

# Companion Diagnostics and Diagnostics – Applications, Limitations and Outlook

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# 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GOÄ severely outdated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

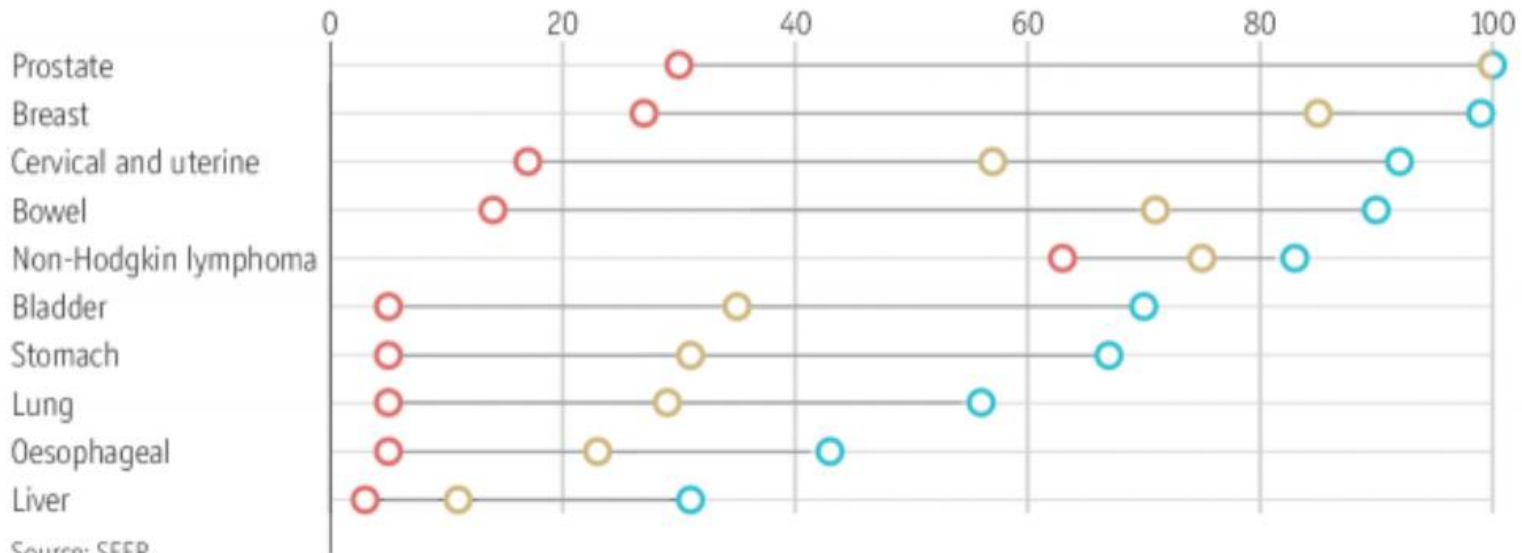
- Each of the  $\sim 10^{13}$  cells in the human body receives tens of thousands of DNA lesions per day
- Strong sunlight can induce  $\sim 100,000$  lesions per exposed cell per hour
- The DNA damages are constantly happening, the vast majority is being repaired, fewer than one mutation in a thousand 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 is our greatest opportunity to improve survival

## First come, first saved

United States, five-year relative survival rate by state at diagnosis, 2016, %

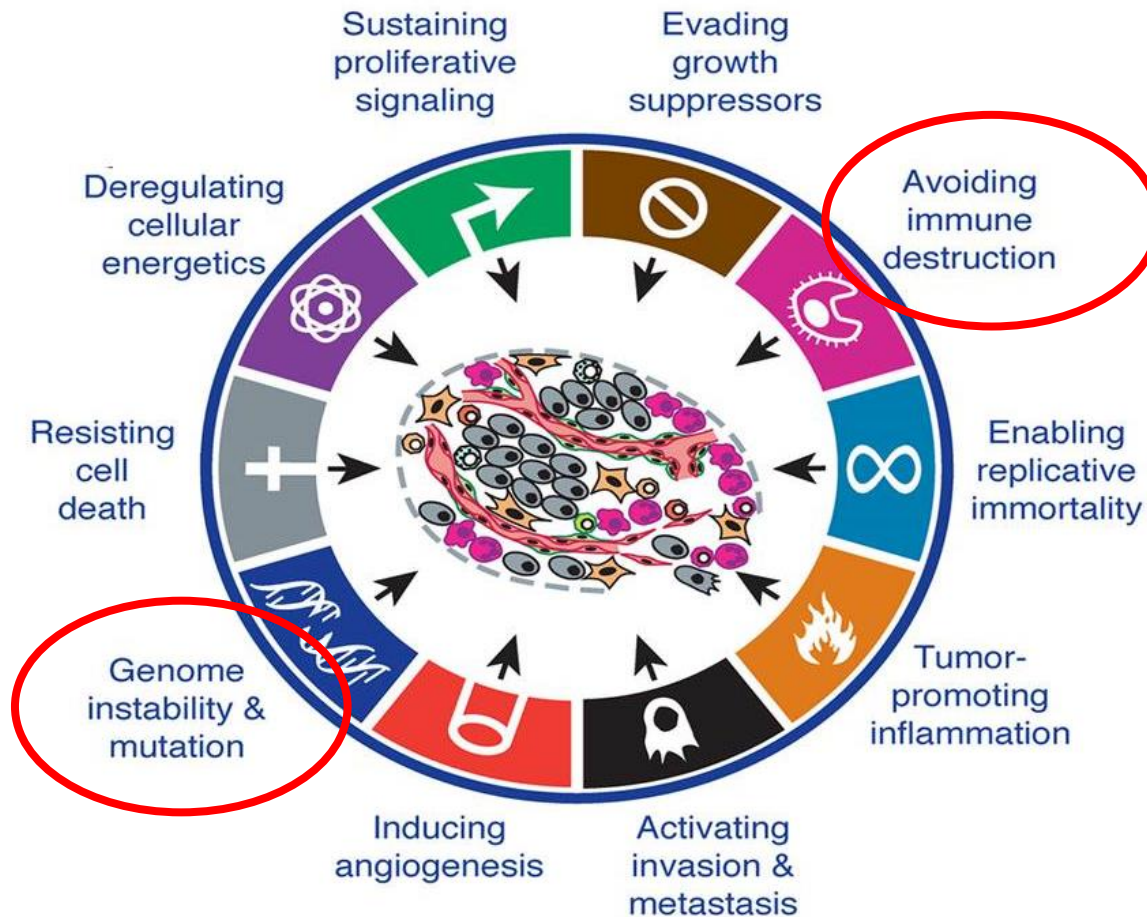
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Source: SEER

Economist.com

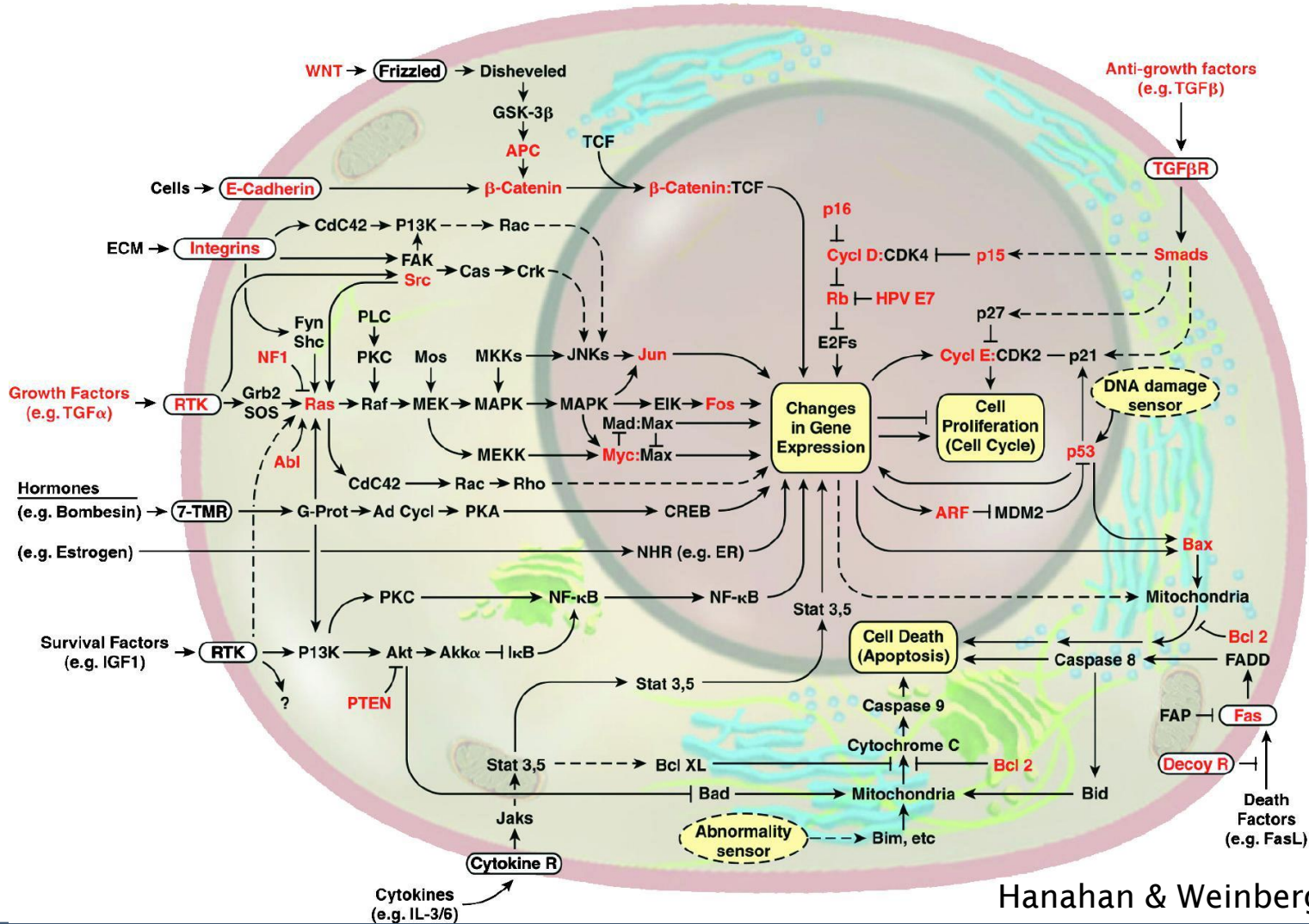
# Tumor biology



Hanahan & Weinberg, Cell 2011



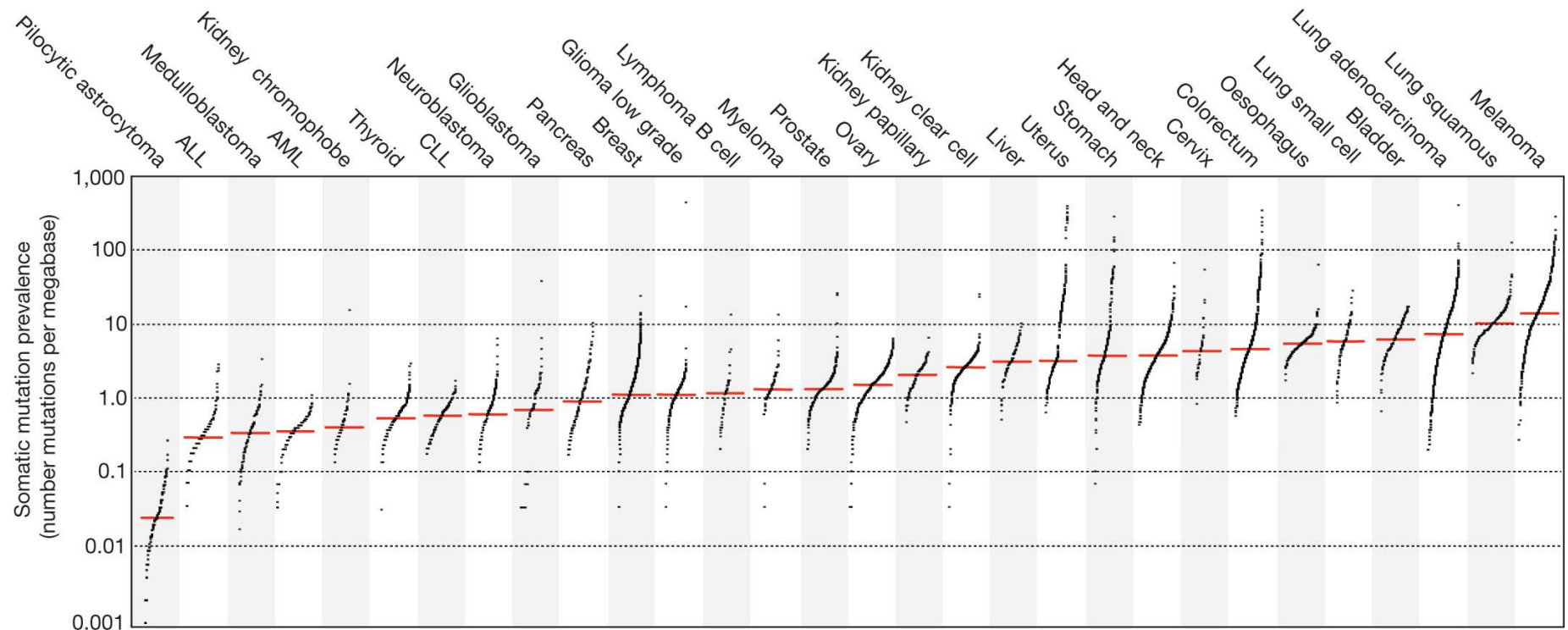
# Driver mutations lead to „Signalopathies“



Hanahan & Weinberg, *Cell* 2000

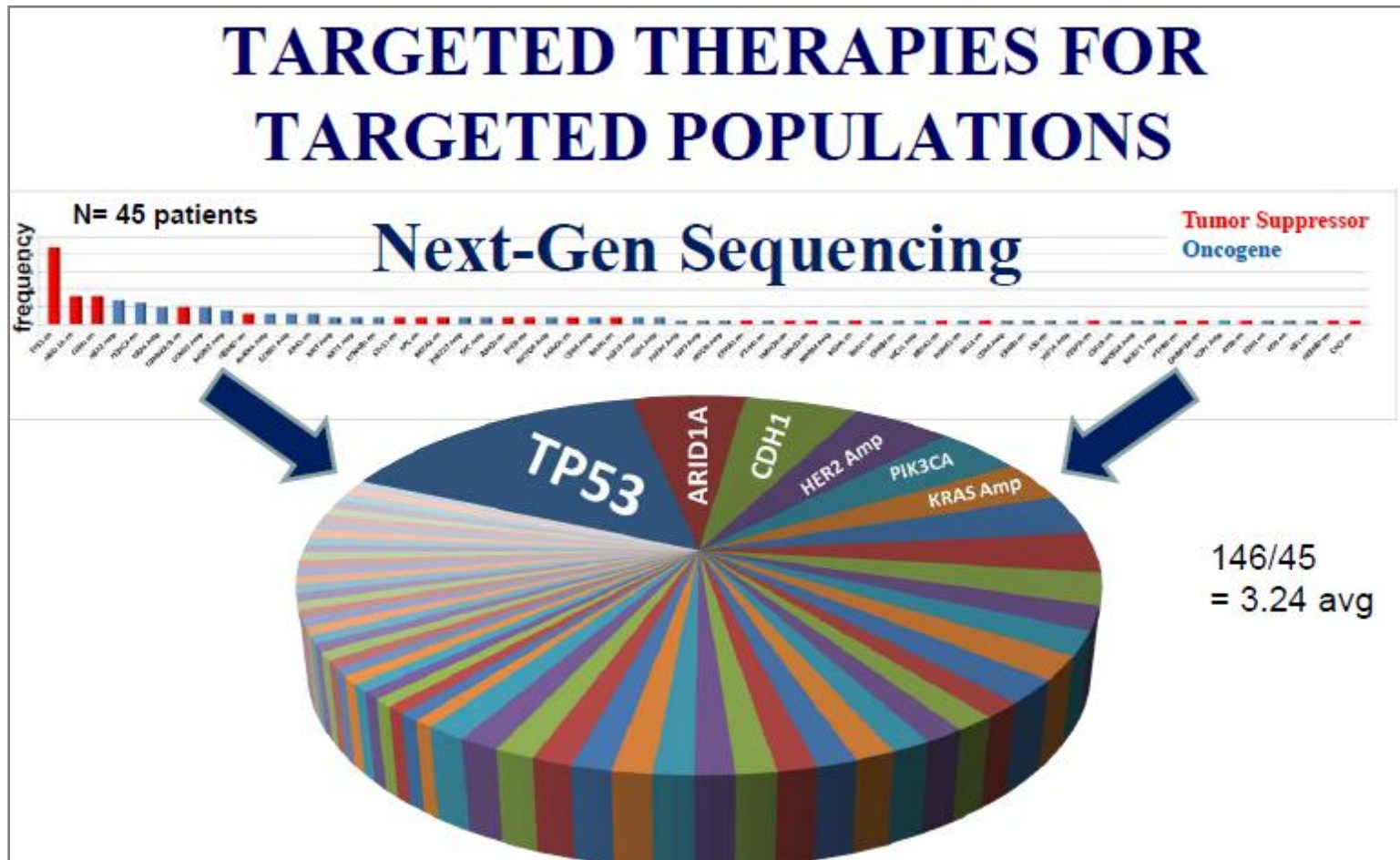


# Mutational Burden



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

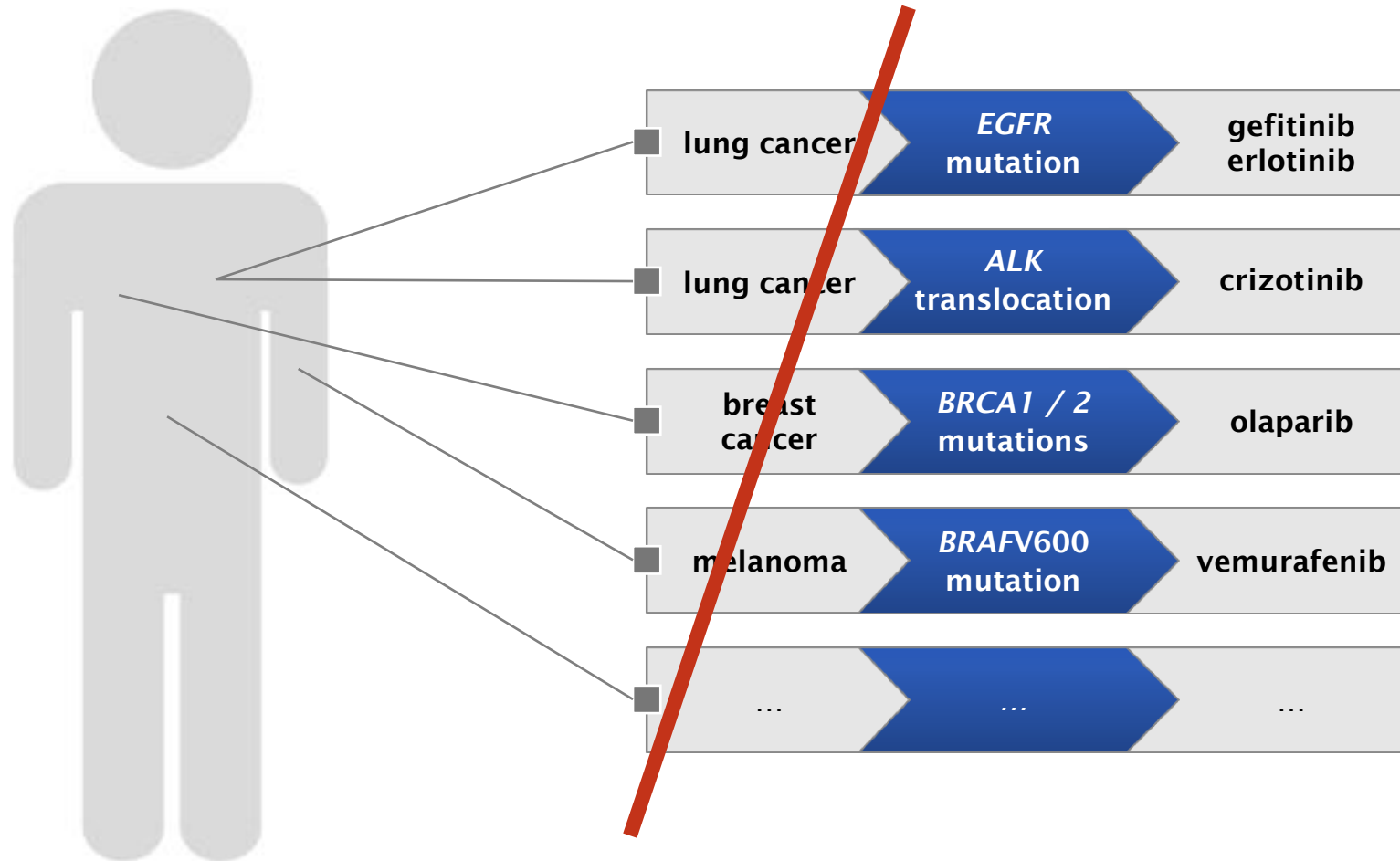


# Targeted Tumor therapy - Olaparib

## **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

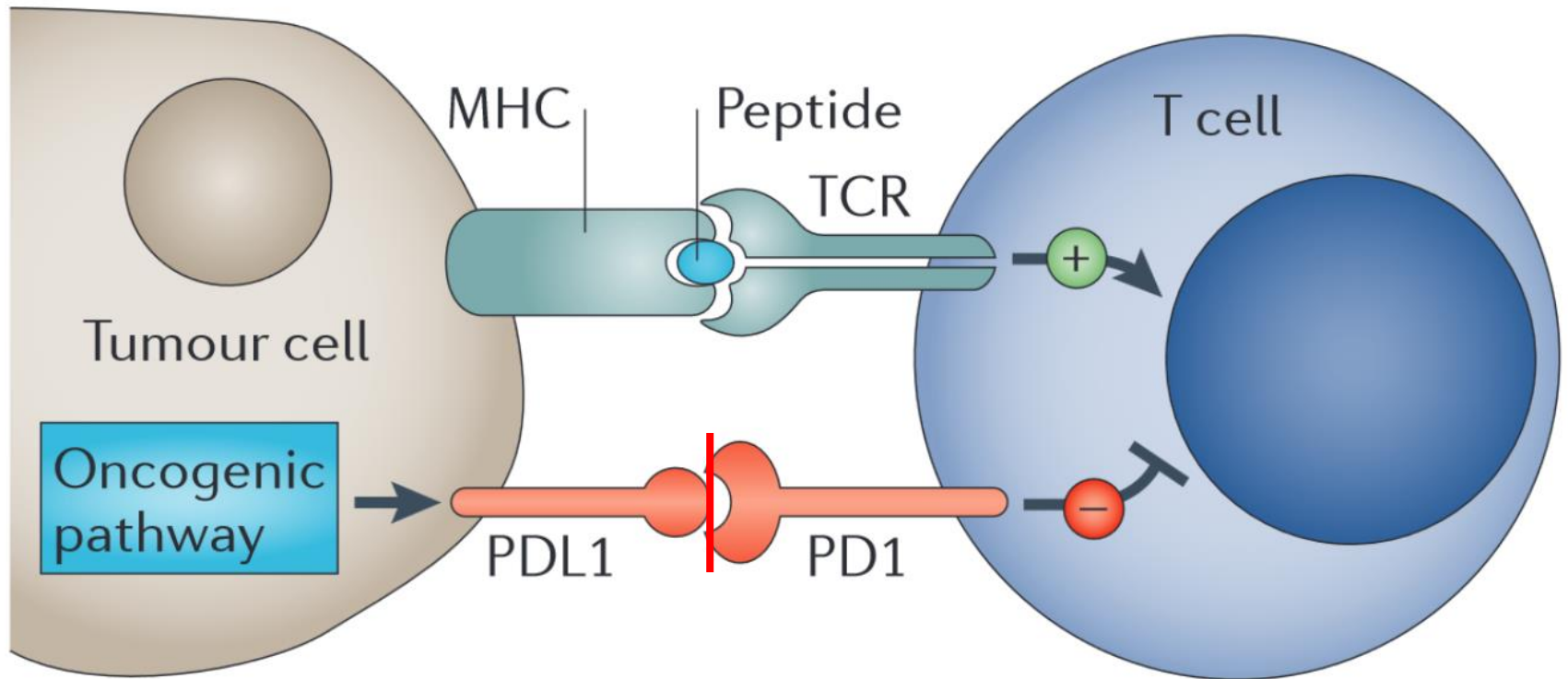


# Targeted Tumor therapy - Larotrectinib

## Larotrectinib

- works for TRK fusion patients (tropomyosin 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
<ul style="list-style-type: none"><li>– ca. 20,000 genes</li><li>– Very sensitive, if tumor content is &gt;50%</li><li>– 150x</li><li>– TAT 2 – 3 weeks</li></ul>	<ul style="list-style-type: none"><li>– 710 tumor-and pharmacogenetic relevant genes</li><li>– Translocations in 29 genes</li><li>– 1,000x</li><li>– TAT 2 – 3 weeks</li></ul>	<ul style="list-style-type: none"><li>– expressed mutations</li><li>– fusion transcripts</li><li>– abundance of altered transcripts</li><li>– PD1/PD-L1 expr.</li><li>– BRCAness</li></ul>
Mutational load Neoepitope prediction	Mutational load, Treatment Decision Support	Treatment Decision Support



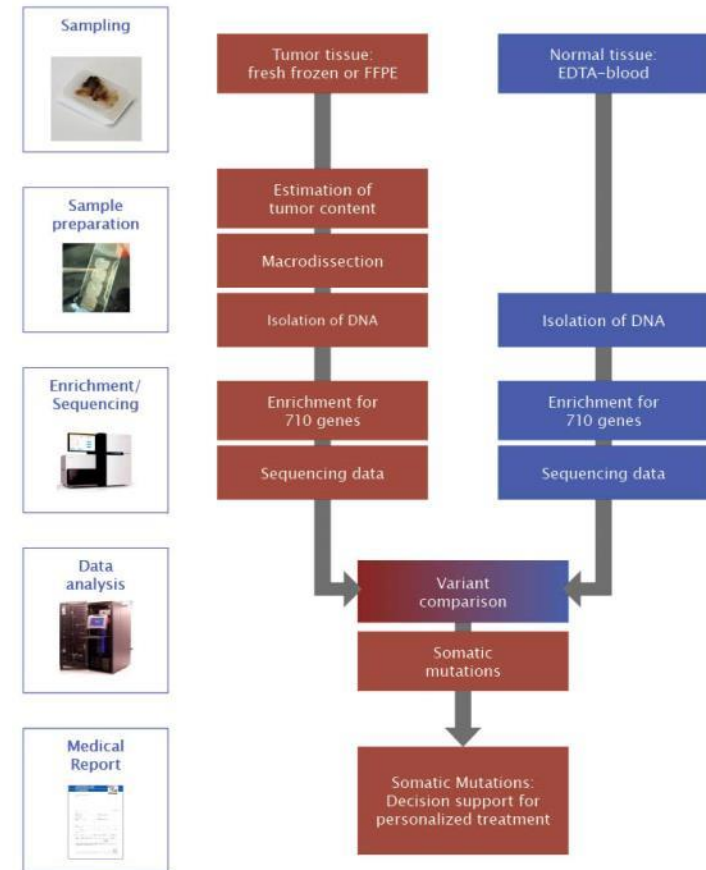
# Somatic Tumor Panel

## 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 achieve 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



# Report – Somatic Tumors

Gene	Functional category	Effect	Transcript ID	NPV
KRAS	oncogene	p.G12D	NM_004465.4	0.26
CDKN2A	stop, gain	p.RB2*	NM_000774.4	0.49
CDKN2A	oncogene	loss of wildtype allele	NM_000774.4	n.s.
TP53	oncogene	p.R572Y	NM_000538.5	0.49
TP53	oncogene	loss of wildtype allele	NM_000538.5	n.s.

concerning copy number variations is Array-CGH analysis, which can be performed upon request.

Somatic mutations classified as having **no** current therapeutic relevance are listed in the supplementary information.

KRAS, c.35G&gt;A; p.G12D, NM\_004353.4

KRAS is an oncogene that encodes K-Ras, a member of the Ras family of membrane proteins that bind GTP and possess GTPase activity. Activation of Ras signaling causes cell growth, differentiation, and possibly activation of the R-RAS/GRB2/PLK1 kinase pathway and the PI3K/Akt pathway.

KRAS mutation has been reported to be one of the most common mutations involved in pancreatic tumorigenesis, with mutations detected as an early event in intraepithelial neoplasms, and found to be

involved in transcriptional/pioneering carcinoma (Murphy et al., 2013, PMID 23912084; Campbell et al., 2007, PMID 17323359; Ewer et al., 2014, PMID 24755586; Kleppel et al., 2013, PMID 25262472; Cheng et al., 2014, PMID 25243635; Ferro and Falasca, 2014, PMID 25152578). KRAS mutations have also been found to be critical to pancreatic tumor maintenance, and to play a role in invasion and cell motility (Almouzni et al., 2011, PMID 21195524; Hultman et al., 2012, PMID 22952003; Rachez et al., 2011, PMID 21564589).

KRAS-G12D is an activating mutation. Activating mutations in KRAS result in activation of downstream pathways, including the Raf/MEK/ERK pathway (Nakano et al., 1994, PMID 8320714; Polyak-Gupta et al., 2011, PMID 21955244). Current guidelines of the European Society for Clinical Oncology (ESMO),

20022658; Ludovisi et al., 2011, PMID 21268260; Sun et al., 2013, PMID 23724088; Pao et al., 2005, PMID 15696205]. The MEK inhibitor trametinib has been approved for use in BRAF V600-mutant melanoma. Trametinib and other MEK inhibitors, alone or in combination therapy, are in clinical trials, as

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### Sample clinical trials

Study ID	
NCT02283803	Title: Lapatinib Plus Trastuzumab in KRAS Mutant Malignancies (M14-76)
Phase I/II	Target: HER, EGFR, KRAS
	Contact: Jan HIR-Schellens, MD PhD: 0031-20-6121847, j.hir@amc.uva.nl
	Study site (Referring)
	The Netherlands Cancer Institute

Phase 1/2

<p>gyn. 065, inst@rzu.nl</p> <p>Contact: Jan-HF Schellekens, MD, PhD, +31 20 512 2440   j.schellekens@rzu.nl</p> <p>Study site: (Reusding)</p> <p>Netherlands Cancer Institute – Antoni van Leeuwenhoek Hospital Amsterdam, Netherlands, 10523 Contact: J.H.M. Schellekens, MD, PhD</p>
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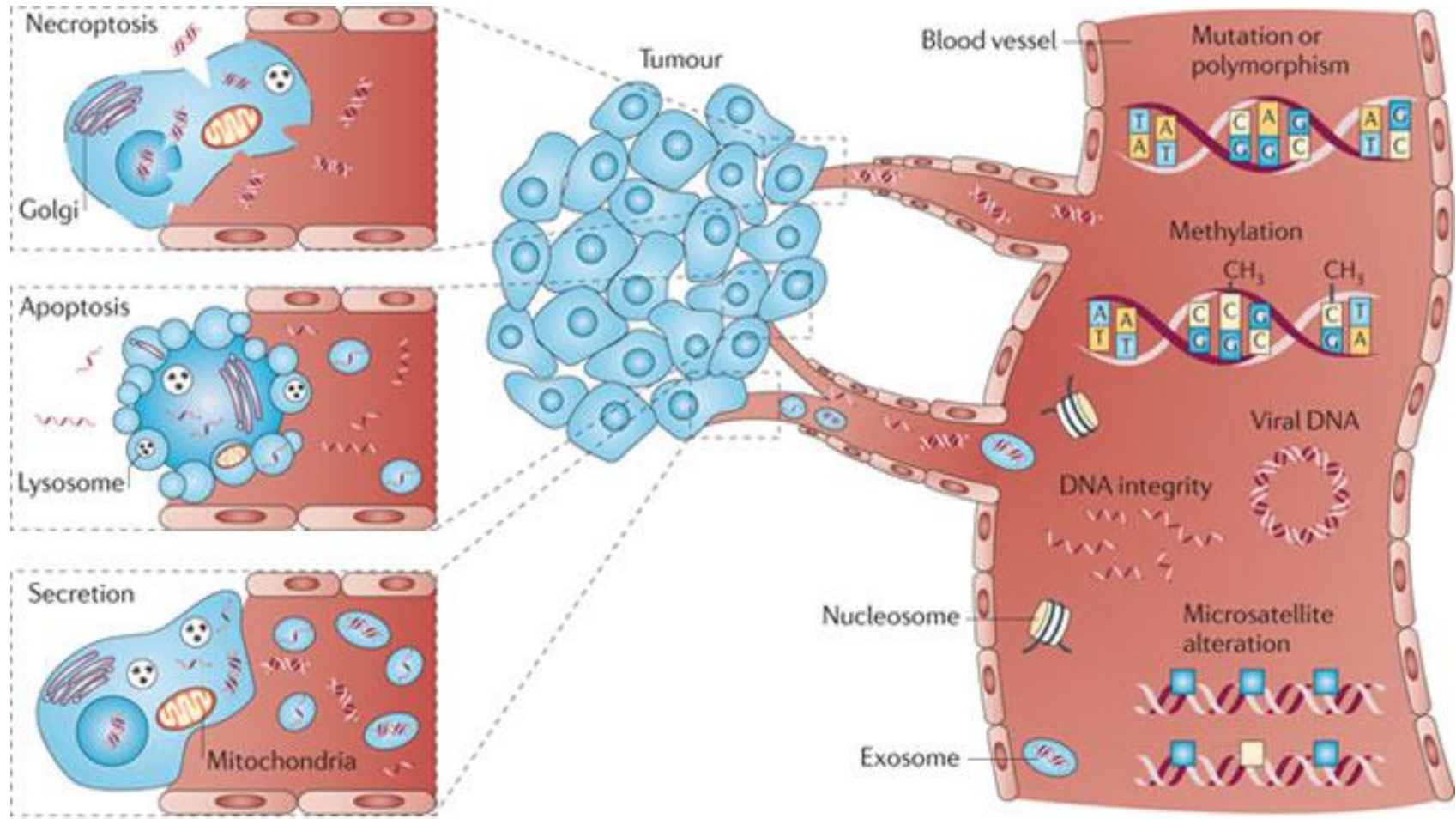
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**Section 2: (3) Details and (4) interpretation of identified variants with potential therapeutic relevance**

Ce / GaT

# The future is now: Liquid Biopsy



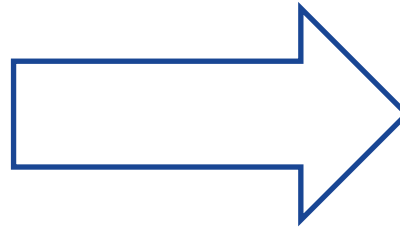
Nature Reviews | Cancer  
Schwarzenbach et al. 2011

# 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

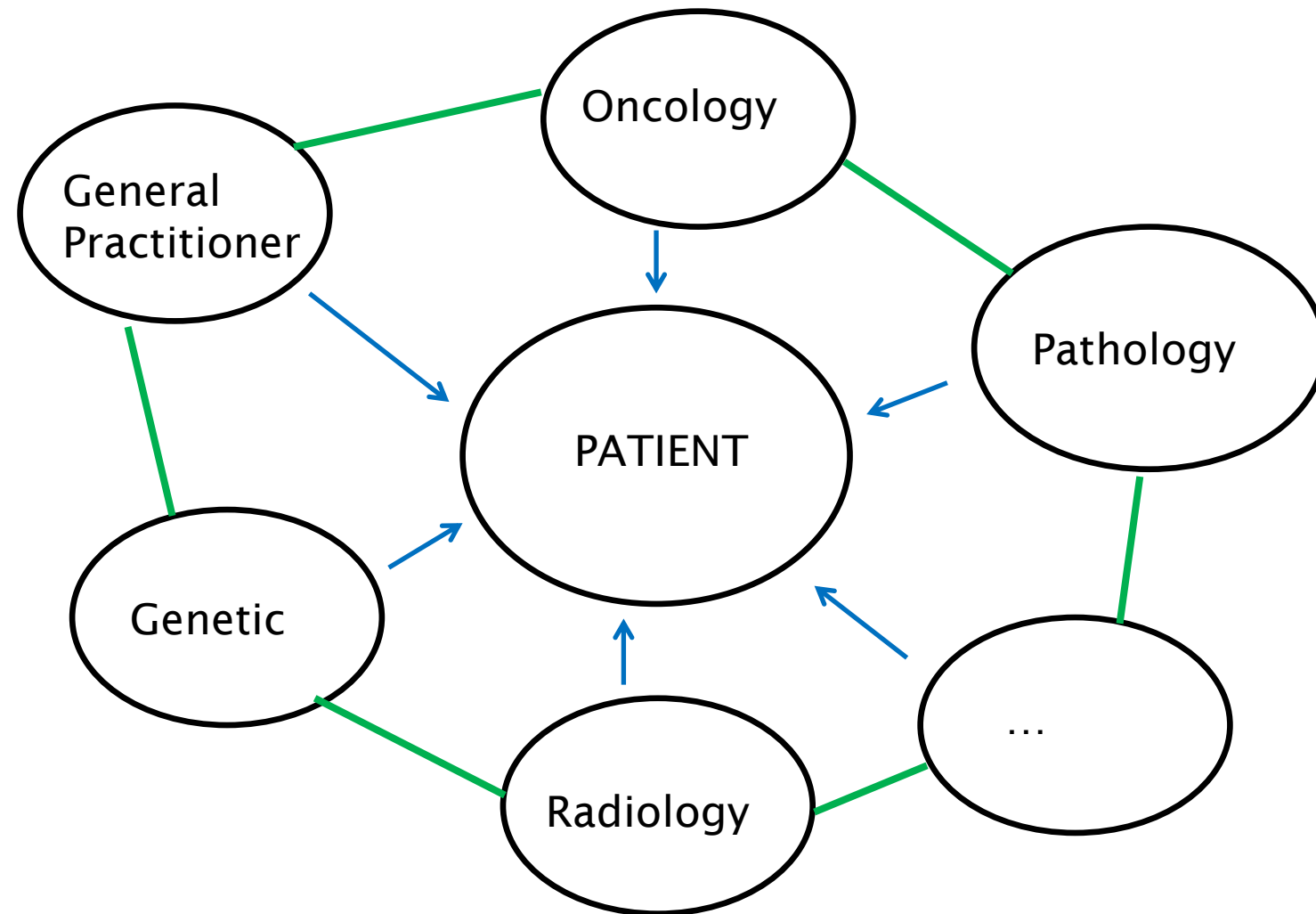


# Summary

- **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 a 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**

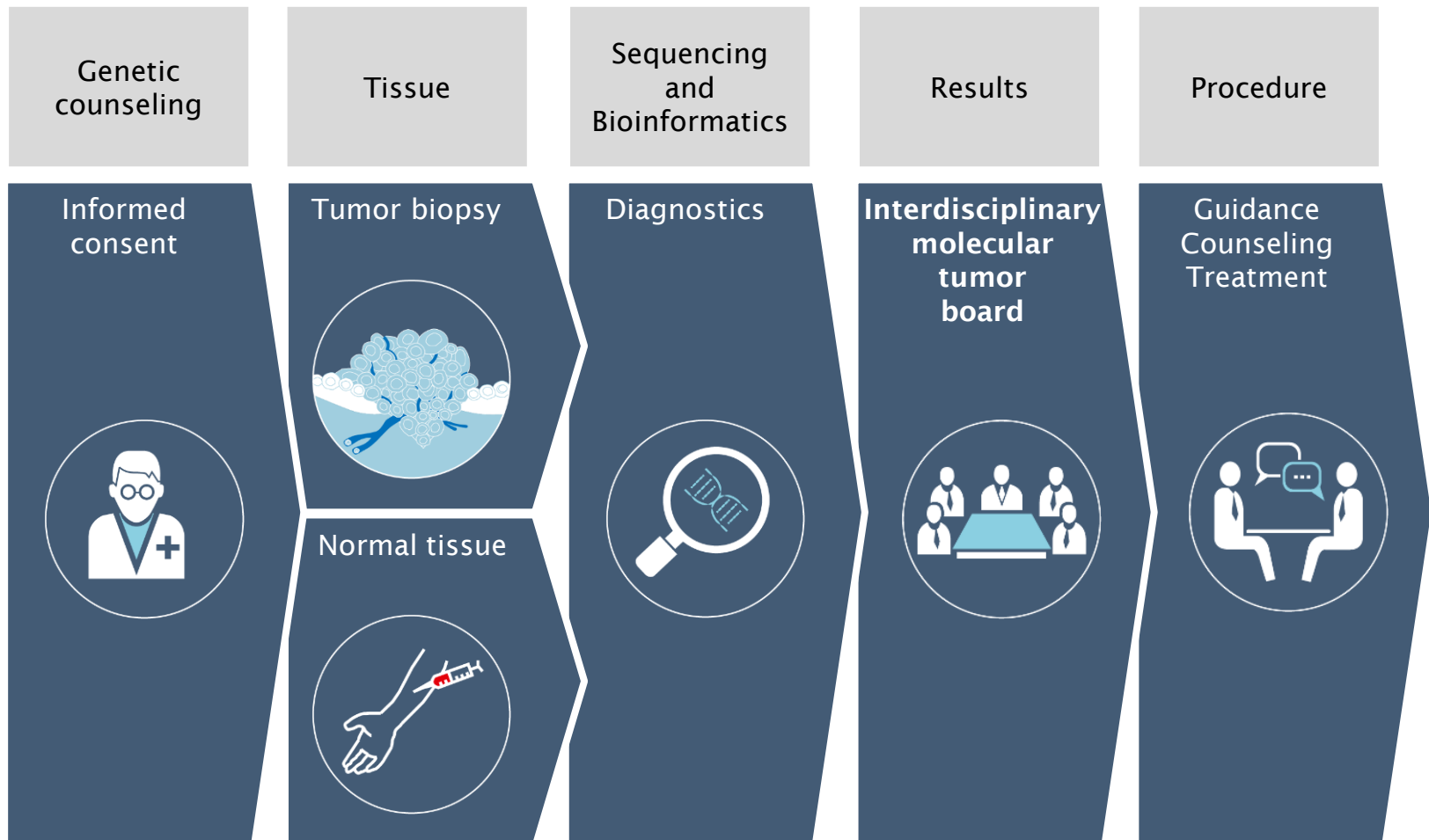
**Prevention**

**Personalized**

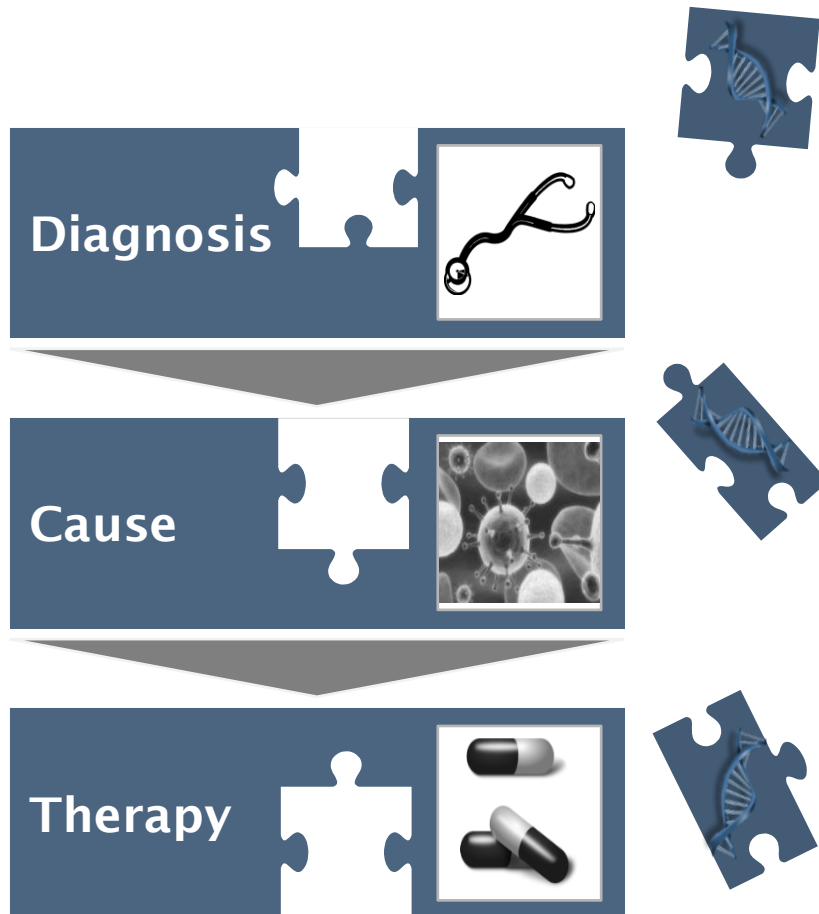
**Participating**

**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.“*



## 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.



# TRANSFERRING CODE TO DIAGNOSIS

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