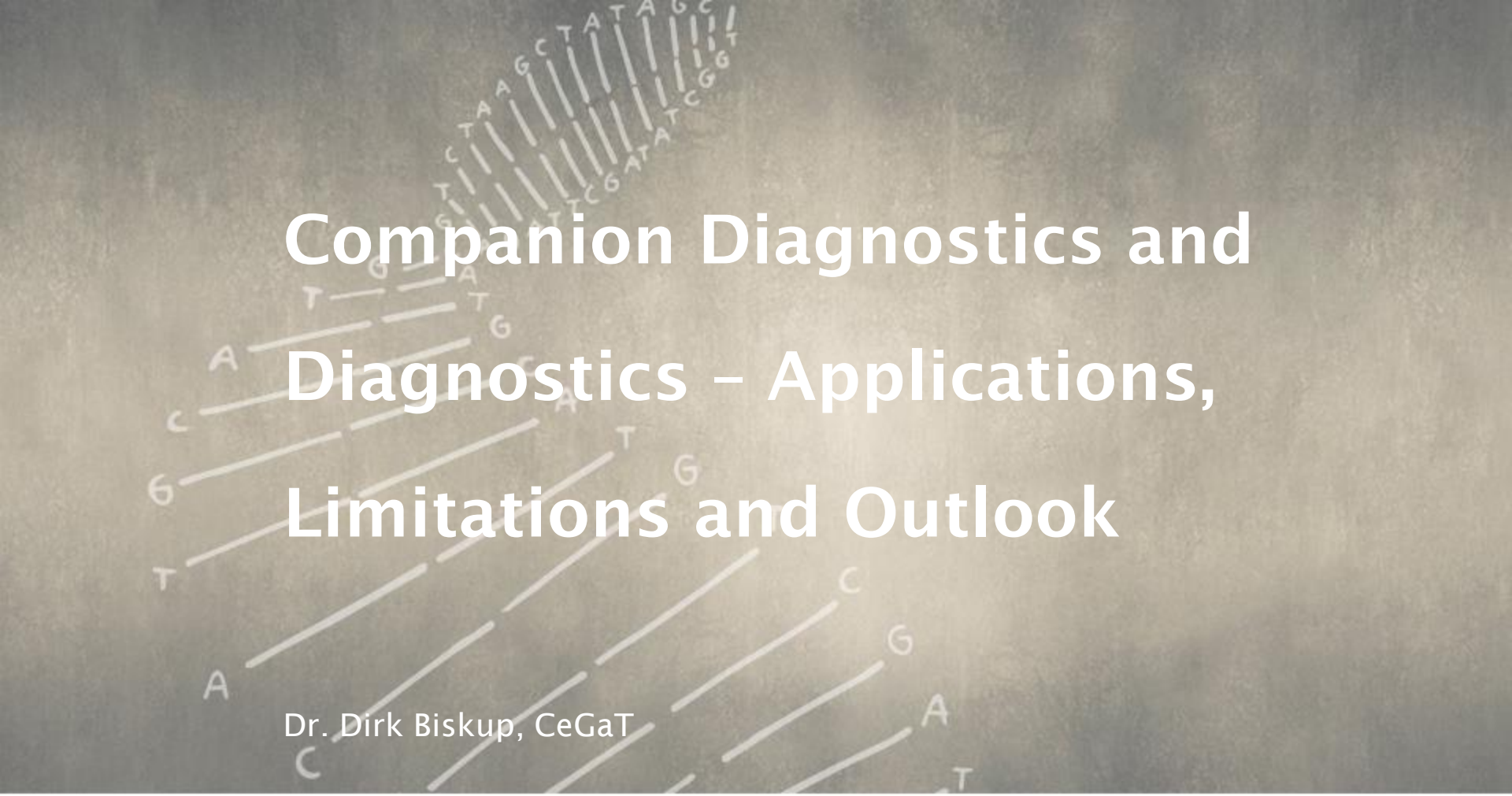


Companion Diagnostics and Diagnostics – Applications, Limitations and Outlook

Dr. Dirk Biskup, CeGaT

CeGaT
Center for Genomics and Transcriptomics

Companion Diagnostics (today)

Definition

… a device specifically intended to select patients with a previously diagnosed condition or predisposition as eligible for treatment with a **specific medicinal product**.

*„… ein Produkt, das **speziell** dafür bestimmt ist festzustellen, ob **eine bestimmte** Therapie für Patienten mit einem bereits diagnostizierten Zustand bzw. einer bereits bekannten Prädisposition geeignet ist.“*

Art. 2 Abs. 6 des Vorschlags für eine EU-Verordnung über In-vitro-Diagnostika

Example

EGFR Mutation

Non-small-cell lung carcinoma (NSCLC)

Result:

1) Gefitinib if Exon 19 is deleted or L858R
2) Osimertinib if T790M

Reimbursement in Germany

EBM (public health insurance)

Chapter 19.4.4 tumor related genetic variants

19451:	point mutation (del/dup)	211 points
19453:	somatic mutations in up to 20 Kb	24,914 points
19456:	BRCA1 and BRCA2	19,643 points

GOÄ (private health insurance)

GOÄ severely outdated

3926:
Sequence analysis
EUR 116.57

Cost estimate and upfront approval necessary

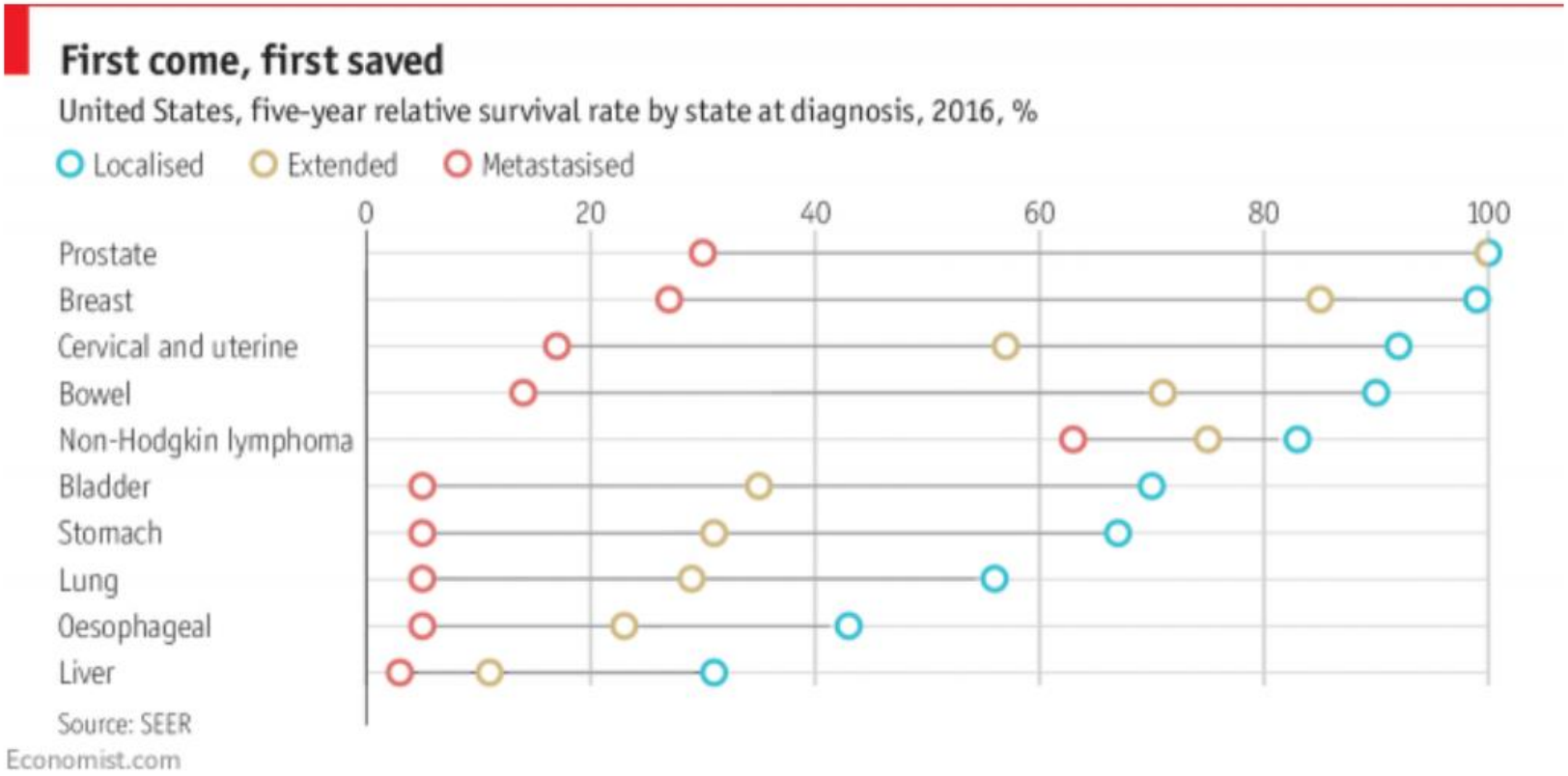
Introductory remarks on Tumor

- Killed 8.8m people in 2015, three-quarters of them in low- and middle-income countries
- Between 2005 and 2015 the number of cases increased by 33% (due to aging and population growth)
- New cases are expected to increase by 70% in the next 20 years
- In rich countries cancer is becoming more survivable (2/3 of patients in the US will survive for 5 or more years)
- Generally speaking: The poor are ill served. But the failures are not limited to poor countries. Cancers due to bad diet, obesity, alcohol abuse and smoking could all be reduced significantly in wealthy countries.
- Surprising exceptions: Vaccination against HPV is routine in Rwanda, it is still limited in America (many cervical cancer could have been avoided).

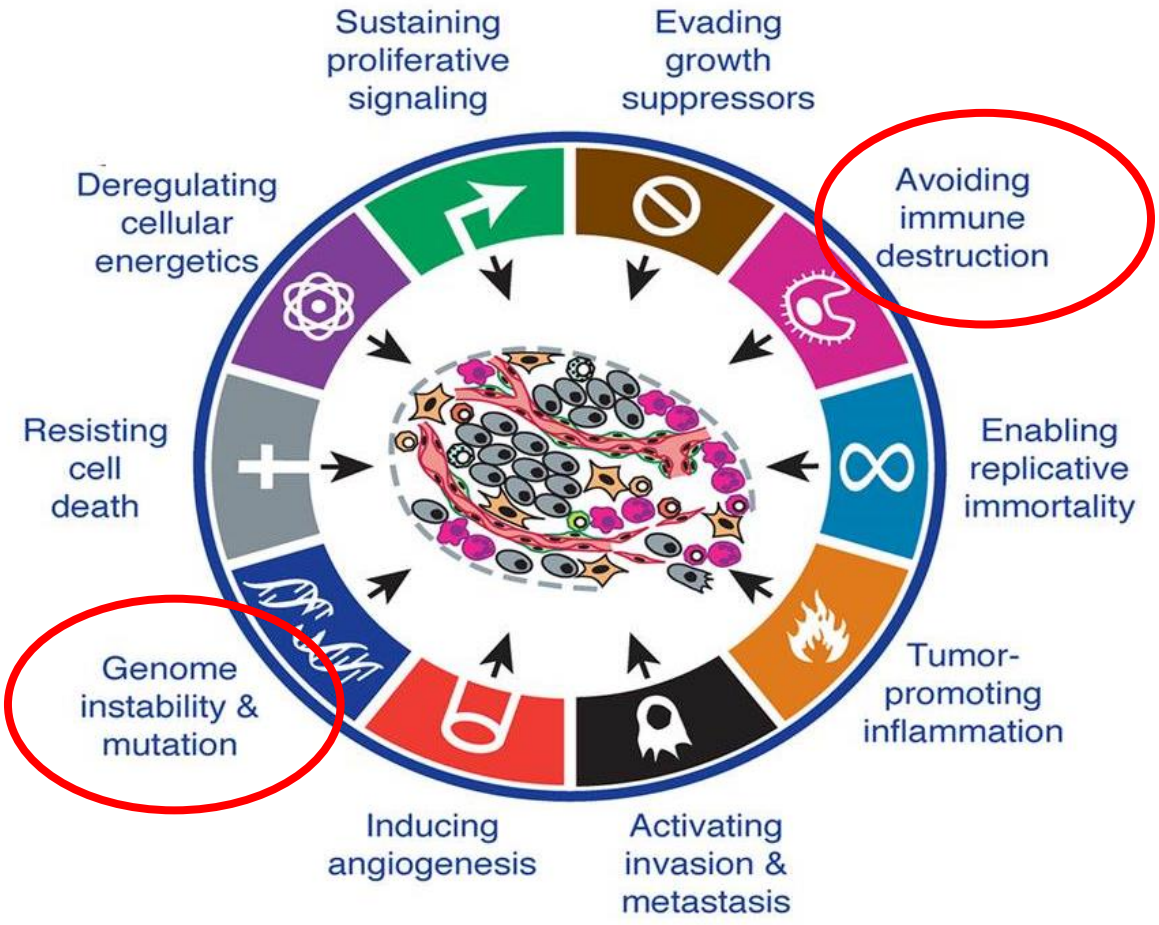
It‘s a numbers game

- Each of the ~10^{13} cells in the human body receives tens of thousands of DNA lesions per day
- Strong sunlight can induce ~100,000 lesions per exposed cell per hour
- The DNA damages are constantly happening, the vast majority is being repaired, fewer than one mutation in a thousand persists
- Over time genetic damages accumulate, the likelihood rises that multiple mutations in one cell accumulate and that the cell develops the ability to grow without check
- This likelihood is not the same for everybody: quirks in the genome can increase the likelihood, e.g. BRCA1 and BRCA2
- Once a cancer has begun its unruly growth it will pick up more and more mutations

Early detection ist our greatest opportunity to improve survival

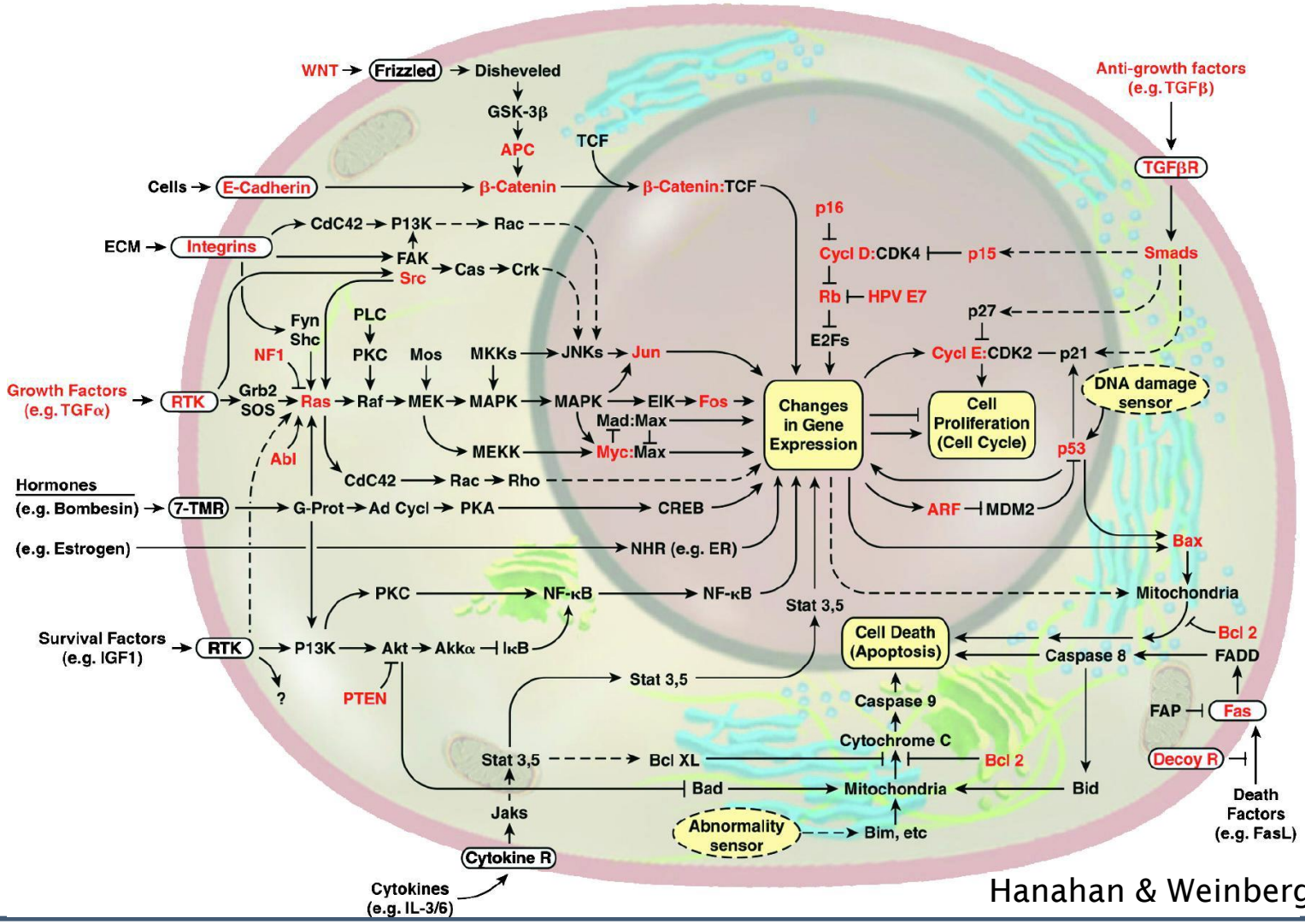


Tumor biology



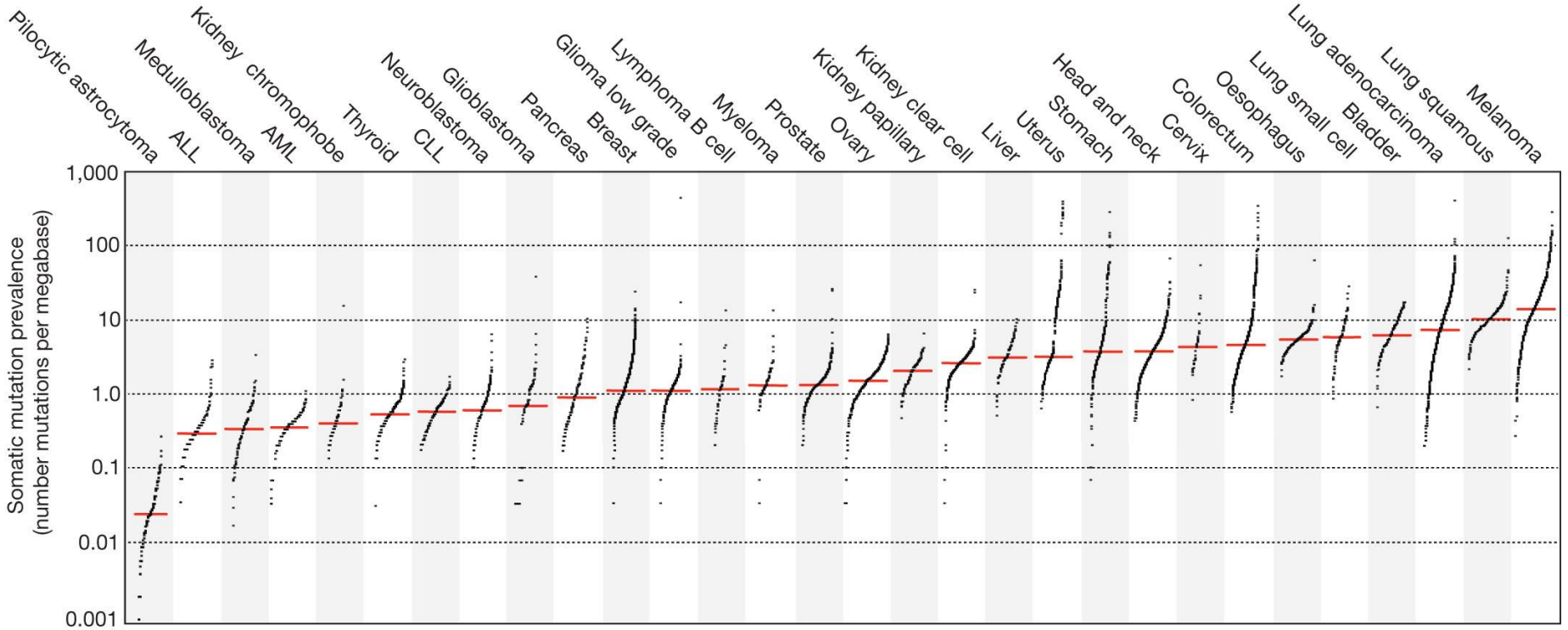
Hanahan & Weinberg, Cell 2011

Driver mutations lead to „Signalopathies“



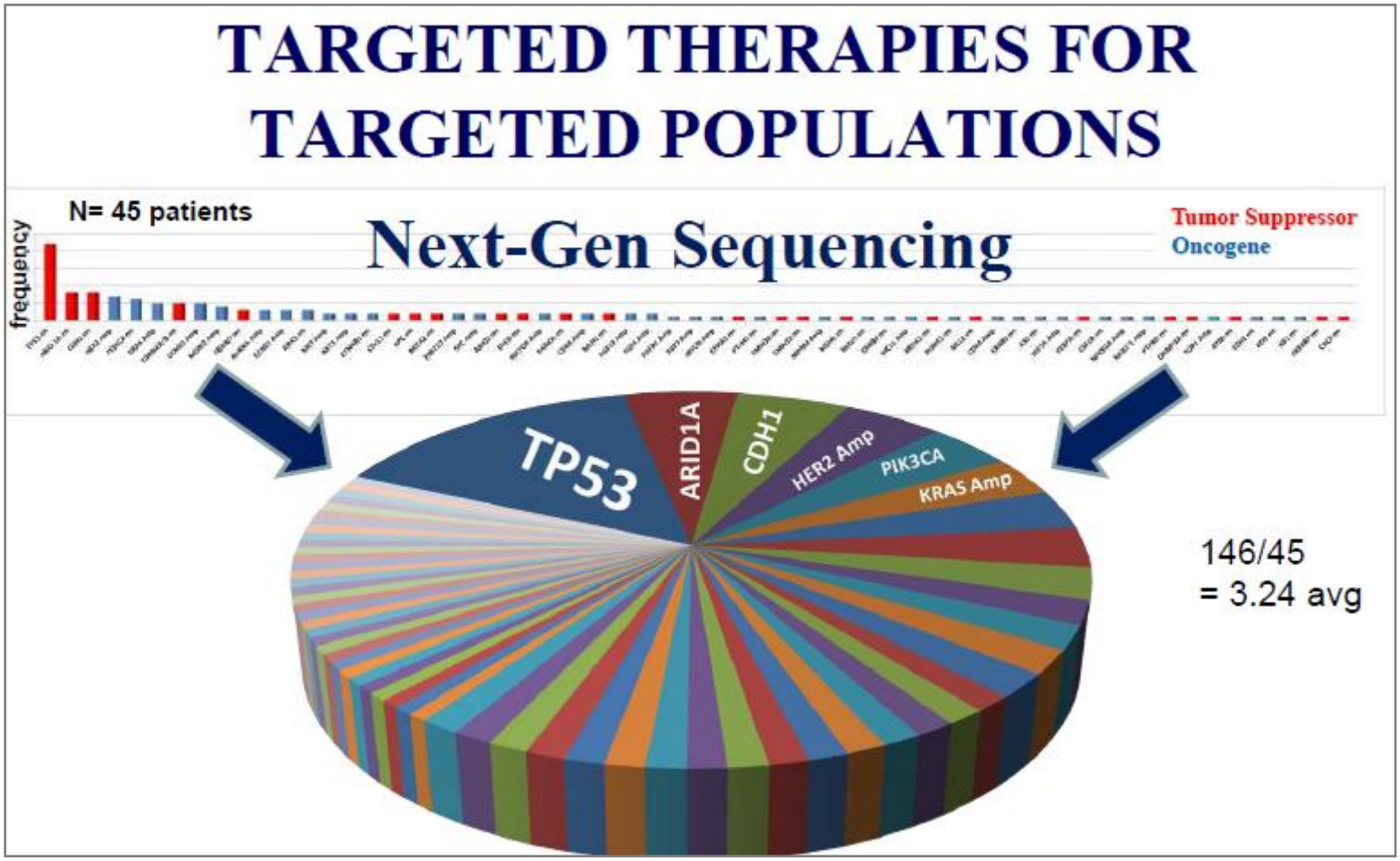
Hanahan & Weinberg, *Cell* 2000

Mutational Burden



Alexandrov et al., Nature 2013

Tumors are heterogeneous and contain different combinations of mutations



Grafik: WINsymposium 2013

Summary

Every individual is different	Germline mutations / develop mutations over time
Every tumor has mutations	A tumor is a genetic disease
Every tumor is unique	Somatic mutations – drivers and passengers

Tumor therapy

- **Surgery** is the first and most common form of cancer treatment
  - Minimal invasive surgery, lasers, cryosurgery, cyberknife
  - Imaging: Ultrasound, magnetic-resonance imaging (MRI), X-ray tomography, Positron-emission tomography (PET)
- **Radiation**: Often surgery goes hand-in-hand with radiation to kill remaining cells or to kill the cancer cells where surgery would be hard
- **Chemotherapy**: used to kill remaining cells
  - Problem 1: cancers can become resistant to chemotherapy
  - Problem 2: severe side-effects as all cells are attacked by chemotherapy

Targeted tumor therapy

- **Targeted therapies:** Targeted cancer therapies are expected to be more effective than older forms of treatments and less harmful to normal cells.
- **Biomarkers** are usually required to aid the selection of patients who will likely respond to a given targeted therapy.

Targeted Tumor therapy - Gefitinib

Gefitinib (Iressa)

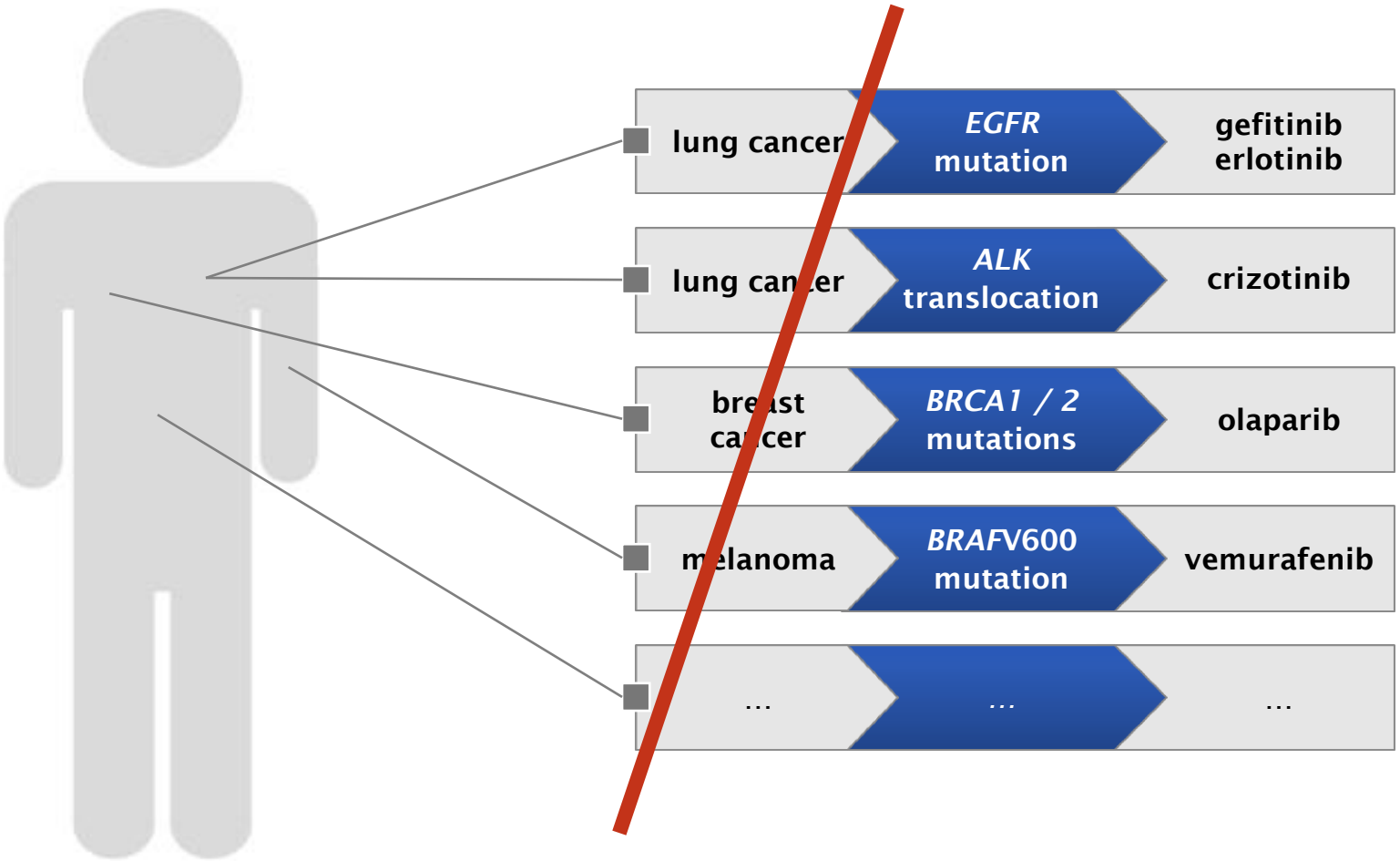
- approved 2002 (Japan) and 2003 (by FDA).
- used for certain breast, lung and other cancers.
- **EGFR inhibitor** which interrupts signaling through the epidermal growth factor receptor (EGFR) in target cells.
- only effective in cancers with mutated and overactive EGFR.
- inhibitor of epidermal growth factor receptor's (EGFR) tyrosine kinase domain.
- Gain of function: If EGFR is overexpressed this leads to inappropriate activation of the anti-apoptotic Ras signalling cascade, eventually leading to uncontrolled cell proliferation.
- These mutations are more commonly seen in Asians, women, and non-smokers

Targeted Tumor therapy - Olaparib

Olaparib (Lynparza)

- approved 2014 for germline BRCA mutated advanced ovarian cancer (three or more prior lines of chemotherapy)
- BRCA1 and BRCA2 (among others) are proteins that are important for the repair of double-strand DNA breaks (homologous recombination)
- if cancers have BRCA1 or BRCA2 mutations, for example, the remaining repair mechanism uses a protein called poly-ADP-ribose polymerase (PARP)
- even a tumor needs residual DNA repair function. Olaparib aims to fully destroy the DNA repair system in a tumor cell
- Olaparib is a PARP inhibitor

Paradigm shift

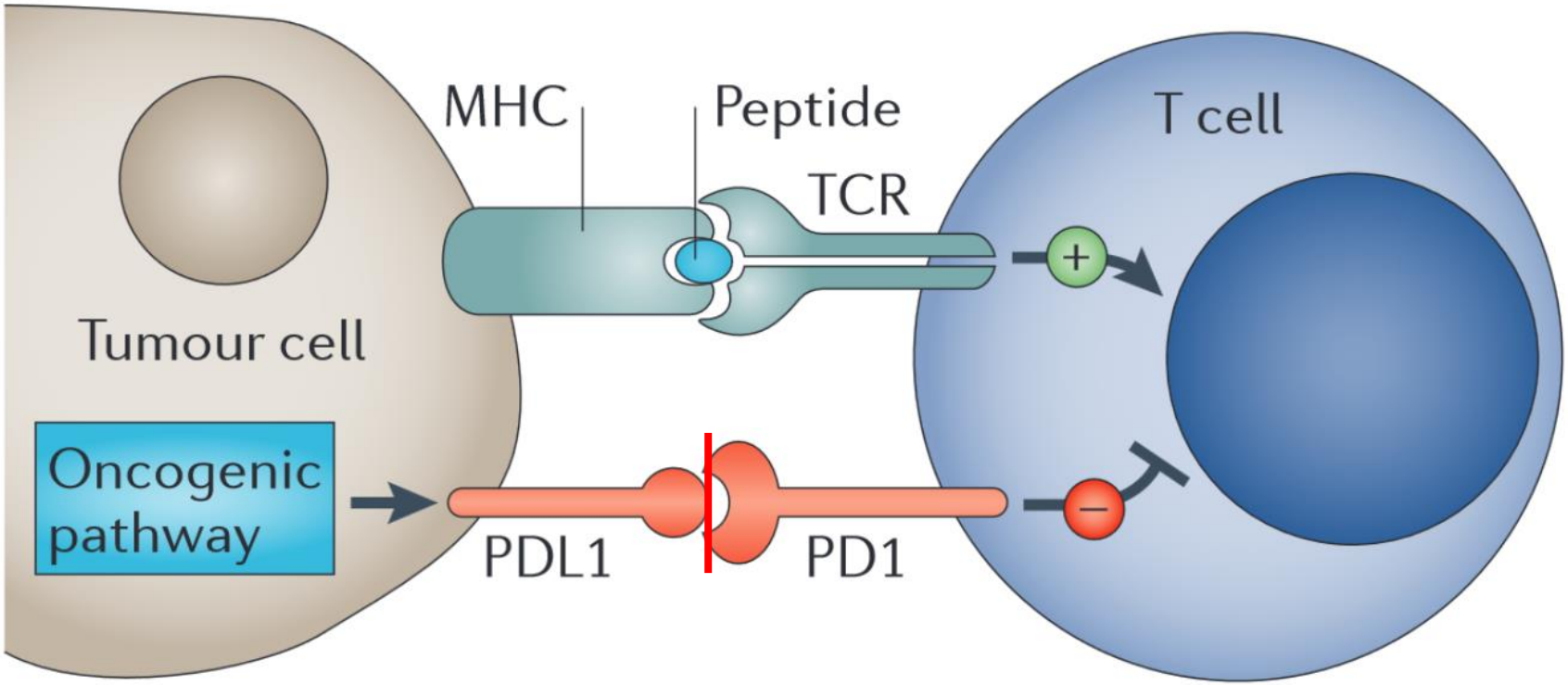


Targeted Tumor therapy - Larotrectinib

Larotrectinib

- works for TRK fusion patients (tropomysin receptor kinase)
- TRK inhibitor
- very rare mutation (5,000 patients in the U.S. have TRK-modulated tumors)
- clinical data 2017: the drug was tested in 50 patients with **17 different tumors**, 78% of the patients responded to the drug

Checkpoint Inhibition



Pardoll, Nature Reviews Cancer 2012

Targeted Tumor therapy - Pembrolizumab

Pembrolizumab (Keytruda)

- approved 2015 for metastatic non-small cell lung cancer (NSCLC) in patients whose tumors express PD-L1 and who have failed treatment with other chemotherapeutic agents
- approved 2017 for use in any cancer with mismatch-repair-gene defects or high MSI – **independent of tumor entity**
- checkpoint inhibitor PD1-PDL1

Clinical criteria for checkpoint inhibition

- mutational load > 100 – large panel
- MSI – PCR based assay
- PDL1 expression - immunohistochemistry

What does this mean for companion diagnostics?

Precision tumor diagnostics

Tumor Exome	Somatic Tumor Panel	Transcriptome
– ca. 20,000 genes – Very sensitive, if tumor content is >50% – 150x – TAT 2 – 3 weeks	– 710 tumor-and pharmacogenetic relevant genes – Translocations in 29 genes – 1,000x – TAT 2 – 3 weeks	– expressed mutations – fusion transcripts – abundance of altered transcripts – PD1/PD-L1 expr. – BRCAness
Mutational load Neoepitope prediction	Mutational load, Treatment Decision Support	Treatment Decision Support

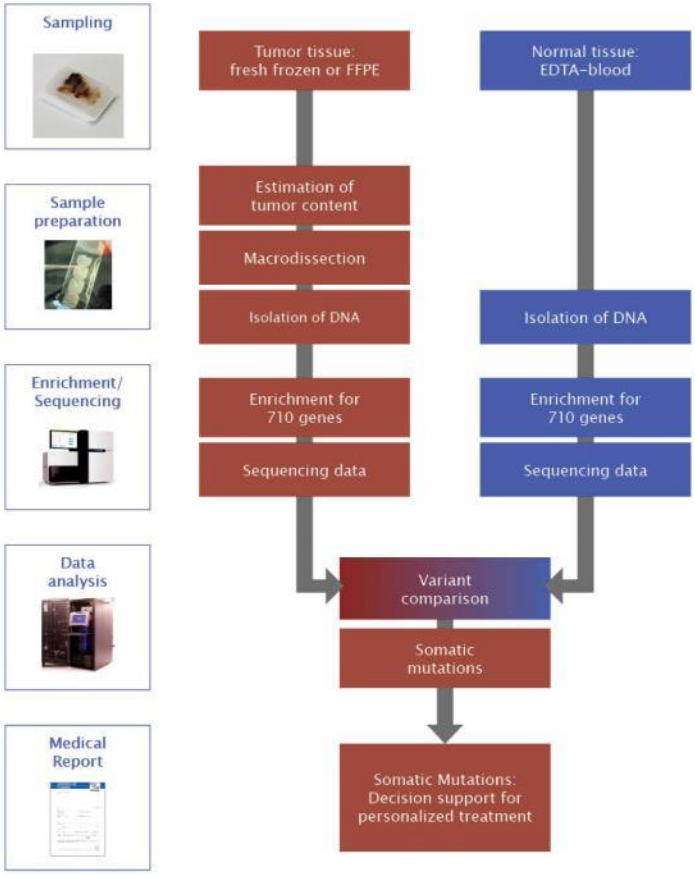
Somatic Tumor Panel

Key facts

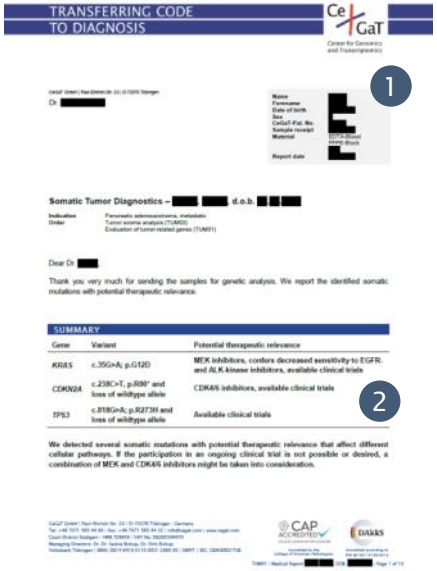
- Comparison of tumor and normal tissue to avoid false-positive results (Jones et al., 2015)
- Assists the selection of the best therapy, incl. off-label use
- Determination of mutational load for decision on immune therapy approaches (Rizvi et al., 2015)
- Provides information on appropriate clinical studies for all patients with solid tumor, leukemia and lymphoma
- 710 genes that have an impact on tumor development and drug response
- Selected translocations in 29 genes
- Analysis of additional samples (e.g. metastases) possible
- Sample requirements for solid tumors:
  Tumor tissue (FFPE or frozen) and normal tissue (EDTA-blood)
  Macrodissection and pathology review to achive a minimum of 20% of tumor content in tumor tissue
- Samples requirements for leukemia/lymphoma:
  Tumor tissue (EDTA-blood/bone marrow) and normal tissue (e.g. saliva)
- Sensitivity > 98,5%* | Specificity > 99.9%

  * Based on high quality sample with 60% tumor content for detection of a heterozygous variant

Workflow



Medical Report – Somatic Tumor Panel

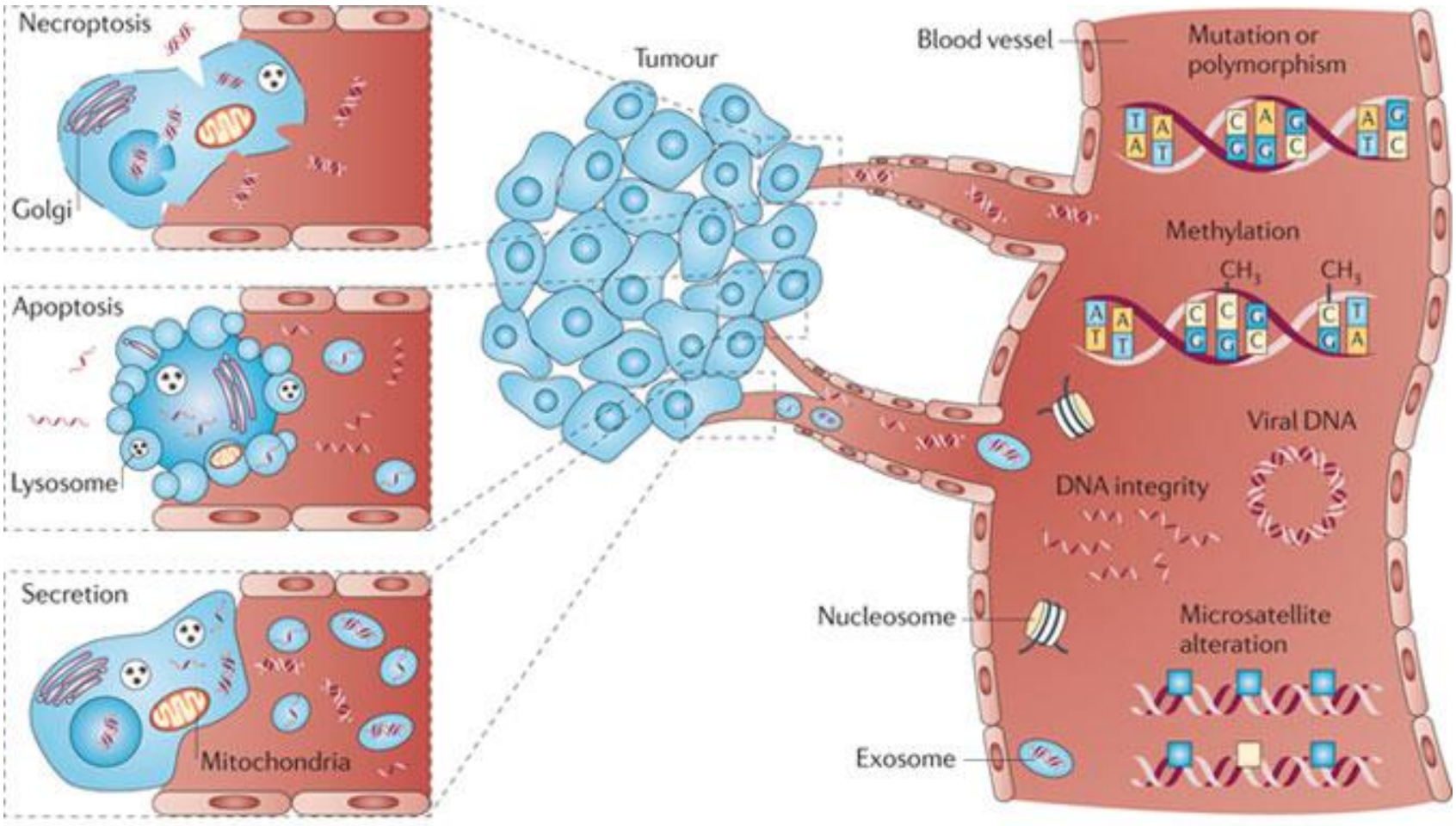


Section 1: **(1)** Patient information and (2) summary of result (variants with potential therapeutic relevance), mutational load

Section 2: **(3)** Details and (4) interpretation of identified variants with potential therapeutic relevance

Section 3: Additional information with methodology (5), somatic mutations classified as having no current therapeutic relevance (6), and possible therapeutic strategies (7)

The future is now: Liquid Biopsy



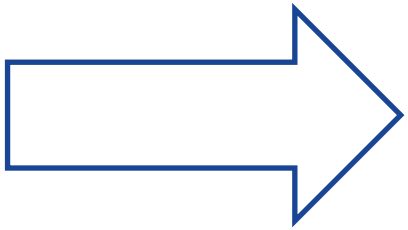
Schwarzenbach et al. 2011

Cell free Tumor DNA / CTCs / DTCs

Somatic Tumor Panel from ctDNA or CTC

- ctDNA should be > 20% of total cfDNA
- 6 or more single tumor cells
- No biopsy needed
- **Once, prior to treatment**

Mutational load, Treatment Decision Support



Digital PCR or Mass Array

- Known mutation(s)
- Individual assay design, highest possible sensitivity
- Once a month, inexpensive
- **Monitoring of disease**

Individualized tumor markers

CNV

CNV track from NGS data difficult as DNA is often degraded (esp. if FFPE)

Del/Dup play a significant role in tumors and are often drivers

No CNV score available as of today

Low Coverage **Genome**

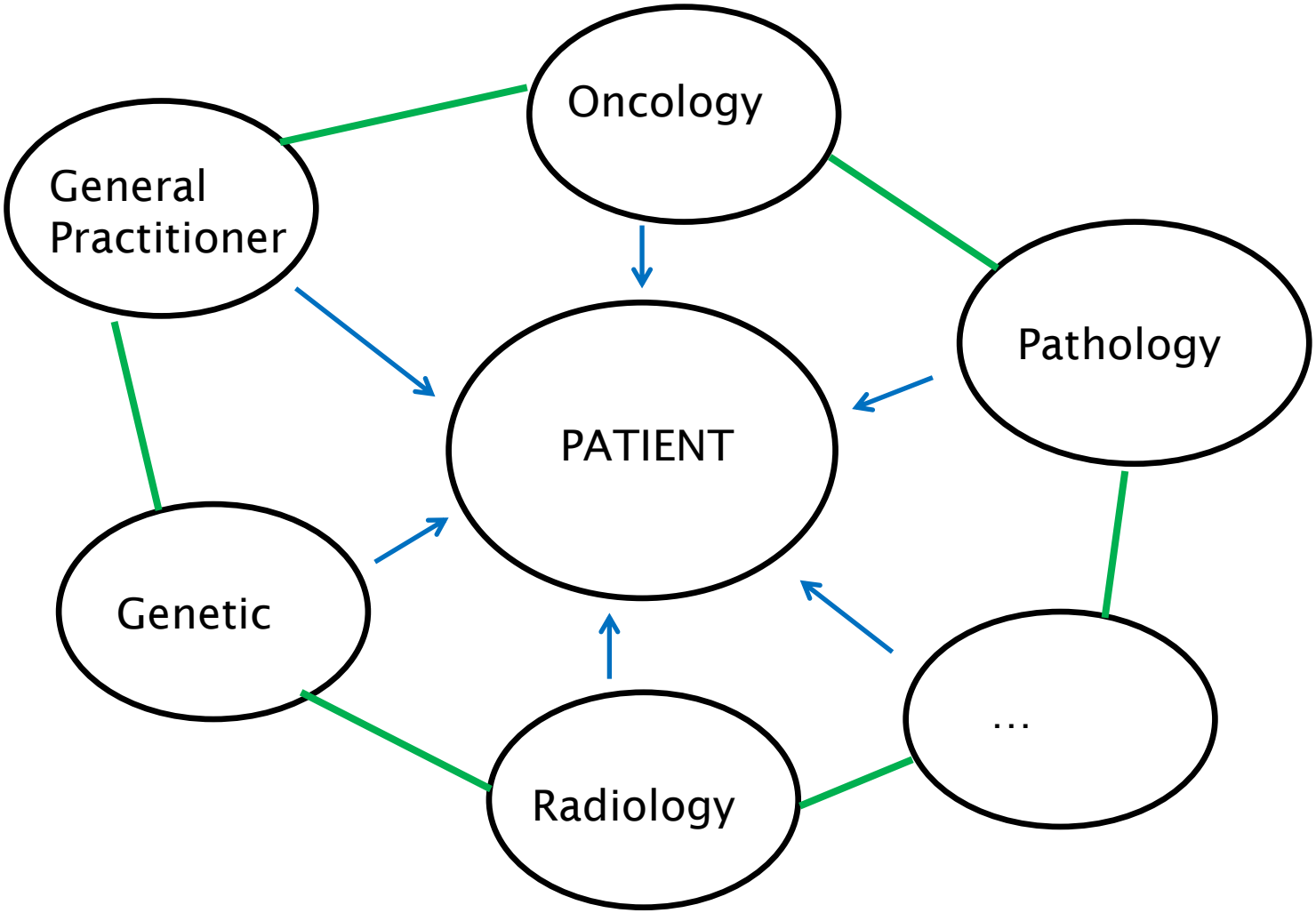
- All 3bn bases
- More sensitive and less expensive than ArrayCGH
- 5x
- TAT 2 – 3 weeks

CNV burden

Summary

- As every tumor is individual, a standard treatment might help but must not help.
- Before treating a tumor, a thorough diagnosis is recommended. This can be s stepwise approach (regular molecular pathology, “famous” genes, full panel).
- Treatment decisions should be based, among others, on a thorough diagnosis and not on the tumor entity.
- Molecular Tumor Boards are highly recommended!
- A regular monitoring of the tumor helps to understand the treatment success.

„Ring of care“



P4

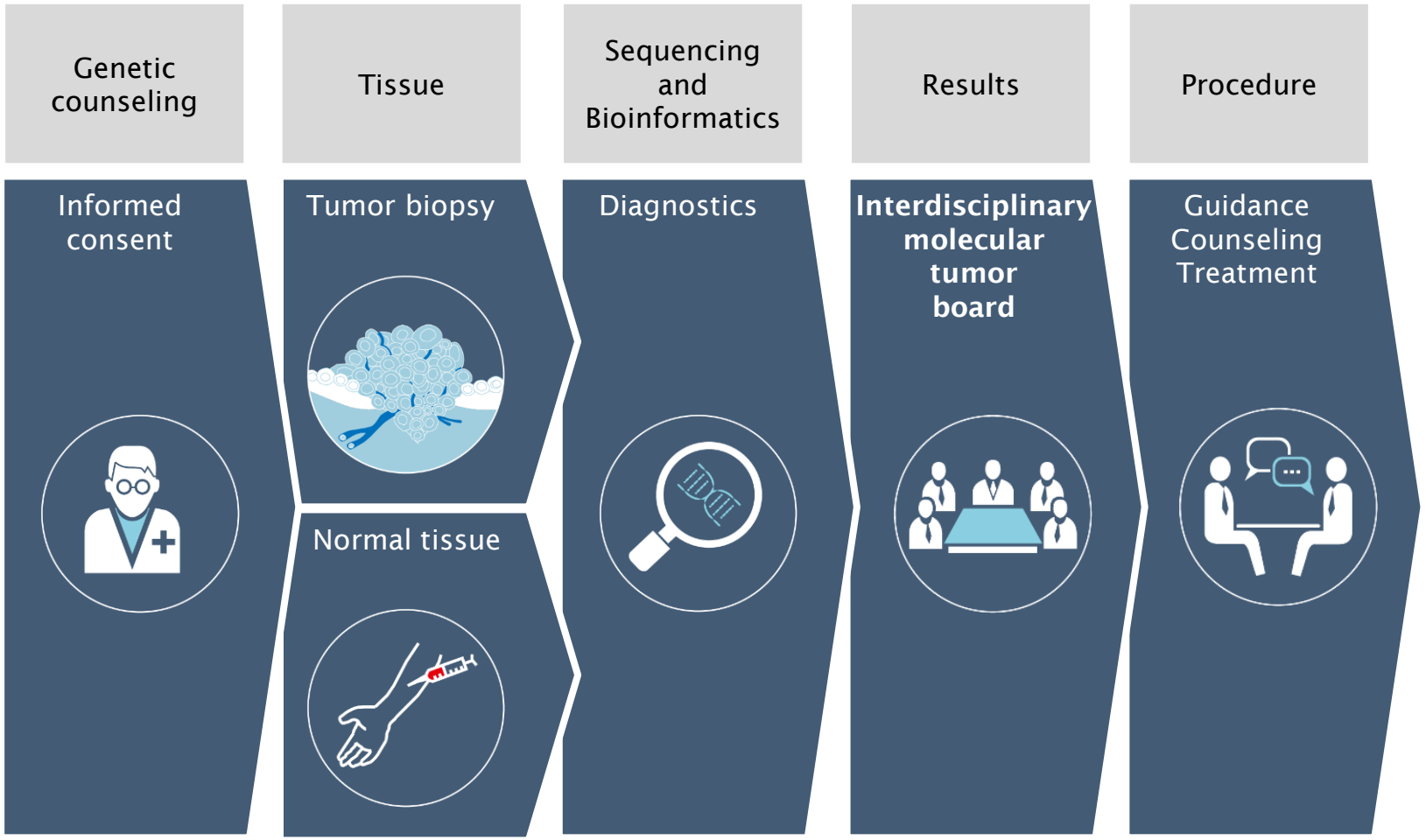
Prevention

Personalized

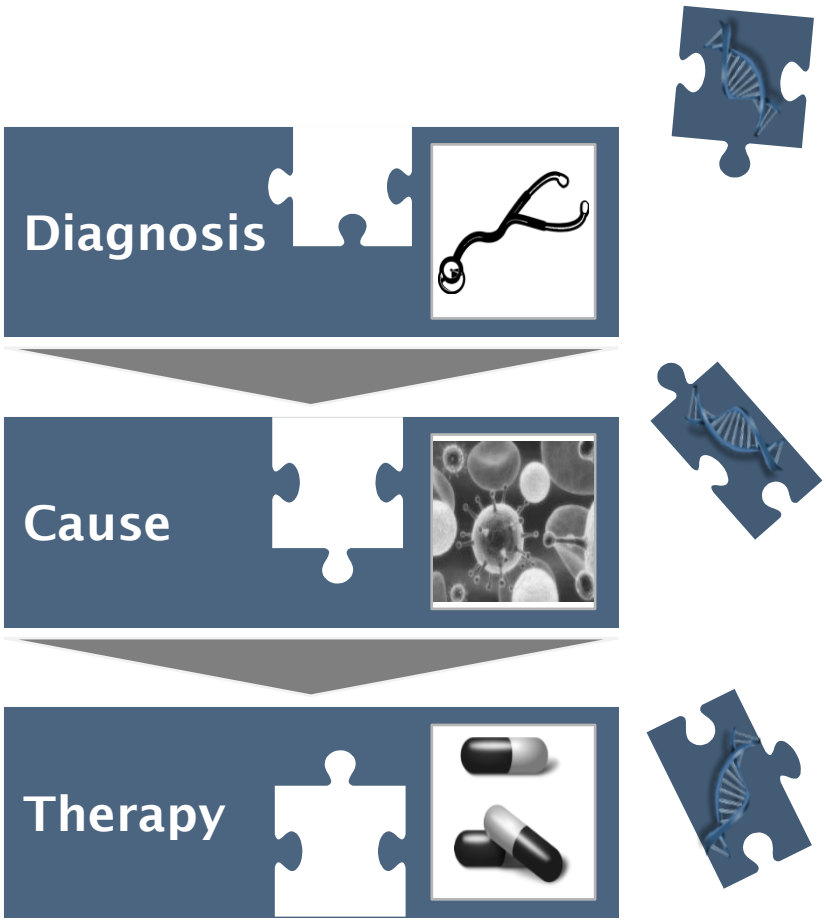
Participating

Precise

Clinical setting



Necessary Changes



- A genetic diagnosis should be one of the first diagnoses.
- Stop thinking „one mutation = one drug“. Have a holistic view on the tumor and the pathways.
- Classify the tumor by molecular variants and not by the entity.

Companion Diagnostics (perspective)

Definition (as of today)

… a device specifically intended to select patients with a previously diagnosed condition or predisposition as eligible for treatment with a **specific medicinal product**.

*„… ein Produkt, das **speziell** dafür bestimmt ist festzustellen, ob **eine bestimmte** Therapie für Patienten mit einem bereits diagnostizierten Zustand bzw. einer bereits bekannten Prädisposition geeignet ist.“*

Art. 2 Abs. 6 des Vorschlags für eine EU-Verordnung über In-vitro-Diagnostika

Definition (suggested)

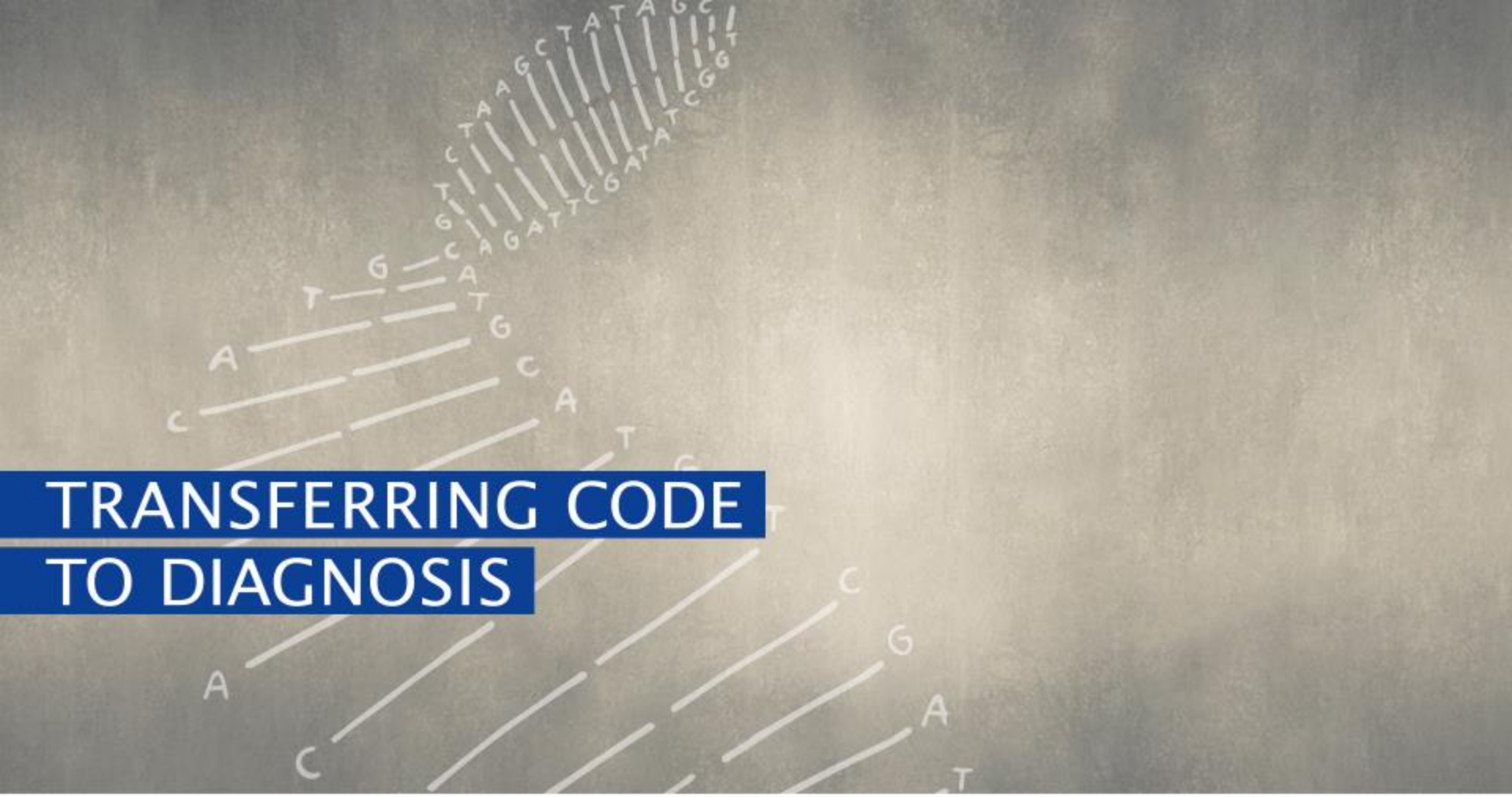
… a device specifically intended to select **the best medicinal product** for patients with a previously diagnosed condition or predisposition.

*„… ein Produkt, das **speziell** dafür bestimmt ist festzustellen, **welche** Therapie für Patienten mit einem bereits diagnostizierten Zustand bzw. einer bereits bekannten Prädisposition **am besten** geeignet ist.“*

Germany

Suggestions

- EBM: Get rid of the 20Kb limitation in 19453 (somatic tumor diagnostic).
- Incorporate liquid biopsy into the EBM.
- Quality: A lab accreditation should be mandatory.



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