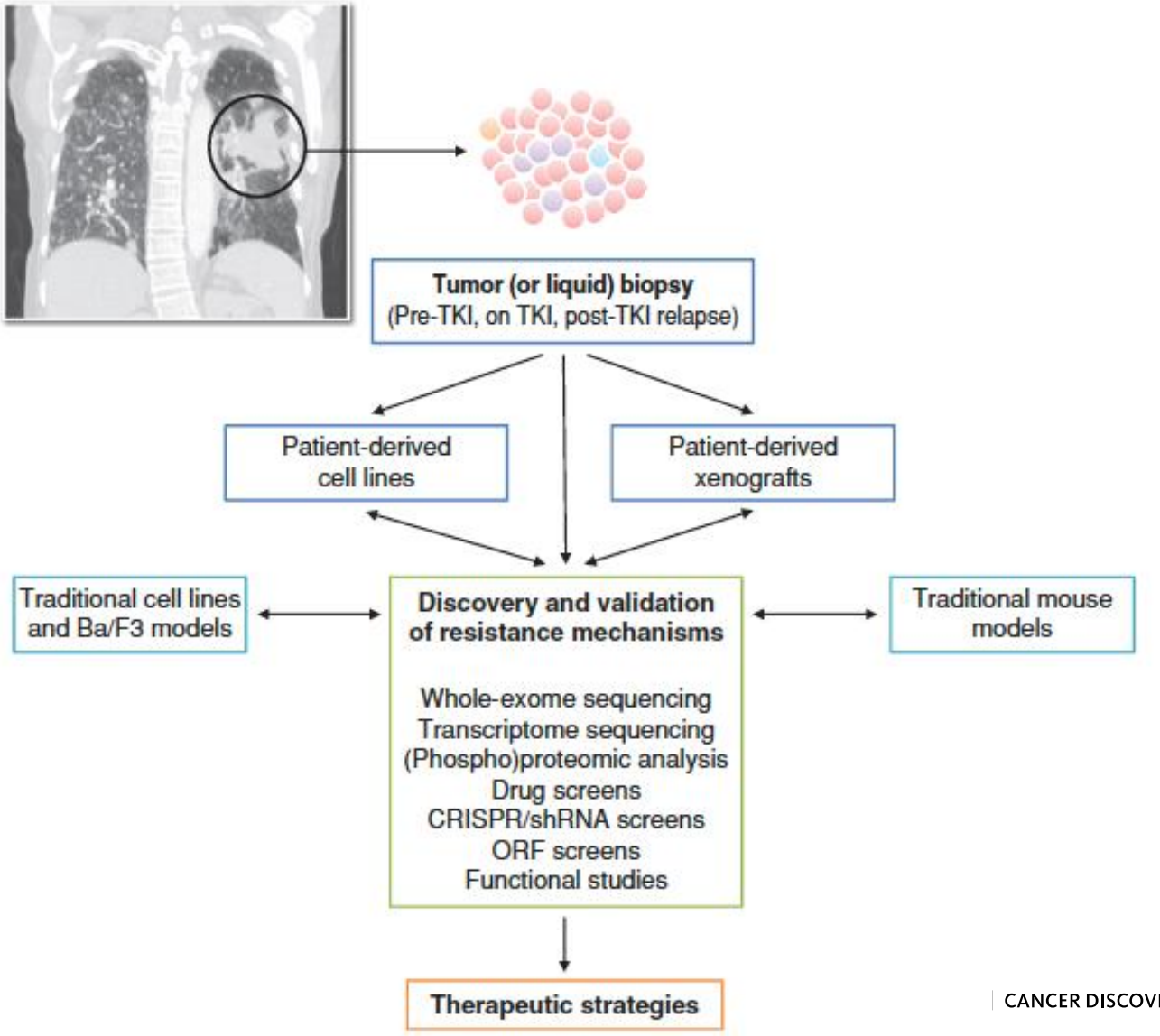
Two phase III trials showed that crizotinib was superior to first- and second-line chemotherapy in advanced ALK-rearranged NSCLC (response rate from 60% to 74%)

Thus two second generation of ALK inibitors, ceritinib and alectinib, were approved for crizotinib-pre-treated ALK-rearranged NSCLC

The second generation of ALK inhibitors are more potent than crizotinib, can overcome crizotinib-resistant ALK-mutations

Despite initial responses, patients treated with ALK TKIs progress within 1 to 2 years due to acquired resistance

# Experimental systems and approaches used for the identification of ALK TKI resistance mechanisms



# Mechanisms of resistance to ALK TKIs

There are two major classes of ALK TKI resistant mechanisms:

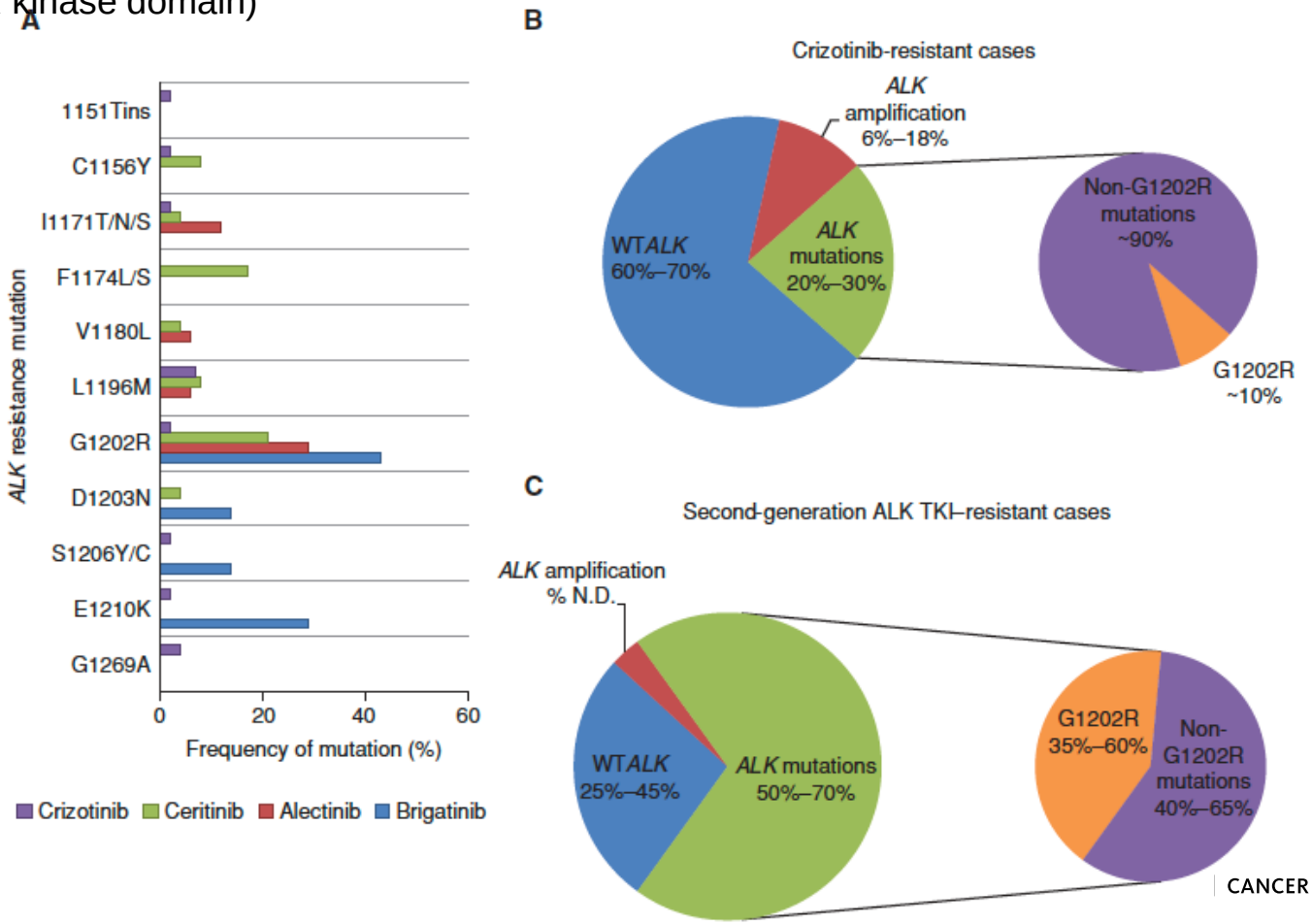
-ALK-dependent mechanisms that include ALK secondary resistance mutations or amplifications (dependency on ALK persists)

-ALK-independent mechanisms that include the activation of bypass tracks and lineage changes (dependency on ALK escape)

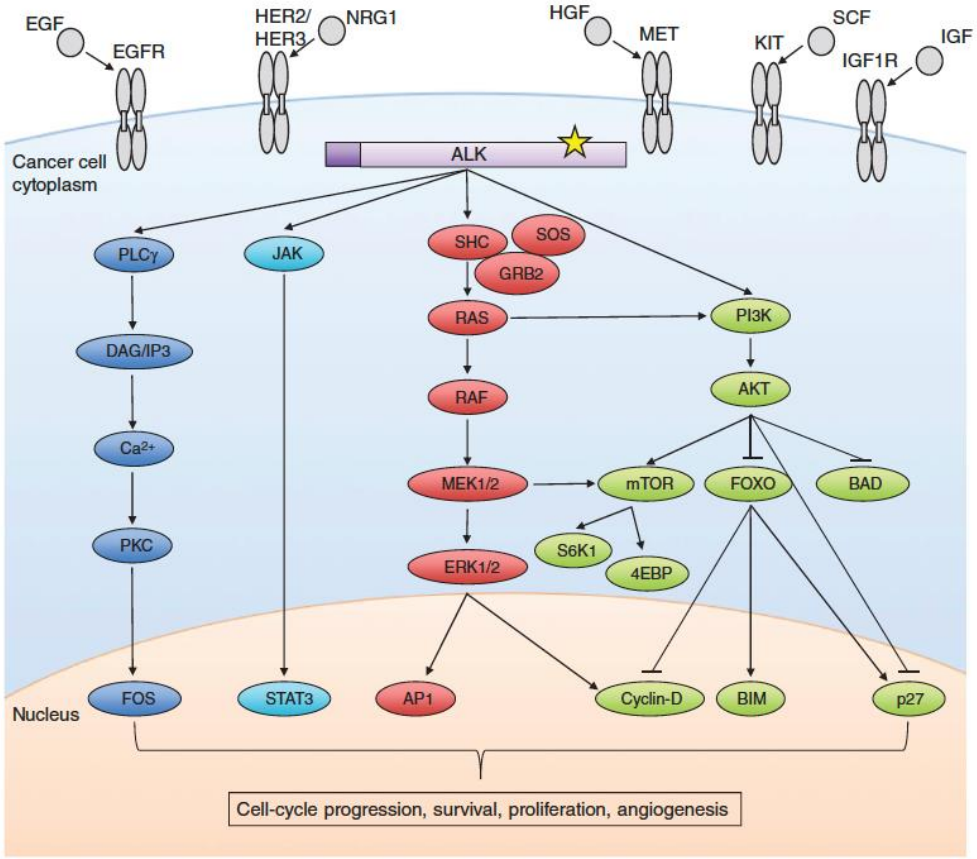
# ALK-dependent resistance

Unlike in EGFR-mutant NSCLC, where the T790M gatekeeper mutation is the predominant, a much broader spectrum of non-target mutations has been identified in ALK-positive NSCLC treated with ALK TKIs

The difference in spectrum of resistance mutations may be attributable to the genetic mechanism of oncogene activation (gene rearrangements involving ALK *versus* activating point mutations within the EGFR kinase domain)



# ALK-independent resistance




**Bypass signaling pathways activated:**
EGFR activation

Neuregulin-1 (NRG1) overexpression

MET amplification

Direct reactivation of downstream effector proteins (MEK)

PIK3CA mutations

**Phenotypic changes:**
Epithelial-to-mesenchimal transition (EMT)

Histologic change

# ALK TKIs currently available and being developed

**Table 2. Pharmacologic properties of ALK inhibitors approved by the FDA or in clinical testing**

| ALK TKI | Crizotinib (PF-02341066) | Ceritinib (LDK378) | Alectinib (RO/CH5424802) | Brigatinib (AP26113) | Lorlatinib (PF-06463922) | Entrectinib (RXDX-101) | Ensartinib (X-396) |
|---|---|---|---|---|---|---|---|
| Manufacturer | Pfizer | Novartis | Genentech | Ariad | Pfizer | Ignyta | Xcovery |
| Targets other than ALK | ROS1<br>MET | ROS1<br>IGF1R<br>IR | GAK<br>LTK<br>RET | ROS1 | ROS1 | NTRK1<br>NTRK2<br>NTRK3<br>ROS1 | ROS1<br>MET<br>AXL |
| Resistance mutations known to be targeted by TKI | L1198F | I1171T/N<br>L1196M<br>S1206C/Y<br>G1269A/S | L1152P/R<br>C1156Y/T<br>F1174C/L/V<br>L1196M<br>S1206C/Y<br>G1269A/S | I1151Tins<br>L1152P/R<br>C1156Y/T<br>F1174C/L/V<br>L1196M<br>G1202R[a]<br>G1269A/S | I1151Tins<br>L1152P/R<br>C1156Y/T<br>I1171T/N/S<br>F1174C/L/V<br>L1196M<br>G1202R[b]<br>S1206C/Y<br>E1210K<br>G1269A/S | C1156Y/T<br>L1196M | C1156Y/T<br>L1196M |
| Reported resistance mutations to the TKI | I1151Tins<br>L1152P/R<br>C1156Y/T<br>I1171T/N/S<br>F1174C/L/V<br>V1180L<br>L1196M<br>G1202R<br>S1206C/Y<br>E1210K<br>G1269A/S | I1151Tins<br>L1152P/R<br>C1156Y/T<br>F1174C/L/V<br>G1202R | I1171T/N/S<br>V1180L<br>G1202R | G1202R[a]<br>E1210K + S1206C<br>E1210K + D1203N | L1198F + C1156Y[c] | G1202R | N.D. |
| Regulatory approval | Approved for 1L and beyond | Approved for crizotinib-pretreated | Approved for crizotinib-pretreated; breakthrough therapy designation for 1L | Breakthrough therapy designation for crizotinib-pretreated | N/A<br>Approved for II or III-line ALK+ metastatic NSCLC | N/A | N/A |
| Phase of testing | Phase III complete | III | III | III | III | II | III |
| References | (3, 4, 74, 78) | (73, 78) | (78, 137, 184) | (78, 124–130) | (74, 78, 131) | (132–134) | (135, 136) |

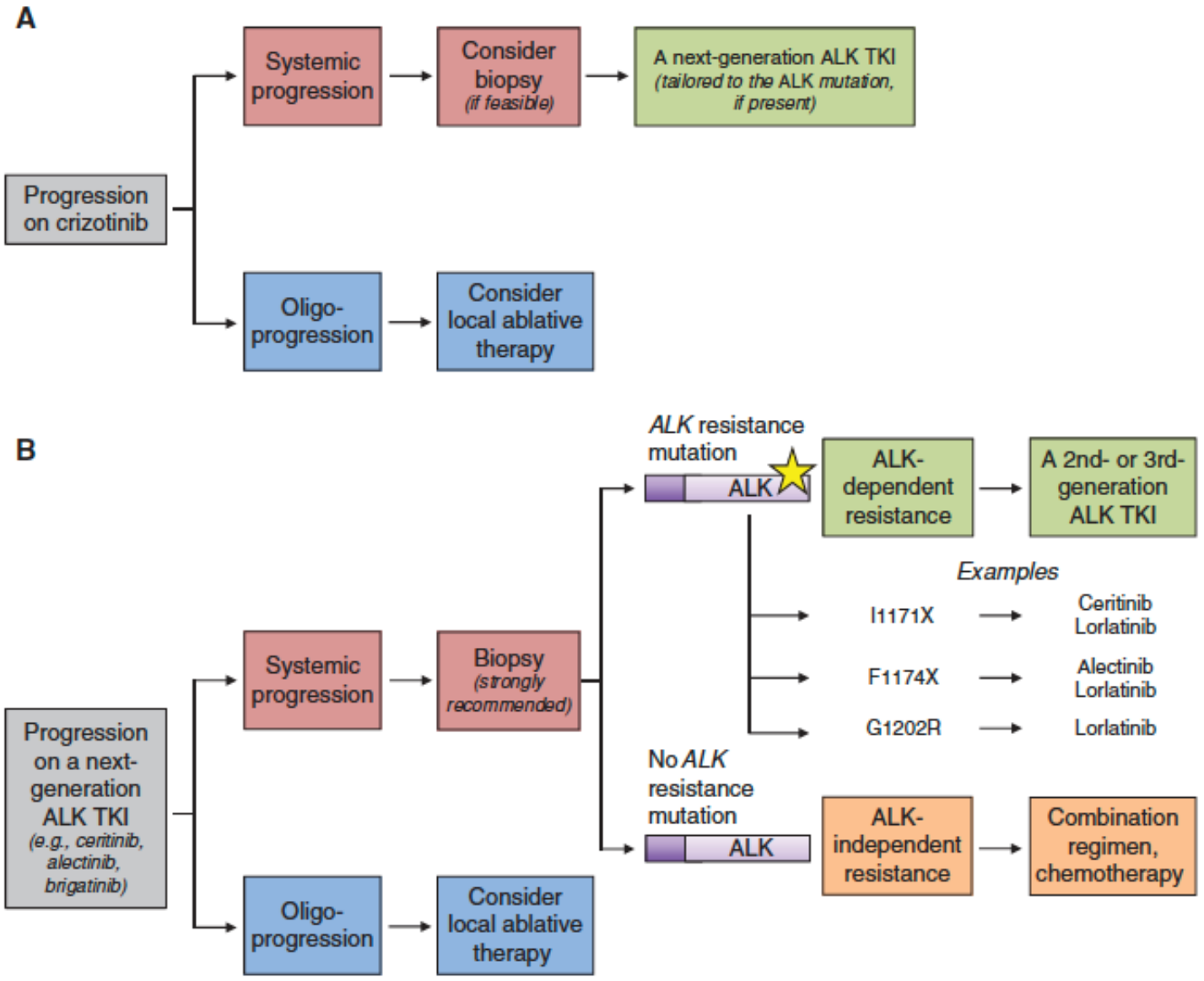
Distinct ALK inhibitors:

1. possess different potencies against resistant ALK mutations
2. differ in target kinase selectivity
3. give rise to a different spectrum of ALK resistance mutations

Critical need for repeat biopsies to guide therapeutic strategies.

The detection of a particular ALK resistance mutation may inform the choice of the next ALK TKI

# Treatment after progression on an ALK TKI



# CONCLUSIONS

ALK is an established therapeutic target in lung cancer and several other hematologic and solid malignancies

Several ALK inhibitors have entered the clinic, and to date, three have become standard therapies for advanced ALK-positive lung cancer

Despite the remarkable responses seen with ALK inhibitors, patients invariably relapse due to acquired resistance

Therefore developing strategies to overcome or prevent resistance is an urgent priority

# Colorectal Cancer

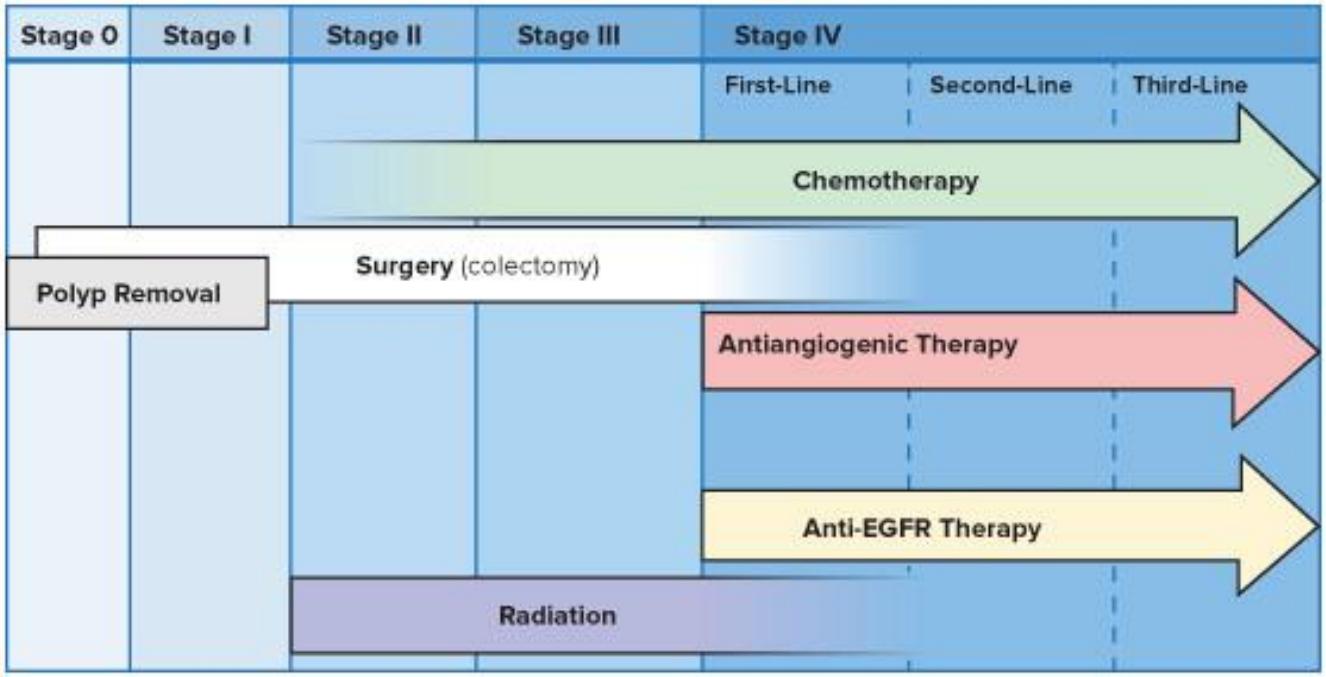
Globally, colorectal cancer (CRC) is the second most common cancer in women (614, 000 cases per year) and the third most common in men (746, 000 cases per year).

The incidence rates are higher in more developed countries (737,000 cases per year) than in less developed ones (624, 000 cases per year).

However, mortality is higher in the latter (52% of total deaths), which indicates poor survival.

In 2015, the GLOBOCAN online analysis tool has predicted 61, 228 new CRC cases for Asia. Accordingly, 25, 816 of these cases are associated with people who are less than 65 years old.

# Treatment option of Colorectal Cancer

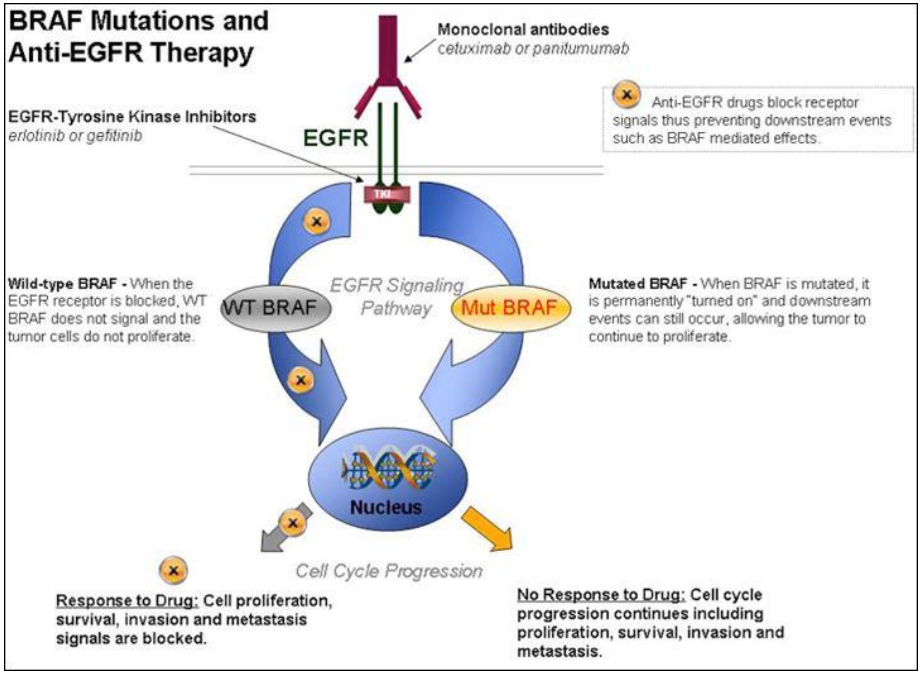


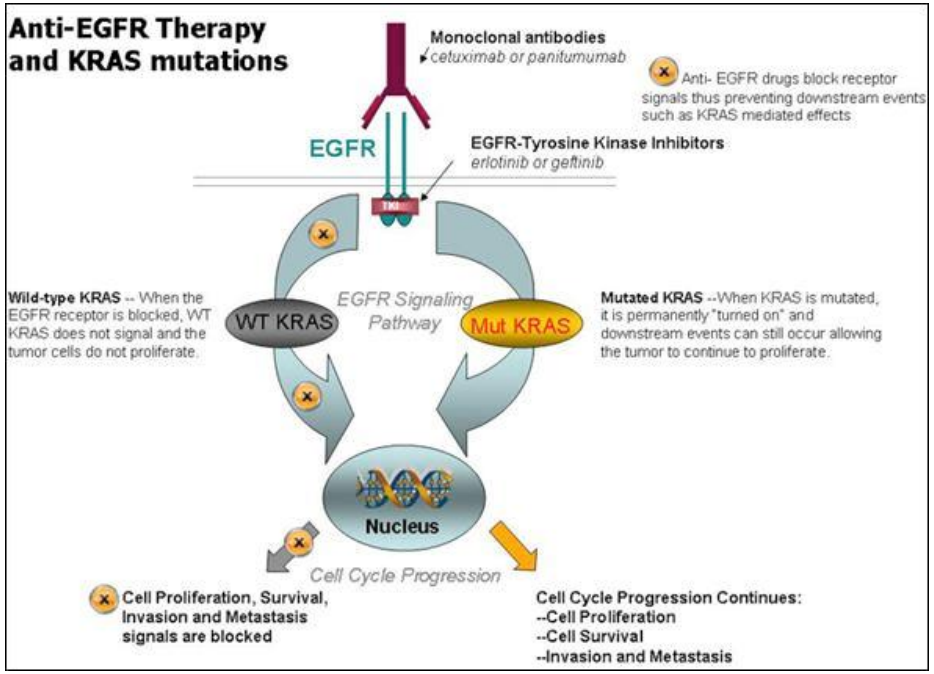

- Chemotherapy: 5-FU + leucovorin
  Capecitabine (oral form of 5-FU)
  FOLFOX: 5-FU + leucovorin + oxaliplatin
  FOLFIRI: 5-FU + leucovorin + irinotecan

- Antiangiogenic therapy: Bevacizumab (anti-VEGF)

- Anti-EGFR therapy (Cetuximab or Panitumumab MoAbs – Erlotinib/Gefitinib TKi)

# The importance of K*RAS* and *BRAF* mutations for treatment decisions in candidates for the combination of chemotherapy and target therapies

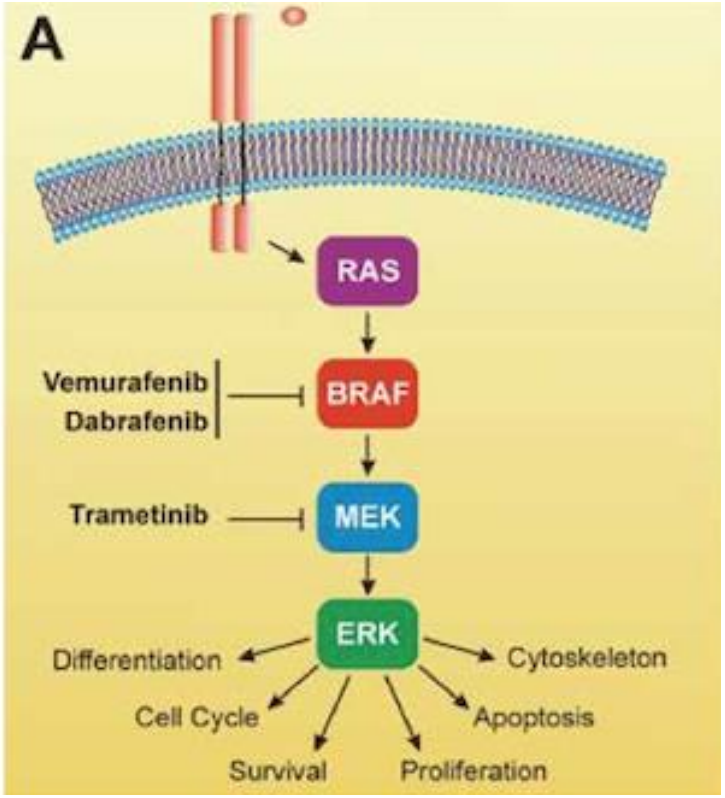



BRAF is mutated in about 15% of colorectal cancer patients

KRAS is mutated in about 50% of colorectal cancer patients

# Overcoming K*RAS* and *BRAF* mutations: the clinical development of MEK inhibitors




A number of highly specific and highly potent MEK1/2 inhibitors have been developed and evaluated in clinical studies

Only one MEK inhibitor, trametinib, has gained FDA approval for clinical use over the past decade

Most of the others agents exhibited only limited efficacy as single-agent therapies and failed to demonstrate substantial clinical activity in most tumour types in which they were studied

Such a lack of response to inhibition of a pathway that is activated in cancer probably results from activation of a secondary pathway that supports cancer cell viability in the presence of the inhibitory drug

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Report

# Intrinsic Resistance to MEK Inhibition in *KRAS* Mutant Lung and Colon Cancer through Transcriptional Induction of *ERBB3*

Chong Sun,[1] Sebastijan Hobor,[2] Andrea Bertotti,[2,3] Davide Zecchin,[2,3] Sidong Huang,[1,4] Francesco Galimi,[2,3] Francesca Cottino,[2] Anirudh Prahallad,[1] Wipawadee Grernrum,[1] Anna Tzani,[1] Andreas Schlicker,[1] Lodewyk F.A. Wessels,[1] Egbert F. Smit,[5] Erik Thunnissen,[6] Pasi Halonen,[1] Cor Lieftink,[1] Roderick L. Beijersbergen,[1] Federica Di Nicolantonio,[3] Alberto Bardelli,[2,3,7,*] Livio Trusolino,[2,3] and Rene Bernards[1,*]

In this work the authors found that MEK inhibition, by selumetinib, results in MYC-dependent transcriptional upregulation of ERBB3, which is responsible for intrinsic drug resistance

# HER3 tyrosine-kinase receptor is strongly involved in the mechanisms of cancer drug resistance

HER3 is the only member of the ErbB receptor family that cannot form a homodimer and lacks the intracellular kinase activity

The C-terminal region of HER3 contains six consensus phosphotyrosine sites which bind the SH2 domain of p85, the regulatory subunit of PI3K

The heterodimer HER-2/HER-3 is the major activator of PI3K/Akt pathway, and is considered an oncogenic unit

The overexpression of HER3 correlates with:

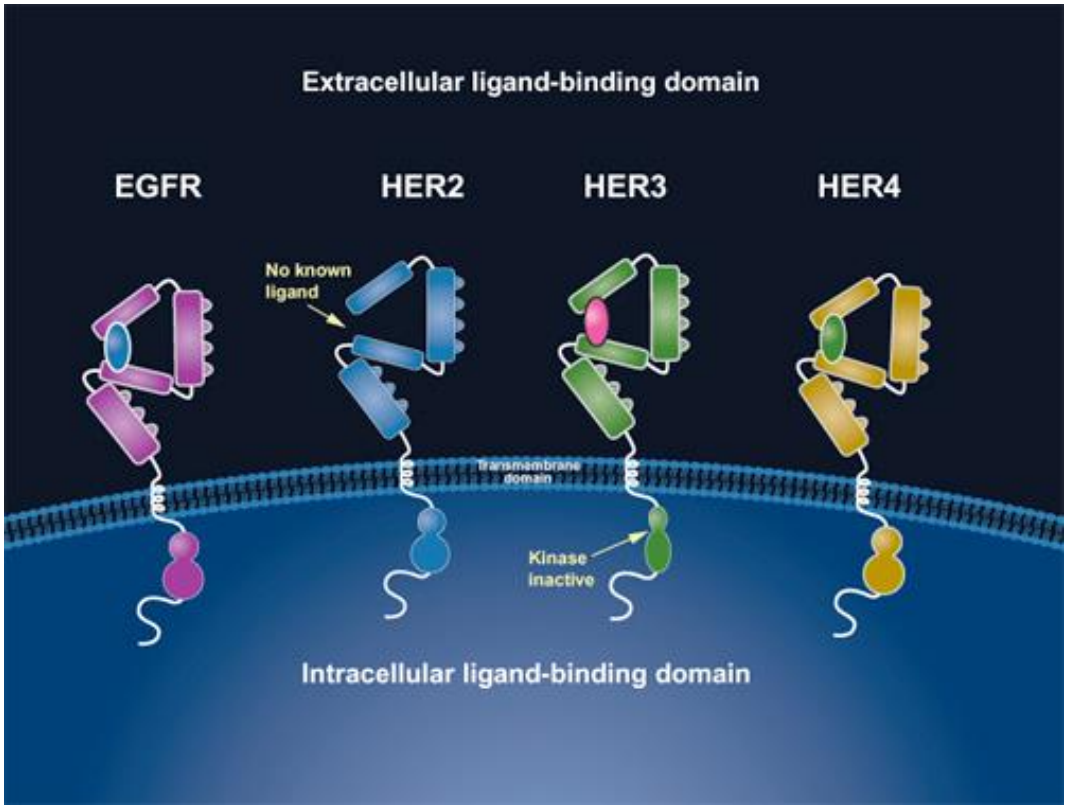
•Resistance to Tamoxifen in hormone-dependent BC

•Limph node metastases and shorter time to progression in CRC

•Resistance to the EGFR inhibitor Gefitinib in SCCHN

•Resistance to Cetuximab in CRC (increased Hrg expression)

- Resistance to Trastuzumab in HER2-overexpressin BC



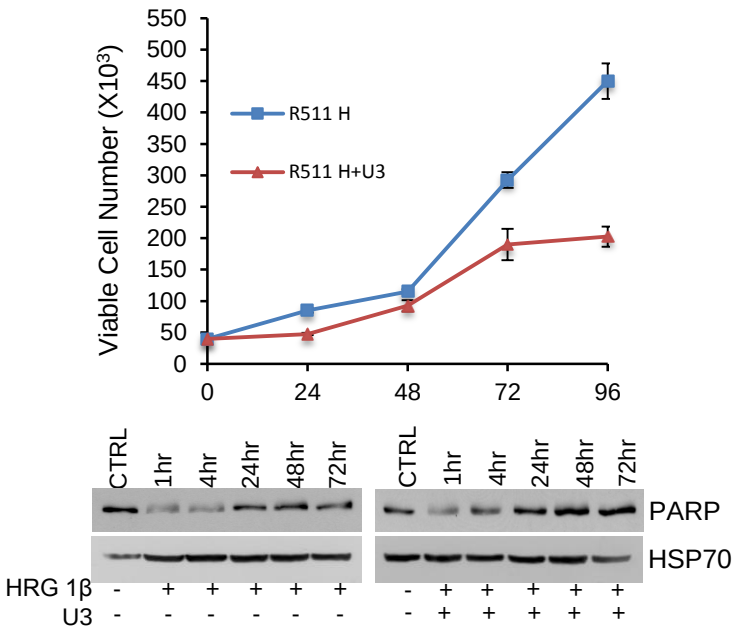
# Targeting HER3 as an Anticancer Therapy

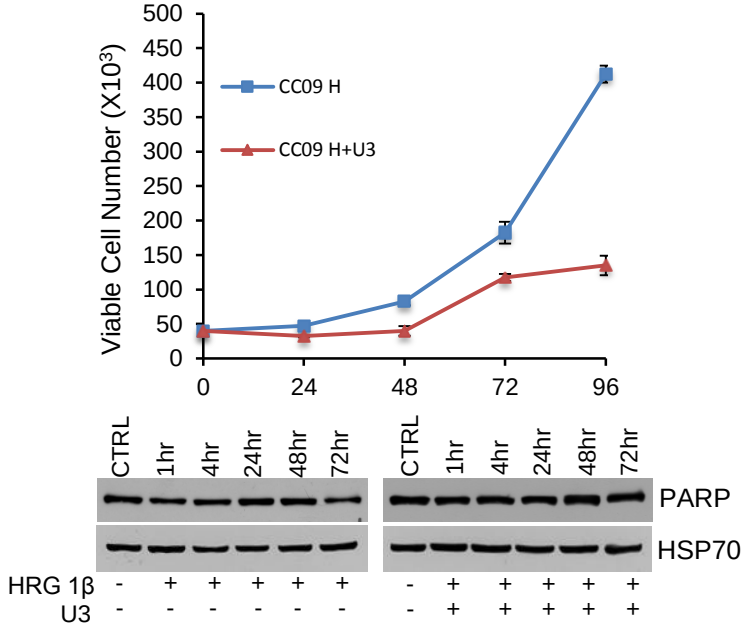
| Drug | Type | Target(s) | Development phase | Sponsor |
|---|---|---|---|---|
| MM-121 | Humanized mAb | HER3 | Phase I/II | Merrimack |
| U3-1287 (AMG 888) | Humanized mAb | HER3 | Phase I | U3 Pharma GmbH |
| MM-111 | Bispecific antibody | HER2-HER3 | Phase I | Merrimack |
| Pertuzumab | Humanized mAb | HER2-HER3 | Phase III | Genentech |
| MEHD7945A | mAb | HER1, HER3 | Phase II | Genentech |
| MP-470 (Amuvatinib) | Pan inhibitor | HER1/2/3 | Phase II | Astex Pharmaceuticals |
| AZD8931 | Pan inhibitor | HER1/2/3 | Phase I/II | AstraZeneca |

Many HER3 inhibitors have been developed and a number of them are in early clinical development

A principal technical challenge of targeting HER3 is that, unlike other HER family members, HER3 lacks enzymatic catalytic activity. Its function cannot be inhibited by ATP binding site inhibitor TKIs

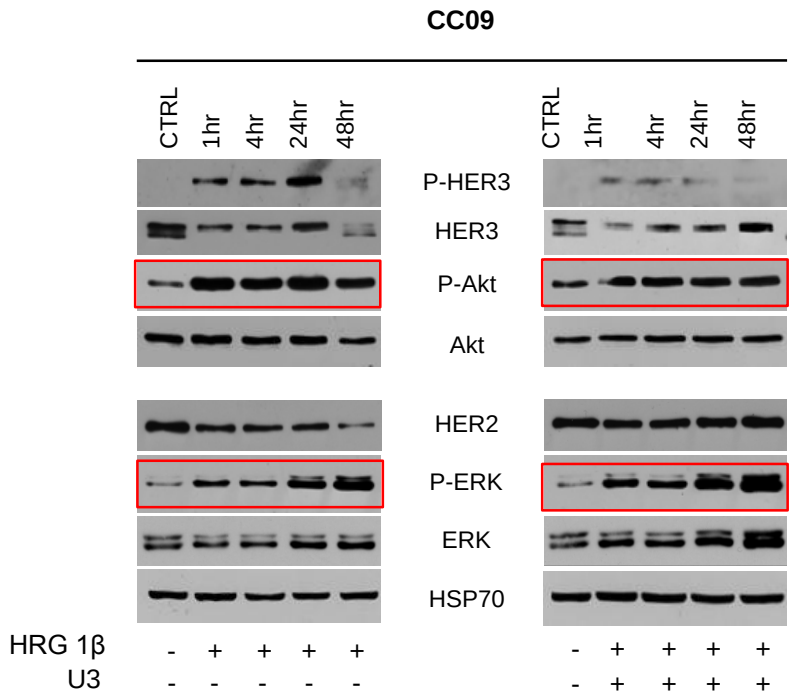
# The anti-HER3 U3 1287 antibody inhibits cell proliferation of patient-derived colon cancer cells



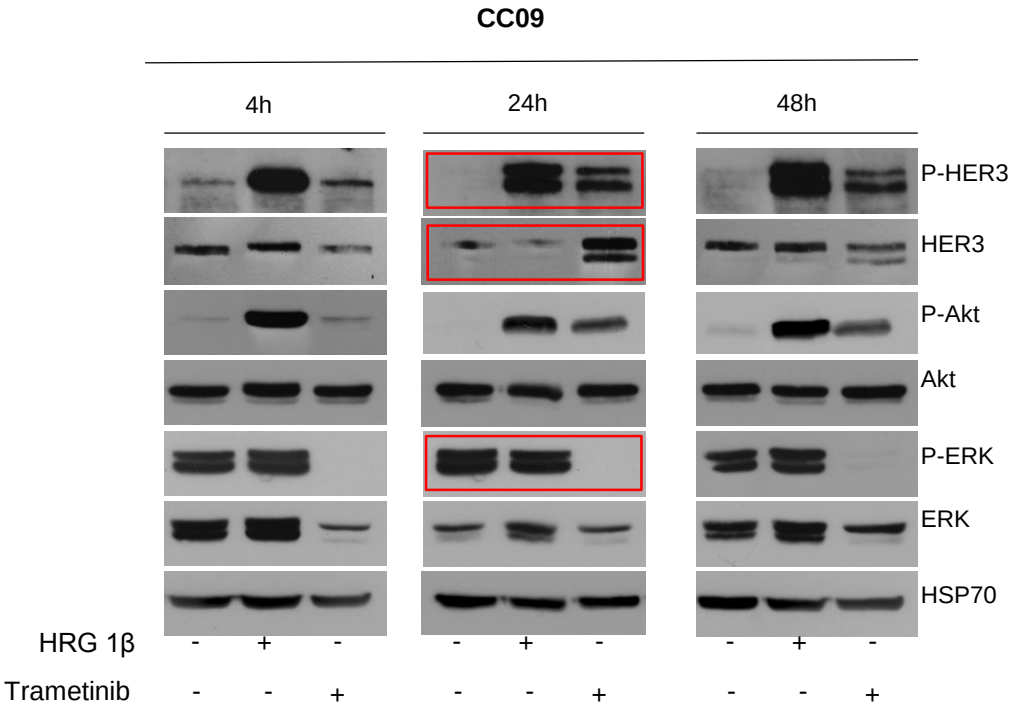




PARPs is an enzyme that plays an important role in various cellular processes, including modulation of chromatin structure, transcription, replication, recombination, and DNA repair.
The inhibition of one of these events, by drugs, causes cleavage of PARP.
The results indicate that U3 1287 antibody induces growth arrest at G1 of the cell cycle.

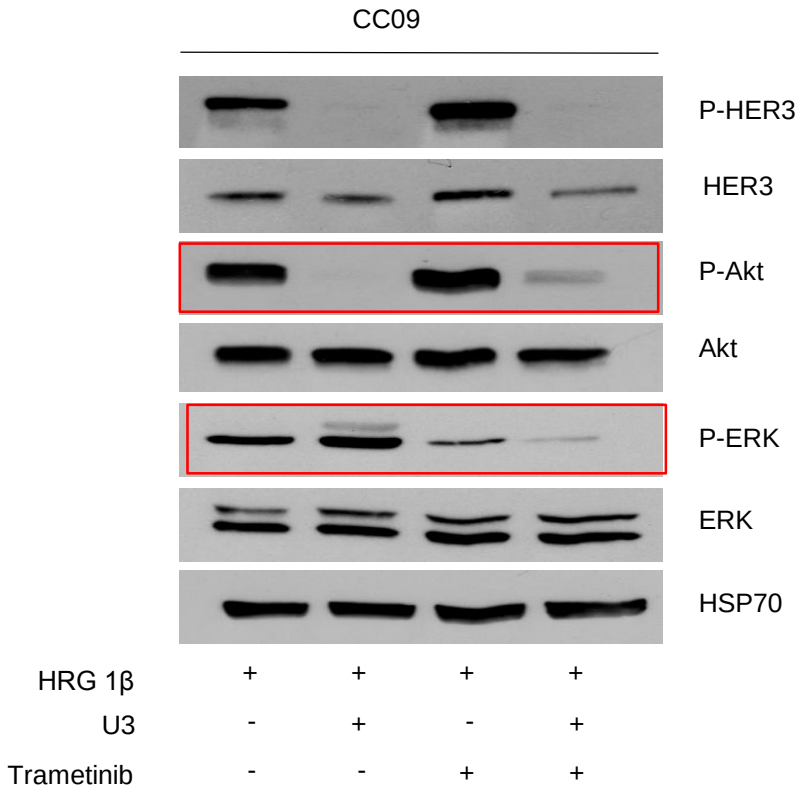
# The treatment with U3 antibody inhibits the PI3K survival pathway but induces the phosphorylation of ERK as a compensatory mechanism



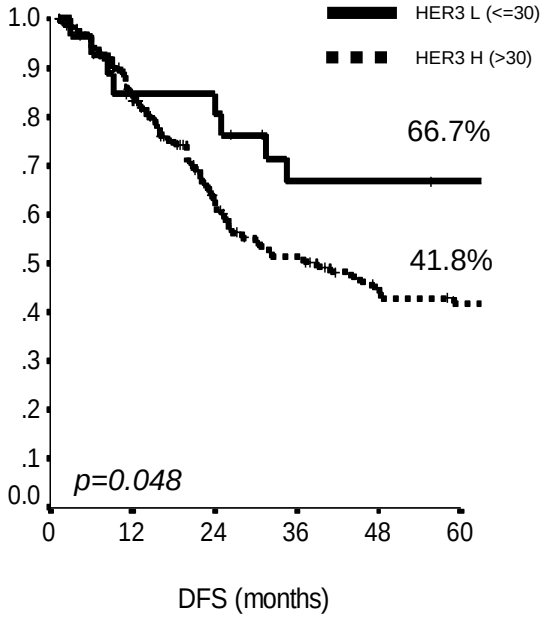
# The MEK-inhibitor Trametinib inhibits MAPK but activates the HER3-dependent PI3K survival pathway as compensatory mechanism

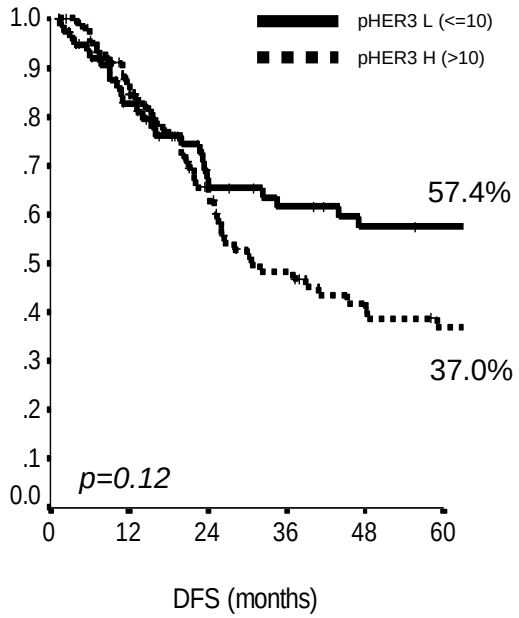


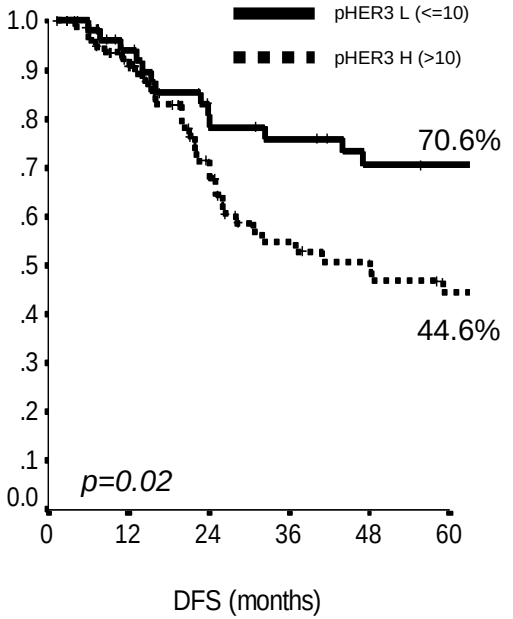
# The combination therapy with U3 antibody and Trametinib abolishes both PI3K and MAPK pathways and induces cell death



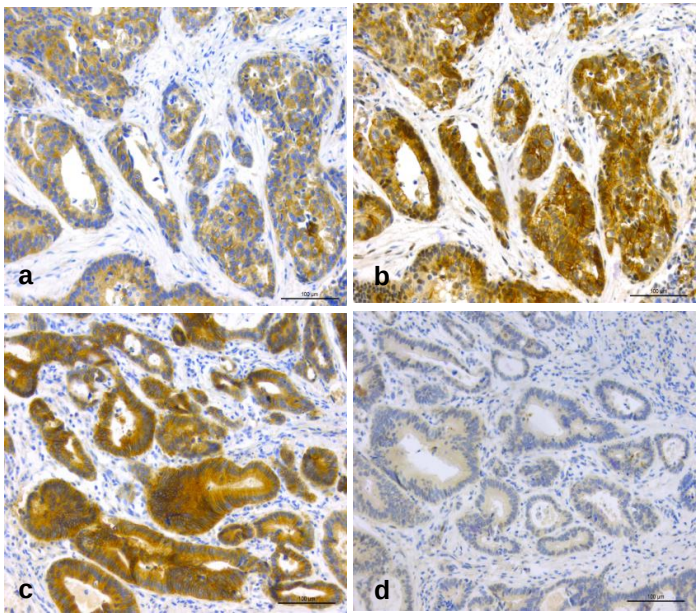
# HER3 and p-HER3 expression correlates with disease-free survival




| Variables | Disease-free survival | | | |
|---|---|---|---|---|
| | Univariate Analysis | | Multivariate Analysis | |
| | HR (CI95%) | p value | HR (CI95%) | p value |
| Age (>67 vs ≤67) | 1.33(0.76-2.32) | 0.31 | | |
| Sex (male vs female) | 1.79(1.0-3.23) | 0.05 | | |
| Site (rectum vs colon) | 2.37(1.40-4.01) | 0.001 | 2.48 (1.39-4.41) | 0.002 |
| pT (34 vs 12) | 1.33(0.71-2.47) | 0.37 | | |
| pN (N+ vs N-) | 1.66(1.0-2.81) | 0.05 | 1.76 (1.0-3.12) | 0.05 |
| Grading (3 vs 1/2) | 1.05(0.50-2.22) | 0.90 | | |
| HER3 (>30 vs <30) | 2.04(0.81-5.14) | 0.13 | | |
| p-HER3 (>10 vs <10) | 1.94(1.06-3.58) | 0.03 | 1.85 (1.0-3.42) | 0.05 |

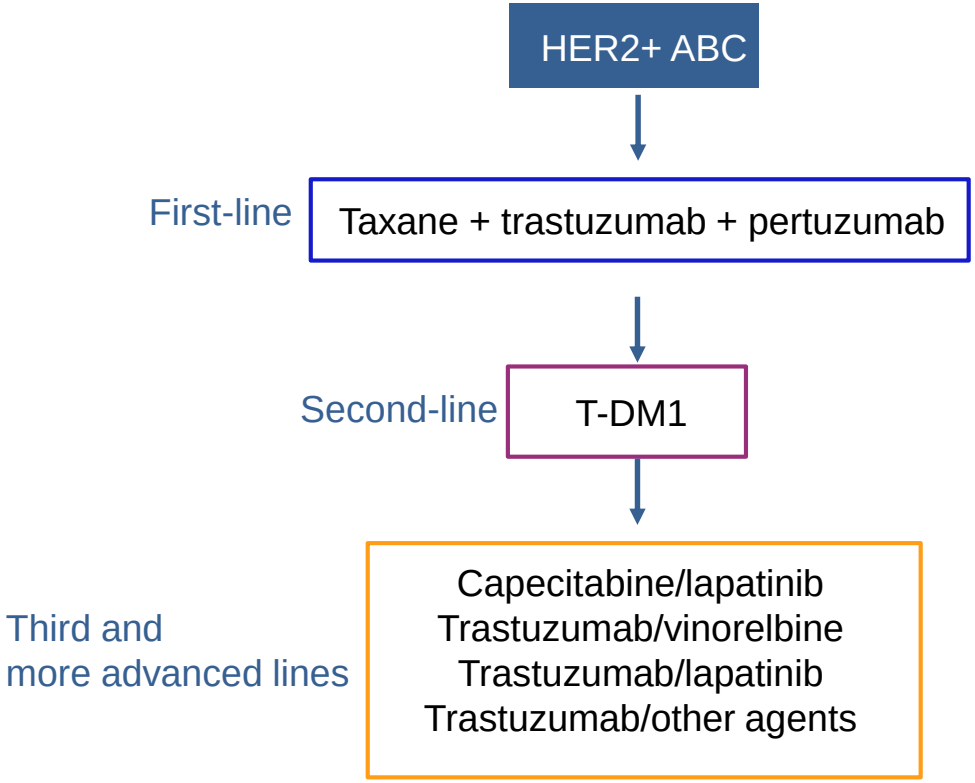


# Conclusions

- HER3 is strongly involved in the mechanisms of resistance to chemotherapy in colon cancer cells
- The anti-HER3 U3 1287 antibody inhibits cell proliferation, induces growth arrest, and inhibits the PI3K survival pathway in colon cancer cells, but induces the phosphorylation of ERK as a compensatory mechanism
- The MEK-inhibitor Trametinib inhibits ERK activity, but induces the phosphorylation of HER3 and activates the HER3-dependent PI3K survival pathway
- The combination therapy with U3 antibody and Trametinib completely abolishes PI3K and MAPK pathways and induces a complete inhibition of cell viability
- Both total- and pHER3 expression correlate with DFS

*Bon et al., Oncotarget 2016*

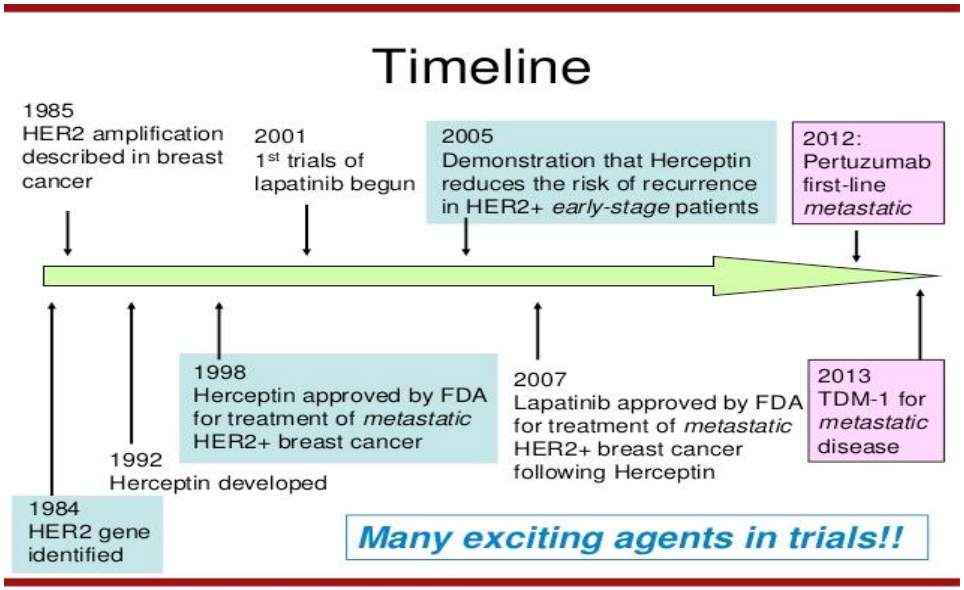
# Current Standard of Care for HER2-positive Advanced Breast Cancer




Trastuzumab has dramatically changed the therapeutic landscape of ErbB2+ advanced breast cancer

The subsequent approval of three additional anti-ErbB2 agents- lapatinib, pertuzumab, and trastuzumab emtansine (T-DM1) -converted ErbB2+ advanced breast cancer into a highly treatable disease, with more favorable long-term outcomes

# Current Standard of Care for HER2-positive Advanced Breast Cancer




**CLEOPATRA trial**

**EMILIA trial**
**THERESA trial**

Unfortunately, evidence on T-DM1 efficacy following trastuzumab/pertuzumab-containing regimens is still limited. None of the patients included in the T-DM1 pivotal trials had received previous pertuzumab, so prospective evidence is lacking

A retrospective study by Dzimitrowicz et al showed lower response rate to T-DM1 in pertuzumab-pretreated patients compared to the rates observed in pivotal trials

In a recent retrospective study Vici et al showed that patients receiving T-DM1 as second-line after trastuzumab/pertuzumab had significantly shorter PFS and OS compared with pertuzumab-naïve patients

# Known mechanisms of resistance to HER2-targeted therapy

| | Mechanisms | Comments |
|---|---|---|
| **Barriers to antibody binding** | Increased expression of p95-HER2 | Constitutively active tyrosine kinase which lacks the extracellular domain and therefore, the binding site of trastuzumab. |
| | Epitope masking | MUC4 is a large cell surface mucin that may be overexpressed and is closely associated with HER2 and thus may prevent antibody binding.<br>CD44 / hyaluron complexes are able to activate PI3K and RAS pathways. |
| **Upregulation of downstream signaling** | PTEN loss | Trastuzumab binding stabilizes and activates PTEN and downregulates PI3K/Akt signaling pathway (39). Loss of PTEN results in constitutive activation of PI3K/Akt and correlates with loss of response. |
| | PI3K mutations | PI3K pathway mutations are common in cancers and activating mutations (eg, activating PIK3R1 and PIK3CA) allow continued signaling irrespective of trastuzumab binding to HER2. |
| | Increased Akt kinase activity | |
| **Crosstalk** | Increased IGF-IR signaling | Results in PI3K activation |
| | Continued signaling from HER2/HER3 heterodimers, or HER3 or EGFR upregulation | Allows continued signalling of PI3K pathway |
| | Increased c-Met expression | Frequently co-expressed with HER2 and results in sustained Akt activation |
| | Upregulation of ER signaling | Activates an alternative survival pathway |
| | Increased AXL signaling | May be particularly important for lapatinib resistance |
| **Failure of ADCC** | Fc receptor polymorphisms | |

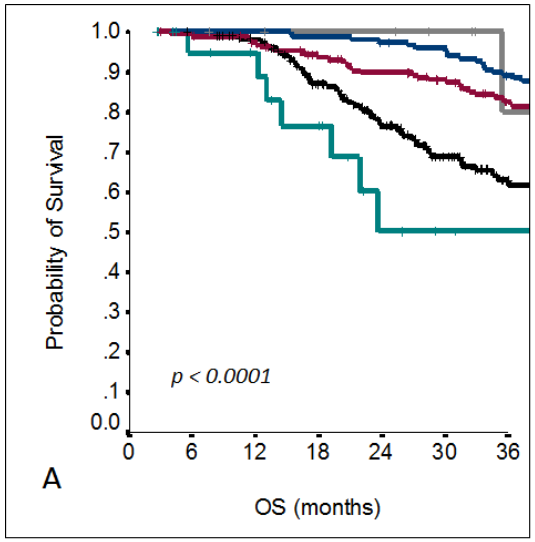
# T-DM1 efficacy following dual ErbB2 blockade by trastuzumab/pertuzumab? Study design

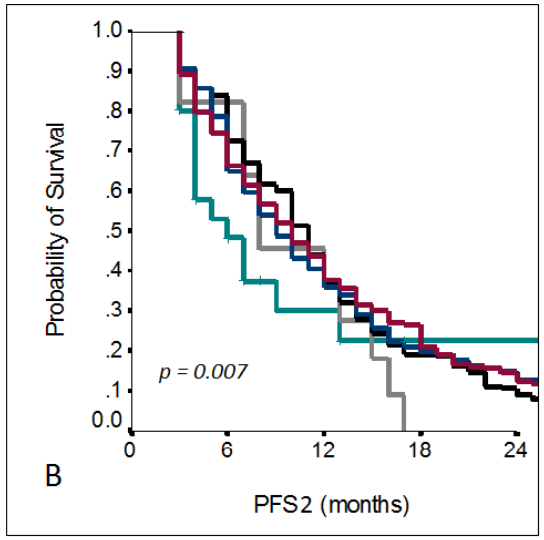
MULTICENTRIC RETROSPECTIVE STUDY
555 ErbB2+ advanced breast cancer patients

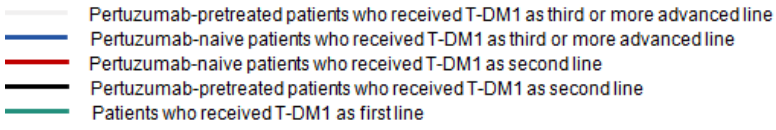
INVESTIGATION OF ERBB2 BIOLOGY *in vitro*
through sequential treatments

Are we  adopting the optimal sequence of administration of HER2 targeting drugs in current use?

# Trastuzumab/pertuzumab-pretreatment significantly affects both median overall survival (mOS) and median progression-free survival to second-line T-DM1 (mPFS2).



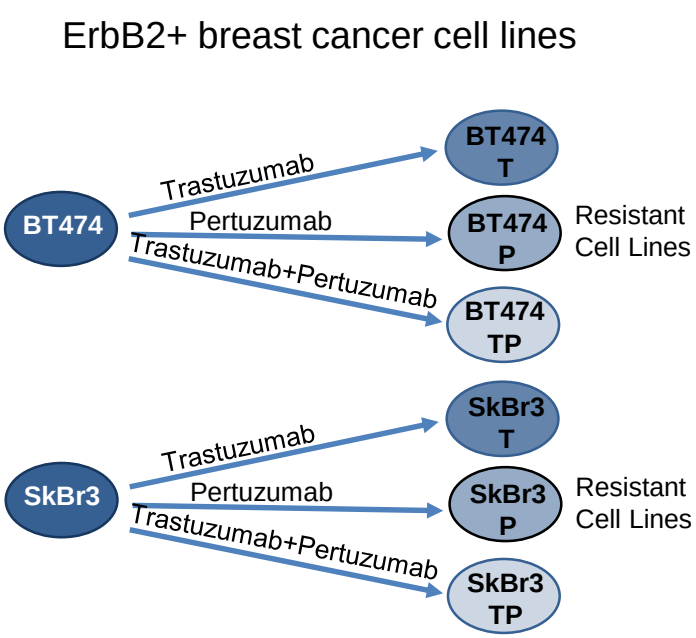



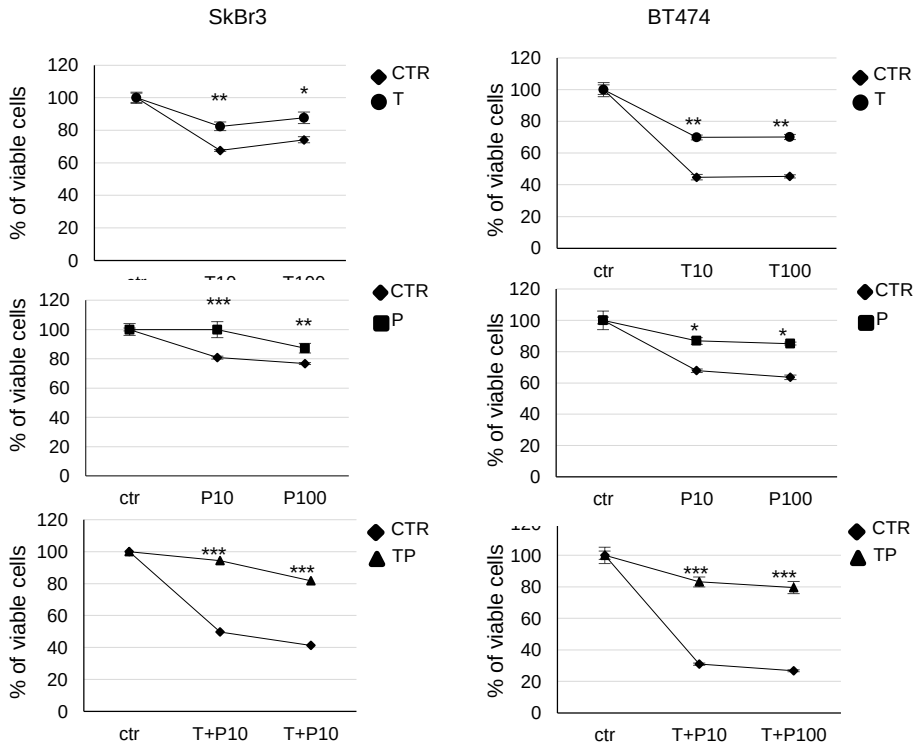




| | | Median Overall Survival (months) | | | |
|---|---|---|---|---|---|
| **Treatment sequences** | Number of patients | Overall | TP | ER or PgR+ | HRs- |
| **Group 1: First-line without pertuzumab/ T-DM1 as second-line** | 194 | 74 (52-97) | 74 (49-100) | 96 (39-152) | 62 (45-79) |
| **Group 2: First-line without pertuzumab/ T-DM1 as third-line or beyond** | 148 | 91 (71-112) | 94 (62-127) | 71 (55-88) | 102 (76-128) |
| **Group 3: First-line with pertuzumab/ T-DM1 as second-line** | 177 | 52 (41-64) | 67 (37-96) | N.R. | 36 (28-44) |
| **Group 4: First-line with pertuzumab/ T-DM1 as third-line or beyond** | 11 | N.R. | N.R. | N.R. | N.R. |
| **Group 5: First-line with T-DM1** | 25 | N.R. | N.R. | N.R. | 22 (14-30) |
| **p – value overall** | - | < 0.0001 | 0.07 | 0.88 | < 0.0001 |
| **p – value group 1 vs group 3** | - | 0.001 | - | - | - |
| **p – value group 2 vs group 4** | - | 0.99 | - | - | - |

Bon et al., under revision

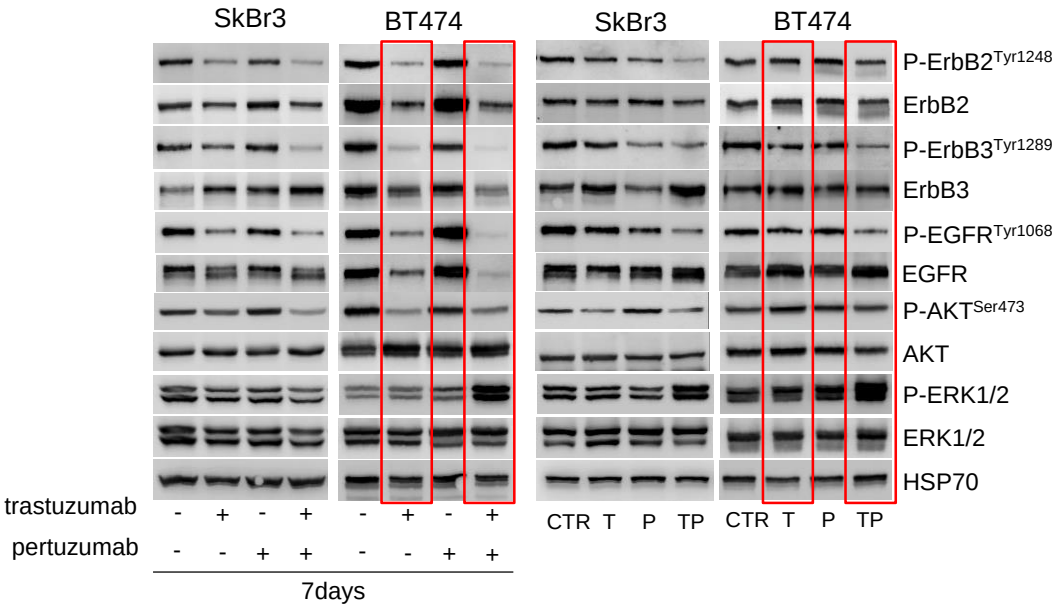
# Generation of ErbB2+ cell lines resistant to trastuzumab, pertuzumab, and trastuzumab/pertuzumab combinaton

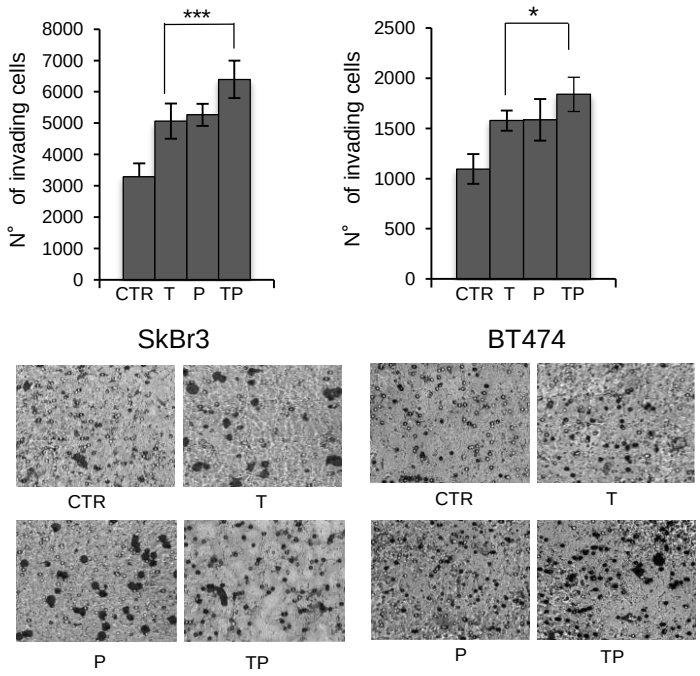



Bon et al., under revision

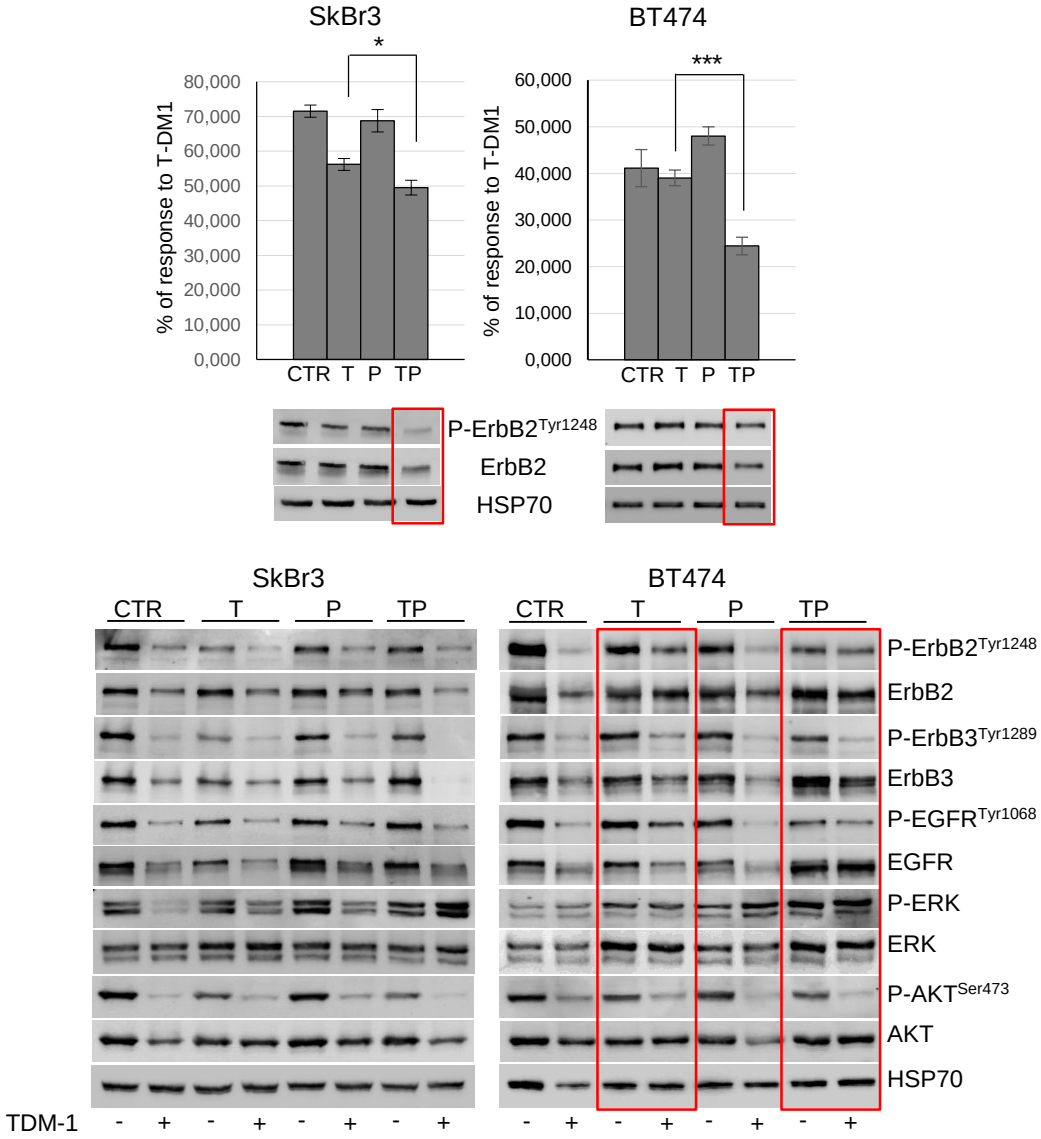
# Trastuzumab+pertuzumab-resistant cell lines exhibit higher invasive capability compared to trastuzumab-resistant cells and induction of ERKs phosphorylation



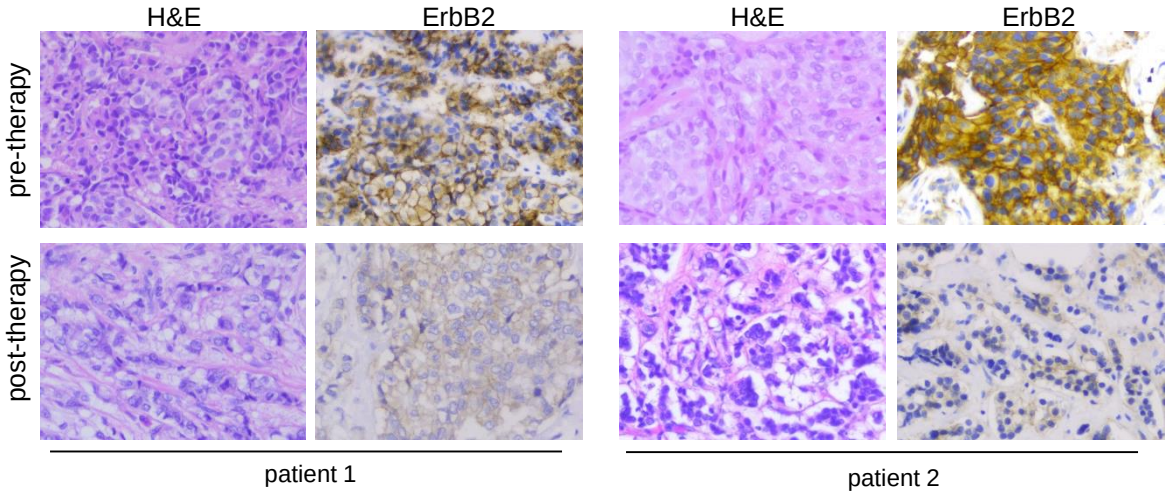

Bon et al., under revision

# Dual HER2 blockade is associated with reduced T-DM1 efficacy due to ErbB2 downregulation.




Bon et al., under revision

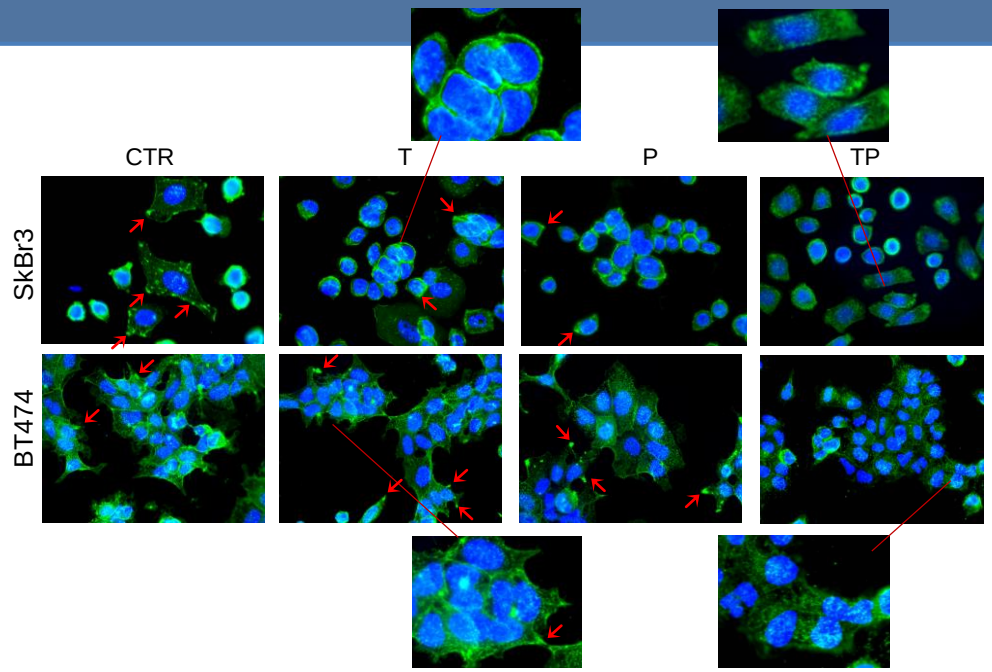
# Trastuzumab + pertuzumab combination determines ErbB2 downregulation *in vivo.*

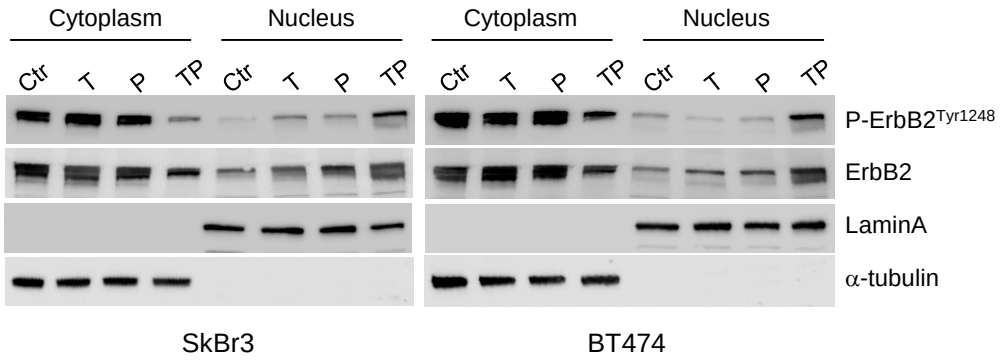



| | Pre-therapy | | | | | Post-therapy | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | ER | PgR | ErbB2 score | ErbB2% | ErbB2% FISH | ER | PgR | ErbB2 score | ErbB2% | ErbB2% FISH |
| **Patient 1** | 90% | 90% | 2+ | 70% | amplified | 90% | 90% | 0 | 0% | - |
| **Patient 2** | 90% | 40% | 2+ | 30% | amplified | 90% | 60% | 1+ | 30% | - |
| **Patient 3** | 46% | 0% | 2+ | 100% | amplified | 75% | 18% | 1+ | 15% | amplified |
| **Patient 4** | 88% | 42% | 3+ | 100% | - | 99% | 1% | 1+ | 60% | amplified |

Bon et al., under revision

# Prolonged trastuzumab+pertuzumab treatment induces ErbB2 nuclear translocation




Bon et al., under revision

# Conclusions

Our multicentric retrospective study involving 555 ErbB2+ advanced breast cancer patients revealed that mOS of patients who received second-line T-DM1 was significantly lower if they were trastuzumab/pertuzumab-pretreated, compared to pertuzumab-naïve patients (52 and 74 months, respectively, p=0.001).

T-DM1 efficacy is markedly reduced in trastuzumab/pertuzumab-resistant cell lines compared to trastuzumab-resistant ones;

We found a marked reduction of ErbB2 in TP-cells compared to T-cell. This data was confirmed *in vivo* by IHC analysis.

Our data indicate a marked nuclear translocation of ErbB2 in TP-cells. Nuclear ErbB2 is phosphorylated, suggesting its active involvement in transcriptional control mechanisms

Our data support the hypothesis that prior exposure to trastuzumab/pertuzumab reduces the amount of ErbB2 available at the plasma membrane for T-DM1.

Moreover, TP-cell lines show a marked induction of ERKs phosphorylation, suggesting a pro-proliferative advantage conferred by prolonged exposure to the combined treatment.