Molekylært målrettet medicinsk kræftbehandling for klinikere
– principper og metoder

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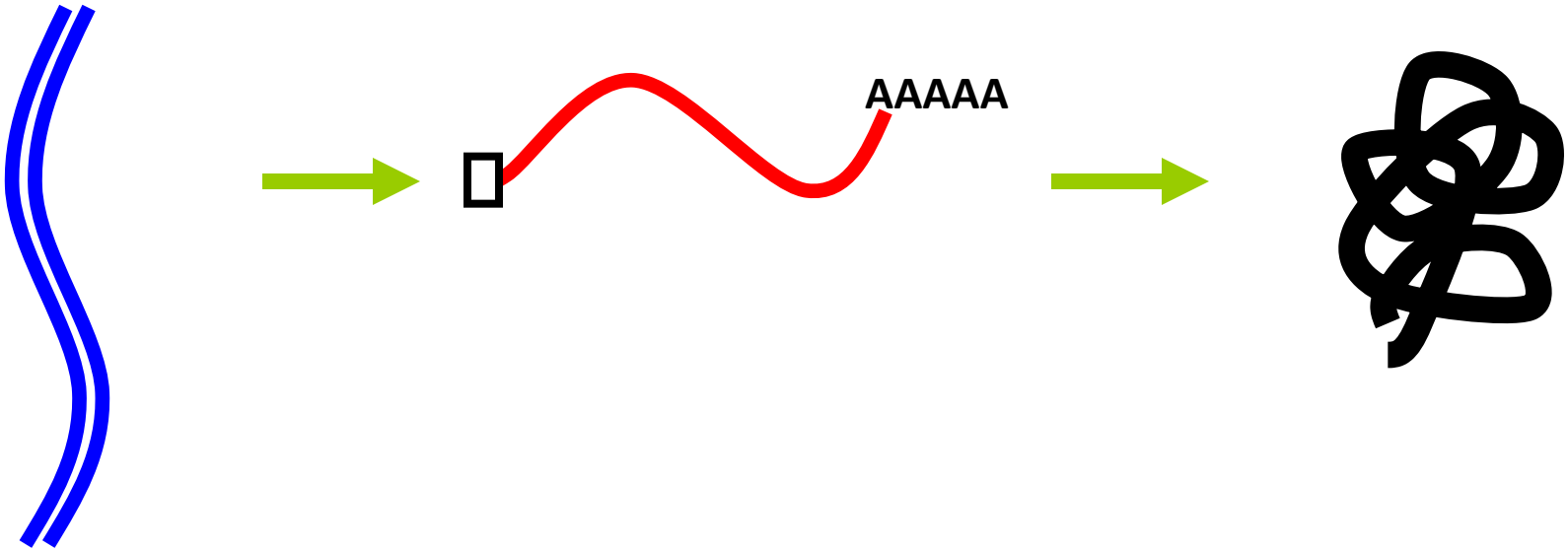
Department of Molecular Medicine (MOMA)
Aarhus University hospital

Outline

- The central dogma
- Regulation of transcription
- Cell signaling pathways
- Oncogenes and Tumor suppressor genes
- What is driving tumor progression?
- Tumor drivers (potential therapeutic targets)
- Methods
- Intra-tumor heterogeneity
- Circulating tumor DNA – the marker of the future?

Det centrale dogme

DNA transkriberes til **RNA** som translateres til **PROTEIN**



Det centrale dogme

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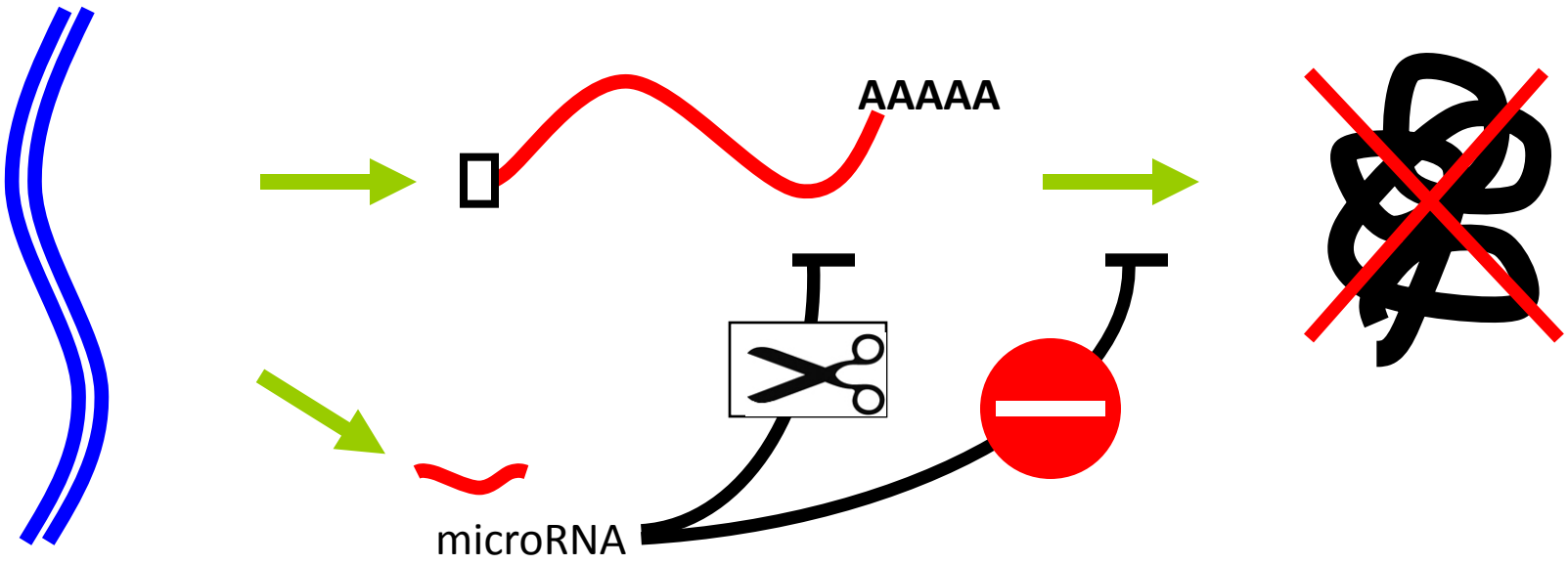
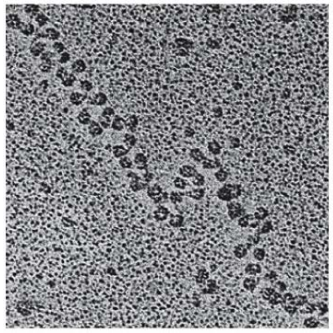




Pakning af DNA

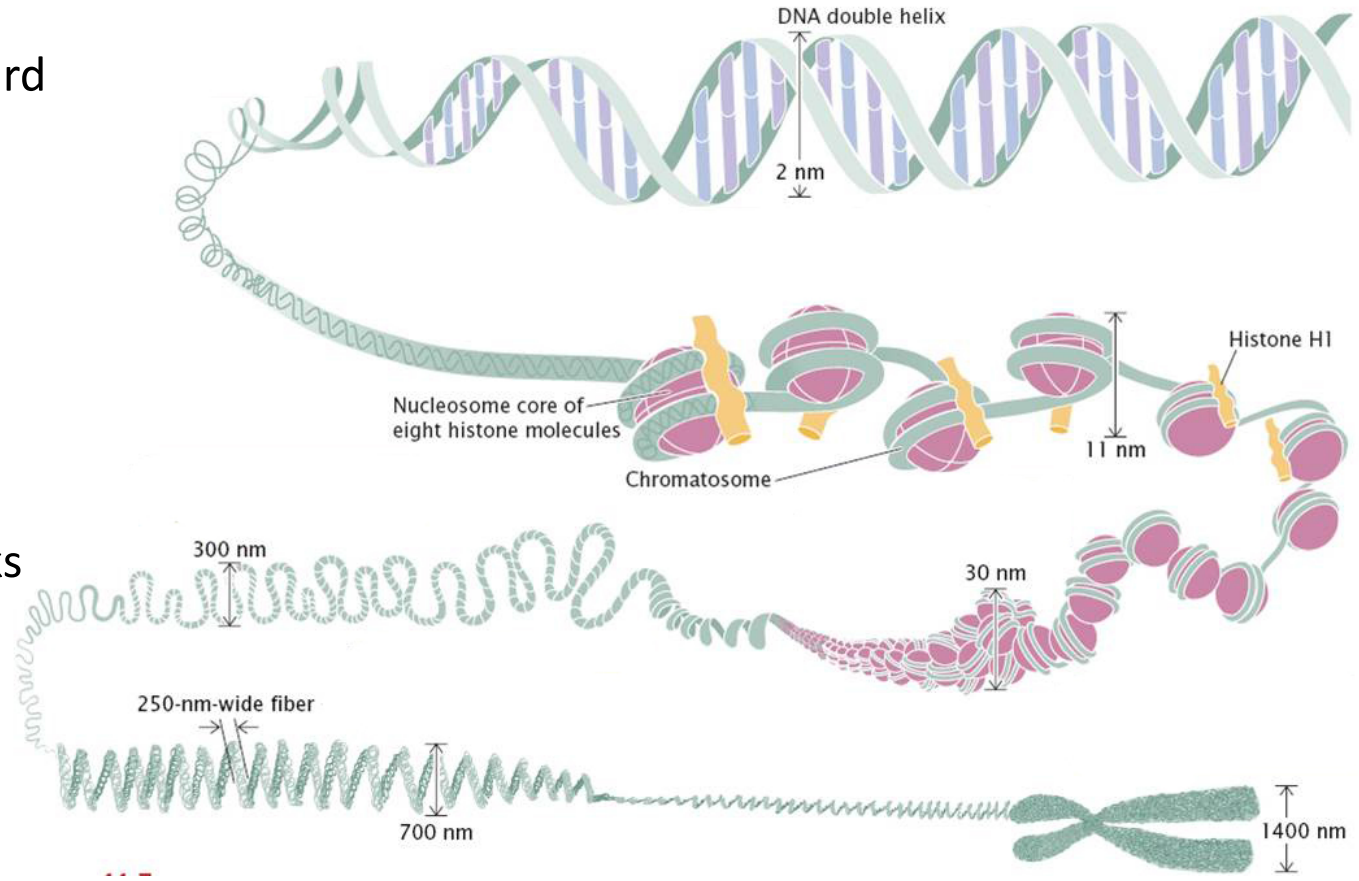
Mennesket: totalt 3mrd bp DNA pr. Genom → 2m DNA!

DNA bindes rundt om proteinkomplekser (**Histoner**)

DNA-protein kompleks = **Kromatin**

Kompakt DNA:

40.000 x kortere end 'udpakket' DNA!

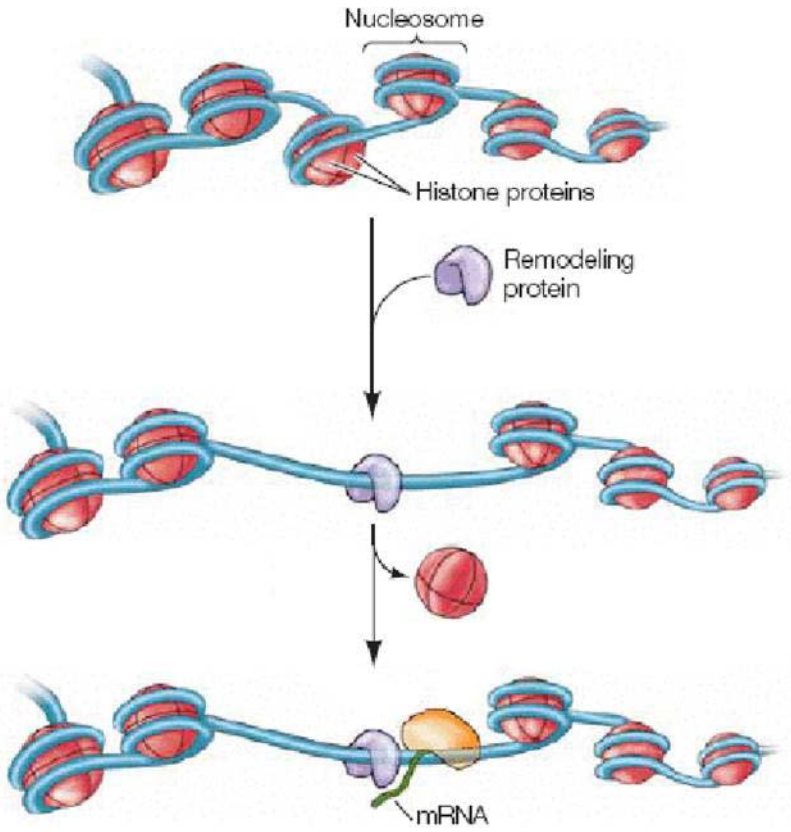


Regulering af transkription

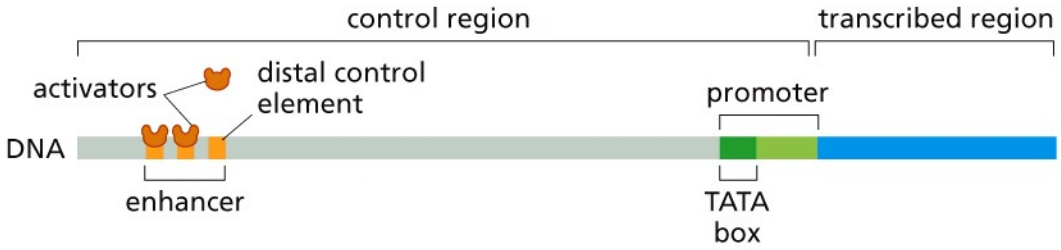
Transkription kræver lokal kromatin remodellering!

To hovedmekanismer gør at DNA sekvensen bliver tilgængelig for polymeraser og andre enzymer:

- Kromatin remodellerings-komplekser
- Enzymatisk modifikation af histoner



Regulation of transcription



Regulation of transcription

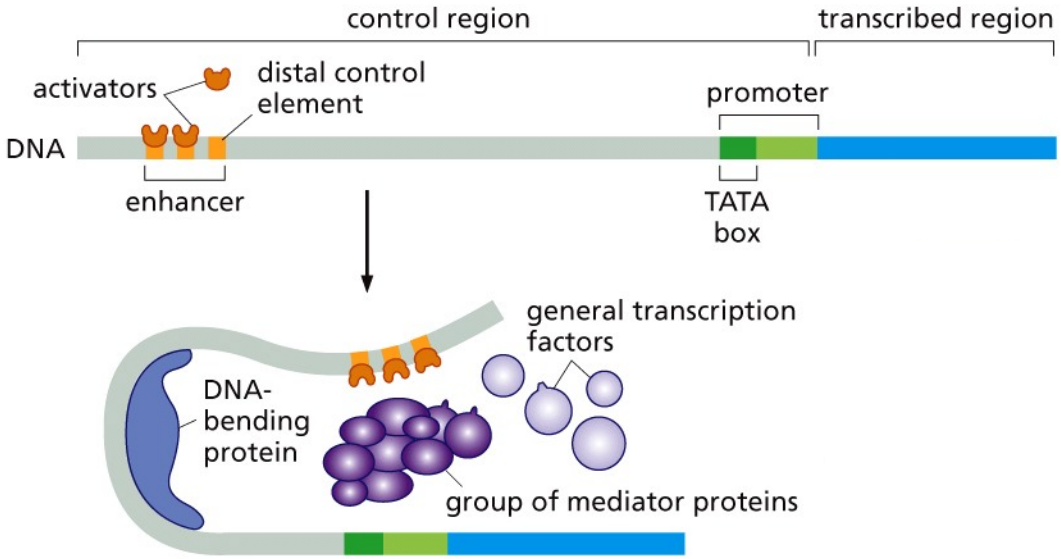


Figure 1.18 The Biology of Cancer (© Garland Science 2014)

Regulation of transcription

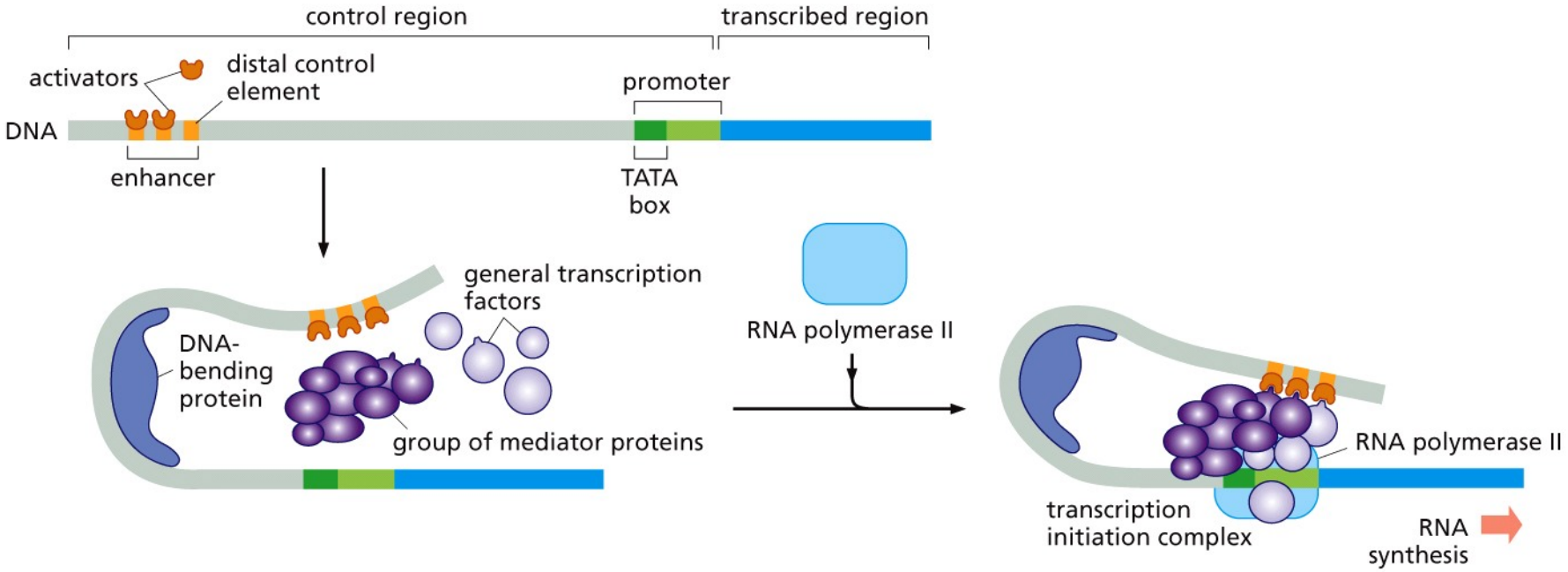


Figure 1.18 The Biology of Cancer (© Garland Science 2014)

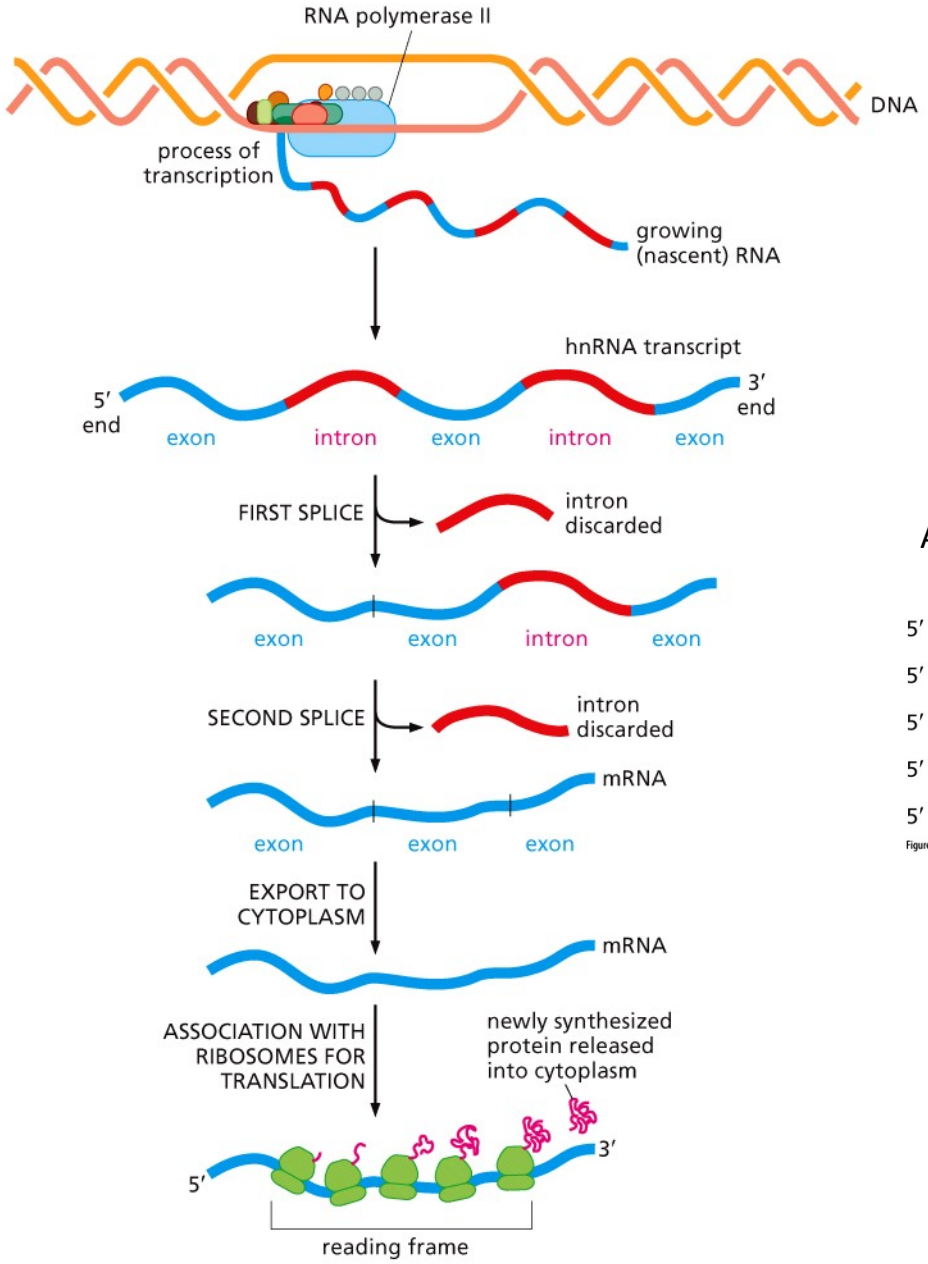


Figure 1.16a The Biology of Cancer (© Garland Science 2014)

Alternative splicing α-tropomysin pre-mRNA

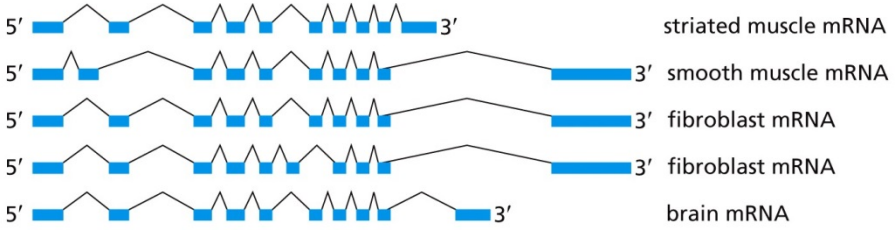


Figure 1.16b The Biology of Cancer (© Garland Science 2014)

Hvad regulerer en normal celles vækst og deling ?

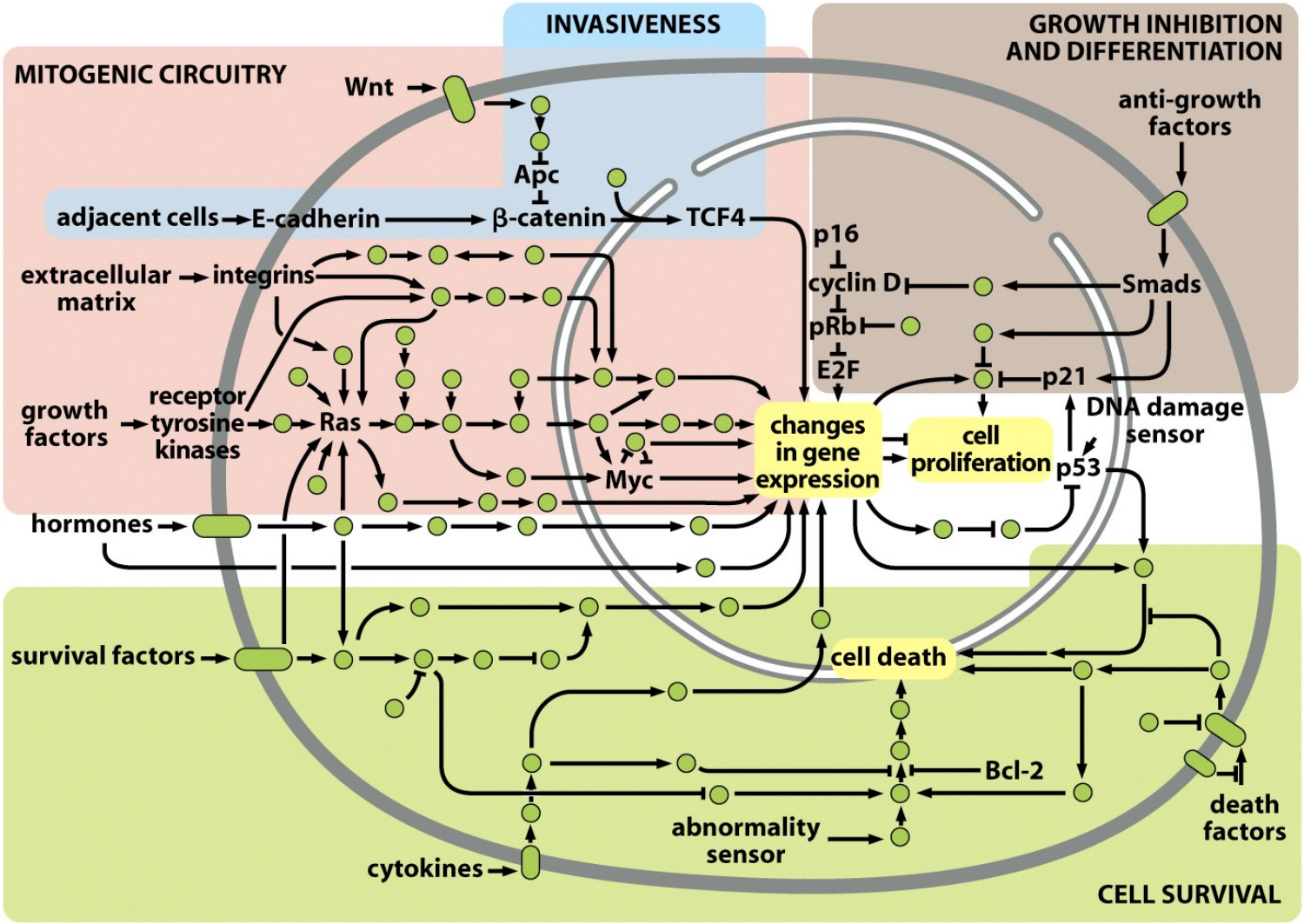
- Kommunikation med omverdenen

  Humane celler er i stand til udsende signaler som andre celler er i stand til at opfatte og respondere på.

  Vækst stimulerende signaler fra omgivelserne processeres i komplexe netværk cellen, som afgør om celle vækst og deling er den passende respons.

Hvad regulerer en normal celles vækst og deling ?

- Kommunikation med omverdenen



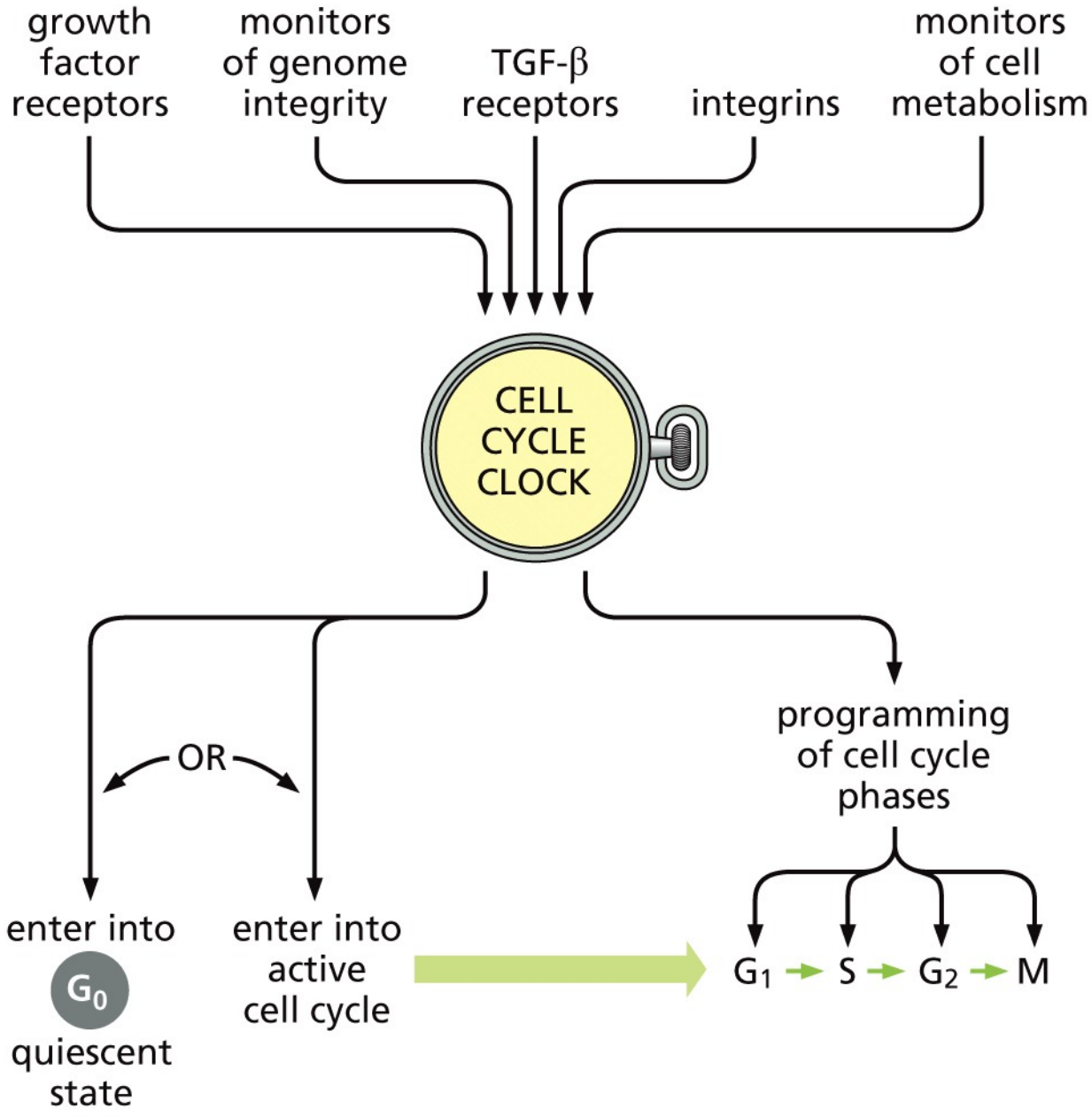
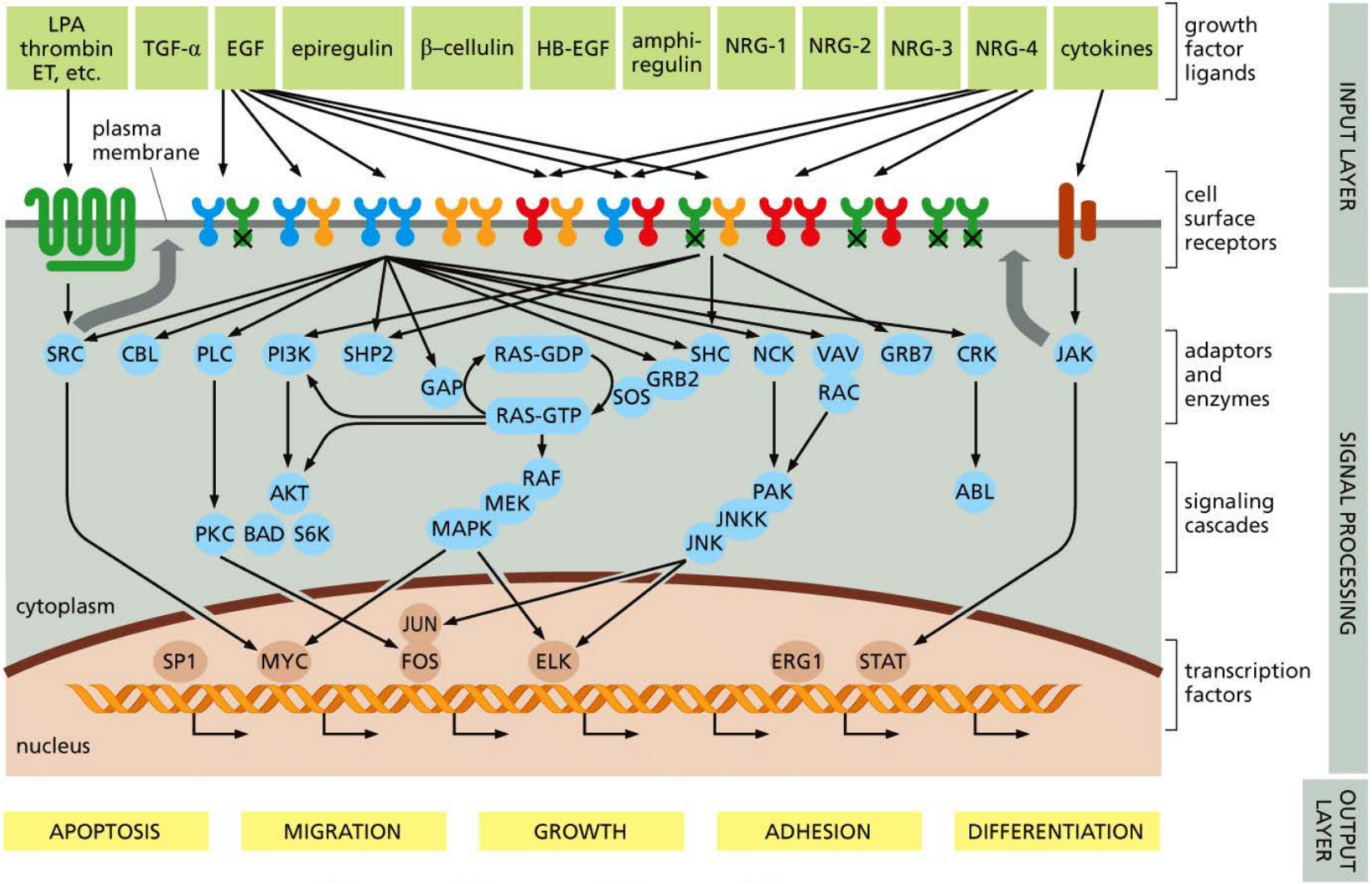


Figure 8.1 The Biology of Cancer (© Garland Science 2014)

The Human EGF receptor signaling network:



Resistance to therapy | **Invasion and metastasis** | **Tumor growth** | **Loss of contact inhibition** | **Stemness, resistance** | **Tumor Output**

An **oncogene** is a gene that has the potential to cause cancer.

In tumor cells, oncogenes are ACTIVATED.

Activated oncogenes can cause cells that ought to die to survive and proliferate instead.

Aproto-oncogene becomes an oncogene

There are three basic methods of activation:

1. A mutation within a proto-oncogene, or within a regulatory region, can cause
    - an increase in protein activity
    - a loss of regulation
2. An increase in the amount of protein, caused by
    - an increase of protein expression (through misregulation)
    - an increase of protein (mRNA) stability, prolonging its existence and thus its activity in the cell
    - gene duplication (one type of chromosome abnormality), resulting in an increased amount of protein in the cell

3) A chromosomal translocation may activate oncogenes (through misregulation)

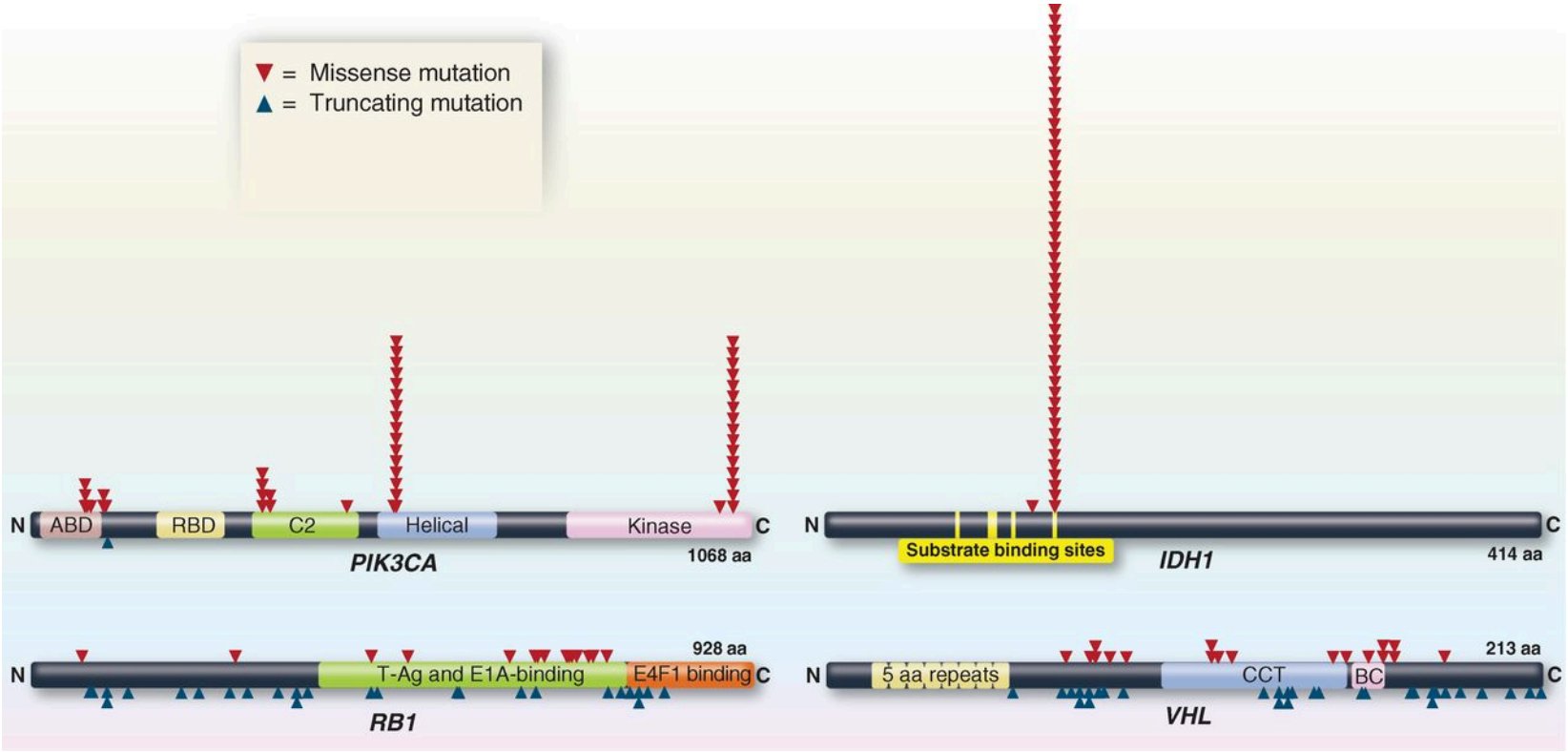
Tumor-suppressor genes, either have a dampening or repressive effect on the regulation of the cell cycle or promote apoptosis, and sometimes do both.

Loss or reduction of the function of a tumor suppressor, enables the cell to progress towards cancer, usually in combination with other genetic changes.

The functions of tumor-suppressor proteins fall into several categories including the following:

1. Repression of genes that are essential for the continuing of the cell cycle.
2. Proteins that couple the cell cycle to DNA damage.
3. If the damage cannot be repaired, the cell should initiate apoptosis (programmed cell death) to remove the threat it poses for the greater good of the organism.
4. Cell adhesion molecules that prevent tumor cells from dispersing, enforce contact inhibition, and inhibit metastasis.
5. DNA repair proteins are classified as tumor suppressors, as mutations in their genes increase the risk of cancer.

Distribution of mutations in: **two oncogenes (PIK3CA and IDH1)**
two tumor suppressor genes (RB1 and VHL).



B Vogelstein et al. Science 2013;339:1546-1558

Science
AAAS

What drives tumor-progression ?

- Genetic instability

- Carcinogens
  - Mutagenic agents (genotoxic)
    - Tumor initiators
  - Non-mutagenic (nongenotoxic)
    - Tumor promoters – both toxic and mitogenic agents

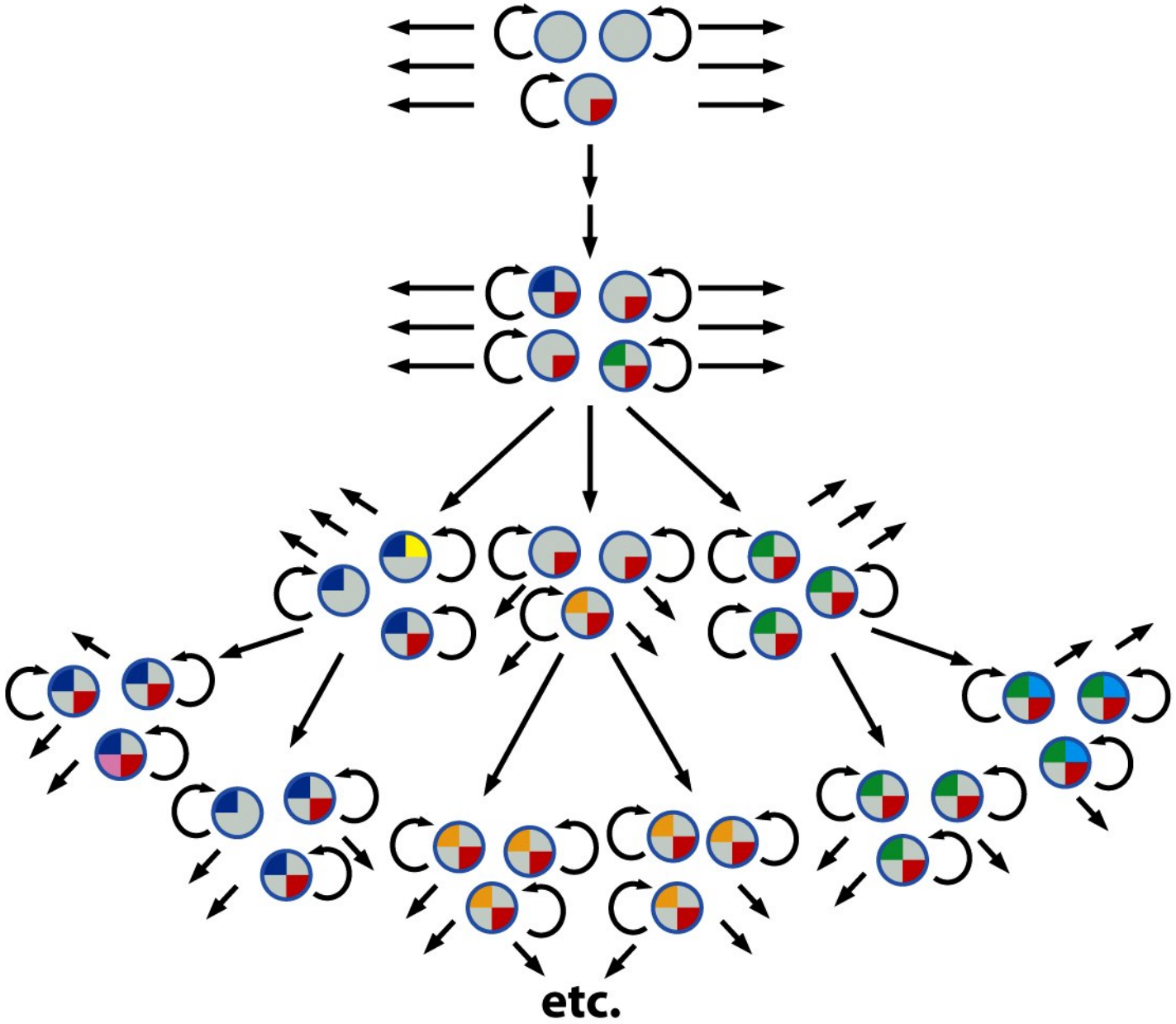


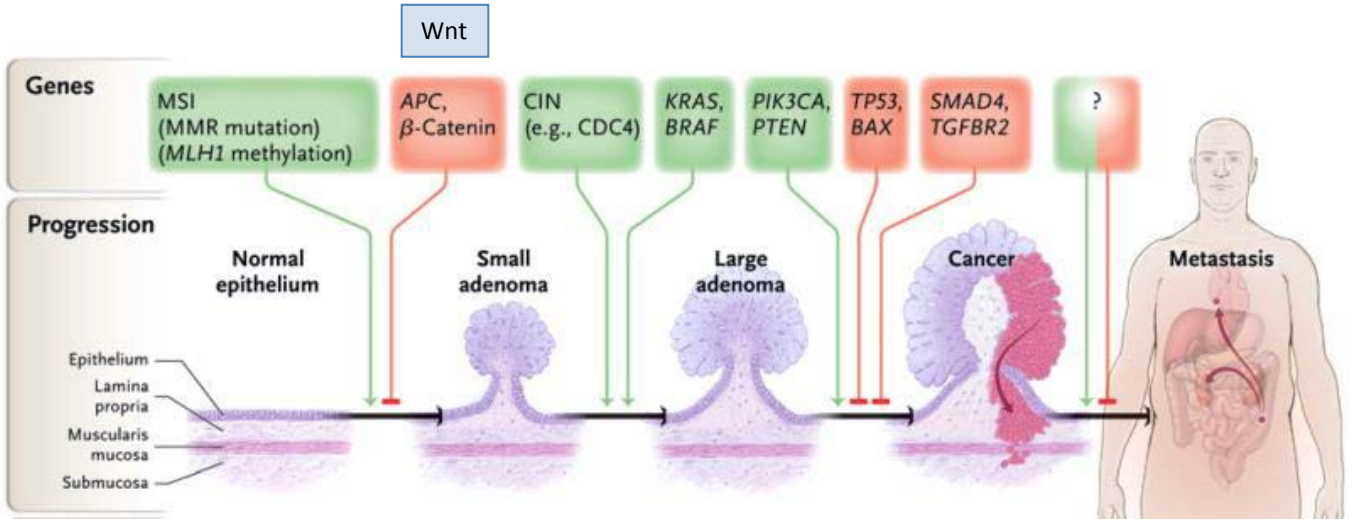
Figure 11.18 *The Biology of Cancer* (© Garland Science 2007)

Consequence of multiple successions of clonal expansion

- Tumors are genetically heterogeneous

- Notice:
  1. Tumor genomes become increasingly unstable as progression advances
  2. The rate of genetic change increases as tumor progression advances
  3. Outpace Darwinian selection

Genetic alterations accumulate during the adenoma to carcinoma sequence



From Markowitz et al 2009 (NEJM. 361; 25)

Multiple paths to colorectal cancer

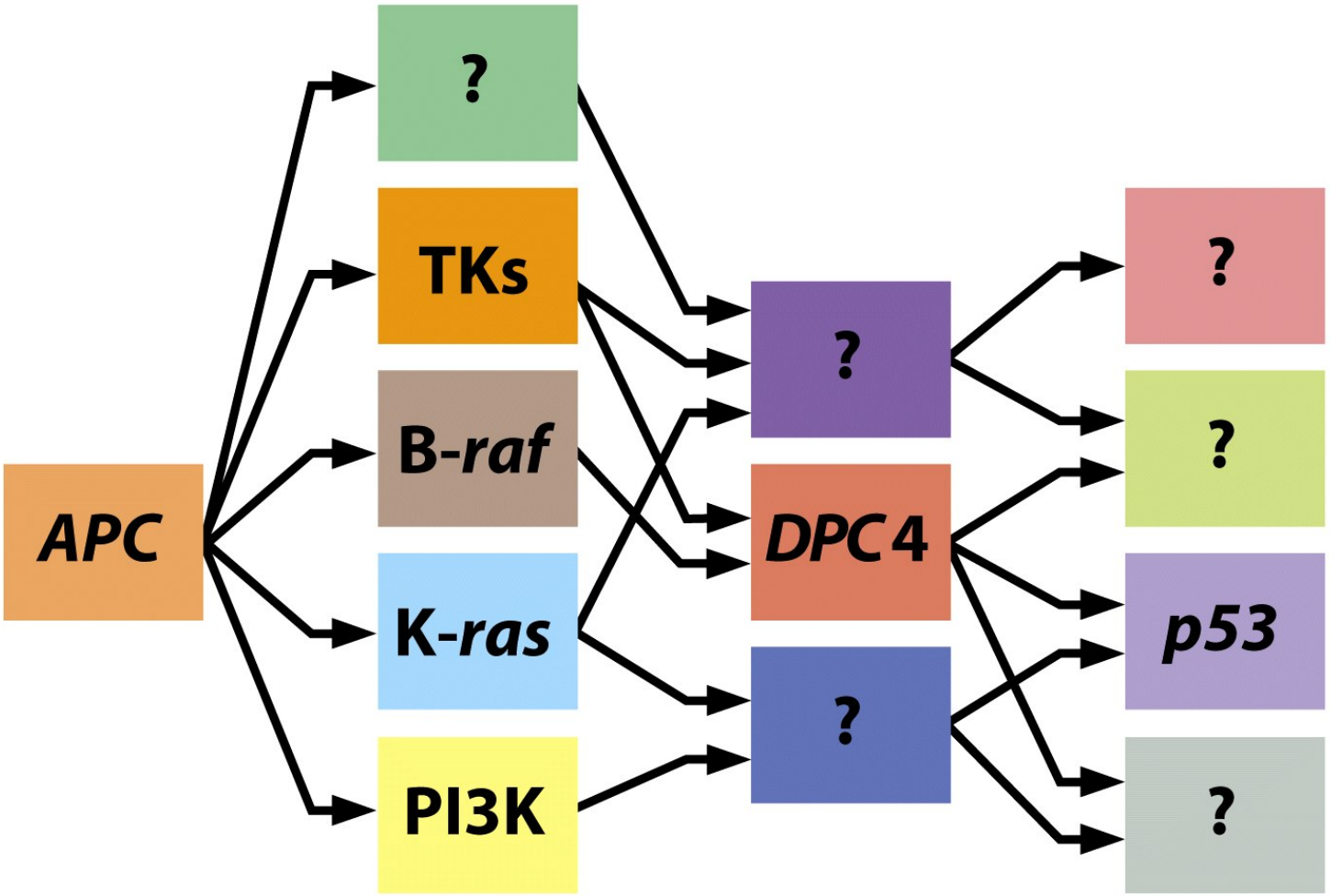


Figure 11.11a *The Biology of Cancer* (© Garland Science 2007)

Tumor progression
En kamp mellem tumor drivere og tumor barrierer

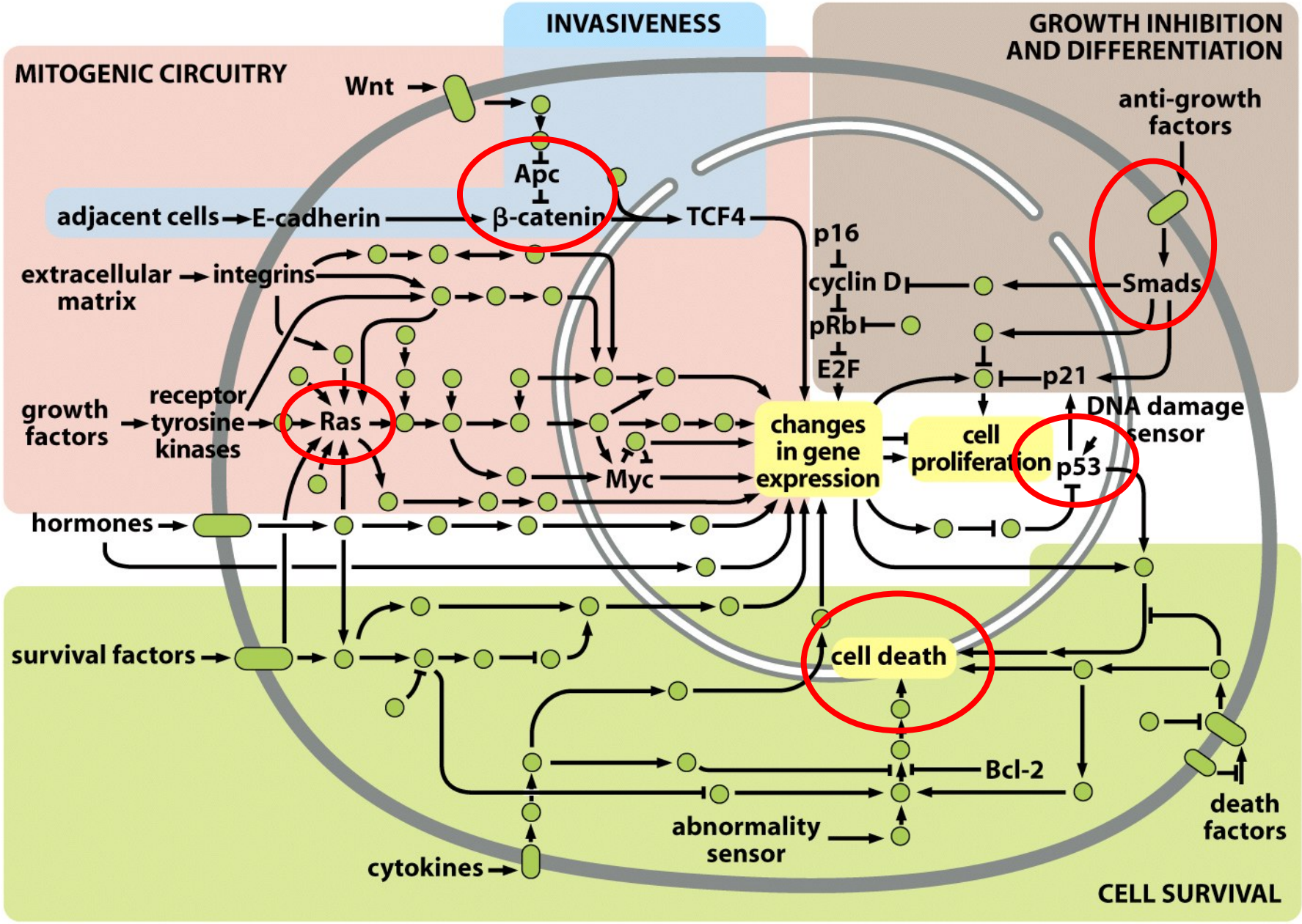
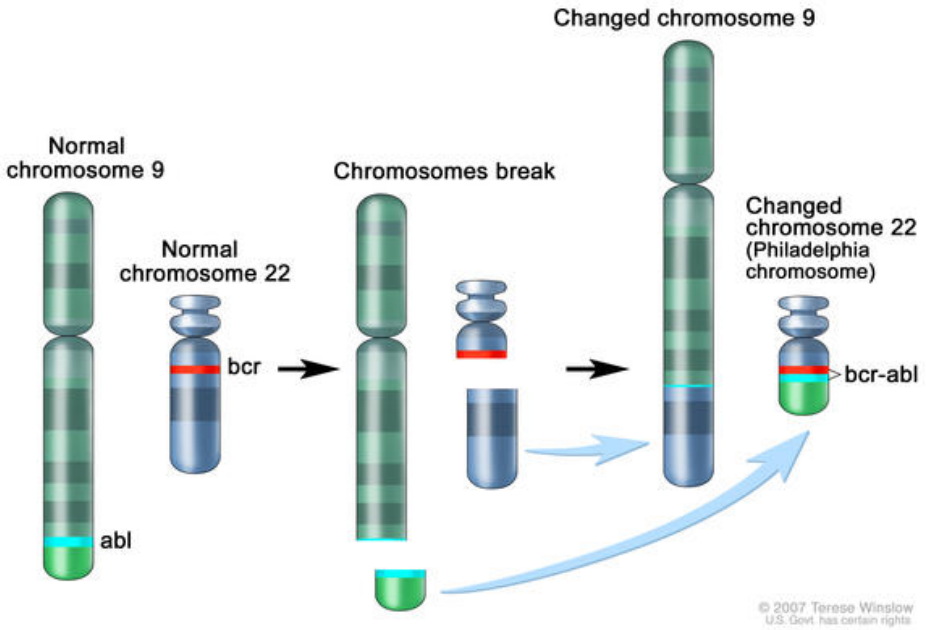


Figure 11.44 *The Biology of Cancer* (© Garland Science 2007)

Examples

Activation of tumor drivers

Formation of the bcr-abl fusion oncogene of Chronic Myelogenous Leukemia



Detection by FISH

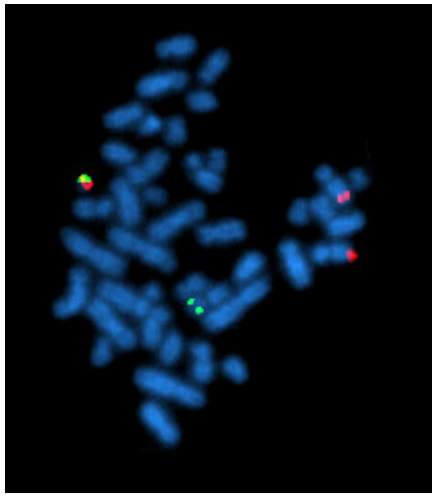


Table 4.5 Translocations in human tumors that cause the formation of oncogenic fusion proteins of novel structure and function

Oncogene	Neoplasm
bcr/abl	chronic myelogenous leukemia; acute lymphocytic leukemia
dek/can	acute myeloid leukemia
E2A/pbx1	acute pre-B-cell leukemia
PML/RAR	acute promyelocytic leukemia
tls/erg	myeloid leukemia
irel/urg	B-cell lymphoma
CBFβ/MYH11	acute myeloid leukemia
aml1/mtg8	acute myeloid leukemia
ews/fli	Ewing's sarcoma
lyt-10/Cα1	B-cell lymphoma
hrx/enl	acute leukemias
hrx/af4	acute leukemias
NPM/ALK	large-cell lymphomas
PAX3/FKHR	alveolar rhabdomyosarcoma
EML4/ALK	non-small-cell lung cancer
MLL/various	acute leukemias

Adapted from G.M. Cooper, Oncogenes, 2nd ed. Sudbury, MA: Jones and Bartlett, 1995. www.jbpub.com. Reprinted with permission.

Table 4.5 The Biology of Cancer (© Garland Science 2014)

Burkitt's lymphoma

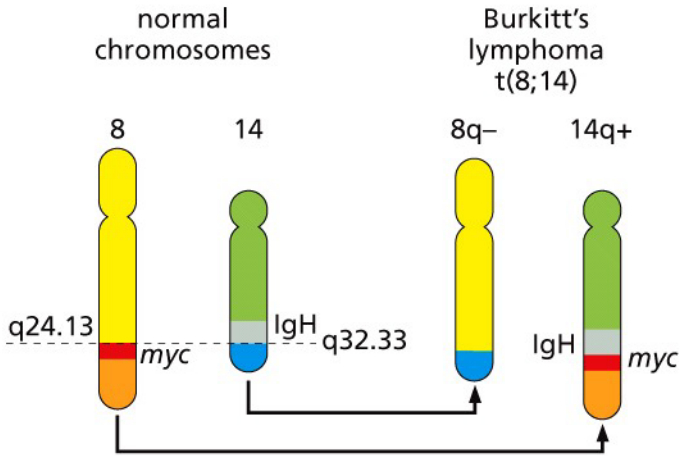


Figure 4.13 The Biology of Cancer (© Garland Science 2014)

Burkitt's lymphoma

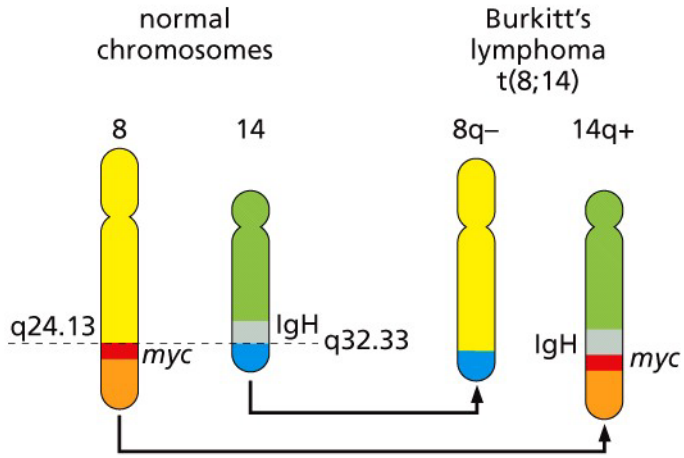
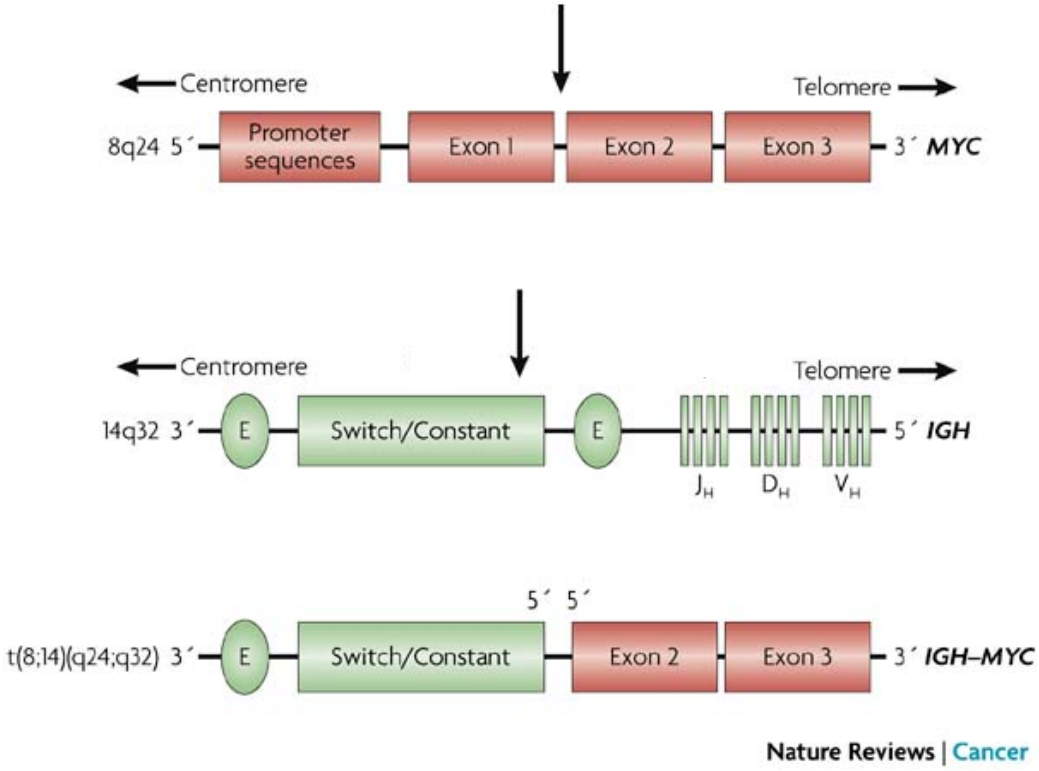


Figure 4.13 The Biology of Cancer (© Garland Science 2014)



Nature Reviews | Cancer

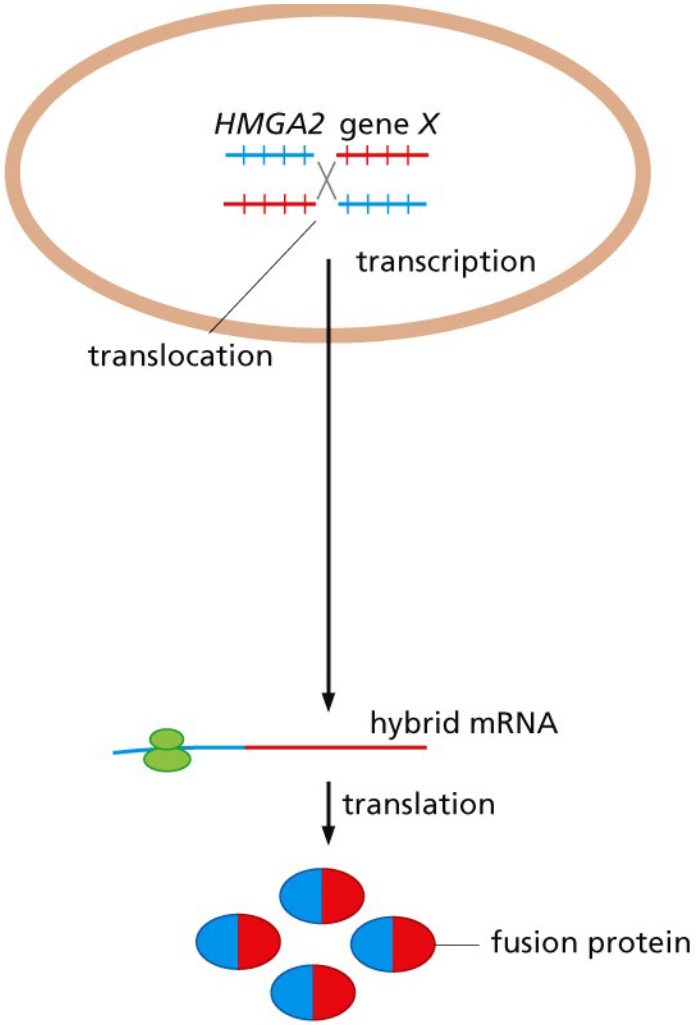


Figure 4.14 The Biology of Cancer (© Garland Science 2014)

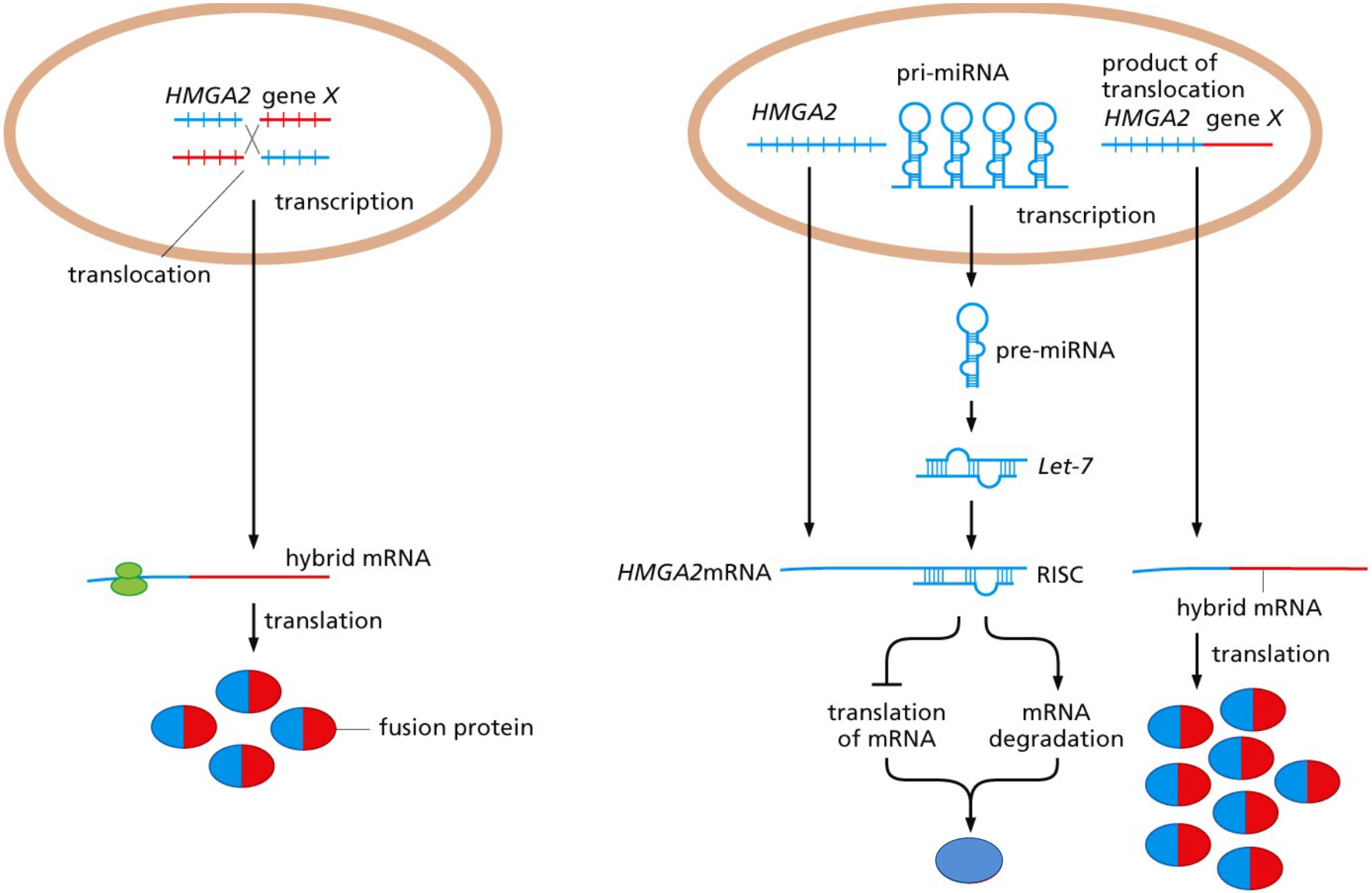


Figure 4.14 The Biology of Cancer (© Garland Science 2014)

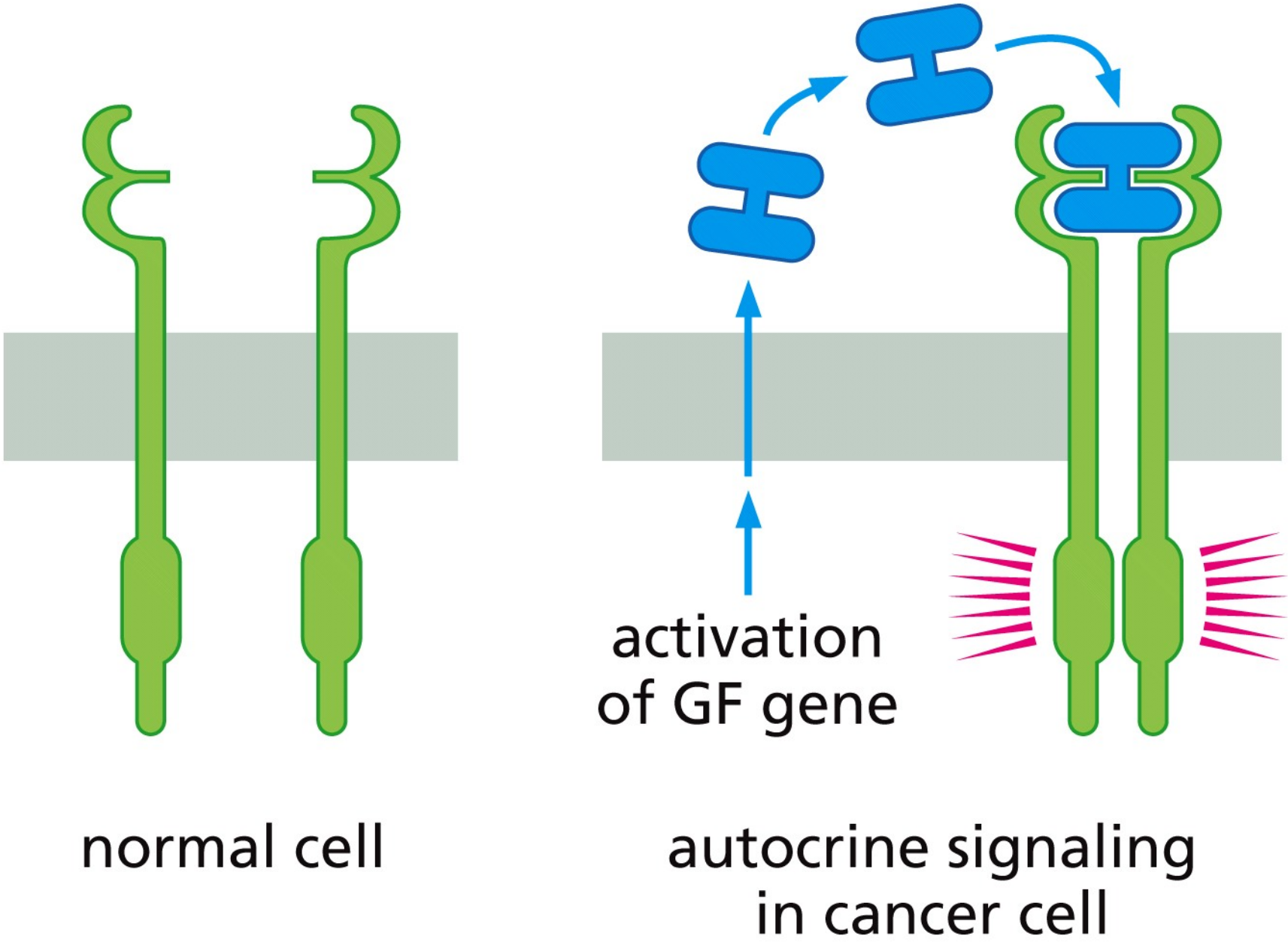


Figure 5.11c The Biology of Cancer (© Garland Science 2014)

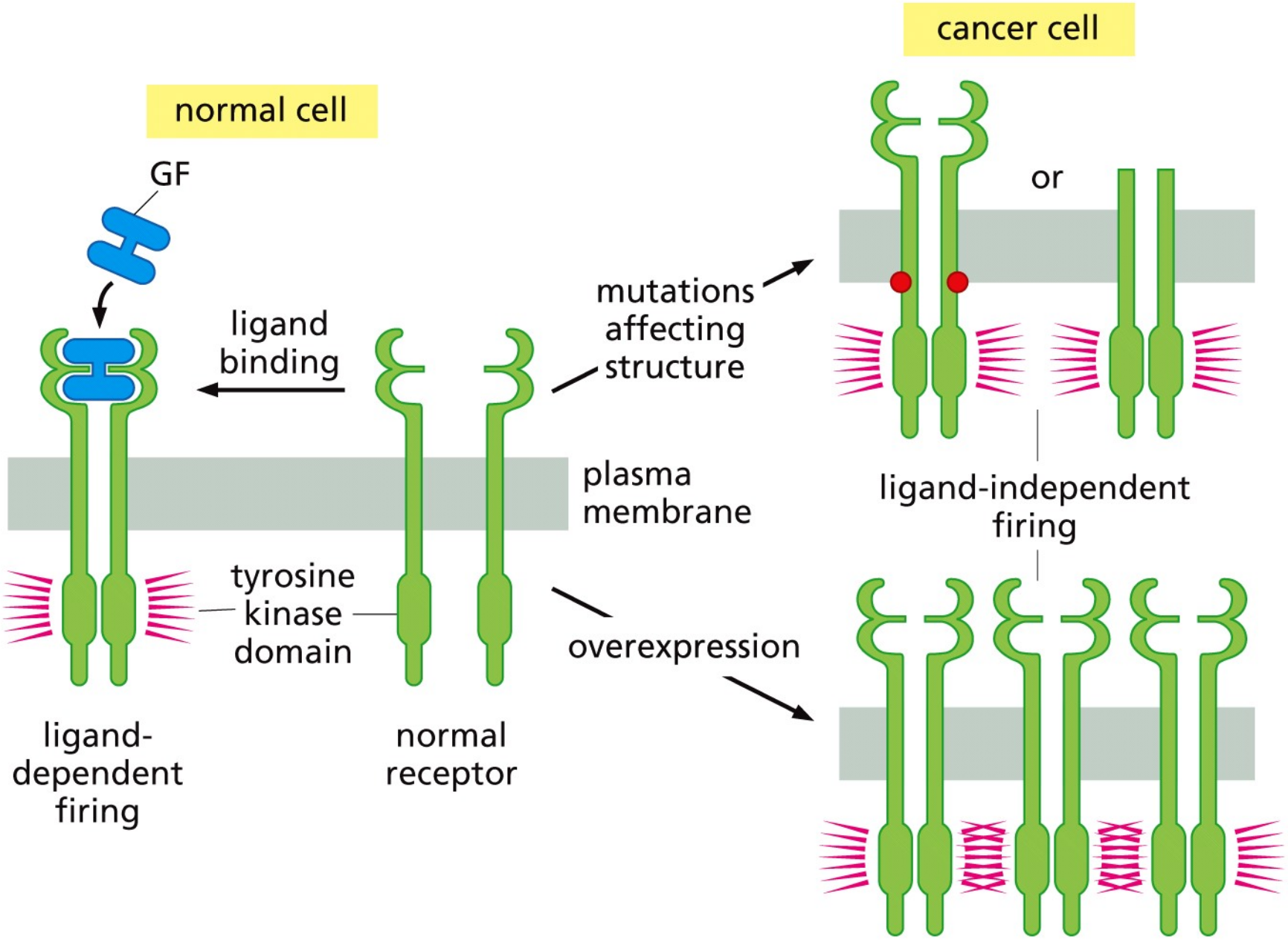


Figure 5.11a The Biology of Cancer (© Garland Science 2014)

Over expression of erbB2/HER2 in mamma cancer is associated with poor prognosis

FISH analysis
erbB2/HER2
CCND1/cyclinD1

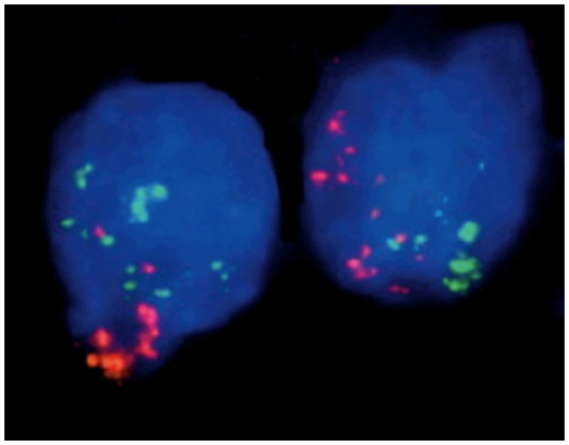


Figure 4.4c The Biology of Cancer (© Garland Science 2014)

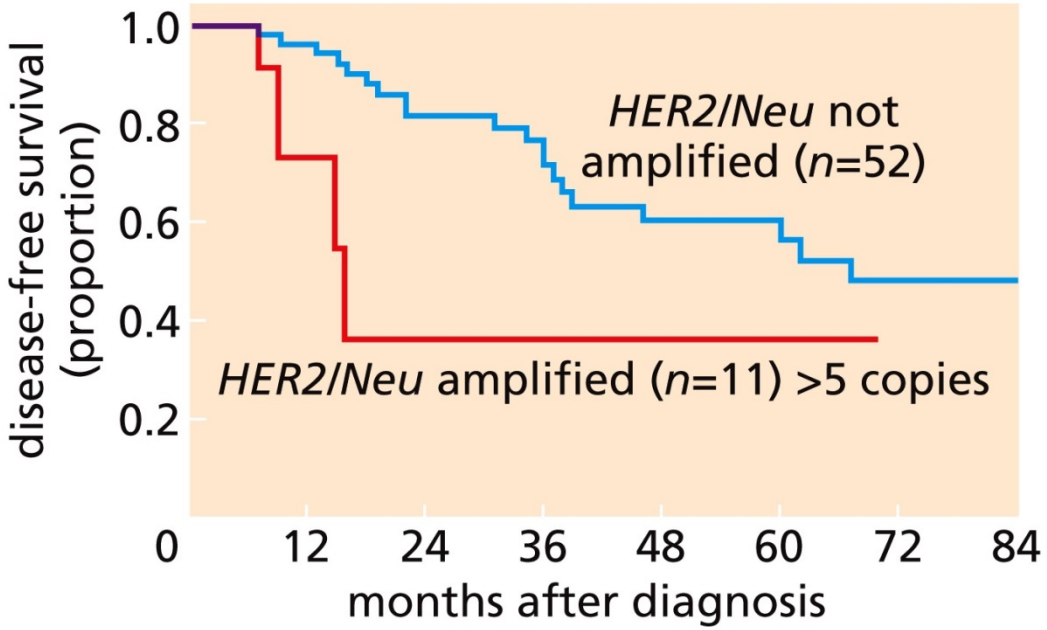
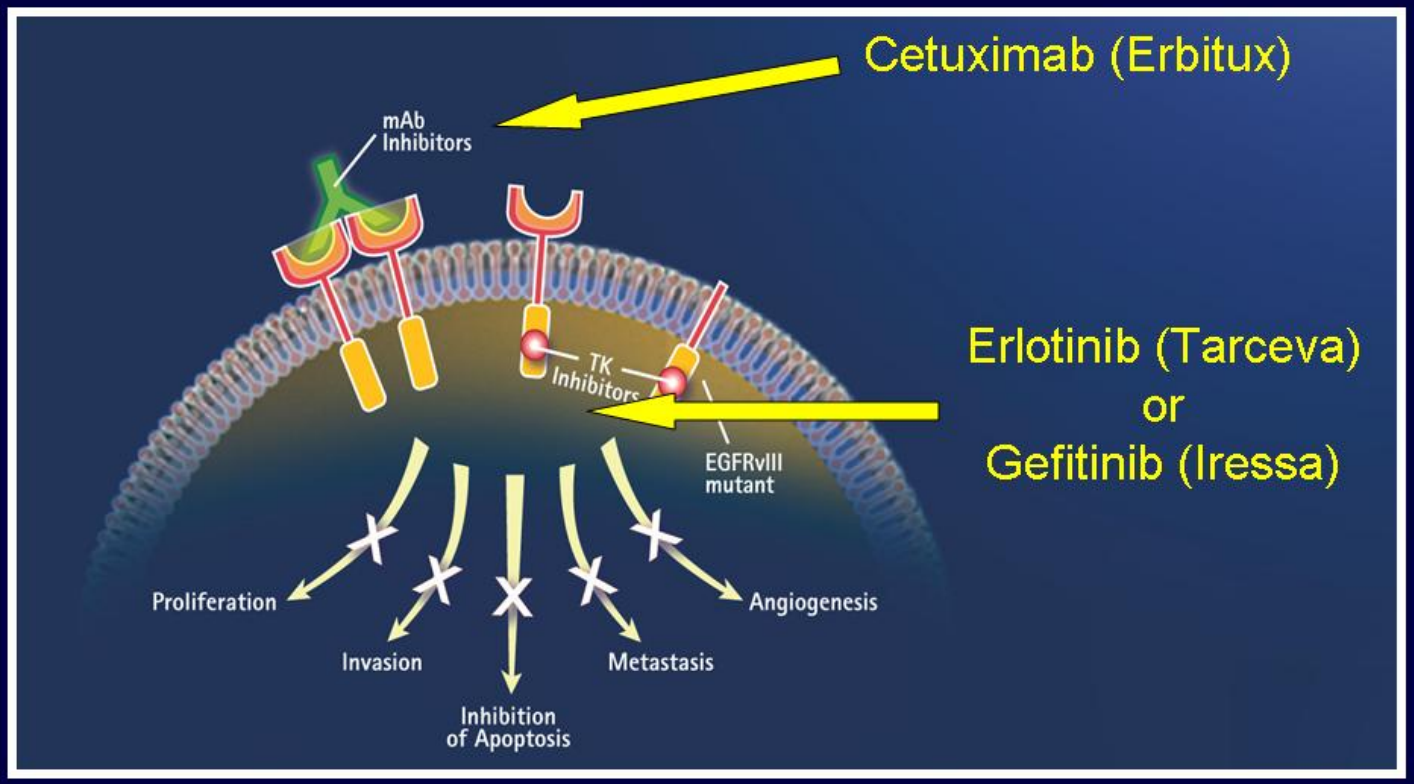


Figure 4.4b The Biology of Cancer (© Garland Science 2014)

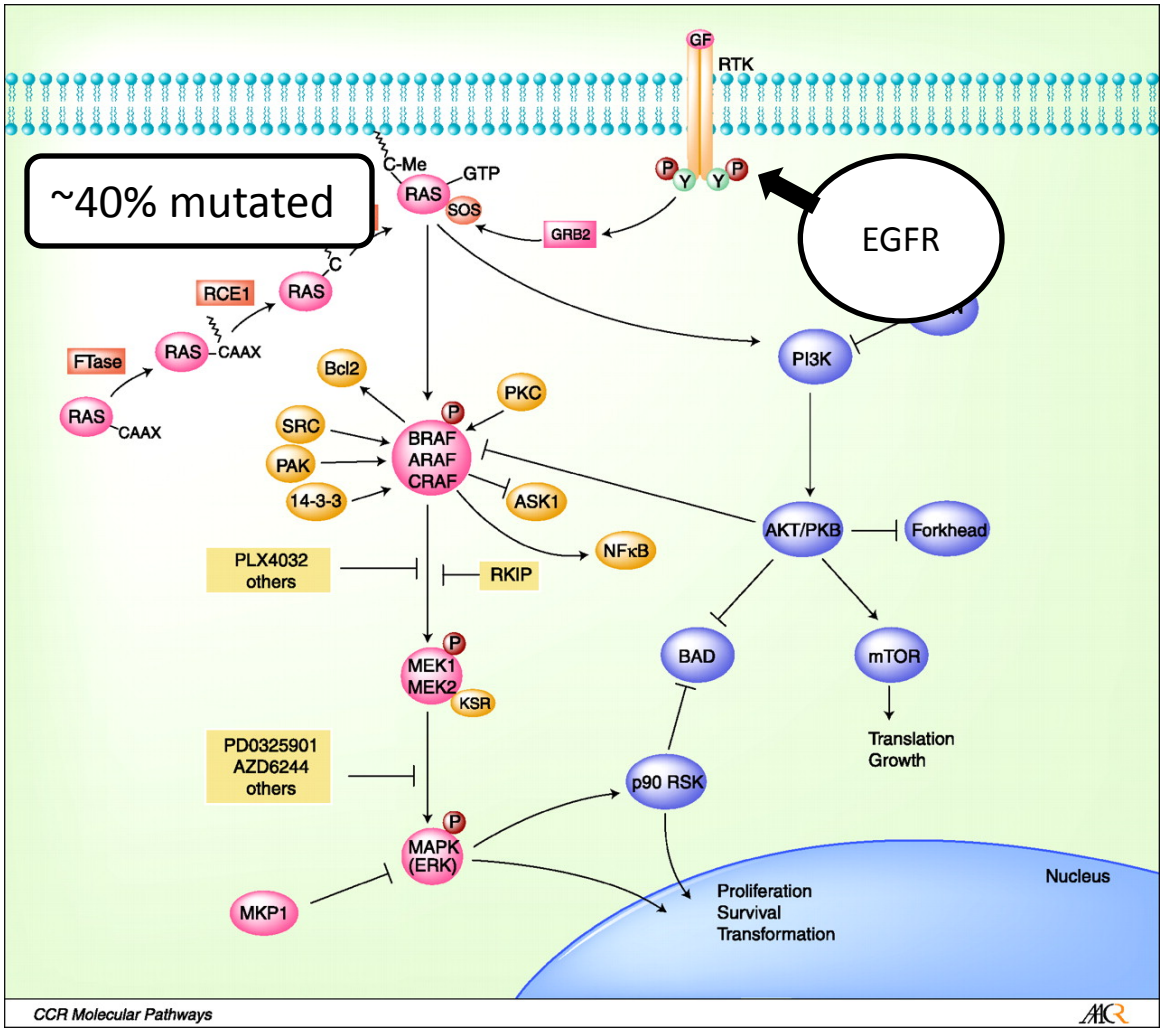
Table 4.3 Some frequently amplified chromosomal regions and the genes they are known to carry

Name of oncogene[a]	Human chromosomal location	Human cancers	Nature of protein[b]
MDM4/MDMX	1q32	breast, colon, lung, pre-B leukemias	p53 inhibitor
PIK3CA	3q26.3	lung SCC, ovarian, breast	PI kinase
erbB1/EGFR	7q12–13	glioblastomas (50%); squamous cell carcinomas (10–20%)	RTK
cab1–erbB2–grb7	17q12	gastric, ovarian, breast carcinomas (10–25%)	RTK, adaptor protein
k-sam	7q26	gastric, breast carcinomas (10–20%)	RTK
FGF-R1	8p12	breast carcinomas (10%)	RTK
met	7q31	gastric carcinomas (20%)	RTK
K-*ras*	12p12.1	lung, ovarian, colorectal, bladder carcinomas (5–20%)	small G protein
N-*ras*	1p13	head and neck cancers (30%)	small G protein
H-*ras*	11p15	colorectal carcinomas (30%)	small G protein
c-*myc*	8q24	various leukemias, carcinomas (10–50%)	TF
L-*myc*	1p32	lung carcinomas (10%)	TF
N-*myc–DDX1*	2p24–25	neuroblastomas, lung carcinomas (30%)	TF
akt-1	14q32–33	gastric cancers (20%)	ser/thr kinase
akt-2	19q13	ovarian carcinomas	ser/thr kinase
cyclin D1–exp1–hst1–ems1	(11q13)	breast and squamous cell carcinomas (25–50%)	G1 cyclin
cdk4–mdm2–sas–gli	12q13	sarcomas (10–30%), HNSCC (40%), B-cell lymphomas (25%)	CDK, p53 antagonist
cyclin E	19q12	gastric cancers (15%)	cyclin
akt2	(19q13)	pancreatic, ovarian cancers (30%)	ser/thr kinase
AIB1, BTAK	(20q12–13)	breast cancers (15%)	receptor co-activator
cdk6	(19q21–22)	gliomas (5%)	CDK
myb	6q23–24	colon carcinoma (5–20%), leukemias	TF
ets-1	11q23	lymphoma	TF
gli	12q13	glioblastomas	TF

Common Approaches for Inhibiting the Epidermal Growth Factor Receptor (EGFR) Axis



GRACE
GLOBAL RESOURCE for ADVANCING CANCER EDUCATION



Sebolt-Leopold, J. S. Clin Cancer Res 2008;14:3651-3656

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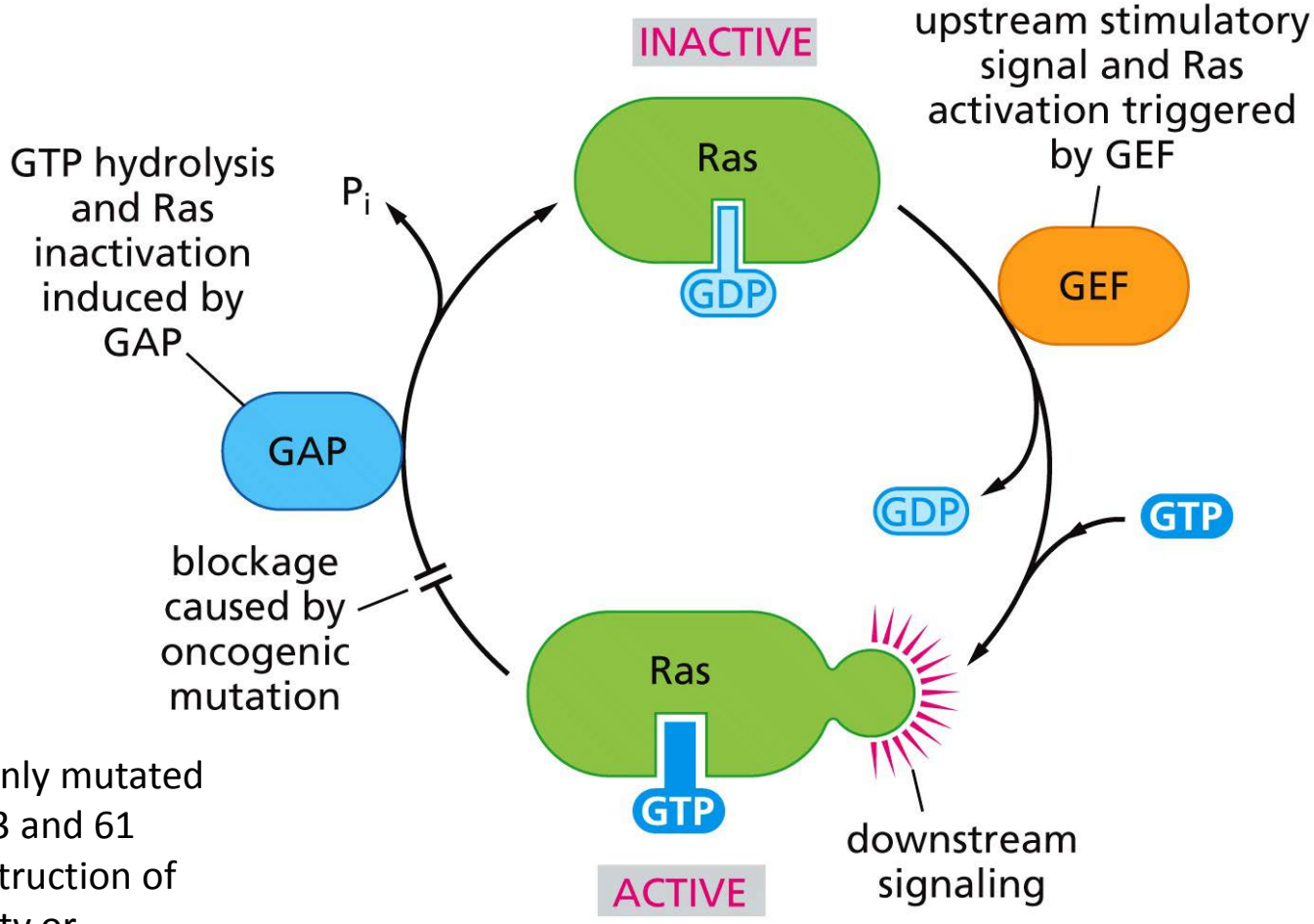
Clinical Cancer Research

RAS signaling



Most commonly mutated codons 12, 13 and 61 results in destruction of GTPase activity or prevention of GAP binding

RAS gets locked in “on” state

GEF: Guanine nucleotide exchange factors
GAP: GTPase-activating protein

Methods

	Target	Purpose
Immunohistochemistry (IHC)	Single	Protein over-expression
Fluorescence in situ hybridization (FISH)	Single	Copy number Structural rearrangements
Reverse-transcription quantitative PCR	Single	RNA Quantification (Fusion transcript)
Mutation detection		
• **Quantitative PCR**	Single	Structural rearrangements
• **Digital PCR**	Single	Structural rearrangements
• **Amplification Refractory Mutation System (ARMS)**	Single	Point mutations
• **Primer extension**	Single	Point mutations
• **Sanger sequencing**	Single	Structural rearrangements Point mutations
• **Next generation sequencing**	Whole genome Whole exome	Structural rearrangements Point mutations

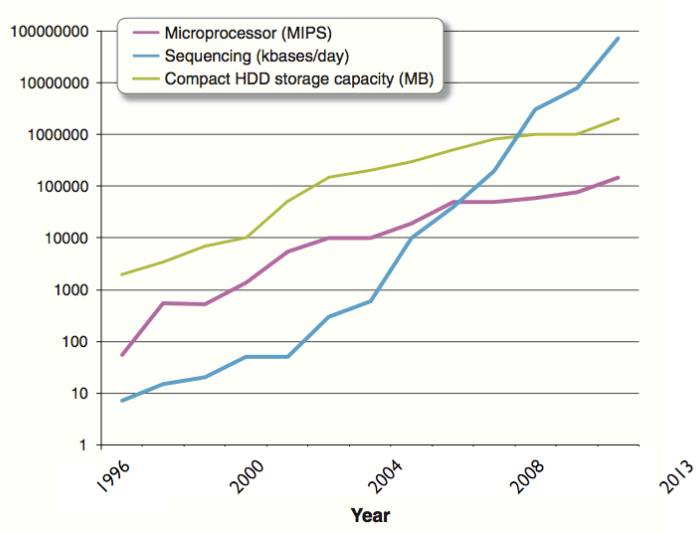
NGS machines

MiSeq

~1.5 Gb /day

~15M reads / day

Sequencing progress versus compute and storage



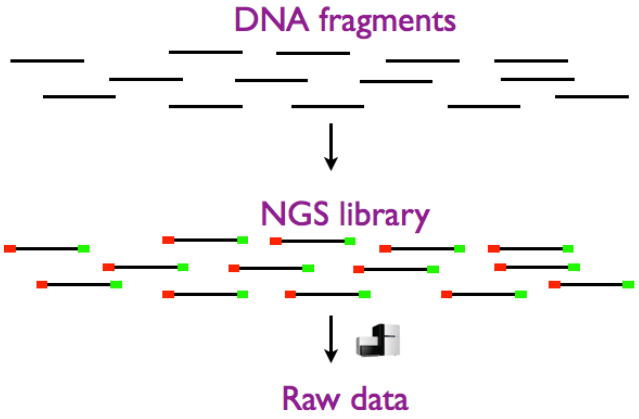
HiSeq 2000

~60 Gb /day

~600M reads / day

Massive parallel sequencing

- Library construction
  - Fragment DNA to ~300 bp
  - Attach adaptors to the ends of the fragments
- Sequencing
  - Attach 8 x 160.000.000 fragments to surface
  - Sequence all fragments in parallel (100 bp from both ends of the fragments)
  - Paired-end sequencing

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+
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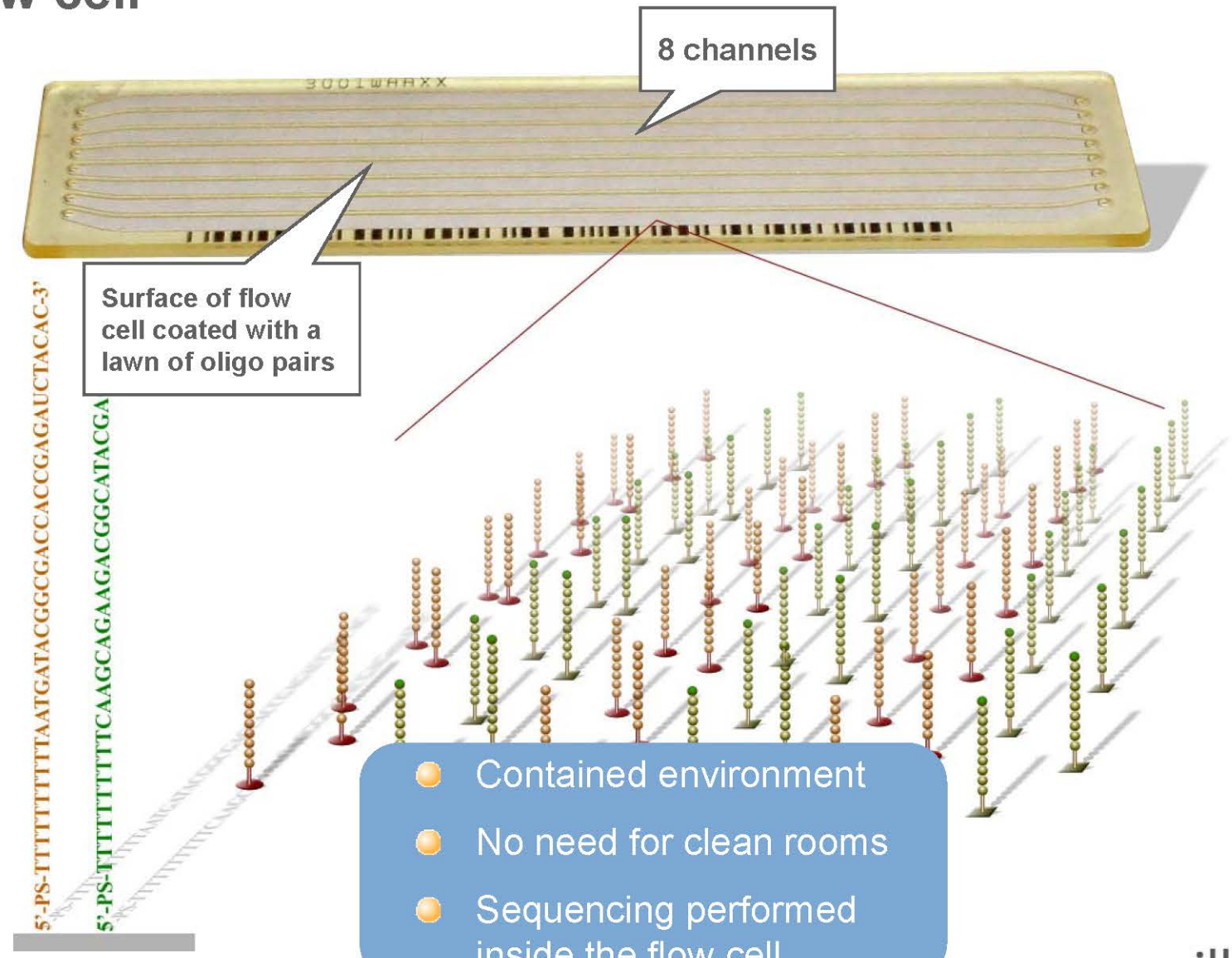
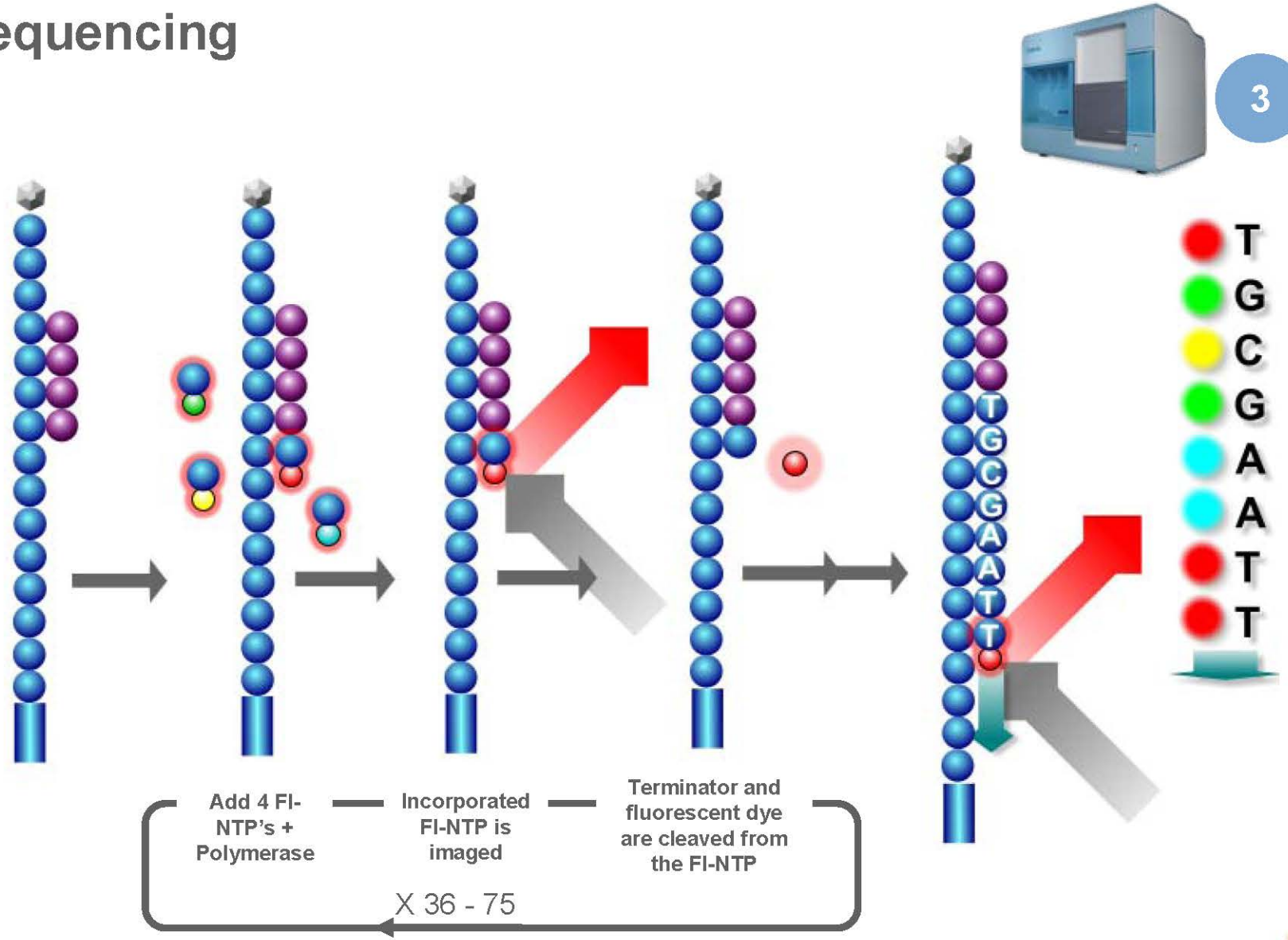
+ many million more lines ...
```

Flow cell



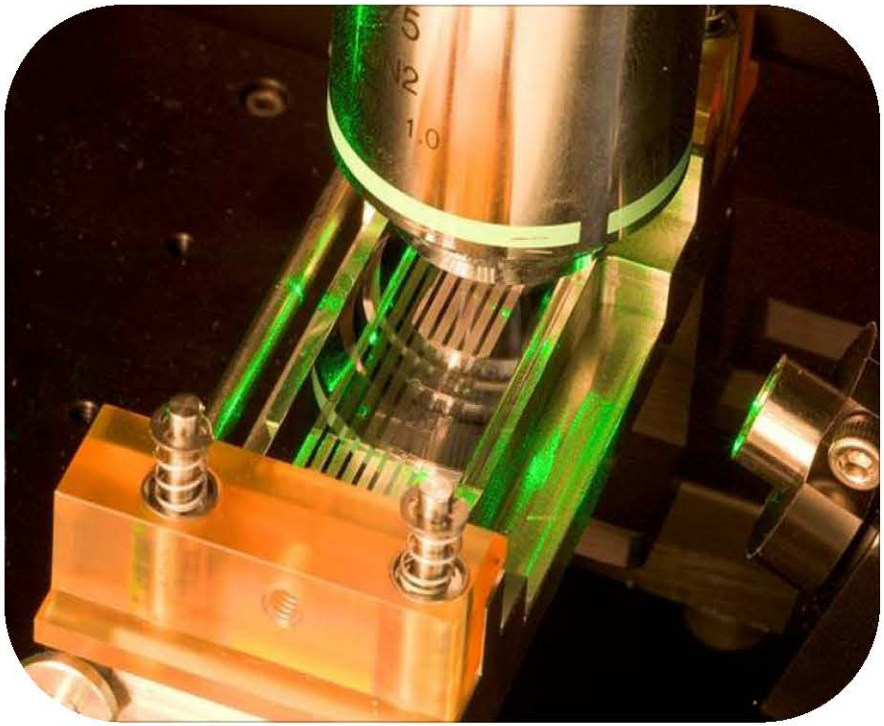
- Contained environment
- No need for clean rooms
- Sequencing performed inside the flow cell

Sequencing

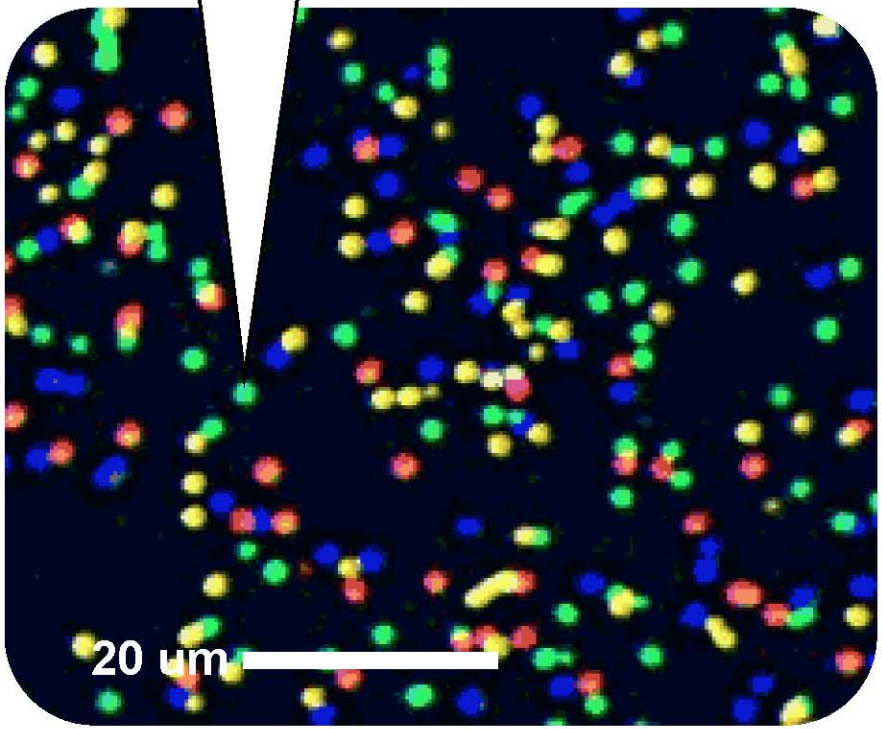


illumina

Sequencing



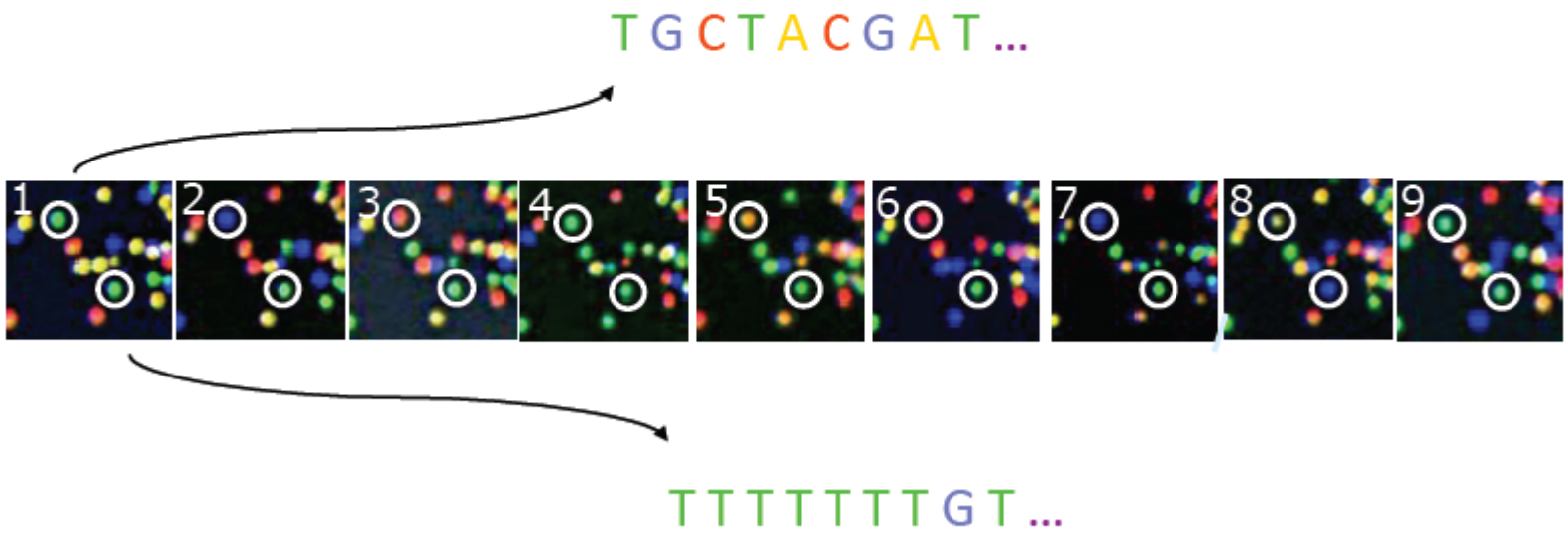
~1000 copies per cluster



>150 Million Clusters Per Flow Cell

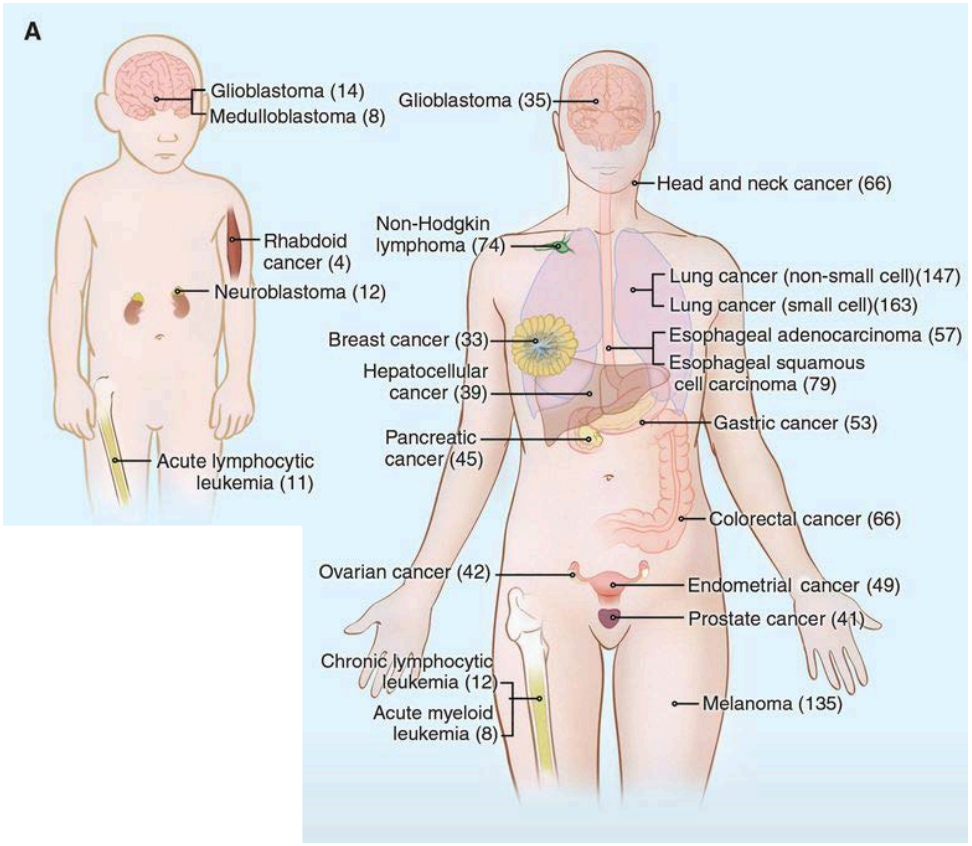
illumina

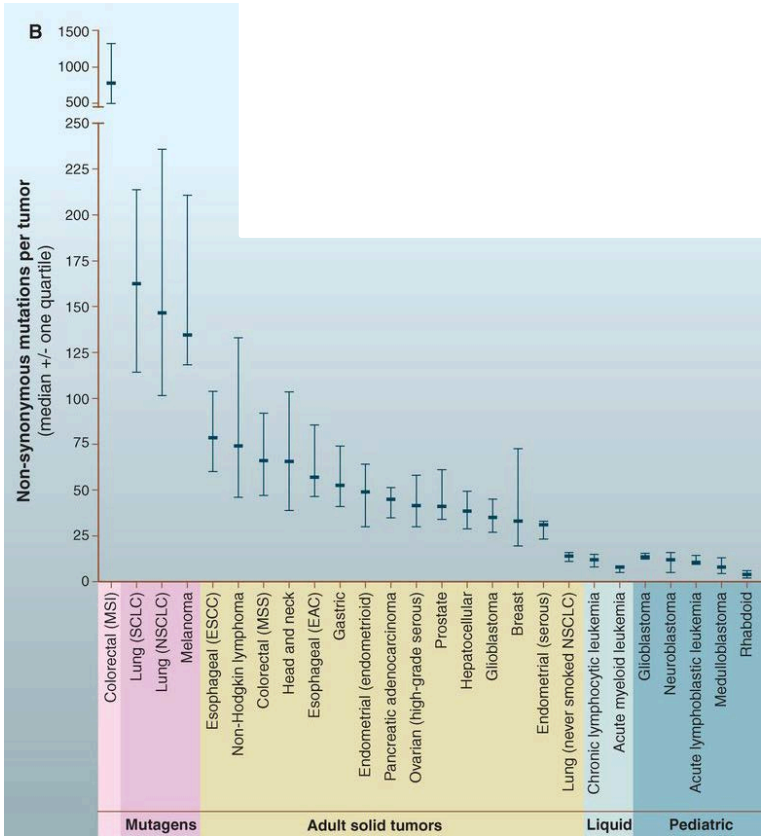
Base Calling from Raw Data



Number of somatic mutations in human cancers

detected by genome-wide sequencing studies

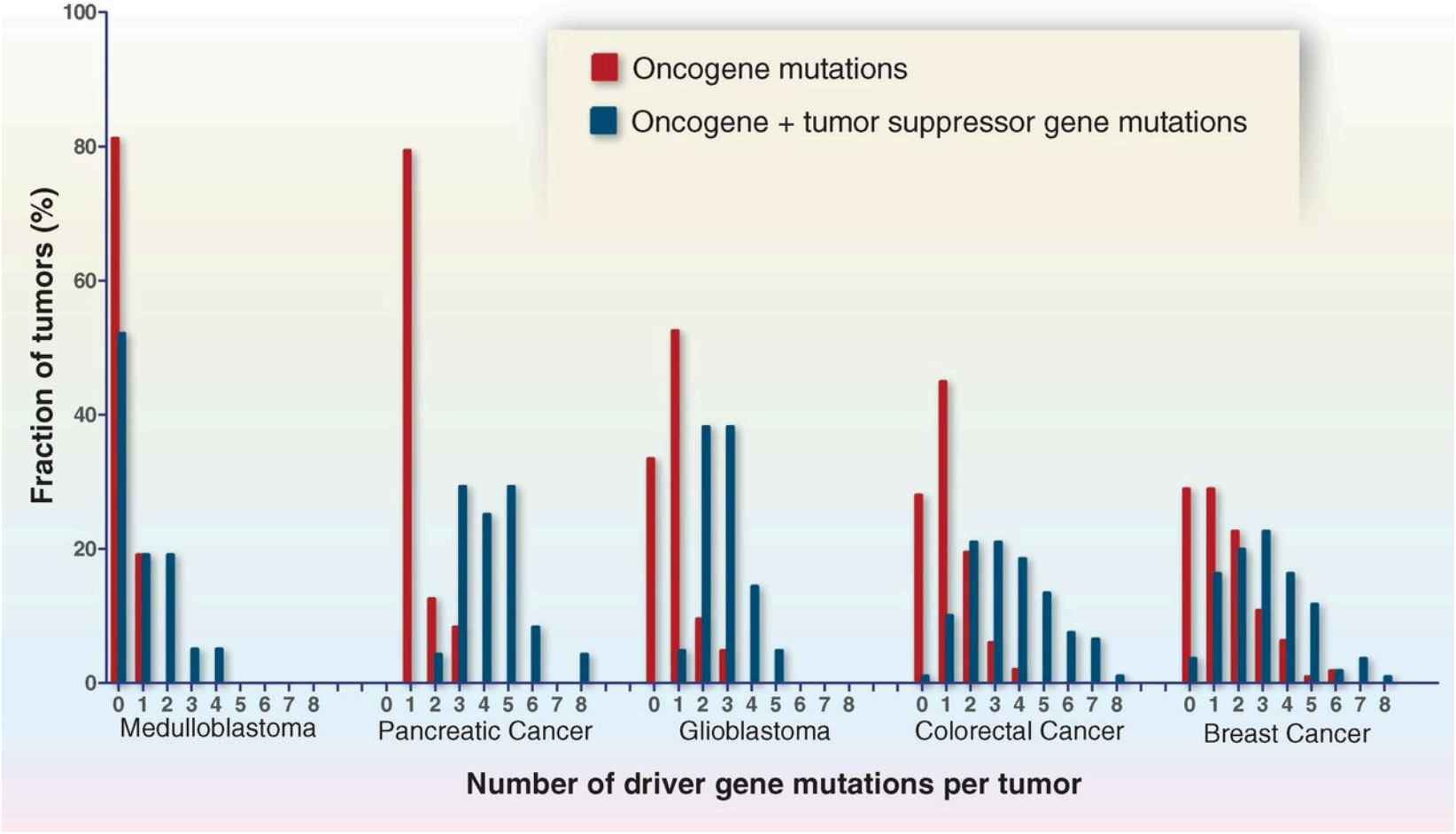




B Vogelstein et al. Science 2013;339:1546-1558

Science
AAAS

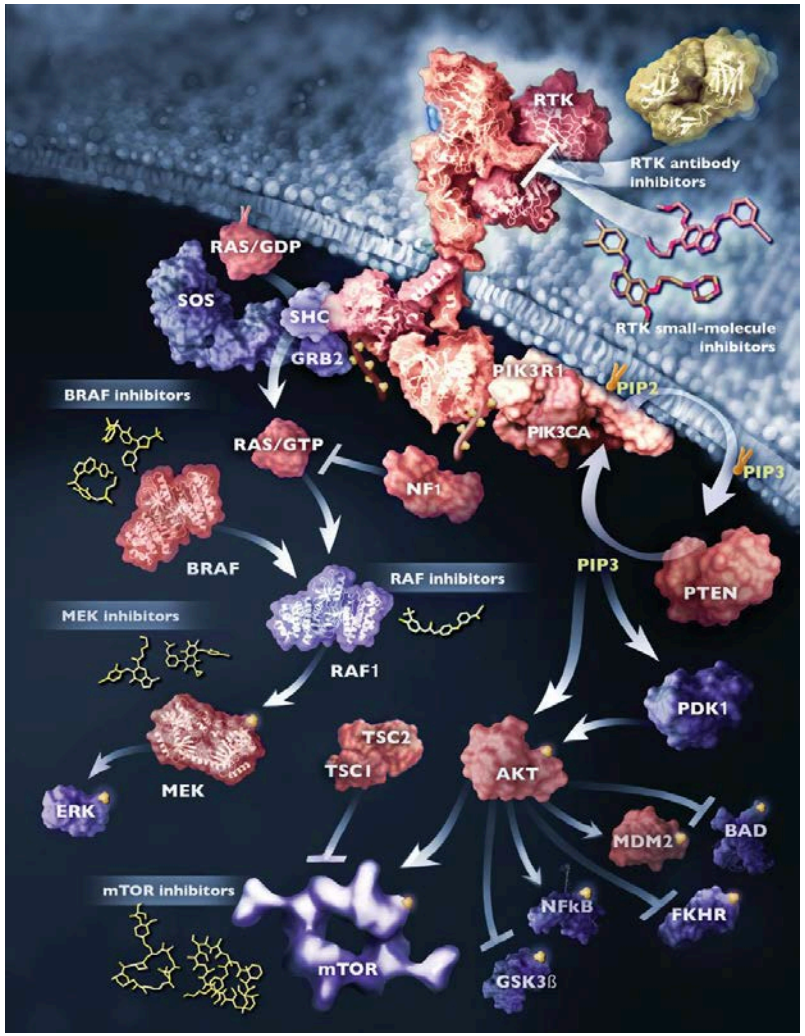
Number and distribution of driver gene mutations in five tumor types.



B Vogelstein et al. Science 2013;339:1546-1558

Science
AAAS

Signal transduction pathways affected by mutations in human cancer.

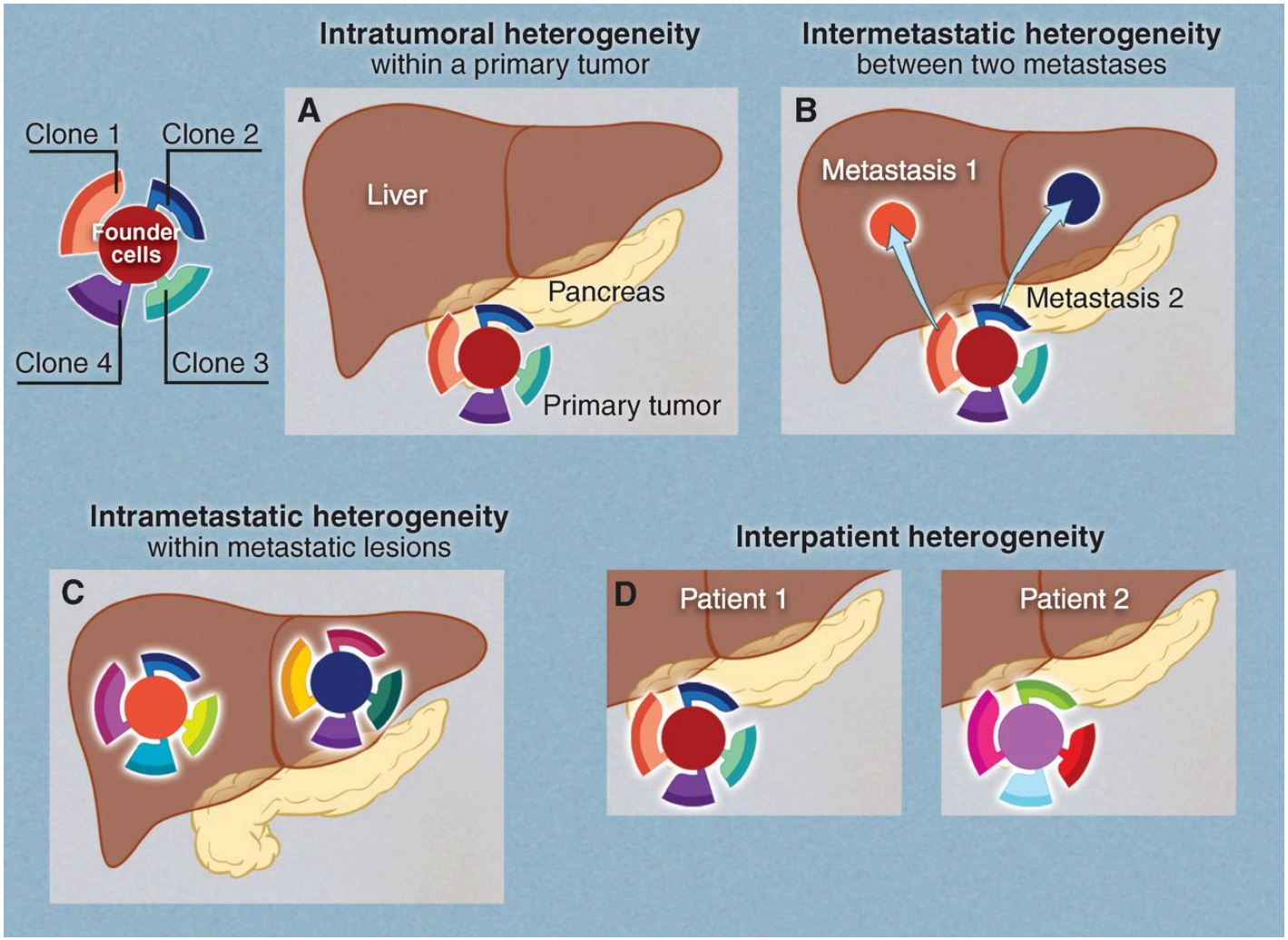


Red = driver genes

B Vogelstein et al. Science 2013;339:1546-1558

Science
AAAS

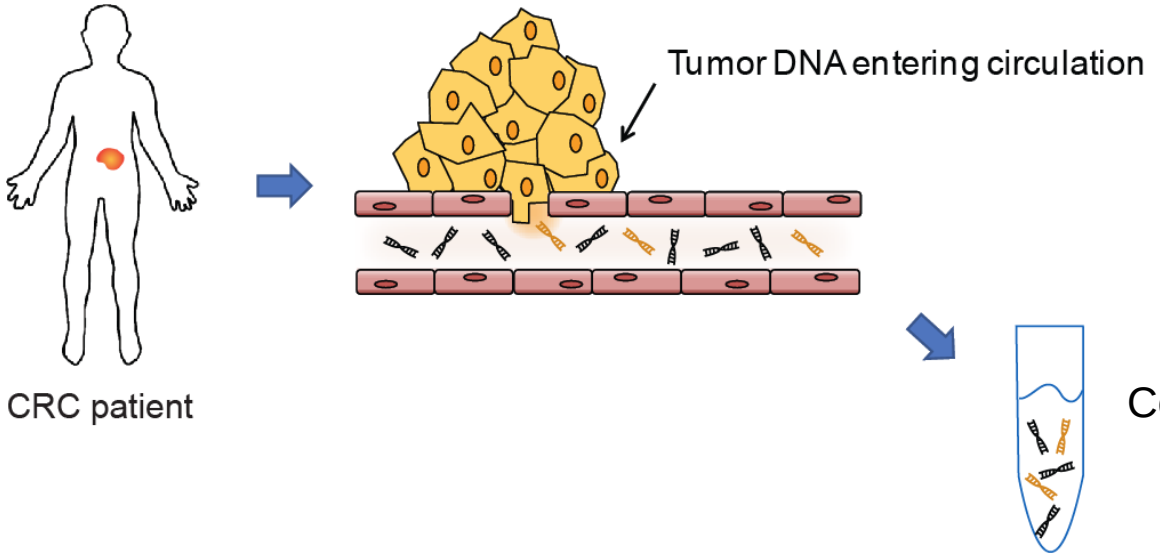
Four types of genetic heterogeneity in tumors



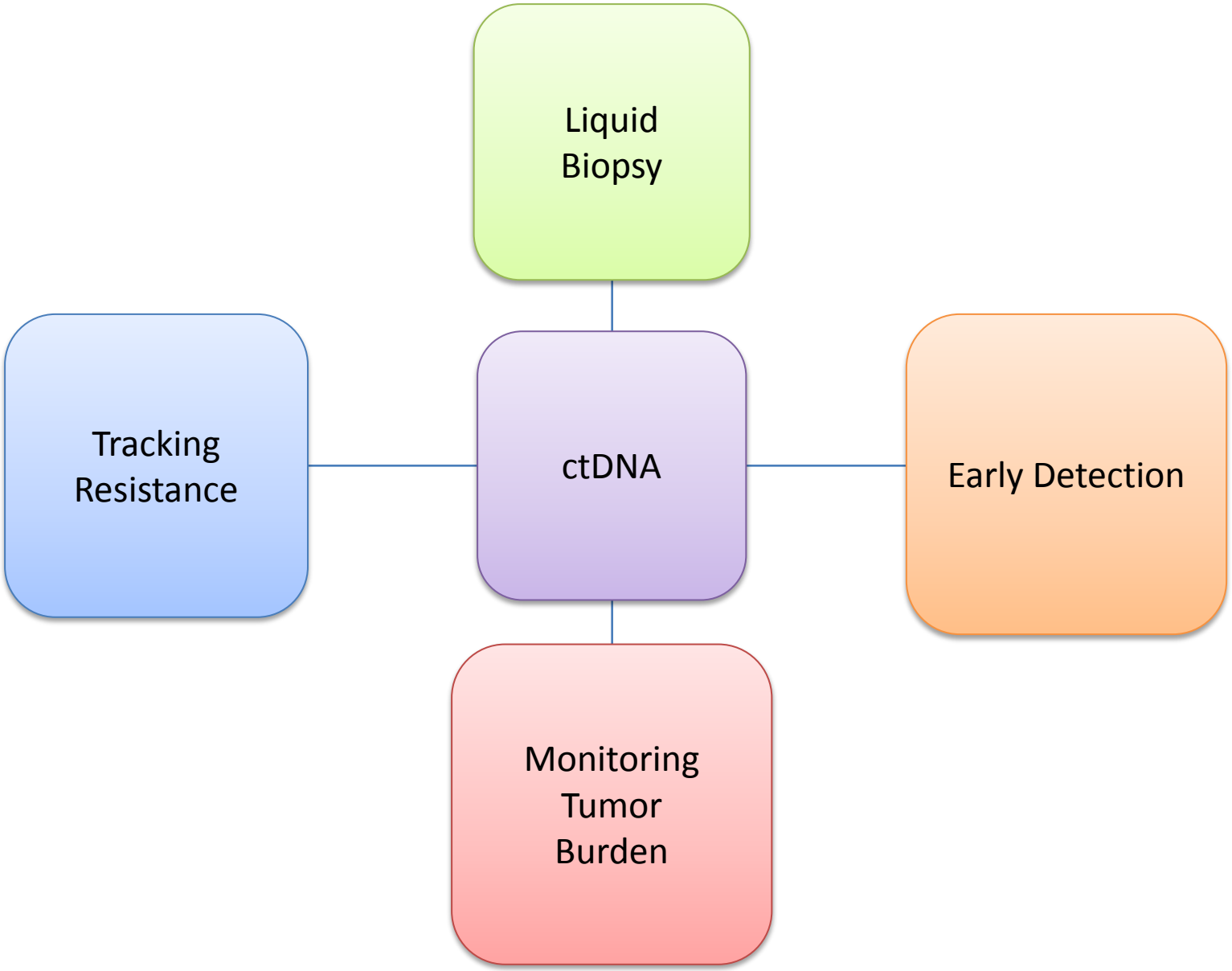
B Vogelstein et al. Science 2013;339:1546-1558

Science
AAAS

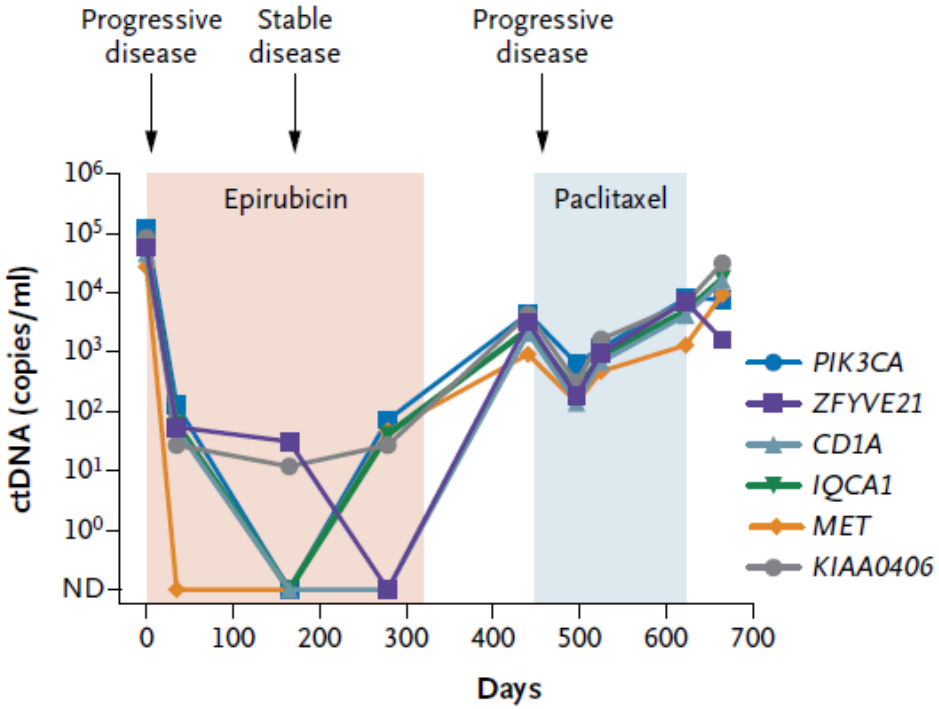
Solid tumors release DNA to the circulation



Circulating tumor DNA, ctDNA


Liquid Biopsy
Tracking Resistance
ctDNA
Early Detection
Monitoring Tumor Burden

Monitoring response to chemotherapy



Metastatic breast cancer patient

RESEARCH ARTICLE www.ScienceTranslationalMedicine.org 28 November 2012 Vol 4 Issue 162 162ra154

DIAGNOSTICS

Detection of Chromosomal Alterations in the Circulation of Cancer Patients with Whole-Genome Sequencing

Rebecca J. Leary,[1]* Mark Sausen,[1]* Isaac Kinde,[1]* Nickolas Papadopoulos,[1] John D. Carpten,[2] David Craig,[2] Joyce O'Shaughnessy,[3] Kenneth W. Kinzler,[1] Giovanni Parmigiani,[4,5] Bert Vogelstein,[1] Luis A. Diaz Jr.,[1†] Victor E. Velculescu[1†]

LETTER

doi:10.1038/nature12065

Non-invasive analysis of acquired resistance to cancer therapy by sequencing of plasma DNA

Muhammed Murtaza[1]*, Sarah-Jane Dawson[1,2]*, Dana W. Y. Tsui[1]*, Davina Gale[1], Tim Forshew[1], Anna M. Piskorz[1], Christine Parkinson[1,2], Suet-Feung Chin[1], Zoya Kingsbury[3], Alvin S. C. Wong[4], Francesco Marass[1], Sean Humphray[3], James Hadfield[3], David Bentley[3], Tan Min Chin[4,5], James D. Brenton[1,2,6], Carlos Caldas[1,2,6] & Nitzan Rosenfeld[1]

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ORIGINAL ARTICLE

Analysis of Circulating Tumor DNA to Monitor Metastatic Breast Cancer

Sarah-Jane Dawson, F.R.A.C.P., Ph.D., Dana W.Y. Tsui, Ph.D., Muhammed Murtaza, M.B., B.S., Heather Biggs, M.A., Oscar M. Rueda, Ph.D., Suet-Feung Chin, Ph.D., Mark J. Dunning, Ph.D., Davina Gale, B.Sc., Tim Forshew, Ph.D., Betania Mahler-Araujo, M.D., Sabrina Rajan, M.D., Sean Humphray, B.Sc., Jennifer Becq, Ph.D., David Halsall, M.R.C.Path., Ph.D., Matthew Wallis, M.B., Ch.B., David Bentley, D.Phil., Carlos Caldas, M.D., F.Med.Sci., and Nitzan Rosenfeld, Ph.D.