Functional Genomics and CRISPR

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February 18, 2020

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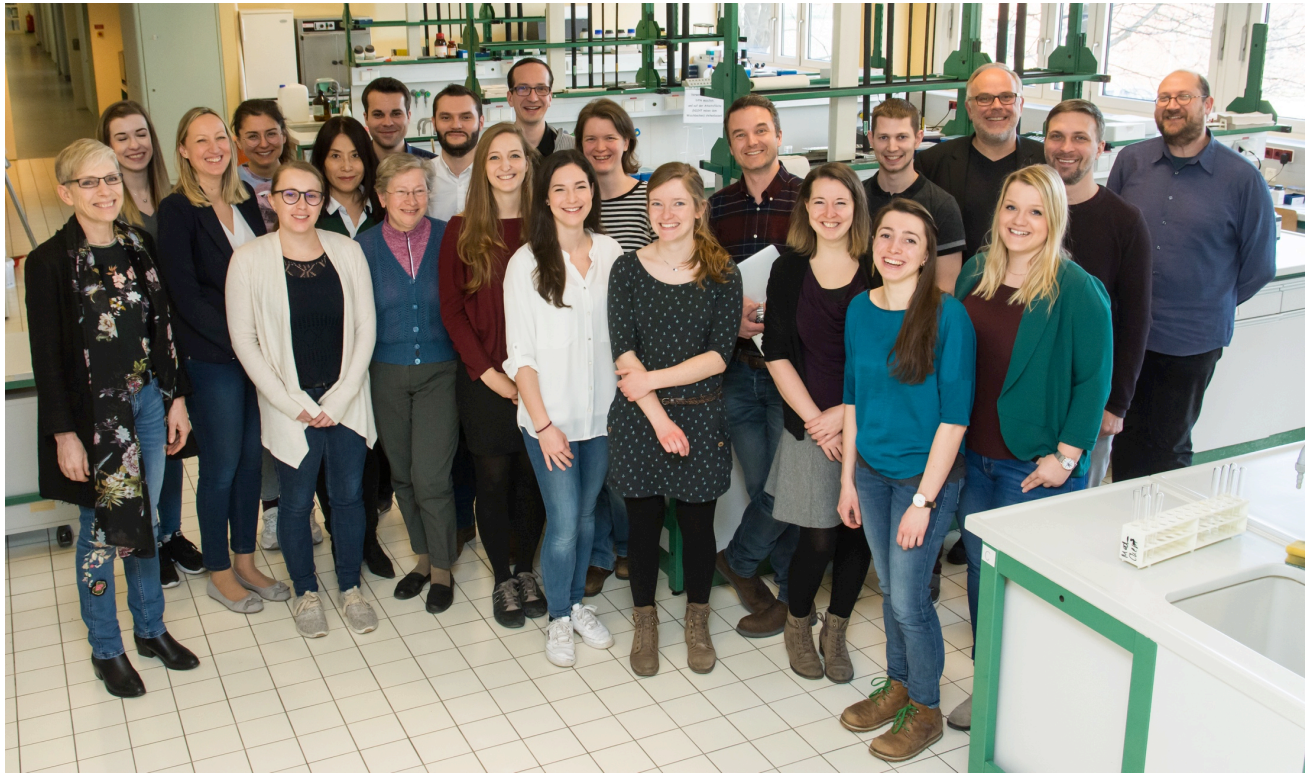
The University of Veterinary Medicine Vienna

The Team

Staff of the Institute of Medical Biochemistry, Vetmeduni Vienna (2019)



Our mission

Investigate molecular mechanisms in

Cancer – Acute Injury – Neurobiology

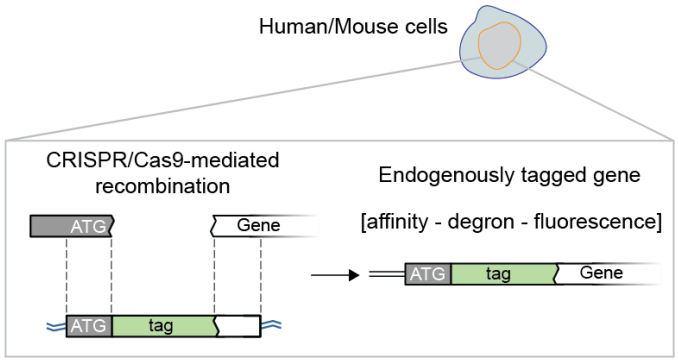
to improve patient management

3 important technological cornerstones of our research:

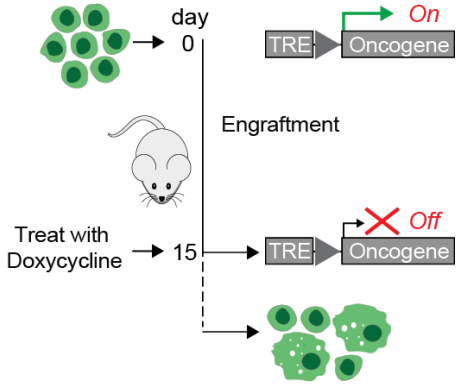
+ *Advanced cell culture and in vivo models*
+ *Global approaches*
+ *Detailed mechanistic studies*

Advanced cell culture and in vivo models

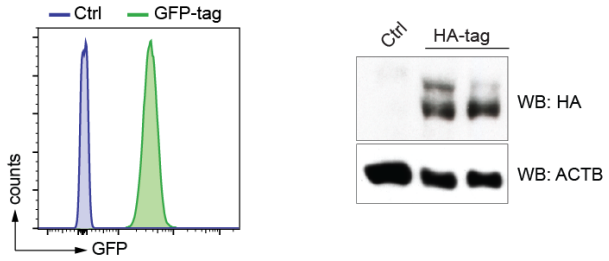
Cellular Models for Functional Studies



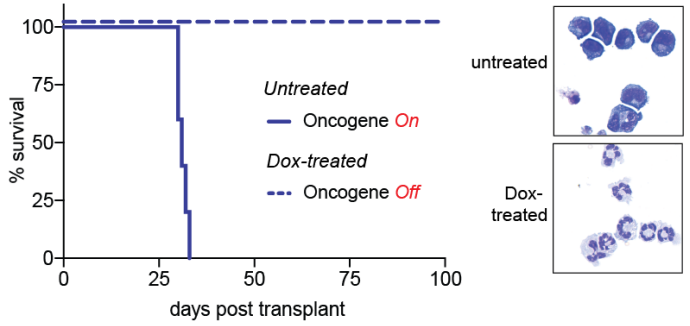
In vivo Models for Controlled Oncogene Expression



Tools for Detection - Isolation - Manipulation of Proteins

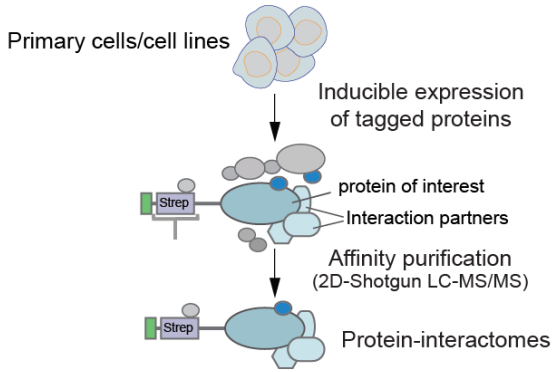


Investigation of *in vivo* Oncogene Dependence in Leukemia

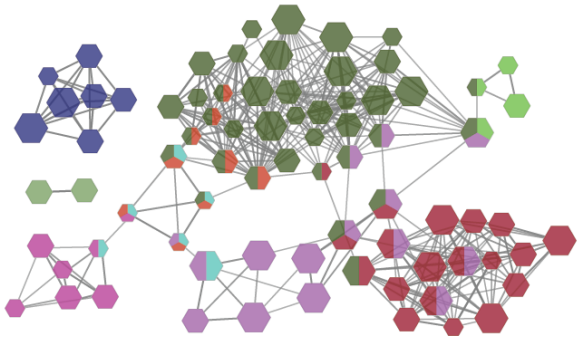


Global approaches

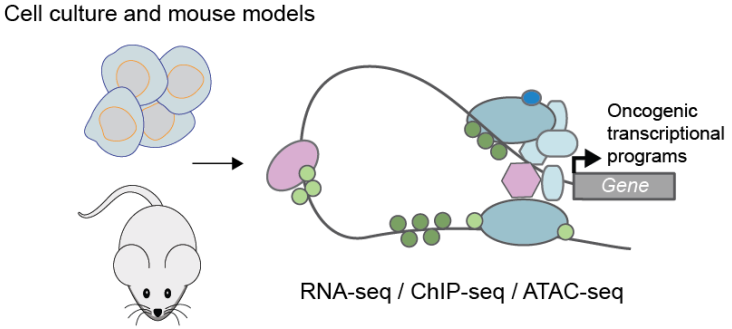
Mass Spectrometry-Based Interaction Proteomics



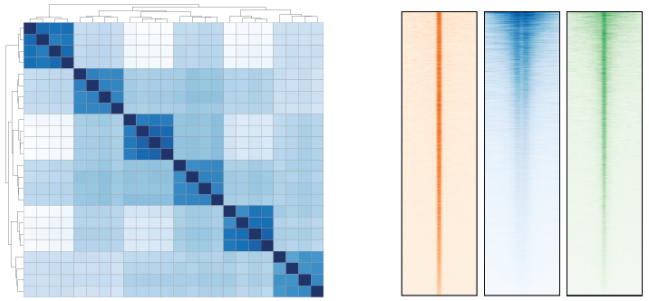
Functionally Annotated Protein Interaction Networks



Global Genomic Analysis of Disease Models

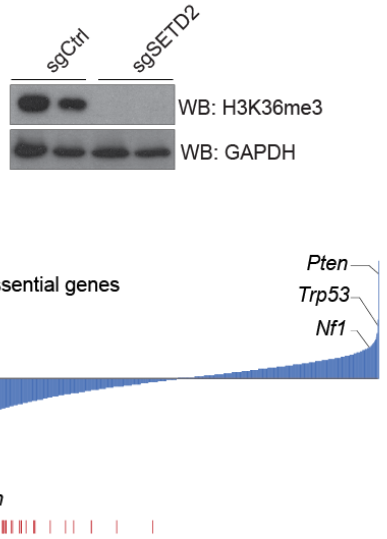


Global Patterns of Transcriptional and (Epi)genomic Alterations

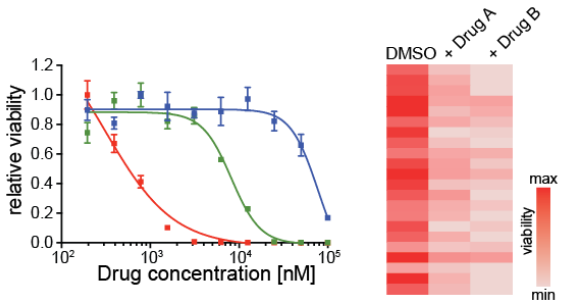


Detailed mechanistic studies

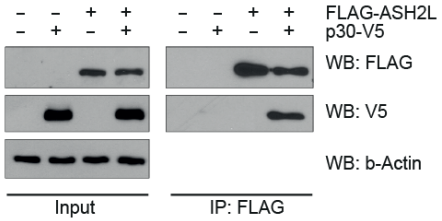
CRISPR/Cas9 Loss-Of-Function Approaches (single genes vs. genomes)



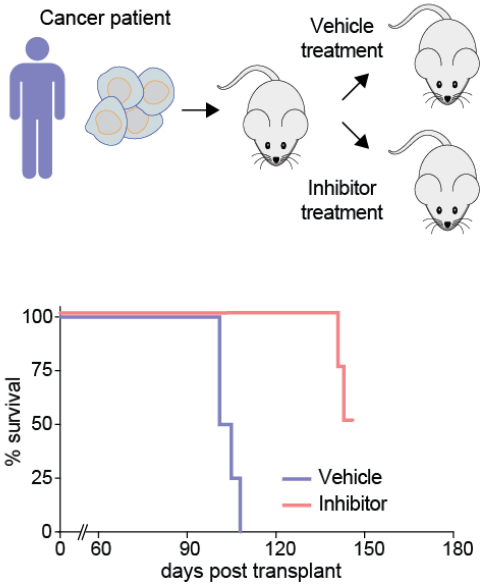
Chemical Biology/Enzymology



Biochemistry

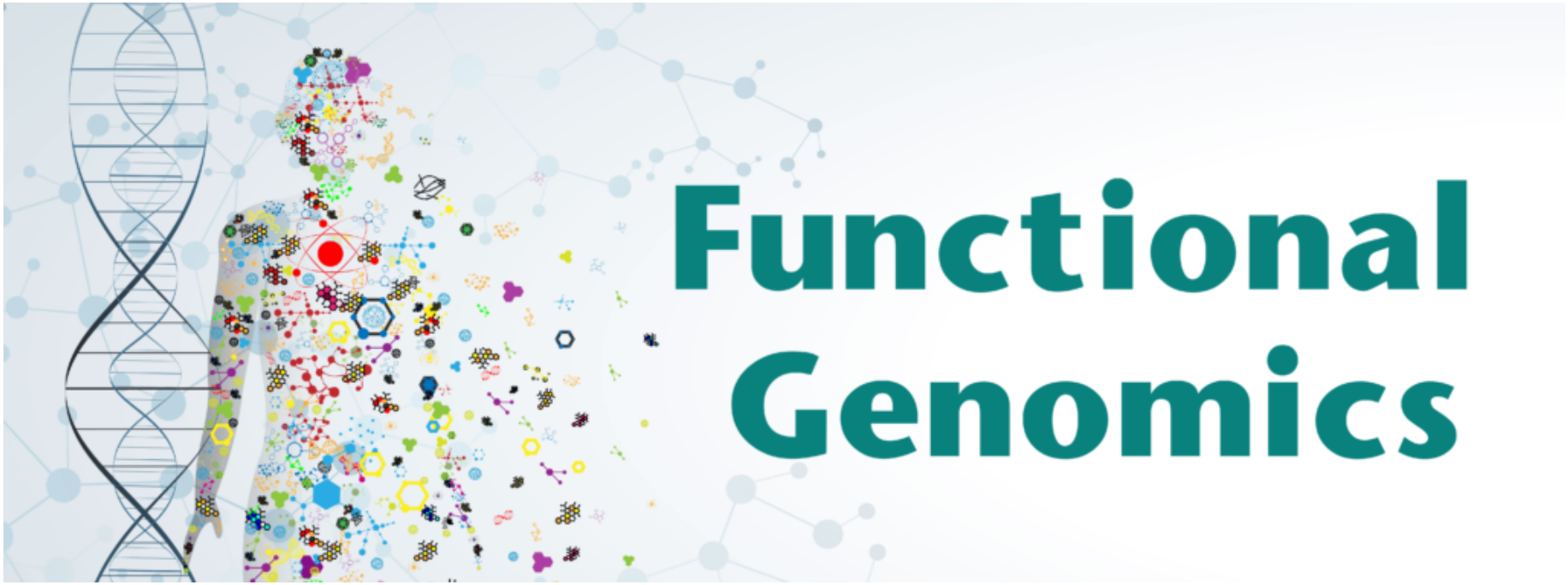


Patient-Derived Xenograft Models



Functional Genomics

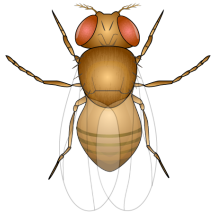
What is functional genomics?



http://nas-sites.org/dels/events/next-steps-for-functional-genomics-a-workshop/

What makes us different?

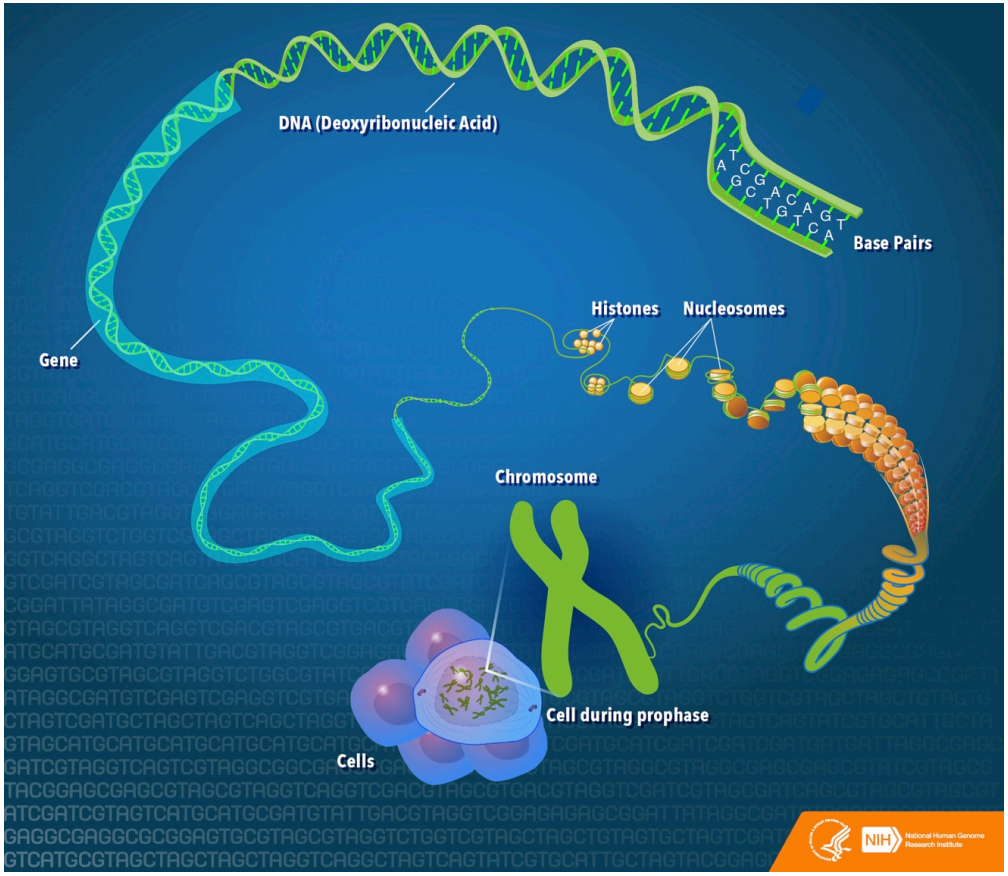
Model organisms



Veterinary Species

Humans

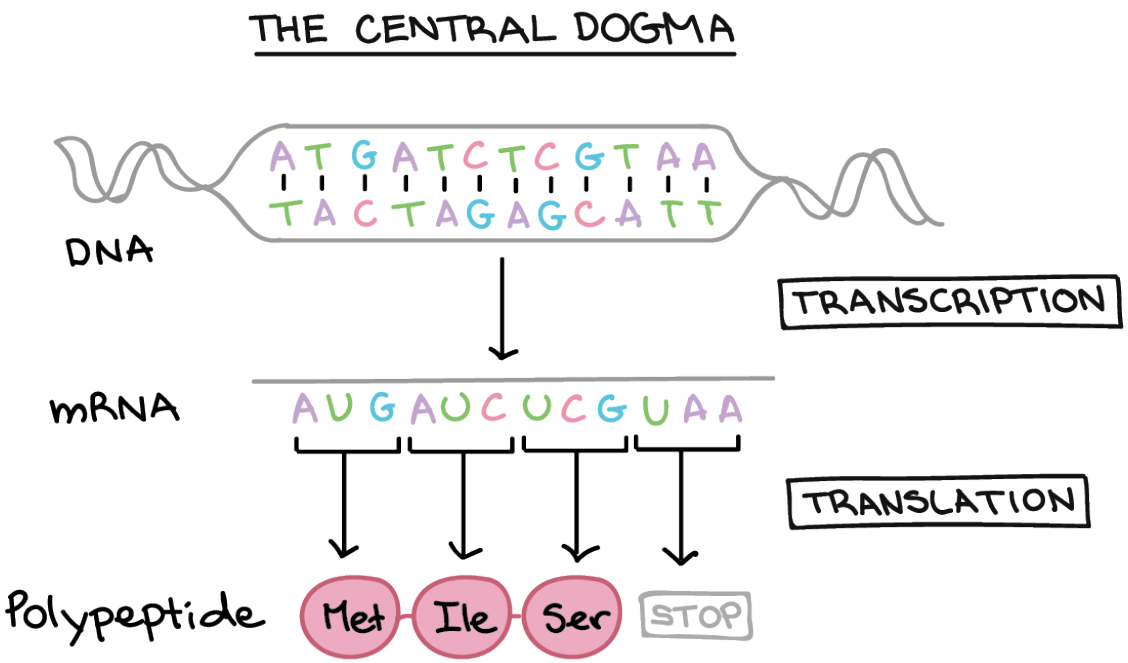
Genomes store information



Genome vs. Epigenome

https://www.genome.gov/about-genomics/fact-sheets/A-Brief-Guide-to-Genomics

“The Central Dogma”



Functional Genomics

Functional genomics is the study of how genes and intergenic regions of the genome contribute to different biological processes.

https://www.medicalnewstoday.com/articles/318764

Functional Genomics

How do the components of a biological system work together to produce a particular phenotype?

Functional Genomics focuses on the dynamic expression of gene products in a specific context, e.g.

- Development
- Disease

→ Linking genotype to phenotype

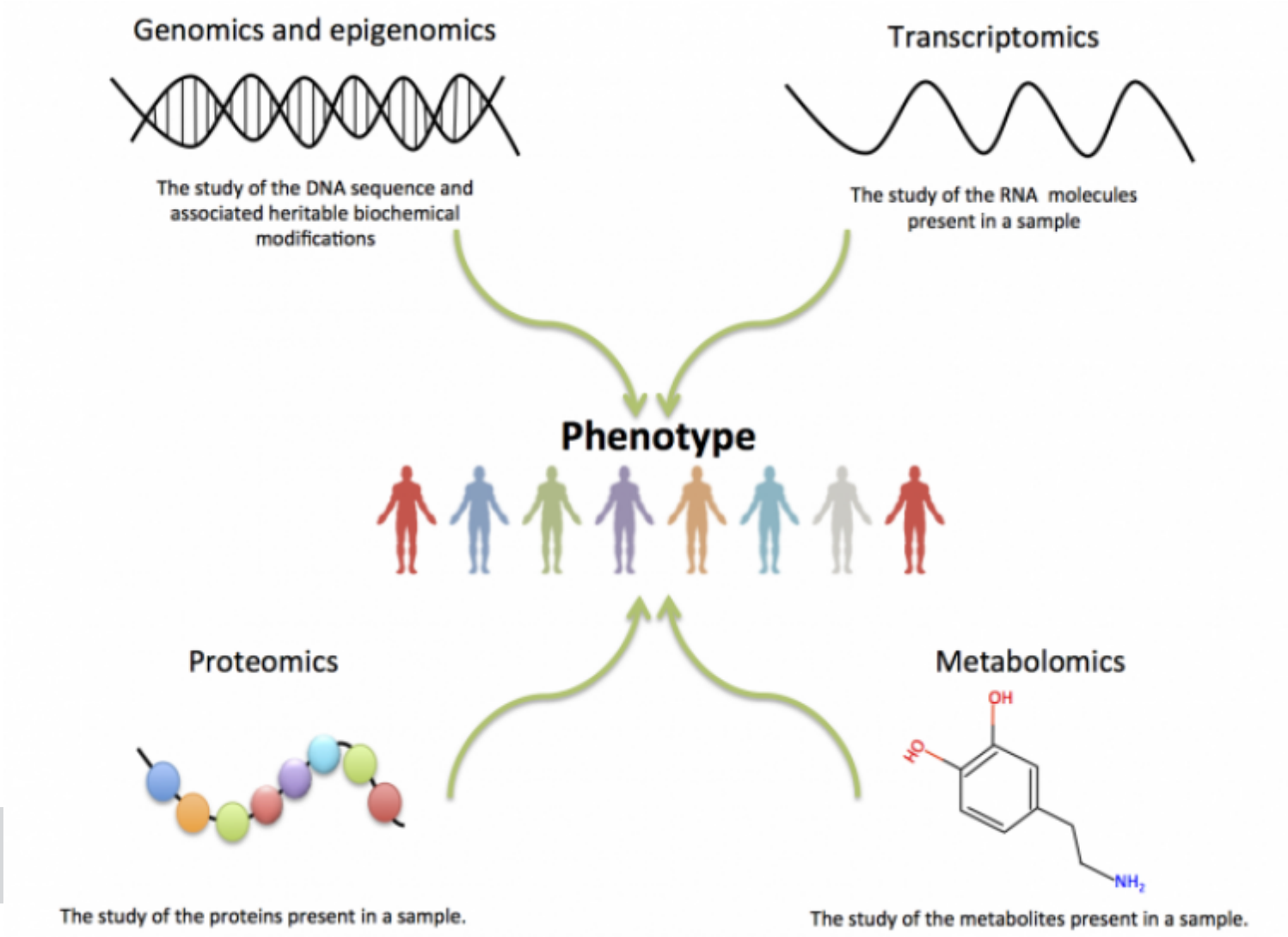
Functional Genomics Approaches

Researchers in the field of functional genomics often study effects in a global level
i.e. ***"genome-wide": -Omics studies***

Approaches:

- DNA level: Genomics / Epigenomics
- RNA level: Transcriptomics
- Protein level: Proteomics
- Metabolite level: Metabolomics

Functional Genomics Approaches

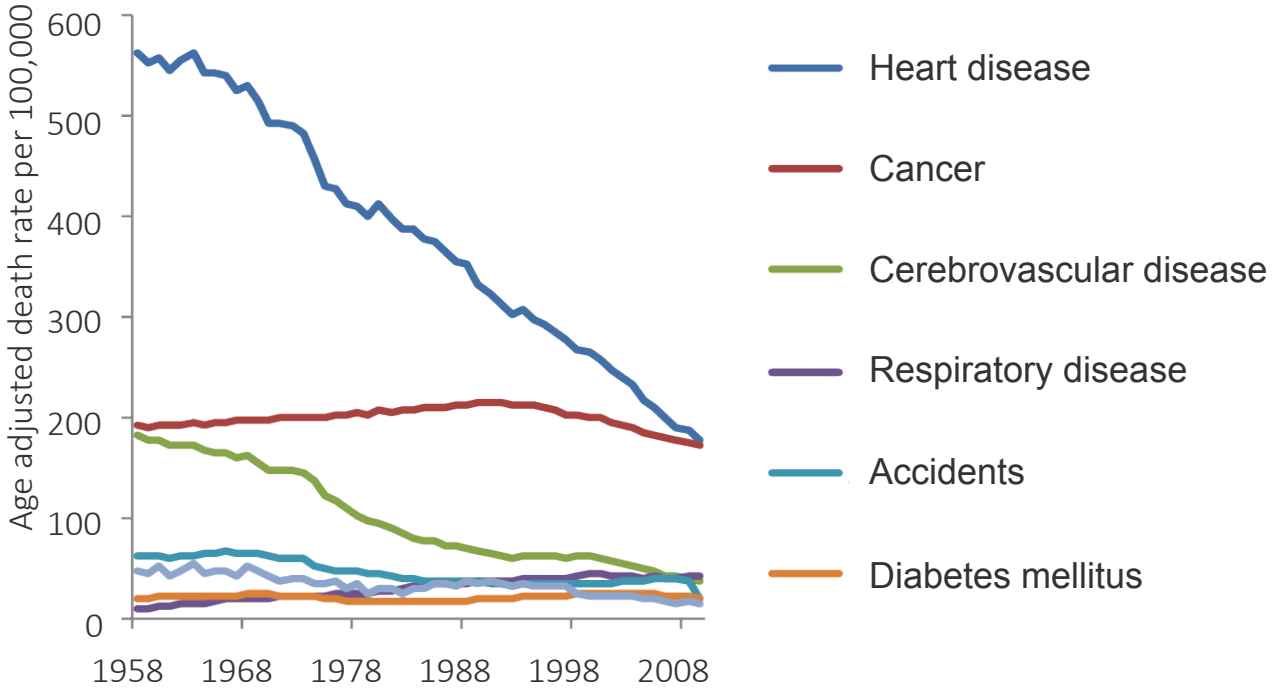


Functional Genomics

Examples of biological questions that can be tackled using functional genomics:

- Why are some cultivars of rice more resistant to drought than others?
- What makes some individuals more susceptible to skin allergies?
- **Why do some cancer drugs only work effectively on a subset of patients with the disease?**

Drug Discovery and Cancer



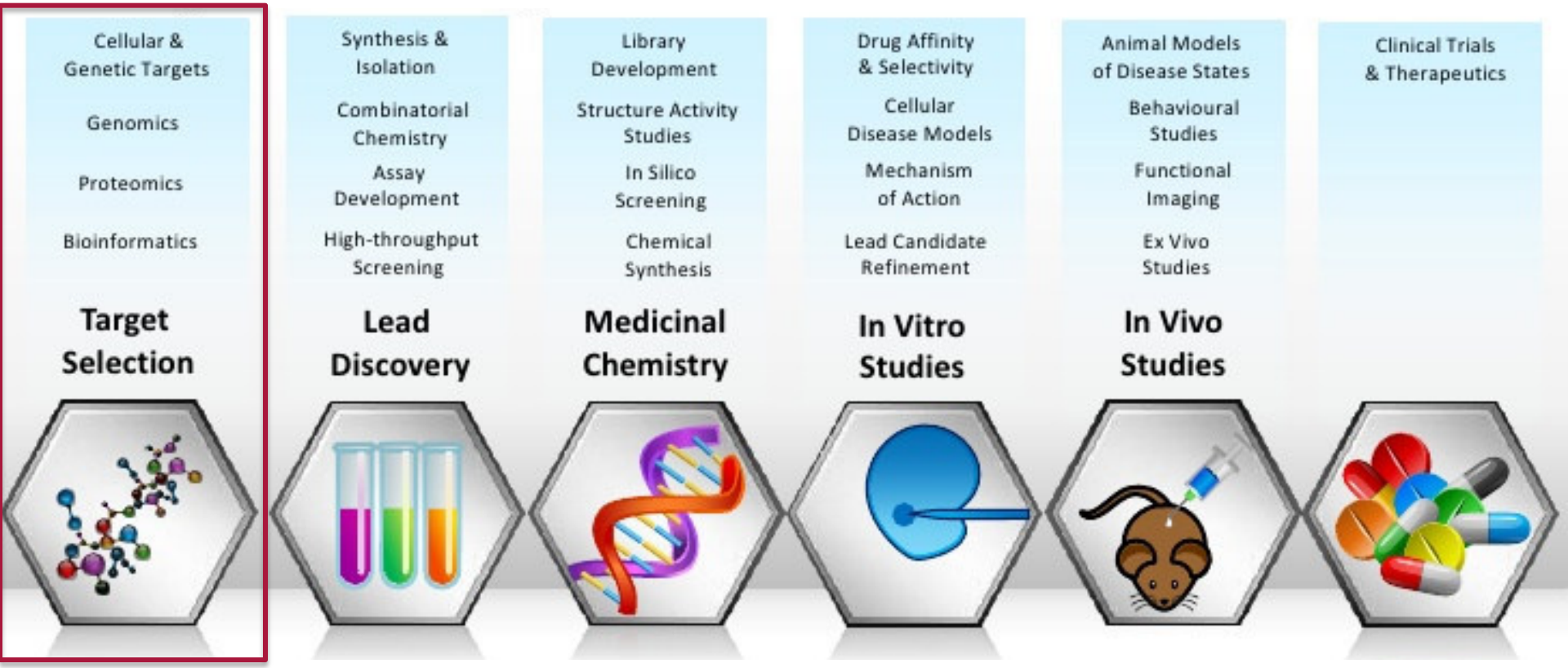
41% **of all people will be diagnosed with cancer at some point in their life**

Since 1971 (Nixon: "War On Cancer") **>1 Trillion $** **spent on cancer research**

Source: WHO, 2017

The Drug Discovery Process

Drug discovery is a multi-step process



https://www.embodi3d.com/gallery/image/900-drug-discovery-process/

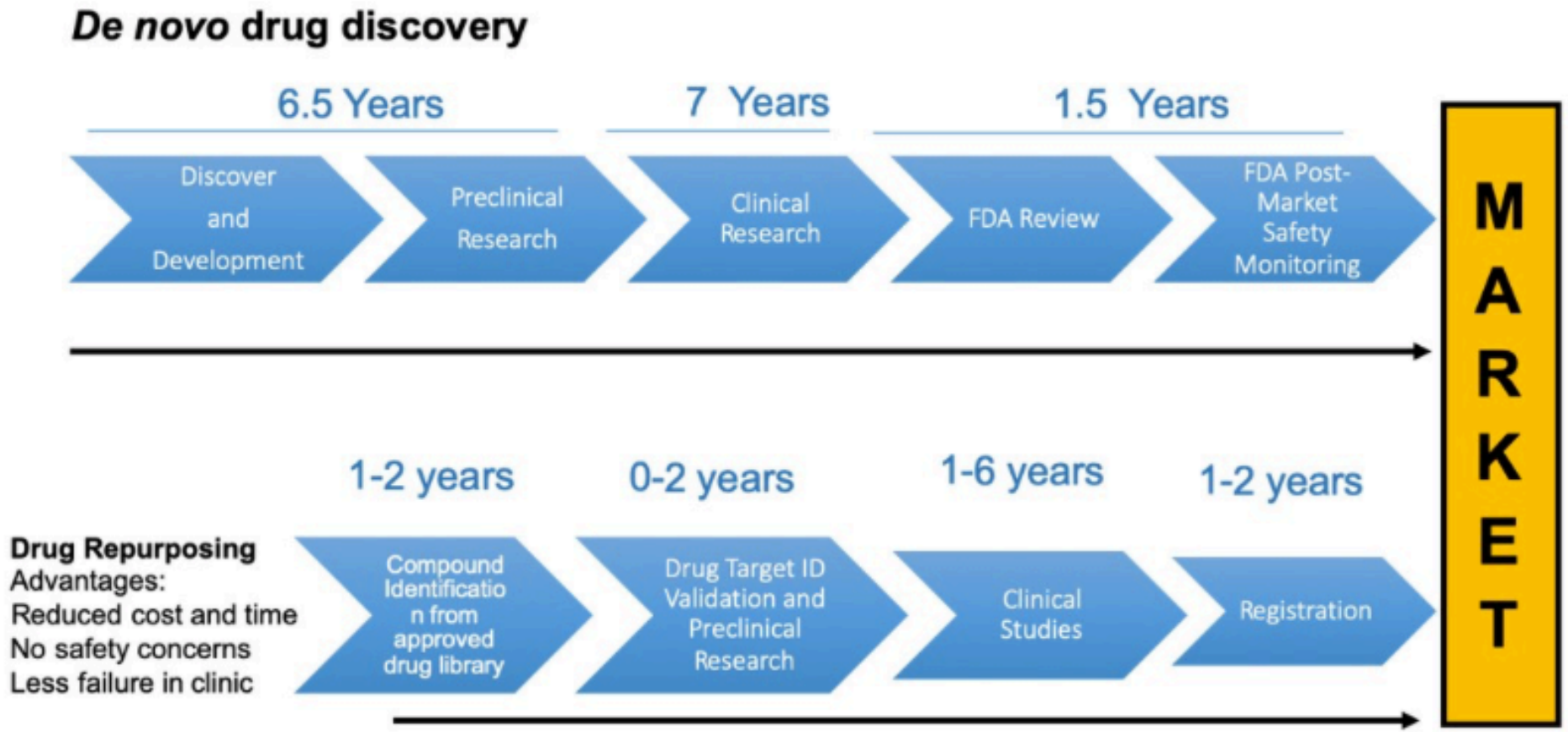
Functional Genomics in Drug Discovery

The first steps in the drug discovery pipeline:

- Characterisation of the disease process
- Identification of drug ('therapeutic') targets

A 'target' is defined as a protein or messenger RNA which, when modified by a drug, favourably affects the outcome of a disease.

The Drug Discovery Process



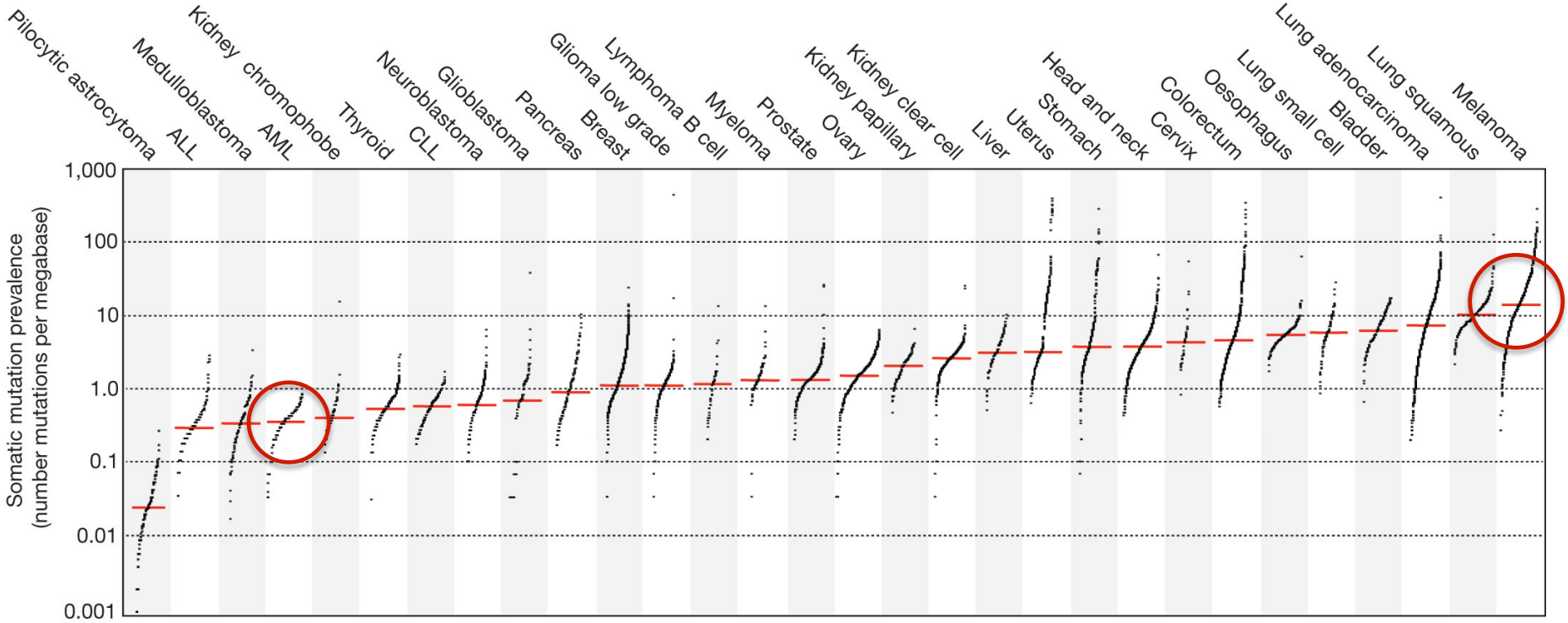
https://www.saintjohnscancer.org/translational-research-departments/drug-discovery/current-research-topics/introduction-to-drug-repurposing-for-rare-diseases/

The Genetic Complexity of Cancer

vetmeduni vienna

COSMIC
Catalogue of somatic mutations in cancer

Analyzed samples: 1,343,214
Coding mutations: 5,366,273



Size of the human genome: 3200 Mb

Lawrence et al., Nature (2013)

Acute Myeloid Leukemia (Blood cancer): 1000 mutations
Melanoma (Skin cancer): 30,000 mutations

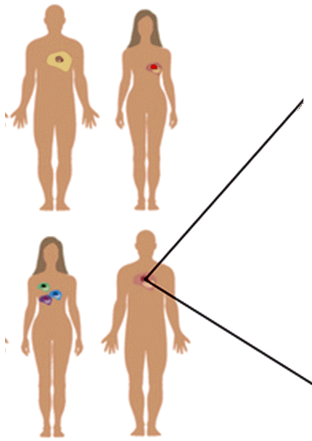
The Genetic Complexity of Cancer

vetmeduni vienna

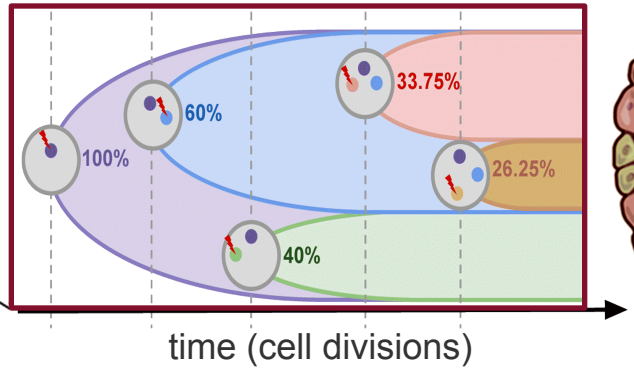
COSMIC
Catalogue of somatic mutations in cancer

Analyzed samples:	1,343,214
Coding mutations:	5,366,273

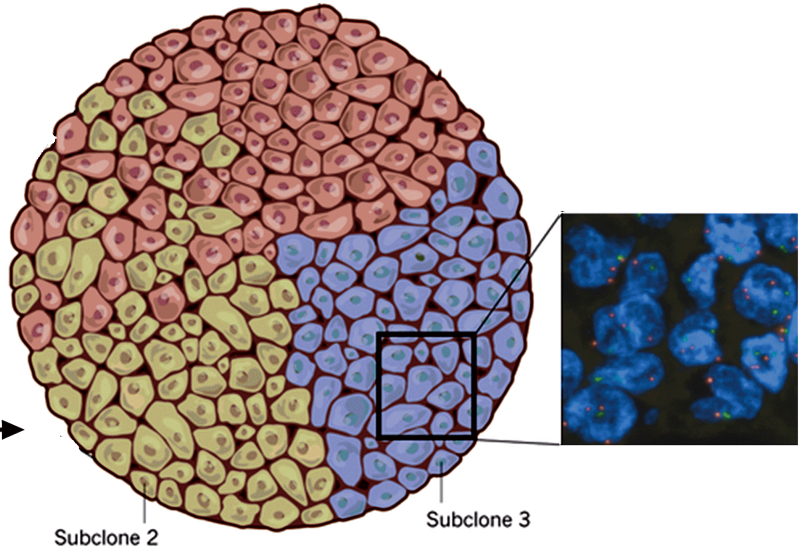
Intertumor heterogeneity



Clonal evolution



Intratumor heterogeneity



Burrell et al., Nature (2013)

Cancer genes total:	572
Driver genes per patient:	5–35

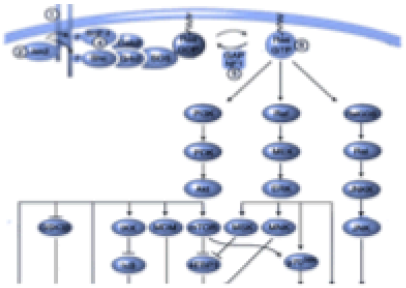
Development of Targeted Therapies

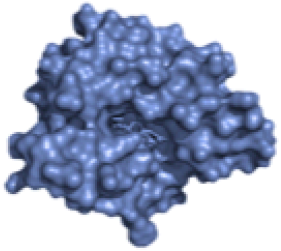
The conventional approach

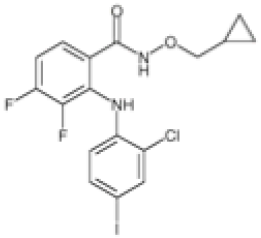
Genetics ▶ Mechanism ▶ Target ▶ Intervention

KRAS^{G12D}

EGFR PTEN p53 RB1 MYC BRAF MLL3 PTCH1 CDKN2A APC ... IDH1

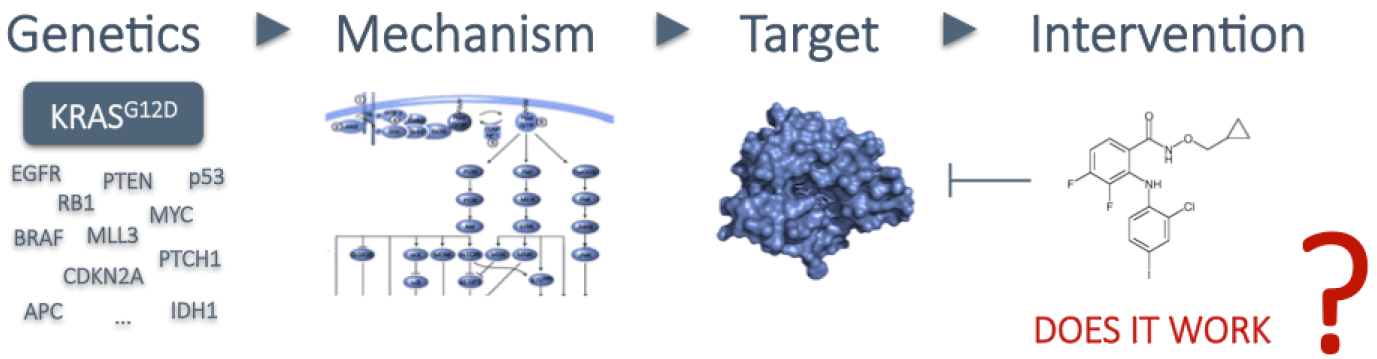




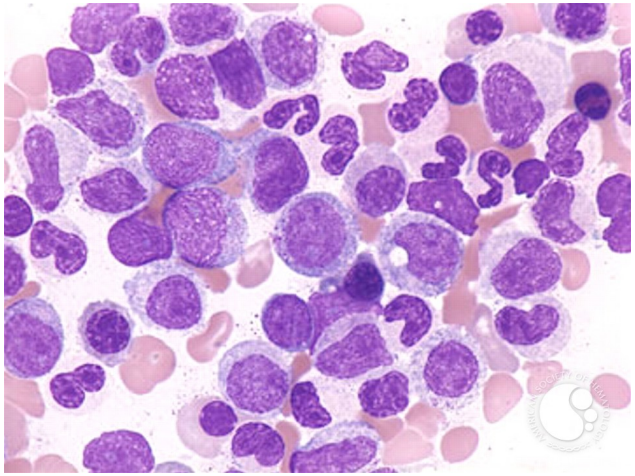


DOES IT WORK ?

Development of Targeted Therapies

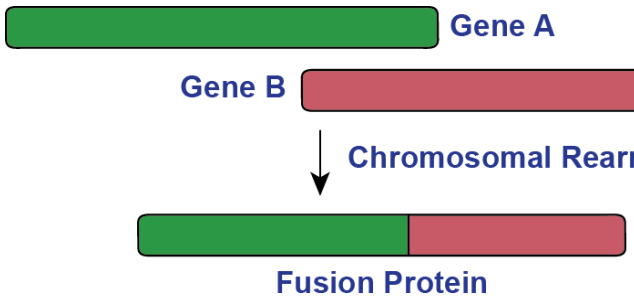


Chronic Myeloid Leukemia (CML)

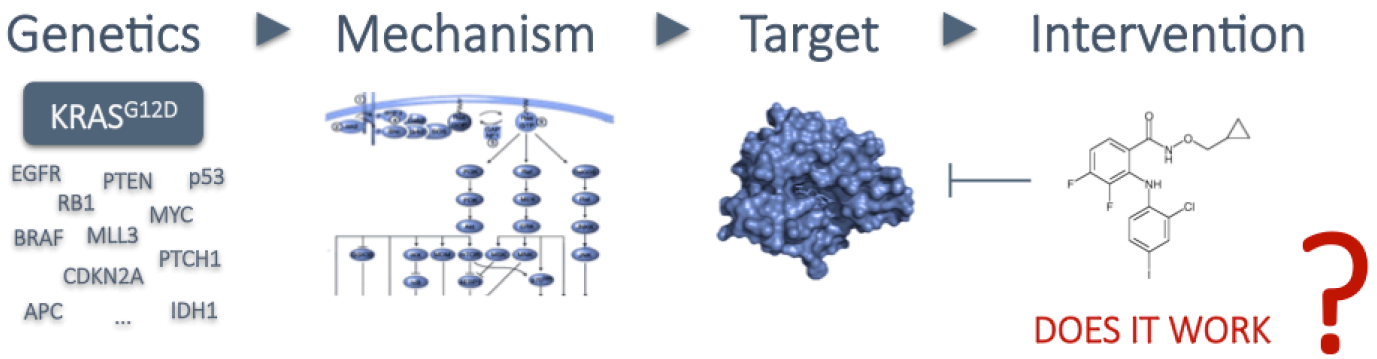


Source: ASH Image Bank

Chromosomal Translocation



Development of Targeted Therapies



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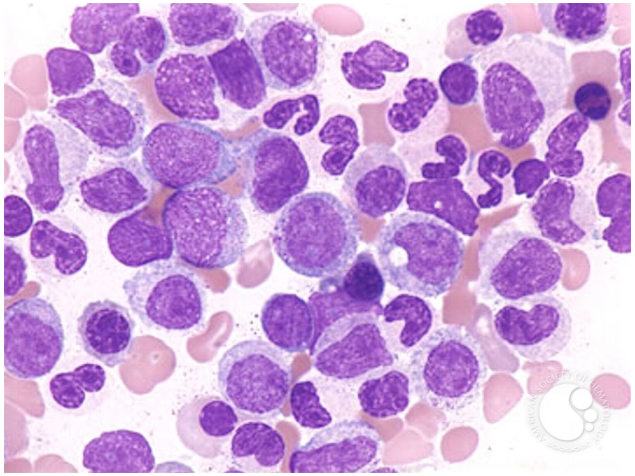
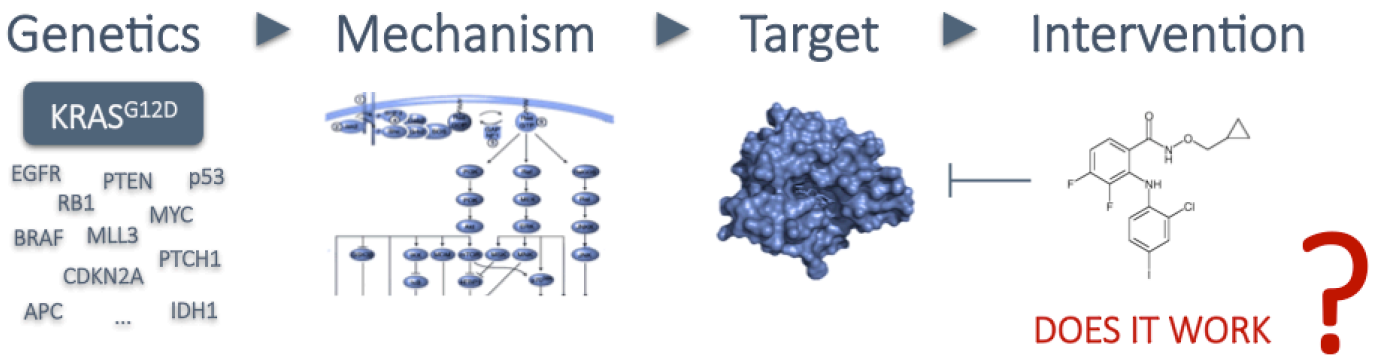


Source: ASH Image Bank

Imatinib

BCR/ABL fusion oncoprotein

Development of Targeted Therapies



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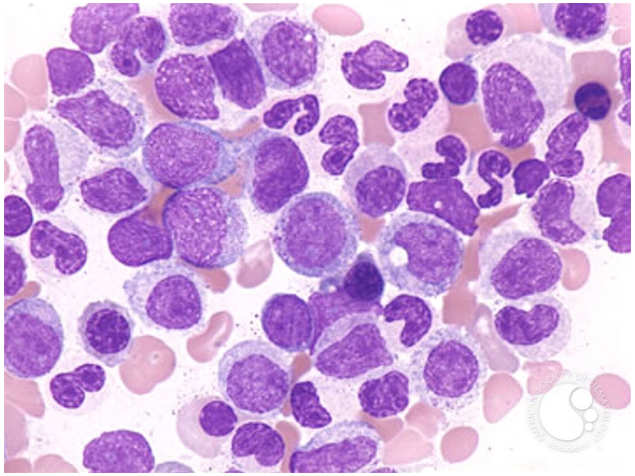

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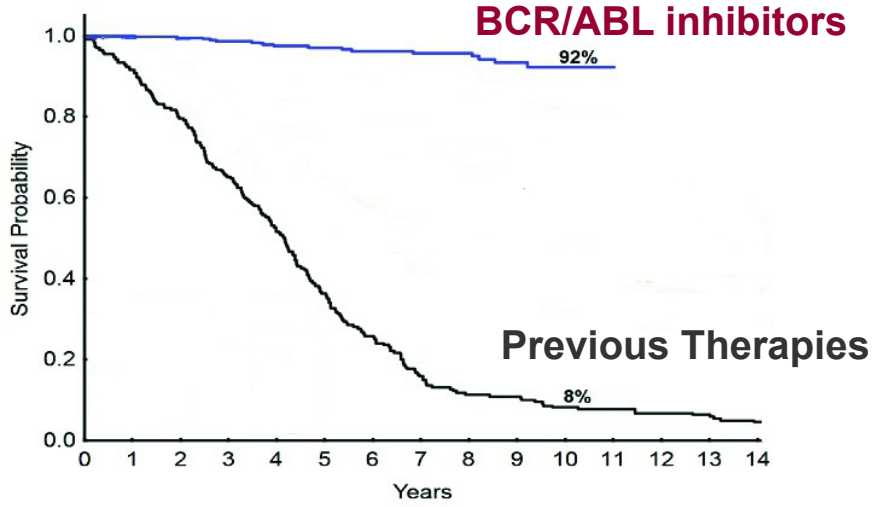
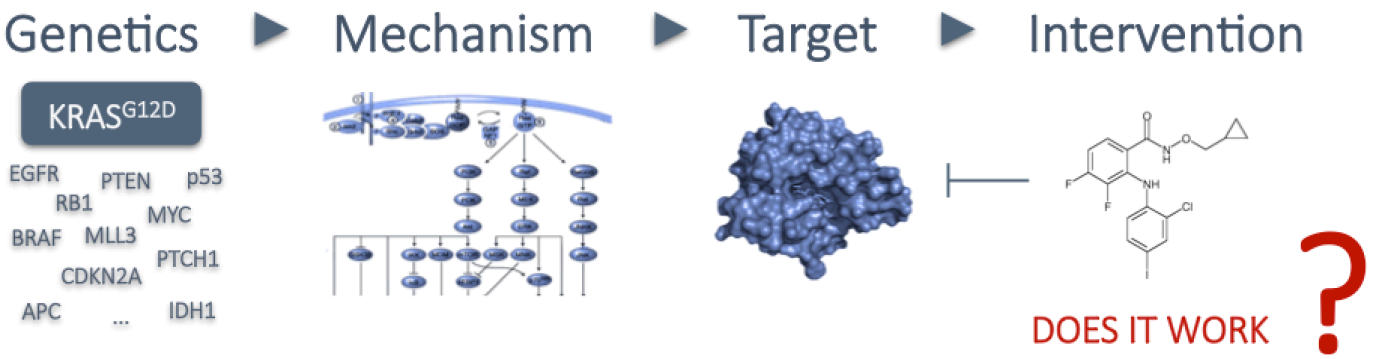
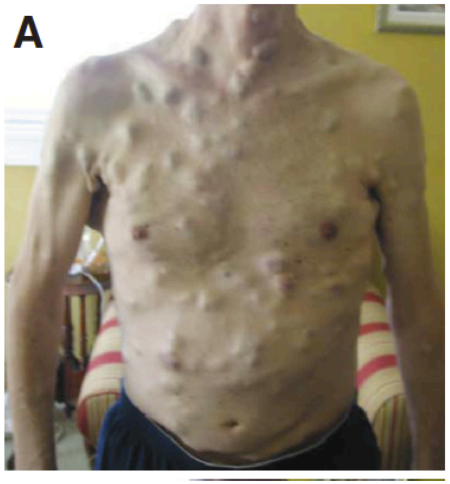


Mughal et al., Haematologica (2016)

Development of Targeted Therapies



Melanoma (Skin cancer)
BRAF-mutated

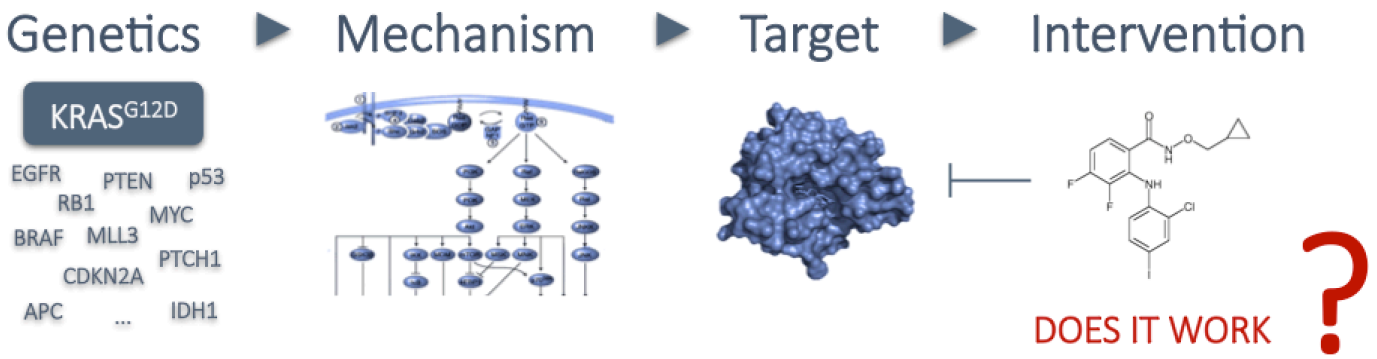


Vemurafenib

Mutated BRAF

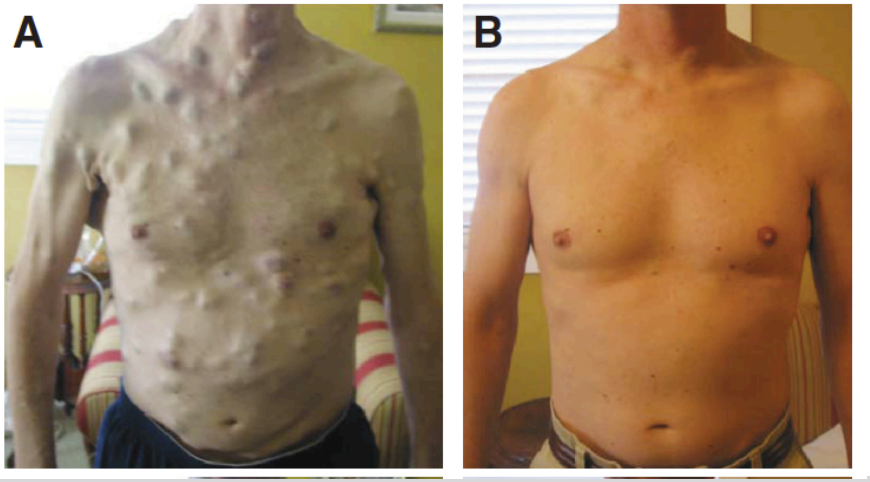
Wagle et al., Journal of Clinical Oncology (2011)

Development of Targeted Therapies



Melanoma (Skin cancer)
BRAF-mutated

15 weeks treatment
Vemurafenib (BRAF-inhibitor)

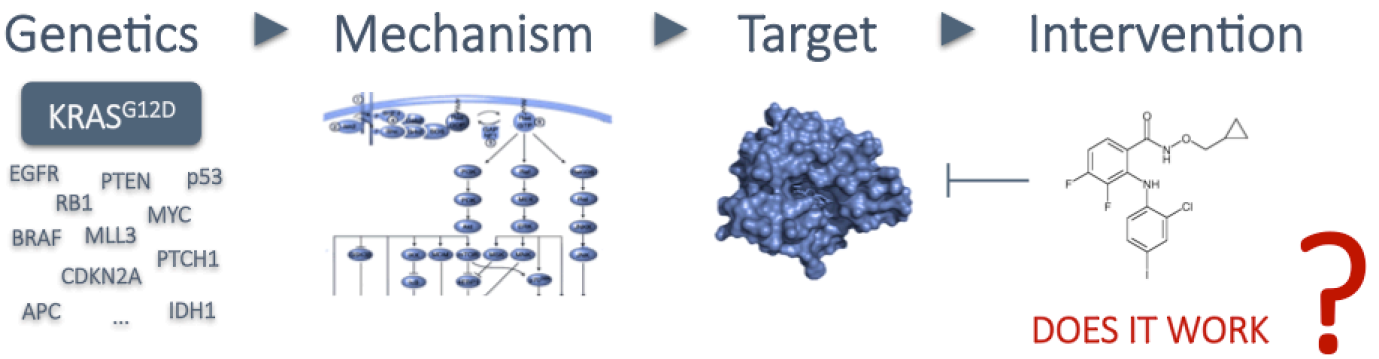


Vemurafenib

Mutated BRAF

Wagle et al., Journal of Clinical Oncology (2011)

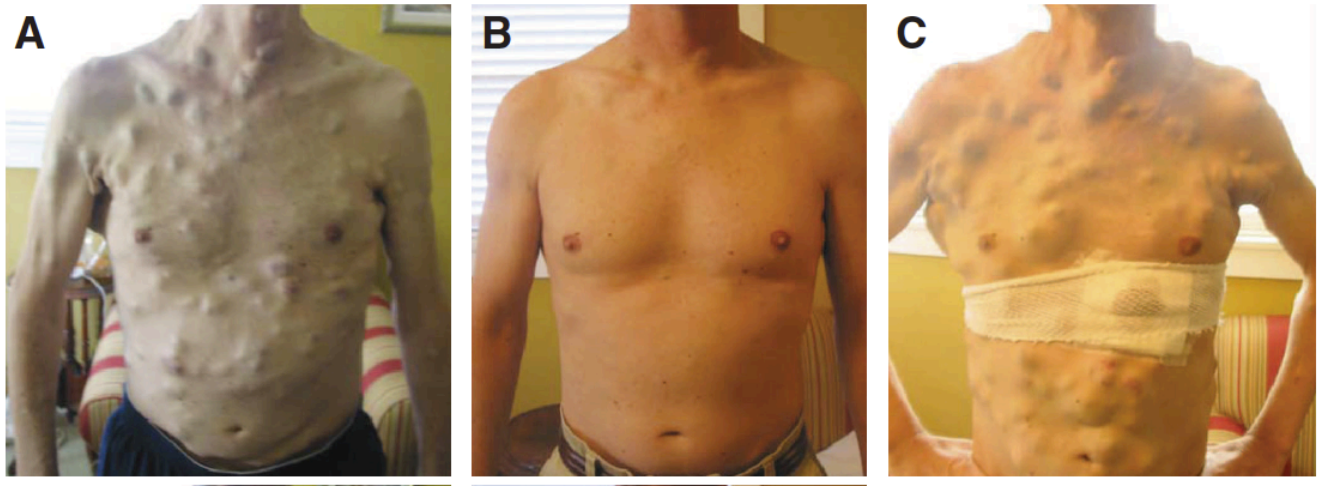
Development of Targeted Therapies



Melanoma (Skin cancer)
BRAF-mutated

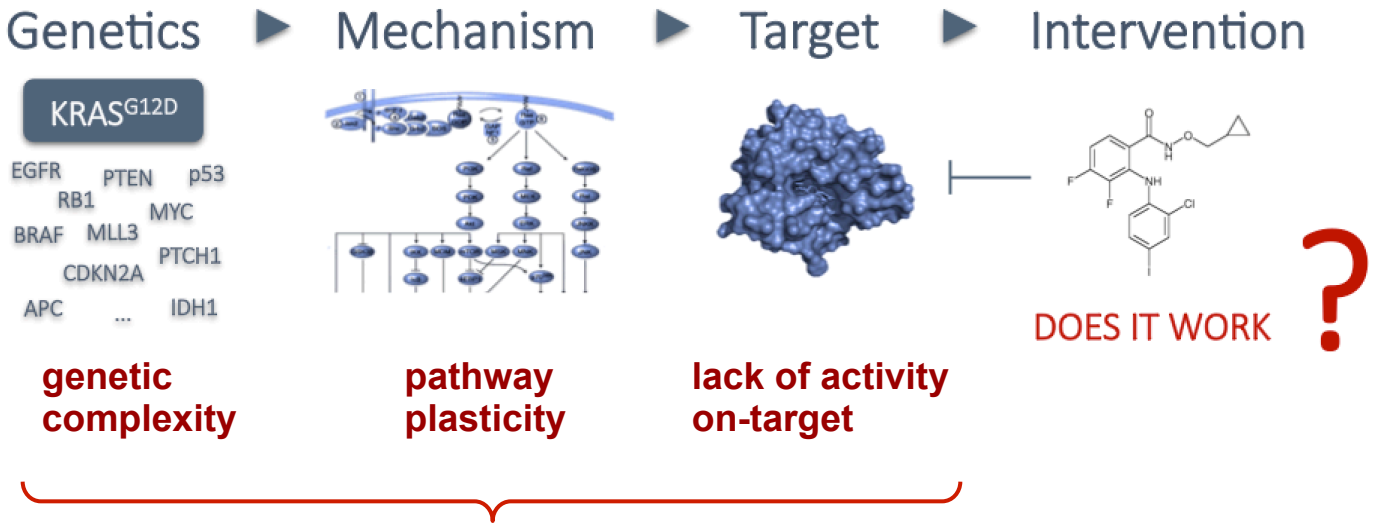
15 weeks treatment
Vemurafenib (BRAF-inhibitor)

23 weeks treatment
Vemurafenib (BRAF-inhibitor)



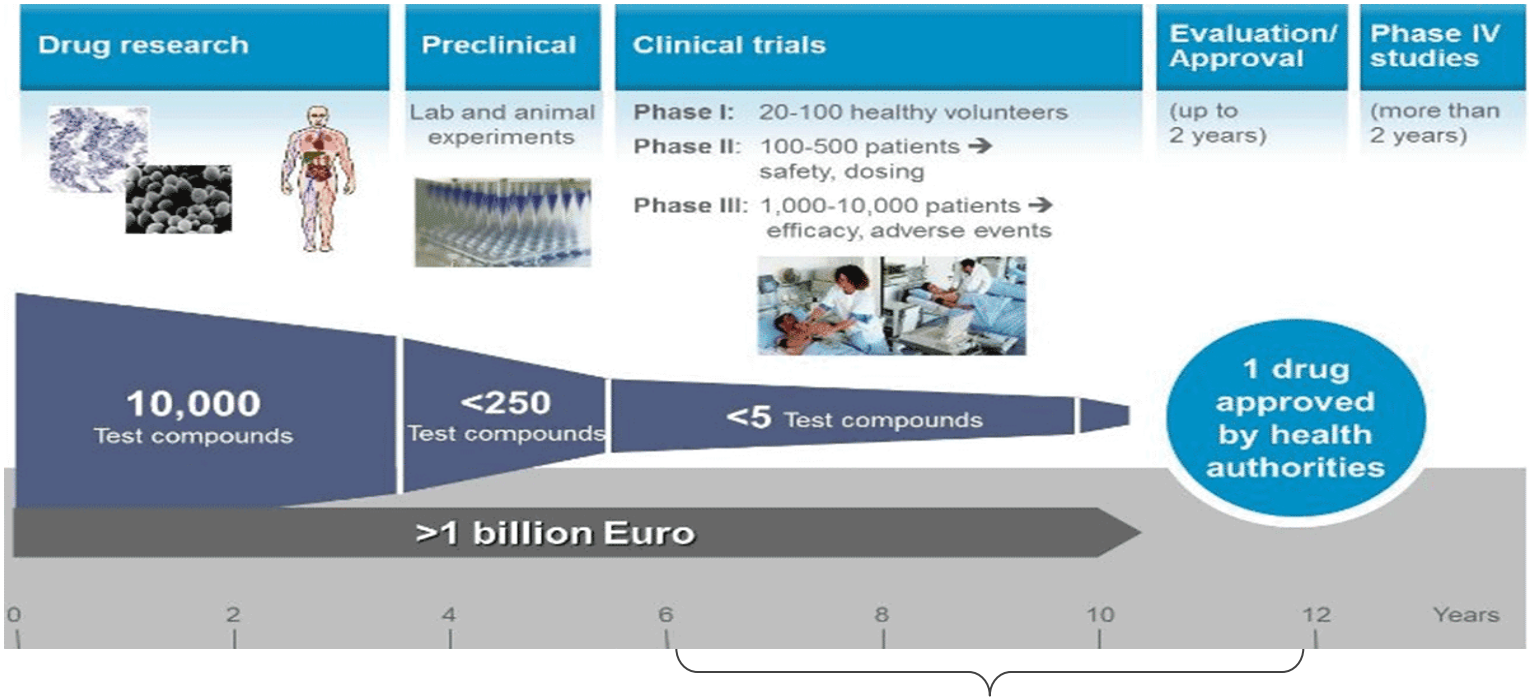
Wagle et al., Journal of Clinical Oncology (2011)

Development of Targeted Therapies



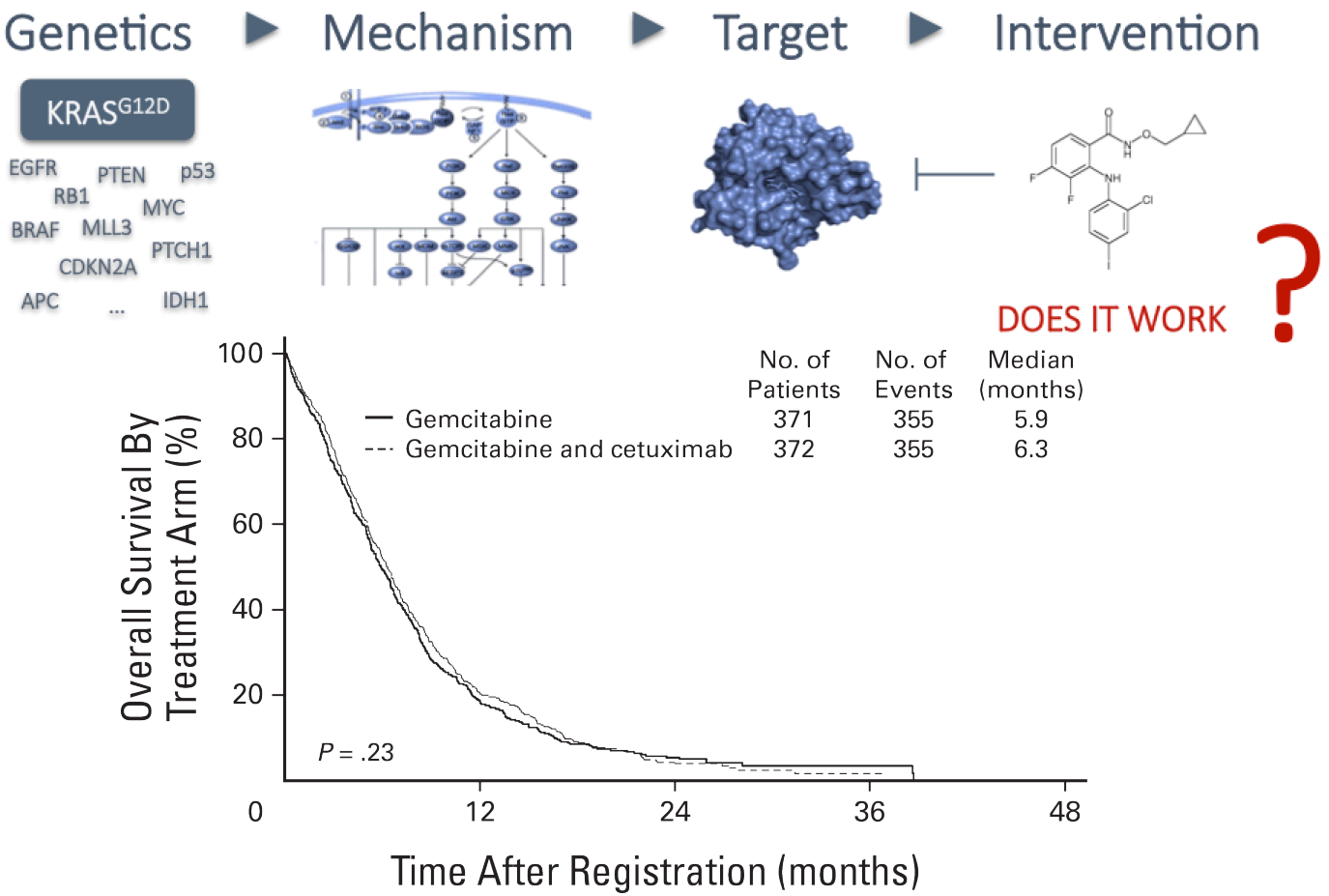
primary ineffectiveness & secondary resistance

Development of Targeted Therapies



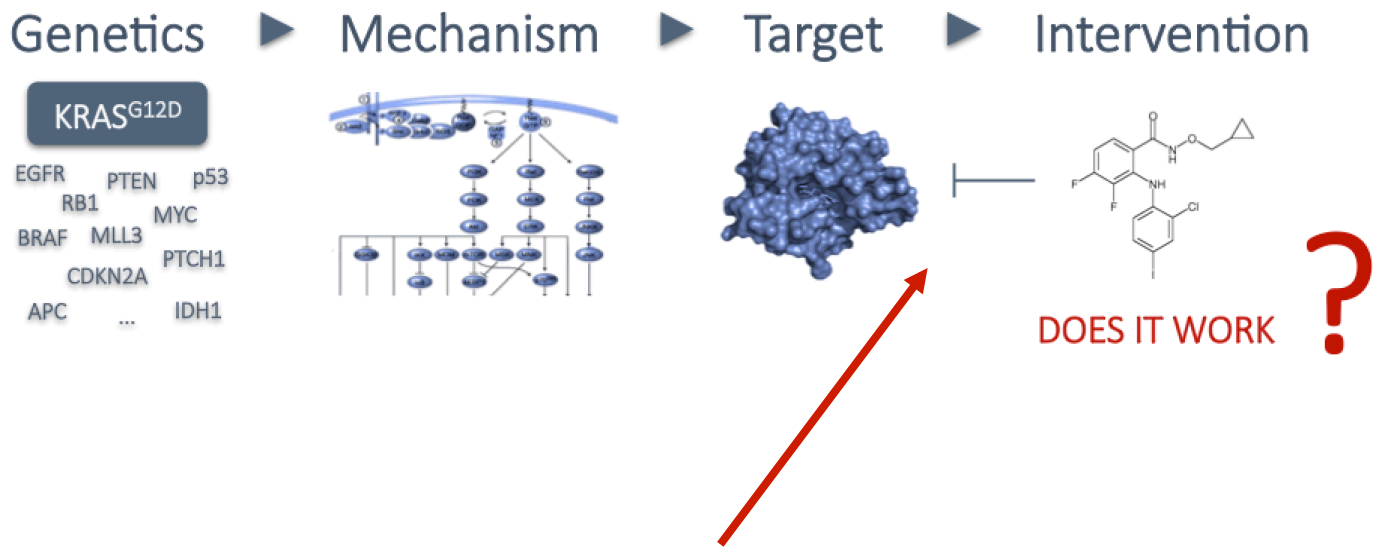
cumulative failure rate in clinical trials: **94%**

Development of Targeted Therapies



Philip et al., JCO (2010)

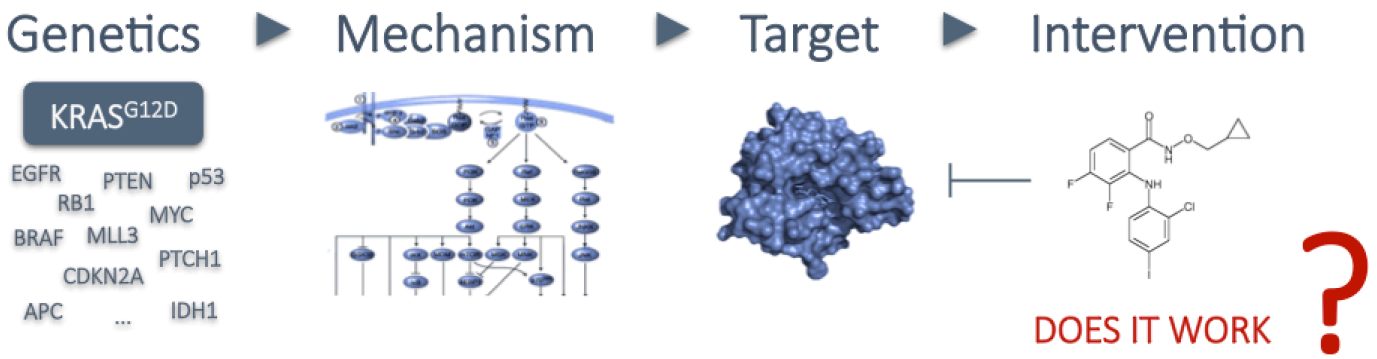
Development of Targeted Therapies



Genetic tools needed
to study potential drug targets systematically

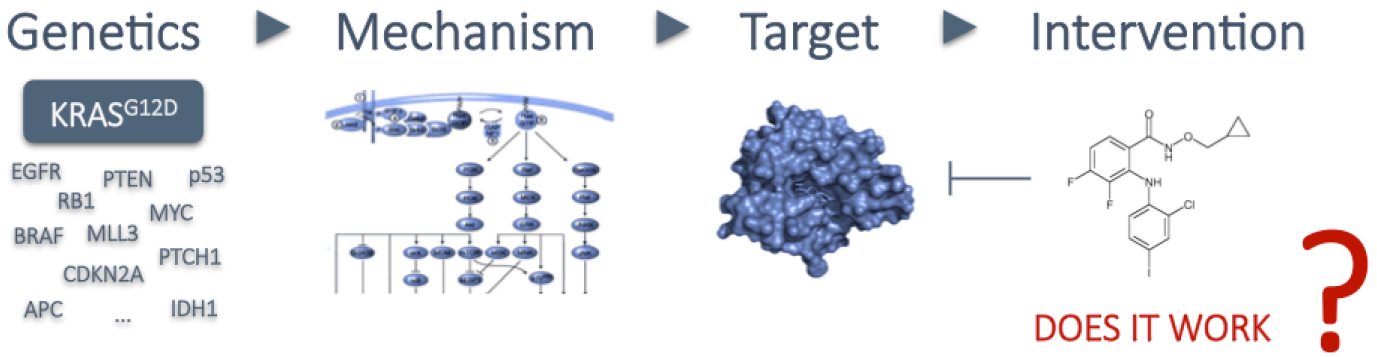
Philip et al., JCO (2010)

Development of Targeted Therapies



1. **Systematically identify the most effective targets**
2. **Study underlying response mechanisms & biomarkers**
3. **Explore candidate targets in combination**
4. **Rigorously test intervention effects *in vivo***
   - on tumor cells: **efficacy?**
   - on normal tissues: **safety?**

Development of Targeted Therapies



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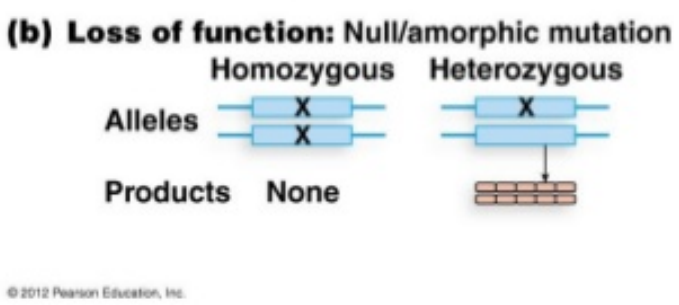
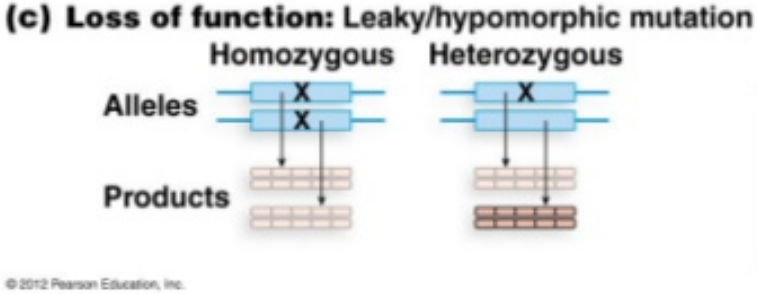
Functional Genomics Experiments
CRISPR/Cas9
Loss-of-Function
Gain-of-Function

Loss-of-Function (LOF)



Null alleles produce no functional product. Homozygous null organisms have mutant (amorphic) phenotype due to absence of the gene product. Heterozygous organisms produce less functional gene product than homozygous wild-type organisms and may have mutant phenotype. See text for discussion of dominant versus recessive mutations.

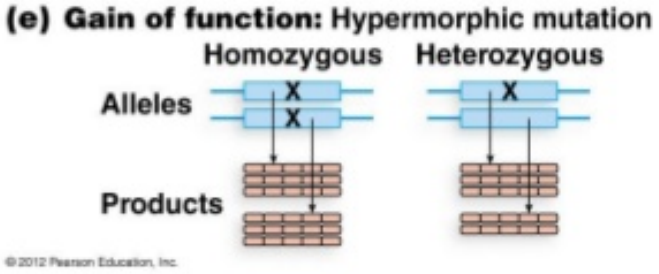
Amorphic = no function



Leaky mutant alleles produce a small amount of wild-type gene product. Homozygous organisms have a mutant (hypomorphic) phenotype. Heterozygous organisms may also be mutant.

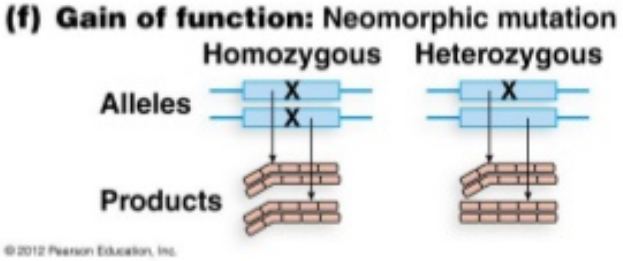
Hypomorphic = less function

Gain-of-Function (GOF)



Excessive expression of the gene product leads to excessive gene action. The mutant phenotype may be more severe or lethal in the homozygous genotype than in the heterozygous genotype.

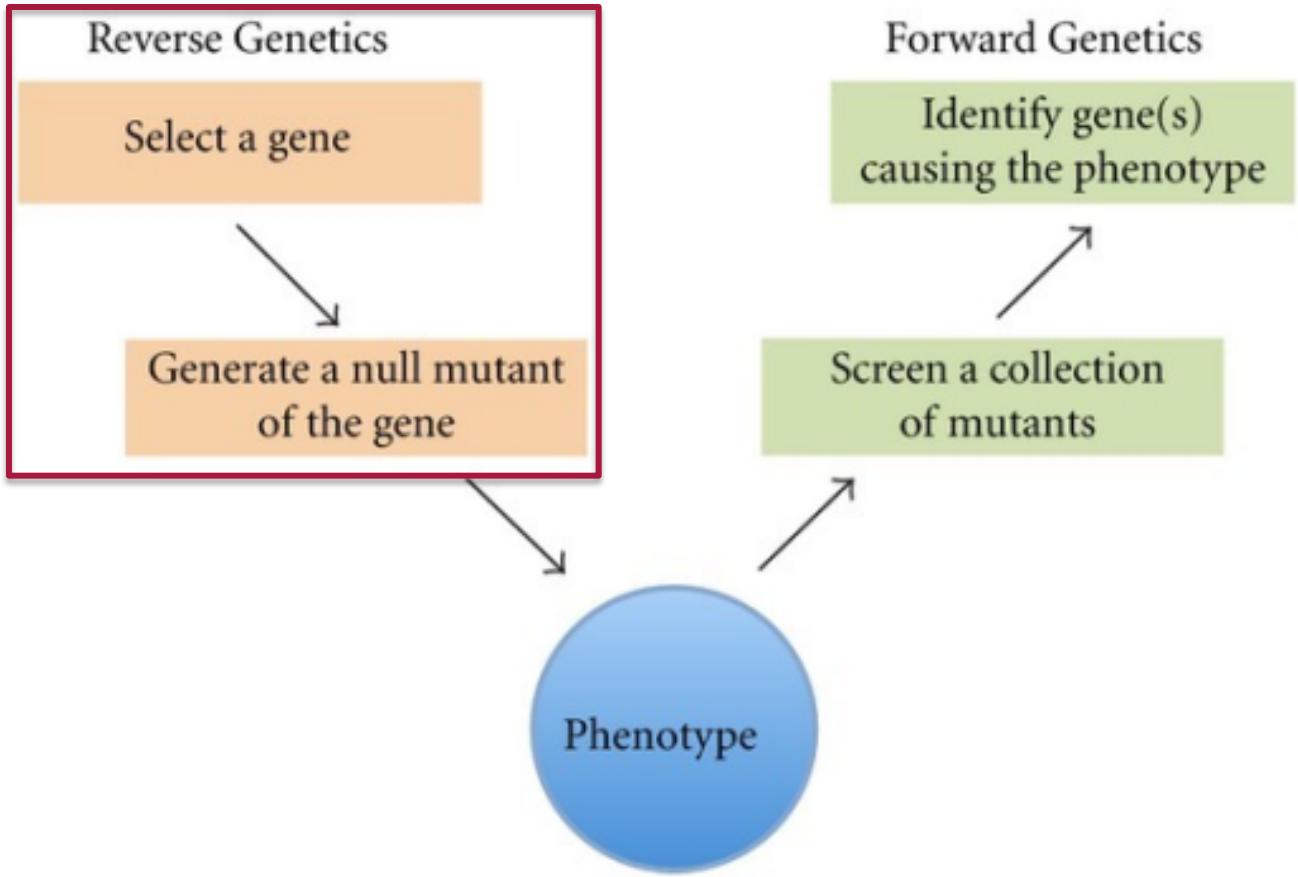
Hypermorphic = more function



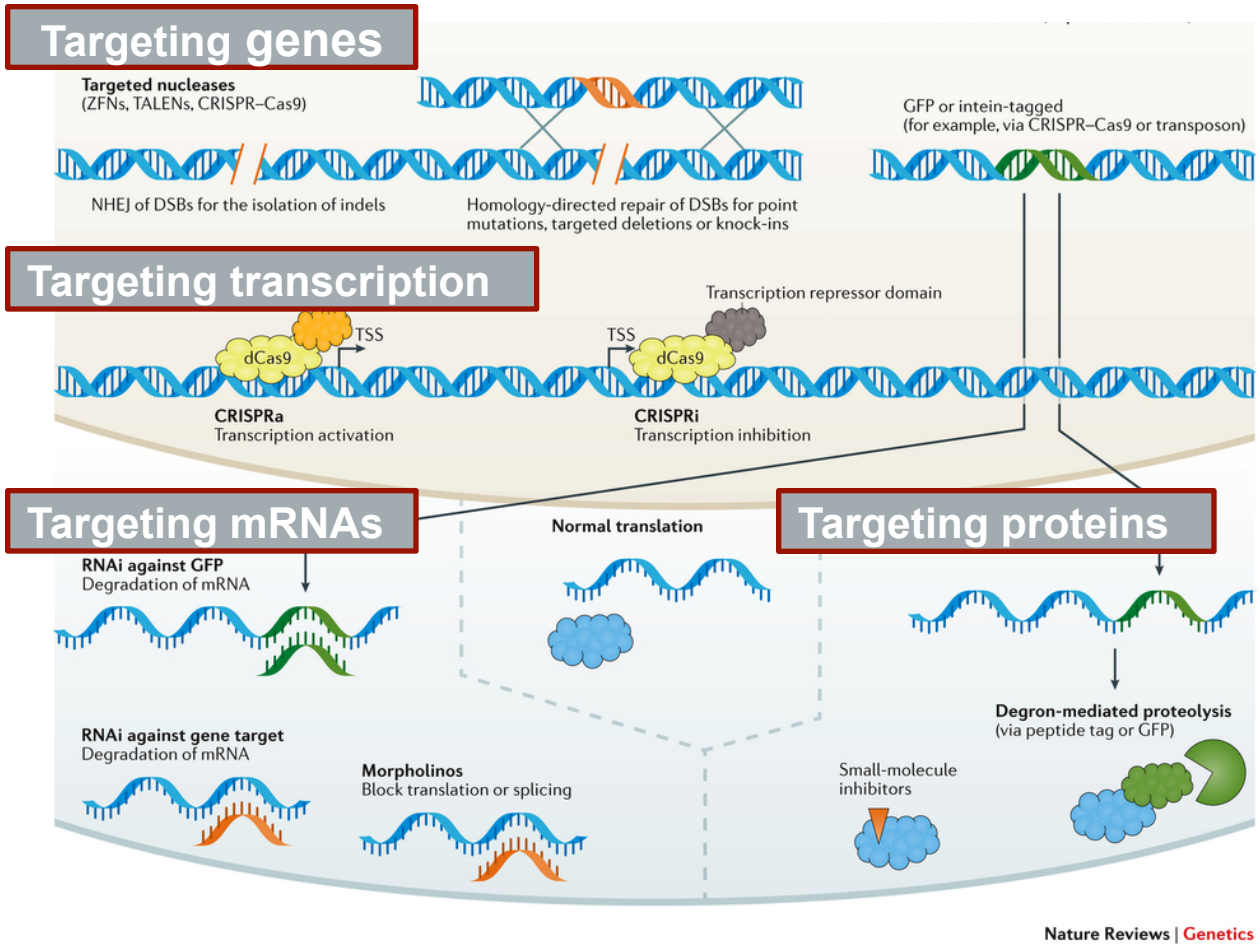
The mutant allele has novel function that produces a mutant phenotype in homozygous and heterozygous organisms, and may be more severe in homozygous organisms.

Neomorphic = *new* function

Forward vs. Reverse Genetics



Functional Genomics Tools



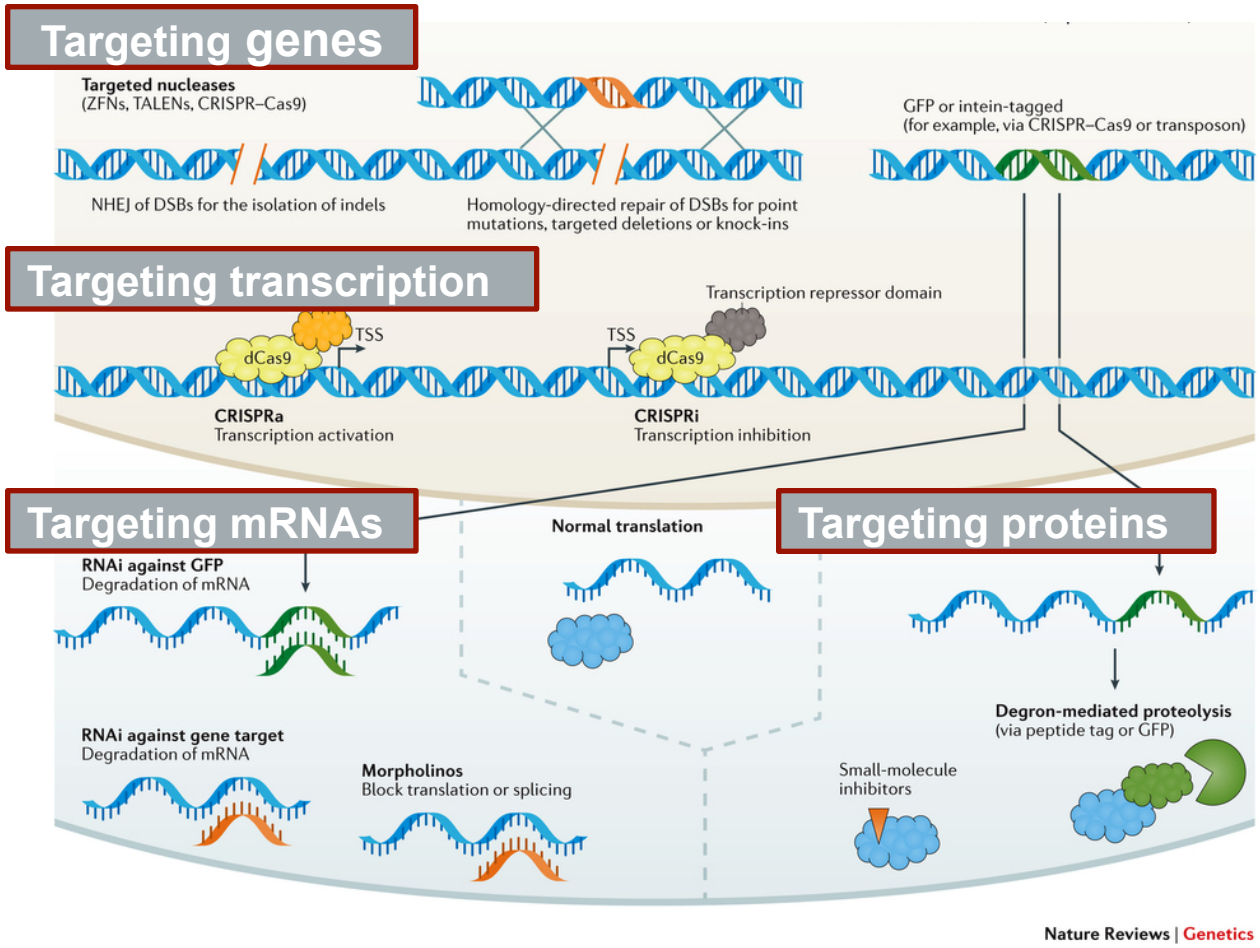
DNA

RNA

Protein

Housden, et al., Nature Reviews Genetics (2017)

Functional Genomics Tools



DNA

RNA

Protein

Housden, et al., Nature Reviews Genetics (2017)

The CRISPR/Cas System

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CRISPR/Cas9 nuclease (CRISPR)

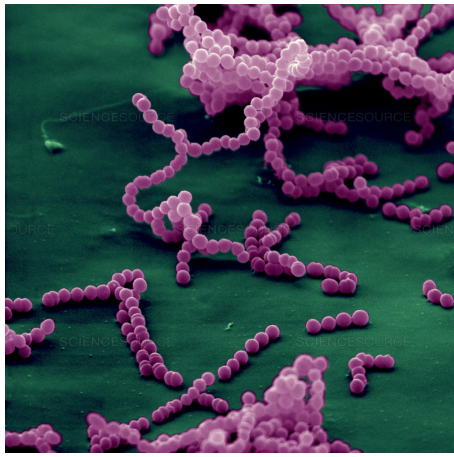
Clustered Regular Interspaced Short Palindromic Repeats

+ ***Natural***
+ ***Ancient***
+ ***component of a „bacterial immune system“***

Defense against invading pathogens

found in:

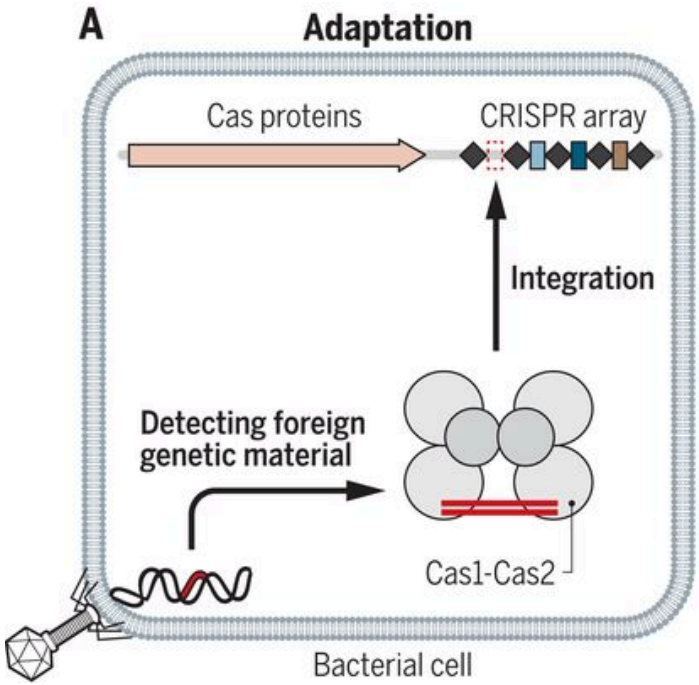
Campylobacter sp.
Staphylococcus sp
Streptococcus sp.
Neisseria sp.
...and many others



https://www.sciencesource.com/Doc/SCS/Media/TR1_WATERMARKED/2/a/7/f/SS2104469.jpg?d63641154958

The CRISPR/Cas System

Prokaryotes use the CRISPR/Cas system to fight pathogens

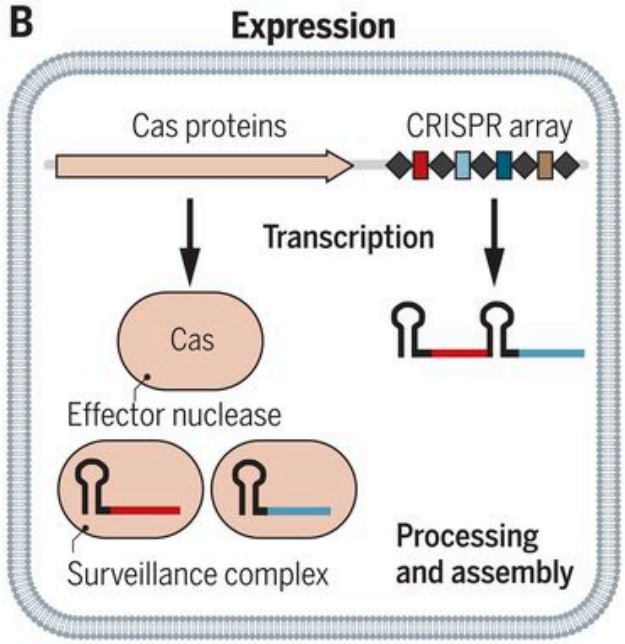


Foreign genetic elements are acquired by Cas1-Cas2 and integrated into the CRISPR array in a process termed adaptation.

Knott and Doudna, Science (2018)

The CRISPR/Cas System

Prokaryotes use the CRISPR/Cas system to fight pathogens

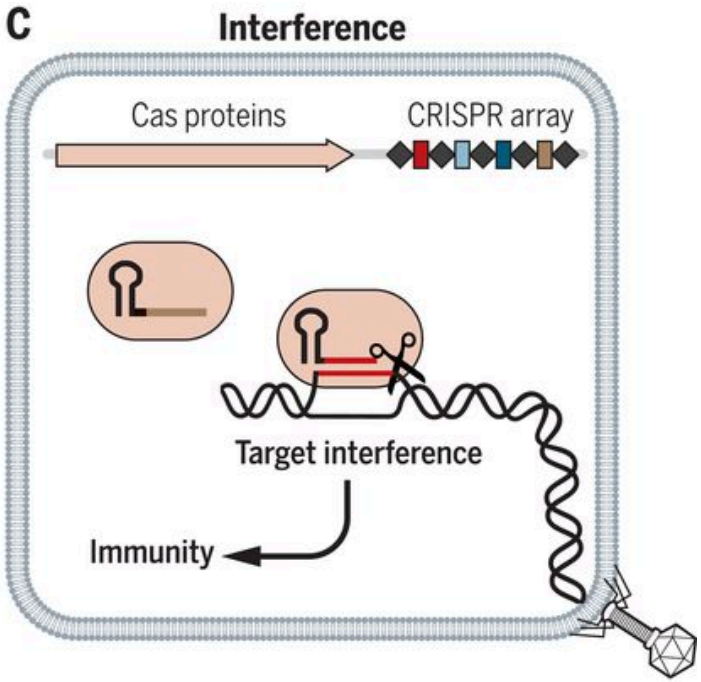


The CRISPR array and associated Cas proteins are expressed.
The CRISPR array is processed and Cas effector nucleases associate with a crRNA to form a surveillance complex.

Knott and Doudna, Science (2018)

The CRISPR/Cas System

Prokaryotes use the CRISPR/Cas system to fight pathogens



The Cas effector nucleases target foreign genetic elements complementary to their crRNA, leading to target interference and immunity.

Knott and Doudna, Science (2018)

The CRISPR/Cas9 System

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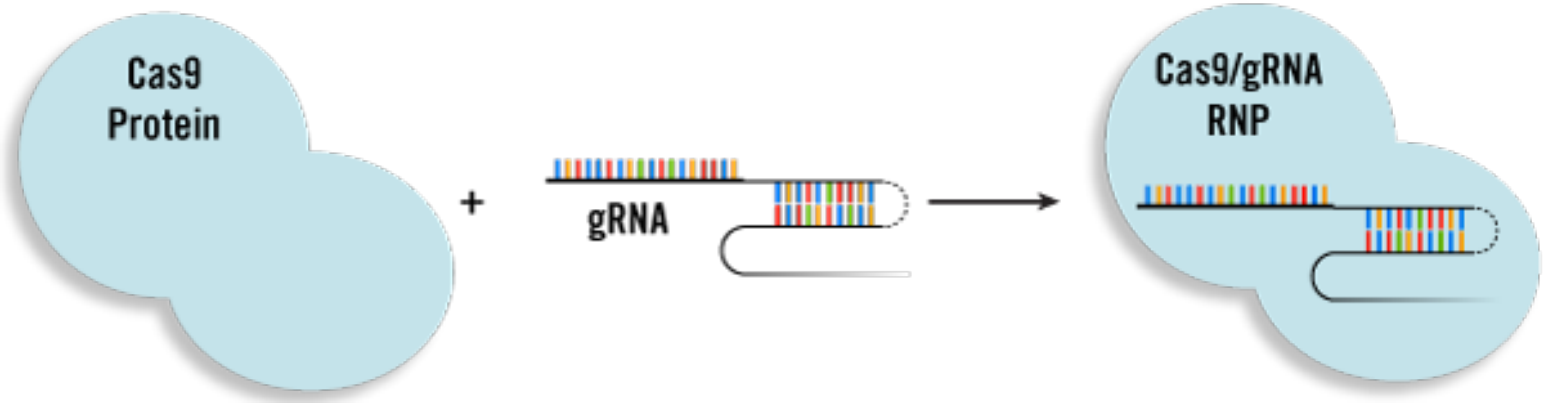
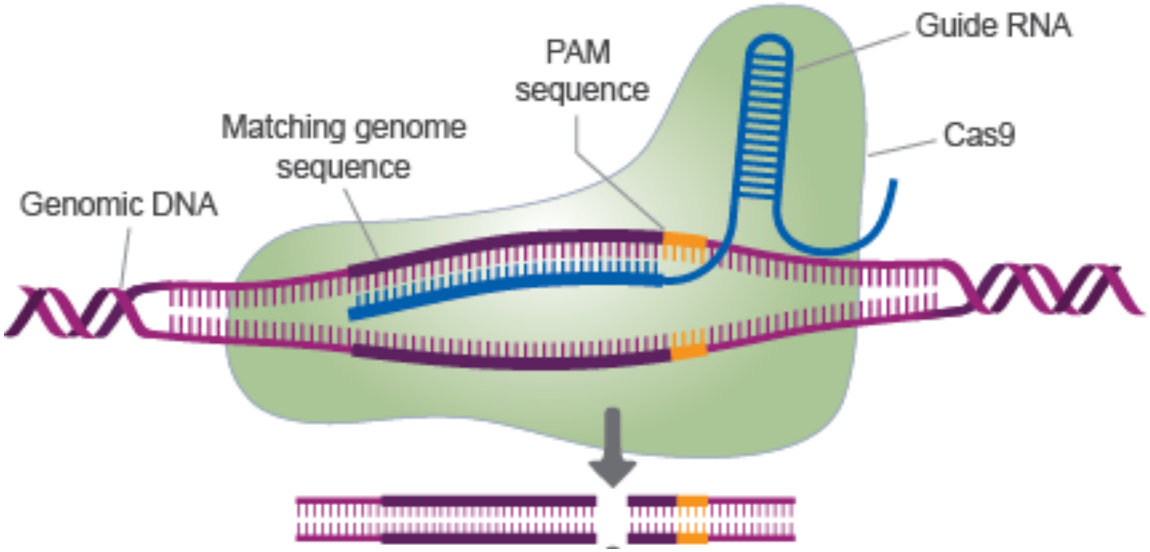
2 Components:

+ Cas9 Nuclease (enzyme that cuts DNA)

+ guide RNA molecule



The CRISPR/Cas9 System



+ The guide RNA targets Cas9 nuclease activity to specific regions in the genome
+ Cas9 induces a DNA double strand near the PAM sequence

The CRISPR/Cas9 System

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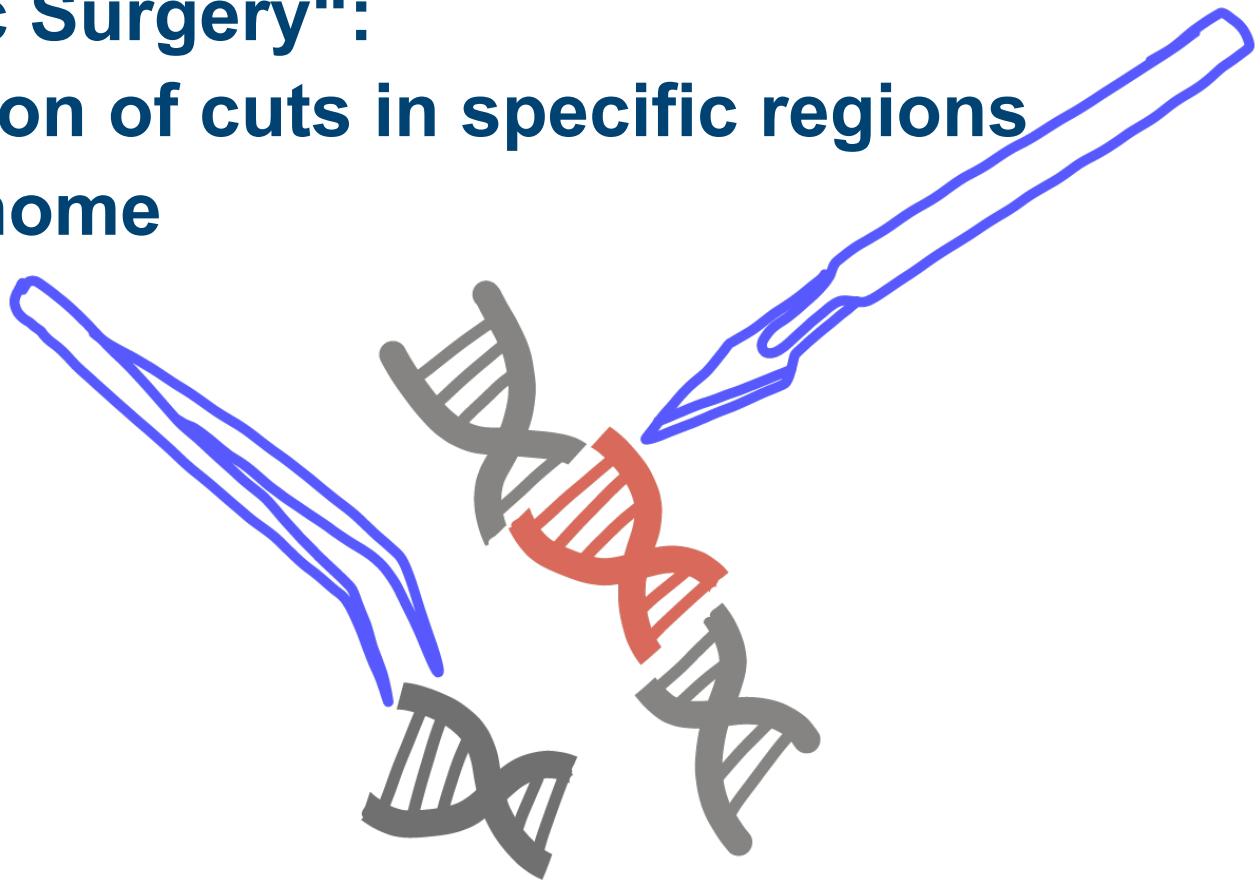
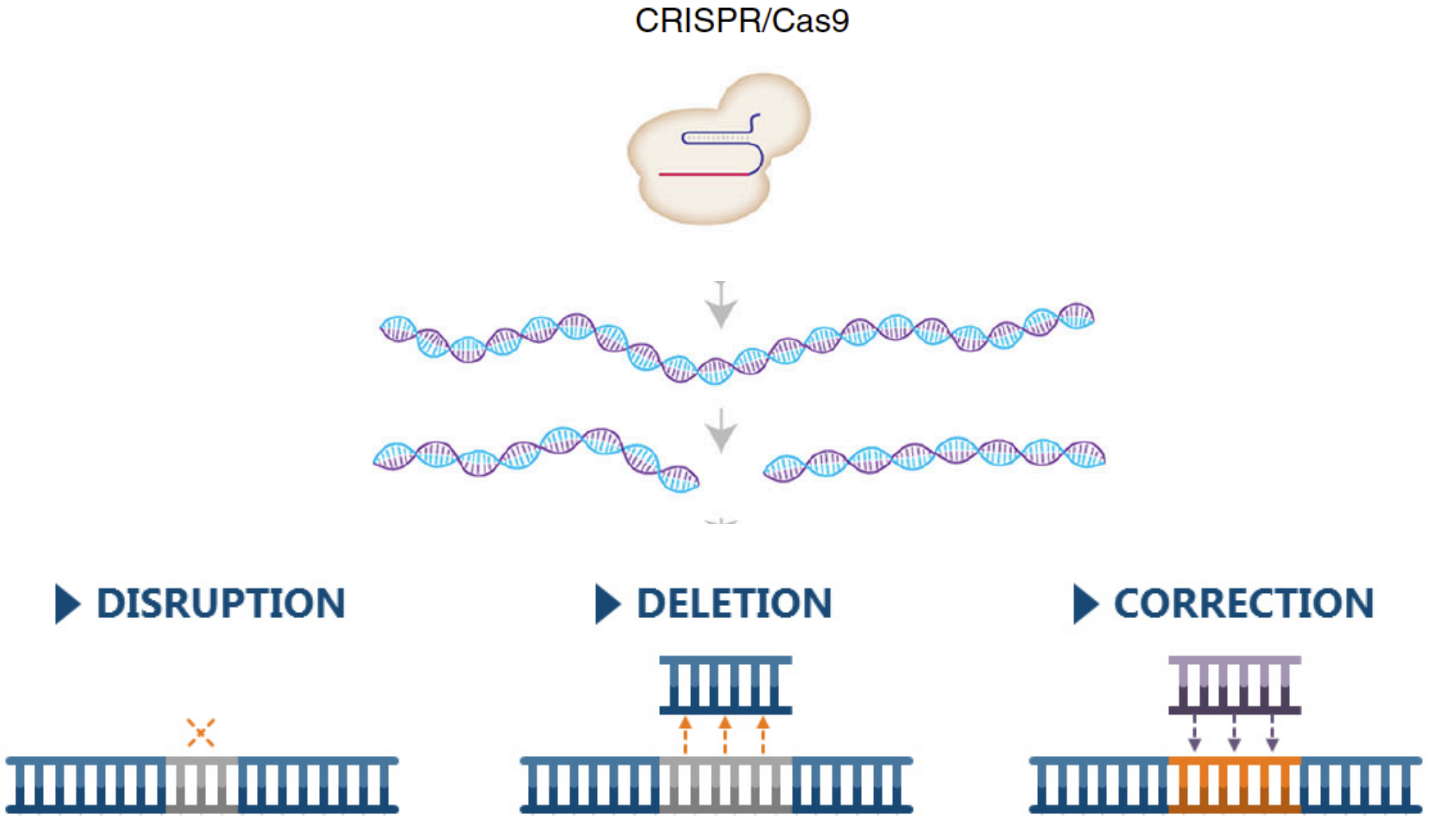
„Genomic Surgery“: Introduction of cuts in specific regions of the genome



https://innovativegenomics.org/glossary/genome-surgery/

The CRISPR/Cas9 System



Non-homologous end joining (NHEJ) vs. homologous recombination

Mazhar Adli, Nature Communications (2018)
http://crisprtx.com

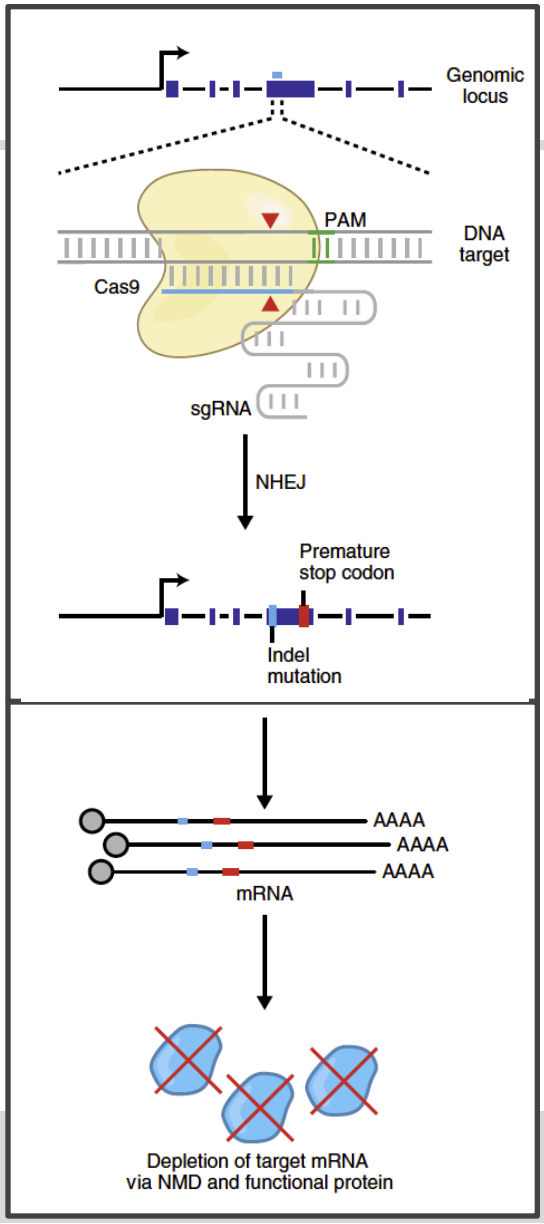
Summary

CRISPR/Cas9

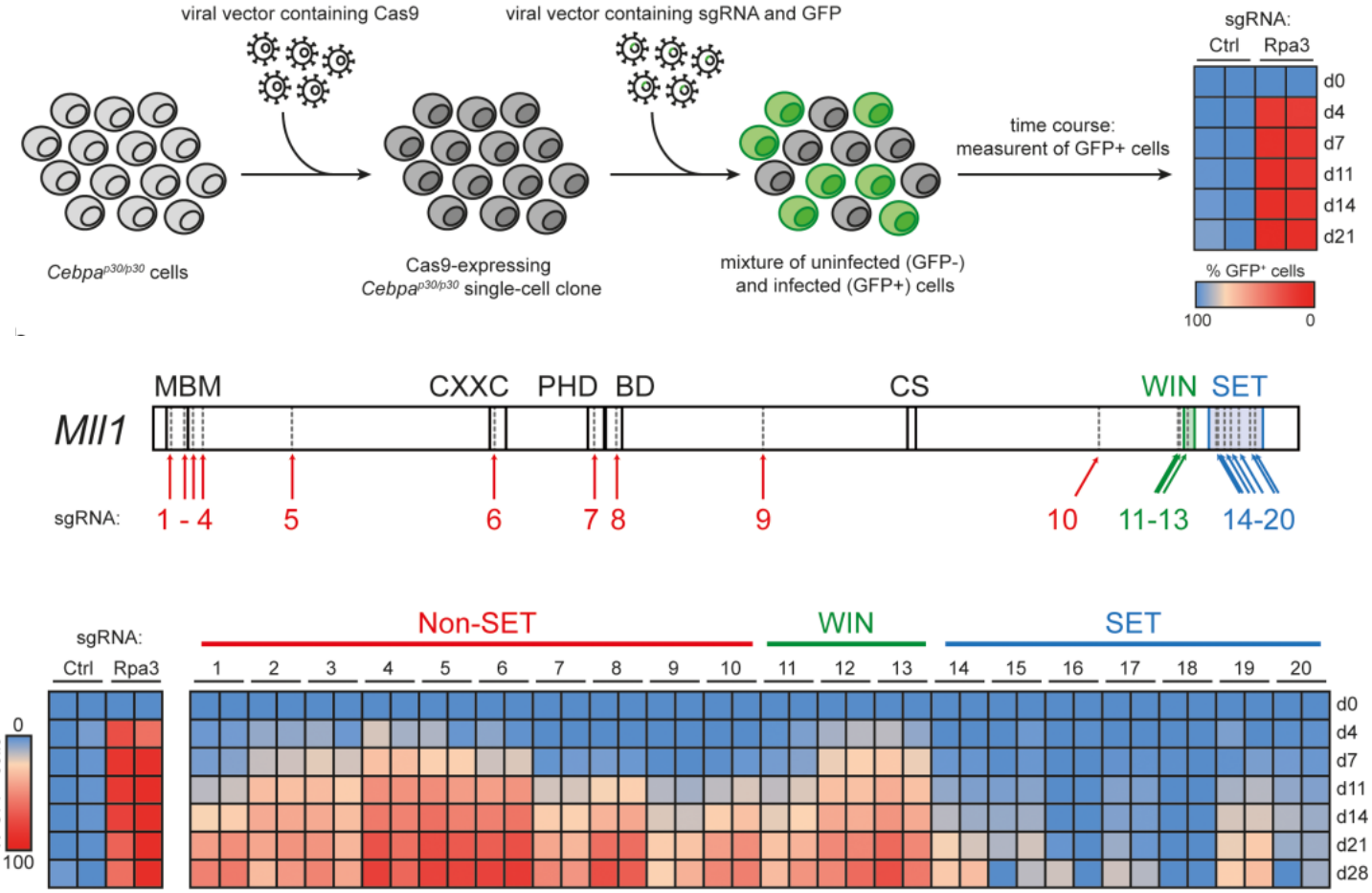
- Recognition: sgRNA-DNA
- Cas9 nuclease loaded with single guide RNA (sgRNA)
- DSB → NHEJ → Insertion/deletion (Indel)
- Knockout of Gene of Interest

  + Targeting N-terminus or specific protein domains
  + Targeting multiple alleles
  + Very high efficiency, scalable
  + High throughput screening feasible!

  - In-frame mutations
  - Not reversible

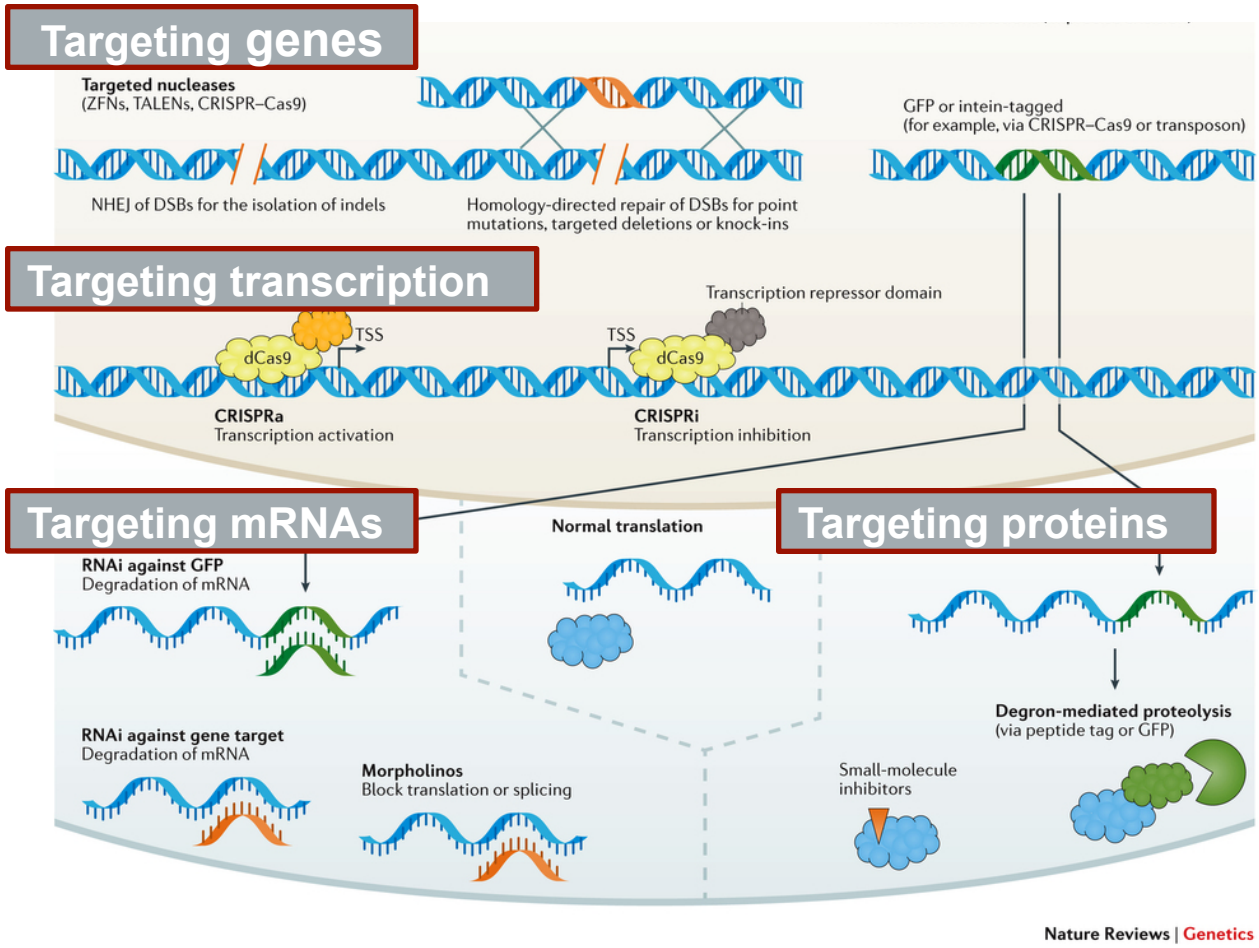


CRISPR/Cas9 – an example



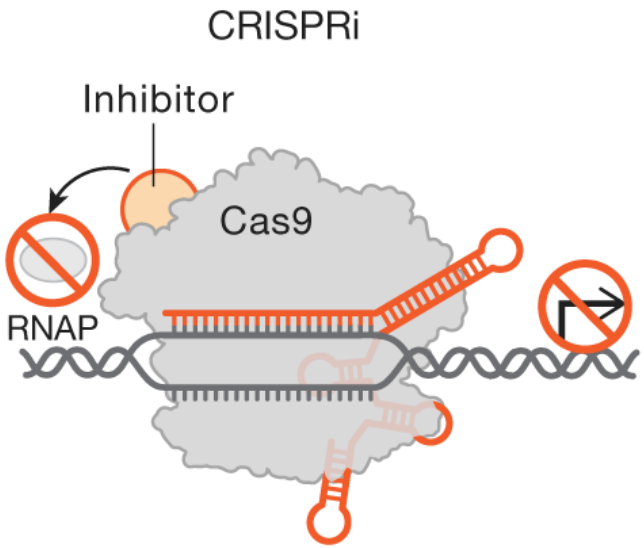
Schmidt, et al., Leukemia (2019)

Functional Genomics Tools



Housden, et al., Nature Reviews Genetics (2017)

CRISPRi/CRISPRa



CRISPR-inhibition (CRISPRi)

Fusion of nuclease-deficient Cas9 to inhibitory domain

Gene repression

Temporary or persistent

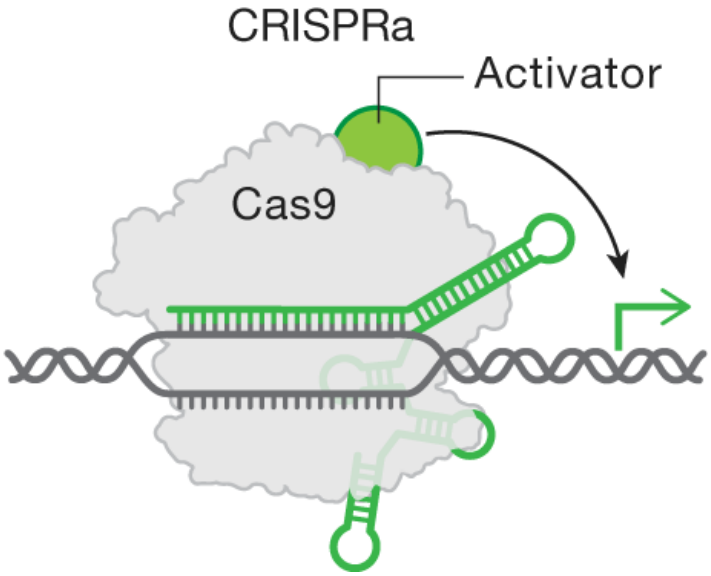
Epigenetic modification or RNA targeting

Doudna, Nature (2020)

CRISPRi/CRISPRa

CRISPR-activation (CRISPRa)

Fusion of nuclease-deficient Cas9 to activating domain



Gene activation

Temporary or persistent

Epigenetic modification

Doudna, Nature (2020)

The CRISPR/Cas9 Transformation of Cancer Research

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In Cancer research, CRISPR/Cas9 has enabled us to:

→ make better models of mutations associated with cancer

→ better interrogate gene function in cancer

+ **Ease:** only few working steps, no special equipment or methodology required
+ **Time:** Knock-out single genes in 3 weeks
+ **Flexibility:** Remove – introduce – modify - visualize
+ **Scalability:** single genes vs. genome-wide, non-coding genome
+ **Access to knowlegde:** reagents (addgene.org), publications (biorxiv.org), Online tools for CRISPR design (see next presentation), Twitter (twitter.com)

Methods for Delivery of Gene-Editing Tools

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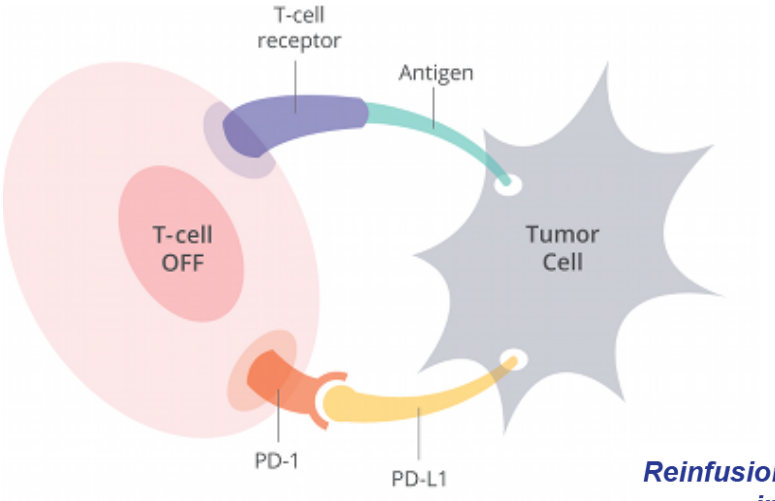
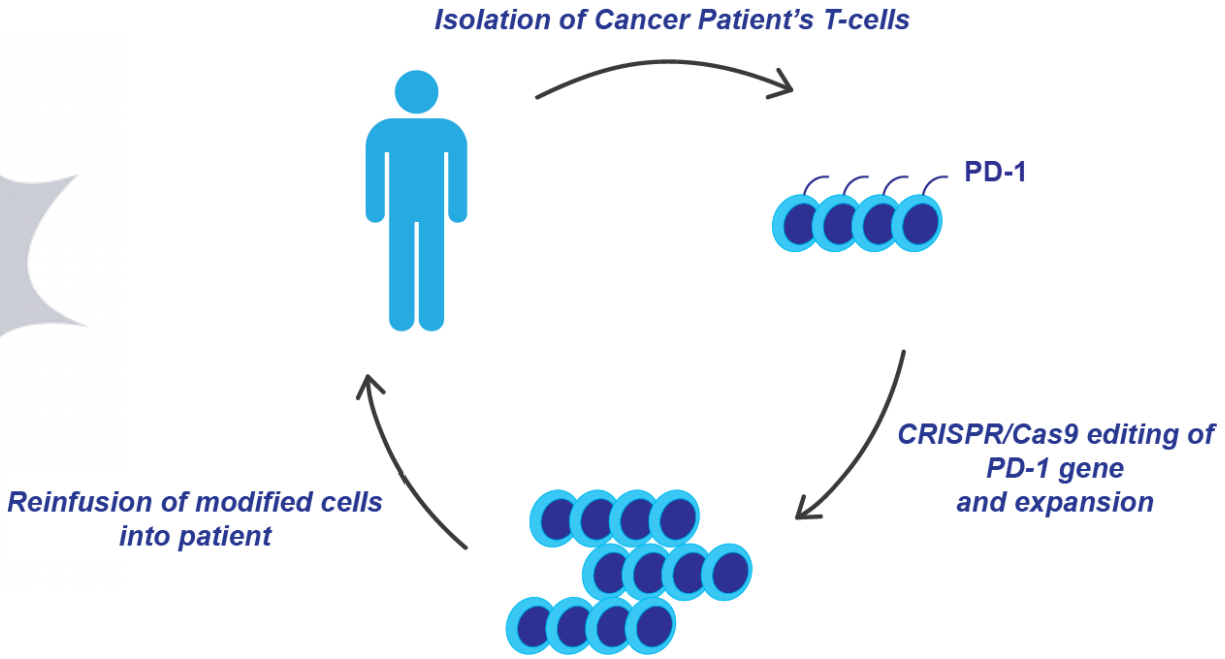
Property	Nanoparticles	Viruses	RNPs
Features and applications	Cationic lipid polymers can be used to encapsulate molecular cargo, facilitating cellular entry.	AAVs are the most commonly used clinical delivery vehicle for gene therapy.	Purified protein and guide RNA can be electroporated into stem cells extracted from patients to treat blood disorders such as sickle cell disease.
Size	50–500 nm	20 nm	12 nm
Payload	mRNA, DNA, RNP (from most to least commonly used)	DNA	Preformed enzyme complexes
Advantages	- Inexpensive and relatively easy to produce - No genomic integration - Low immunogenicity	- Broad tissue targeting possibilities - Clinically established method - Efficient	- No genomic integration - No long-term expression and fewer off-target effects
Disadvantages	- Limited capacity for tissue targeting	- Limited cargo size - Undesired integration risk - Sustained expression can lead to off-target effects - Immunogenicity - High cost and manufacturing challenges	- Will not enter cells without engineering or additional reagents - Potential immunogenicity in vivo - Unprotected RNPs are at risk of degradation
Targets	Liver	Liver, eyes, brain, lungs and muscle	Oocytes, stem cells and T cells

Doudna, Nature (2020)

Can CRISPR be used to combat diseases?

Cancer Immunotherapy



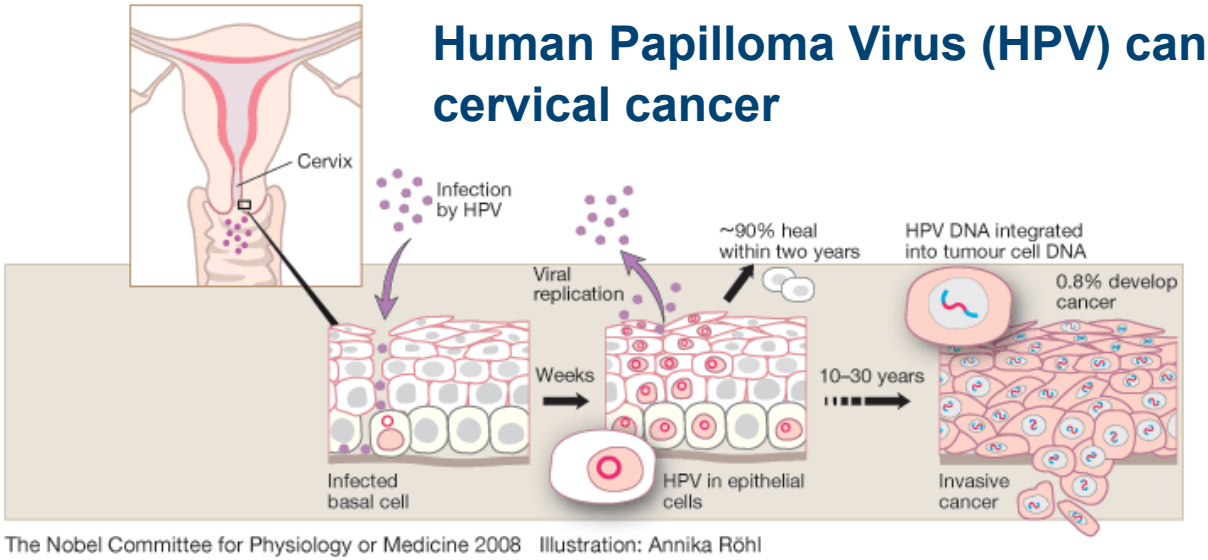


1st clinical trial started in October 2016, Chengdu, China, *Lung Cancer*

>10 more clinical trials currently recruiting in China,
Breast, Prostate, Bladder, Oesophageal, Kidney, Colorectal Cancer

+ additional trials started in China, USA, UK etc.

Human Papilloma Virus



The Nobel Committee for Physiology or Medicine 2008 Illustration: Annika Röhl

Human Papilloma Virus (HPV) can cause cervical cancer

+ HPV proteins E6 and E7 lead to oncogenic transformation

+ E6 and E7 deletion leads to death of HPV-positive cells

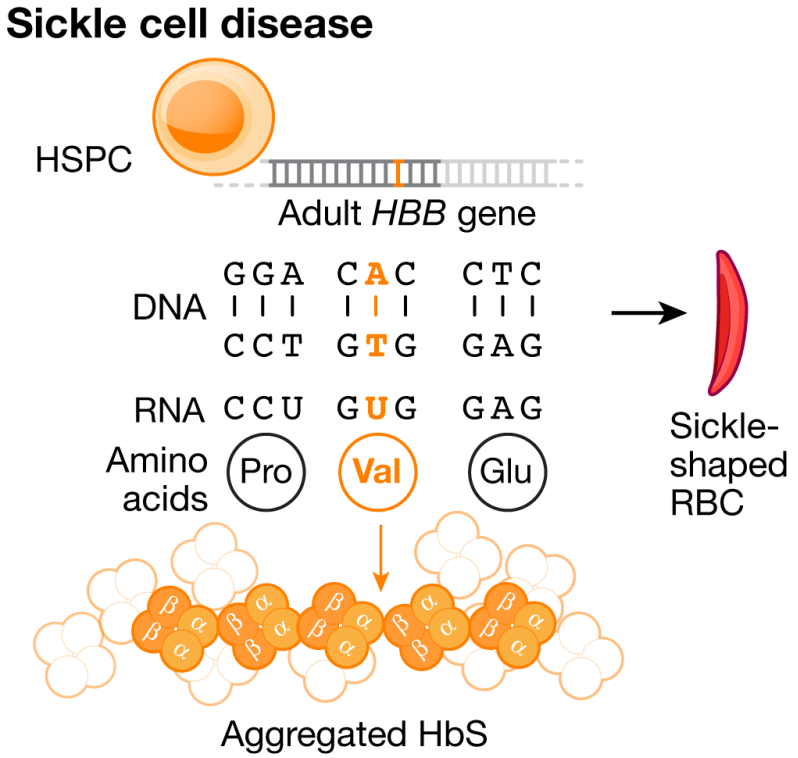
***NCT03057912*: CRISPR-mediated disruption of HPV E6 and E7 genes to treat HPV persistence.**

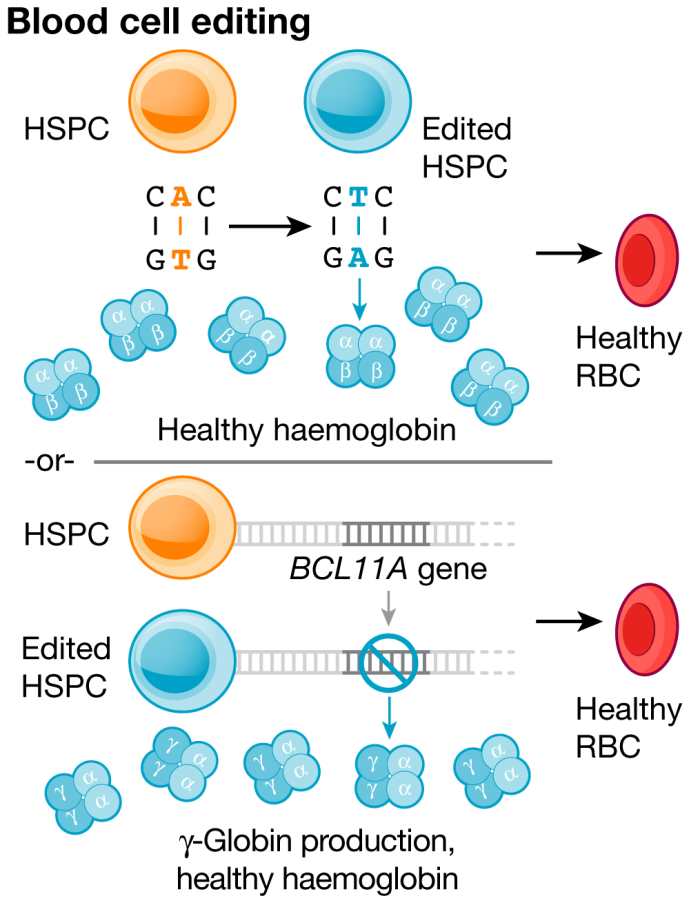
Application of a gel containing CRISPR reagents to the cervix.

1st clinical trial ever to delete genes while they are inside the body

Start Date: January 2018, Sun Yat-Sen University, China

Future Applications of CRISPR: Sickle Cell Disease





Patients with Sickle Cell Disease have a homozygous Glu→Val mutation in the *HBB* gene

Repair of HBB gene defect or re-activate expression of fetal globin?

Two future Nobel laureates?

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The discoverers of the CRISPR system

Emmanuelle Charpentier Jennifer Doudna

Thank you for your attention!

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