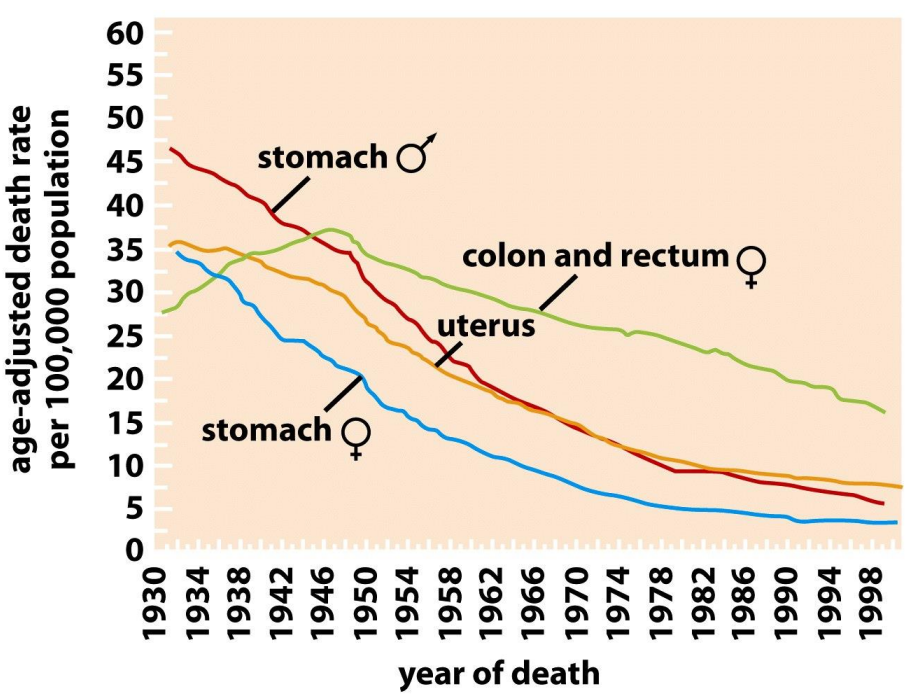
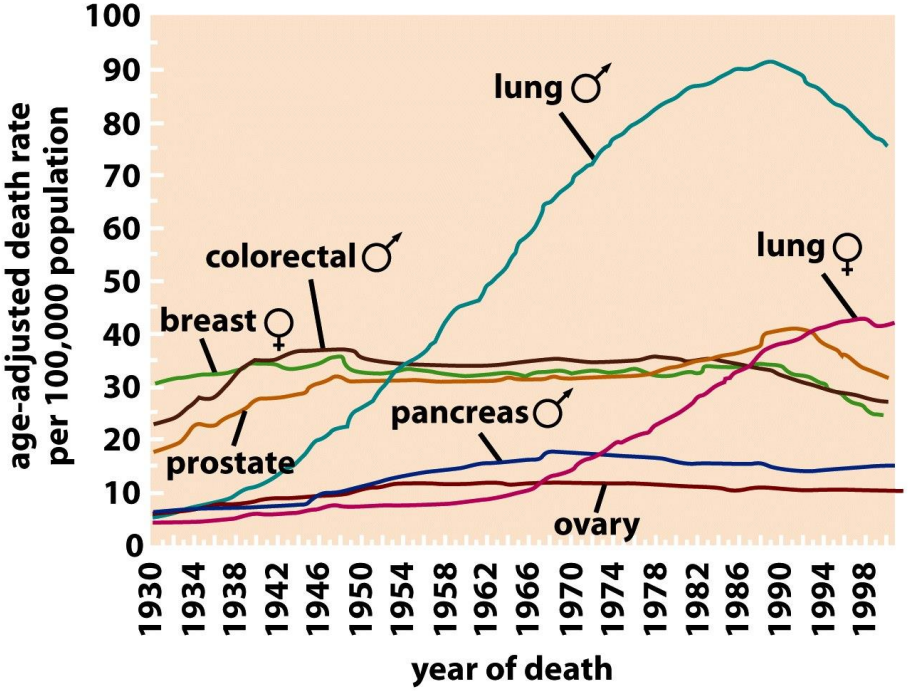
# Anti-cancer therapies: Targets, mechanisms, and resistance

# Cancer mortality rates




The decline of death rates for certain cancer types is largely due to improved diagnostic tools, health conditions (e.g., decreased *H. pilori* and HPV infections) and habits, not necessarily better cancer therapies.

Figure 16.1a *The Biology of Cancer* (© Garland Science 2007)

# Conventional and targeted anticancer drugs

## Conventional

**Objectives**

The drug targets and kills proliferating cells

**Molecules**

Various agents (DNA alchylating, crosslinking and intercalating; topoisomerase inhibitors; microtubule-stabilizing agents; etc...); radiation

**Targets**

Cellular components required for cell proliferation (DNA; DNA-modifying enzymes; microtubules)

**Advantages**

In general, broadly applicable to cancer patients with a certain cancer, irrespective of specific “driver mutation(s)”

**Disadvantages**

Highly toxic; frequently lead to resistance; can cause cancer

## Targeted

**Objectives**

The drug targets cancer cells (spares “normal” cells)

**Molecules**

Small molecules;
Antibodies

**Targets**

Typically one gene product, which needs to be “druggable” and important for the survival of the cancer cell;
Specific to the cancer cell

**Advantages**

Only targets cancer cells
Allows for the development of personalized therapies

**Disadvantages**

Leads to resistance (almost invariably)

# Conventional anti-cancer therapies

- Surgery
- Radiotherapy
- Chemotherapy

# Surgical oncology

- Surgery is the oldest cancer treatment

- Main principle of surgery in cancer is to remove the cancerous tissue/organ.

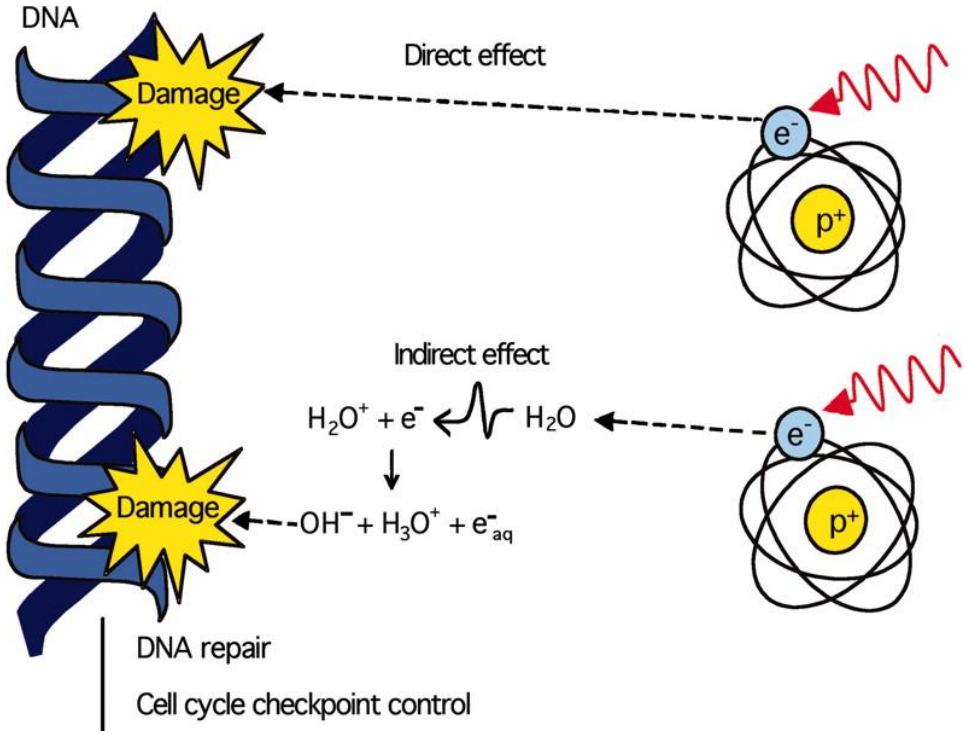
- Rationale:
  - Small tumors will grow into large tumors.
  - Removing the primary tumor will reduce the risk of recurrence of the disease (including metastasis).

- Surgery is usually combined with chemo-/radiotherapy
  - **Neoadjuvant therapy**: therapy *before* surgery to shrink the tumor and make it easier to remove
  - **Adjuvant therapy**: therapy *after* surgery to kill the remaining cancer cells

# Radiotherapy

- Main principle of radiotherapy is to damage the DNA of the cancer cells, so to inhibit their further proliferation.
  - Charged particles (protons or ions; direct DNA damage)
  - Photons (indirect DNA damage through free radicals)

- Radiation can damage the DNA directly or indirectly. Indirectly, radiation can ionize oxygen or water molecules in the cell leading to the formation of free radicals that damage the DNA.

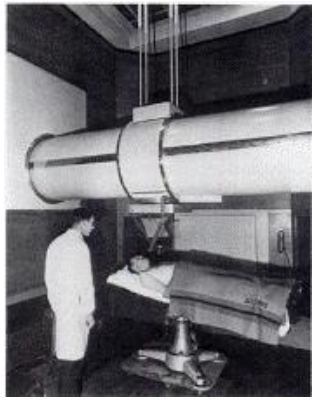


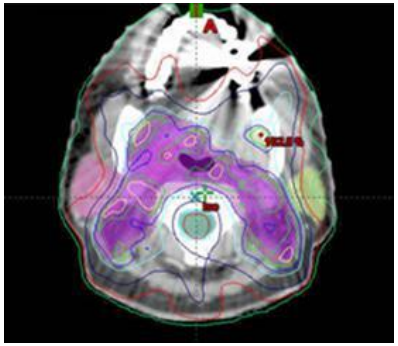

Morgan W. & Sowa M. B PNAS 2005;102:14127-14128

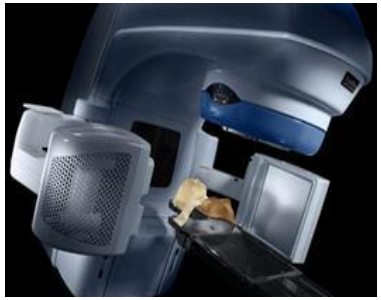
- Tumor hypoxia limits the efficacy of radiation therapy

# History of radiotherapy

- Roentgen discovered X-rays in 1895.
- Radiation therapy was first used in 1896 by Dr. Emil Grubbe for breast cancer.
- Early radiotherapy consisted of **high doses of radiation** in limited number of treatments.
- With the **fractionation** approach established by Claude Regaud, radiation therapy became more effective with less side effects.
- High energy, deeply penetrating beams generated by linear accelerators enable to reach the tumors inside the body without major damaging of skin and normal tissues.
- With the development of Intensity Modulated Radiation Therapy (IMRT), tumors can be targeted in **3 dimensions** resulting in decreased side effects.

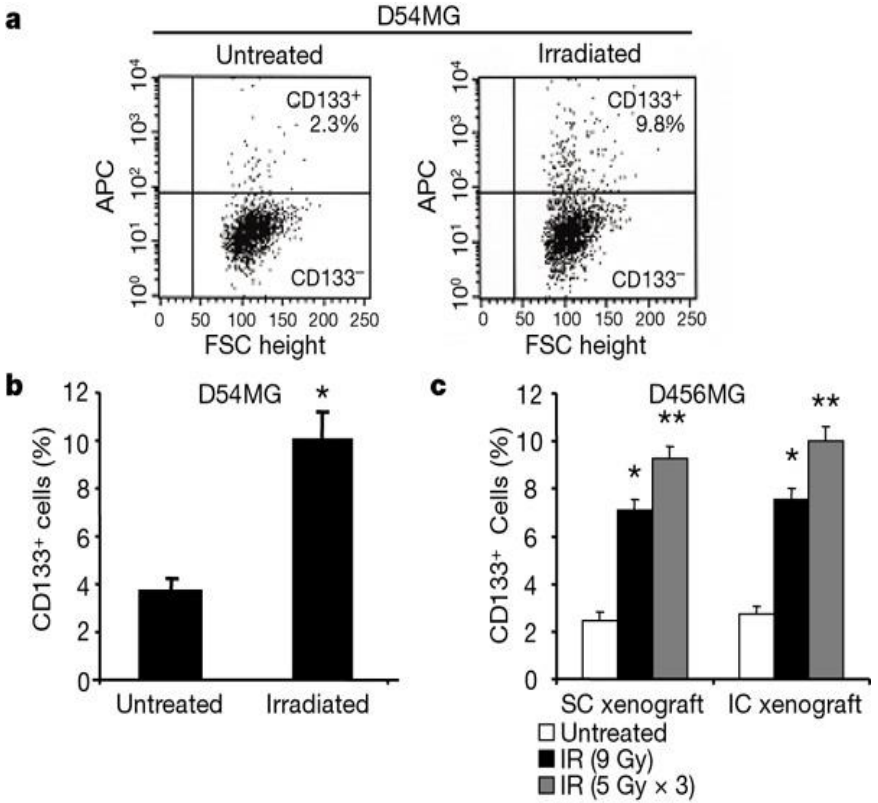


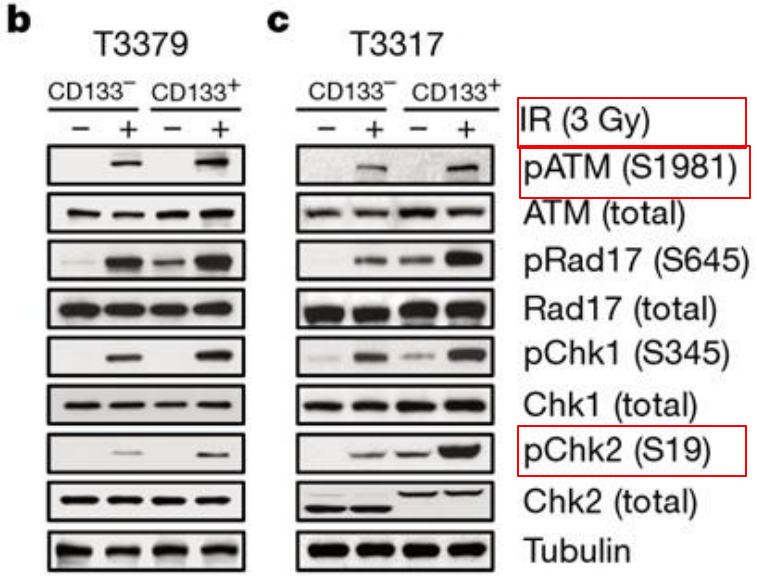




# Resistance to radiotherapy

- Radiotherapy provides transient efficacy in glioblastoma due to radioresistance.
- In response to irradiation, $CD133^+$ cancer stem cells (CSCs) were enriched in tumors. CSCs activate DNA damage checkpoint proteins like ATM (which activates checkpoint kinase 2 (CHK2) or p53), leading to cell cycle arrest to repair DNA damage induced by radiation and/or apoptosis.

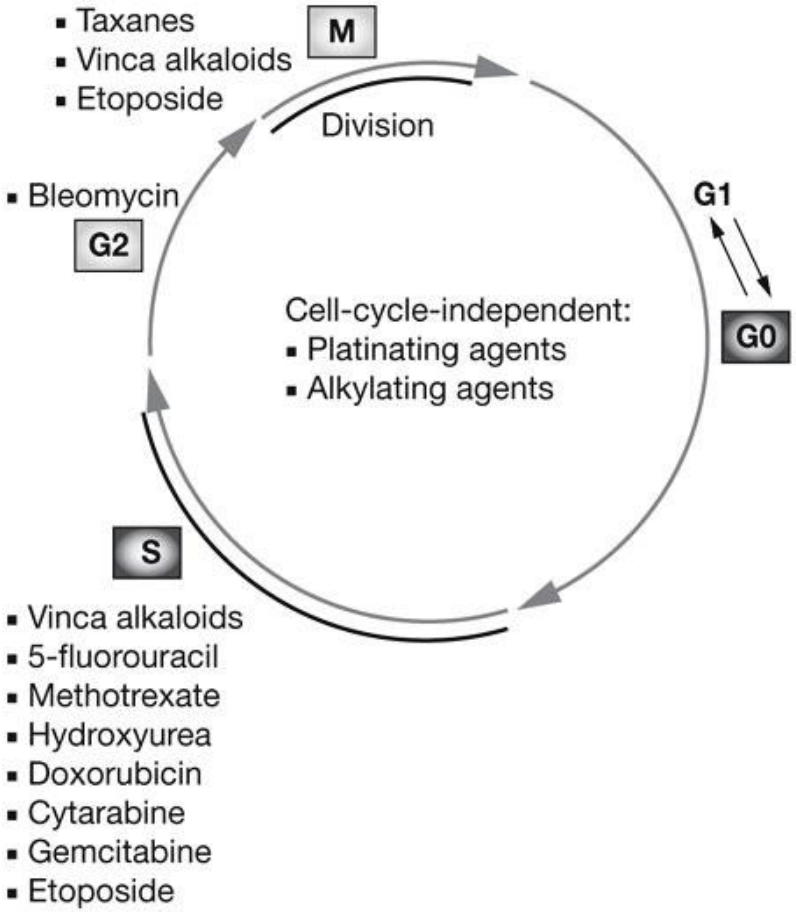


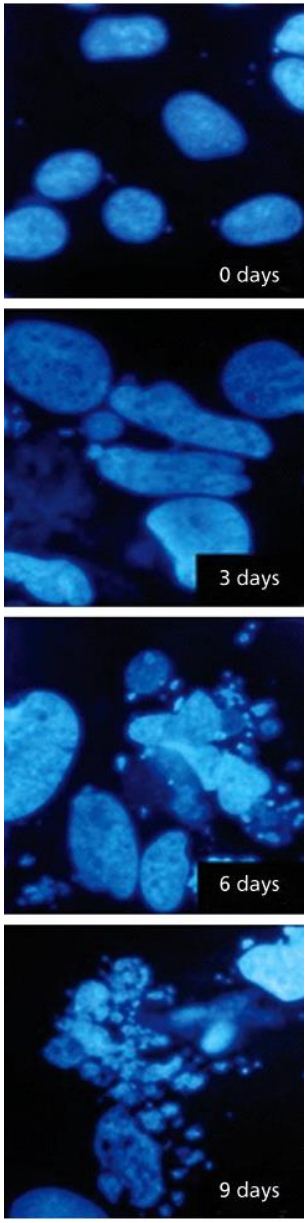

Bao S. et al., Nature 2006;444:756-760

# Principles of chemotherapy

- Main principle of chemotherapy is to induce cancer cell death via apoptosis.
- Chemotherapies may target different stages of the cell cycle, or affect the cancer cells independent of cell cycle.

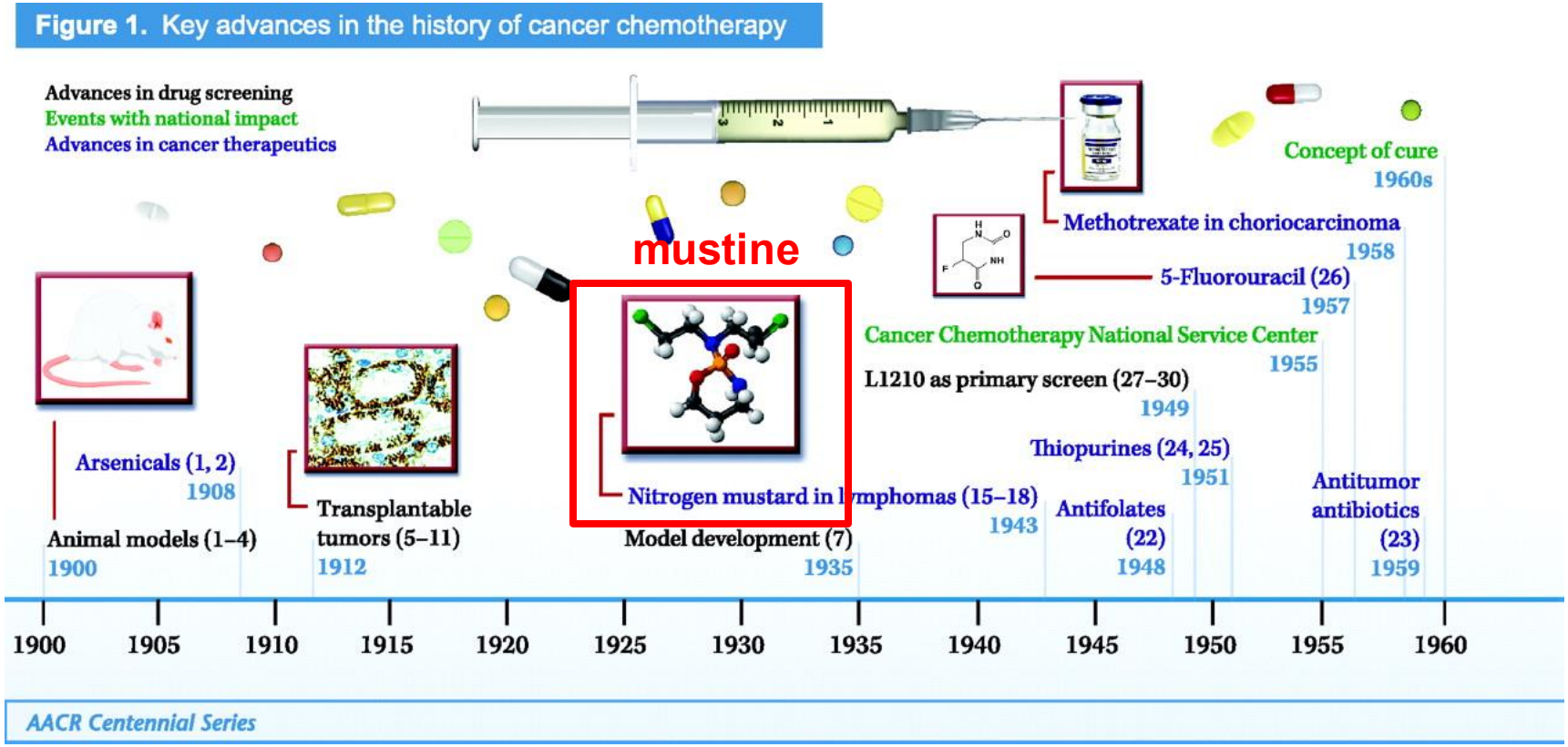



Many chemotherapeutic drugs damage the DNA of cancer cells. Cancer cells often lack $G_2$/M checkpoint controls (e.g., via p53 loss or mutations), so they can go into mitosis without repairing DNA damage, resulting in aneuploidy, polyploidy, formation of micronuclei, and eventual cell death.




igure 16.8 The Biology of Cancer (© Gar

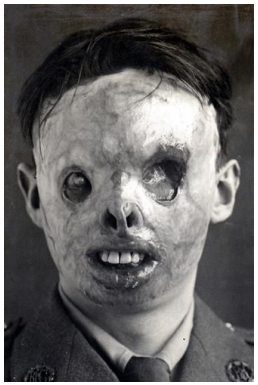
# History of chemotherapy

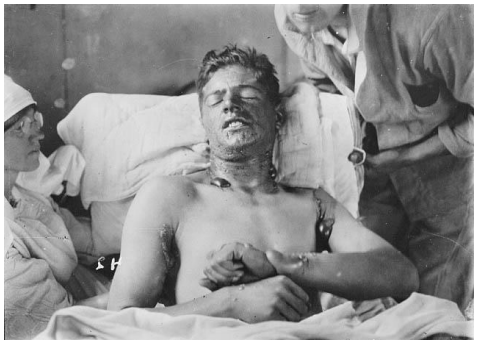



DeVita V T , and Chu E Cancer Res 2008;68:8643-8653

# History of chemotherapy

WWI:





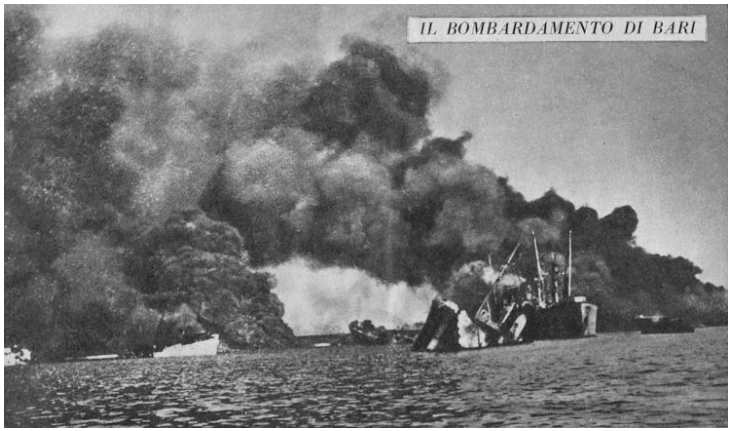

1919:

THE BLOOD AND BONE MARROW IN YELLOW CROSS GAS (MUSTARD GAS) POISONING.

CHANGES PRODUCED IN THE BONE MARROW OF FATAL CASES.*

E. B. KRUMBHAAR, M.D., Ph.D., RECENTLY MAJOR, M.C., U.S.A., AND HELEN D. KRUMBHAAR.

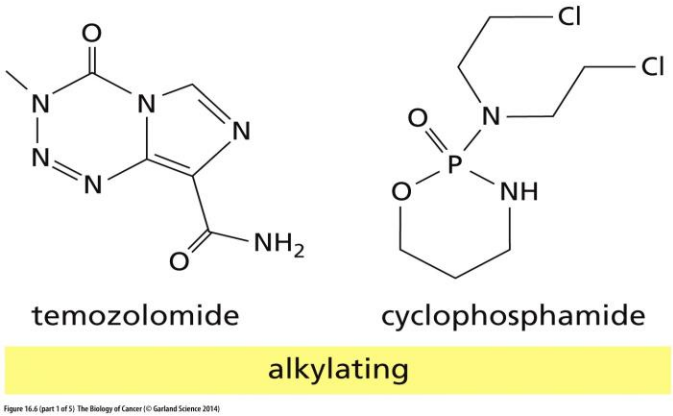
WWII - 1943:

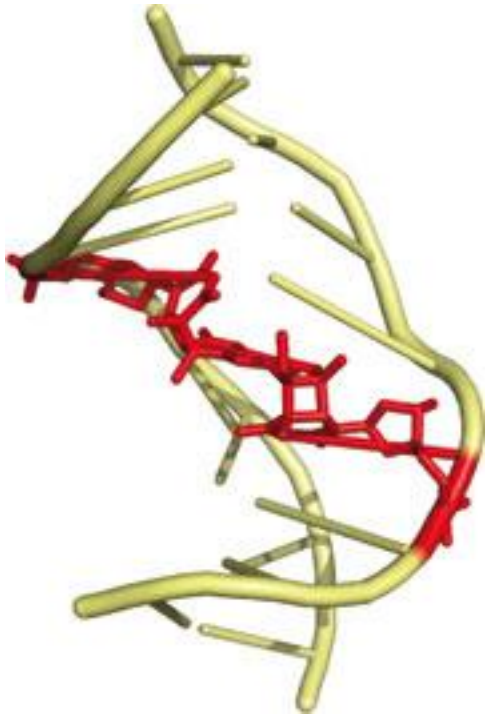



# Types of chemotherapy

## Alkylating agents

- They attach alkyl groups covalently to the DNA bases
- Mustine (derived from mustard gas) was the prototype
- First use in the 1940's for lymphoma



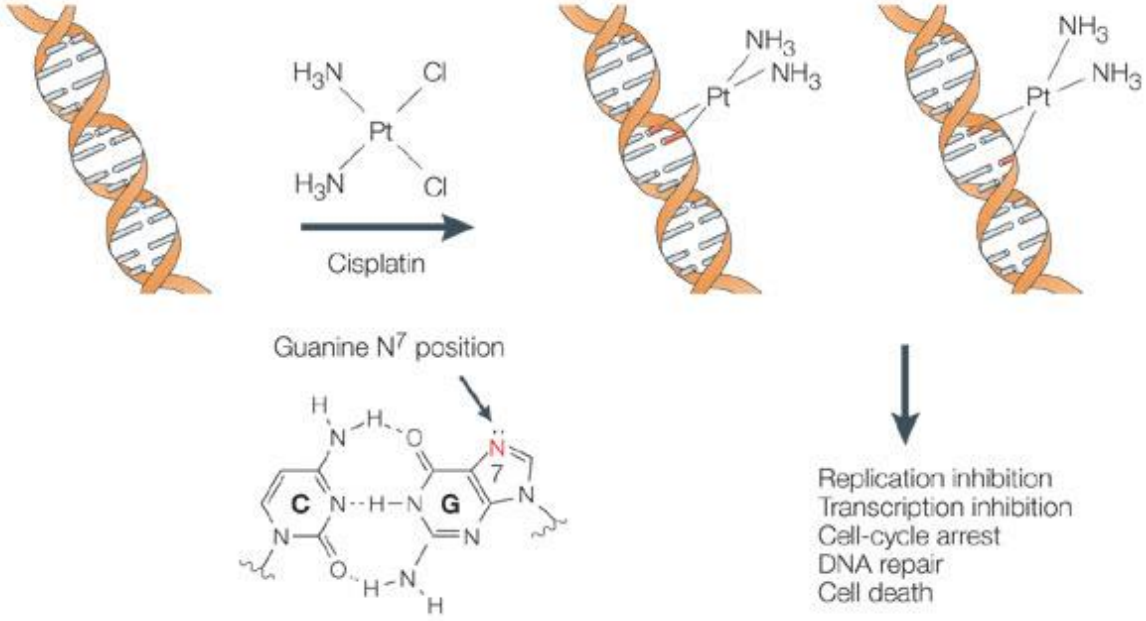



- They work by binding to DNA, crosslinking two strands and preventing cell duplication. They bind to the N7 nitrogen on the DNA base guanine (imidazole group).
- They are highly mutagenic, may cause secondary tumors after therapy. Same mechanism of action of mustard gas.

# Types of chemotherapy

## Platinum derivatives

• In 1965, cisplatin was discovered by Barnett Rosenberg as an antibacterial agent formed at platinum electrodes.




Wang L. & Lippard S., Nat Rev Dru Discov, 4, 307-320, 2005

- The platinum atom of cisplatin binds covalently the $N^7$ position of purines to form 1,2- or 1,3-intrastrand and interstrand crosslinks. Cisplatin–**DNA adducts** cause various cellular responses, such as DNA replication arrest, transcription inhibition, cell-cycle arrest, DNA repair, and apoptosis.

# Types of chemotherapy

## Antimetabolites

• They interfere with the normal functioning of specific enzymes/macromolecules that participate in DNA replication.

**Table 16.2** Examples of antimetabolites used to treat cancer

| Name | Chemical structure | Targeted reaction | Examples of clinical use |
|---|---|---|---|
| methotrexate | folate analog | formation of tetrahydrofolate | breast cancer, lymphomas |
| 6-mercaptopurine | purine analog | purine biosynthesis | leukemia, NHL |
| doxorubicin | natural product[a] | intercalating agent, inhibits topoisomerase | wide range |
| thioguanine | guanine analog | purine biosynthesis | acute granulocytic leukemia |
| fludarabine | purine analog | ribonucleotide reductase, DNA replication | chronic lymphocytic leukemia, NHL |
| cladribine | adenosine analog | adenosine deaminase | hairy-cell leukemia |
| bortezomib | peptide analog | proteasomal degradation | multiple myeloma |
| paclitaxel | natural product[a] | microtubule destabilization | lung, ovarian, breast cancer |
| etoposide | natural product[a] | DNA unwinding | lung cancer, sarcomas, glioblastoma |
| mitoxantrone | topoisomerase inhibitor | DNA unwinding | AML, breast cancer, NHL |
| irinotecan | topoisomerase inhibitor | DNA unwinding | colorectal carcinoma |
| vinblastine | natural product[a] | microtubule assembly | Hodgkin's lymphoma |
| vorinostat | hydroxamic acid | histone deacetylation | cutaneous T-cell lymphoma |
| azacitidine | pyrimidine analog | DNA methylation | myelodysplastic syndrome |

Abbreviations: NHL, non-Hodgkin's lymphoma; AML, acute myelogenous leukemia.
[a]Complex structure.

Table 16.2 The Biology of Cancer (© Garland Science 2014)

Microtubule-interfering agents

DNA-intercalating → topoisomerase inhibitors

Microtubles and DNA: https://youtu.be/IvJrDsRuWxQ

# Resistance to chemotherapy

**Table 16.5** Mechanisms of acquired resistance to anti-cancer therapies[a]

| Nature of resistance | Mechanism of resistance |
|---|---|
| Multi-drug resistance[b] | increased expression of drug export pumps |
| Pan-drug resistance[c] | unknown |
| Drug detoxification[d] | enzymatic detoxification of drug molecule |
| Acquired drug resistance | refuge of cancer cells in drug-protected anatomical sites[e]<br>failure of tissue to convert pro-drug into active form<br>refuge of cancer cells in an anatomical site that provides protective trophic signals[f]<br>massive stromalization[g]<br>emergence of mutant, structurally altered cellular target[h]<br>amplification of gene encoding targeted protein<br>emergence of cells bearing alterations in genes whose products are functionally redundant with drug target[i]<br>loss of drug importer[j]<br>passage through an EMT[k]<br>activation of anti-apoptotic regulators |
| **Physiologic activation of compensatory adaptive mechanisms** | |
| Resistance to EGF-R inhibition | up-regulation of IGF-1R signaling<br>amplification of *Met* gene<br>mutational activation of a *ras* gene |
| Resistance to Smoothened inhibition | amplification of *Gli2* gene |
| Resistance to Bcr-Abl inhibition | amplification of *Bcr-Abl* gene |

Table 16.5 The Biology of Cancer (© Garland Science 2014)

Concomitant resistance to paclitaxel, doxorubicin, etoposide and vinblastine was observed in cancer cells overexpressing P-glycoprotein, a drug export transporter, on the plasma membrane.

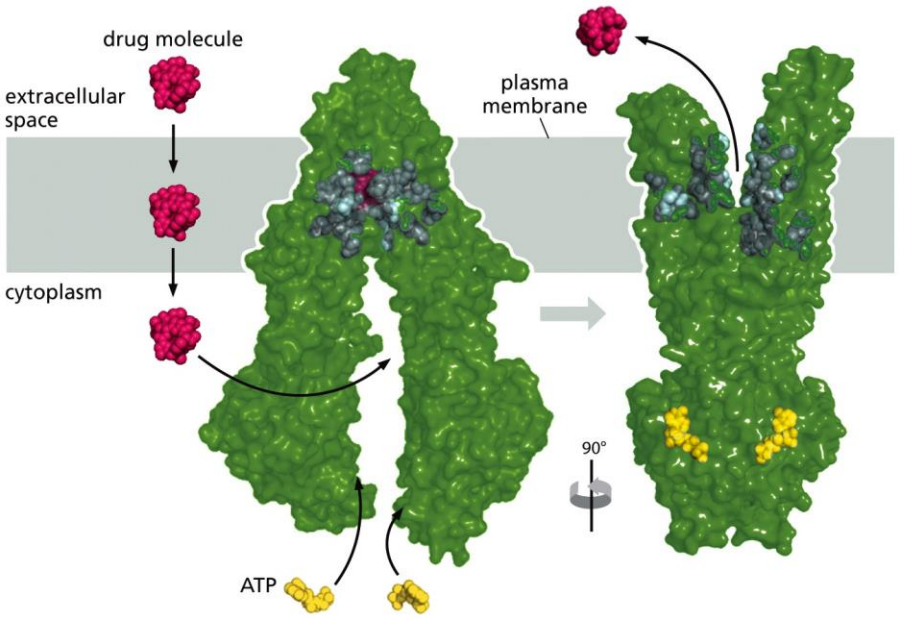



Figure 16.21 The Biology of Cancer (© Garland Science 2014)

# Conventional and targeted anticancer drugs

## Conventional

**Objectives**

The drug targets and kills proliferating cells

**Molecules**

Various agents (DNA alchylating, crosslinking and intercalating; topoisomerase inhibitors; microtubule-stabilizing agents; etc...); radiation

**Targets**

Cellular components required for cell proliferation (DNA; DNA-modifying enzymes; microtubules)

**Advantages**

In general, broadly applicable to cancer patients with a certain cancer, irrespective of specific “driver mutation(s)”

**Disadvantages**

Highly toxic; frequently leads to resistance; can cause cancer

## Targeted

**Objectives**

The drug targets cancer cells (spares “normal” cells)

**Molecules**

Small molecules;
Antibodies

**Targets**

Typically, one gene product, which needs to be “druggable” and mportant for the survival of the cancer cell;
Specific to the cancer cell

**Advantages**

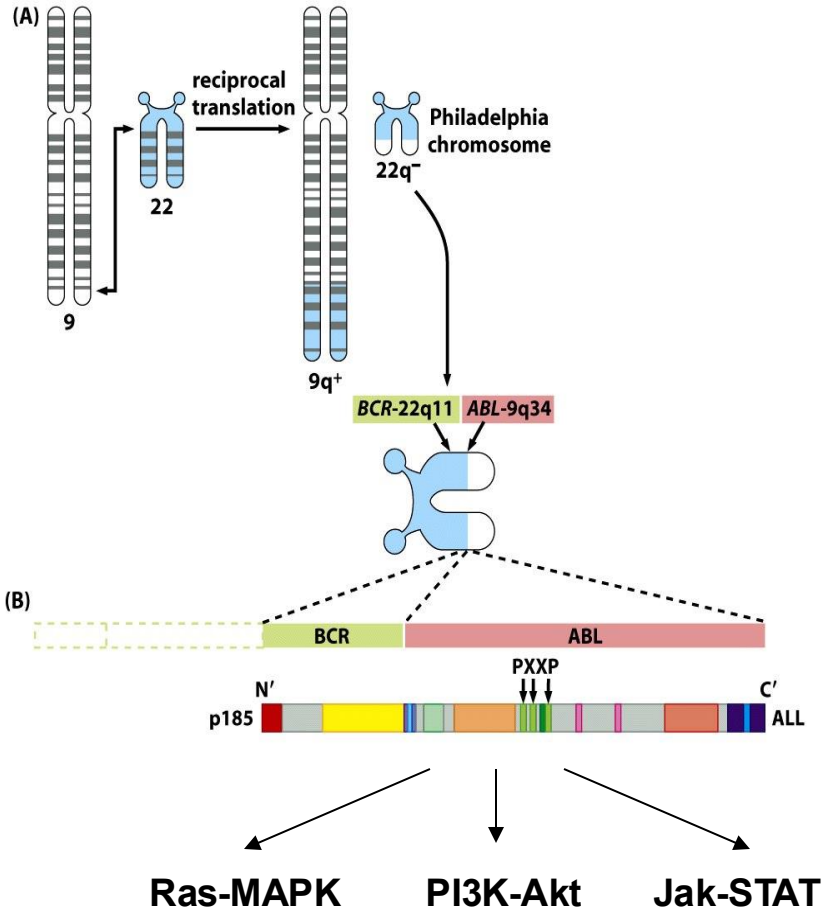
Only targets cancer cells
Allows the development of personalized therapies

**Disadvantages**

Leads to resistance (almost invariably)

# Oncogene-targeted therapies

# History of the first targeted drug




**Chronic myeloid leukemia (CML)**

- **1914**, First hypothesis that chromosomal aberrations may be associated with cancer

- **1960**, It is found that CML cells frequently display a short chr. 22 (Philadelphia)

- **1972**, Identification of a translocation (9-22) in the Philadelphia chromosome

- **1982**, Identification of Abl as involved in the 9-22 translocation (Abl is normally on Chr. 9)

- **1984**, It is found that the breakpoint cluster region (BCR)-Abl is a fusion protein with constitutive kinase activity

- **1990**, It is found that the BCR-Abl can cause CML in mice

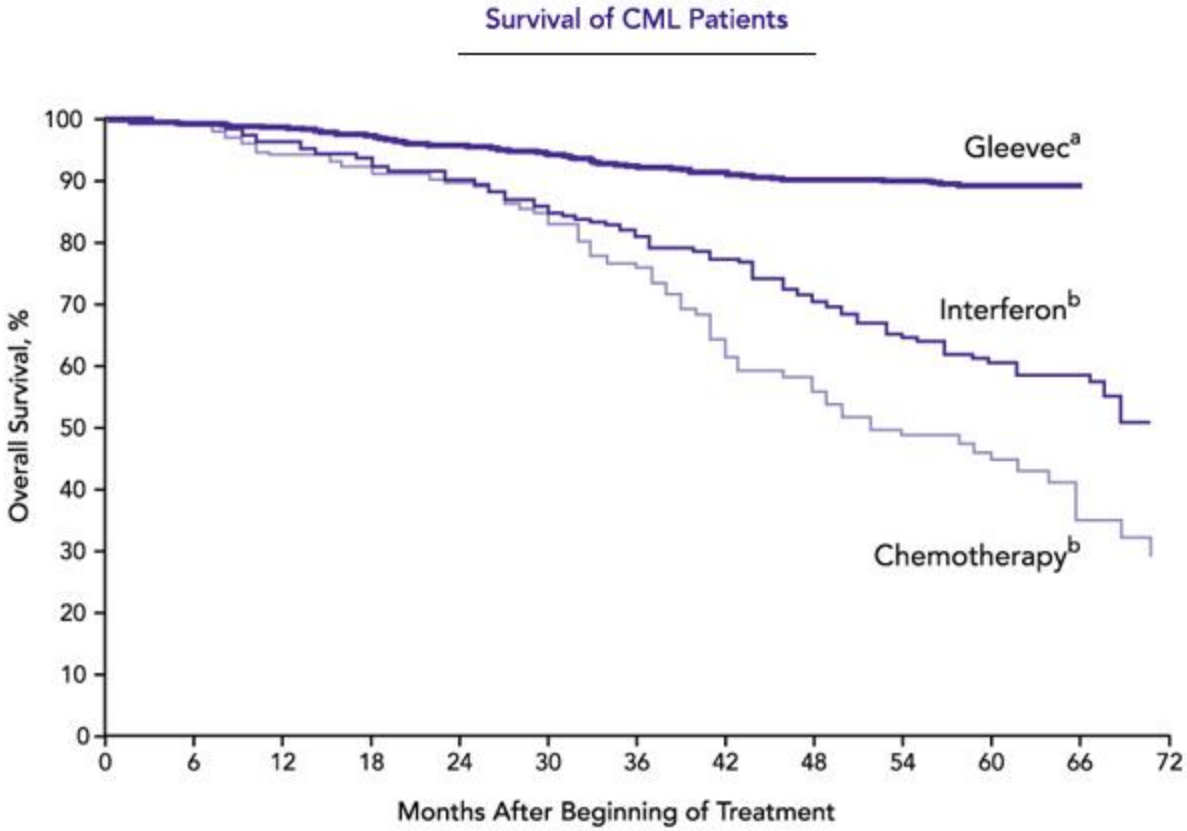
- **1990-96**, Gleevec (imatinib) is developed, a specific Abl inhibitor, which induces apoptosis of CML but not normal bone marrow cells

- **1998**, First clinical trial with Gleevec, extraordinary results

- **2000**, The expression "oncogene addiction" is coined

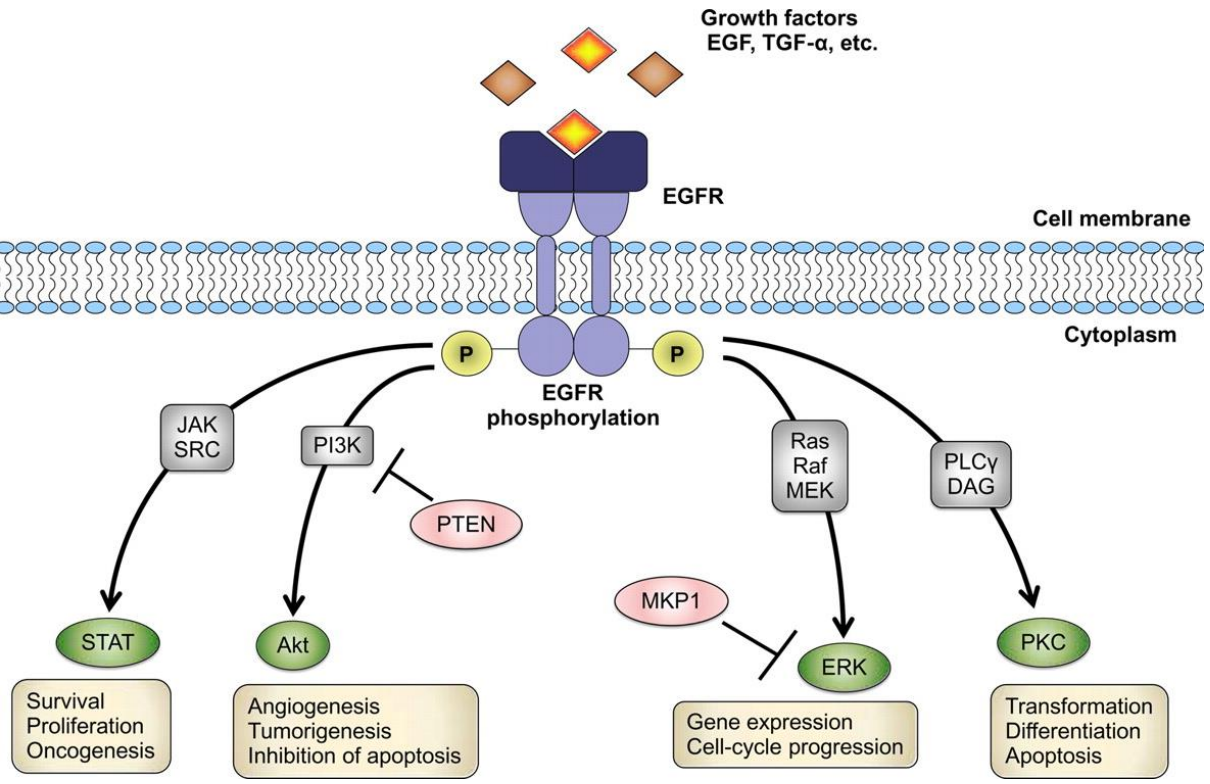
Figure 16.24 *The Biology of Cancer* (© Garland Science 2007)

# Efficacy of Gleevec in CML




[a] From Druker BJ, Guilhot F, O'Brien SG *et al. N Engl J Med.* (2006) **355**:2408-2417.
[b] From The Italian Cooperative Study Group On Chronic Myeloid Leukemia. *N Engl J Med.* (1994) **330**:820-825.

# Targeting EGFR in NSCLC




**NSCLC (non small cell lung cancer)**

- EGFR is upregulated or mutated in more than 60% of NSCLCs

- Expression of EGFR may be predictive of a worse prognosis

- EGFR is a well known and characterized proto-oncogene

**Targeted drugs:**

- gefitinib (Iressa)

- erlotinib (Tarceva)

Both have high affinity for the ATP-binding site of EGFR and inhibit the EGFR TK

# Targeting EGFR in NSCLC

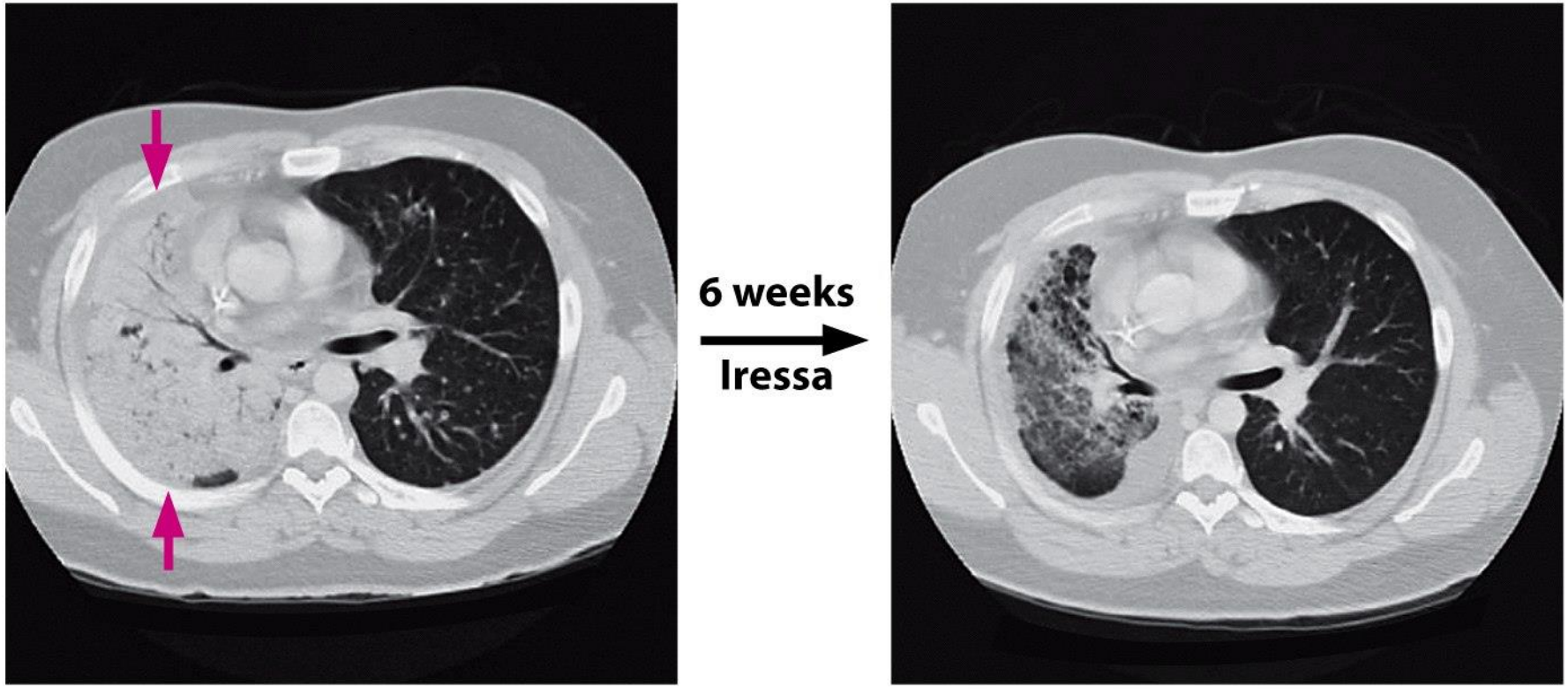
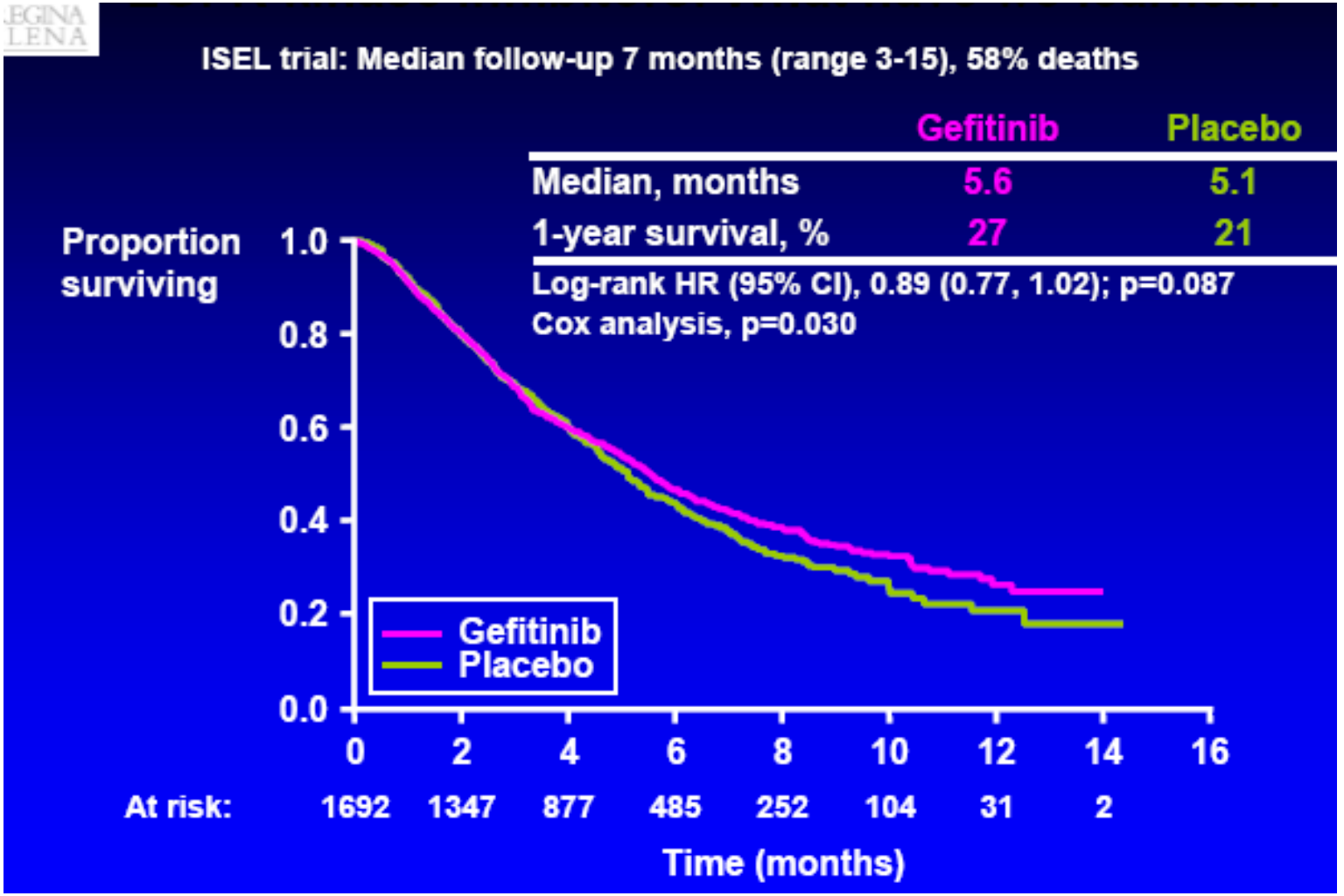



Figure 16.33a *The Biology of Cancer* (© Garland Science 2007)

# Targeting EGFR in NSCLC: first trials




*: patients who failed previous therapies

**Limited benefits... Why???**

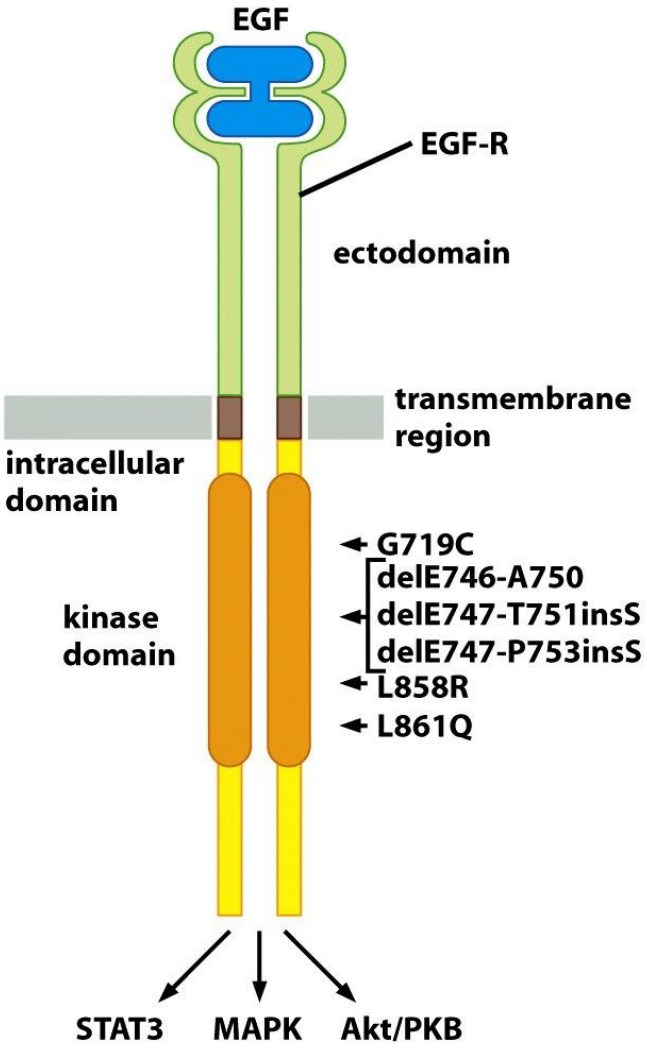
# Targeting the EGFR in NSCLC

In the first trials, only 10% of the patients with EGFR overexpression responded to Iressa....

But in those few cases, tumor responses were quite dramatic!

1) Why?
2) How to select patients that are more likely to respond?

# Specific mutations in the EGFR confer sensitivity to EGFR inhibitors

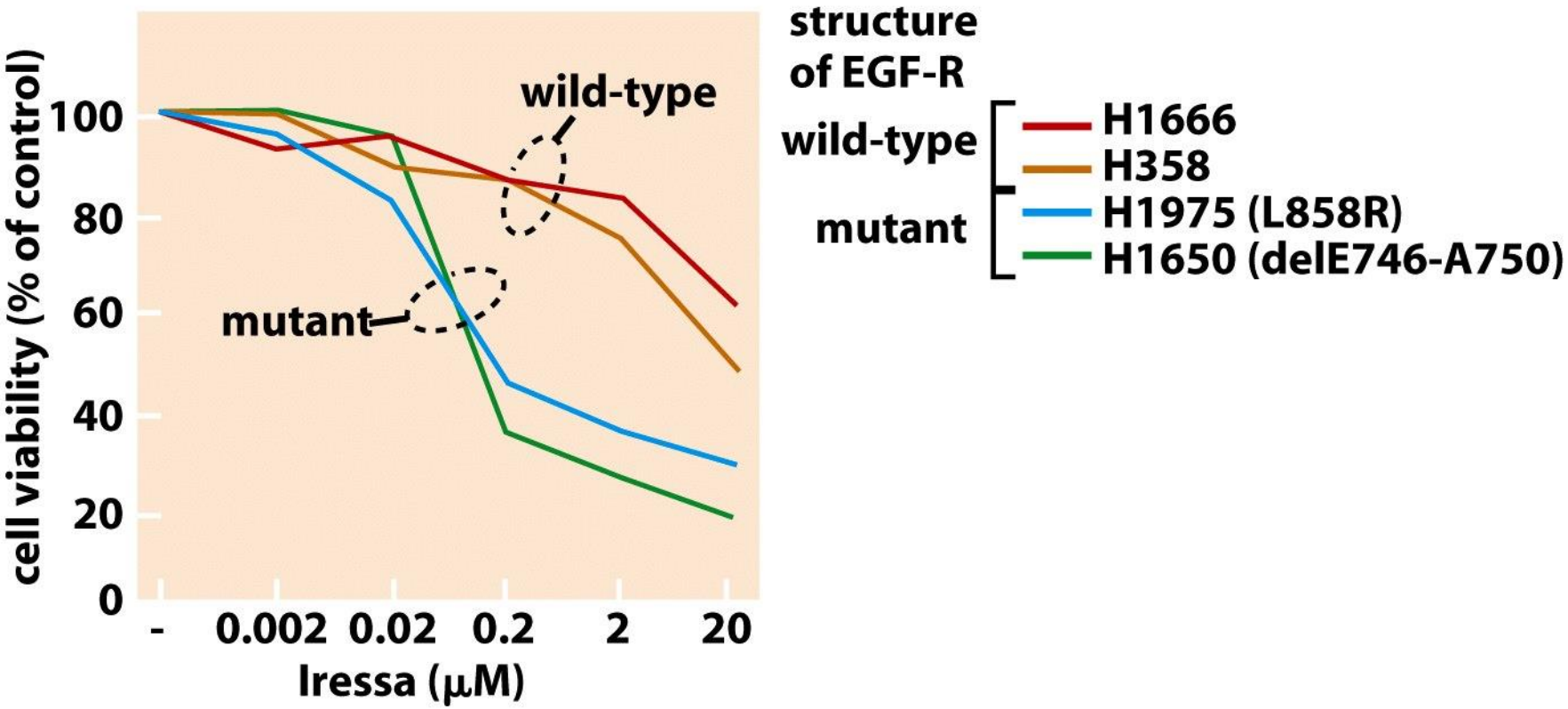



First sequencing of the cancer genomes of responders and non-responders:

It led to the dentification of **mutations in the TK domain that may constitutively activate the tyrosine kinase of EGFR** and promote uncessant downstream signaling (which may also protect the cancer cells from apoptosis induced by genotoxic stress).

Figure 16.33b *The Biology of Cancer* (© Garland Science 2007)

# NSCLCs with activating mutations in EGFR are *more* sensitive to Iressa




**Oncogene addiction**: The data suggest that cancer cells with a mutated (constitutively active) EGFR are more dependent on EGFR signaling than cancer cells with a wild-type EGFR

Figure 16.34a *The Biology of Cancer* (© Garland Science 2007)

# NSCLCs with activating mutations in EGFR respond to Iressa

| Publication | N | Mutation positive | | Wild type | |
|---|---|---|---|---|---|
| | | N (%) | N (%) responders | N (%) | N (%) responders |
| Lynch et al 2004 | 16 | 8 (50%) | 8 (100%) | 8 (50%) | 1 (12.5%) |
| Paez et al 2004 | 9 | 5 (55%) | 5 (100%) | 4 (45%) | 0 (0%) |
| Pao et al 2005 | 60 | 17 (28%) | 17 (100%) | 43 (72%) | 5 (12%) |
| Huang et al 2004 | 16 | 8 (50%) | 7 (87.5%) | 8 (50%) | 2 (25%)* |
| Kosaka et al 2004 | 59 | 33 (55.9%) | 24 (72.7%) | 26 (44.1%) | 2 (7.7%) |
| Koyama et al 2004 | 21 | 8 (38.1%) | 8 (100%) | 13 (61.9%) | 6 (46.2%) |
| Kodo et al 2004 | 7 | 3 (42.8%) | 3 (100%) | 4 (57.2%) | 1 (25%) |
| Rosell et al 2004 | 68 | 19 (28%) | 16 (84%) | 49 (72%) | 7 (14%) |
| Han et al, 2005 | 90 | 17 (18.9%) | 11 (64.7%) | 73 (81.1%) | 10 (13.7%) |
| **Total** | **346** | **118 (34.1%)** | **99 (83.9%) responders** | **228 (65.9%)** | **34 (14.9%) responders** |

# Evidence for "oncogene addiction" in other cancer types

**Table 16.1 Effects of shutting down expression of an initiating oncogenic transgene in tumor-prone mice[a]**

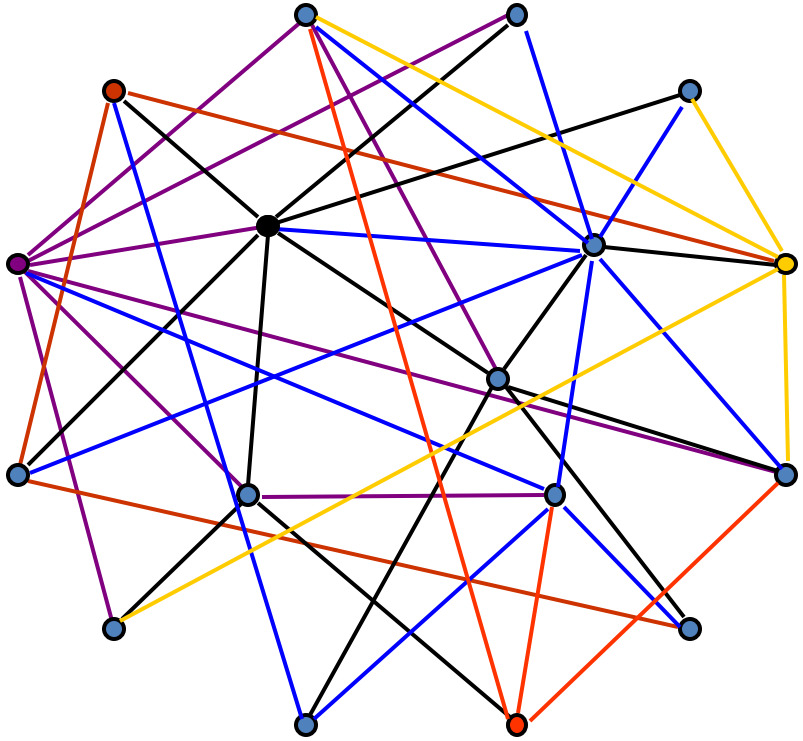
| Transgenic oncogene | Response of tumors |
|---|---|
| **Permanent regression after shutdown of transgene** | |
| H-*ras* | melanoma collapsed |
| K-*ras* | lung adenocarcinoma regressed |
| *bcr-abl* | B-cell leukemia regressed |
| *myc* | T-cell lymphoma, acute myelogenous leukemia regressed |
| *fgf-7* | lung epithelial hyperplasia regressed |
| SV40 large T | salivary gland hyperplasia regressed if transgene expressed < 4 months |

What are the molecular/biochemical bases of such responses?

Table 16.1 *The Biology of Cancer* (© Garland Science 2007)

# Mechanisms of oncogene addiction

# Networks of biochemical reactions in cell homeostasis



Normal cell

External factors can influence and perturb cell homeostasis:

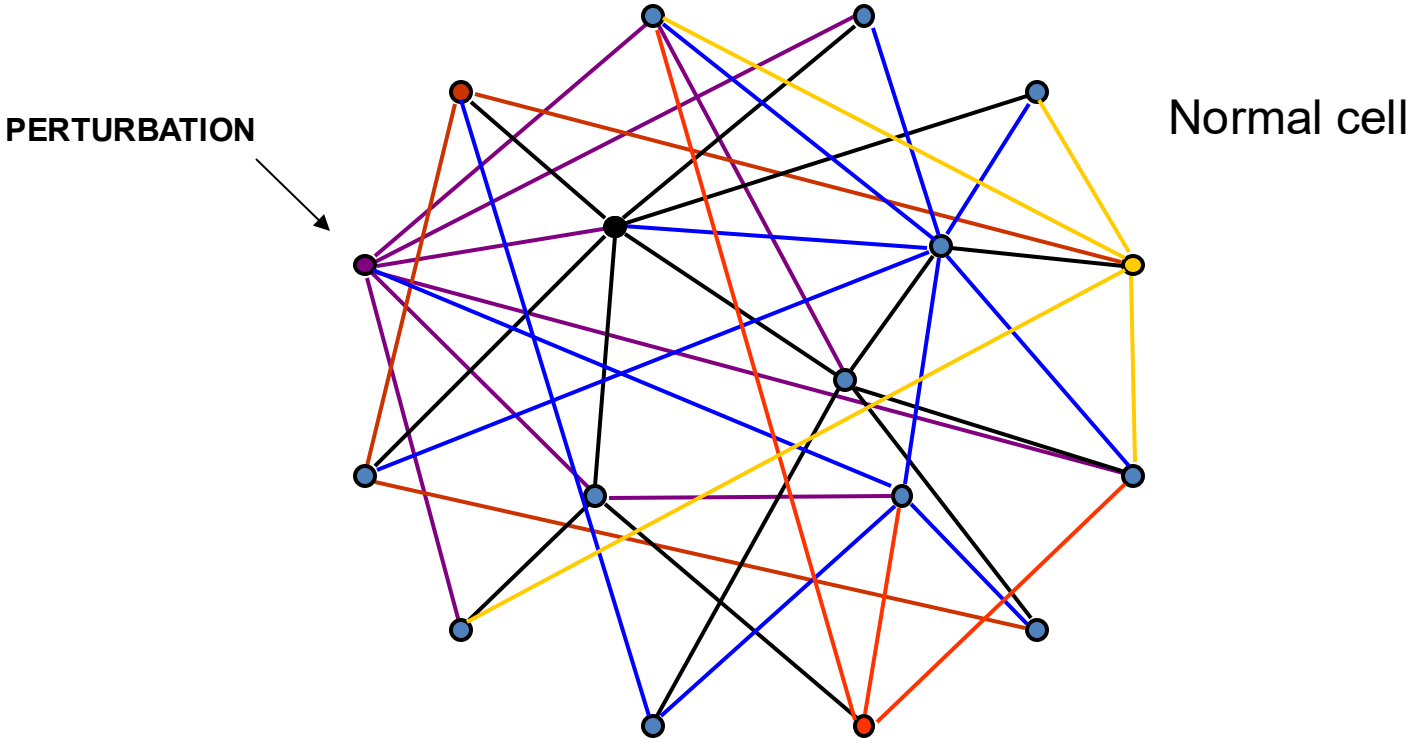
- Directly (e.g, deprivation of nutrients or growth factors)
- Indirectly (e.g., mutagens that affect the function of enzymes and other proteins)

Beyond a certain threshold, perturbations to the system can lead to cell collapse:

- Excessive genetic damage may lead to cell apoptosis
- Excessive toxic stress may lead to cell necrosis

Collapse threshold

# Networks of biochemical reactions in cell homeostasis

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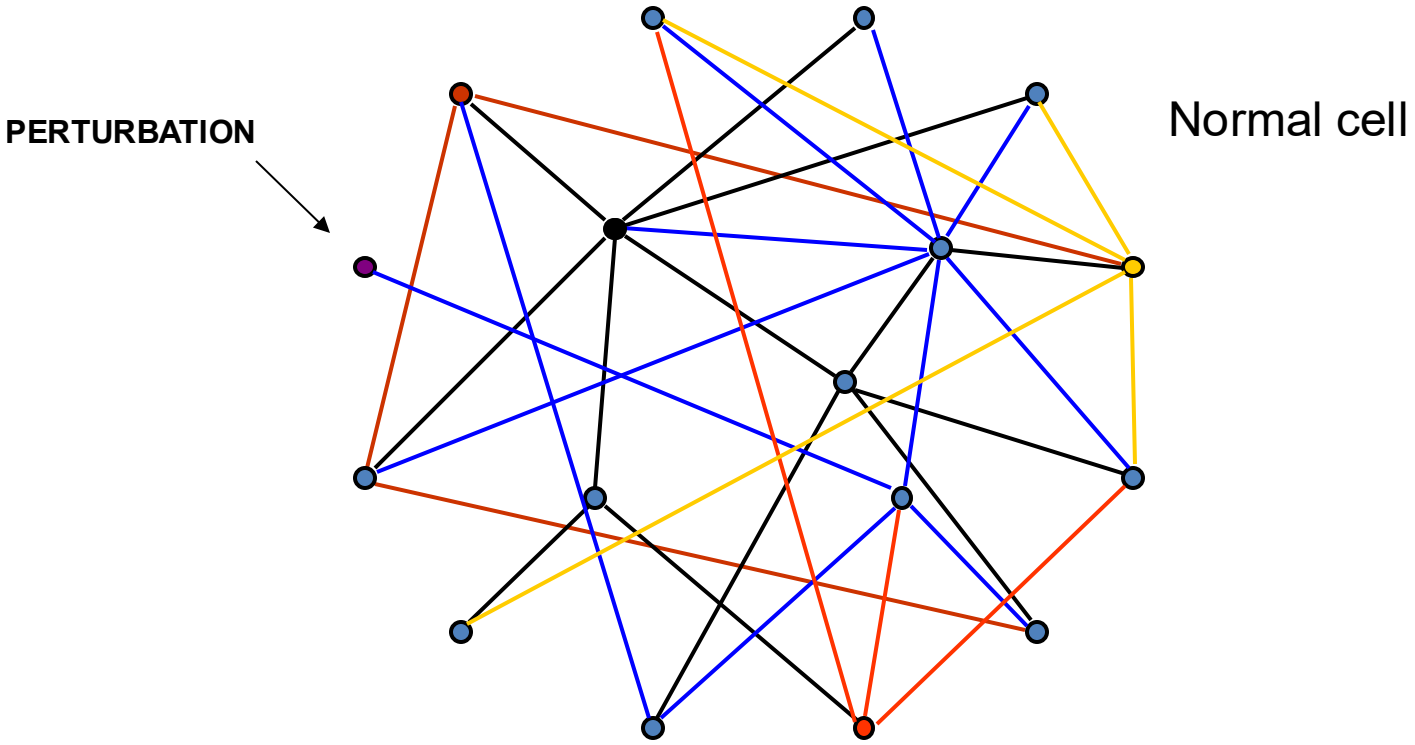
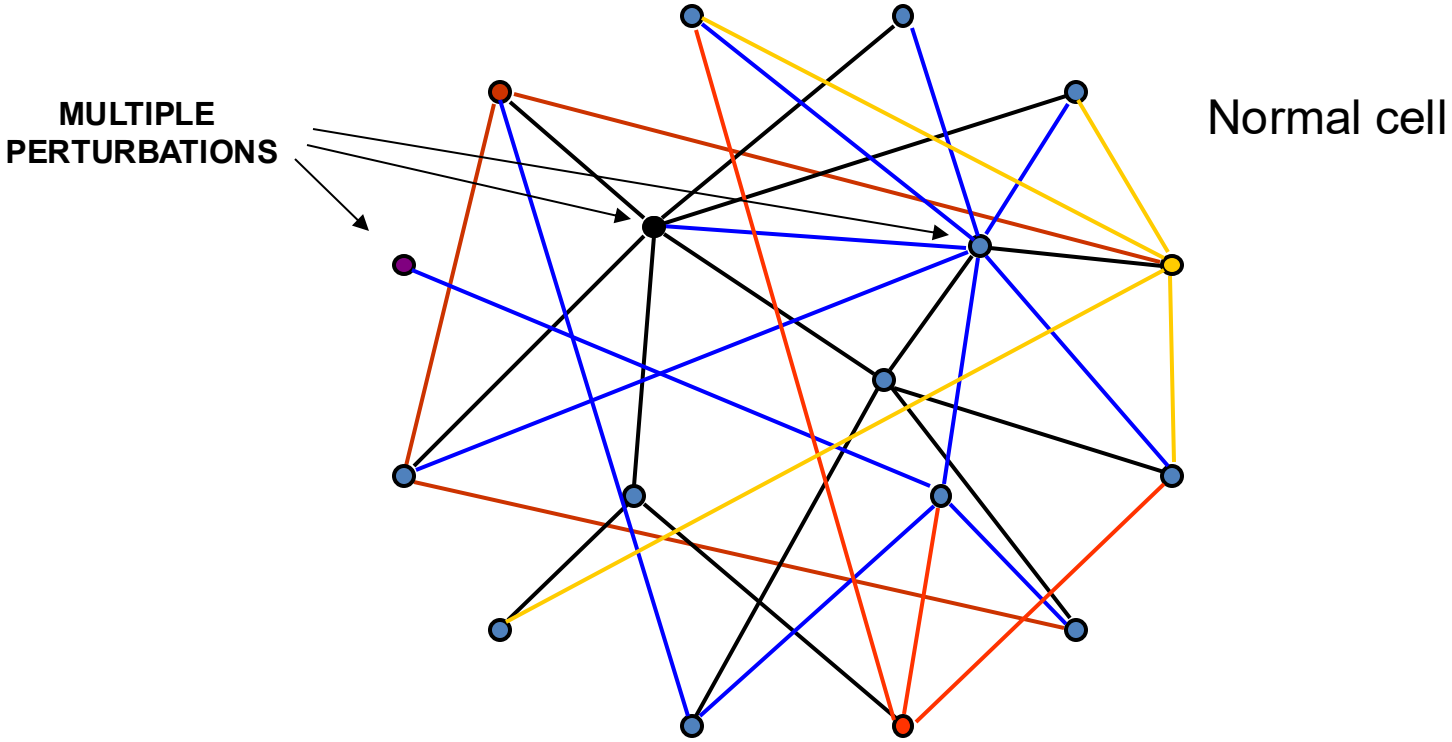
- Indirectly (e.g., mutagens that affect the function of enzymes and other proteins)

# Networks of biochemical reactions in cell homeostasis



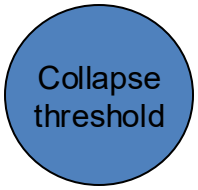

**The system is redundant:**
**Below a certain degree of perturbation, the system remains in equilibrium....**

# Networks of biochemical reactions in cell homeostasis

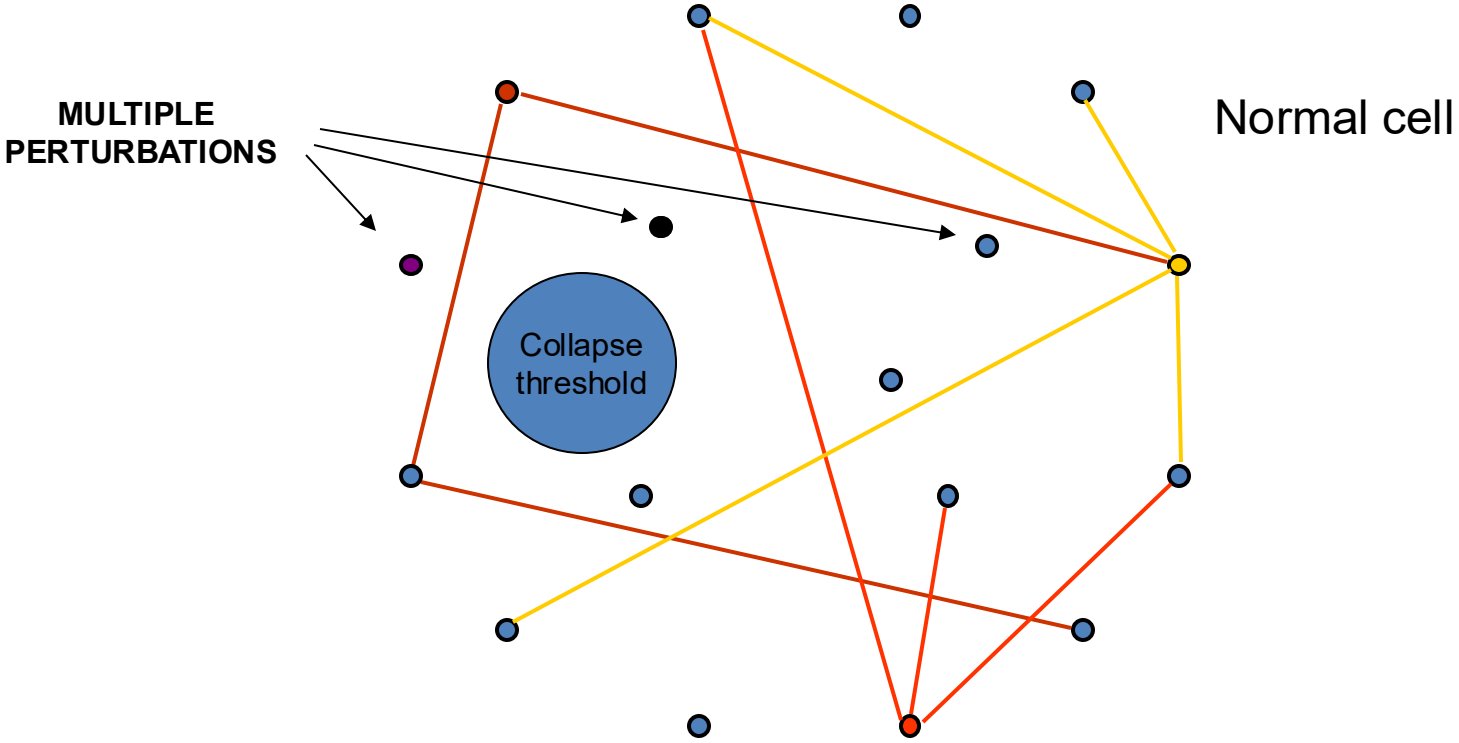



Above a certain threshold, perturbations to the system can lead to cell collapse:

- Excessive genetic damage may lead to cell apoptosis
- Excessive toxic stress may lead to cell necrosis



# Networks of biochemical reactions in cell homeostasis

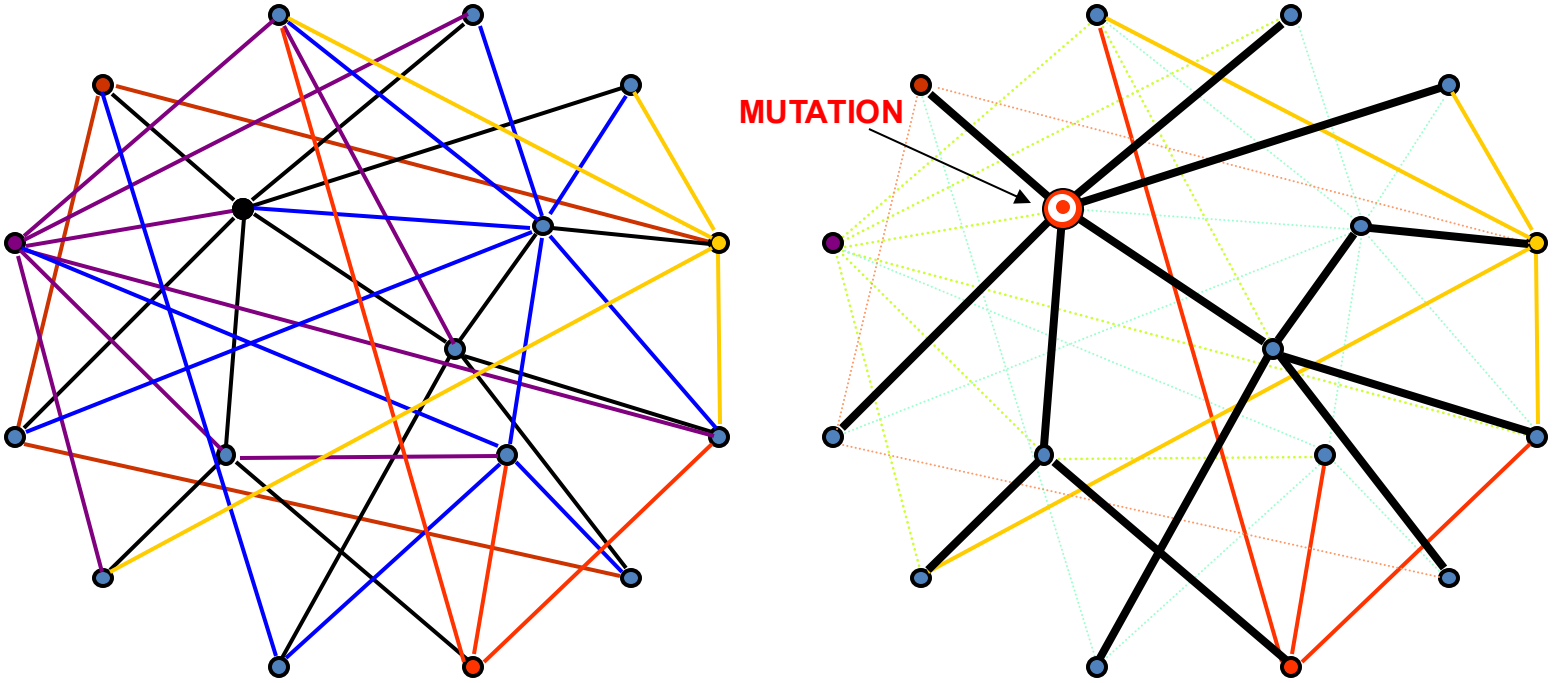



**The cell collapses because too many perturbations have occurred, leading to cell apoptosis or necrosis**

# Mutations perturb biochemical reactions in cancer cells

Normal cell

Cancer cell

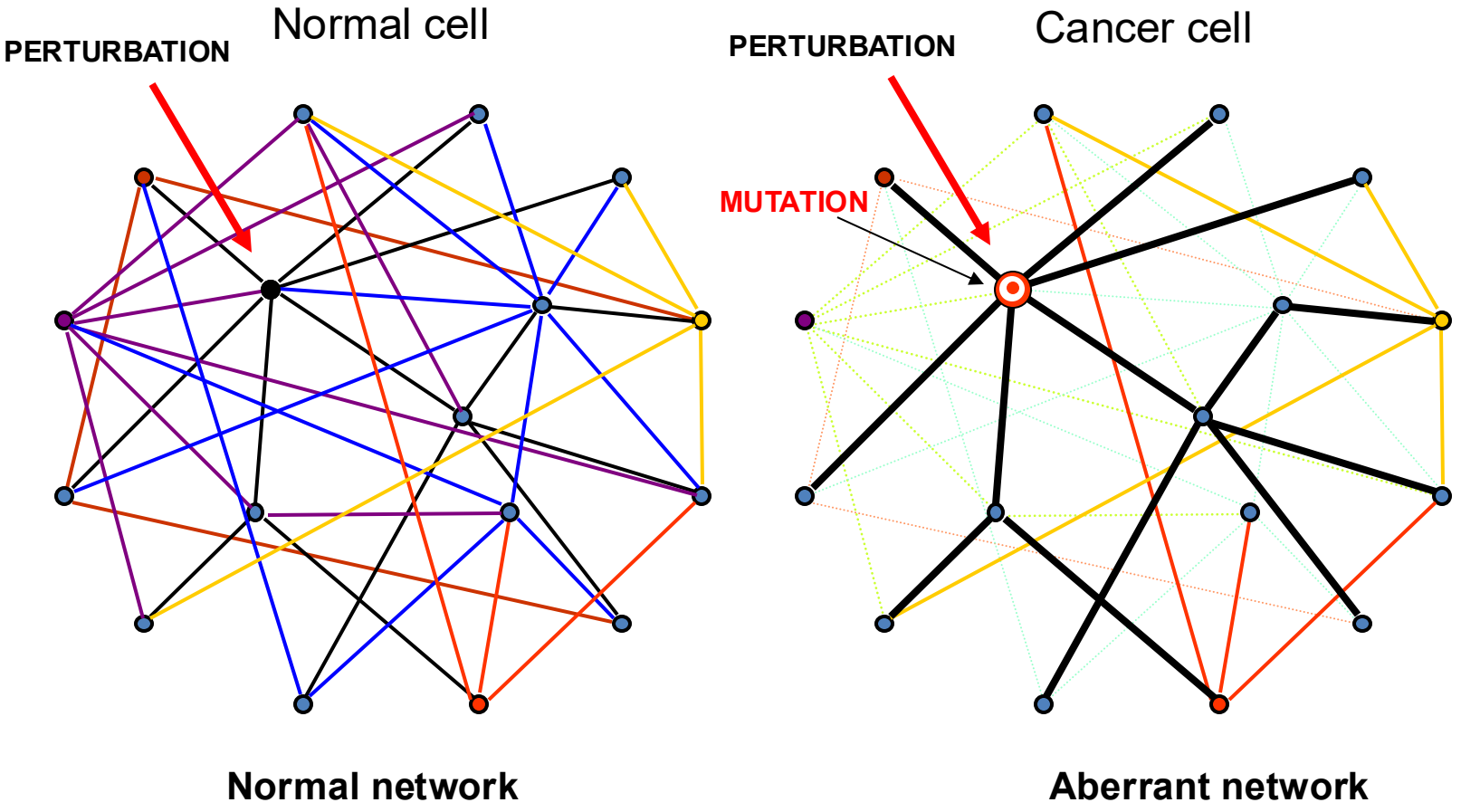



**Normal network**

**Aberrant network**

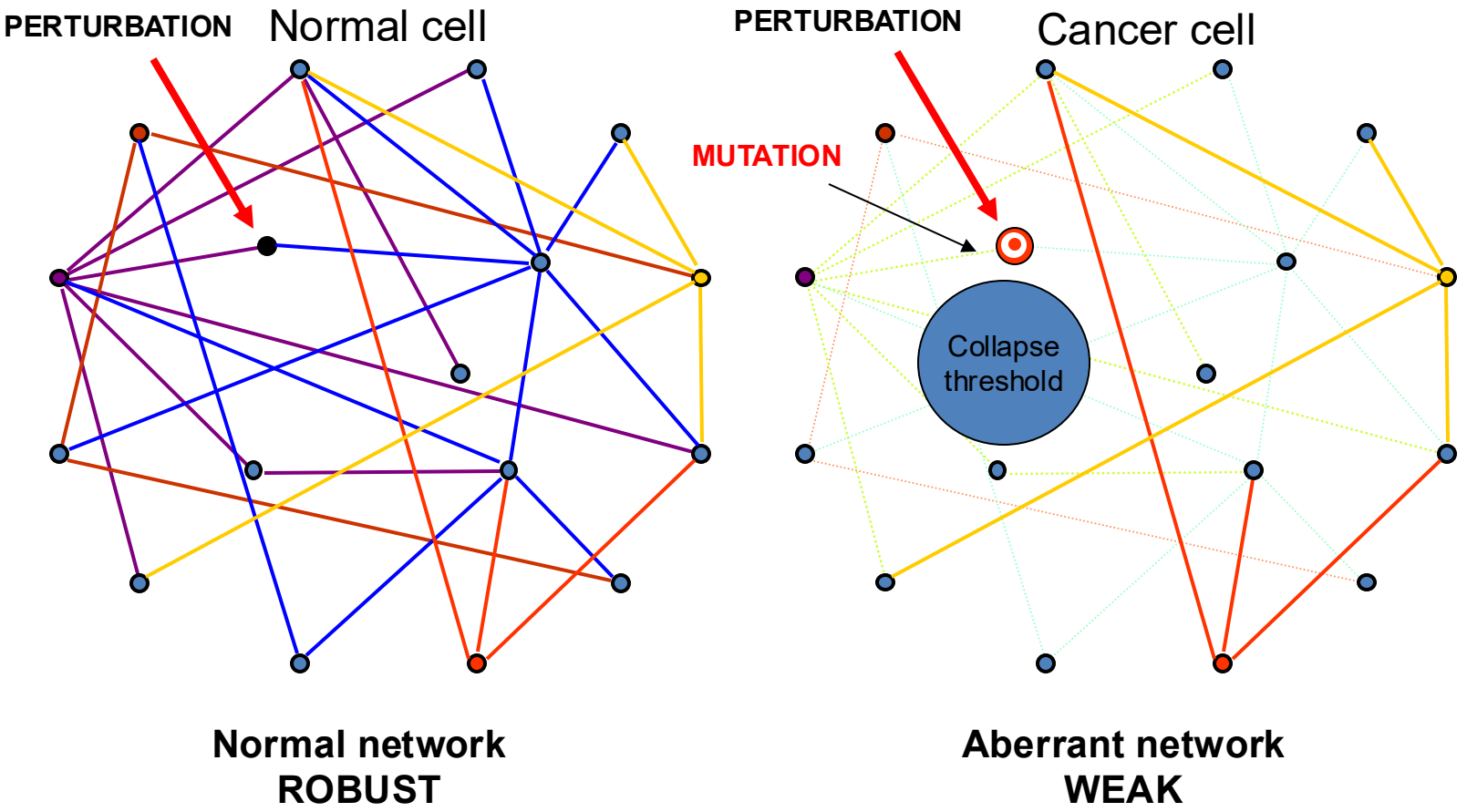
**Mutations is cancer cells strengthen some biochemical interactions while weakening others....**

# Mutations perturb biochemical reactions in cancer cells



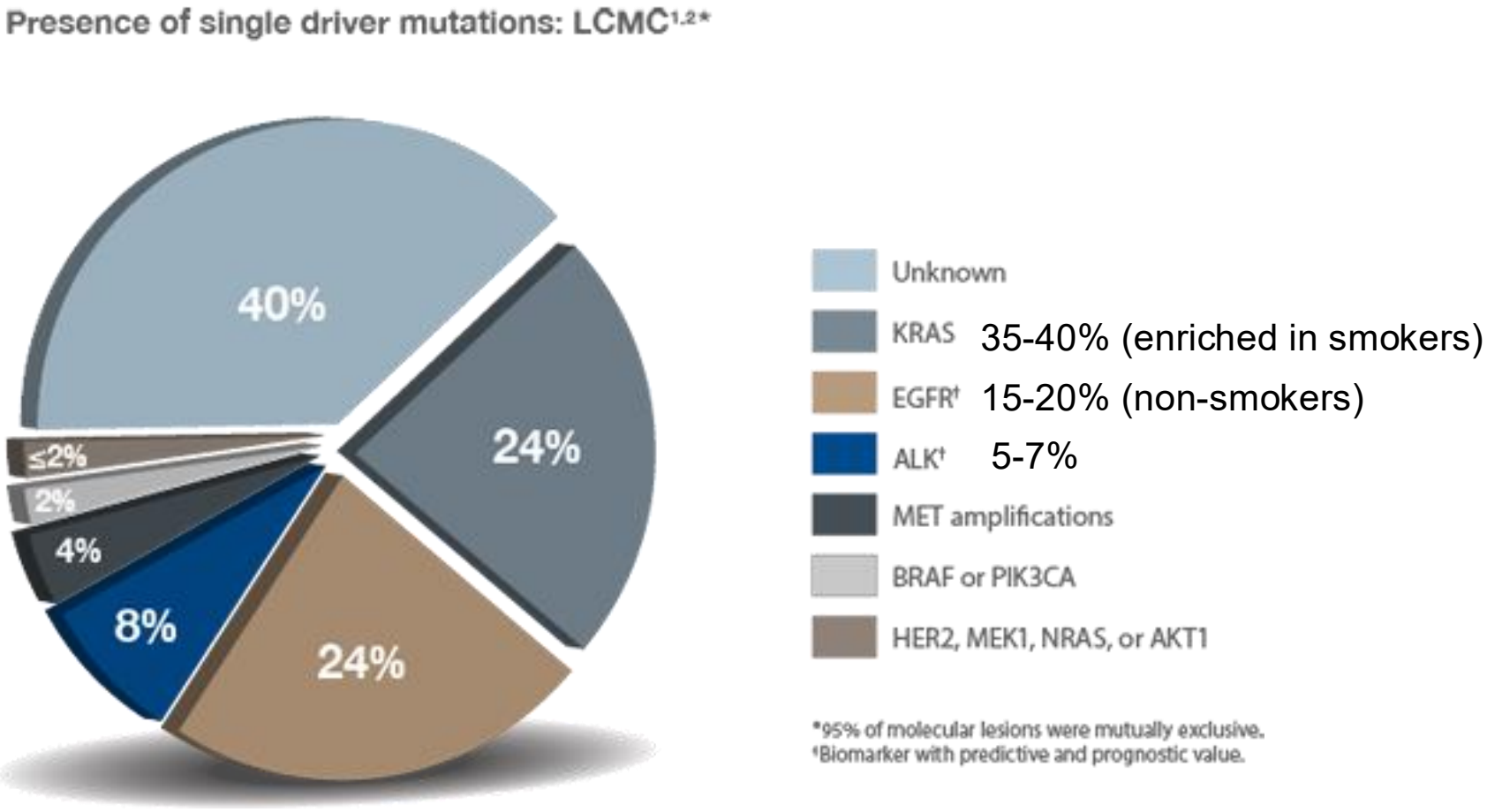

Effects of perturbations to normal and aberrant biochemical networks….

# Mutations perturb biochemical reactions in cancer cells



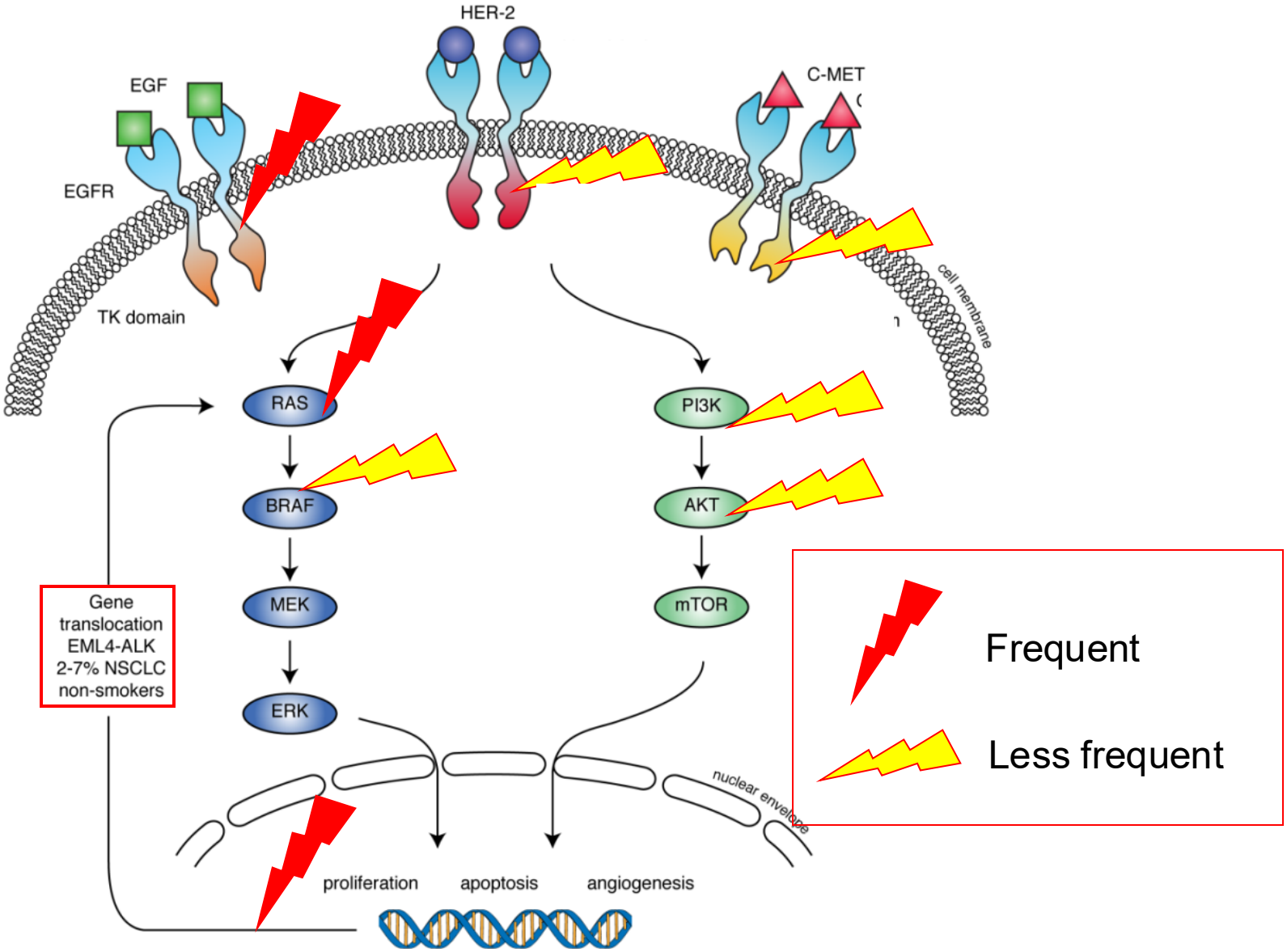

**ONCOGENE ADDICTION: The cell has become dependent on a main (driver) mutation whose product (generally, an oncogenic protein) is required to maintain cell homeostasis in metabolically corrupted cancer cells**

# Evidence for driver mutations/oncogene addiction in NSCLC

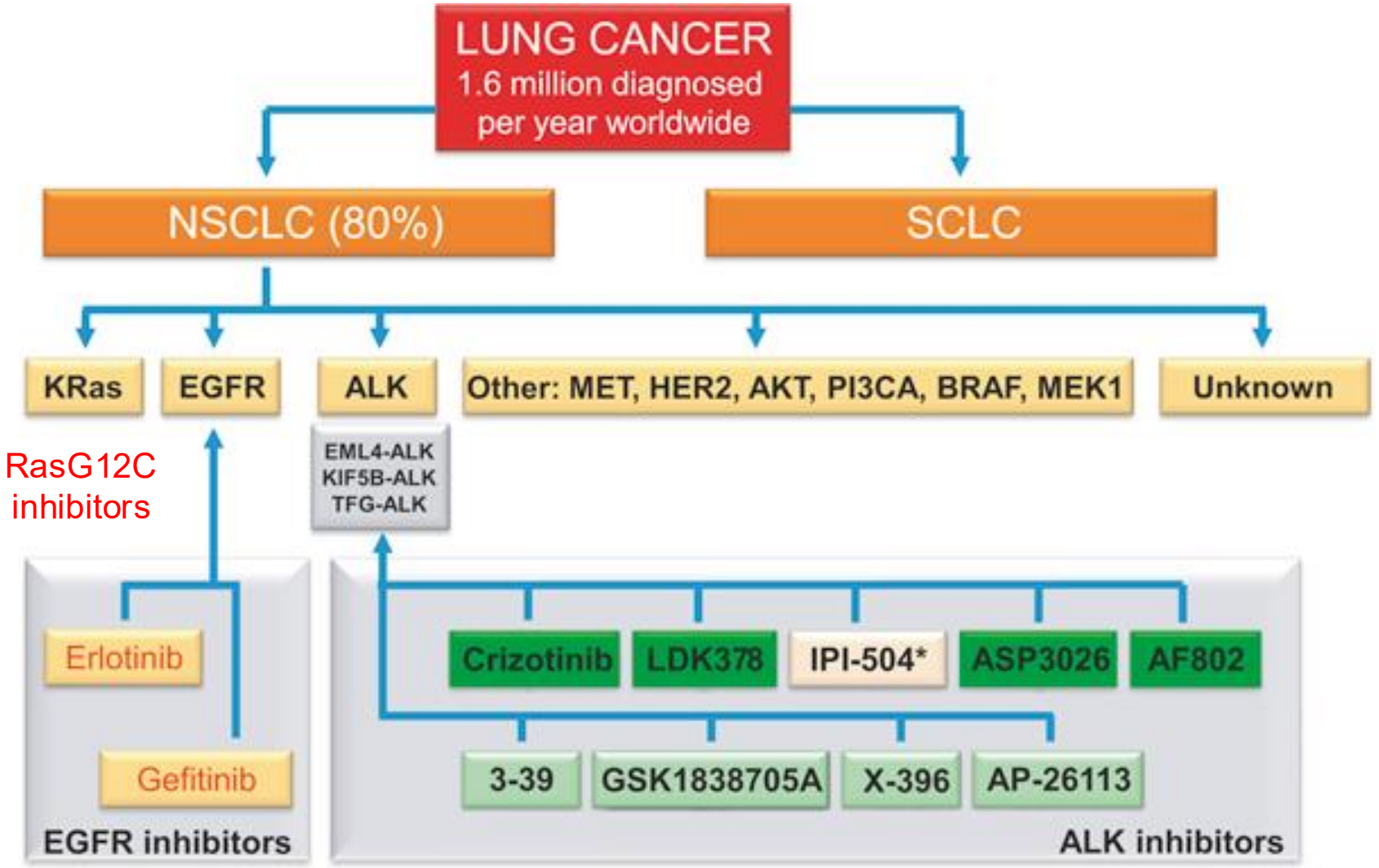



**Driver mutations are frequently mutually exclusive (at least in NSCLC)**

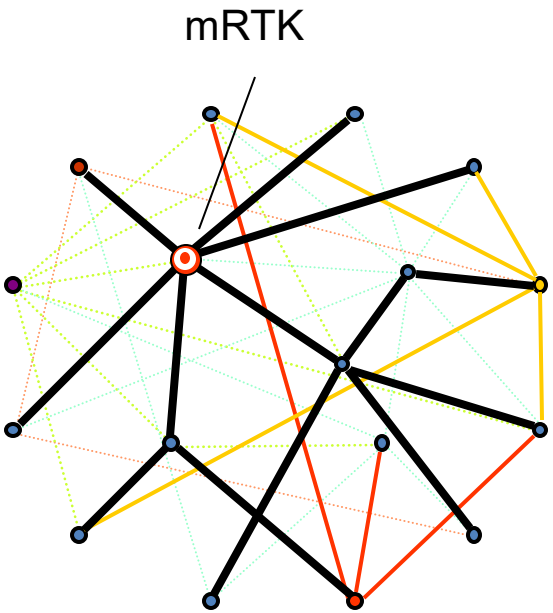
# Driver mutations/oncogene addiction in NSCLC

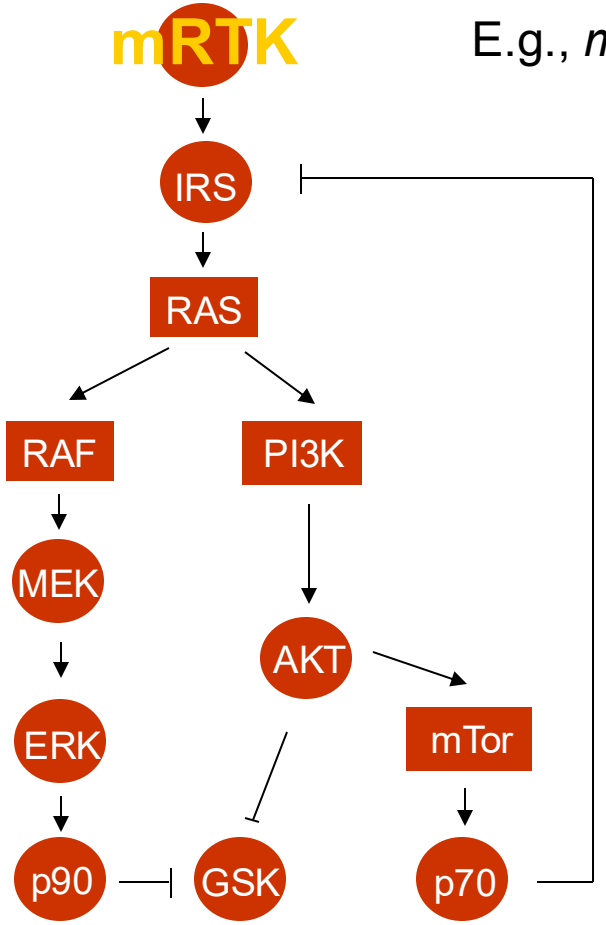


# Targeting driver mutations in NSCLC

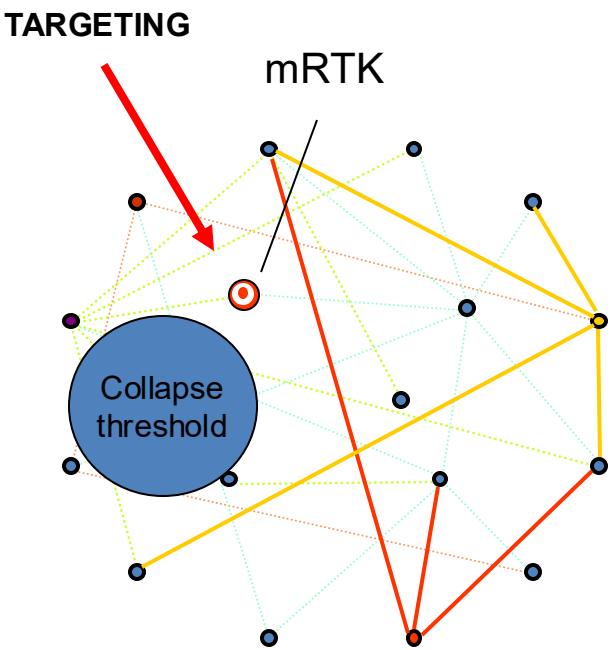


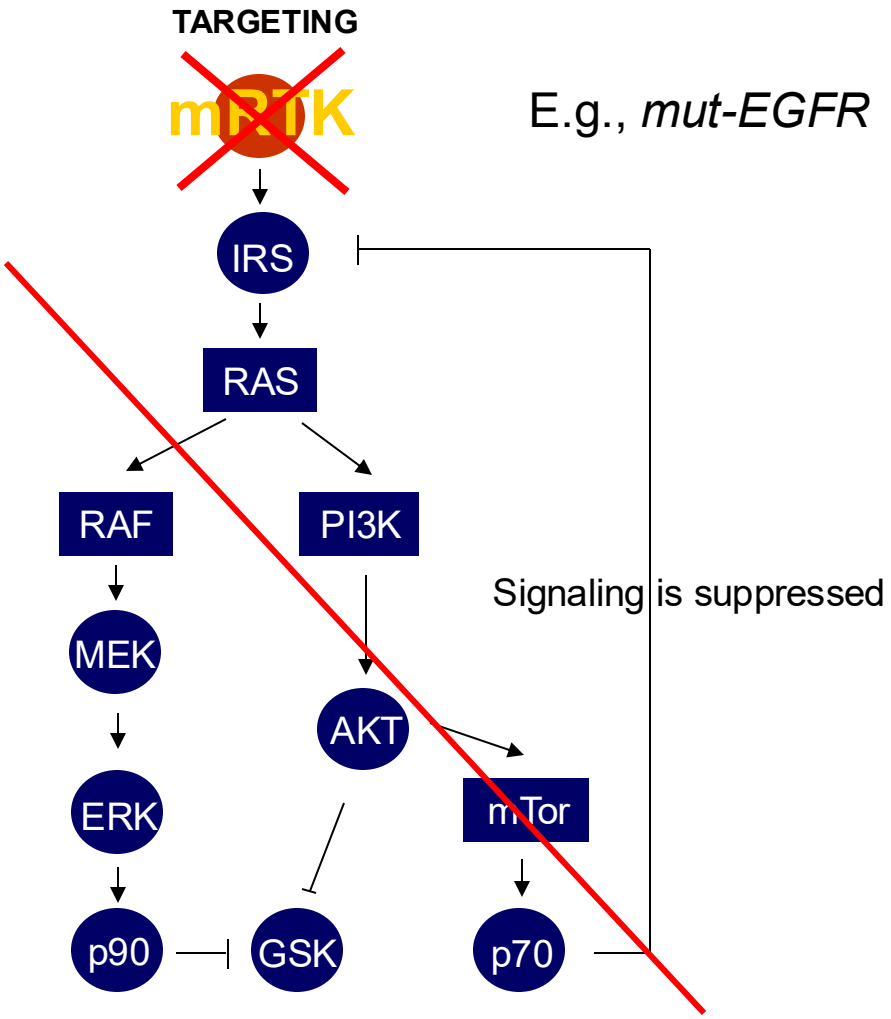
# Drug sensitivity by targeting mutant receptor tyrosine kinases (mRTKs)



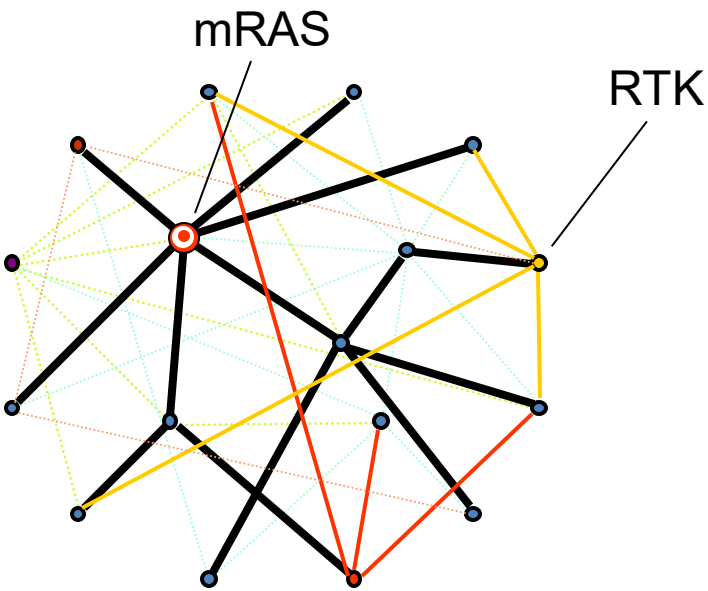
# Drug sensitivity by targeting mutant receptor tyrosine kinases (mRTKs)

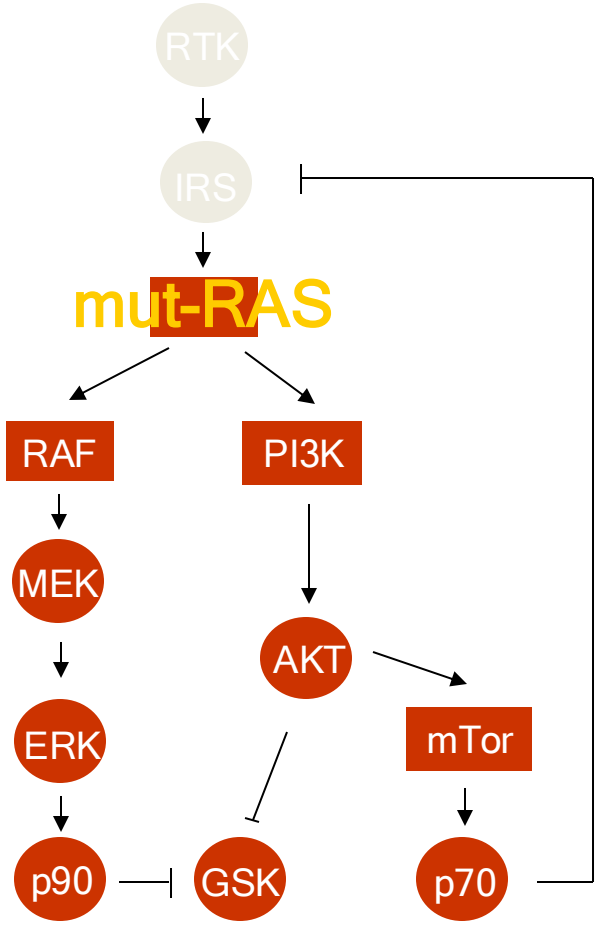


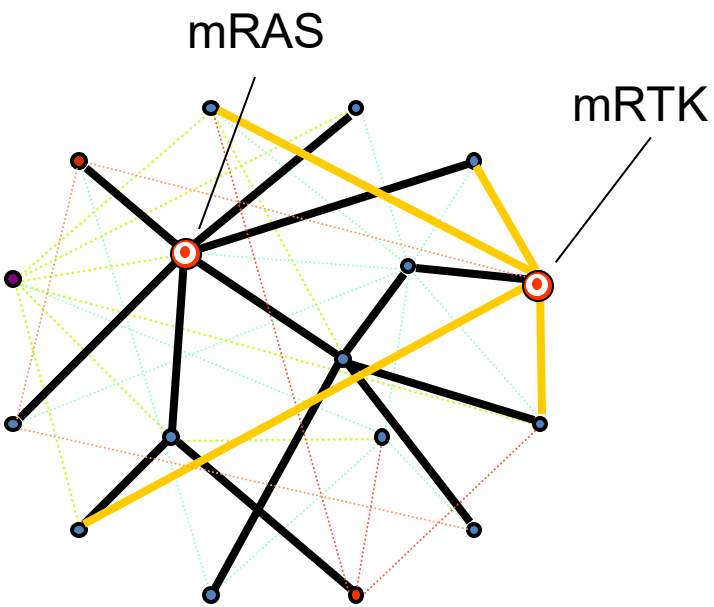

The cancer cell collapses because it is dependent on sustained EGFR signaling to survive (unlike a normal cell)

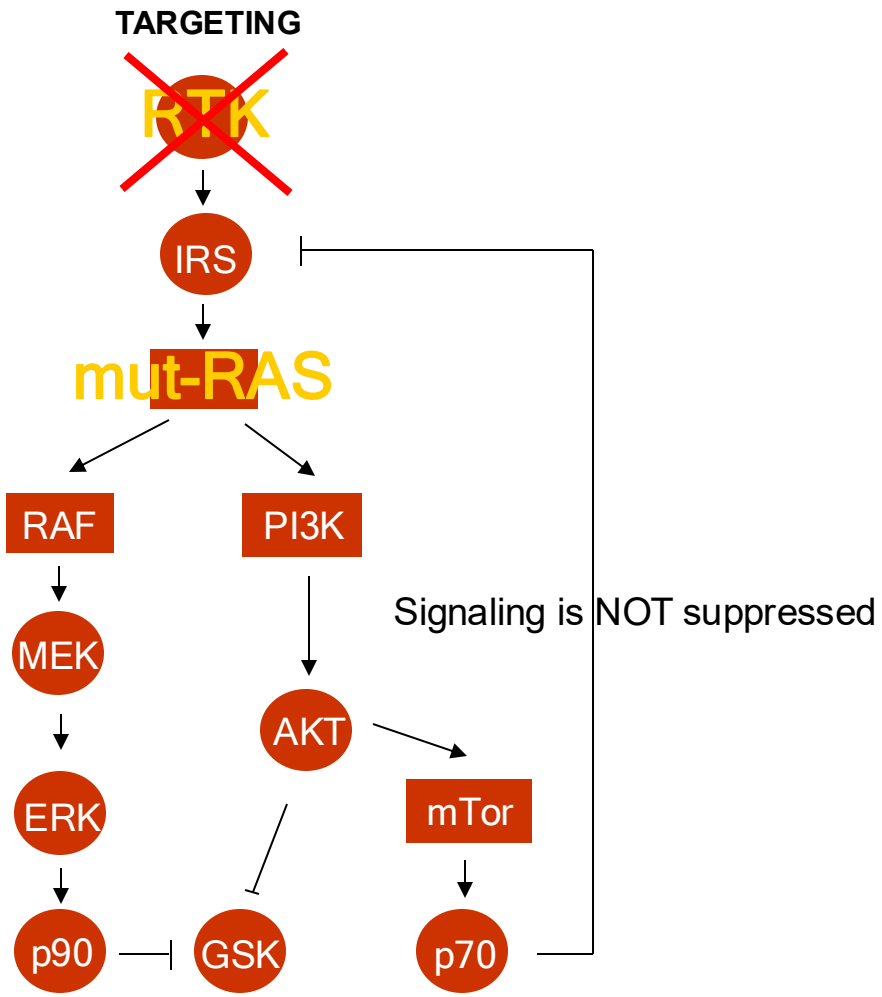
# In non-mutant RTKs, constitutive activation of the pathway may occur downstream, e.g., by mutant RAS



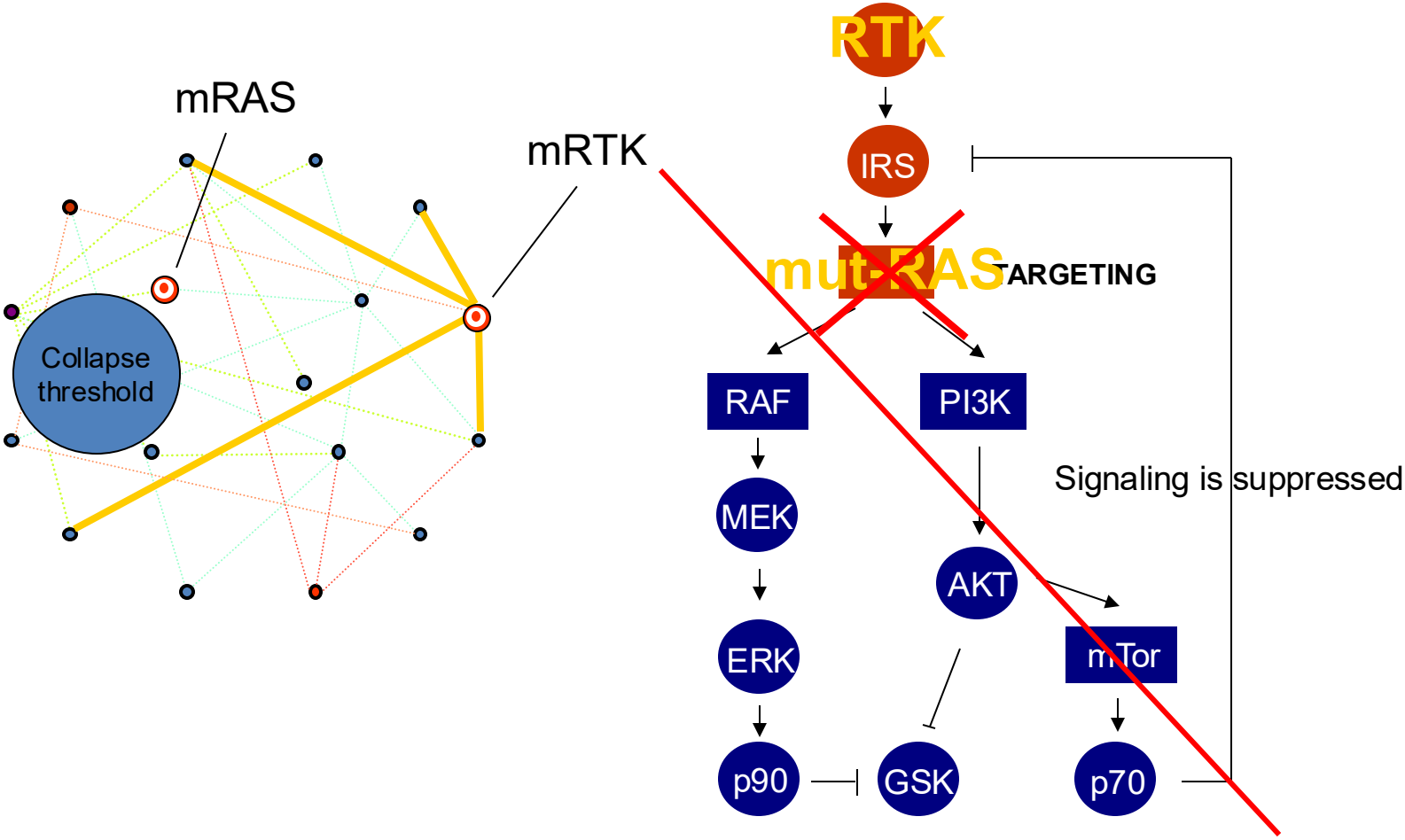



# … leading to PRIMARY DRUG RESISTANCE to the RTK inhibitor



# Targeting downstream signaling may overcome primary drug resistance



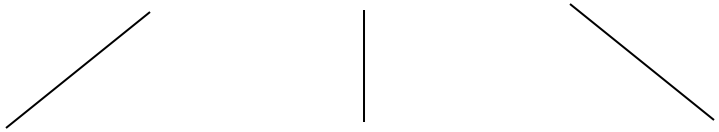

Note that RAS is very rarely mutated in NSCLC with mutant EGFR (no selective advantage!!)

# How to identify patients amenable to targeted therapies?

# How to identify patients amenable to targeted therapies?

**BIOMARKERS (GENES):**

- Prognostic (survival)
- Predictive (response to therapy)
- Pharmacodynamic (drug activity)



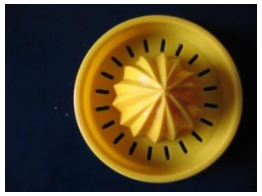
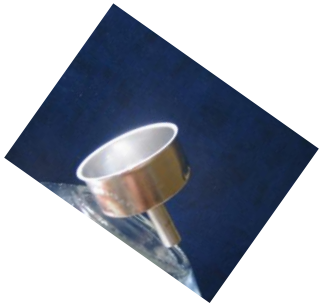
**Genetic profiles (eg, mutations as deiscussed above)**

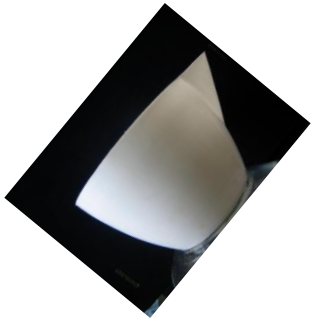
**Gene *expression* signatures**

**Proteomic profiles**

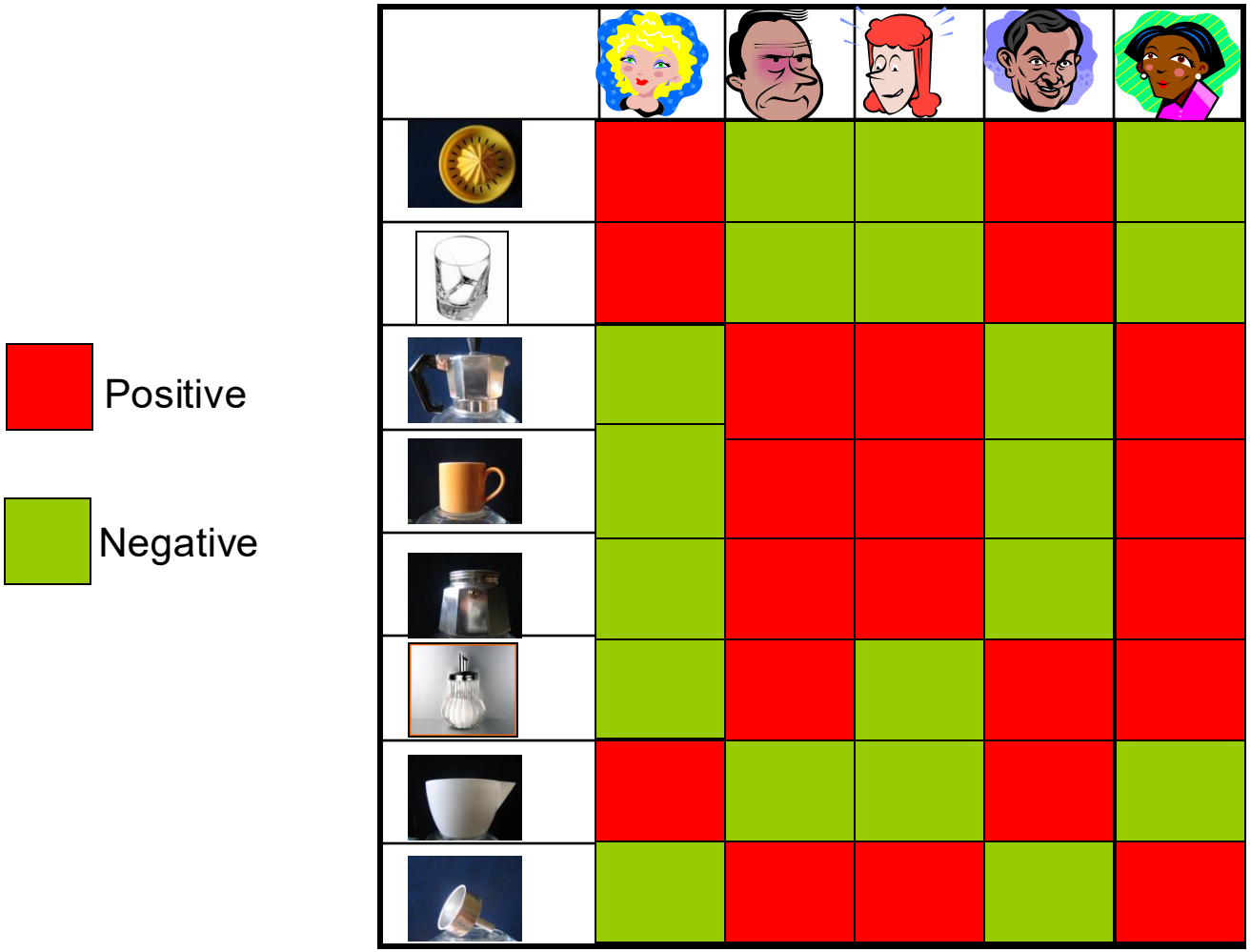
# Unsupervised stratification of patients based on biomarkers

Biomarkers

# Unsupervised stratification of patients based on biomarkers



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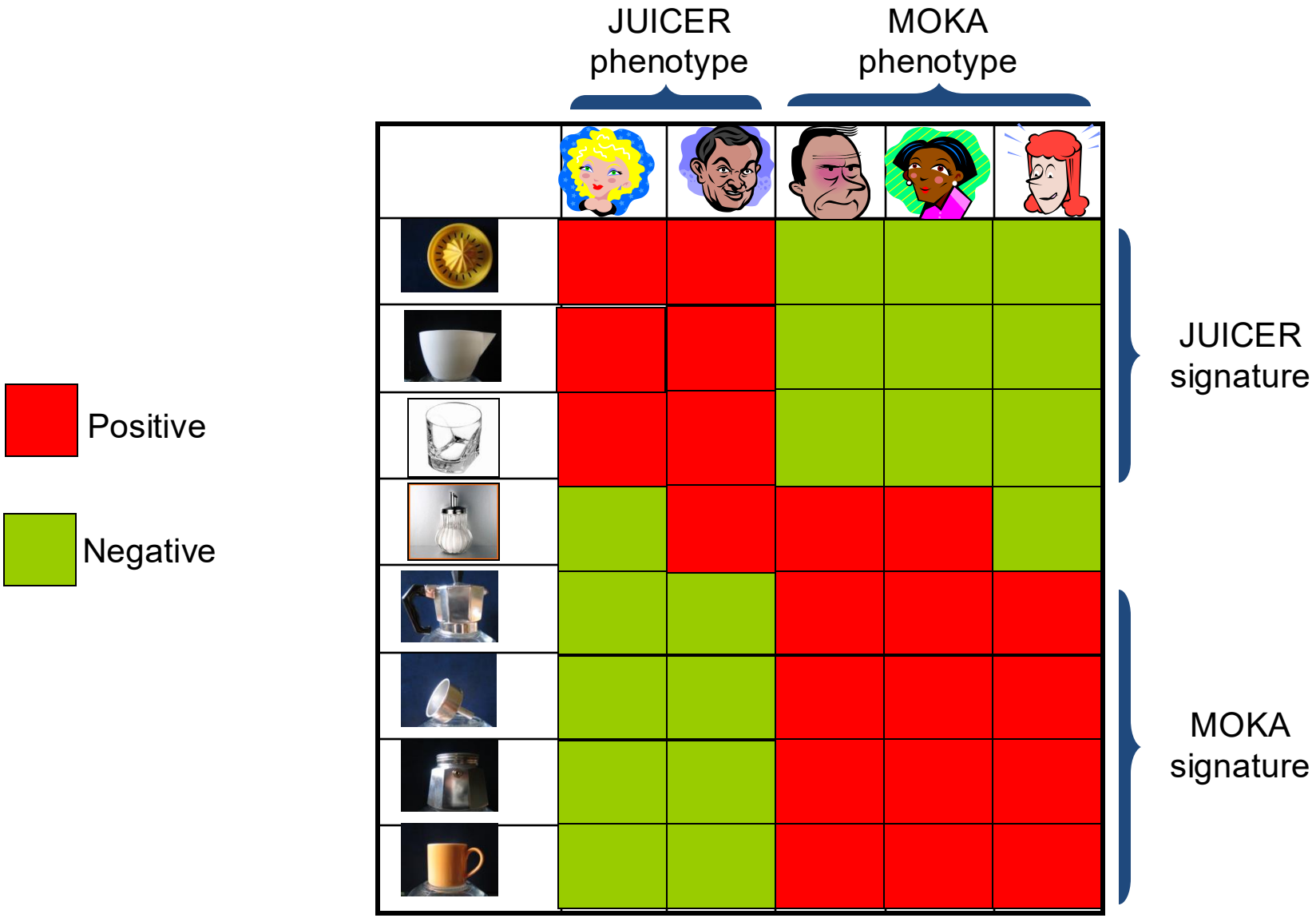
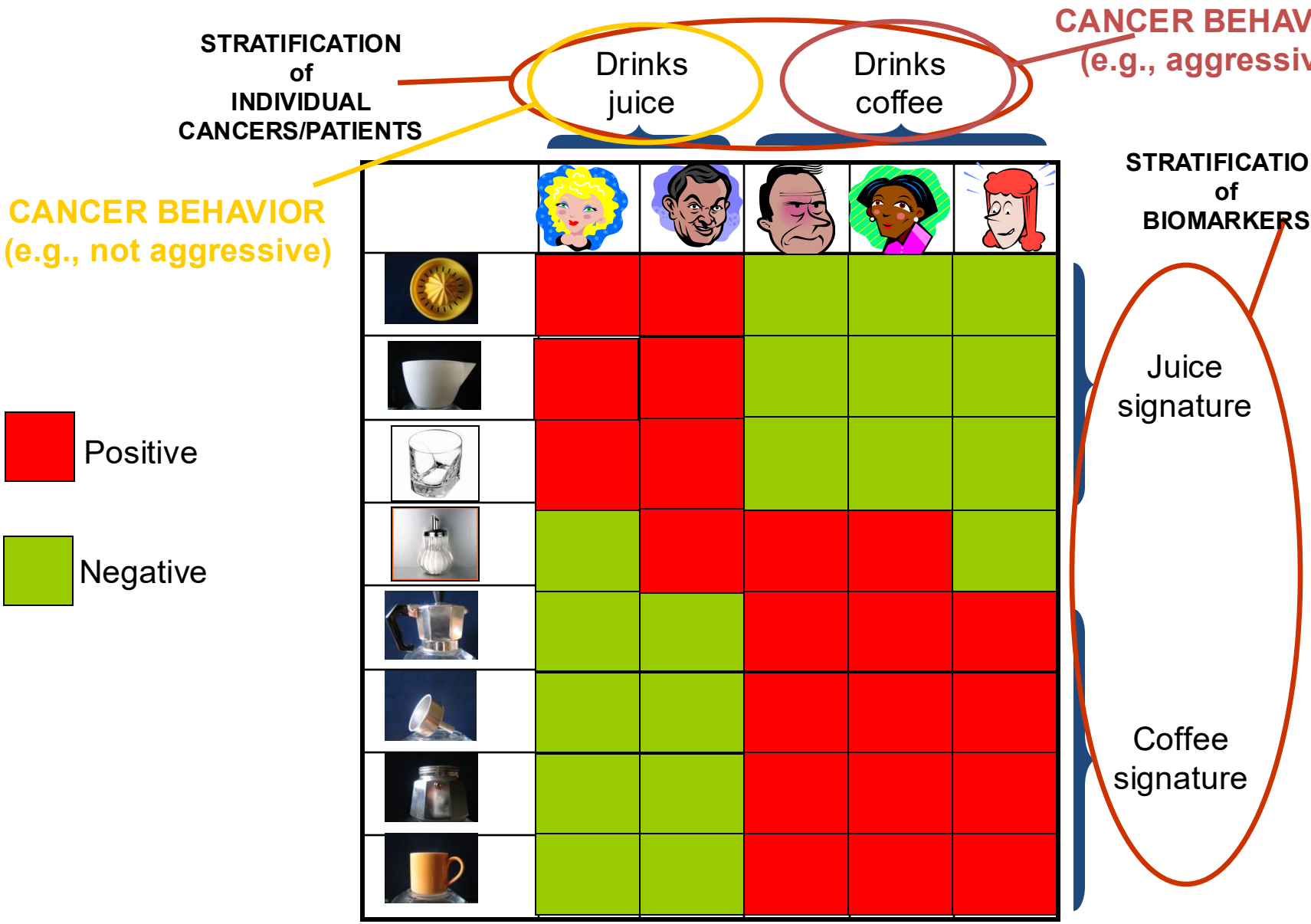
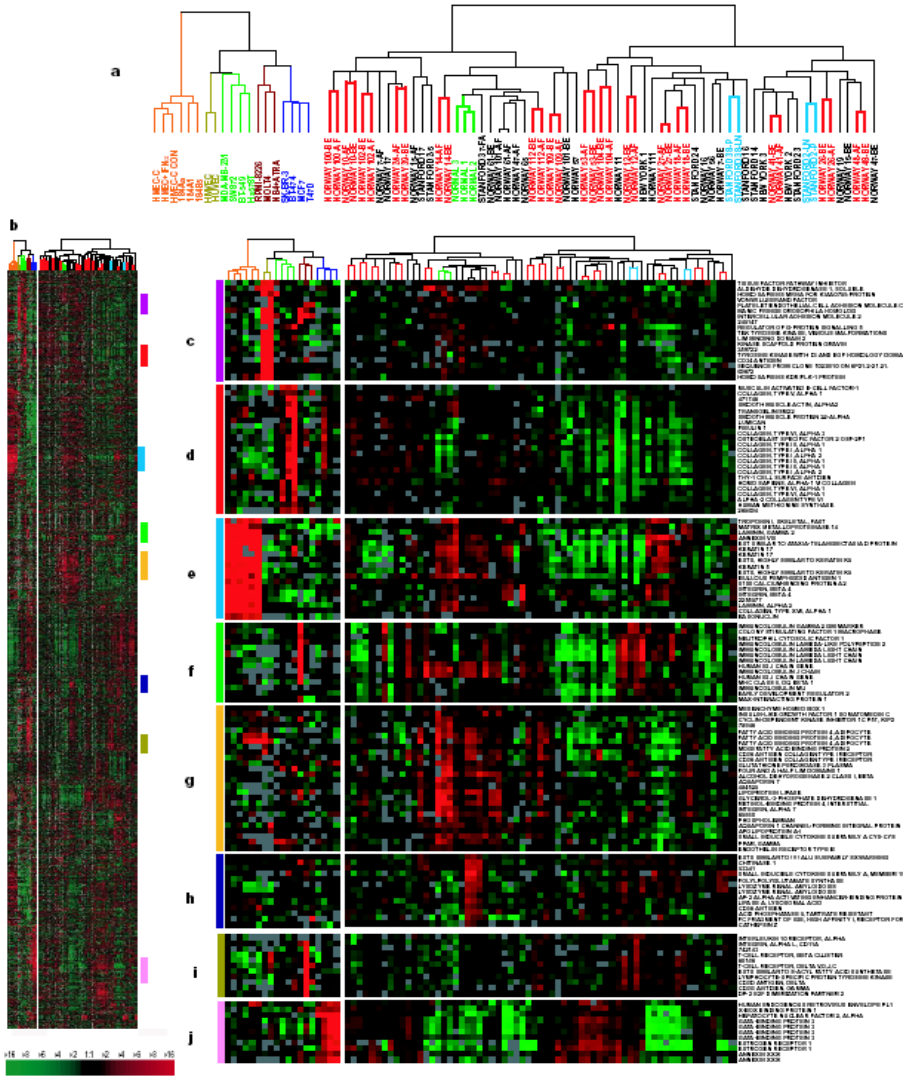


# Unsupervised stratification of patients




Making associations between biomarkers (e.g., gene expression) and a certain behavior (e.g., tumor malignancy, response to therapy, etc.)

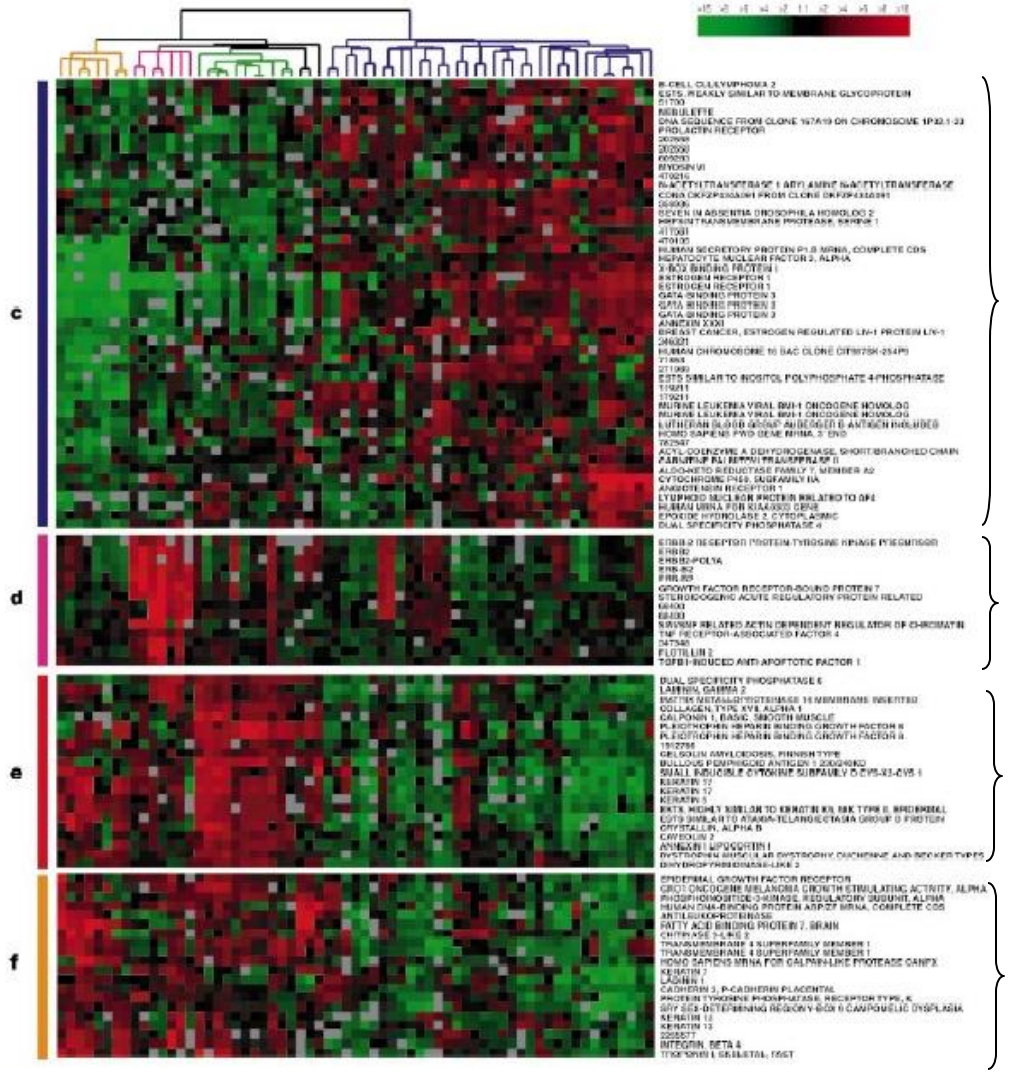
# Unsupervised stratification of cancers based on *gene expression* signatures



"We proposed that the phenotypic diversity of breast tumours might be accompanied by a corresponding diversity in gene expression patterns that we could capture using cDNA microarrays."

Perou et al., Nature 2000

# Molecular classification of **breast cancer** subtypes based on unsupervised analysis




**ER+ and/or PR+ (luminal type): 40-60%**

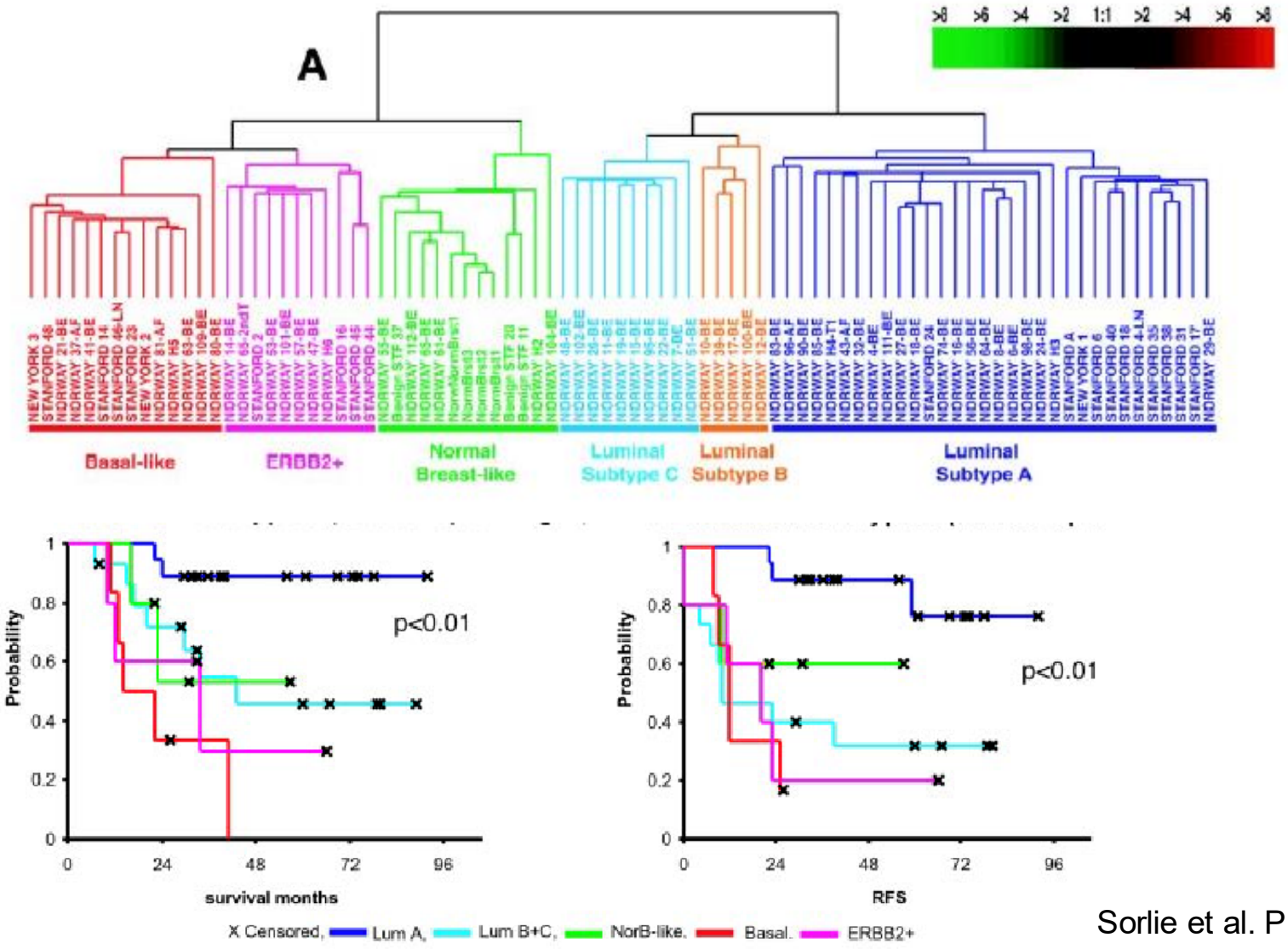
**HER2 (ERBB2)+: 15%**

**Basal-like**

**Normal breast-like**

**Triple negative: 15-20%**

# Prognostic significance of the molecular classification of breast cancer subtypes

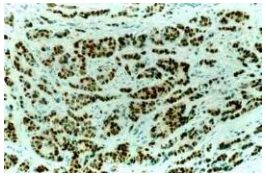



Sorlie et al. PNAS 2001

# Molecular classification of breast cancer may help identify therapeutic targets

**Major Biological Classes of Breast Cancer**

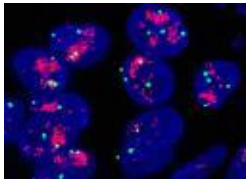
**Potentially endocrine dependent**



**ER and/or PR expression**

- Endocrine therapies
- Tamoxifen
- Aromatase inhibitors
- Fulvestrant

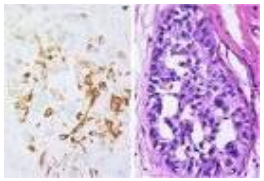
**HER2-driven**



**HER2 amplification**

- Trastuzumab
- Lapatinib
- Pertuzumab

**Triple-negative**



**ER, PR and HER2-Negative**
**Include BRCA1 mutated**

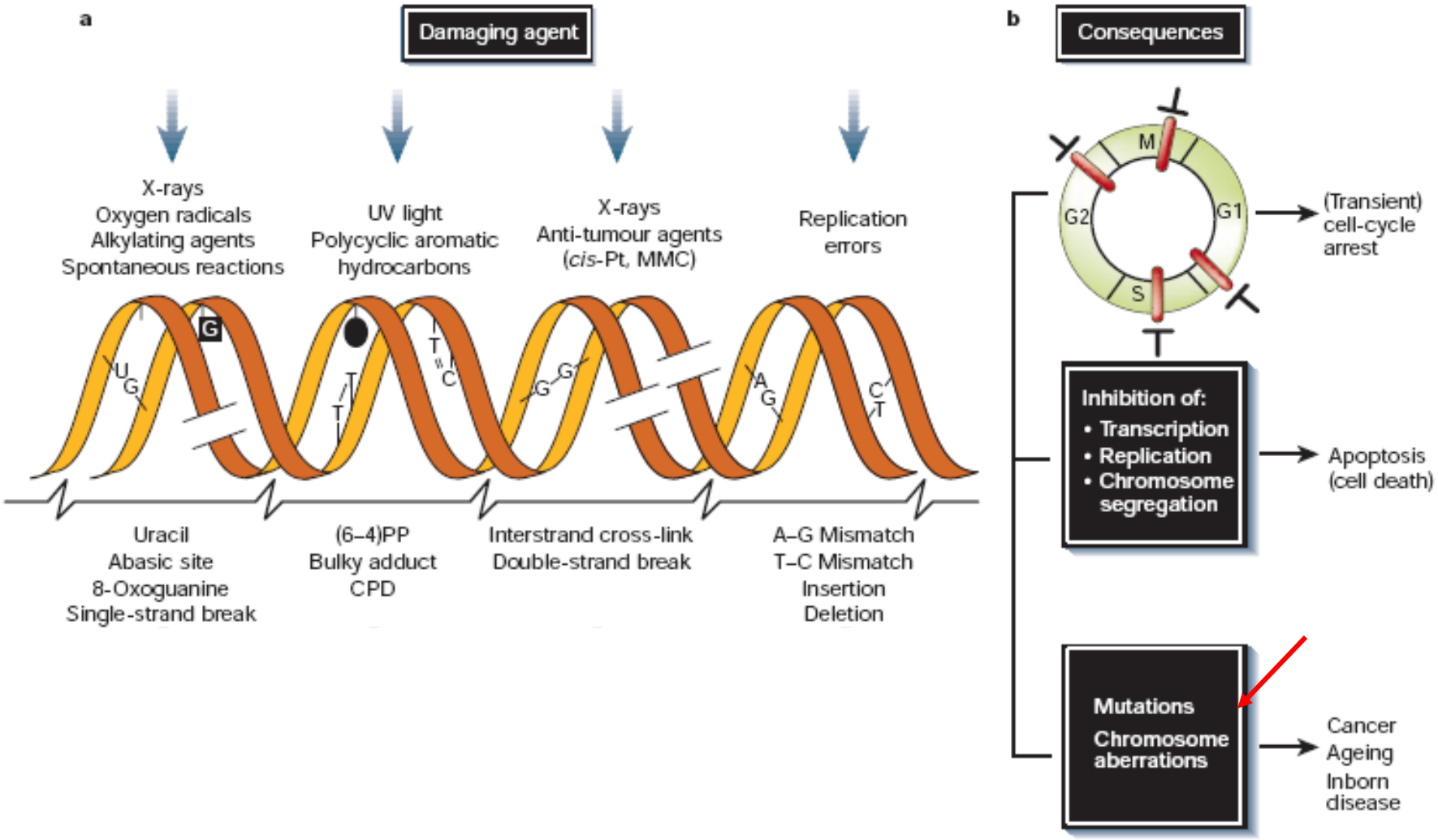
Challenges:

**Overcome primary and acquired resistance**

**Find suitable targets!**

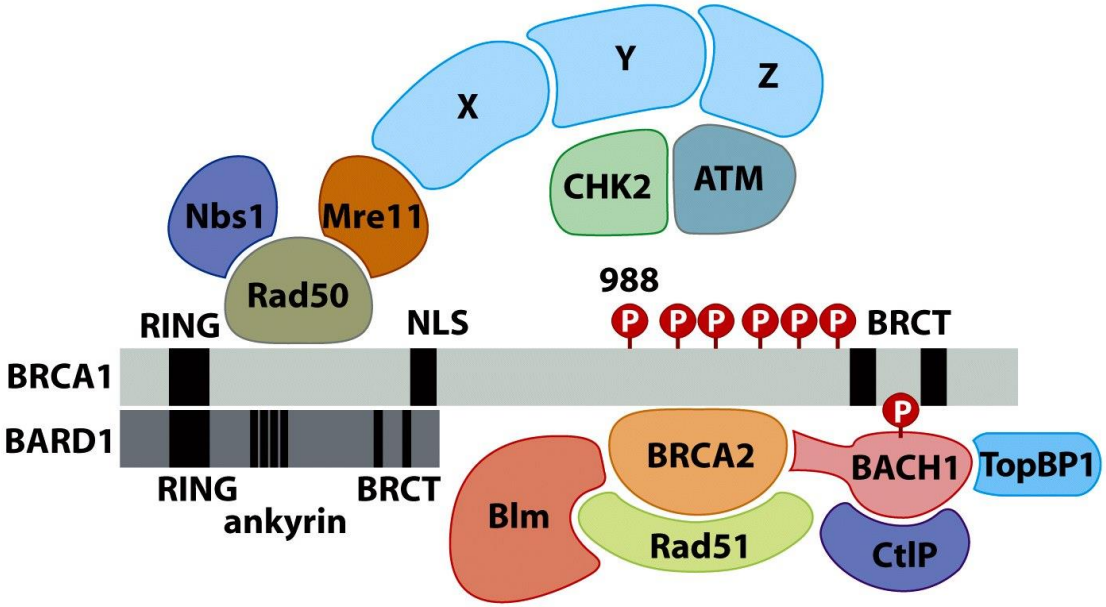
# Synthetic lethality

# DNA mutations and cancer (a reminder…)



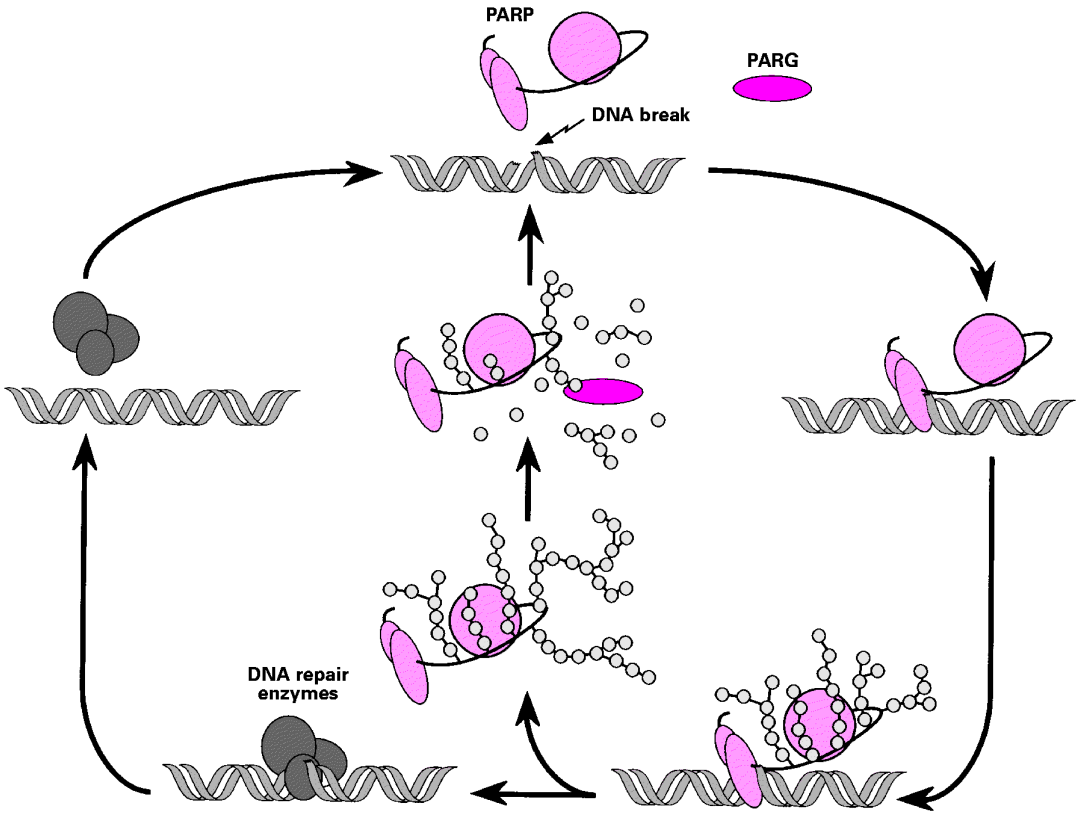

Hoeijmakers, Nature 411; 366, 2001

# Breast cancer susceptibility 1 (BRCA1)



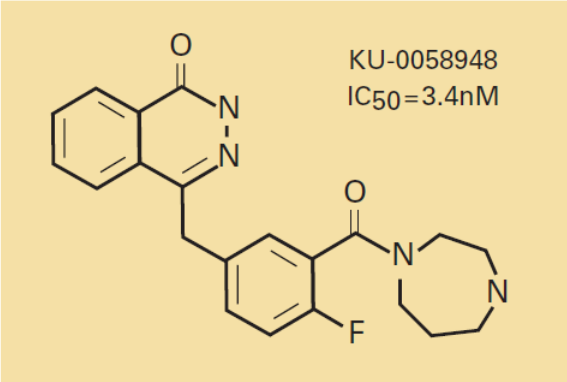

- BRCA1 is part of a large protein complex that binds DNA
- BRCA1 repairs double-strand DNA breaks by HDR
- **BRCA1 is frequently mutated in basal-like, triple-negative breast cancer**

# Poly(ADP-ribose) polymerase (PARP)



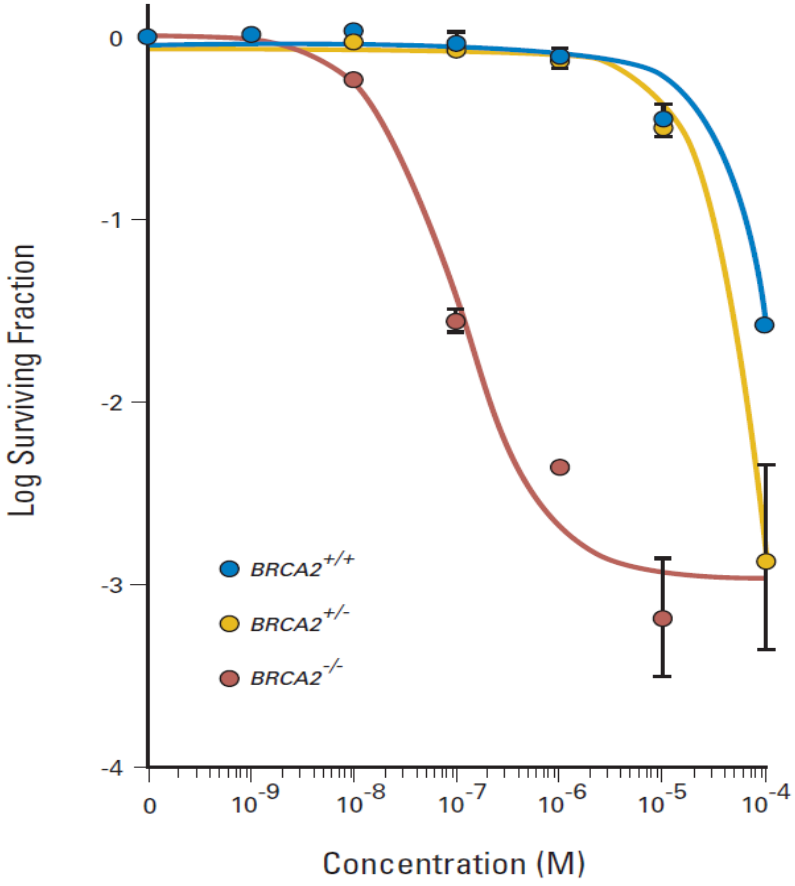

- PARP recognizes SSB proteins bound to single-strand DNA breaks
- It binds DNA and catalyzes the synthesis of poly-ADP-ribose chains
- Poly-ADP-ribose chains trigger the recruitment of DNA rapair enzymes

# Cells with mutant BRCA1 or 2 are dependent on PARP to avoid collapse



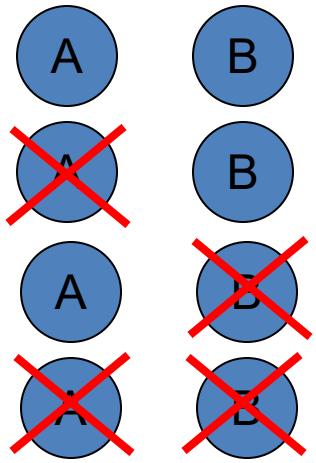

PARP inhibitor

Cancer cells may cope without BRCA1 or 2 (can still use NHEJ to fix double strand breaks), but cannot make it without both BRCA1/2 and PARP as too many DS breaks will occur → catastrophe




Ashworth, J Clin Oncol 26:3785, 2008

# The concept of synthetic lethality

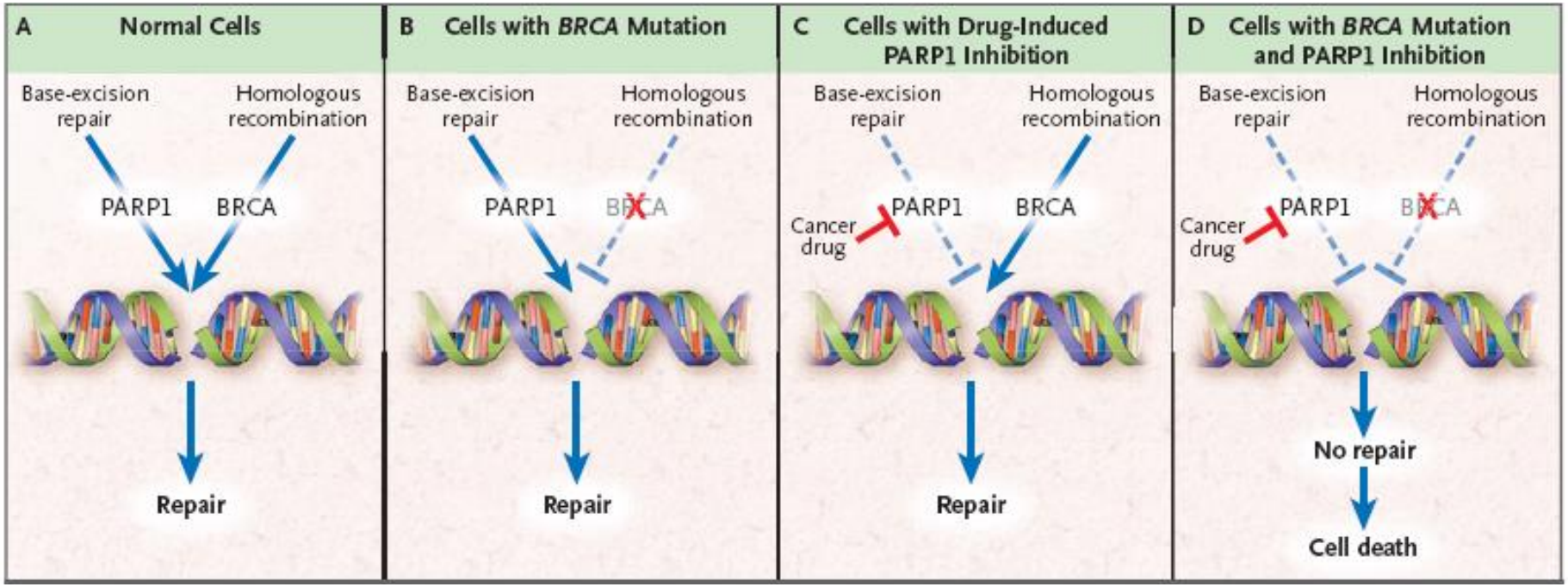



Normal function

B compensates for A deficiency

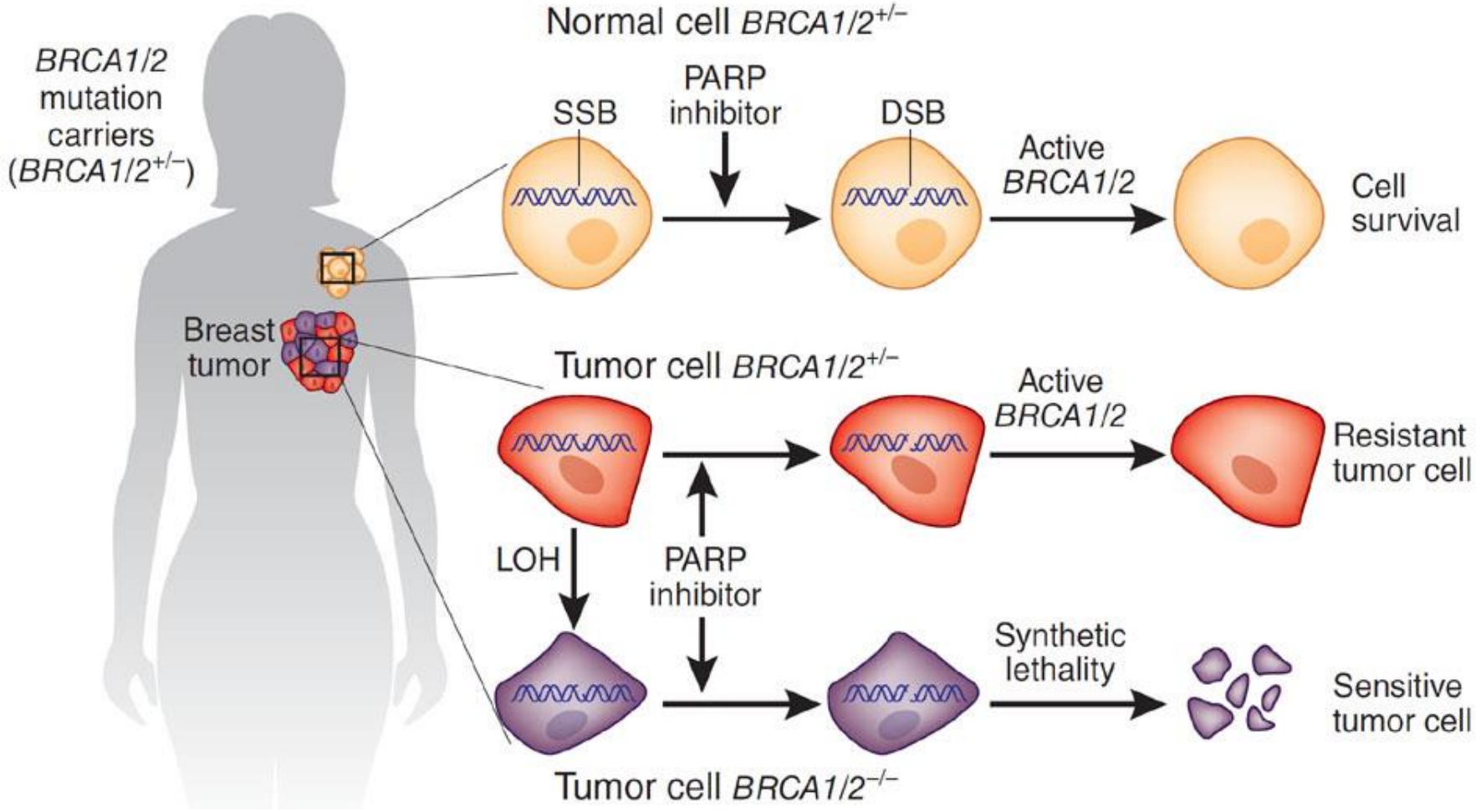
A compensates for B deficiency

Lethality



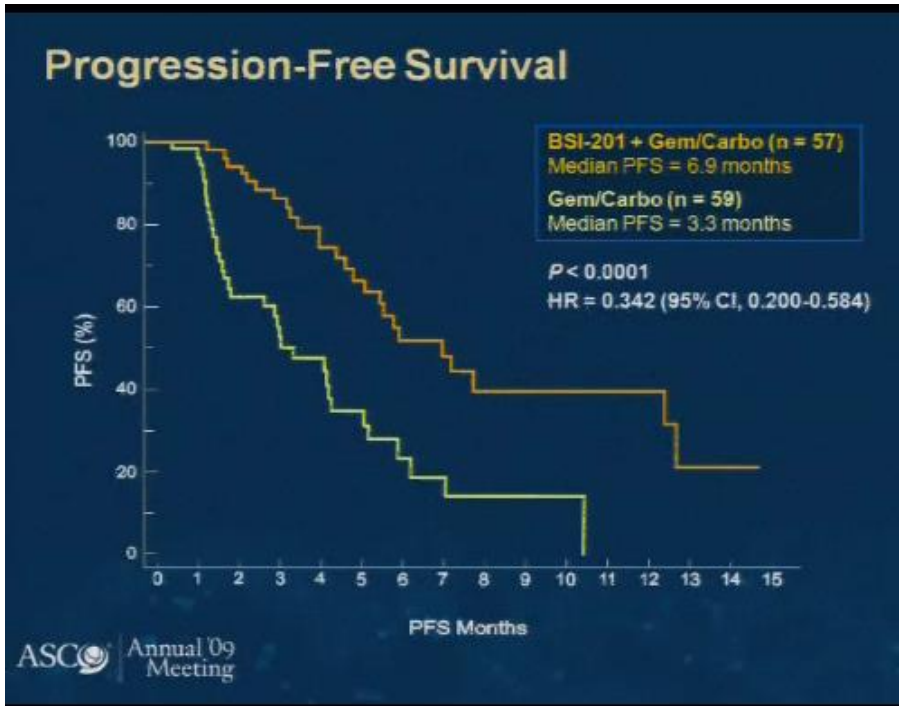

Iglehart, New Engl J Med 361;189, 2009

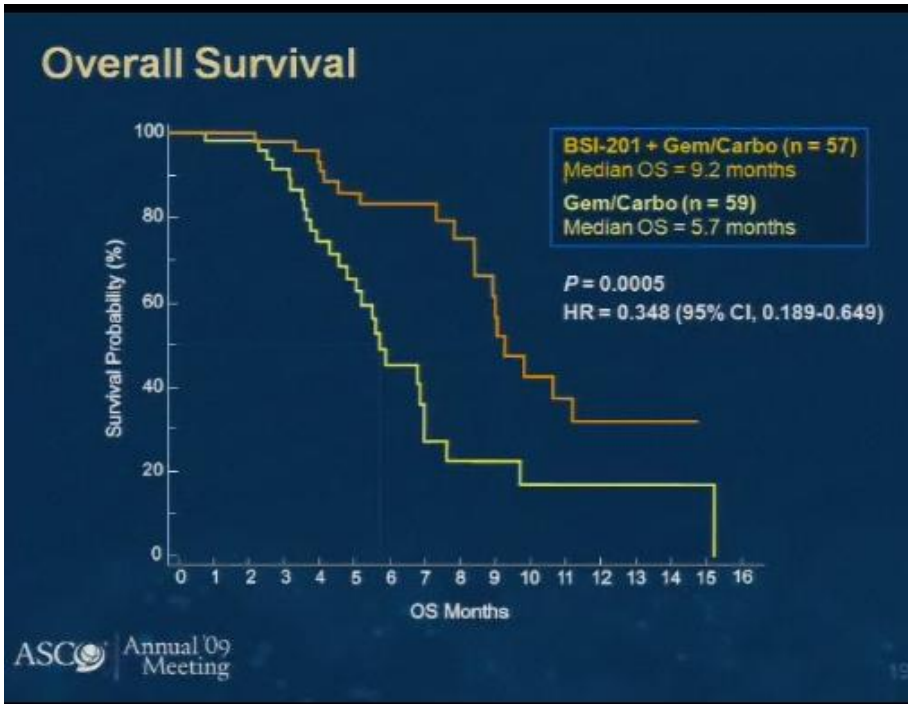
# The concept of synthetic lethality



# Basal-like tumors respond to PARP inhibitors

| | | Chemo | PARP inh + Chemo | *P*-value |
|---|---|---|---|---|
| Therapy | 1st line | 38/59 (64%) | 32/57 (56%) | |
| | 2nd line | 13/59 (22%) | 19/57 (33%) | |
| | 3rd line | 8/59 (14%) | 6/57 (11%) | |
| Objective Response Rate (%) | | 7/44 (16%) | 20/42 (48%) | 0.002 |
| Clinical Benefit Rate (%) | | 9/44 (21%) | 26 (62%) | 0.0002 |



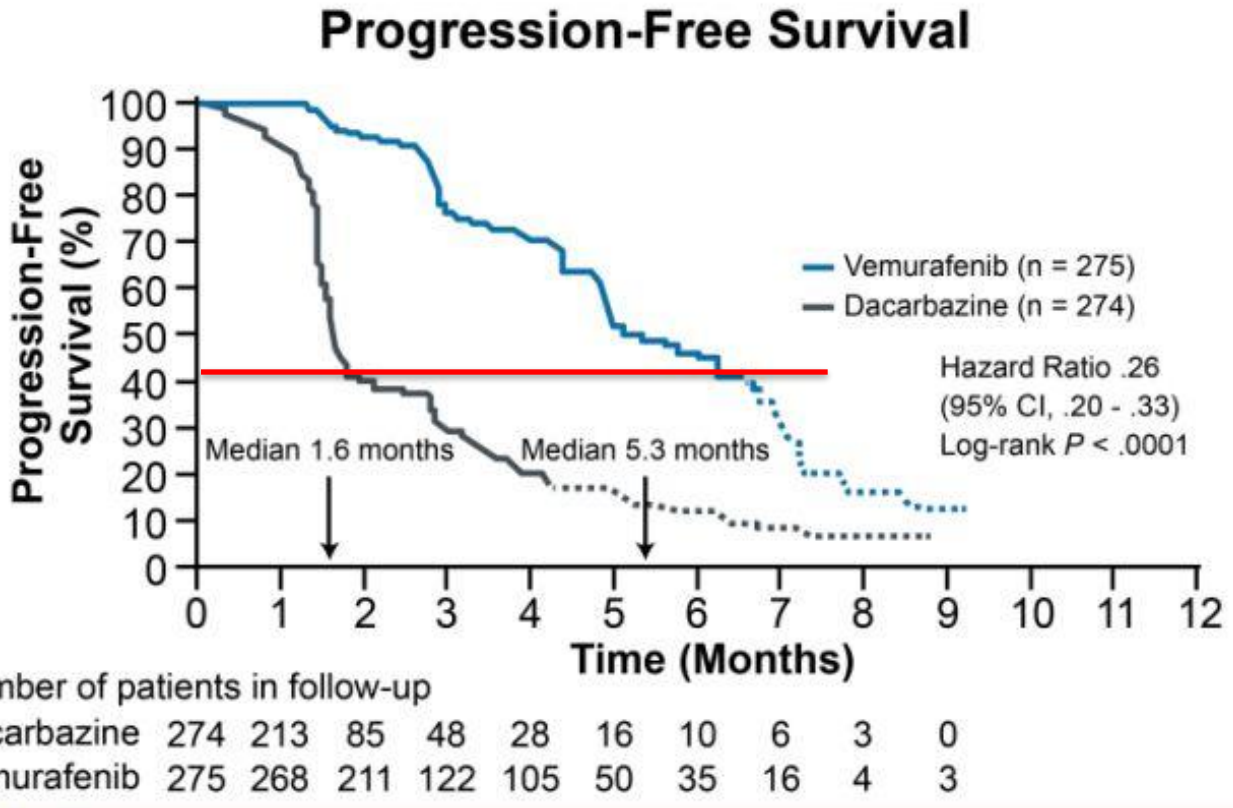
# Resistance to oncogene-targeted therapies

# Druggable BRAF mutations in melanoma

Almost 50% of metastatic melanomas harbor an activating mutation in BRAF (**BRAFV600**)

• These tumors, which are refractory to most available drugs, respond dramatically to the BRAF inhibitor, vemurafenib.

• This has been a revolution in melanoma care.



# Invariable emergence of adaptive resistance to targeted drugs

• Targeted drugs may achieve dramatic antitumor responses (partial to complete responses in most of the patients)

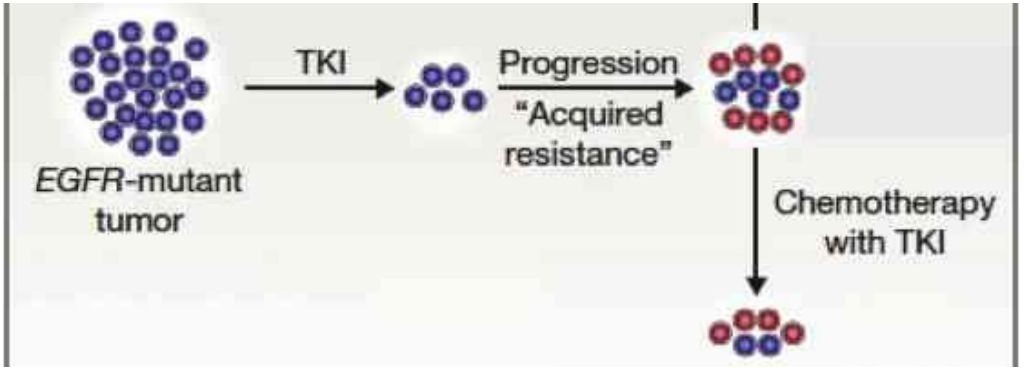
• However, secondary (adaptive) resistance occurs ALMOST INVARIABLY

• Depending on the driver mutation and tumor type, the response phase can be variably long

• Hematological tumors (e.g., CML) are less prone than solid cancers to rapidly develop adaptive resistance (lower mutational burden?)

• Secondary resistance generally occurs via POSITIVE SELECTION OF SUBCLONAL MUTATIONS IN THE TARGETED ONCOGENE, but also through other mutations in parallel signaling pathways

• The “new” mutations can be either **pre-existent** or, more rarely, *de novo* mutations. Both are expanded through Darwinian selection




Second/third generation RTK inhibitors (e.g., irreversible TK inhibitors) have been developed.

# Adaptive resistance to BRAF inhibition in melanoma

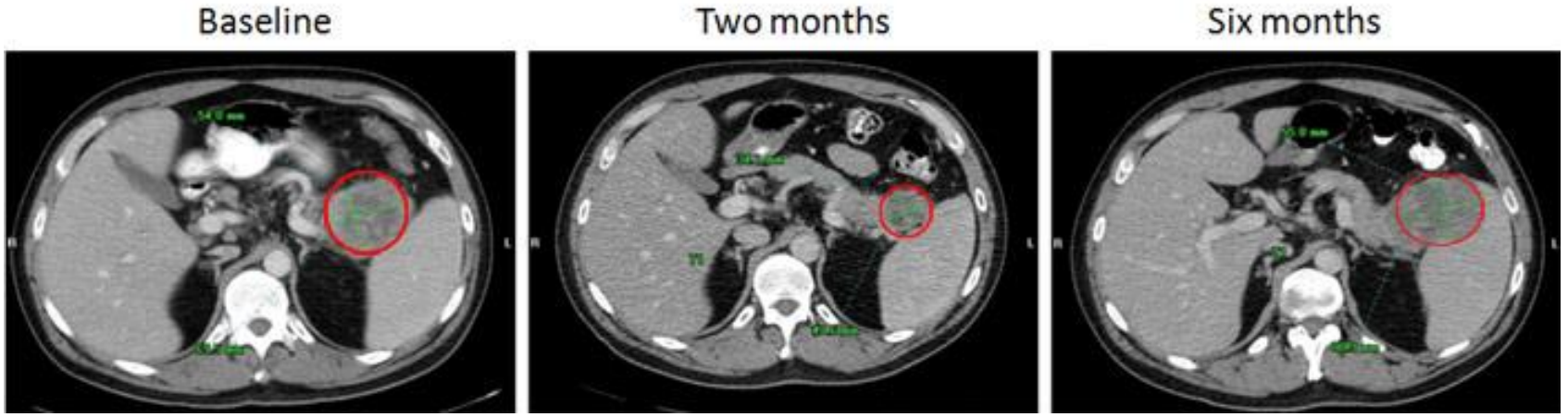
Almost 50% of metastatic melanomas harbor an activating mutation in BRAF (BRAFV600)

• These tumors, which are refractory to most available drugs, respond dramatically to the BRAF inhibitor, vemurafenib.

• This has been a revolution in melanoma care

• **However, acquired resistance occurs almost invariably after a dramatic yet transient response phase...**

• Resistance may be due to new mutations in BRAF, which make it insensitive to vemurafenib




CT scans of an abdominal mass (circled in red) in a patient with advanced melanoma. The cancer responded to vemurafenib but progressed after 6 months of therapy (NCI Bullettin Nov 2011; Image courtesy of Dr. Keith Flaherty, Massachusetts General Hospital)

# Adaptive resistance to BRAF inhibition in melanoma

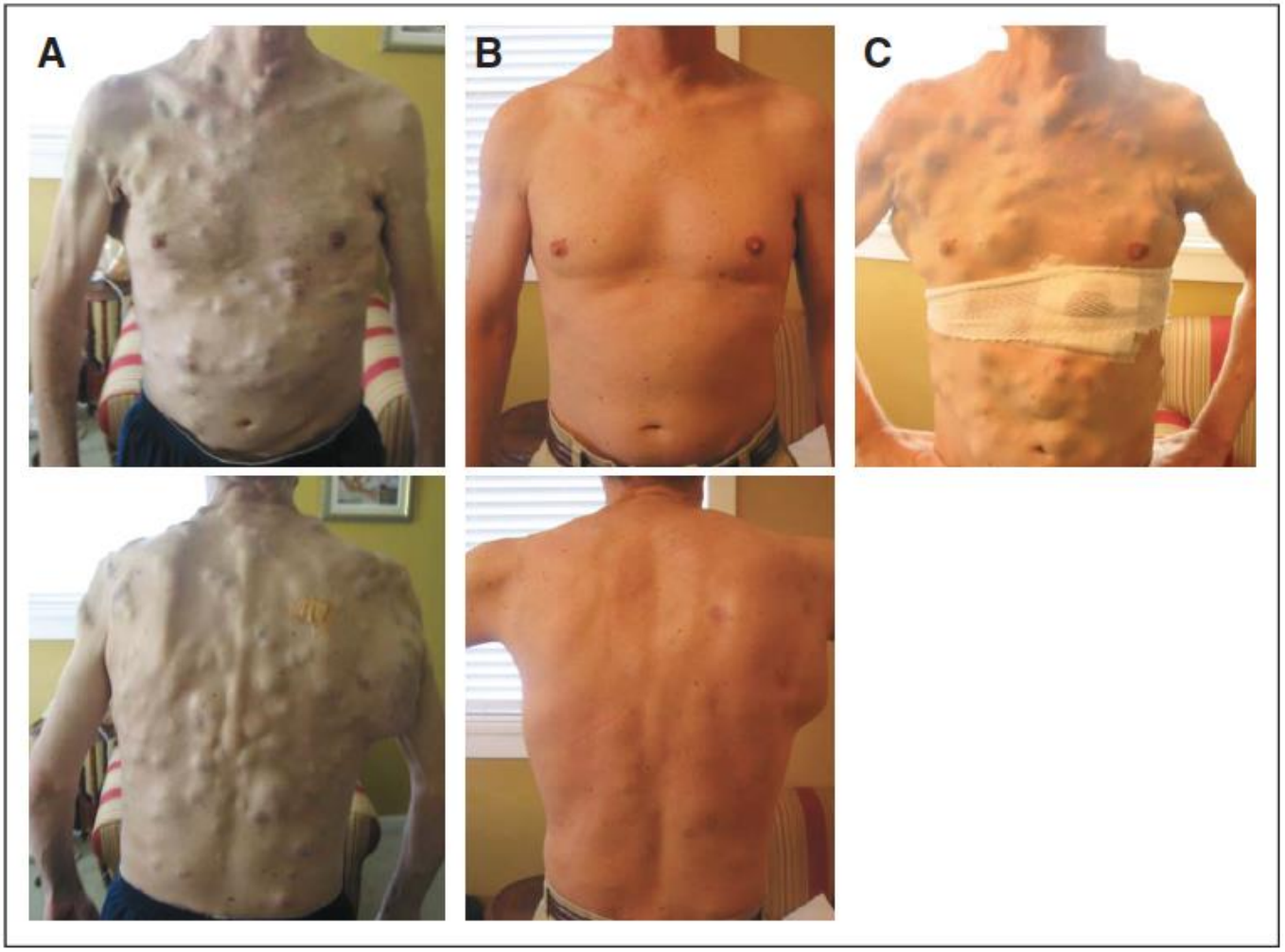
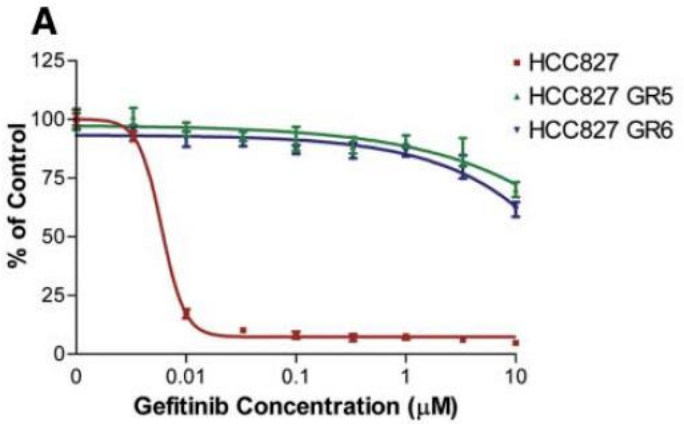
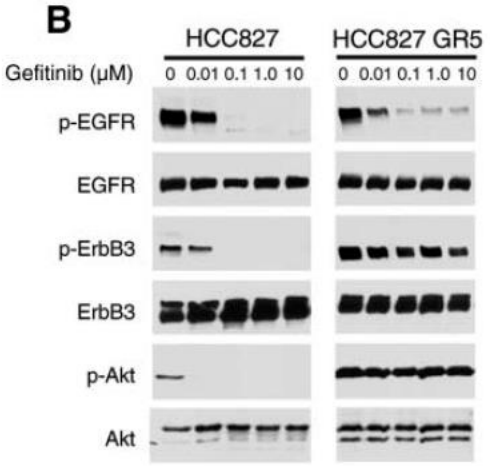


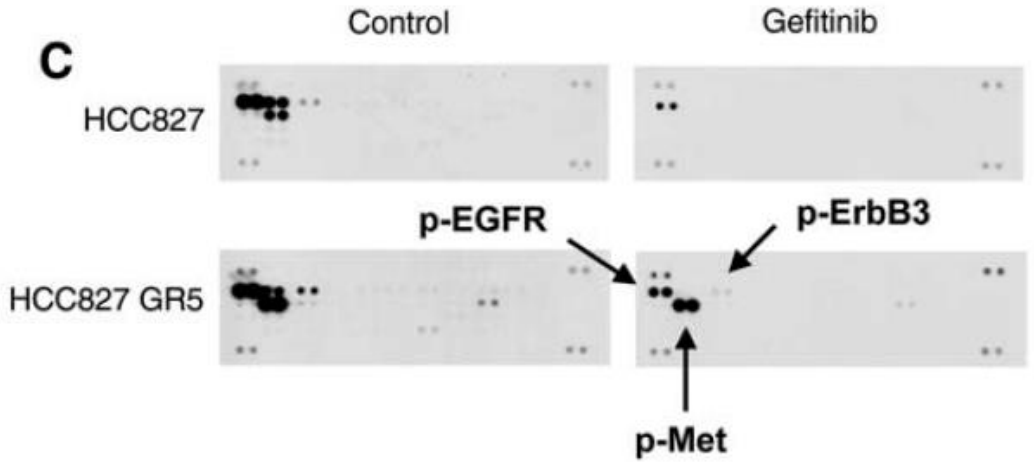

**Fig 2.** A 38-year-old man with *BRAF*-mutant melanoma and miliary, subcutaneous metastatic deposits. Photographs were taken (A) before initiation of PLX4032, (B) after 15 weeks of therapy with PLX4032, and (C) after relapse, after 23 weeks of therapy.

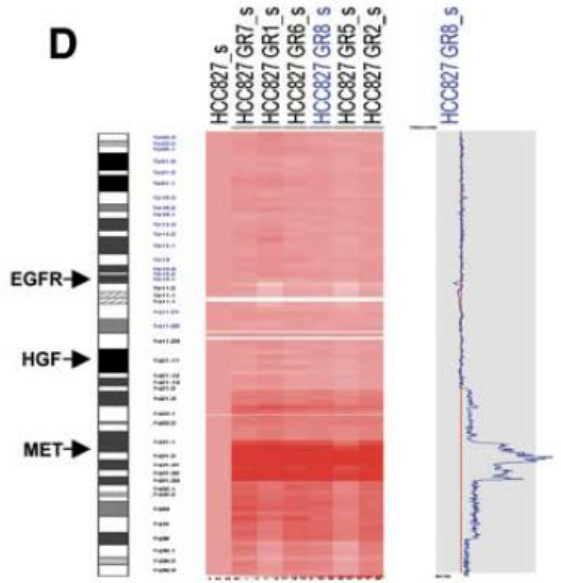
# MET amplification may drive adaptive resistance to EGFR inhibition in NSCLC




The GR5 and 6 clones were obtained by growing the EGFR mutant parental line in the presence of gefitinib.




Engelman et al., Science 2007