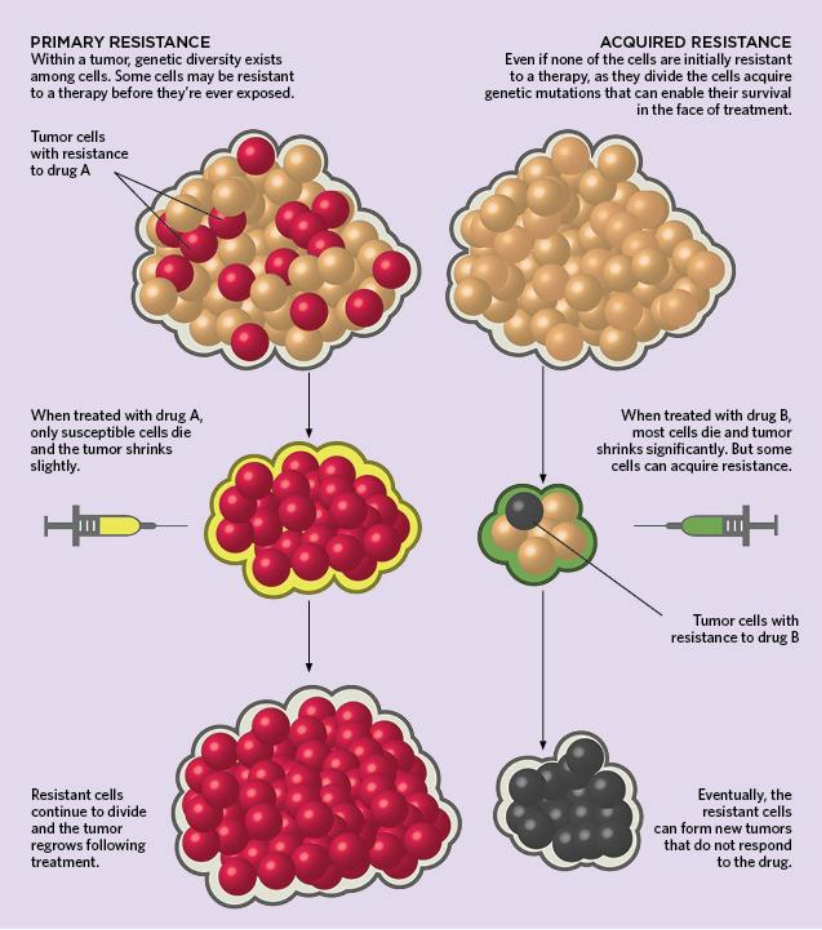
Cancer Treatment and Therapy Resistance

- Most tumors show initial response to therapy (radiation, chemotherapy, immunotherapy) yet cure rates are limited by the development of secondary resistance conductive of progression under therapy.
- Therapy resistance is particularly prominent in the context of targeted therapies and represents a significant barrier in precision medicine.
- Resistance mechanisms are actionable targets for improved therapeutics:
  - -Guide design of more effective inhibitors
  - -Guide combination therapies to prevent or circumvent resistance by targeting downstream or parallel cellular programs conductive of resistance.



Drug combinations and increased dose intensity deliver improved efficacy but more is not always better:

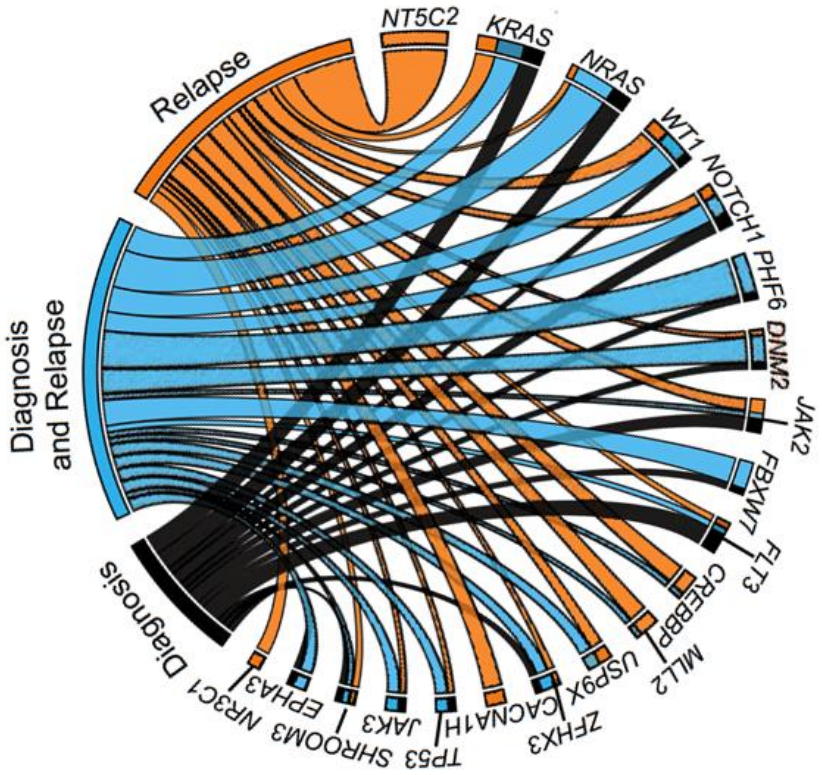
Early success with single agent chemotherapy treatments in highly sensitive tumor types was rapidly followed by relapse and therapeutic failure as result of secondary resistance.

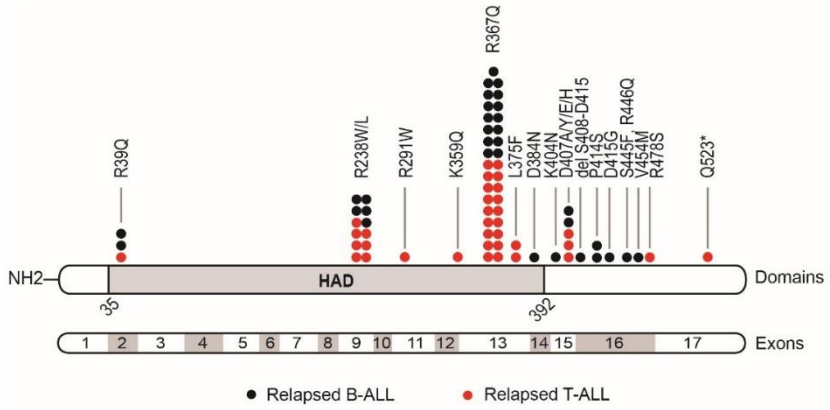
The initial solution to the problem of resistance to single-agent chemotherapy—the combined administration of agents with non-overlapping mechanisms of action which worked remarkably well in some hematologic tumors, breast cancer and testicular cancer.

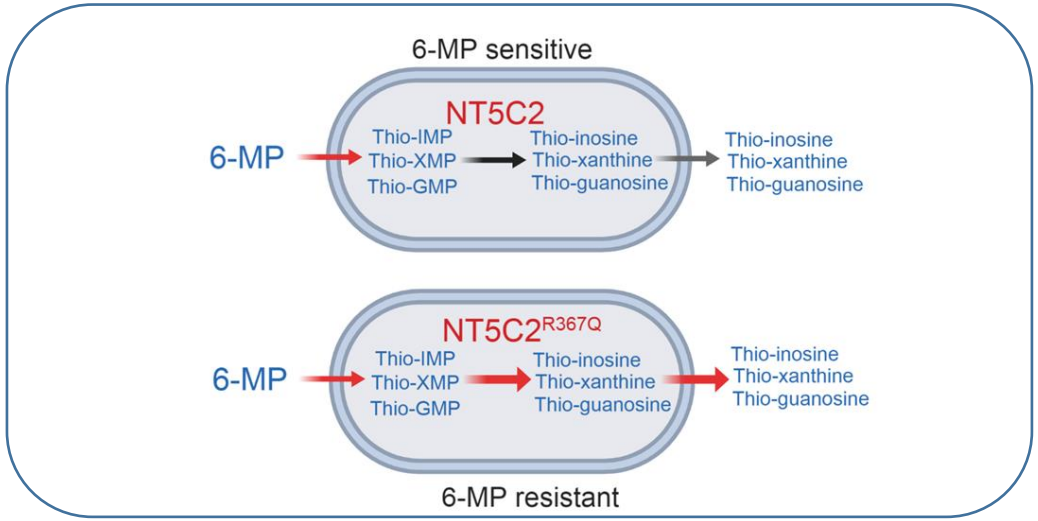
In addition, a number of different approaches to dose intensity, including shorter-interval administrations of chemotherapy or higher doses of chemotherapy with growth factor support to prevent continued bone marrow suppression, have resulted in improved success of these therapies by preventing early regrowth of tumors.

Cumulative toxicities and secondary resistance with loss of therapeutic index at the time of relapse limit the efficacy of polychemotherapy in cancer treatment.

6-MP inactivation by gain of function mutations in NT5C2 in relapsed acute lymphoblastic leukemia







Cellular mechanisms contributing to drug resistance

Increased drug export: multidrug resistance (MDR) drug efflux pumps

Increased drug metabolism: NT5C2 mutations clear 6-MP in relapsed leukemia

Overexpression of drug targets: DHFR amplification in methotrexate treatment

Mutational disruption of the drug binding sites: BCR-ABL1 mutations driving resistance to ABL1 kinase inhibitors; alternative splicing of CD19 with loss of epitope recognized by CAR T cells in acute lymphoblastic leukemia.

Loss of downstream drug effector pathways: TP53 mutations, BCL2 overexpression

Activation of compensatory pathways: increased DNA repair

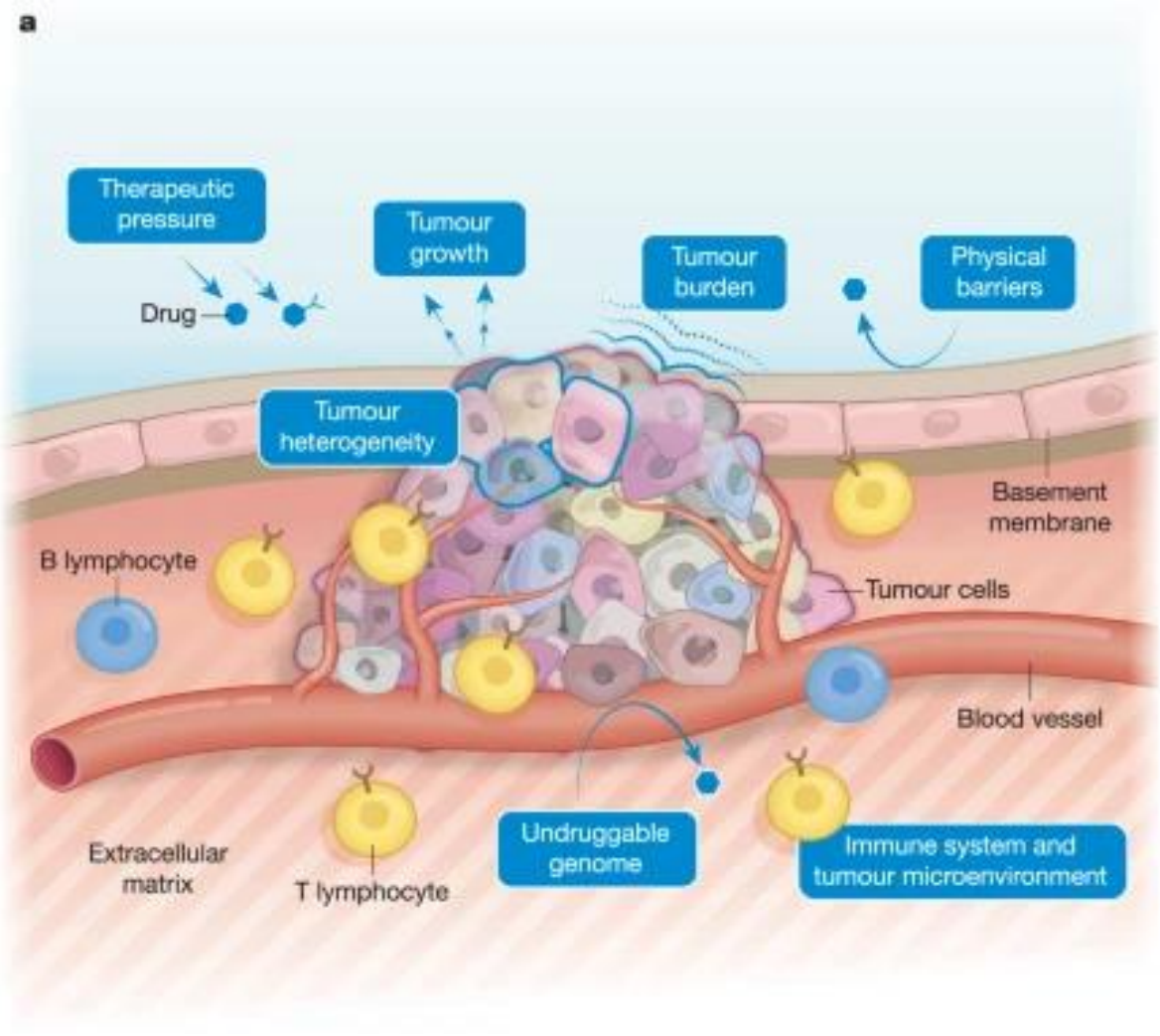
Loss or compensation of targeted cancer vulnerabilities:

Gene conversion in BRCA mutant tumors treated with PARP inhibitors and in Fanconi Anemia tumors treated with alchylating agents

MAPK activating mutations in BRAF inhibitor therapy

Neuroendocrine reprogramming in castration resistance prostate cancer and non small cell lung cancer treated with EGFR inhibitors

Biological determinants of therapy response



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

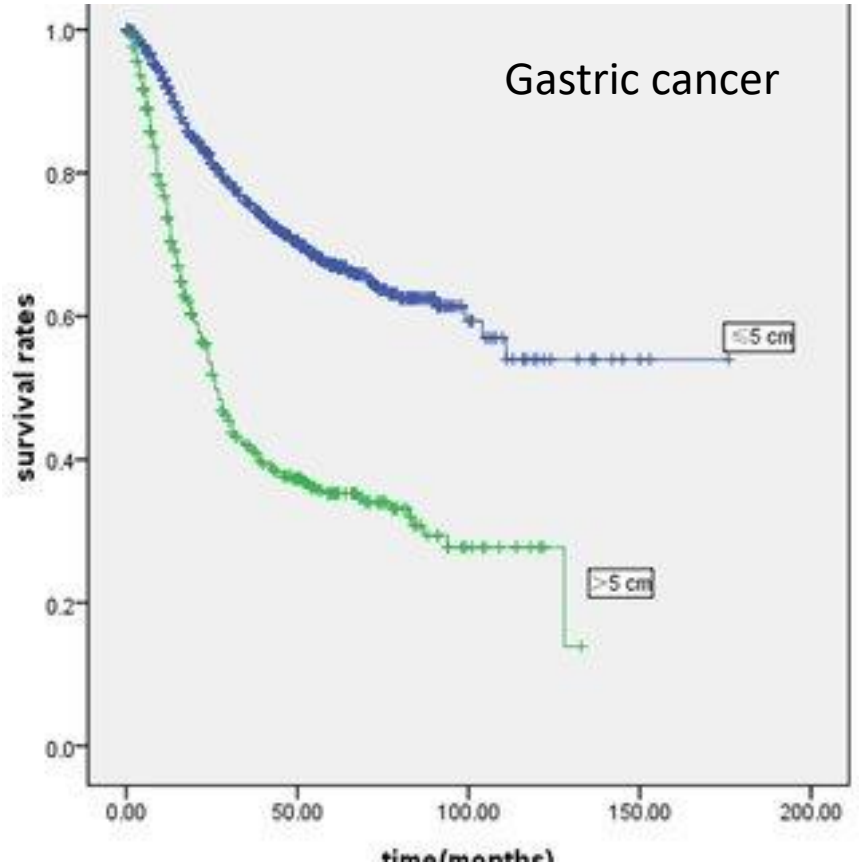
Immune system and tumour microenvironment
- Enhancing tumour recognition by the immune system
- Anti-angiogenic therapy
- Cellular therapies

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

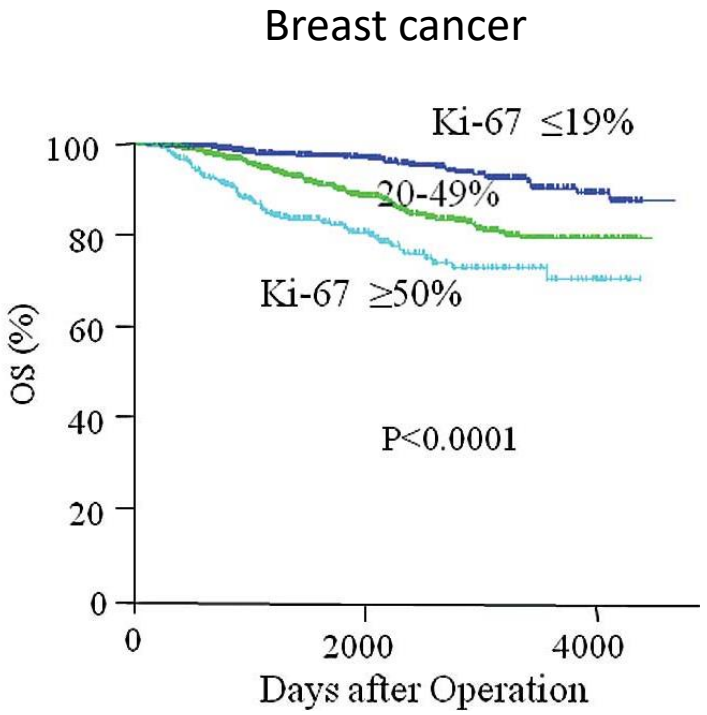
Biological determinants of therapy response: tumor burden

- There is a strong correlation between tumor burden and curability.
- In many tumor types, the size of the tumor at diagnosis is used to estimate prognosis and larger tumors correlate with increased metastatic risk.
- The Goldie–Coldman hypothesis: The probability that a cancer contains drug-resistant clones depends on the mutation rate and the size of the tumor.
- Alternating non-cross-resistant combinations of chemotherapy, rather than administering all therapies at once (which is often limited by toxicity), allows the tumor to be exposed to a greater number of total drugs by an earlier time point and is superior in preventing drug resistance as compared to sequential therapies.



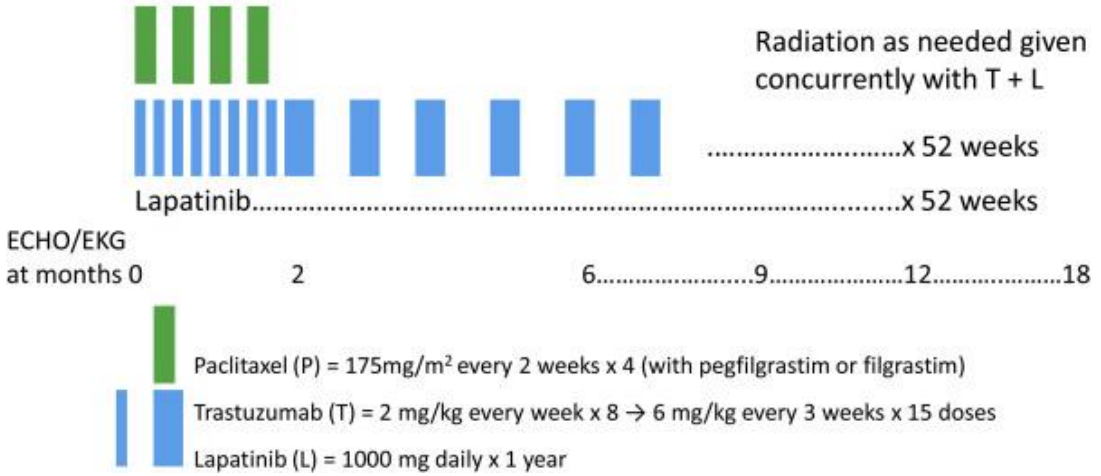
Biological determinants of therapy response: growth kinetics

- Tumor growth kinetics are highly variable ranging from indolent to highly aggressive.
- Rapidly proliferating tumors typically show more rapid responses to therapeutics delivering DNA damage and targeting proliferating mechanisms but also show more rapid progression and commonly have worse prognosis.
- The Norton–Simon hypothesis: Tumors grow in a sigmoidal manner—exponentially faster at low tumor burdens and subsequently approaching a plateau with slower growth rates as they reach a larger size.



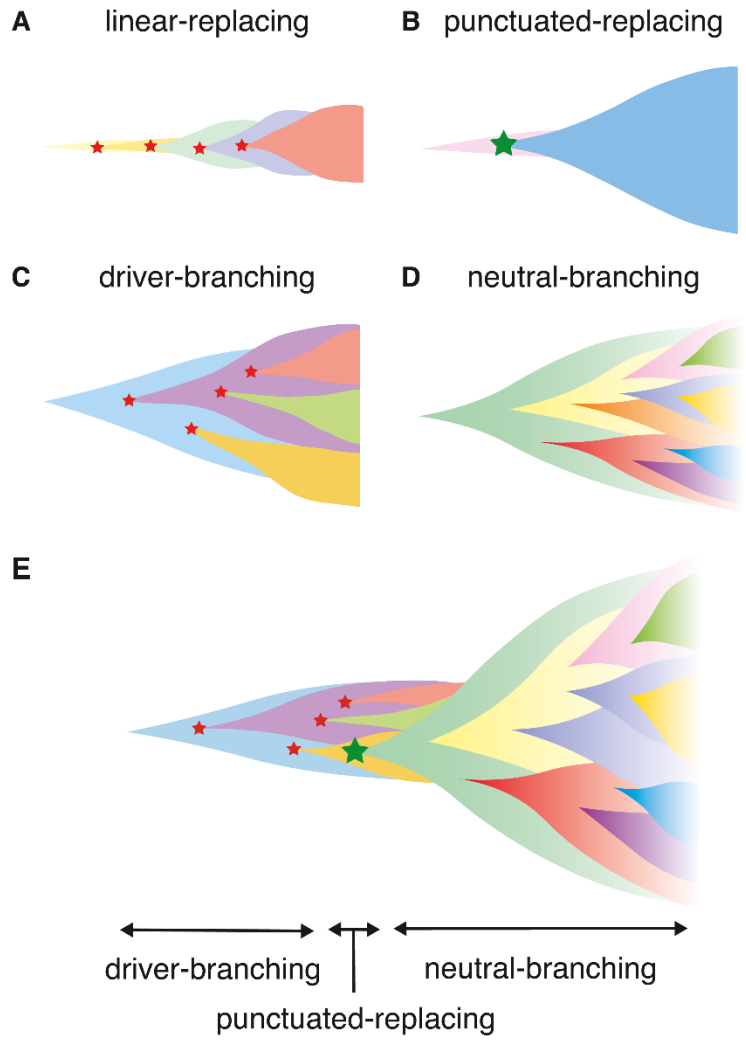
Biological determinants of therapy response: growth kinetics

- Because drugs reduce the size of tumors, they affect growth kinetics. After a single administration of chemotherapy, the remaining tumor fractions may resume their early phase of exponential growth. Following this logic, the probability of eradication is maximized by preventing rapid regrowth of the tumor between treatments.
- This led to the concept of dose-dense chemotherapy, an approach in which the most effective dose level of a drug is given over as short a time interval as possible.
- Clinical proof of concept of dose density has been demonstrated in early breast and ovarian cancer, for which chemotherapy that is administered more frequently has in select circumstances improved overall survival.



Biological determinants of therapy response: genetic heterogeneity

- Cancer cells acquire genomic alterations through a variety of mutational processes that generate spatial and temporal genetic diversity.
- Mutation rates are differ largely between tumor types (slow age-related mutation driven malignancies, fast in hypermutator cancer with defects in DNA repair) in and may change during the natural history of disease with bursts induced by genomic instability, chromothripsis and chromosomal instability.
- In some cases, therapies can induce a state of genomic instability as in low-grade gliomas, in which chemotherapy with temozolomide can result in hypermutated tumours at recurrence and favor transformation to highly aggressive glioblastoma multiforme.
- Darwinian selection of chemotherapy resistance driving mutations operates on a genetic heterogeneous ecosystem of cancer cells. Pediatric low mutation rate tumors may hold less genetic heterogeneity, while hypermutator phenotype driven malignancies may show much more complex clonal architectures and rapid evolutionary trajectories.



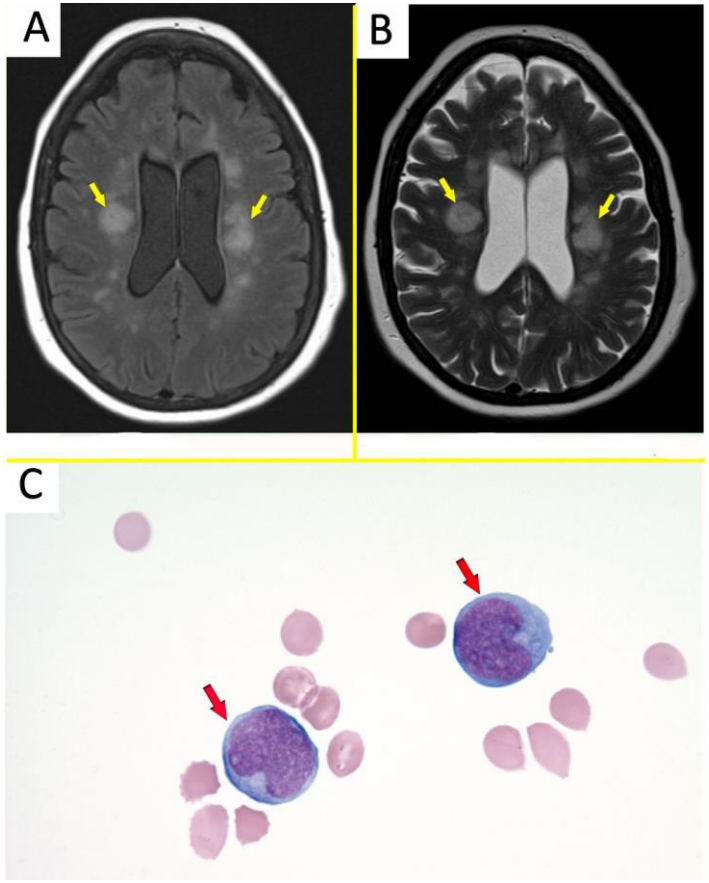
Biological determinants of therapy response: physical barriers

Sanctuary sites: Cancer cells may colonize anatomical spaces in which systemically administered drugs do not reach therapeutic concentrations

The blood–brain barrier limits the reach of chemotherapy to the central nervous system making this a preferential site of relapse in acute lymphoblastic leukemia.

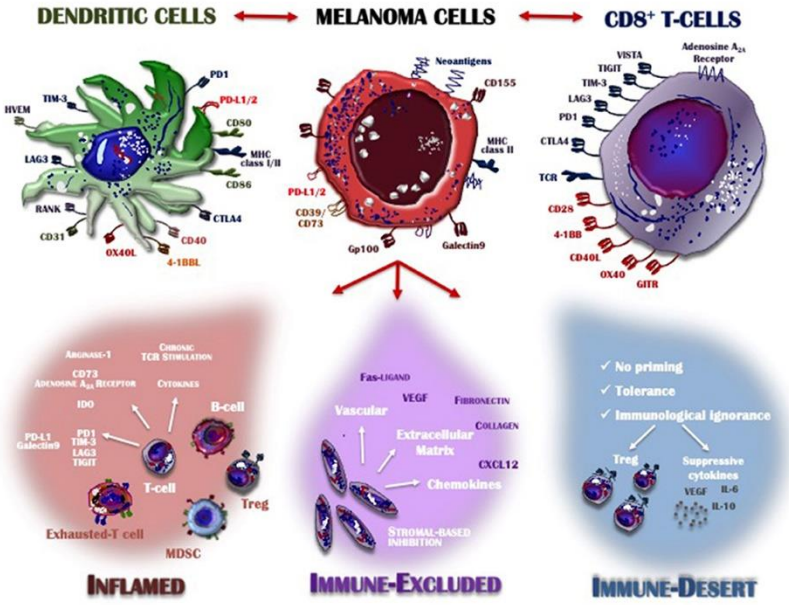
Microenvironment barriers to drug distribution: Cancer cells can create spatial gradients within tumors that prevent adequate blood flow, thereby creating a pro-tumorigenic hypoxic environment and decreasing the effective exposure of a tumor to drugs.

Pancreatic cancer shows a desmoplastic stroma that limits access of chemotherapy drugs to the tumor.



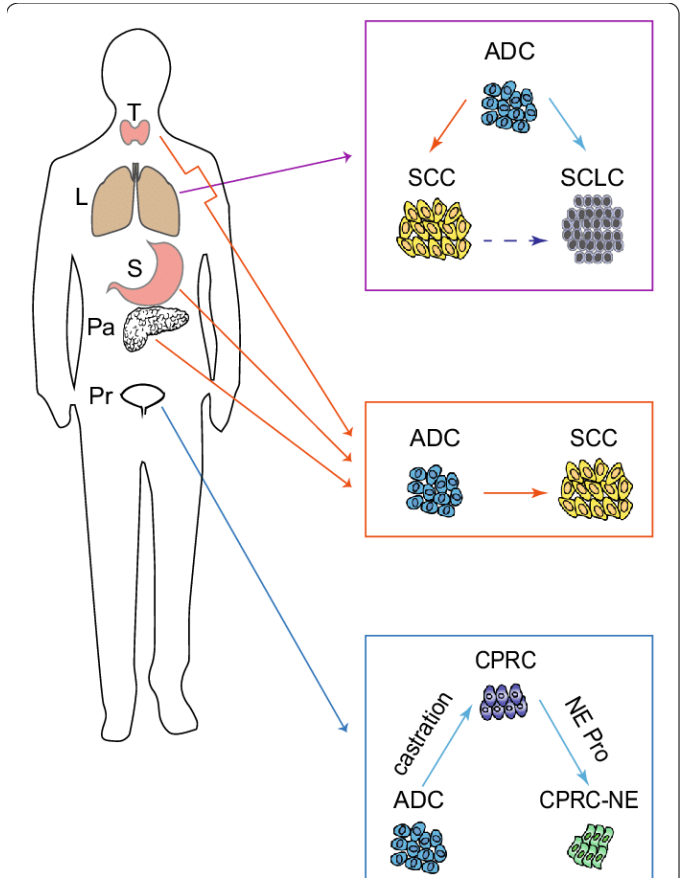
Biological determinants of therapy response: microenvironment sanctuaries

- The tumor microenvironment can functionally alter tumor response by stimulating paracrine growth factors to signal cancer cell growth and survival and by preventing immune clearance of tumor cells by immunotherapy.
- Paracrine signaling can activate oncogenic signaling pathways that bypass the effect of targeted kinase inhibitor therapies: HGF signaling activation of MET kinase receptor as mechanism of persistence and resistance to BRAF inhibitor therapies.
- Immunosuppressive cancer microenvironments—so-called ‘immune deserts’— are a major impediment to checkpoint inhibitors, owing to the presence of regulatory T cells, myeloid-derived suppressor cells, tumor-associated macrophages, cytokines and chemokines—all of which can inhibit immune-mediated anti-tumor effects



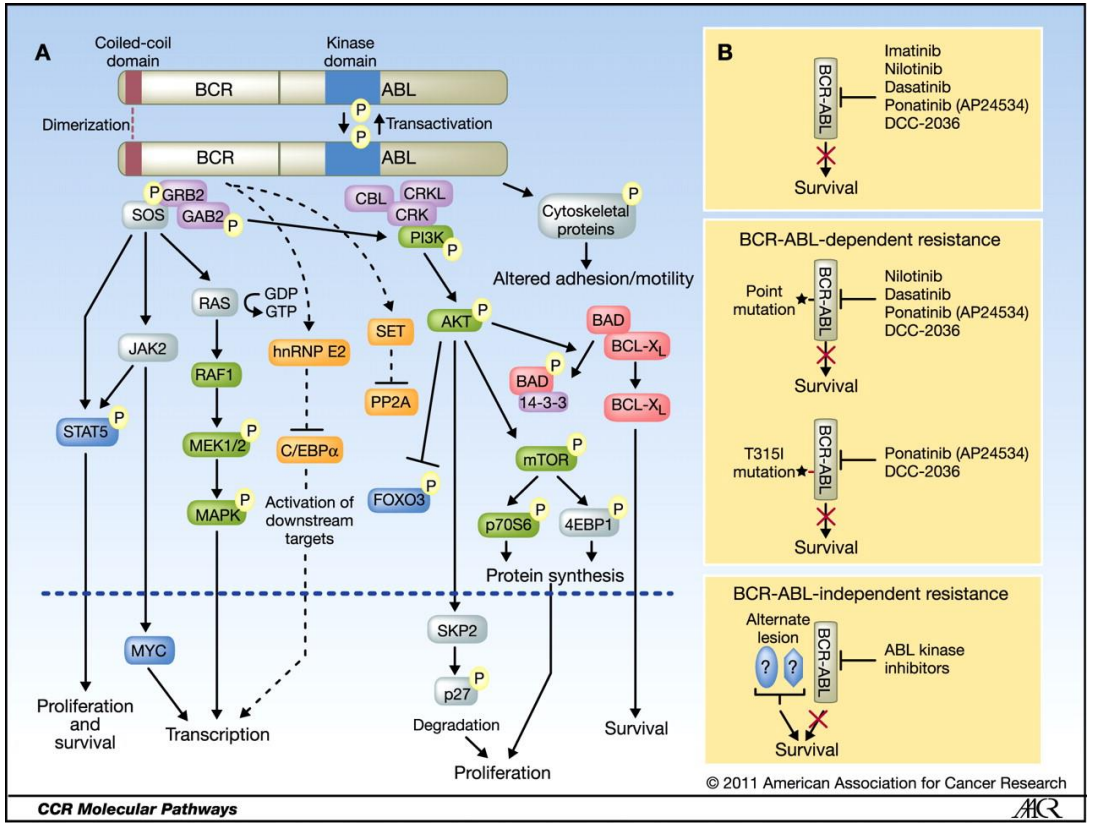
Biological determinants of therapy response: cellular plasticity

- Phenotypic changes may occur that result in the evolution of treated tumors into new histological types.
- Transformation to aggressive clinical neuroendocrine phenotypes has been noted in prostate tumors that are initially responsive to antiandrogens, and in *EGFR*-mutant NSCLC that is initially responsive to TKIs.
- Myeloid conversion with loss of the B-cell specific CD19 marker is found in B-precursor leukemias treated with anti CD19 CAR T-cells.



Chronic myeloid leukemia: Resistance to kinase inhibitor therapy driven by restoration of oncogenic signaling

- An striking example of successful targeted therapy in cancer was the use of the ABL-kinase inhibitor imatinib in the treatment of chronic myelogenous leukemia.
- Early studies of patients who relapsed through imatinib treatment made the critical observation that BCR-ABL kinase activity was frequently restored in this setting.
- This highlighted a fundamental mechanism of resistance to targeted therapy: direct restoration of the biologic function that was disrupted by the applied small molecule drug.
- Preexisting mutant clones harboring gatekeeper mutations in the BCR-ABL kinase drive tumor progression.
- Second and third generation kinase inhibitors effective in the context of gatekeeper mutant oncogene expression are effective in delivering secondary responses.



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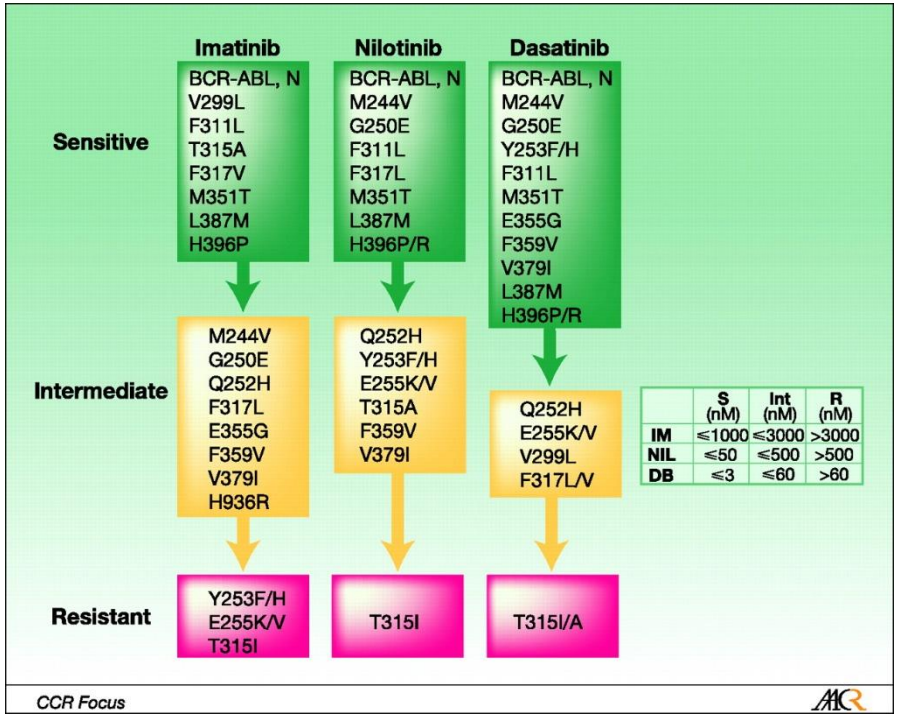
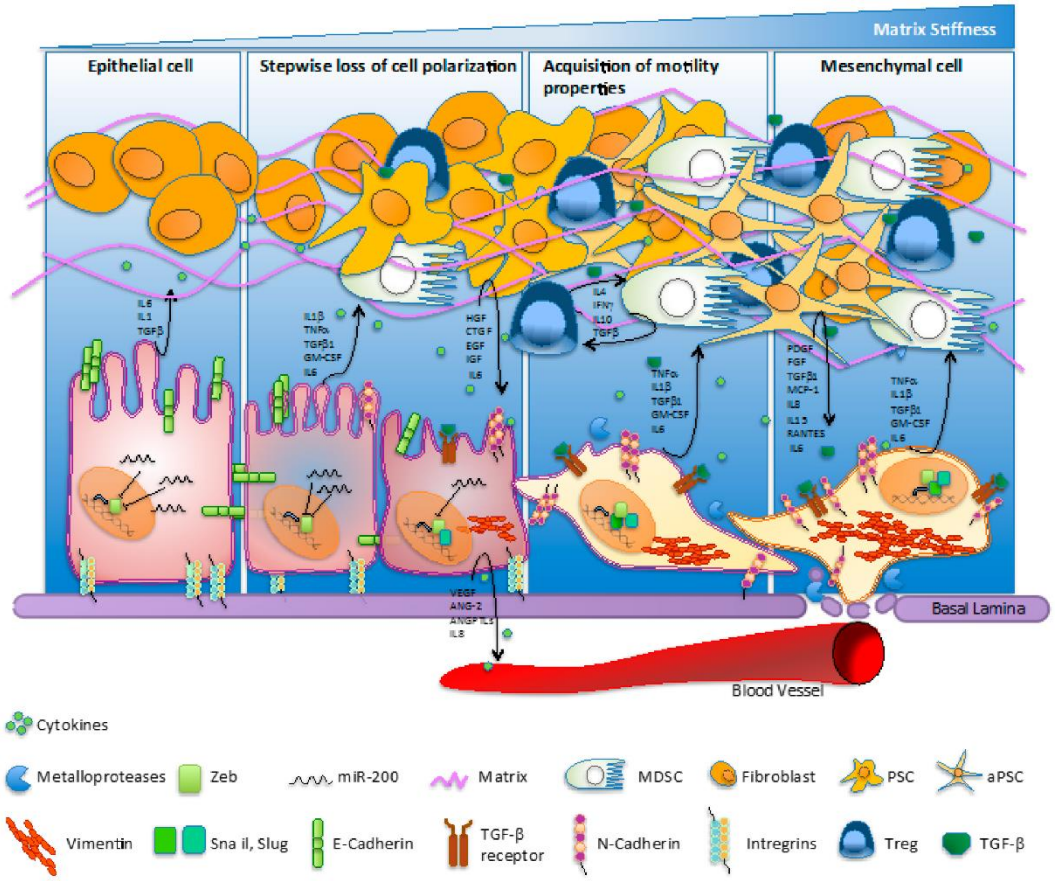


Biological determinants of therapy response: cellular plasticity

- Epithelial to mesenchymal transition (EMT) is observed in NSCLC and other epithelial cancers during targeted therapy or conventional chemotherapy but does not have a clear genetic basis.
- Drivers of the EMT transcriptional program (SNAI1, ZEB1, and TWIST1) repress the expression of canonical epithelial markers such as E-cadherin, and cooperate with TGF-α and WNT signaling to increase the expression of mesenchymal markers such as vimentin.

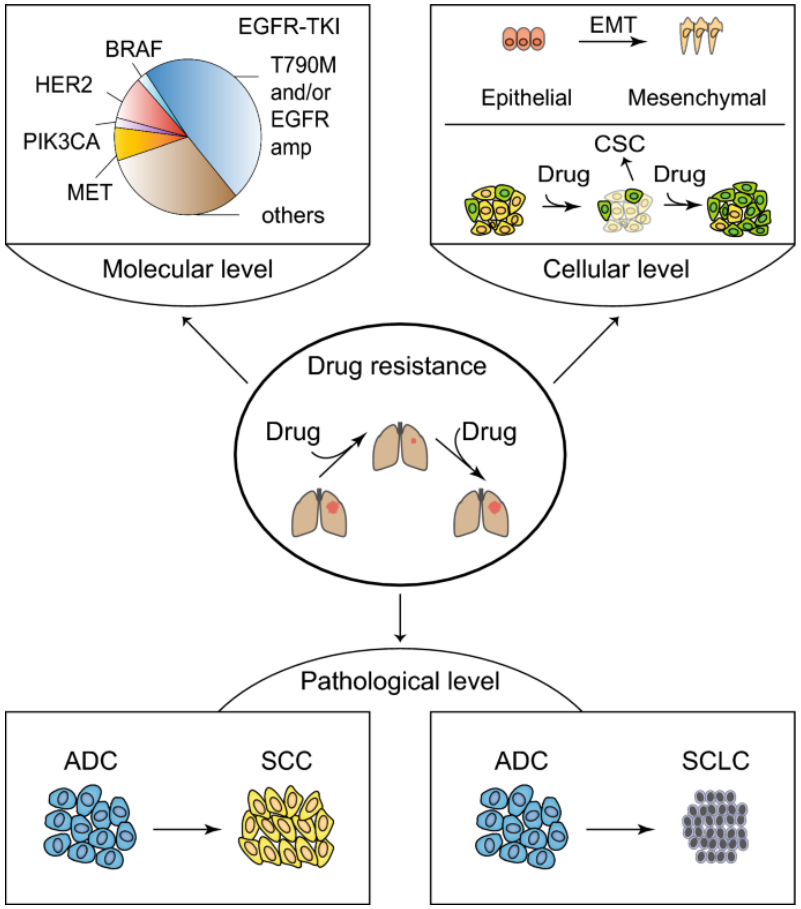


Targeted therapies and resistance in NSCLC

Genetic drivers of Non small cell lung cancer (NSCLC) include activating mutations in KRAS as well as mutations or translocations involving receptor tyrosine kinases: epidermal growth factor receptor (EGFR) and ALK which drive signaling through MAP kinase, PI3K and JAK/STAT pathways.

Tyrosine kinase inhibitors (TKIs) targeting EGFR and ALK induce significant improvement in progression free survival of patients with biomarker-positive, advanced stage disease.

Half of advanced-stage EGFR mutant cancer patients treated with a TKI will have disease progression within one to two years and some will rapidly progress due to intrinsic resistance that prevents drugs from eliciting an initial anti-tumor response.

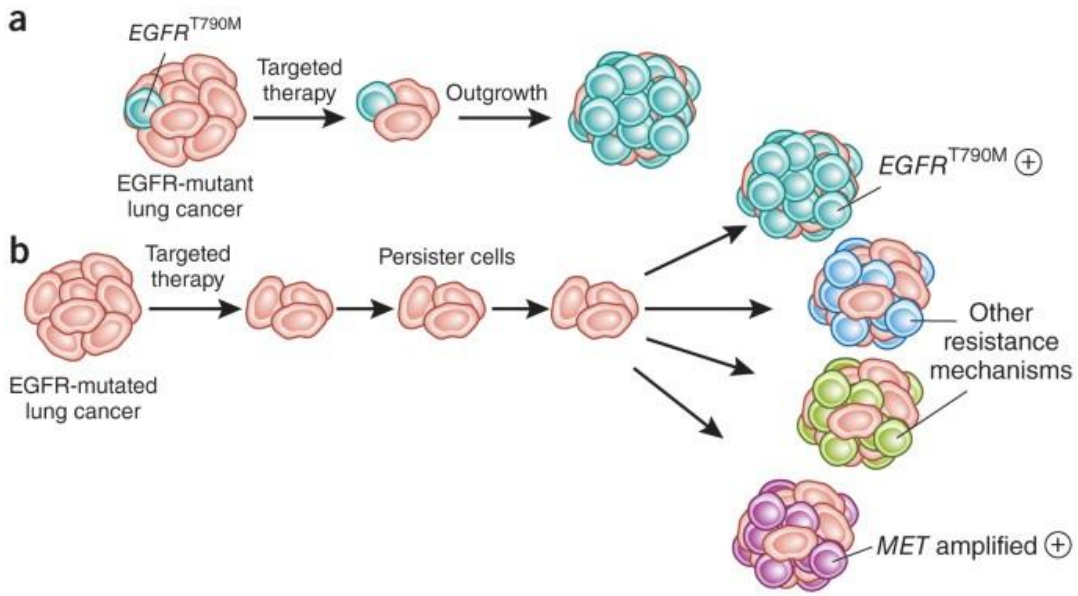


Targeted therapies and resistance in NSCLC

The most common resistance-inducing mutation arising in EGFR mutant NSCLC patients treated with first-generation TKIs is a secondary “gatekeeper” T790M mutation (>50% cases).

The third generation EGFR inhibitor osimertinib, inhibit T790M-mutant EGFR and has shown significant activity in NSCLC patients who progress after initial EGFR inhibitor therapy.

Osimertinib use in previously untreated EGFR mutant NSCLC had similar response rates when compared to first generation EGFR inhibitors (erlotinib or gefitinib); however, patients treated with osimertinib had two-fold improvement in both median progression free survival and duration of response.

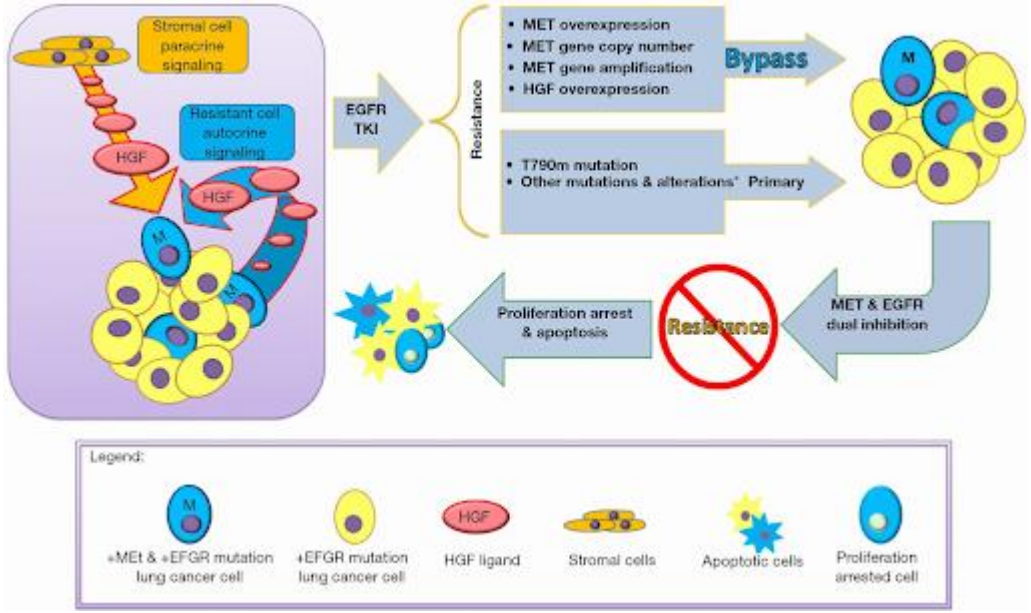


Targeted therapies and resistance in NSCLC

Oncogenic signaling networks are not necessarily tied to single inputs, and activation of parallel signaling could bypass requirements for a specific oncogenic activity in response to targeted therapies.

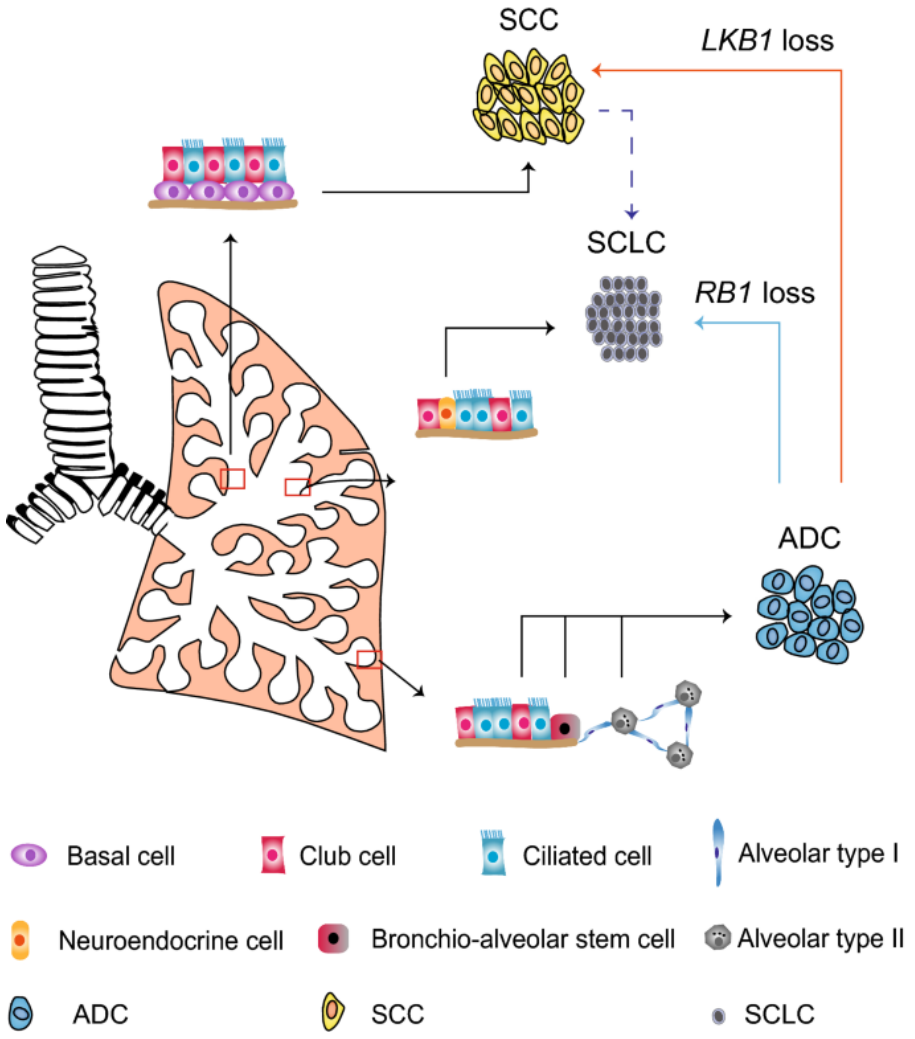
Acquired resistance to first generation EGFR inhibitors in NSCLC resulted in cell lines that maintained ERBB3 phosphorylation and PI3K signaling independent of EGFR inhibition, suggesting a parallel route to ERBB3 activation.

Focal amplification of the MET locus, MET overexpression and paracrine HGF activation of MET drives sustained signaling and resistance.



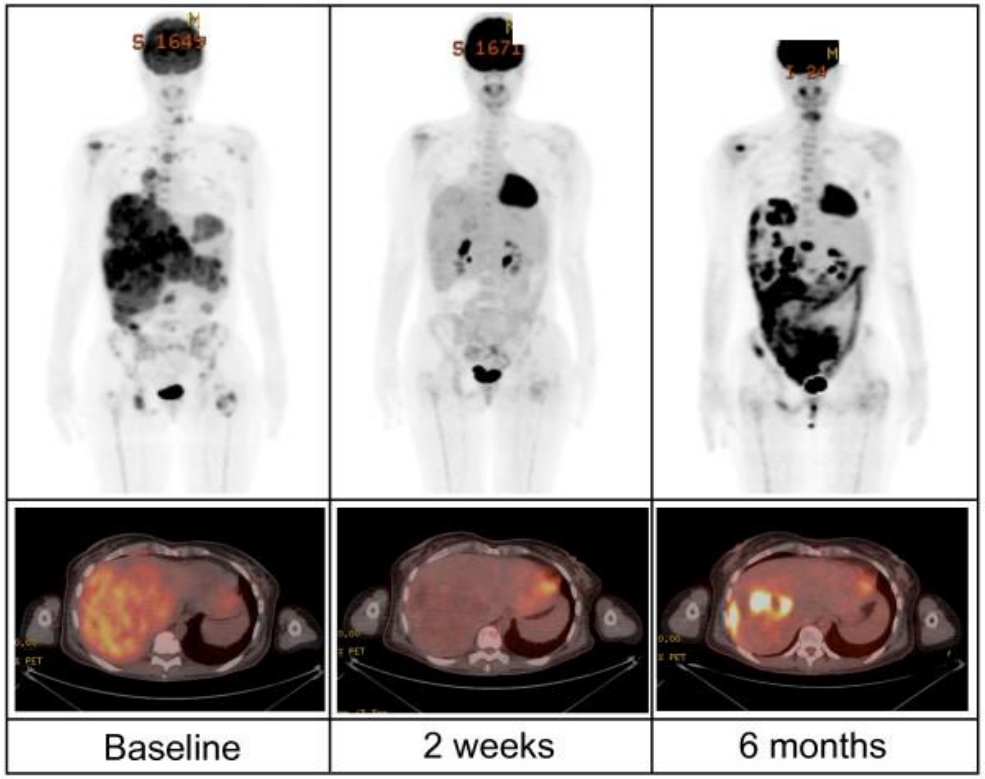
Targeted therapies and resistance in NSCLC

- Targeted cancer therapy resistance can also emerge from loss of dependence on the founding genetic identity of a cancer cell.
- 3-10% of patients who progress on NSCLC EGFR inhibitors develop small cell histology with loss of expression of EGFR.
- Combined loss of RBI and the tumor suppressor TP53 induces development of small cell histology and precedes clinical detection of small cell transformation.



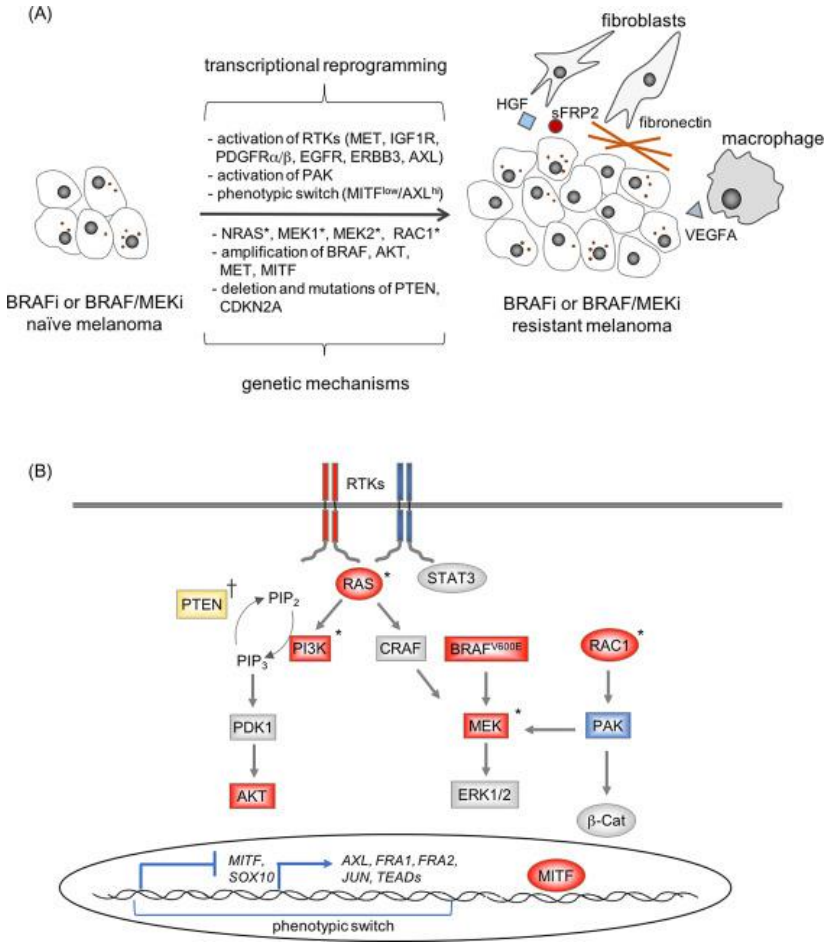
BRAF inhibitor resistance as paradigm of cellular adaptation to evolving signaling networks

- >80% malignant melanomas harbor MAK activating mutations, most commonly in the BRAF gee with the p.V600E allele accounting for 35-50% of cases
- Targeted inhibition of BRAF with the kinase inhibitors (vemurafenib, dabrafenib) induces tumor shrinkage in advanced disease and can prolong 6-month survival from 64% with chemotherapy to 84%.
- However disease progression is observed within seven months on average, and BRAF inhibitor monotherapy is not curative.



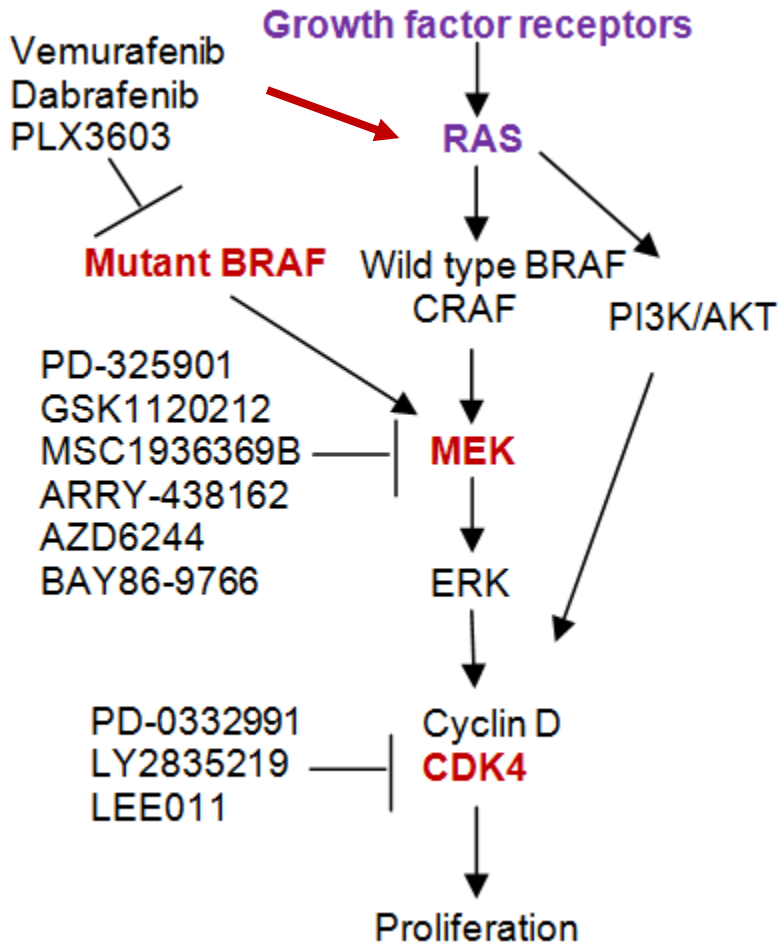
BRAF inhibitor resistance as paradigm of cellular adaptation to evolving signaling networks

- Efforts to characterize mechanisms of resistance to BRAF inhibitors failed to identify second-site mutations in BRAF.
- In vitro analysis of melanoma identified stable expression of a splice variant, BRAFp61, which can restore BRAF signaling through enhanced dimerization in spite of RAF inhibitor treatment.
- Resistance was associated with restoration of MAP kinase signaling via multiple paths leading to reactivation MAP kinase signaling, including mutation or amplification of RAS and loss of the tumor suppressor gene neurofibromatosis-1 (NF1), a negative regulator of RAS activation.



BRAF inhibitor resistance as paradigm of cellular adaptation to evolving signaling networks

- An unanticipated complication of BRAF inhibitor treatment provided further rationale to address resistance caused by MAP kinase reactivation.
- Up to 25% of patients treated with BRAF inhibitors developed keratoacanthomas or squamous cell carcinomas with recurrent RAS mutations.
- BRAF inhibitors actually enhance MAP kinase signaling in cells that lack a BRAF V600E mutation through RAF homo- and heterodimerization.
- Combination of BRAF and MEK inhibition delivers improved progression free survival (up to 10 months) and decreased incidence of second cutaneous malignancies.

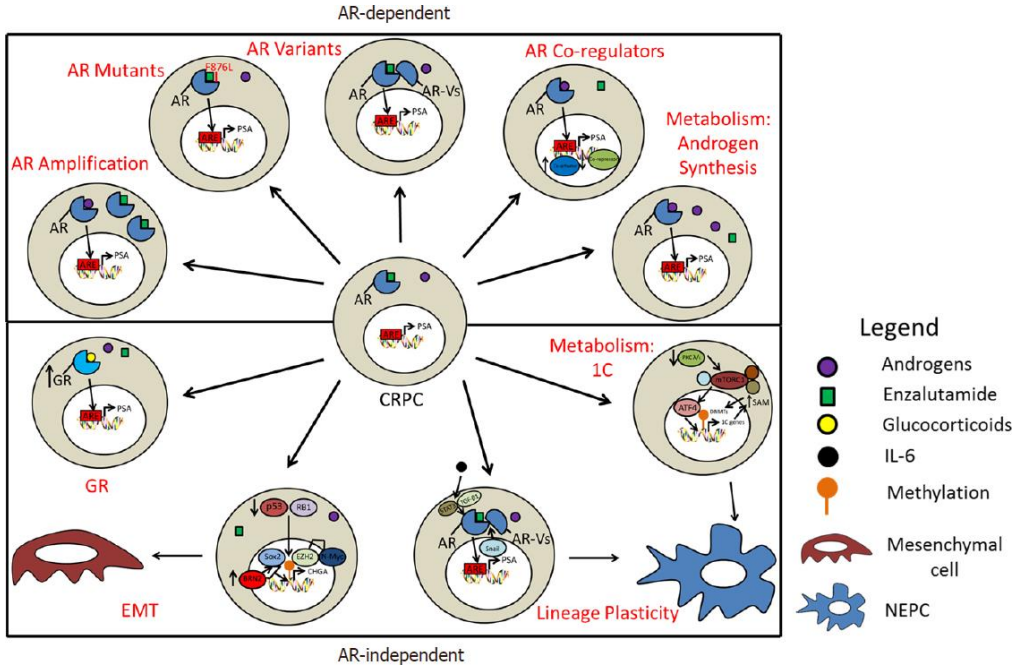


Androgen deprivation therapy in prostate cancer

Androgen signaling is essential for prostate cancer cell survival and androgen deprivation therapy improved the overall survival of patients with high risk, lymph node-involved disease.

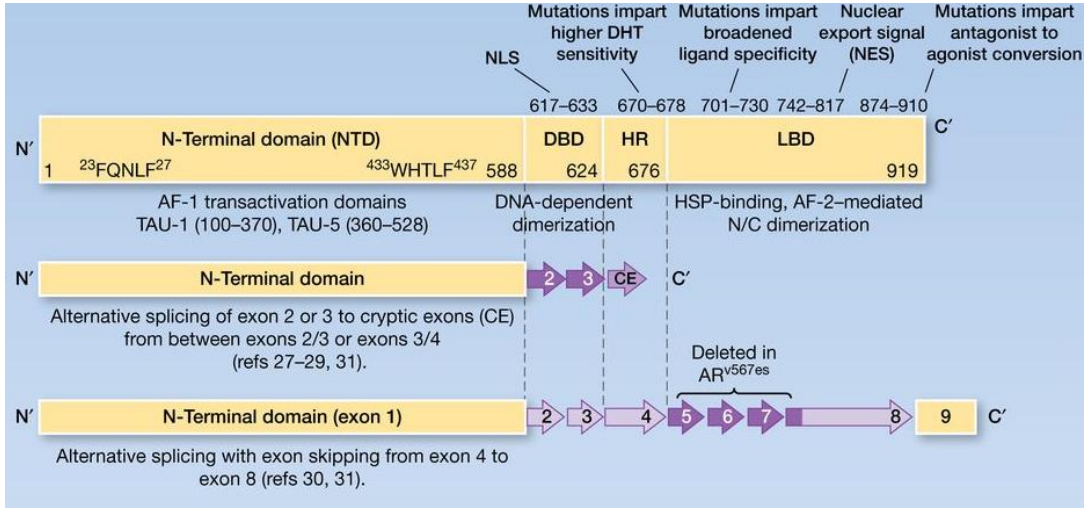
Following radical prostatectomy and lymphadenectomy, patients randomized to early androgen deprivation therapy had a median survival of 13.9 years, compared to 11.3 years in those receiving observation alone.

However 20% of patients receiving early androgen deprivation therapy progressed within 5 years to so-called castration resistant prostate cancer (CRPC), wherein anti-androgen treatment is ineffective.



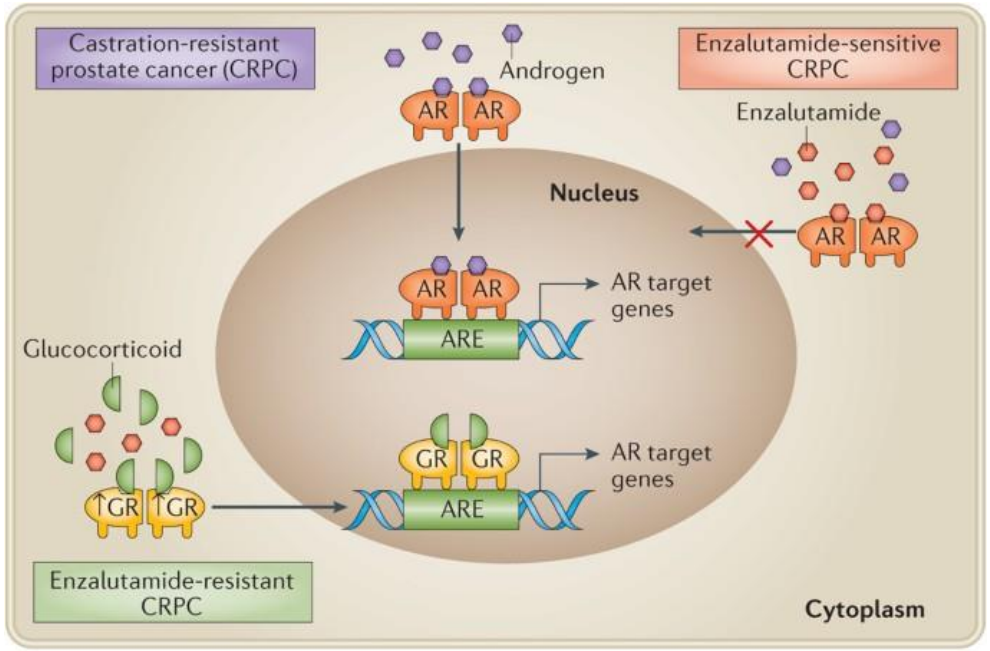
Prostate cancer resistance to antiandrogenic therapy

- Androgen receptor mutations can alter the response to antiandrogenic therapy by altering ligand-receptor interactions and transcriptional output.
- A common mechanism of enzalutamide resistance eliminates the drug-binding domain of AR through alternative splicing.
- This switch appears to be under the control of NF-κB signaling, highlighting how inflammatory signaling can restore interfere with precision therapy.



Prostate cancer resistance to antiandrogenic therapy

- Prostate cancers that develop resistance to the antiandrogen enzalutamide show robust induction of the glucocorticoid receptor, NR3C1.
- Androgen and glucocorticoid receptors can share DNA binding sites, the transcriptional targets of AR and GR overlap, and GR activation with dexamethasone was able to confer resistance to anti-androgens.
- Importantly, glucocorticoid therapy is commonly used in prostate cancer supporting a need to revisit its therapeutic use.



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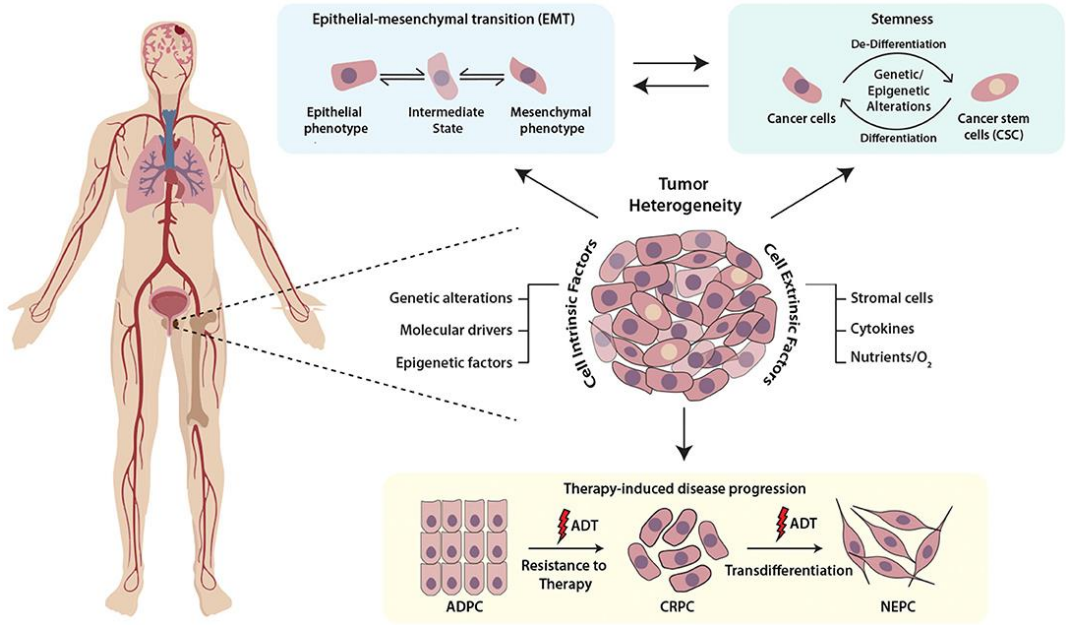
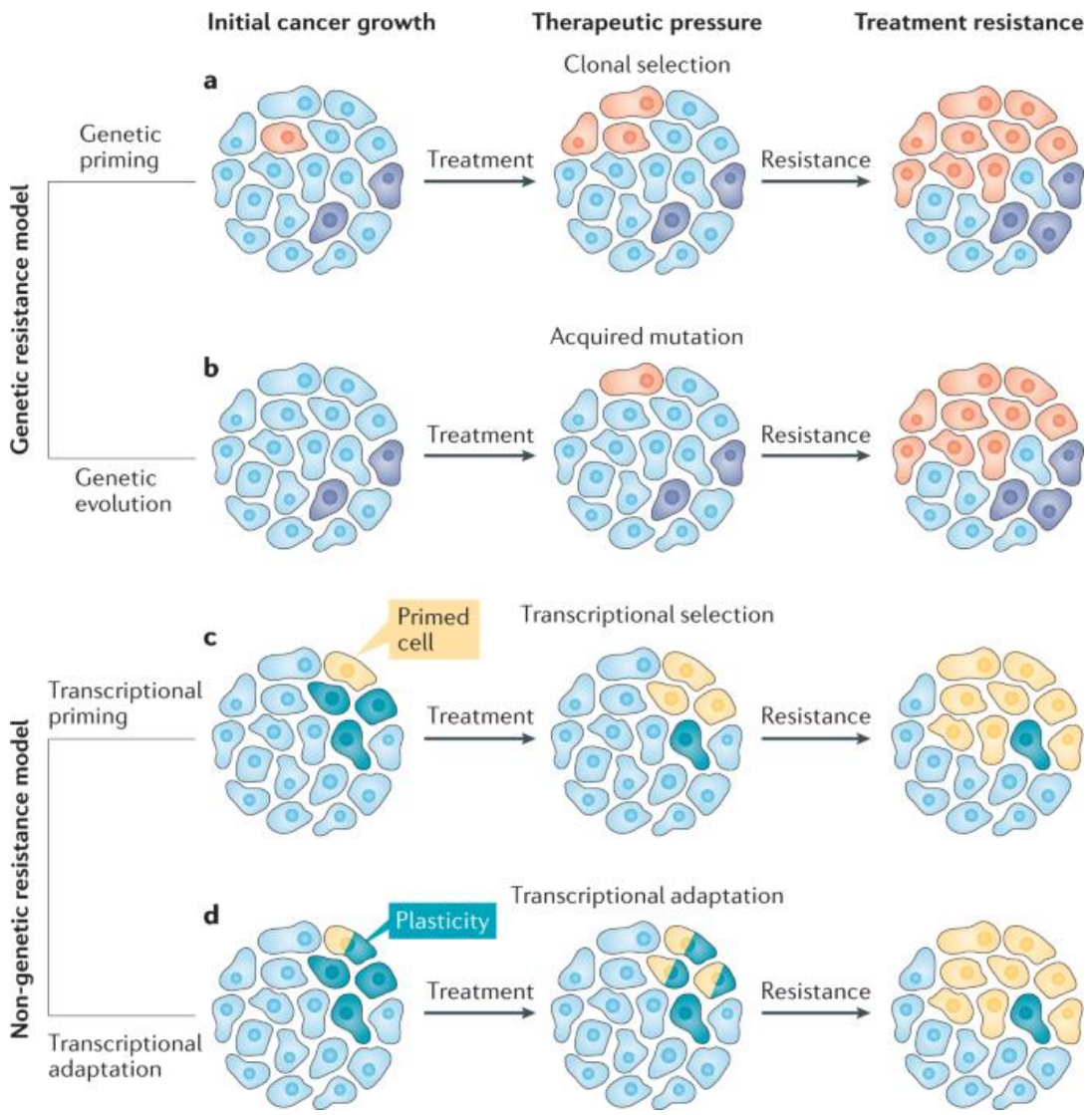
Prostate cancer resistance to antiandrogenic therapy

- Histological transformation occurs in at least 20% of prostate adenocarcinomas on targeted therapy.
- RB1 and p53 deficiency are implicated in — but not sufficient for — neuroendocrine transformation.
- AKT pathway activation and aberrant activity of the MYC and SOX families of transcriptional regulators have been implicated as being effectors of histological transformation.



Genetic selection vs epigenetic/transcriptional/metabolic plasticity



Lineage plasticity in cancer: a shared pathway of therapeutic resistance
Review Nat Rev Clin Oncol . 2020 Jun;17(6):360-371. doi: 10.1038/s41571-020-0340-z.

Principles of Resistance to Targeted Cancer Therapy: Lessons from Basic and Translational Cancer Biology
Trends Mol Med . 2019 Mar;25(3):185-197. doi: 10.1016/j.molmed.2018.12.009.

A view on drug resistance in cancer.
Nature. 2019 Nov;575(7782):299-309. doi: 10.1038/s41586-019-1730-1.

The cellular origins of drug resistance in cancer
Nat Med . 2016 Mar;22(3):232-4. doi: 10.1038/nm.4058.

Non-genetic mechanisms of therapeutic resistance in cancer
Nature Reviews Cancer volume 20, pages743–756(2020)

Question

Mutational analyses of relapsed acute promyelocytic leukemia (Lehmann-Che J, Bally C, Letouzé E, Berthier C, Yuan H, Jollivet F, Ades L, Cassinat B, Hirsch P, Pigneux A, Mozziconacci MJ, Kogan S, Fenaux P, de Thé H. Dual origin of relapses in retinoic-acid resistant acute promyelocytic leukemia. Nat Commun. 2018 May 24;9(1):2047. doi: 10.1038/s41467-018-04384-5.) reveal different mechanisms and evolutionary paths conductive of relapse and resistance to therapy.

Summarize and discuss the key findings of this study in terms of: (i) mutational mechanisms of resistance (RARA, PML, NT5C2), (ii) role of genetic diversity and clonal evolution as diver of relapse, and (iii) rationale for efficacy of combination therapies (ATRA + arsenic trioxide).