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

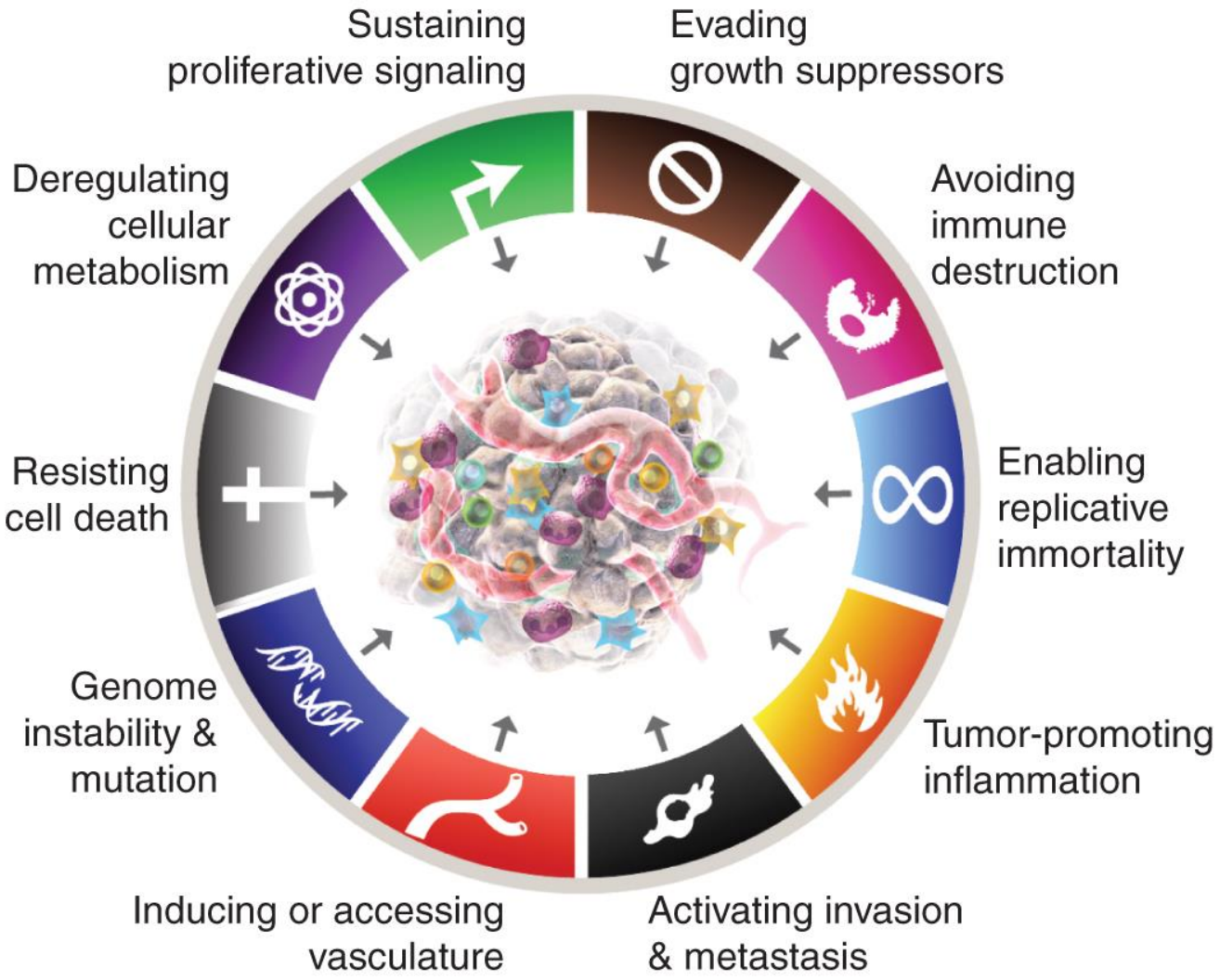
Genomics and predictive medicine

Cancer, a disease of the genome

Towards genome-based predictive cancer medicine

Genomic medicine enabling personalized medicine

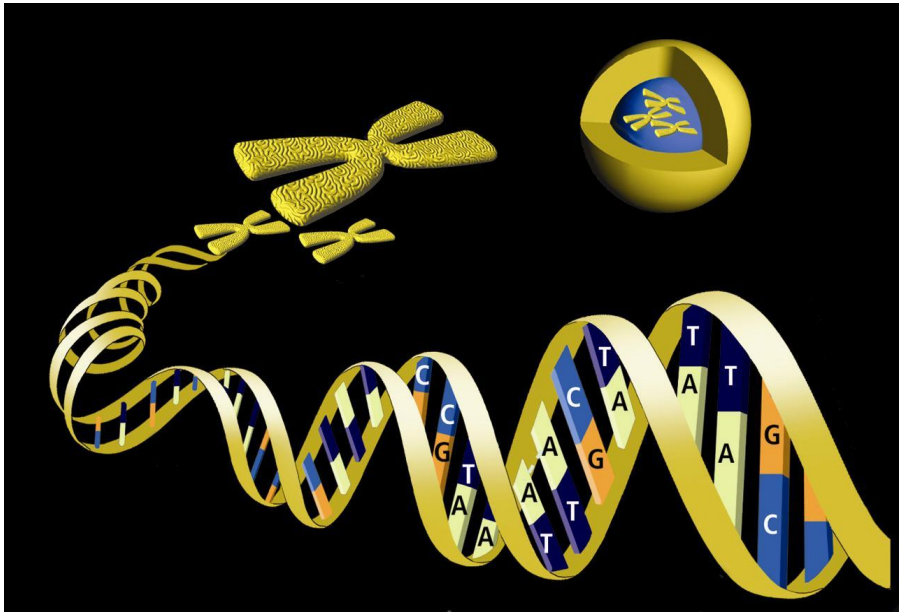
Hallmarks of cancer



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

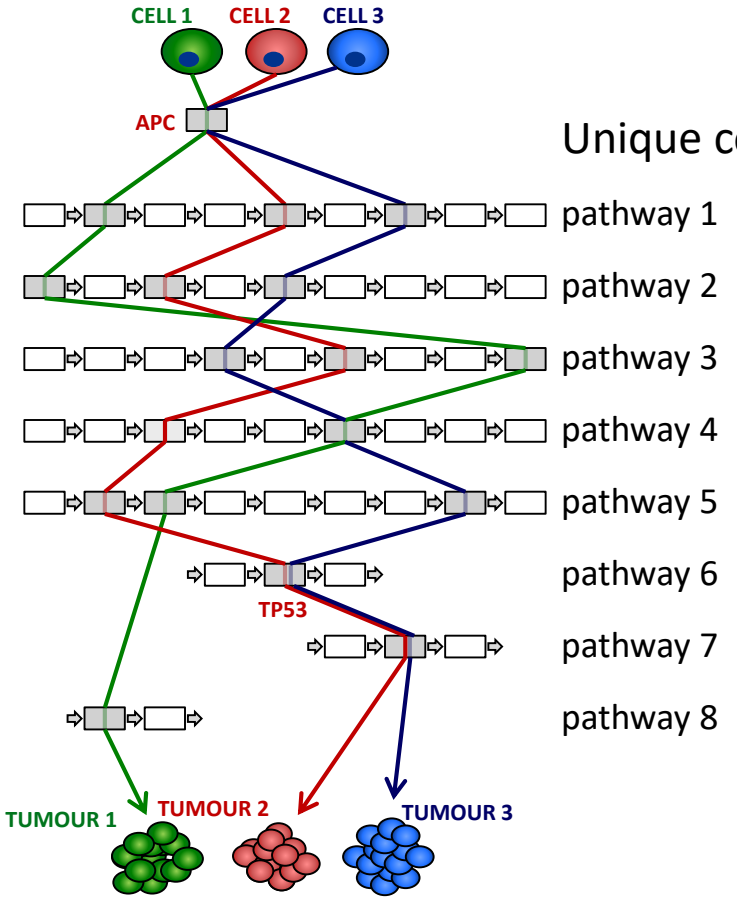
Cancer, a disease of the genome

- 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

.. and cancer genomes are much more unique than illustrated 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

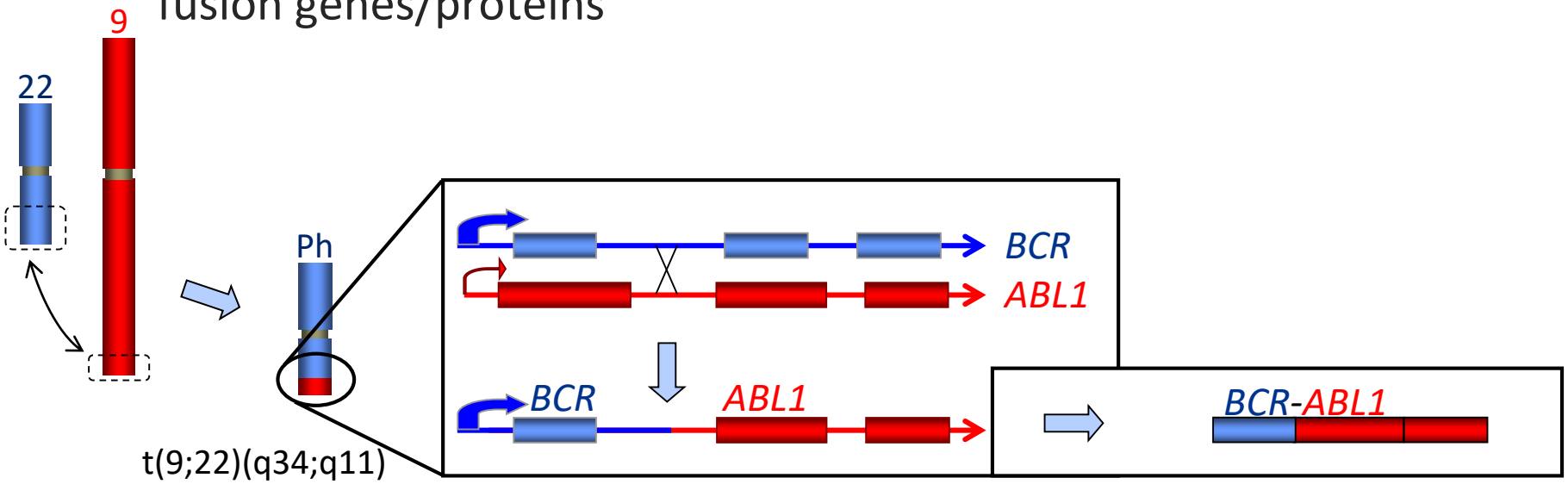
Genome-based predictive medicine
Aims to understand the relevant characteristics underlying a particular individual's disease (both disease and host factors), and then tailor therapy to that individual/disease

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

- Leukemia
- …

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

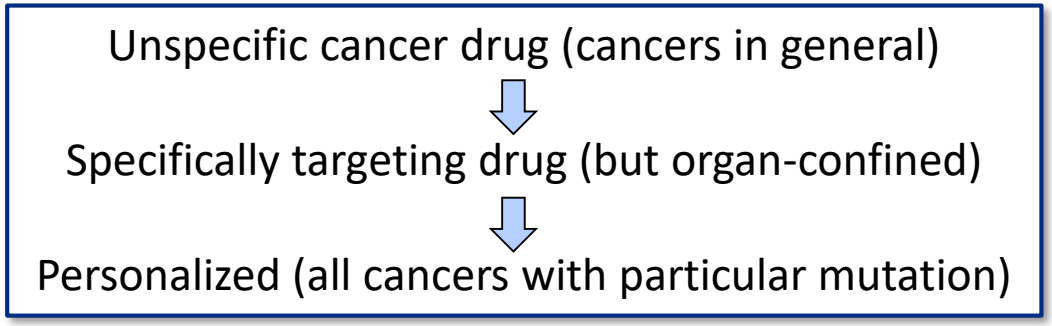


t(9;22)(q34;q11)
alias the Philadelphia chromosome

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

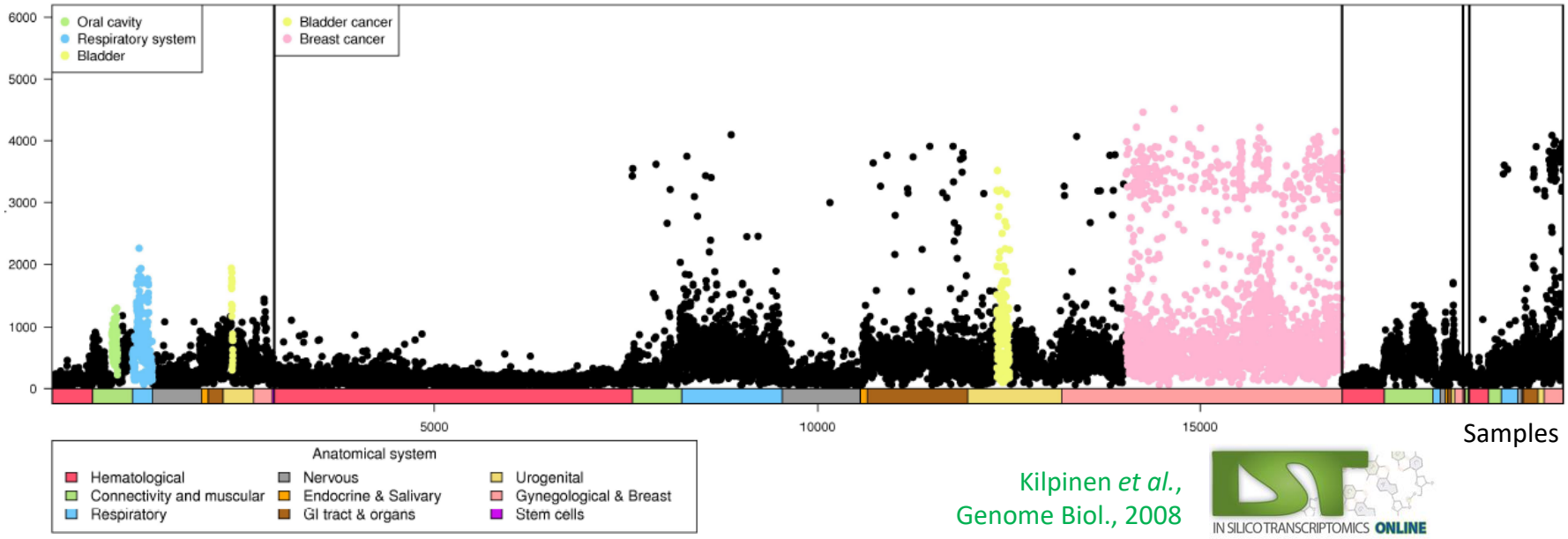


Nowell & Hungerford, Science 1960; Rowley, Nature 1973; Heisterkamp *et al.*, Nature 1983; Groffen *et al.*, Cell 1984; Druker *et al.*, Nat. Med. 1996 and NEJM 2001

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)

ERBB2 (HER2) mRNA levels

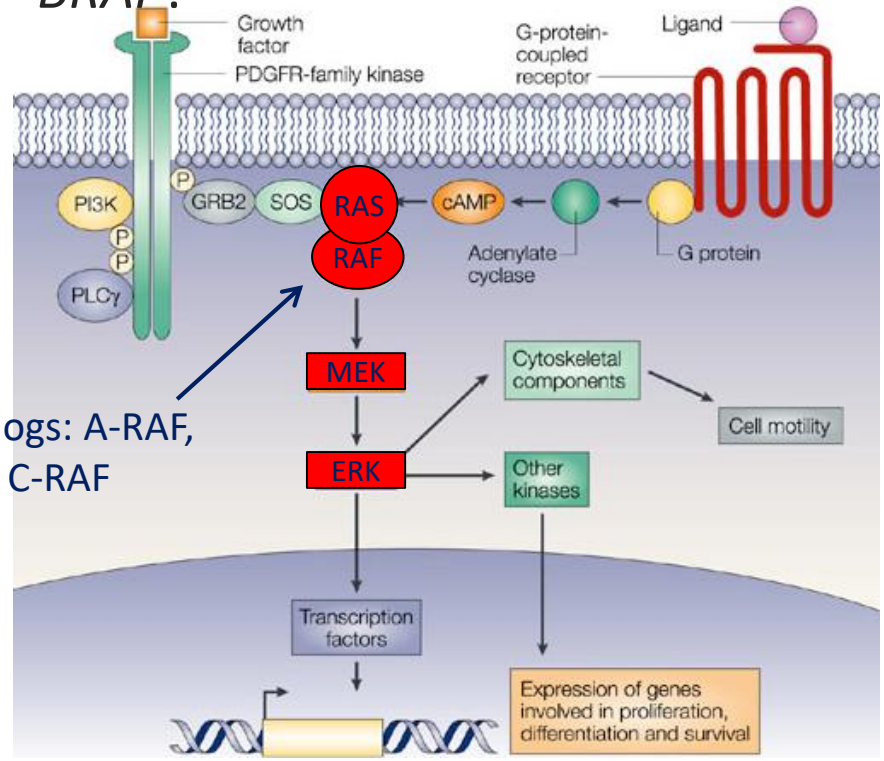


Kilpinen *et al.*, Genome Biol., 2008

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

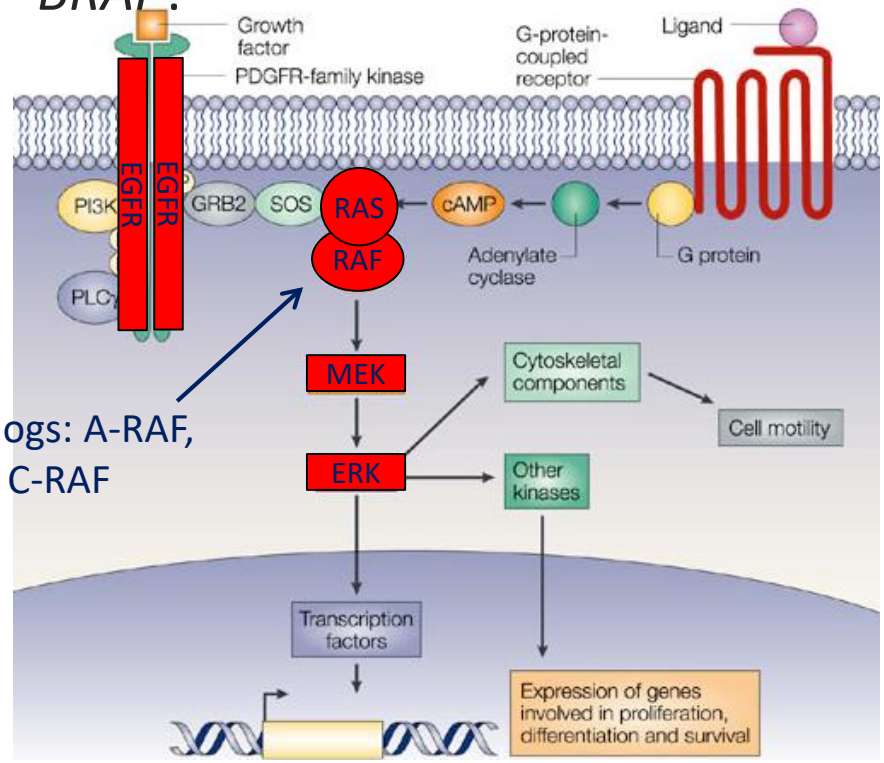
Part of the RAS-RAF-MEK-ERK (alias MAP-kinase) cell signalling pathway, commonly activated in cancer cells

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

Towards genome-based predictive cancer medicine

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

..and having low levels of EGFR



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

nature

Unresponsiveness of colon cancer to BRAF(V600E) inhibition through feedback activation of EGFR

Anirudh Prahallad, Chong Sun, Sidong Huang, Federica Di Nicolantonio, Ramon Salazar, Davide Zecchin, Roderick L. Beijersbergen, Alberto Bardelli & René Bernards

Affiliations | Contributions | Corresponding author

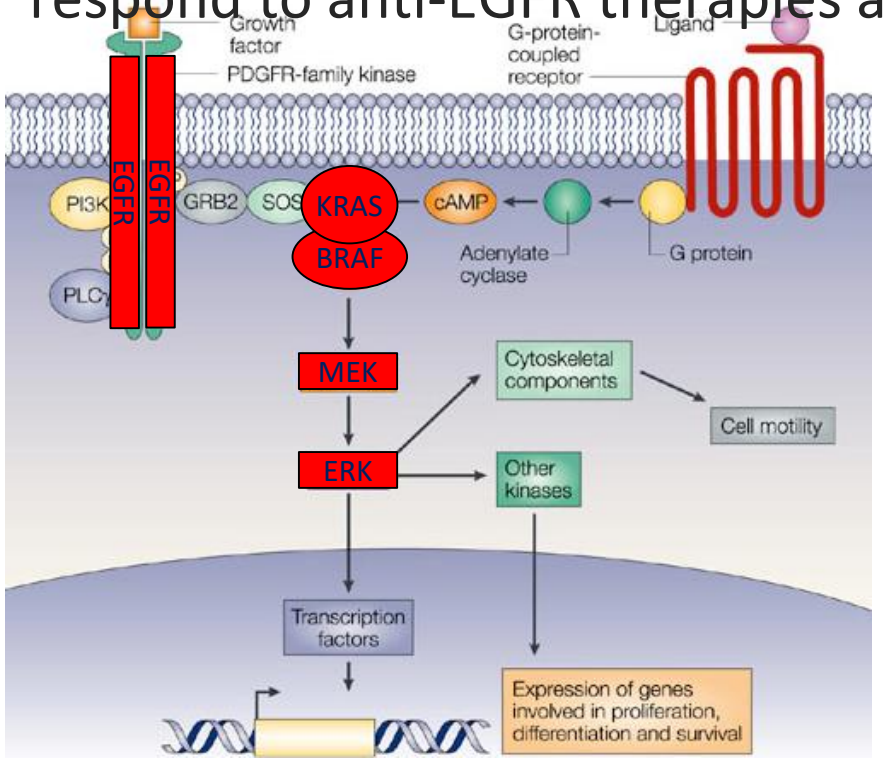
Nature **483**, 100–103 (01 March 2012) | doi:10.1038/nature10868

Received 28 September 2011 | Accepted 18 January 2012 | Published online 26 January 2012

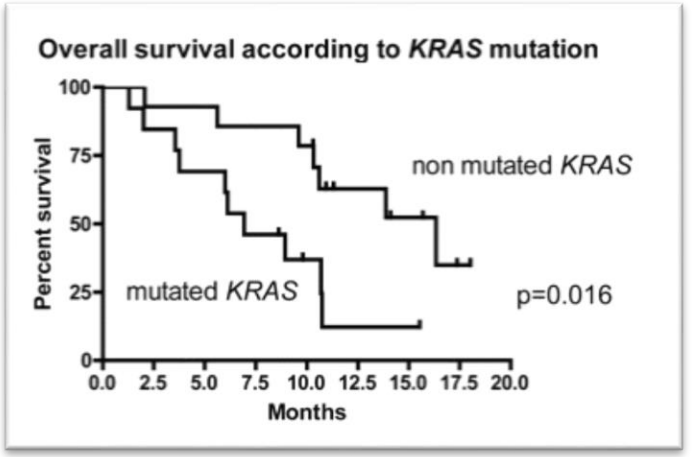
Inhibition of the BRAF(V600E) oncoprotein by the small-molecule drug PLX4032 (vemurafenib) is highly effective in the treatment of melanoma[1]. However, colon cancer patients harbouring the same *BRAF(V600E)* oncogenic lesion have poor prognosis and show only a very limited response to this drug[2, 3, 4]. To investigate the cause of the limited therapeutic effect of PLX4032 in *BRAF(V600E)* mutant colon tumours, here we performed an RNA-interference-based genetic screen in human cells to search for kinases whose knockdown synergizes with BRAF(V600E) inhibition. We report that blockade of the epidermal growth factor receptor (EGFR) shows strong synergy with BRAF(V600E) inhibition. We find in multiple *BRAF(V600E)* mutant colon cancers that inhibition of EGFR by the antibody drug cetuximab or the small-molecule drugs gefitinib or erlotinib is strongly synergistic with BRAF(V600E) inhibition, both *in vitro* and *in vivo*. Mechanistically, we find that BRAF(V600E) inhibition causes a rapid feedback activation of EGFR, which supports continued proliferation in the presence of BRAF(V600E) inhibition. Melanoma cells express low levels of EGFR and are therefore not subject to this feedback activation. Consistent with this, we find that ectopic expression of *EGFR* in melanoma cells is sufficient to cause resistance to PLX4032. Our data suggest that *BRAF(V600E)* mutant colon cancers (approximately 8–10% of all colon cancers[2, 3, 5]), for which there are currently no targeted treatment options available, might benefit from combination therapy consisting of BRAF and EGFR inhibitors.

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



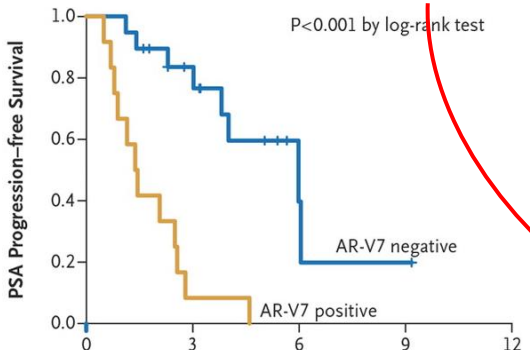
Lièvre *et al.*, Cancer Res., 2006

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

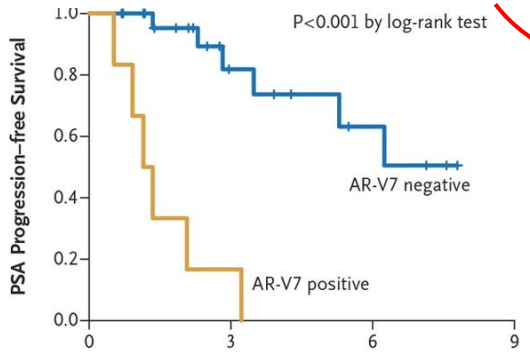
Towards genome-based predictive cancer medicine

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

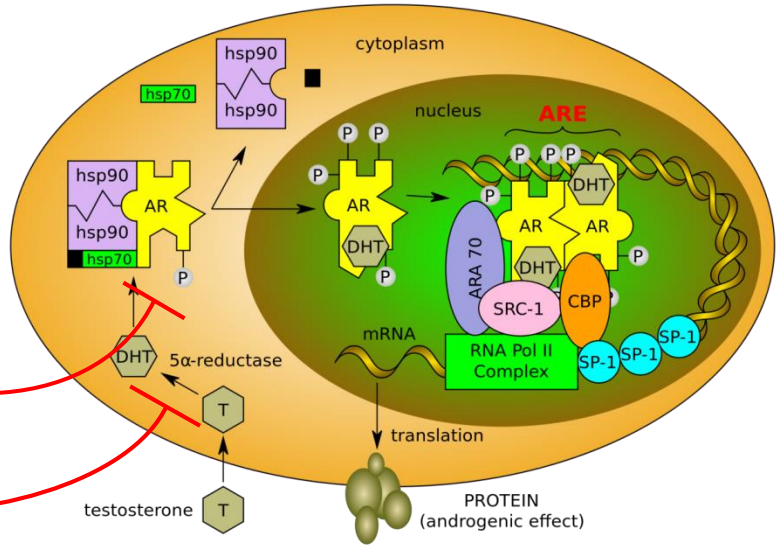
Patients treated with Enzalutamide



Patients treated with Abiraterone

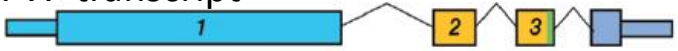


Antonarakis *et al.*, NEJM 2014



AR full length transcript

AR-v7 transcript



Towards genome-based predictive cancer medicine

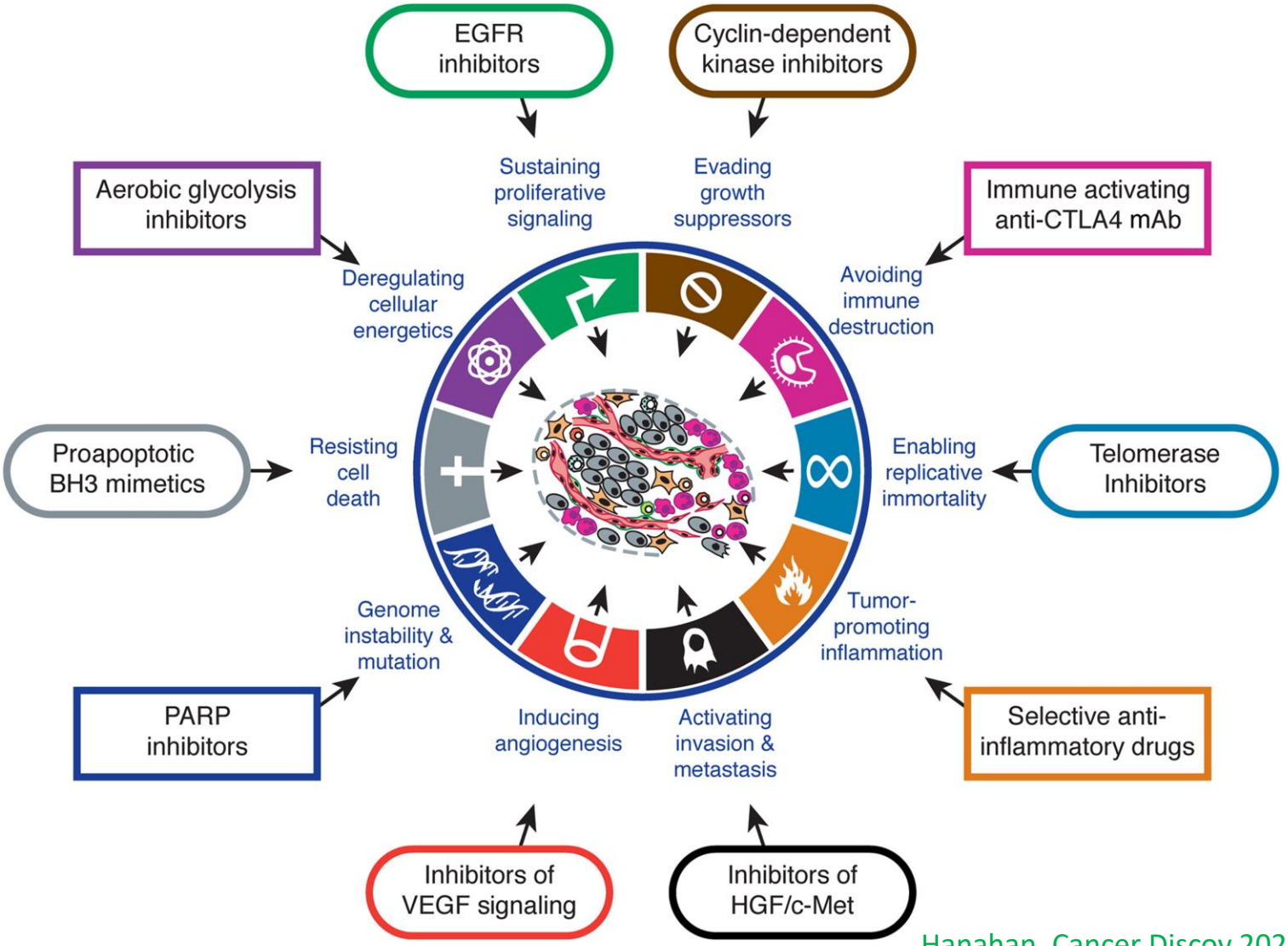
Growing list of targeted drugs with predictive biomarkers across several cancer types

- 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	**Genome-based PCM**
⇩	⇩
Targeting individual actionable genes relevant to the cancer in question	Genome analyses, testing all of DNA/RNA

Therapeutic targeting of the Hallmarks of cancer

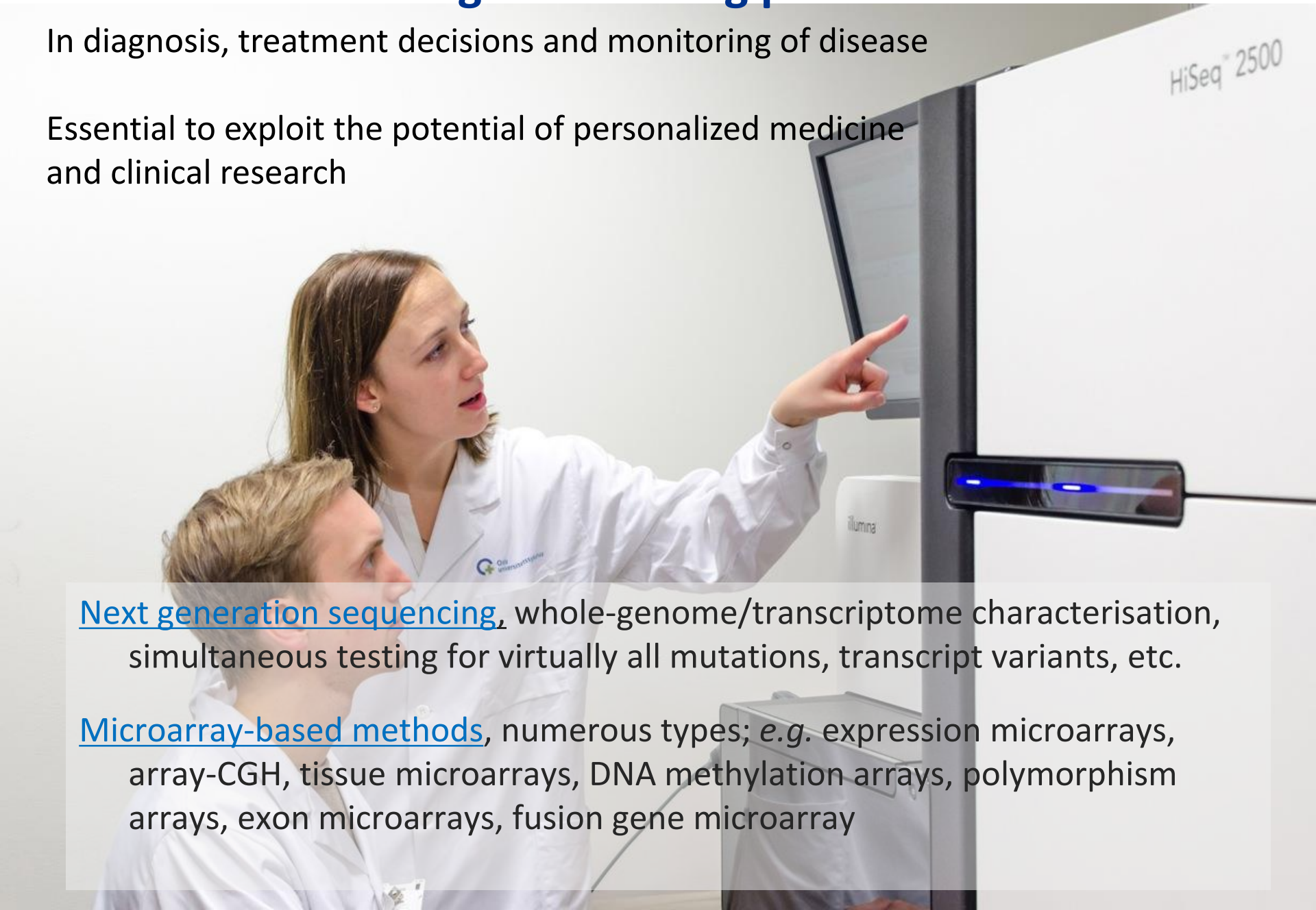


Hanahan, Cancer Discov 2022
Hanahan & Weinberg, Cell 2000 & 2011

Genome technologies: Enabling personalized medicine

In diagnosis, treatment decisions and monitoring of disease

Essential to exploit the potential of personalized medicine and clinical research

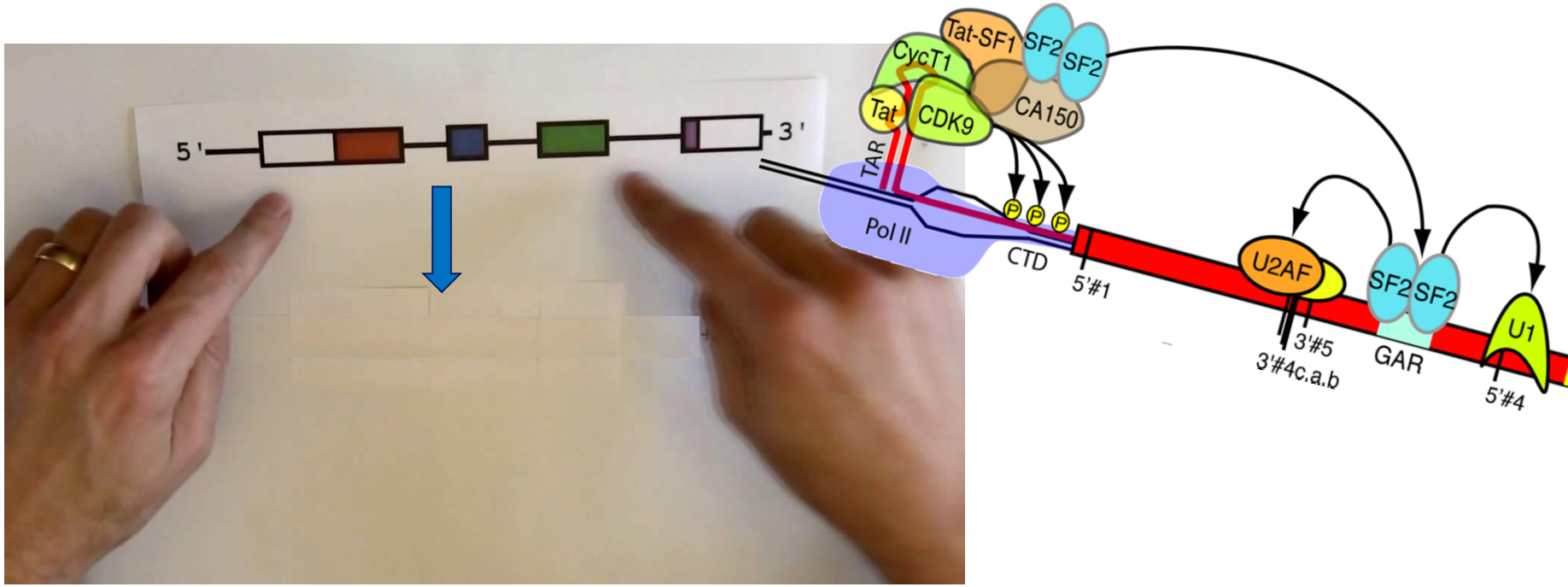


Next generation sequencing, whole-genome/transcriptome characterisation, simultaneous testing for virtually all mutations, transcript variants, etc.

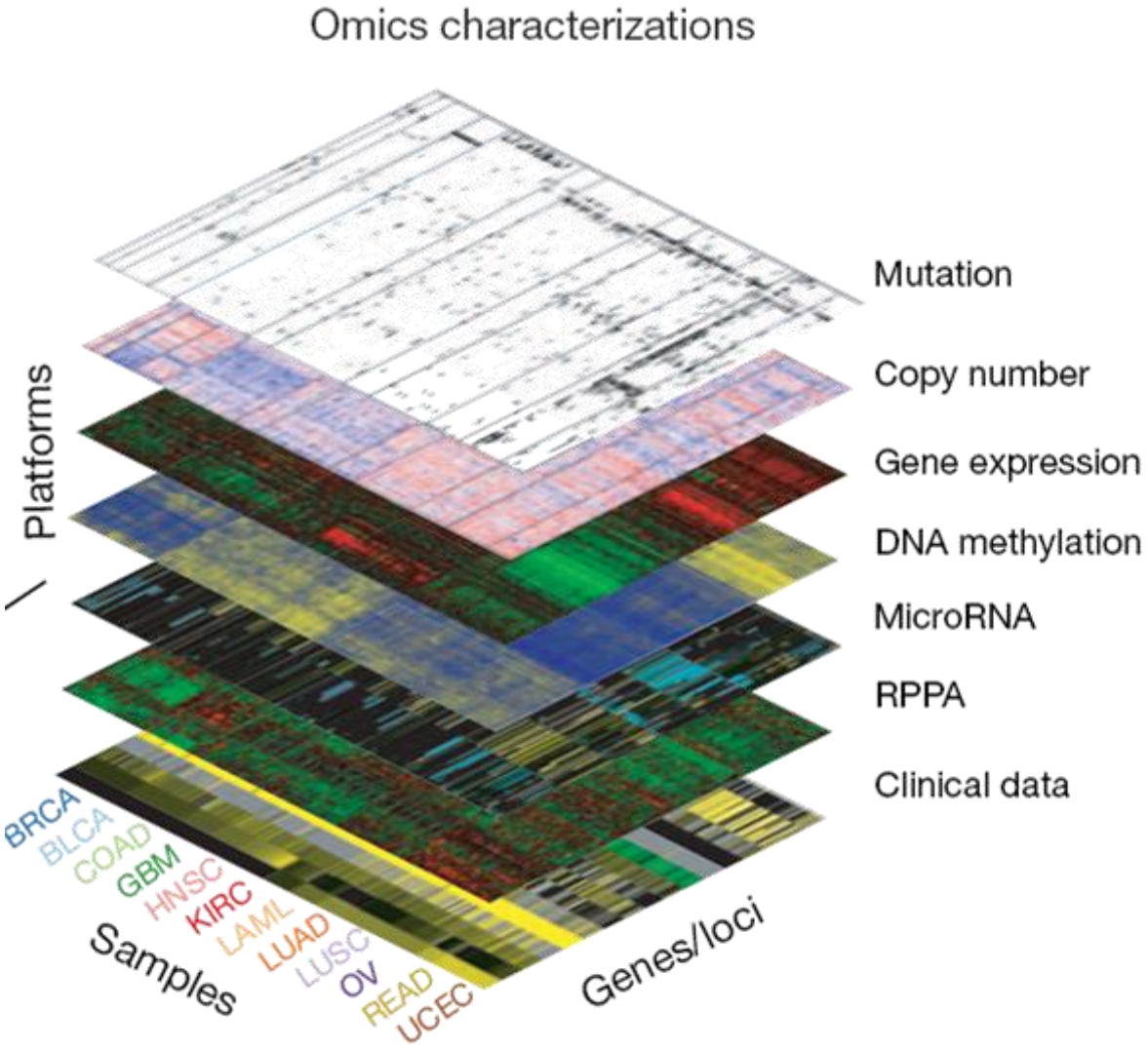
Microarray-based methods, numerous types; *e.g.* expression microarrays, array-CGH, tissue microarrays, DNA methylation arrays, polymorphism arrays, exon microarrays, fusion gene microarray

Importance of RNA in canceromics

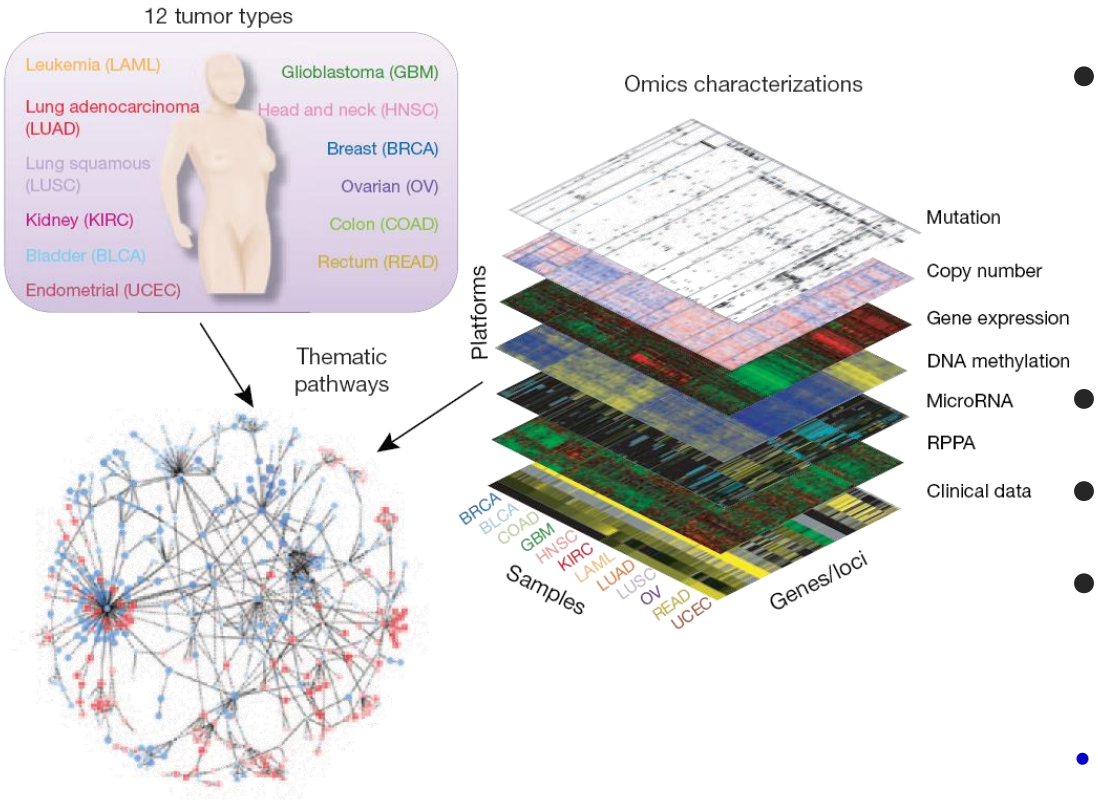
- Whereas DNA holds information on what the cell is capable of, RNA may reveal what it is actually doing
- Distorted RNA-processing cannot easily be inferred from DNA
  - Mutations at splice sites, mutation of splicing factors, chimeric RNAs, ..



Multilayer omics-data



The Cancer Genome Atlas Pan-Cancer analysis project



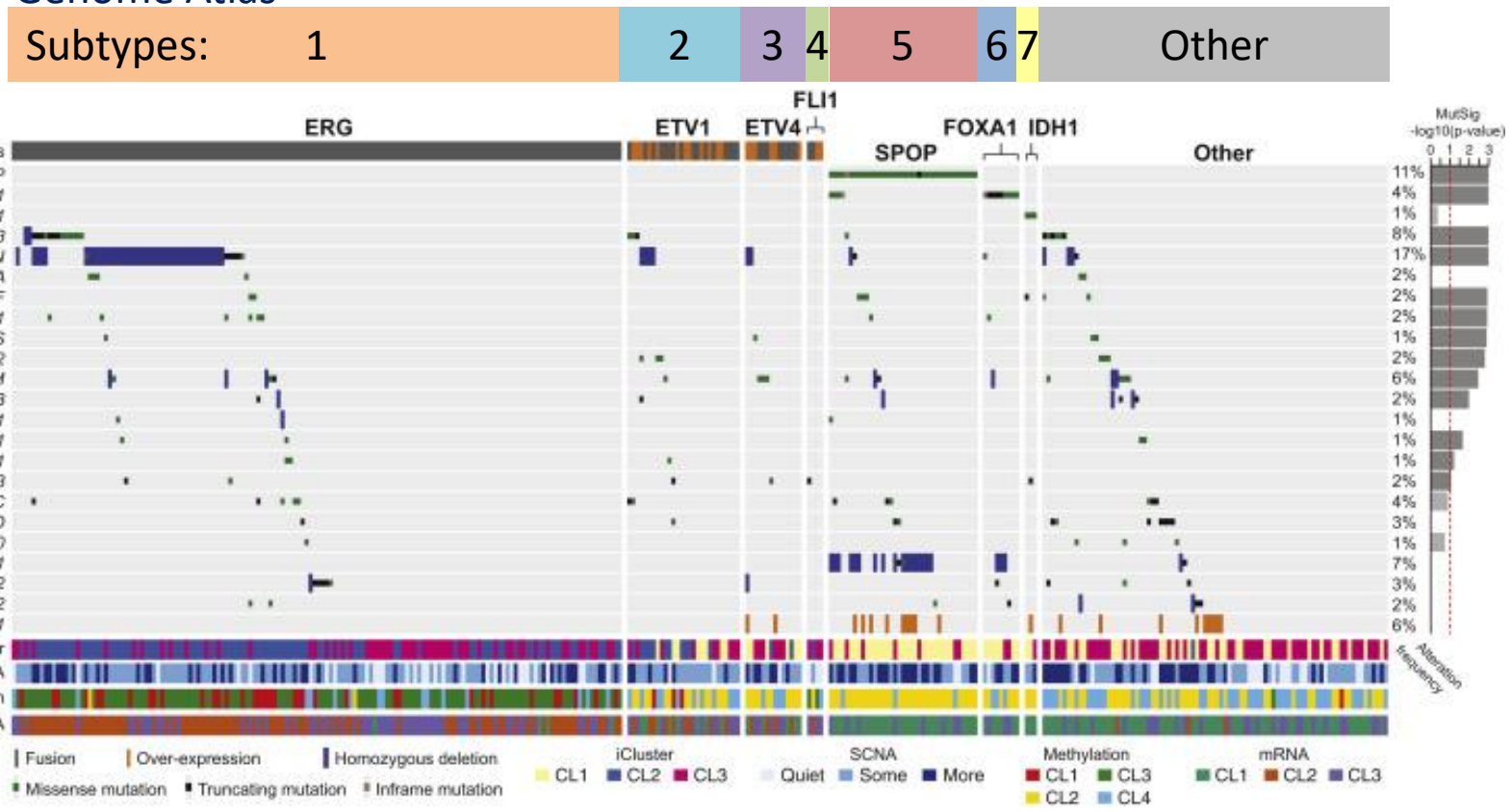
- Molecular patterns of 11091 patients/cancers representing 33 tumor types
- 2.5 petabytes
- 7 different data types
- Pan-cancer study:
  - 12 cancer types

- 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

Available cancer genomics raw data

Project renewal

#2313: Cancer specific transcripts for biomarker discovery

OMB control number: 0925-0670 Expiration date: March 31, 2016

SO: Peder Utne

8173-4	AML Sequencing Project (phs000159.v6.p4) General Research Use (phs000159.v6.p4.c1), NHGRI	SO review revised,GRANTED	2015-02-03	view
8174-5	TCGA - The Cancer Genome Atlas (phs000178.v8.p7) General Research Use (phs000178.v8.p7.c1), TCGA	SO review revised,GRANTED	2015-02-03	view
8175-4	The Cancer Genome Characterization Initiative (phs000235.v6.p1) Cancer Research and General Methods (phs000235.v6.p1.c1), eNCI DAC	SO review revised,GRANTED	2015-02-04	view
8177-4	Genentech whole-genome sequencing of a non-small cell lung carcinoma (phs000299.v2.p1) Health/Medical/Biomedical (MDS) (phs000299.v2.p1.c1), eNCI DAC	SO review revised,GRANTED	2015-02-04	view
13643-4	Characterization of complex chromosomal aberrations in primary prostate cancer genomes (phs000330.v1.p1) For general medical research, for non-profit only (phs000330.v1.p1.c1), NHGRI	SO review revised,GRANTED	2015-02-03	view
13644-4	Discovery of Non-ETS Gene Fusions in Human Prostate Cancer using Next Generation RNA Sequencing (phs000310.v1.p1) For general medical research, for non-profit only (phs000310.v1.p1.c1), NHGRI	SO review revised,GRANTED	2015-02-03	view
13645-4	FusionSeq: a Modular Framework for Finding Gene Fusions by Analyzing Paired-End RNA Sequencing Data (phs000311.v1.p1) For general medical research, for non-profit only (phs000311.v1.p1.c1), NHGRI	SO review revised,GRANTED	2015-02-03	view
13646-4	Genomic Sequencing of Colorectal Adenocarcinomas (phs000374.v1.p1) General Research Use (phs000374.v1.p1.c1), NHGRI	SO review revised,GRANTED	2015-02-03	view
13647-4	Epigenetic Profiling of Human Colorectal Cancer (phs000385.v1.p1) General Research Use (phs000385.v1.p1.c1), eNCI DAC	SO review revised,GRANTED	2015-02-04	view
19362-3	Prostate Cancer Genome Sequencing Project (phs000447.v1.p1) General Research Use (phs000447.v1.p1.c1), NHGRI	SO review revised,GRANTED	2015-02-03	view
19363-3	Prostate Cancer Genome Sequencing Project (phs000447.v1.p1) Cancer Research Only (phs000447.v1.p1.c2), NHGRI	SO review revised,GRANTED	2015-02-03	view
19364-3	MPC_Transcriptome sequencing to identify non-coding RNAs in prostate cancer (phs000443.v1.p1) Cancer Research and General Methods (phs000443.v1.p1.c1), eNCI DAC	SO review revised,GRANTED	2015-02-04	view
19365-3	Genomic Sequencing of Medulloblastoma (phs000504.v2.p2) Disease-Specific (Cancer) (phs000504.v2.p2.c1), NHGRI	SO review revised,GRANTED	2015-02-03	view
19366-3	RNA sequencing of human glioma stem cells (phs000505.v2.p1) General Research Use (MDS) (phs000505.v2.p1.c1), eNCI DAC	SO review revised,GRANTED	2015-02-04	view
25620-2	Somatic L1 Retrotransposition of Colorectal Tumors (phs000536.v1.p1) General Research Use (phs000536.v1.p1.c1), NIGMS	SO review revised,GRANTED	2015-02-07	view
25621-2	Germline Sequencing For Aggressive Prostate Carcinoma (phs000661.v1.p1) Disease-Specific (Prostate Cancer) (phs000661.v1.p1.c1), NHGRI	SO review revised,GRANTED	2015-02-03	view
35060-1	Whole exome sequencing of circulating tumor cells (CTCs) as a window into metastatic cancer (phs000717.v1.p1) Disease-Specific (Prostate Cancer, MDS) (phs000717.v1.p1.c1), eNCI DAC	SO review		view

Competetive edge from own data!

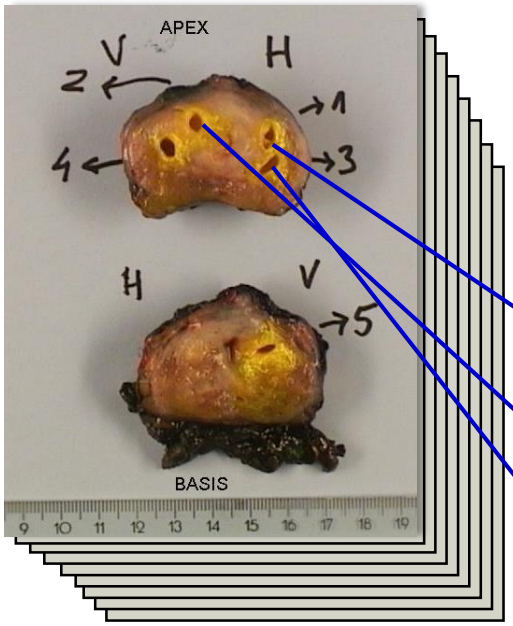
- Clinical data, including follow-up
- Supplmementary analyses from same samples
  - Technical wet-lab validation
  - Complementary molecular data
- Additional biopsies and longitudinal blood sampling
- Relevance to own population, home institution, *etc.*

Data storage and computation

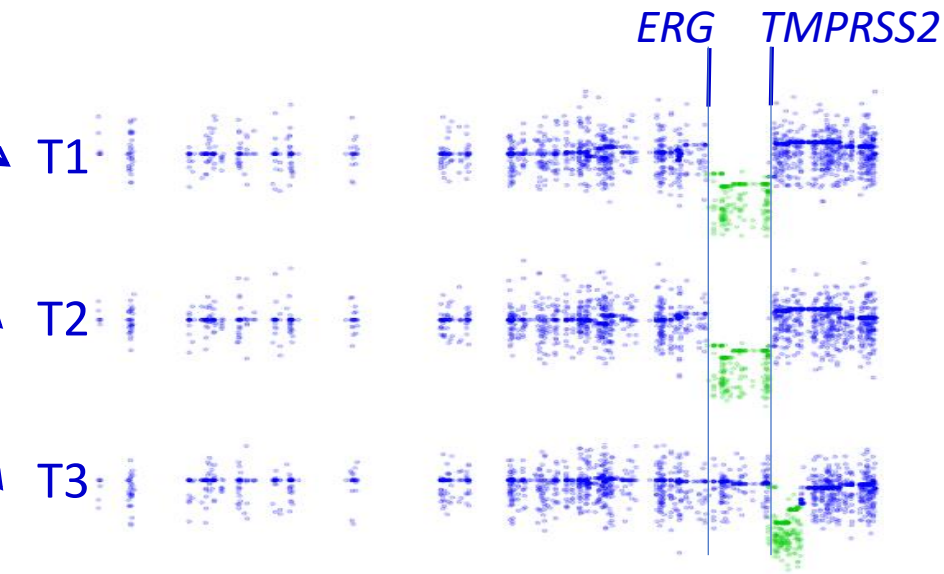
- Exome sequencing raw data
  - Approx 100 Gb fastq files per patient
  - N=100 => 10 Tb data to be transferred (weeks) to secure server at TSD@USIT, UiO
- Processed data
  - High-performance computer Colossus (weeks)
  - Fastq => SAM => BAM files, approx 30 Gb / patient
  - Mutation calling and annotation

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

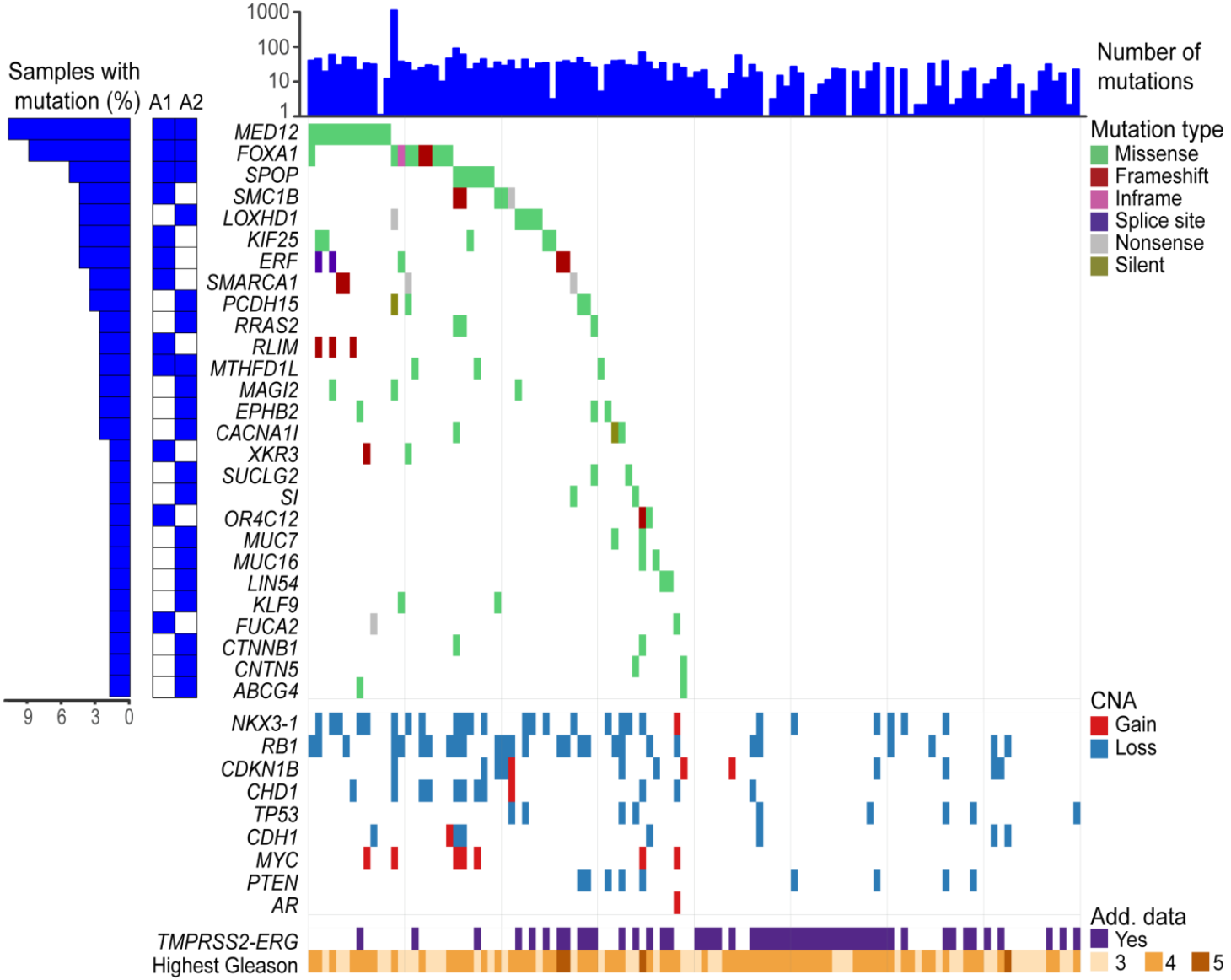


- Cohort with 571 patients (2010-2012), 67 % multifocal
- 3 to 8 frozen tissue samples from each
- Histopathological & clinical data (median 8.7 years follow-up)
- Molecular data (genome-scale seq of DNA & RNA, *etc.*)



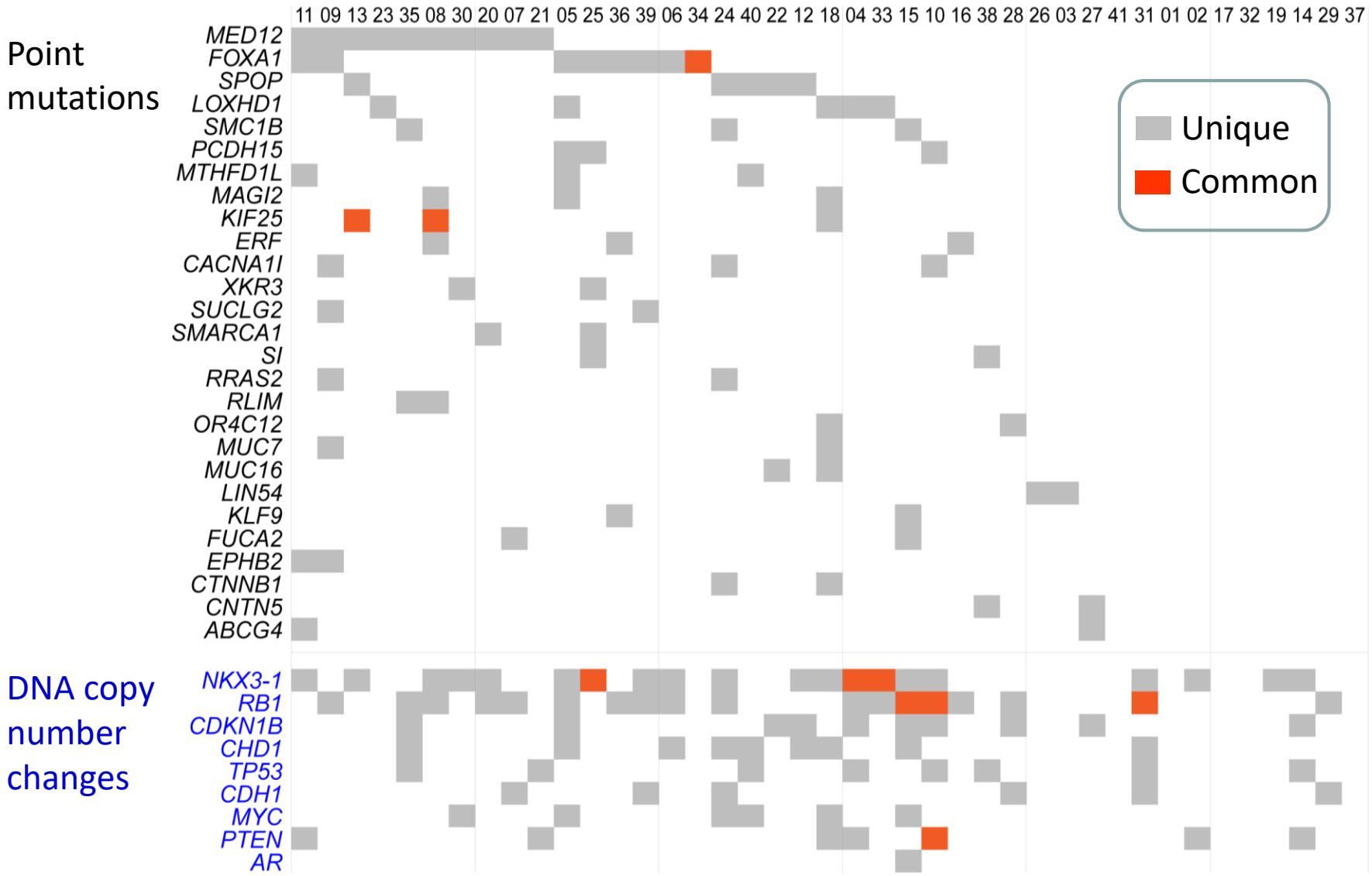
DNA copy numbers along chromosome 21

Point mutations and DNA copy number changes



Løvf *et al.*, Eur. Urol. 2019

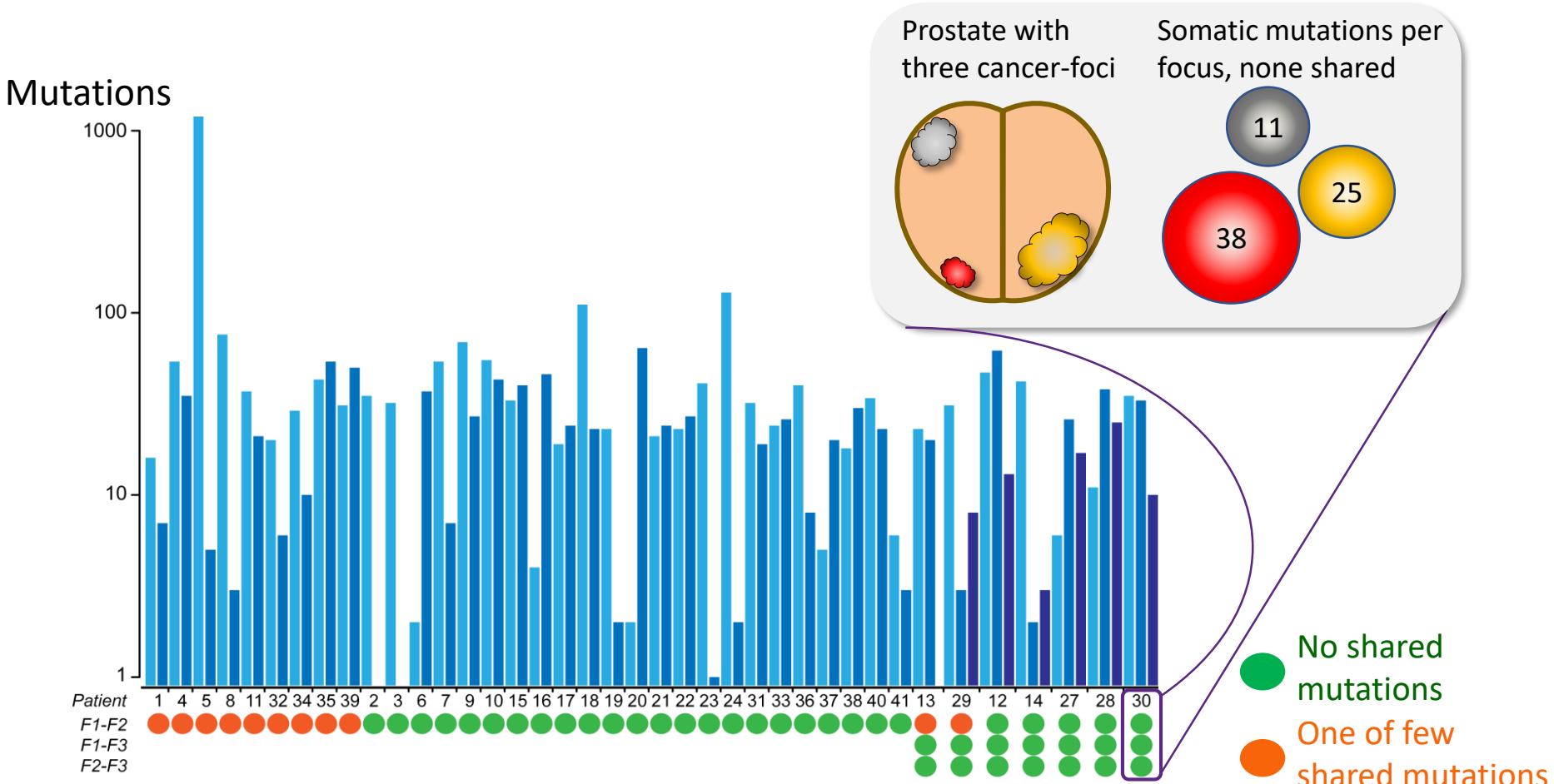
Separate foci have separate sets of somatic mutations



Løvf *et al.*, Eur. Urol. 2019

Separate foci have separate sets of somatic mutations

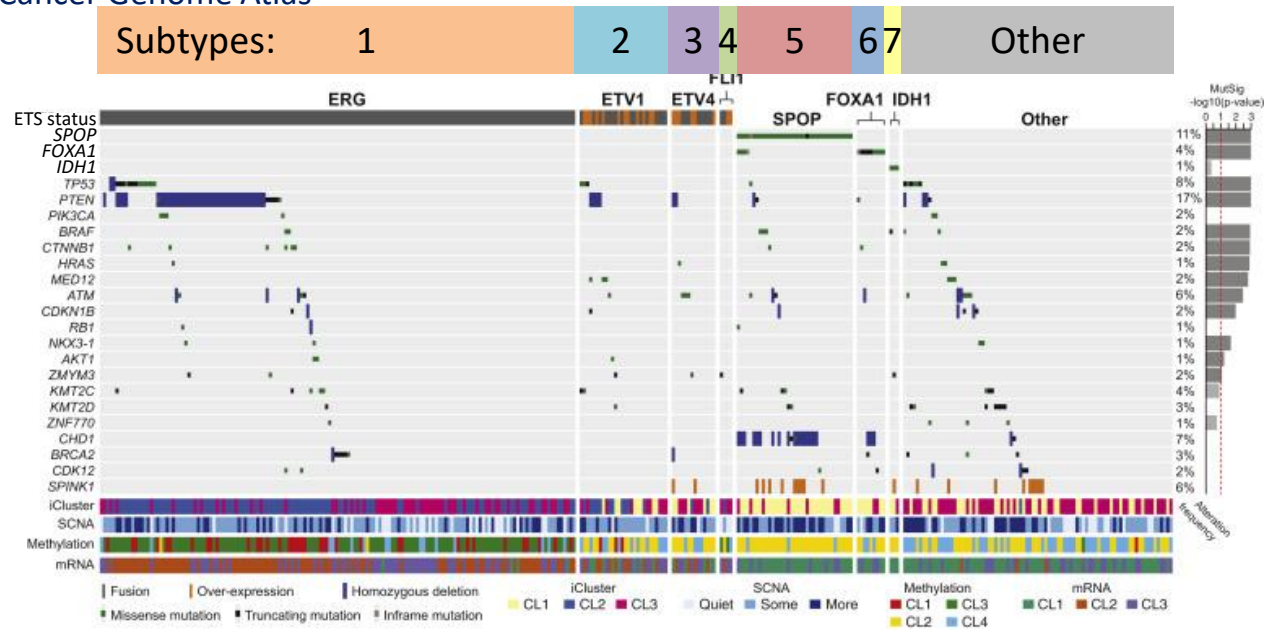
Molecular biomarkers from a random tissue sample can be irrelevant for the most significant cancer focus



Løvf *et al.*, Eur. Urol. 2019

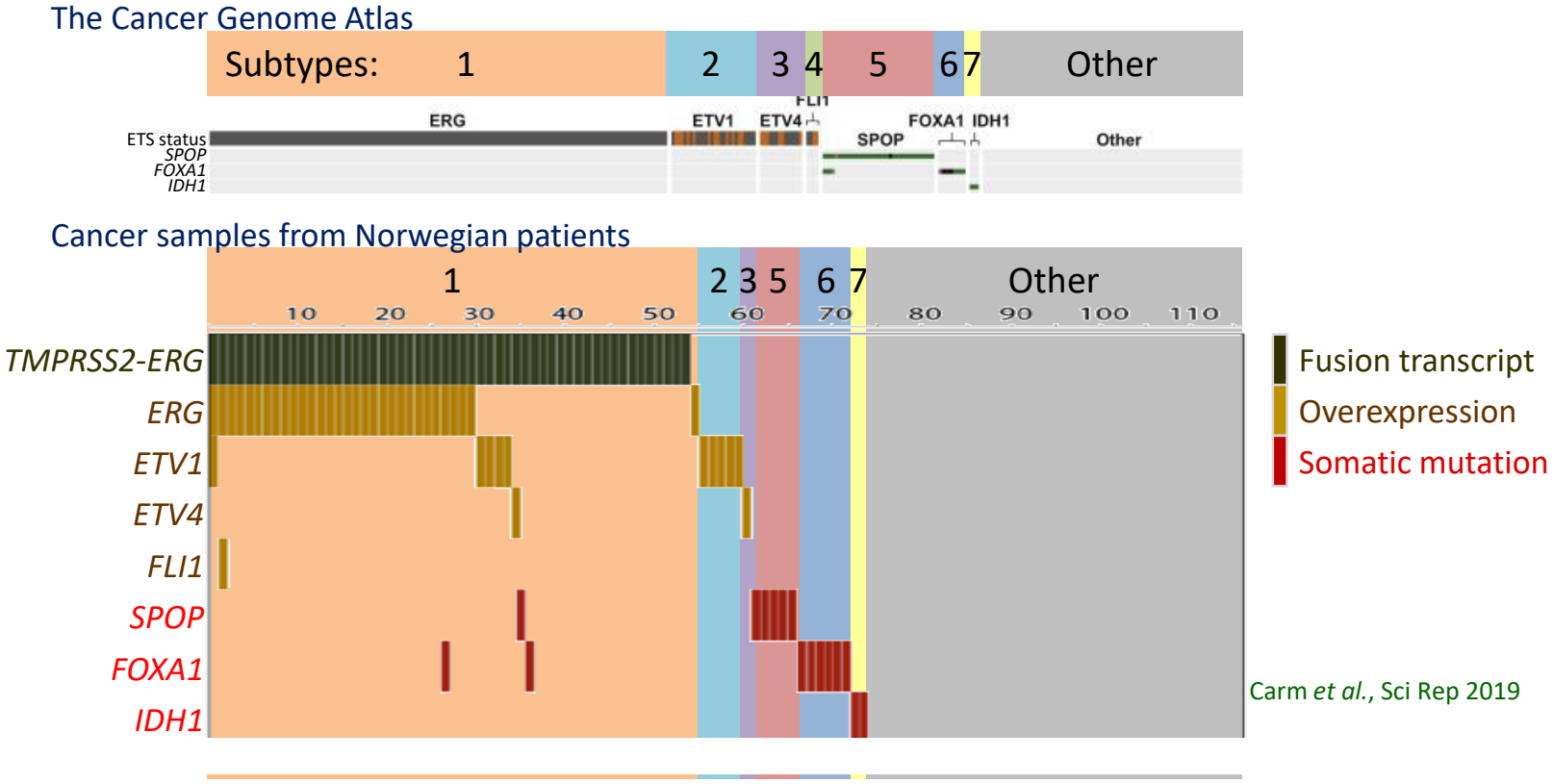
Different molecular subtypes of prostate cancer?

The Cancer Genome Atlas



The Cancer Genome Atlas, Cell 2015

Different molecular subtypes of prostate cancer?



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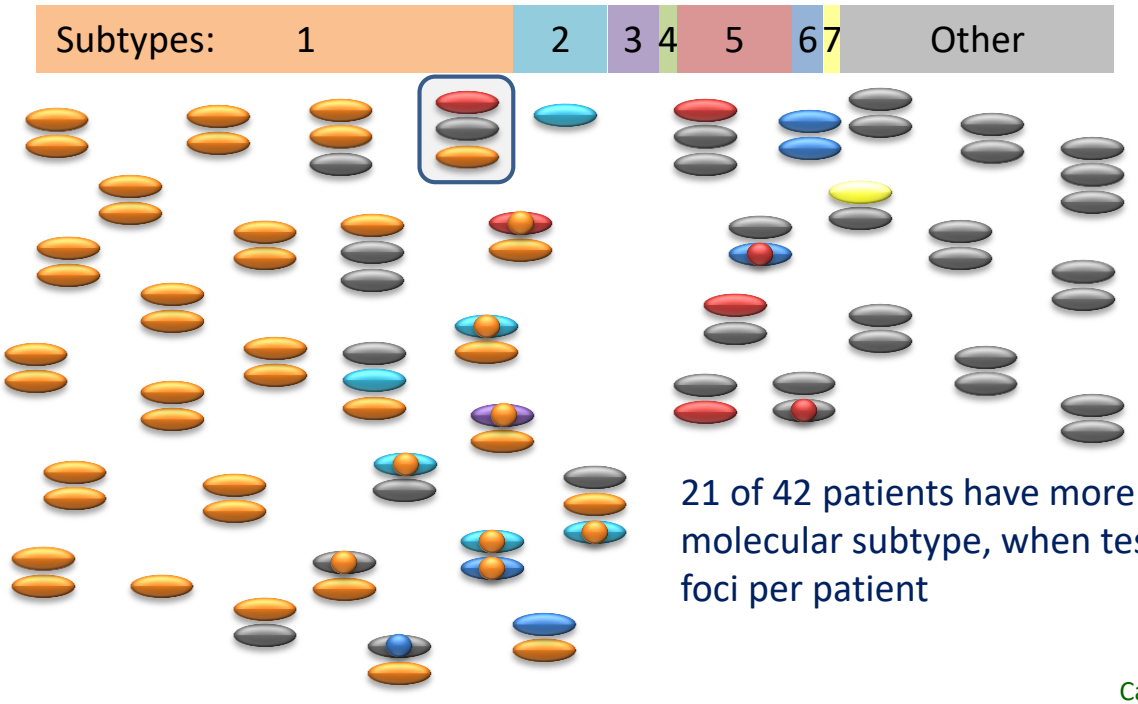
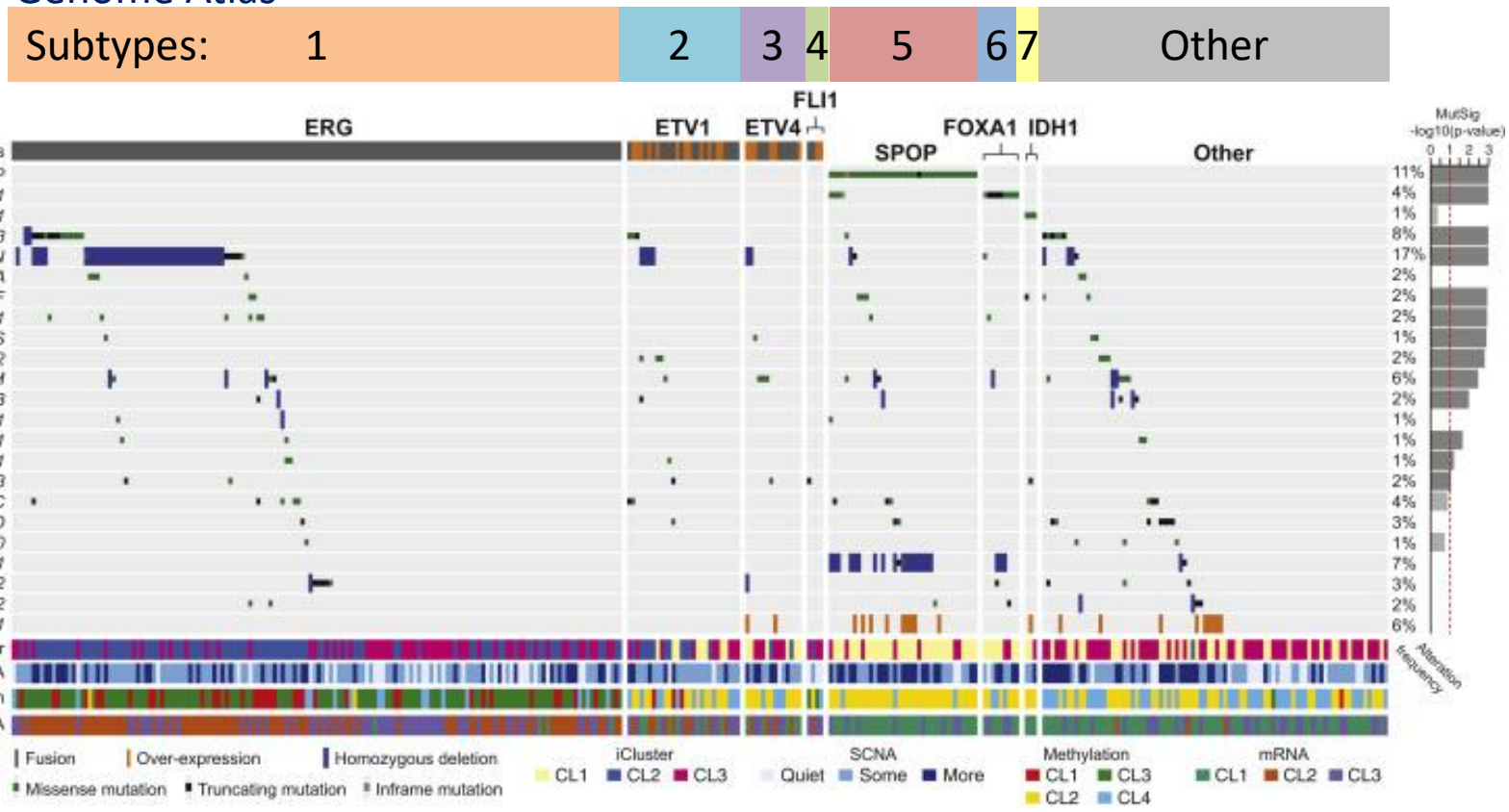


21 of 42 patients have more than one molecular subtype, when testing 2-3 foci per patient

Carm *et al.*, Sci Rep 2019

Different mulecular subtypes of prostate cancer?

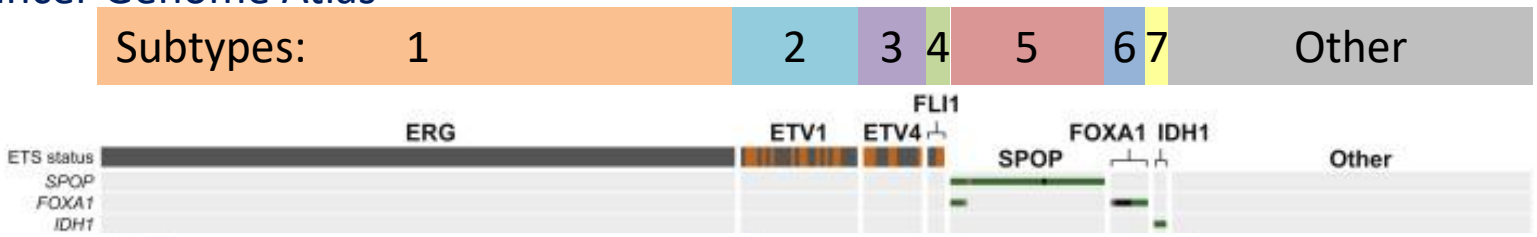
The Cancer Genome Atlas



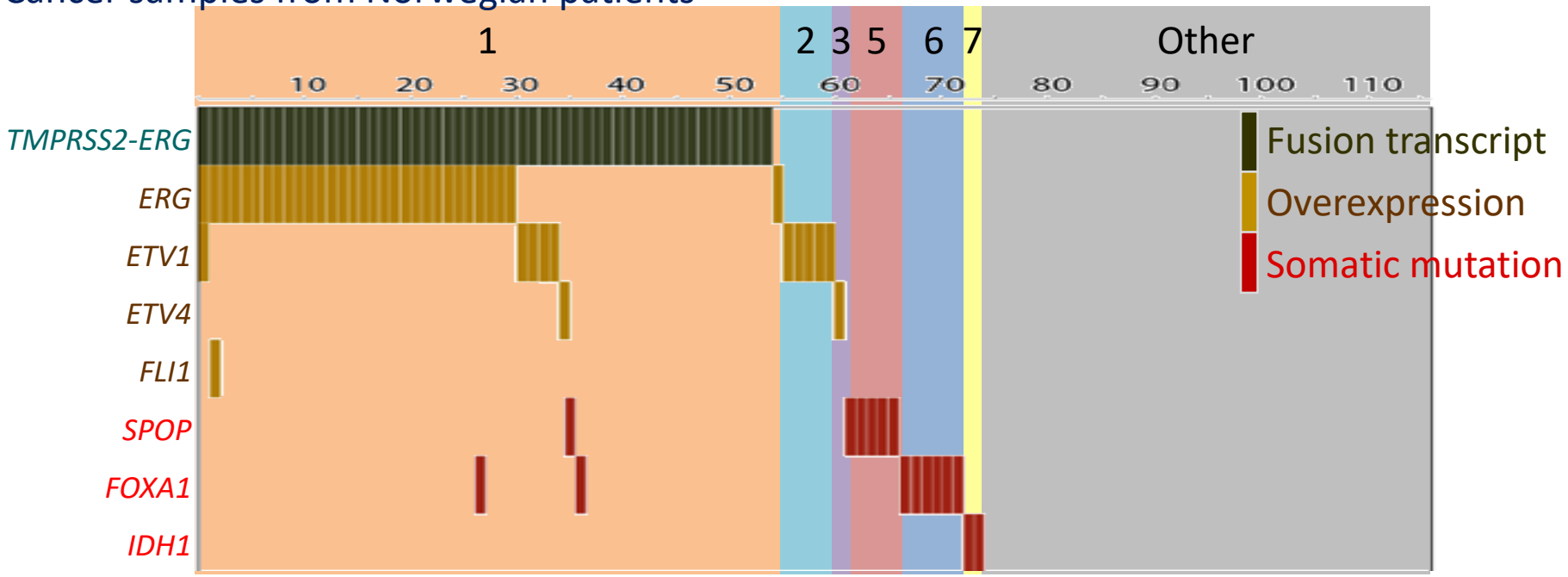
The Cancer Genome Atlas, Cell, 2015

Different mulecular subtypes of prostate cancer?

The Cancer Genome Atlas

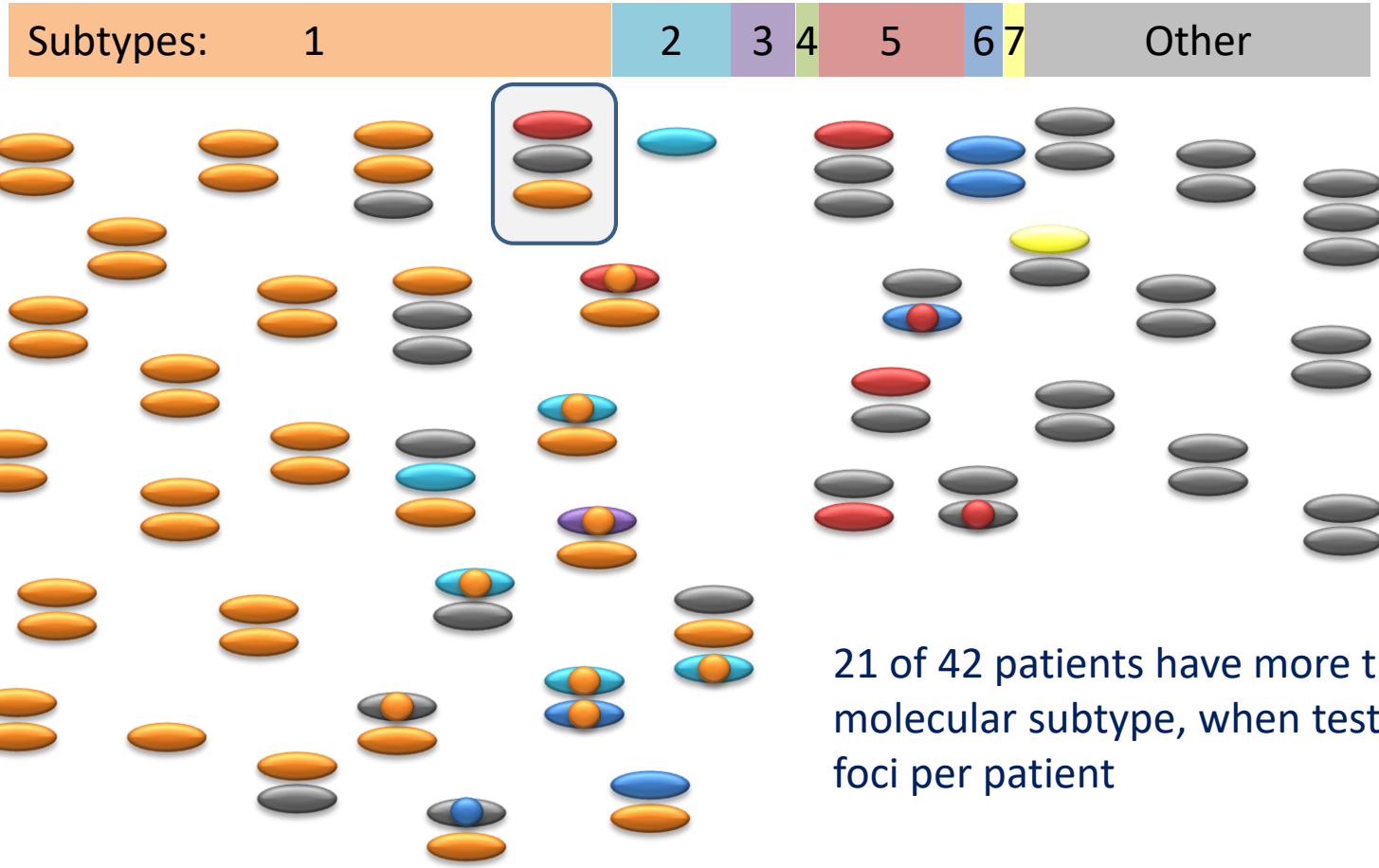


Cancer samples from Norwegian patients



Carm *et al.*, Sci Rep 2019

Molecular classification – per focus

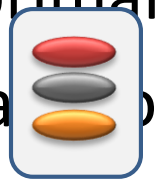


21 of 42 patients have more than one molecular subtype, when testing of 2-3 foci per patient

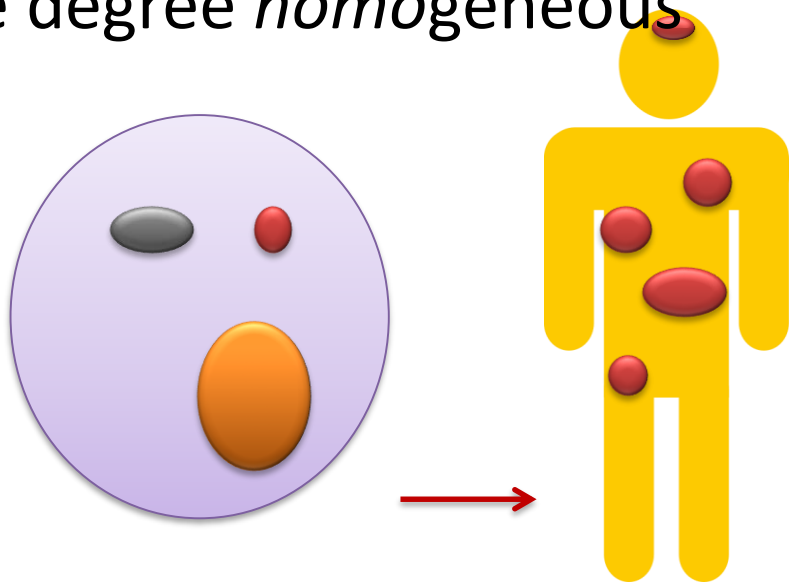
Carm *et al.*, Sci Rep 2019

Heterogenity in prostate cancer

- Tumour foci in primary cancers are *hetero*geneous
- Metastatic foci a[illegible] a large degree *homo*geneous



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



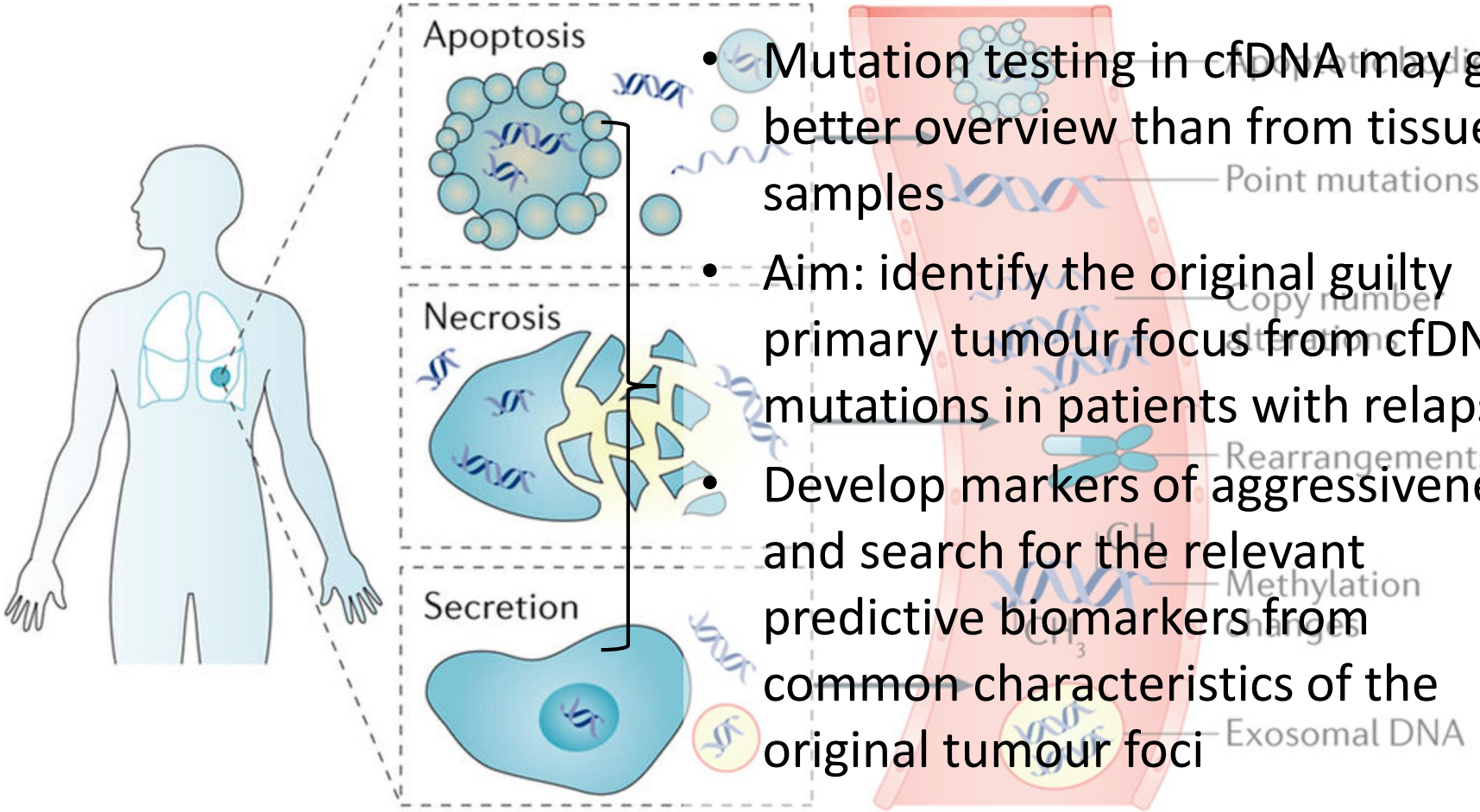
Løvf *et al.*,
Eur Urol 2019

Carm *et al.*,
Sci Rep 2019

Liu *et al.*,
Nat Med 2009

Kumar *et al.*,
Nat Med 2016

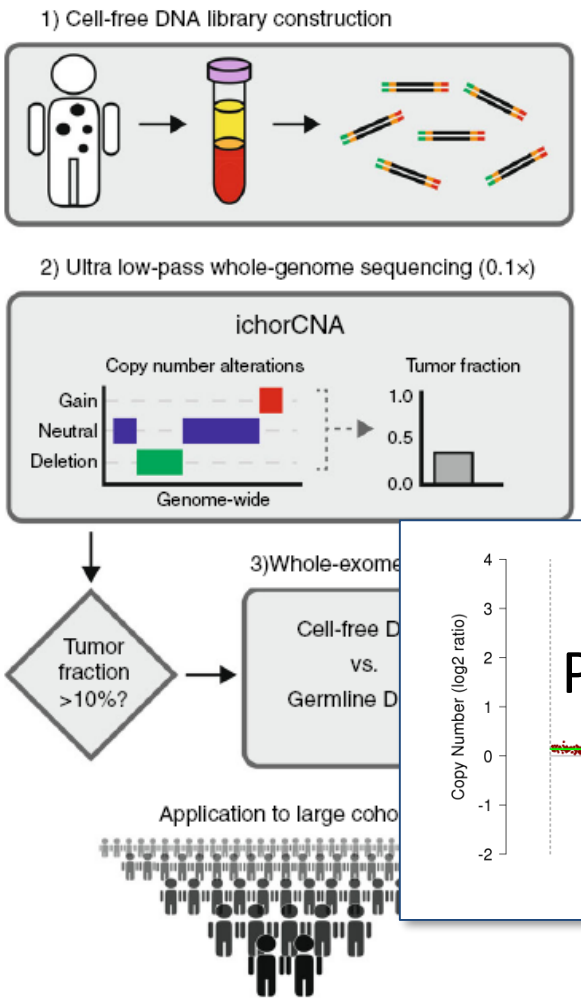
Liquid biopsies

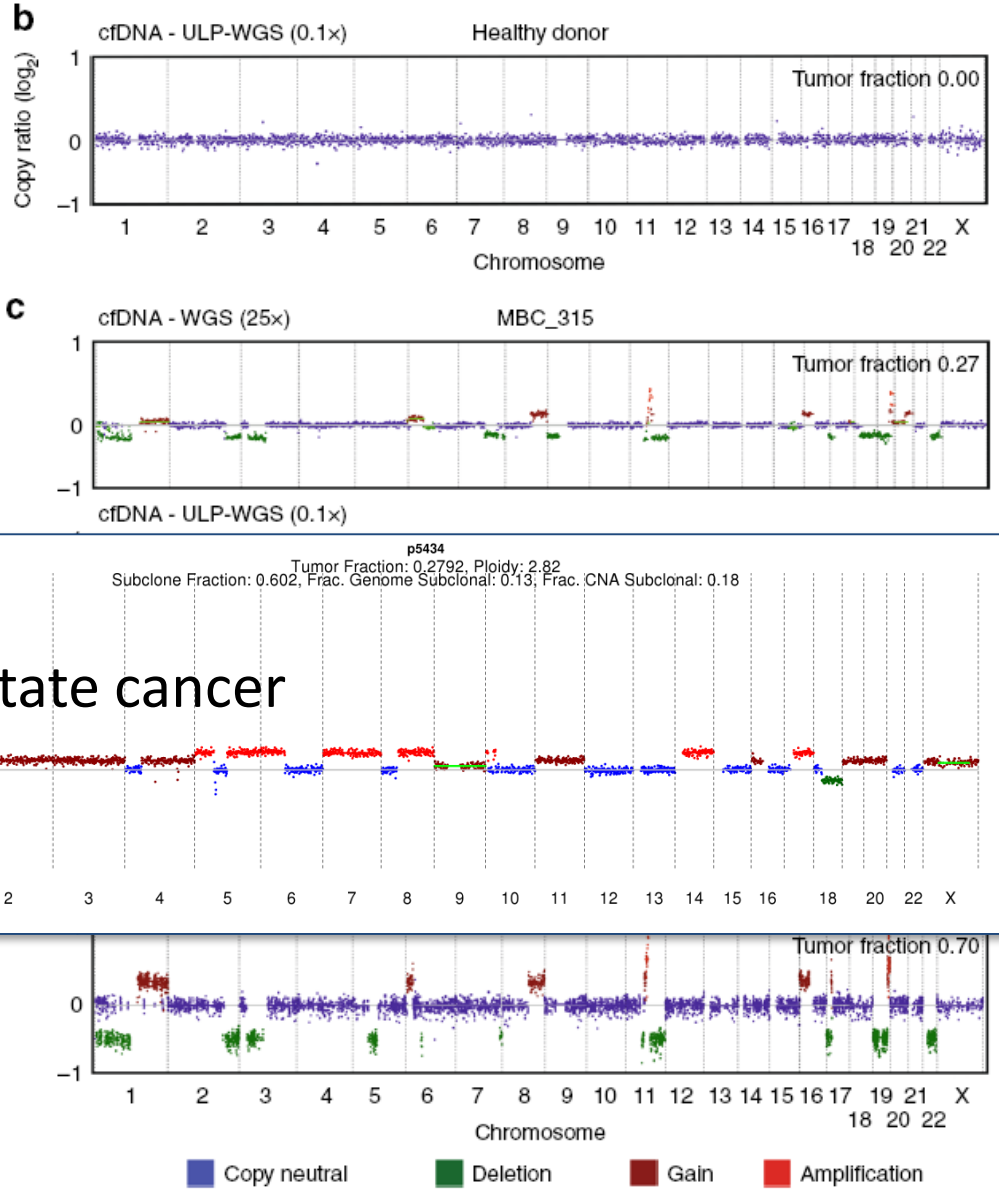


- Mutation testing in cfDNA may give better overview than from tissue samples
- Aim: identify the original guilty primary tumour focus from cfDNA mutations in patients with relapse
- Develop markers of aggressiveness and search for the relevant predictive biomarkers from common characteristics of the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