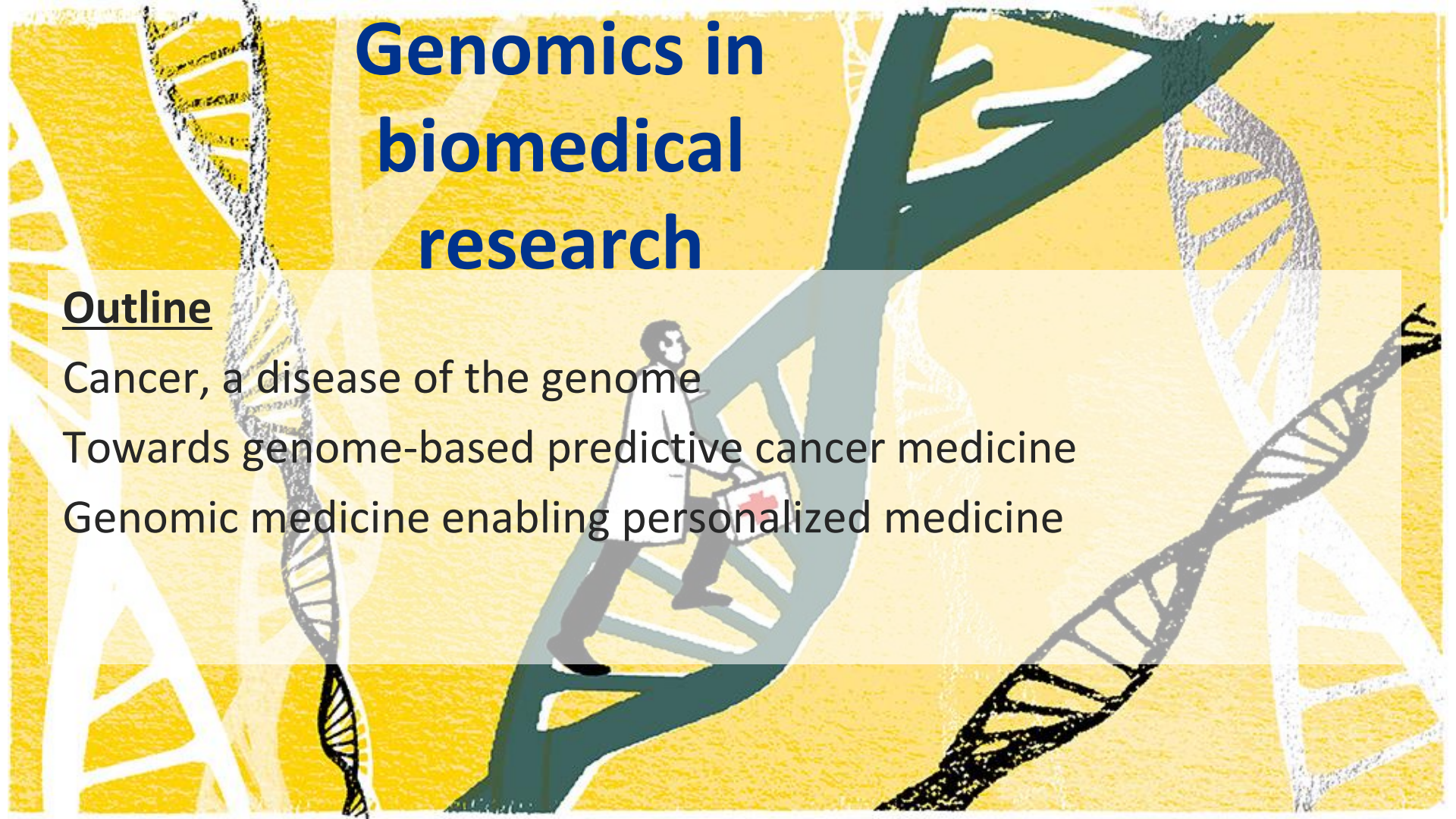
Fundamentals of Molecular Biology

IN-BIOS 5000/9000

1. A guided tour of the human genome
2. From DNA to biological function
3. Genomics in biomedical research

Genomics in biomedical research



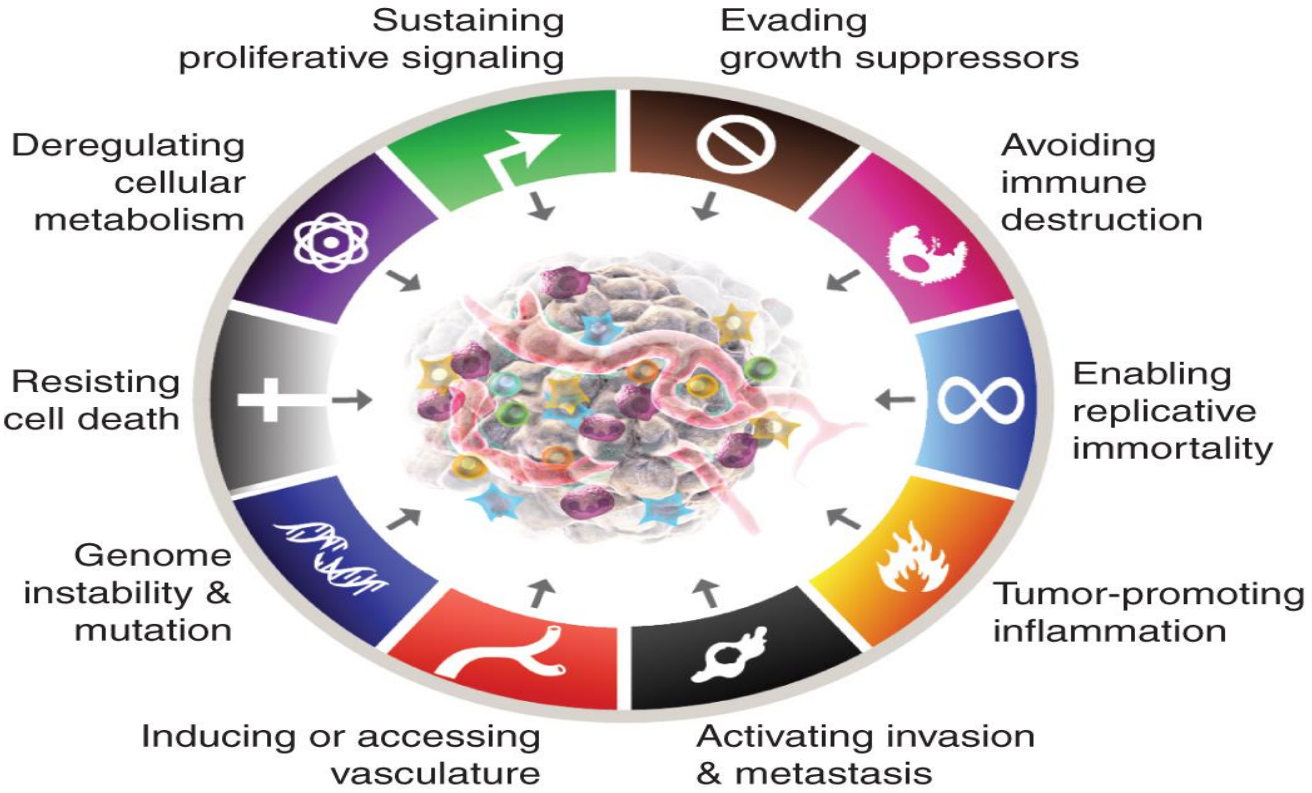
Outline

Cancer, a disease of the genome

Towards genome-based predictive cancer medicine

Genomic medicine enabling personalized medicine

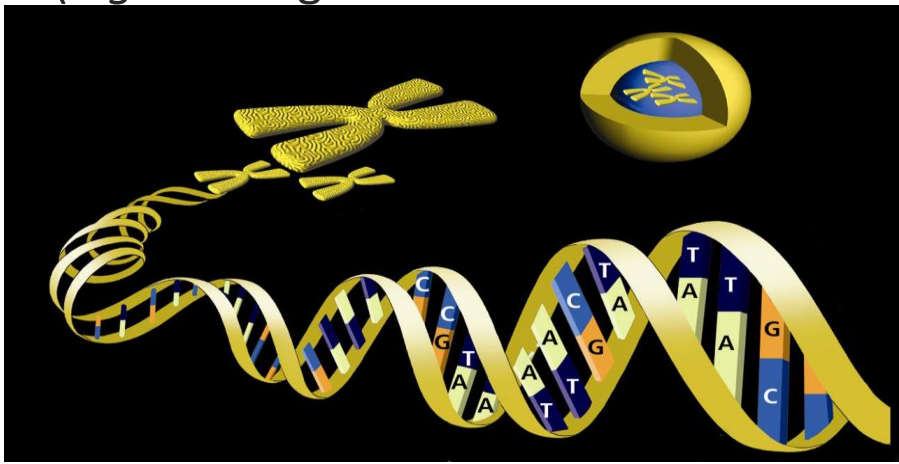
Hallmarks of cancer



Hanahan, Cancer Discov 2022
Hanahan & Weinberg, Cell 2000 & 2011

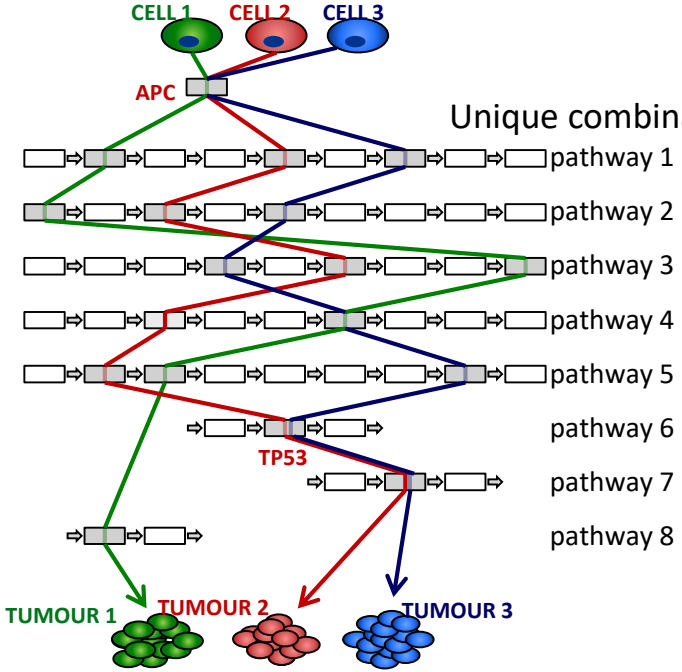
Cancer, a disease of the genome

- Cancer arises as a result of an accumulation of genetic and epigenetic aberrations that are either acquired or inherited
  - Numerical and structural chromosome changes (amplifications, deletions, inversions, translocations)
  - Nucleotide-level variants or mutations (*e.g.* causing amino acid substitutions)
  - Epigenetic changes



Cancer, a disease of the genome

- Cancer arises as a result of an accumulation of genetic and epigenetic aberrations that are either acquired or inherited
- The set of mutations is unique to each cancer



Unique combination of driver mutations in cancer

.. cancer genomes are even more unique because:

- although the mutated *genes* are the same, the *particular* mutations are different
- large amount of passenger mutations, which rarely are shared between individual cancers

Cancer, a disease of the genome

- Cancer arises as a result of an accumulation of genetic and epigenetic aberrations that are either acquired or inherited
- The set of mutations is unique to each cancer
- Mutation status of particular genes is relevant in predictive medicine, *e.g.* through targeting of driver genes and proteins

Companion diagnostics

Enabling targeting of individual actionable genes relevant to the cancer in question

Cancer, a disease of the anatomical site?

Traditionally, physicians and pathologists define types of cancers and subcategories based on anatomic site of origin, clinical behaviour, and histopathologic appearance

- Brain cancer
- Liver cancer
- Breast cancer

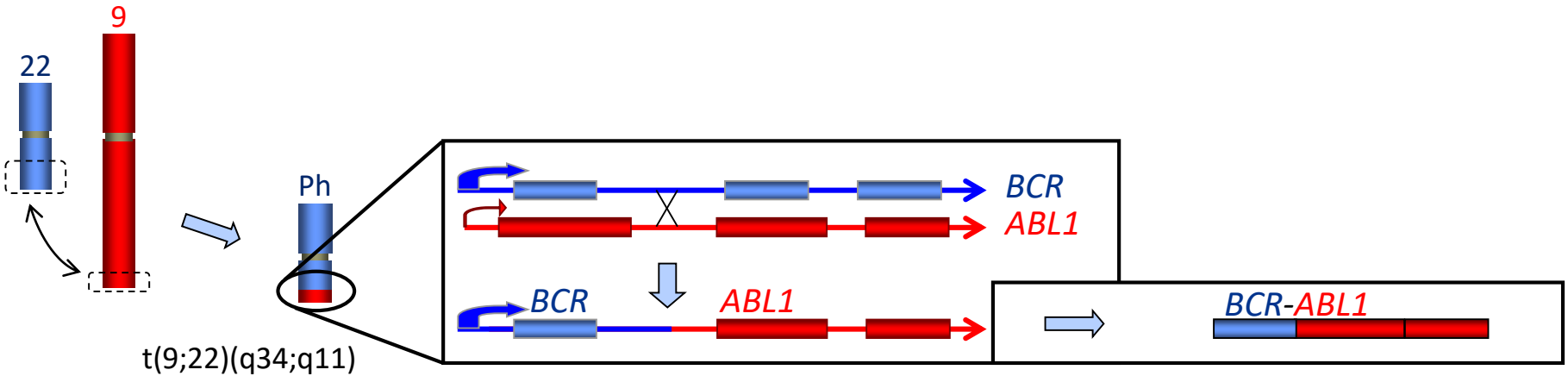
Genome-based predictive medicine

Aims to understand the relevant characteristics underlying a particular individual's disease (both disease and host factors), and then tailor therapy to that individual/disease

In the context of genome-based predictive medicine, cancers are increasingly being classified by driving molecular events, rather than by organ site

Towards genome-based predictive cancer medicine

- Gleevec (imatinib), drug for treatment of leukaemia with *BCR-ABL* fusion gene
  - and drug for treating cancers, *as such*, being driven by *BCR-ABL* fusion genes/proteins



t(9;22)(q34;q11)
alias the Philadelphia chromosome

Nowell & Hungerford, Science 1960; Rowley, Nature 1973; Heisterkamp *et al.*, Nature 1983; Groffen *et al.*, Cell 1984; Druker *et al.*, Nat. Med. 1996 and NEJM 2001

Towards genome-based predictive cancer medicine

- Gleevec (imatinib), drug for treatment of leukaemia with *BCR-ABL* fusion gene
  - and drug for treating cancers, *as such*, being driven by *BCR-ABL* fusion genes/proteins
  - First cancer drug, specifically targeting a certain cancer-critical enzyme, rather than non-specifically killing all rapidly dividing cells
  - Also functional against 4 other activated tyrosine kinase receptors, such as mutated *KIT* in gastrointestinal stromal tumours (GIST)
  - Approved to treat ten different cancers

Unspecific cancer drug (cancers in general)

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Specifically targeting drug (but organ-confined)

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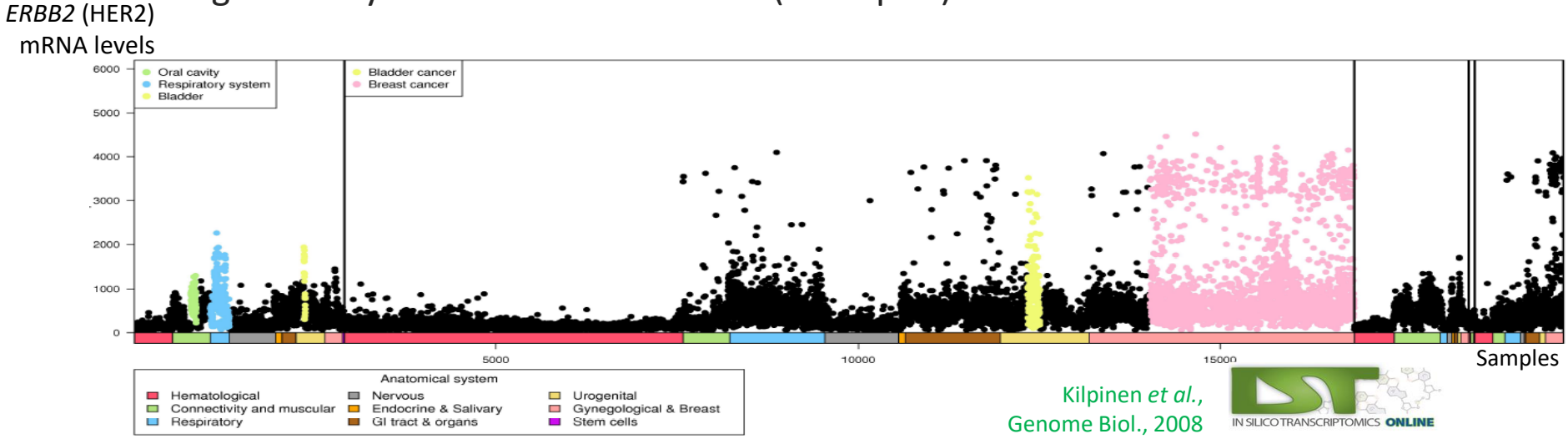
Personalized (all cancers with particular mutation)

Nowell & Hungerford, Science 1960; Rowley, Nature 1973; Heisterkamp *et al.*, Nature 1983; Groffen *et al.*, Cell 1984; Druker *et al.*, Nat. Med. 1996 and NEJM 2001

Towards genome-based predictive cancer medicine

- *ERBB2 (HER2)*, a breast cancer gene?
  - or a gene overexpressed in a subset of cancers - which are most commonly located in the breast?
  - targetable by monoclonal antibodies (herceptin)

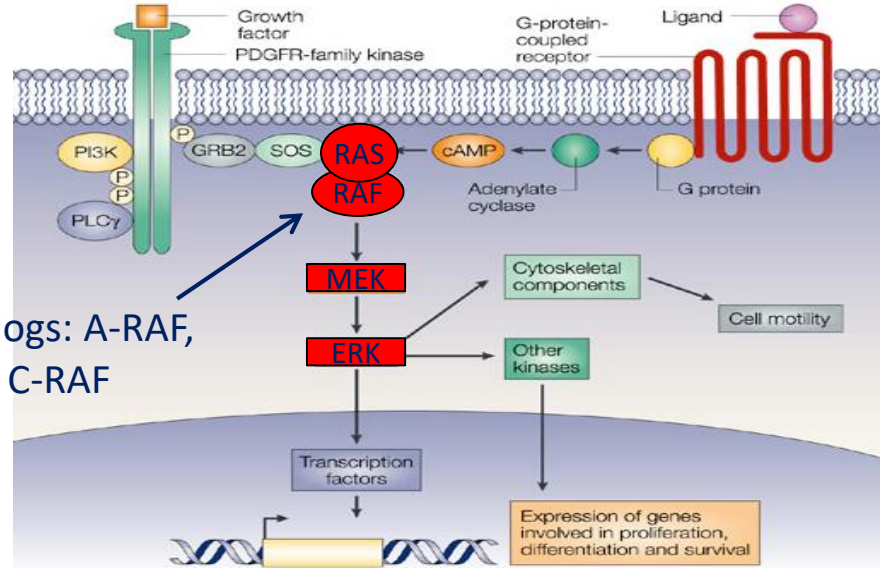


Kilpinen *et al.*, Genome Biol., 2008

IN SILICO TRANSCRIPTOMICS ONLINE

Towards genome-based predictive cancer medicine

- BRAF-inhibitors: originally treatment of melanoma with *BRAF*-mut
  - and drugs for treating cancers, *as such*, being driven by mutated *BRAF*?



Homologs: A-RAF, B-RAF, C-RAF

Part of the RAS-RAF-MEK-ERK (alias MAP-kinase) cell signalling pathway, commonly activated in cancer cells

One targeting inhibitor: vemurafenib, has been tested for other cancers with V600E mutated *BRAF*

Towards genome-based predictive cancer medicine

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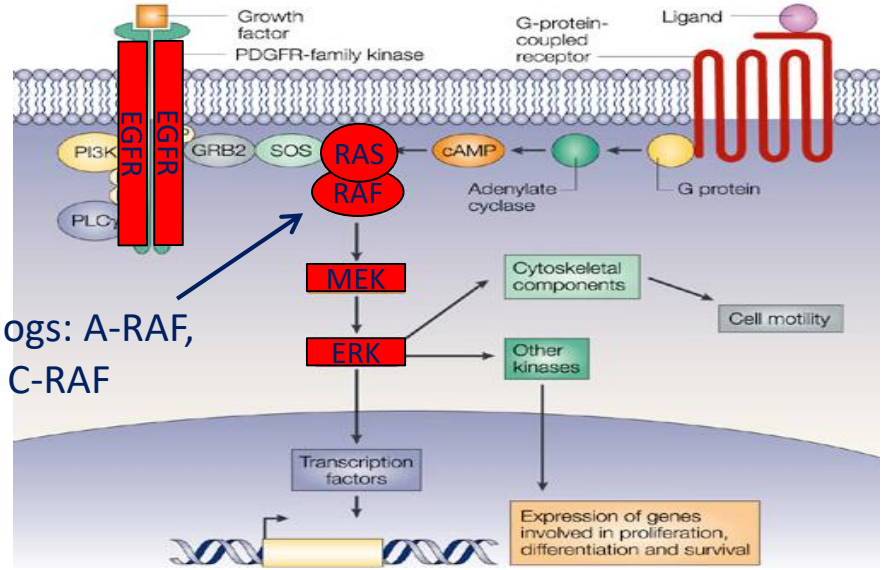
  - and drugs for treating cancers, *as such*, being driven by mutated *BRAF*?



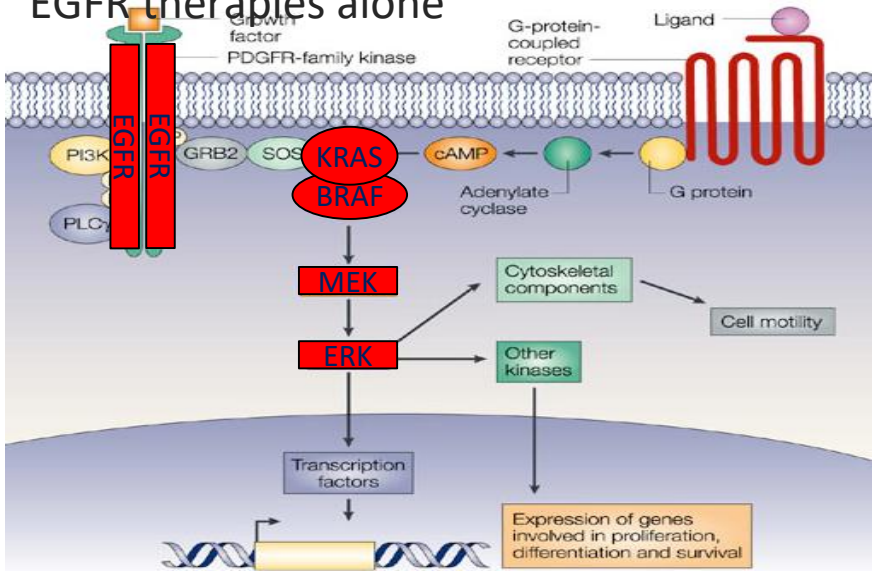
Homologs: A-RAF, B-RAF, C-RAF

Part of the RAS-RAF-MEK-ERK (alias MAP-kinase) cell signalling pathway, commonly activated in cancer cells

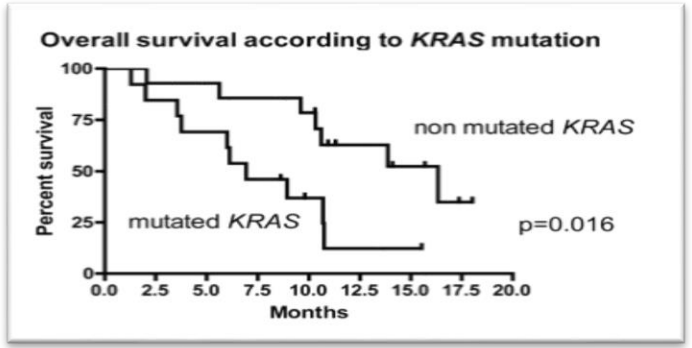
..and having low levels of EGFR

Towards genome-based predictive cancer medicine

- Colorectal cancer actionable genes
  - Patients/cancers with mutated *KRAS* or *BRAF* are less likely to respond to anti-EGFR therapies alone



Part of the RAS-RAF-MEK-ERK (alias MAP-kinase) cell signalling pathway, commonly activated in cancer cells



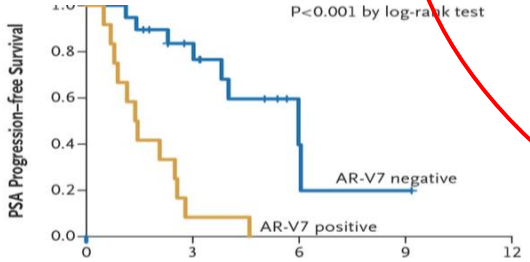
Lièvre *et al.*, Cancer Res., 2006

Later, the same has been observed in much larger patient series, and also for cancers with *BRAF* mutation

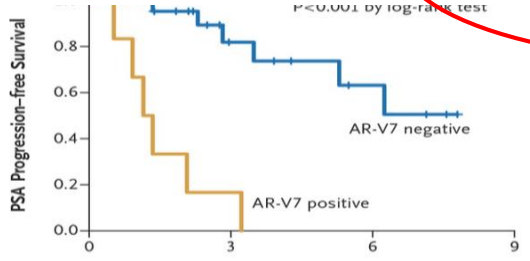
Towards genome-based predictive cancer medicine

- Androgen receptor transcript variant 7 (*AR-v7)* predicts resistance to inhibition of androgen signalling in prostate cancer

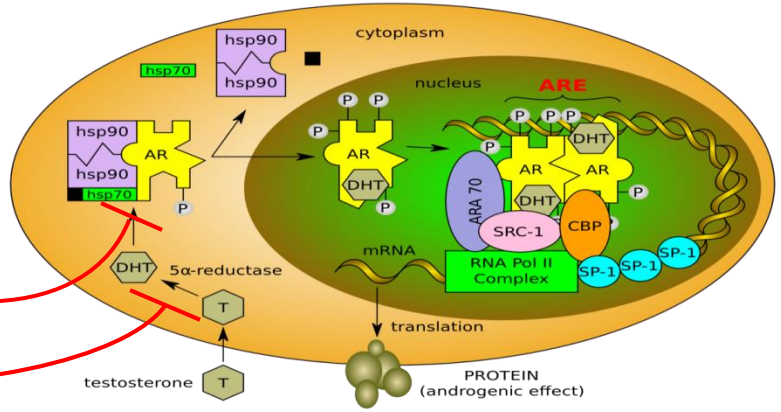
Patients treated with Enzalutamide

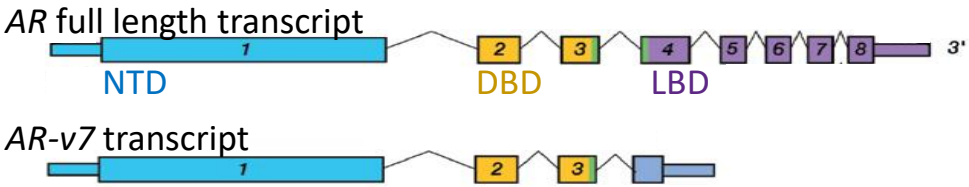


Patients treated with Abiraterone



Antonarakis *et al.*, NEJM 2014





Towards genome-based predictive cancer medicine

Growing list of targeted drugs with predictive biomarkers across several cancer types

- GIST with *KIT*-mutations => leukaemia medicine (Gleevec)
- Bladder cancer with *HER2*/*ERBB2*-overexpression => breast cancer medicine (Herceptin)?
- Prostate or colon cancer with *RAF* rearrangements => melanoma RAF inhibitors (vemurafenib)?
- Prostate cancer with *AR-V7* transcripts => resistant to androgen inhibition

Is it feasible to test for *all* possibilities?

Companion diagnostics

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Targeting individual actionable genes relevant to the cancer in question

Genome-based PCM

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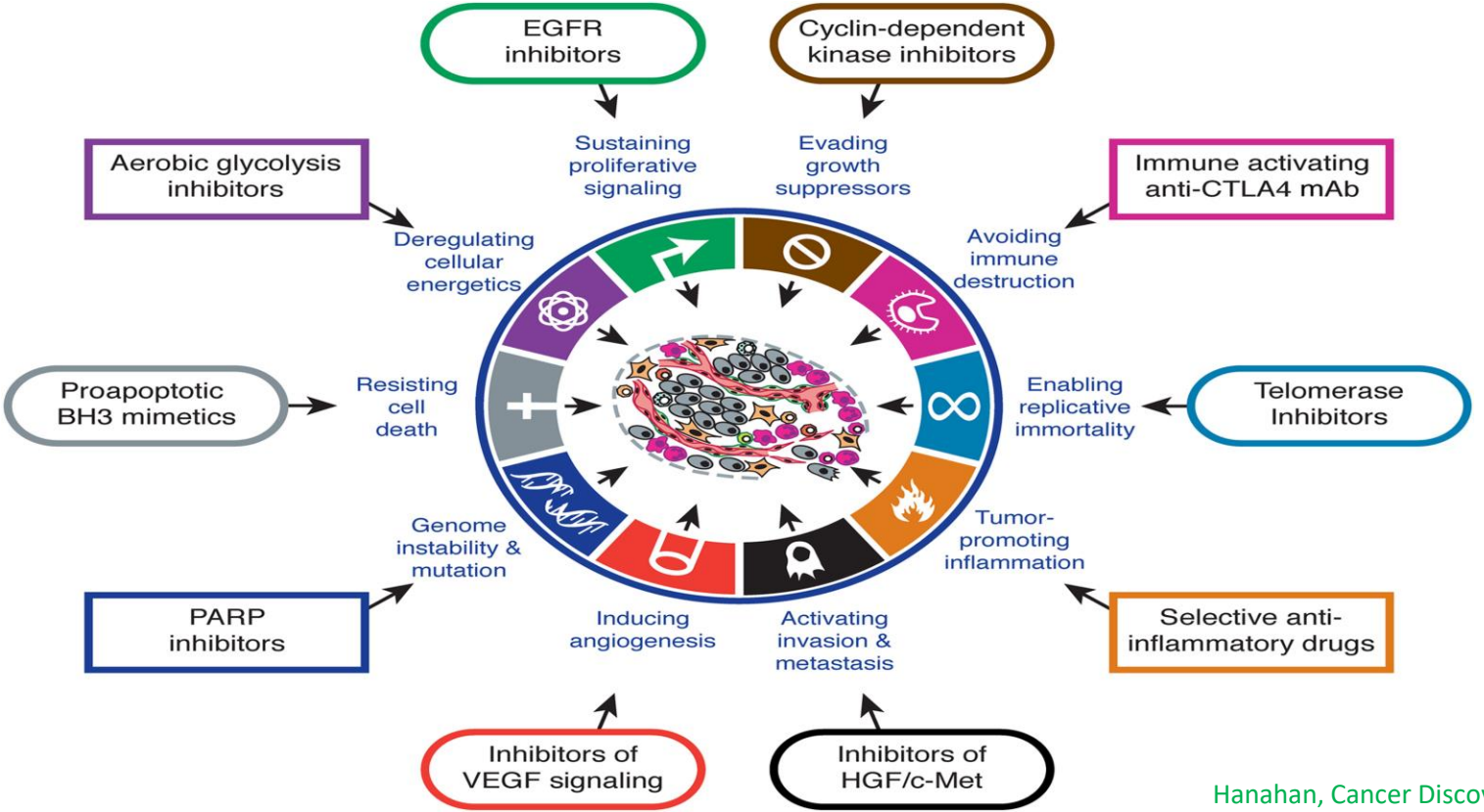
Genome analyses, testing all of DNA/RNA

Therapeutic targeting of the Hallmarks of cancer



Hanahan, Cancer Discov 2022
Hanahan & Weinberg, Cell 2000 & 2011

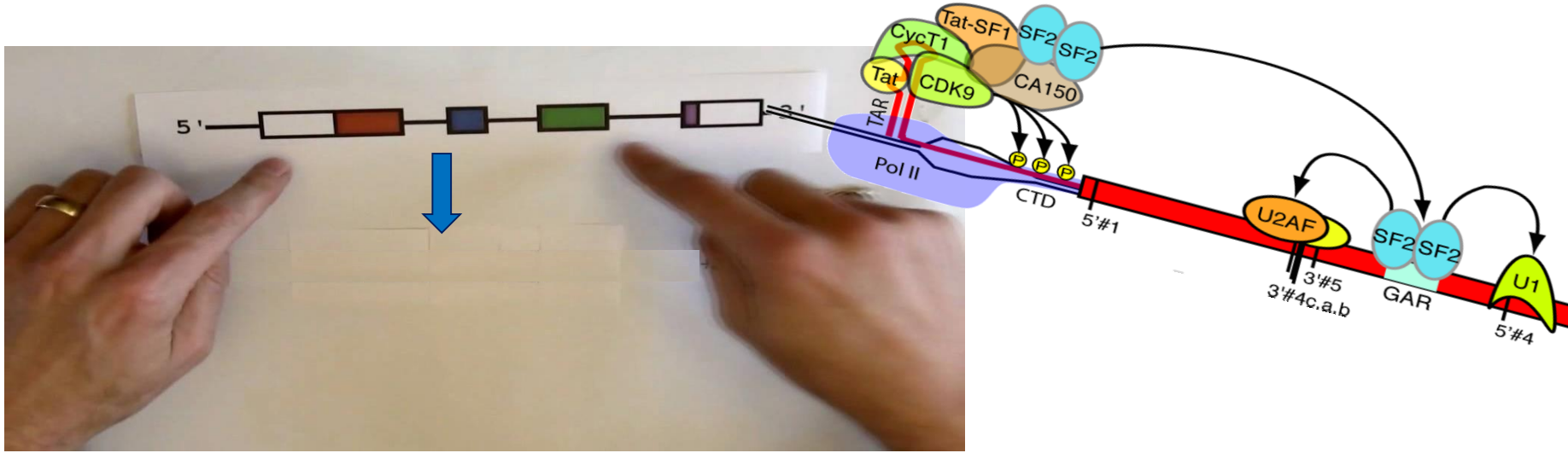
Genome technologies: Enabling personalized medicine

In diagnosis, treatment decisions and monitoring of disease

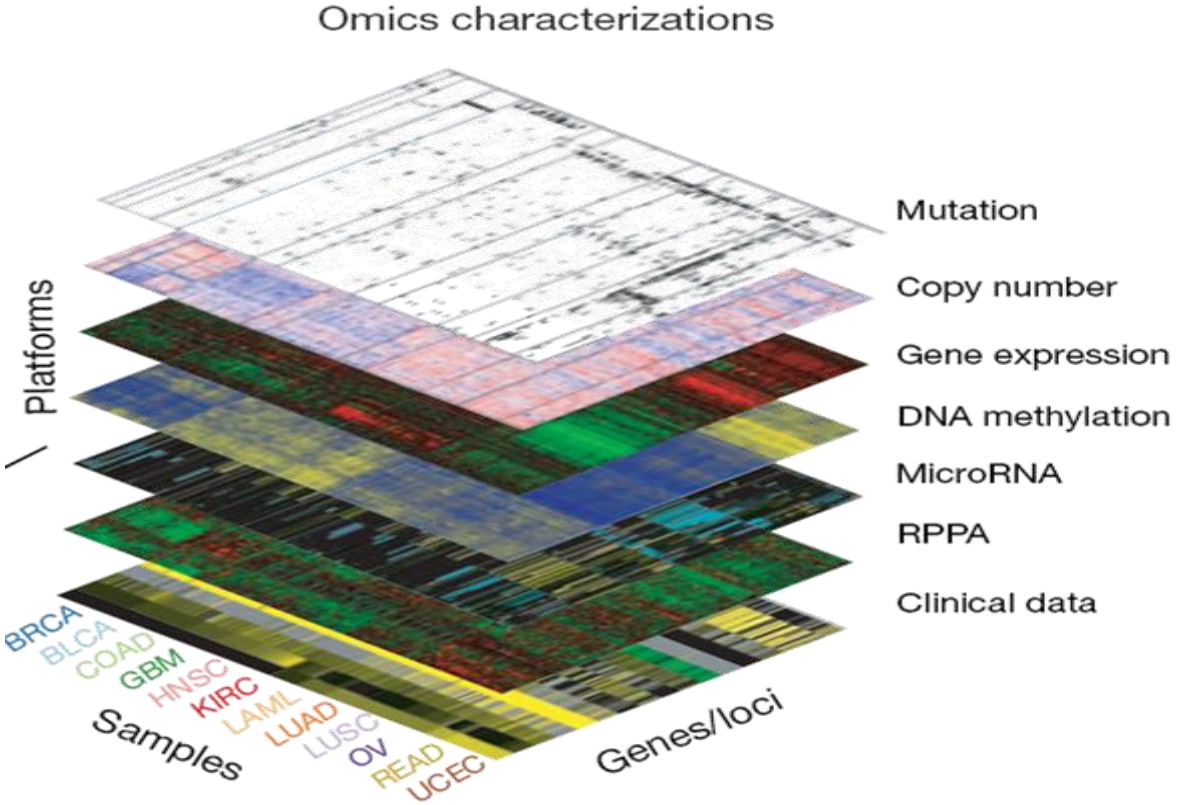
Next generation sequencing, whole-genome/transcriptome characterisation, simultaneous testing for virtually all mutations, transcript variants and abundancies, *etc.*

Importance of RNA in canceromics

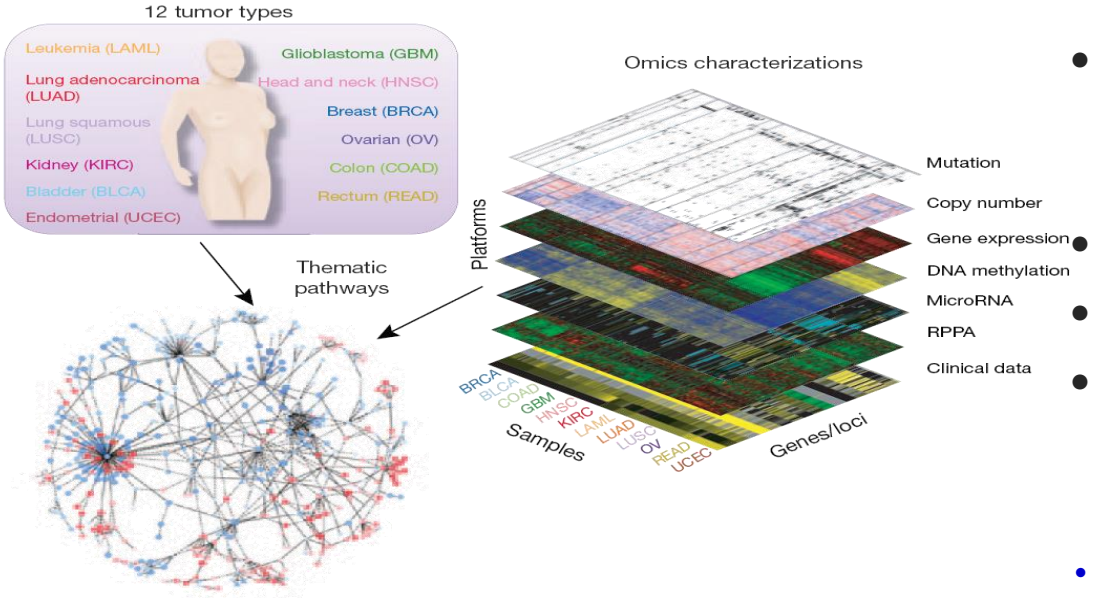
- Whereas DNA holds information on what the cell is capable of, RNA may reveal what it is actually doing
- Distorted RNA-processing cannot easily be inferred from DNA
  - Mutations at splice sites, mutation of splicing factors, chimeric RNAs, ..



Multilayer omics-data



The Cancer Genome Atlas Pan-Cancer analysis project



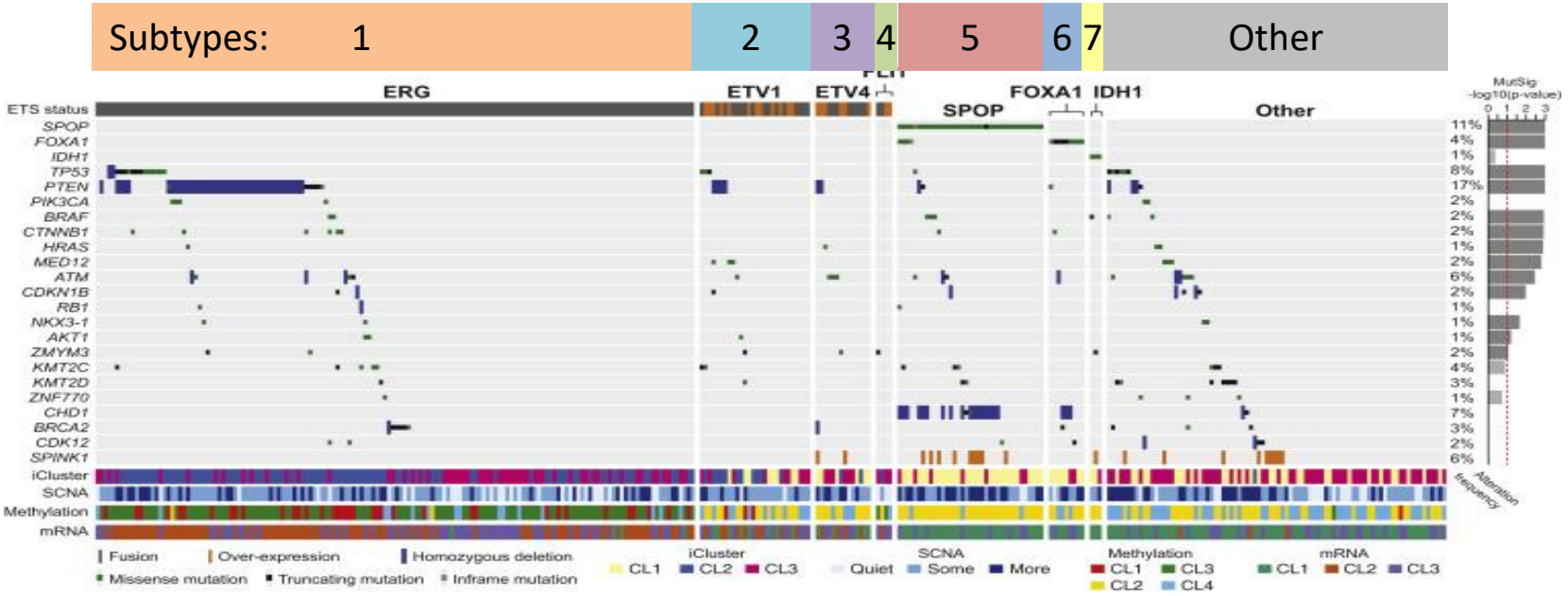
- Molecular patterns of 11091 patients/cancers representing 33 tumor types
- 2.5 petabytes
- 7 different data types
- Pan-cancer study:
  - 12 cancer types

- cancergenome.nih.gov
- nature.com/tcga
- intogen.org

Important to exploit such resources in conjunction with own research!

Different mulecular subtypes of prostate cancer?

The Cancer Genome Atlas



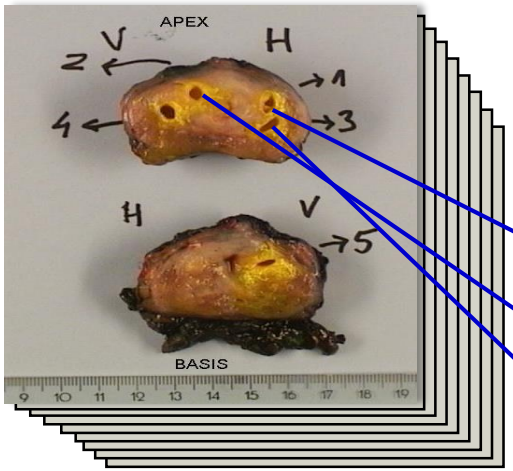
Cell, 2015

Competetive edge from own data!

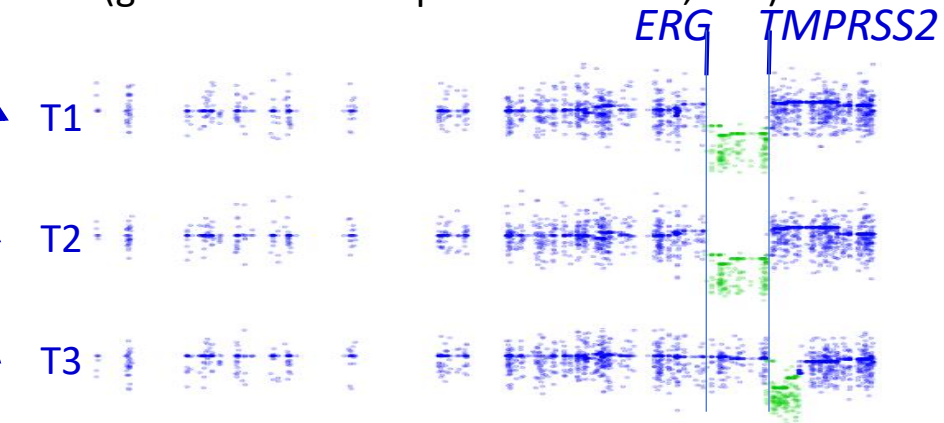
- Clinical data, including follow-up
- Supplmementary analyses from same samples
  - Technical wet-lab validation
  - Complementary molecular data
- Additional biopsies and longitudinal blood sampling
- Relevance to own population, home institution, *etc.*

Local research project: Enabling genome-based predictive medicine in multifocal prostate cancer

Multisample biobank enables heterogeneity aware analyses, in the development of diagnostic and prognostic biomarkers

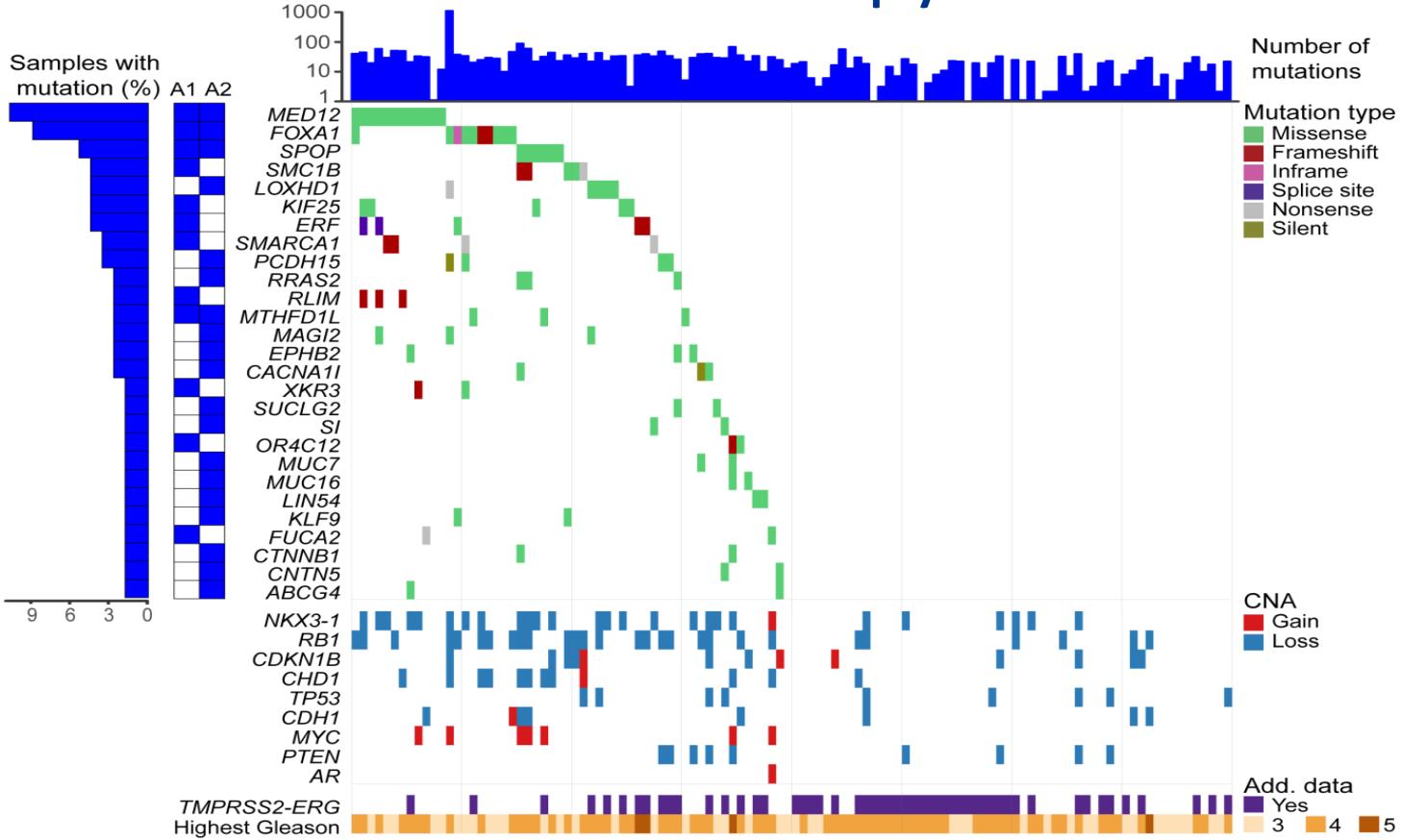


- Cohort with 571 patients (2010-2012), 67 % multifocal
- 3 to 8 frozen tissue samples from each
- Histopathological & clinical data (median 10 years follow-up)
- Molecular data (genome-scale seq of DNA & RNA, *etc.*)



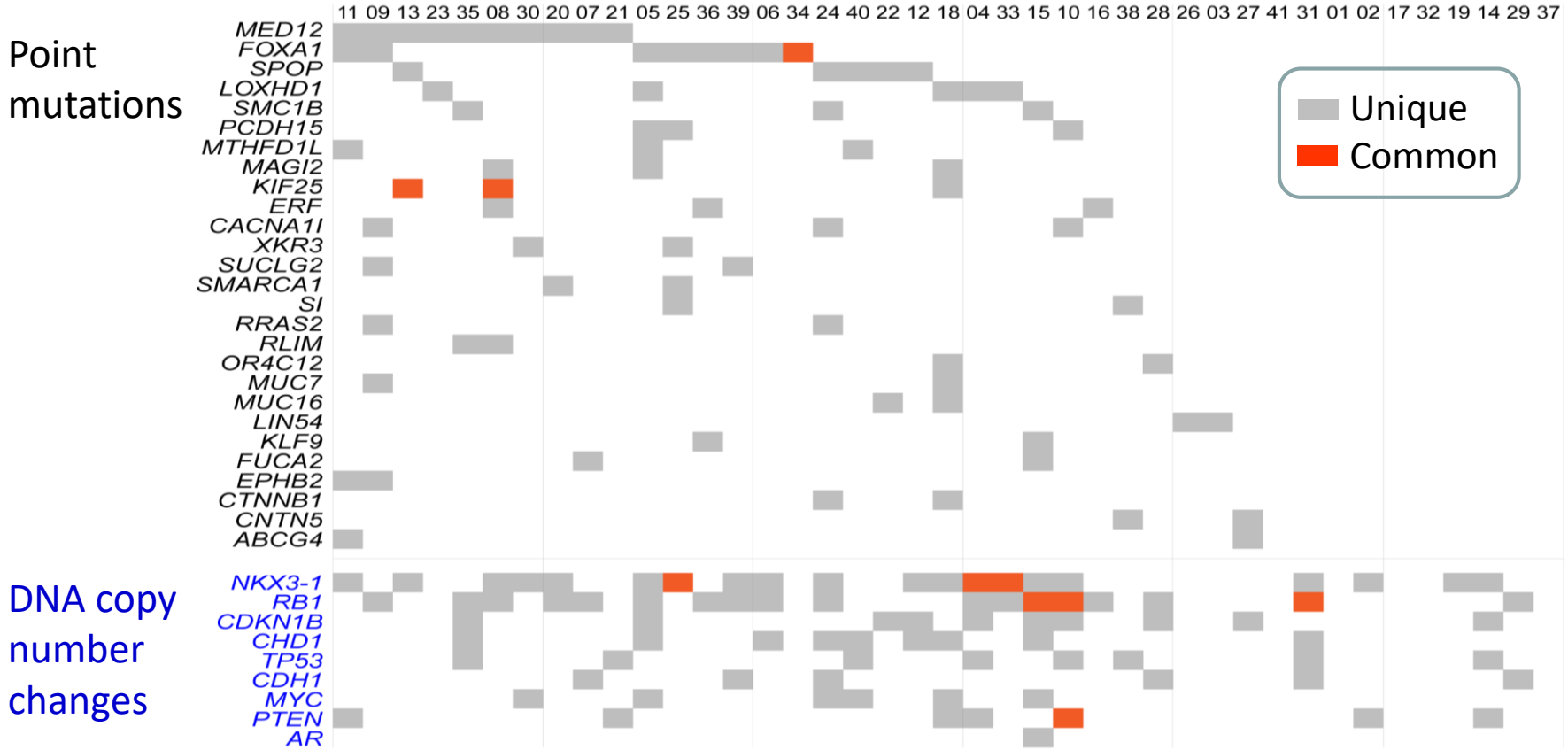
DNA copy numbers along chromosome 21

Point mutations and DNA copy number changes



Løvf *et al.*, Eur. Urol. 2019

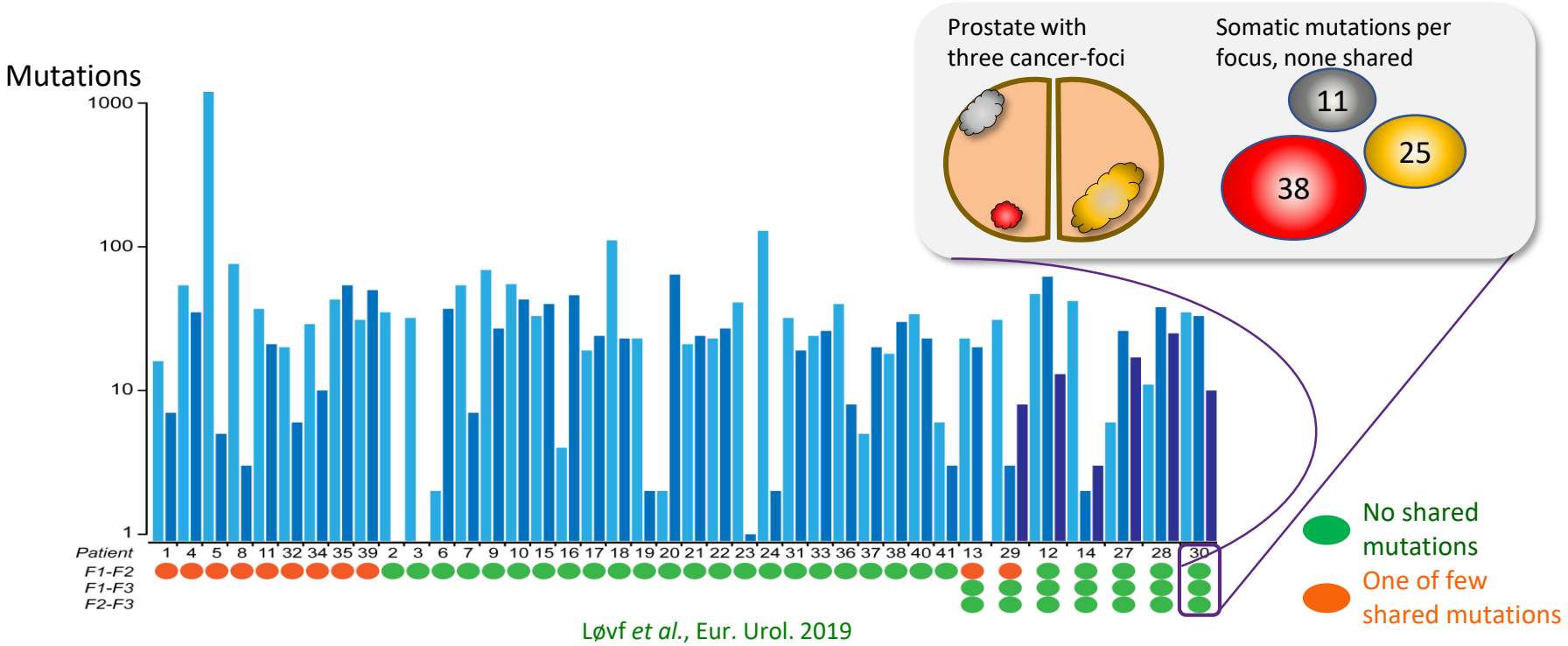
Separate foci have separate sets of somatic mutations



Løvf *et al.*, Eur. Urol. 2019

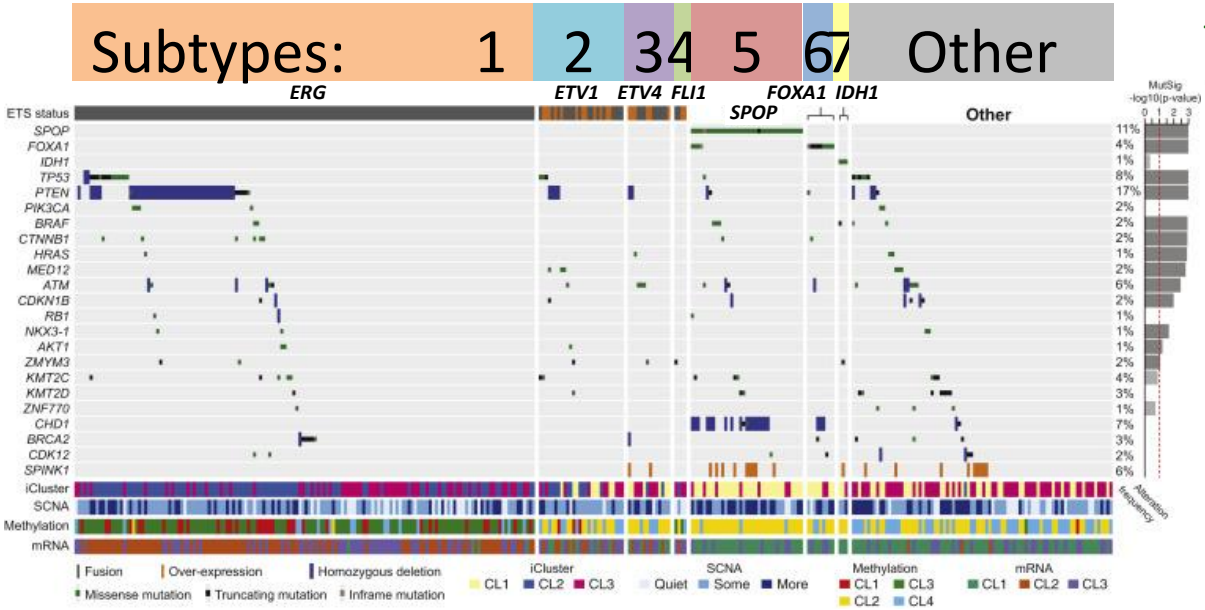
Separate foci have separate sets of somatic mutations

Molecular biomarkers from a random tissue sample can be irrelevant for the most significant cancer focus



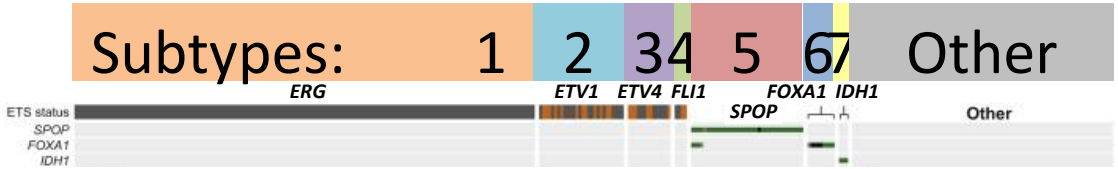
Løvf *et al.*, Eur. Urol. 2019

Molecular subtypes of prostate cancer?

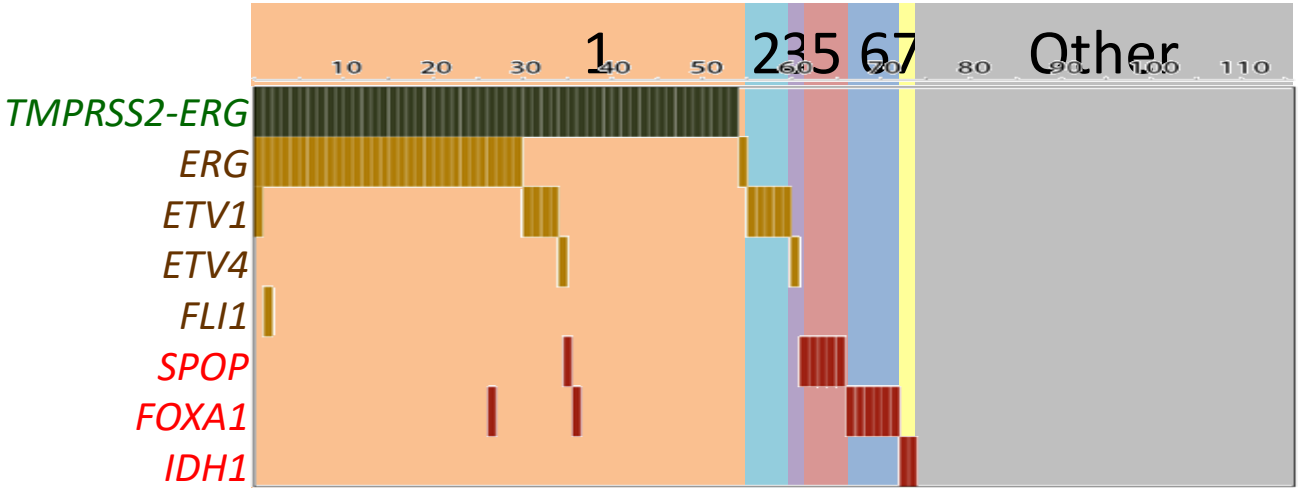


The Cancer Genome Atlas, Cell 2015

Molecular subtypes of prostate cancer?

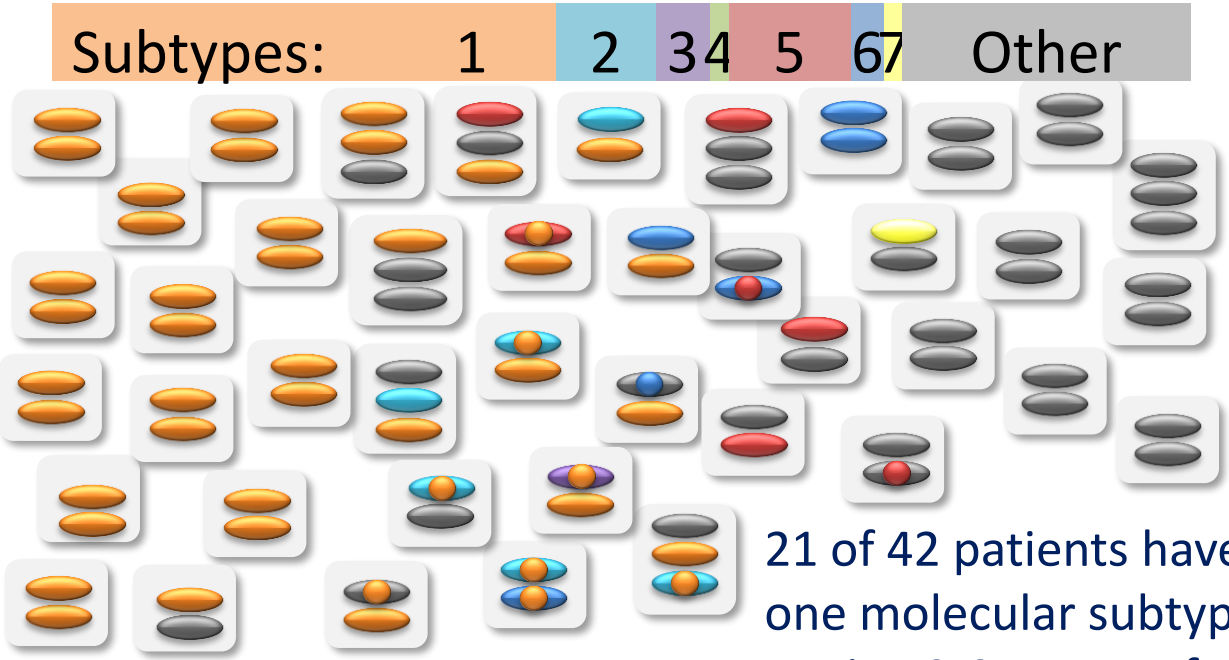


The Cancer Genome Atlas, Cell 2015



Norwegian cohort
Carm *et al.*, Sci Rep 2019

Molecular subtypes – per focus



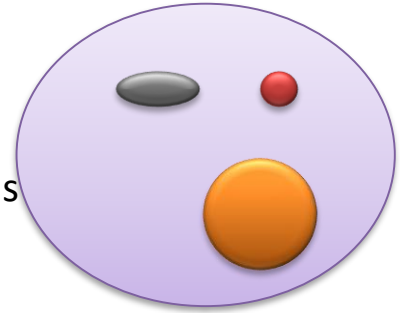
21 of 42 patients have more than one molecular subtype, when testing 2-3 tumour foci per patient

Carm *et al.*, Sci Rep 2019

Heterogenity in prostate cancer

- Tumour foci in primary cancers are *hetero*geneous
- Metastatic foci are to a  degree *homo*geneous

Molecular biomarkers from a random tissue sample can be irrelevant for the most significant cancer focus



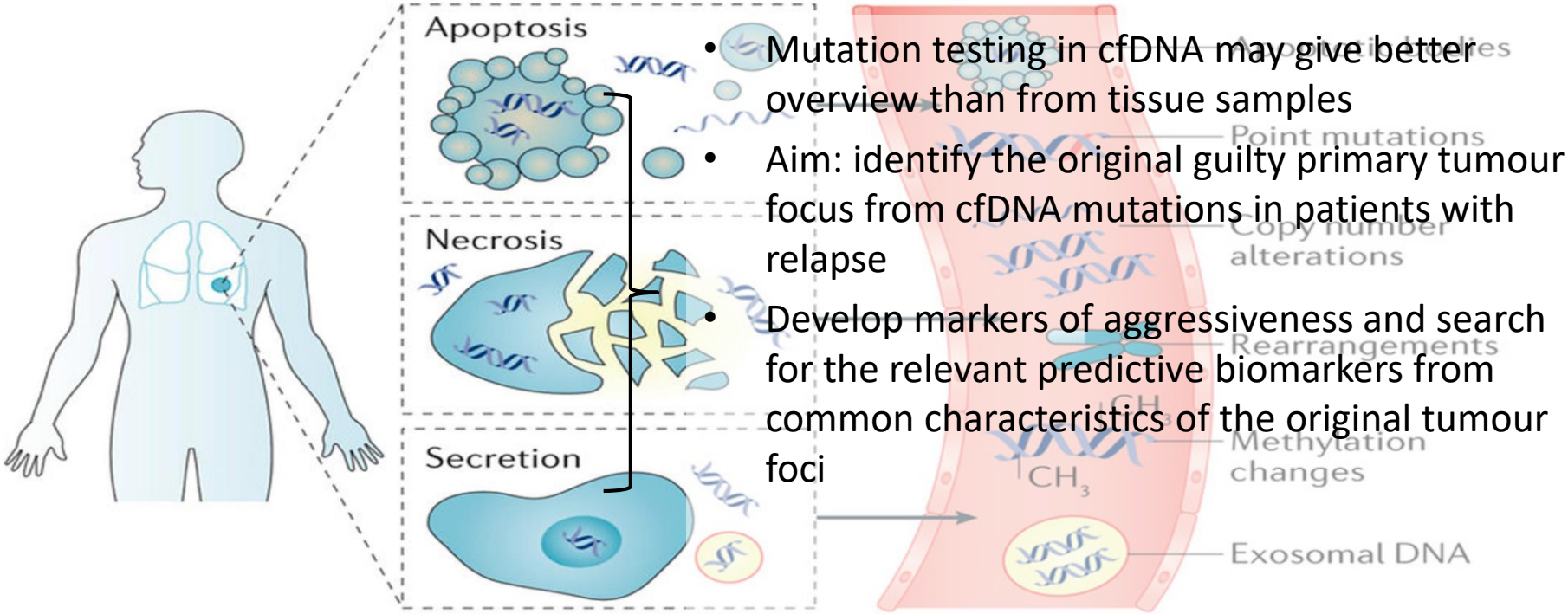
Løvf *et al.*,
Eur Urol 2019

Carm *et al.*,
Sci Rep 2019

Liu *et al.*,
Nat Med 2009

Kumar *et al.*,
Nat Med 2016

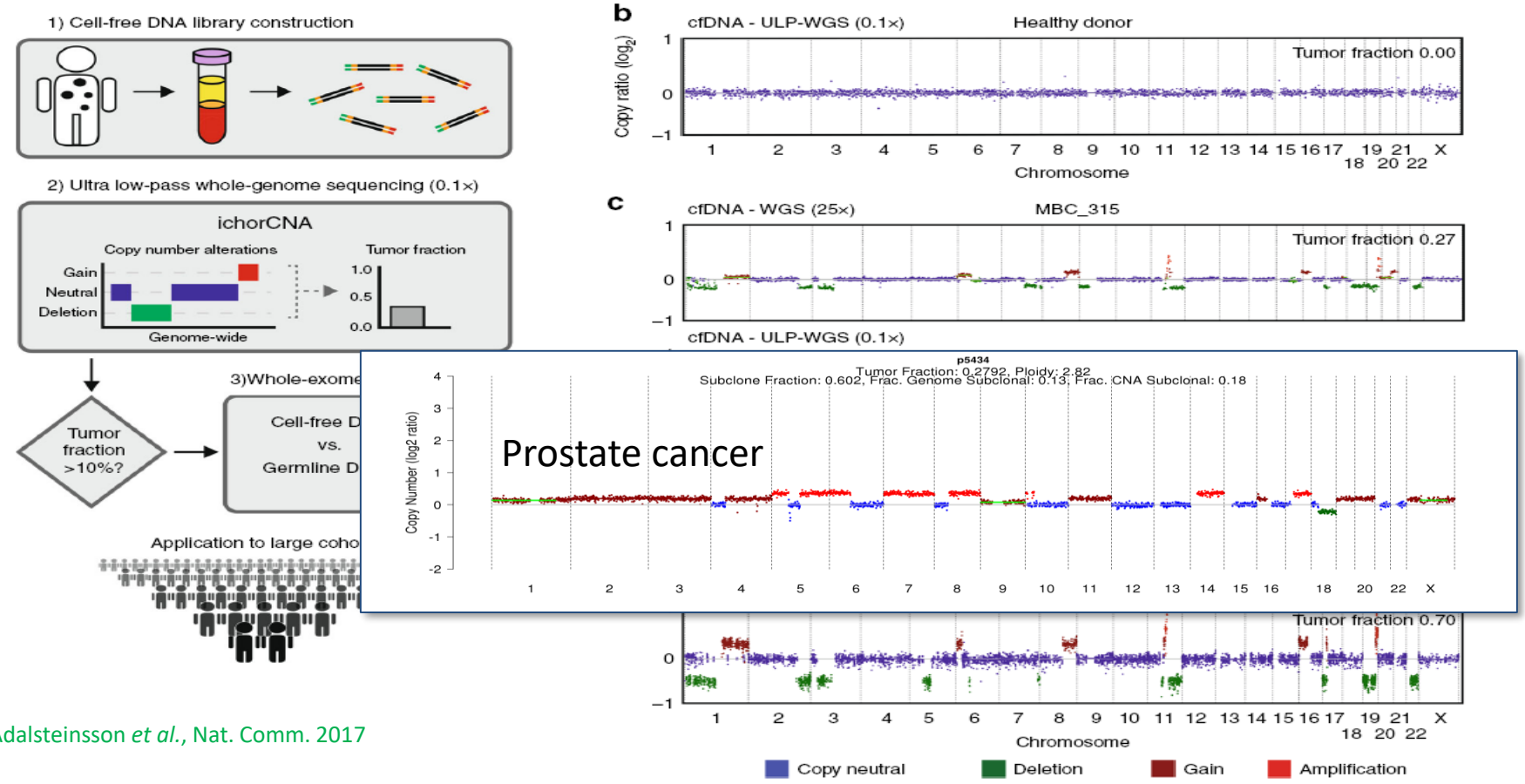
Liquid biopsies



- Mutation testing in cfDNA may give better overview than from tissue samples
- Aim: identify the original guilty primary tumour focus from cfDNA mutations in patients with relapse
- Develop markers of aggressiveness and search for the relevant predictive biomarkers from common characteristics of the original tumour foci

Wan *et al.* Nature Reviews Cancer 2017

Whole-exome seq in cell-free DNA



Adalsteinsson *et al.*, Nat. Comm. 2017

Some challenges to genome-based personalized cancer medicine

- Separation of driver *vs.* passenger mutations
- Development of specific targeted drugs is slow
- Tumours are heterogeneous
- Mutational spectrum changes throughout cancer development
- Unknown effects of combination therapies
- Handling of large amounts of patient sensitive genome sequence data