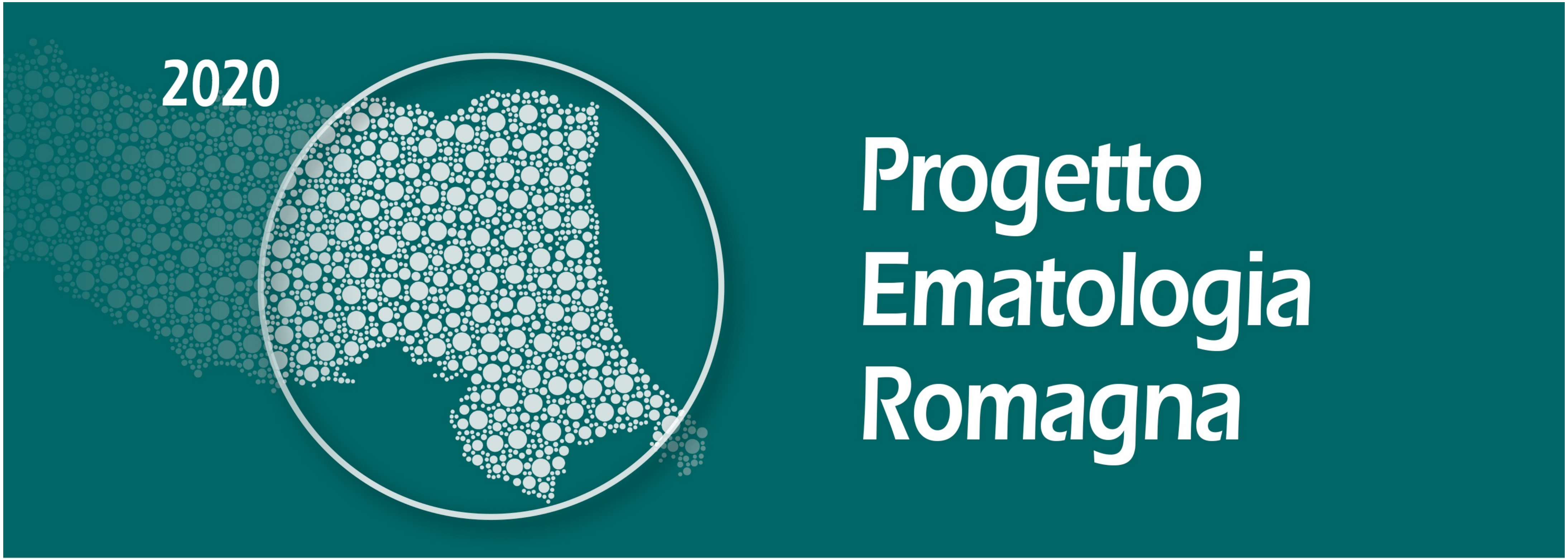


Le Conoscenze in Biologia Corrono!

Simona Soverini
Istituto di Ematologia «Lorenzo e Ariosto Seràgnoli», Università di Bologna

Disclosures

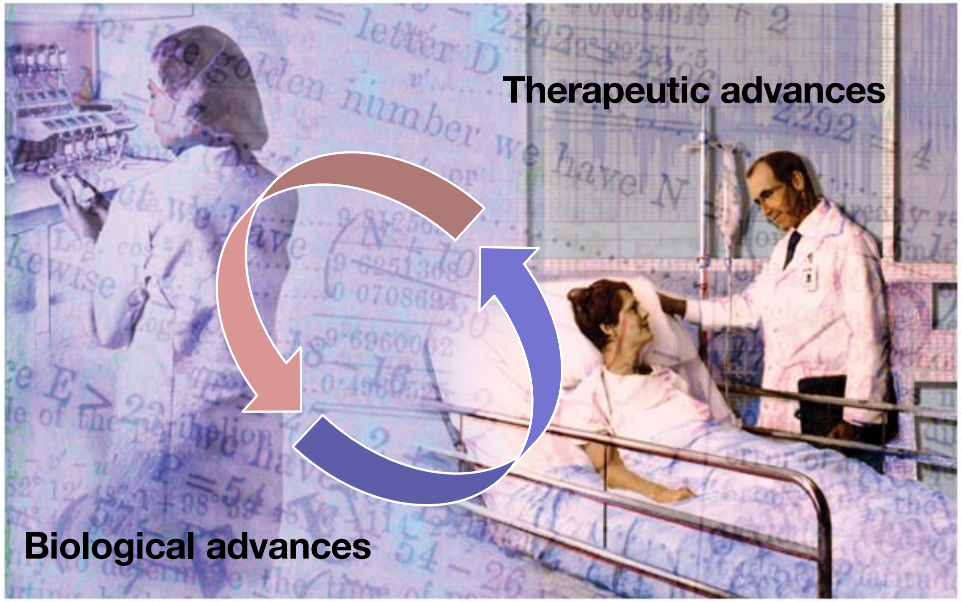
- Incyte Biosciences: consultancy

How biology may fuel precision medicine

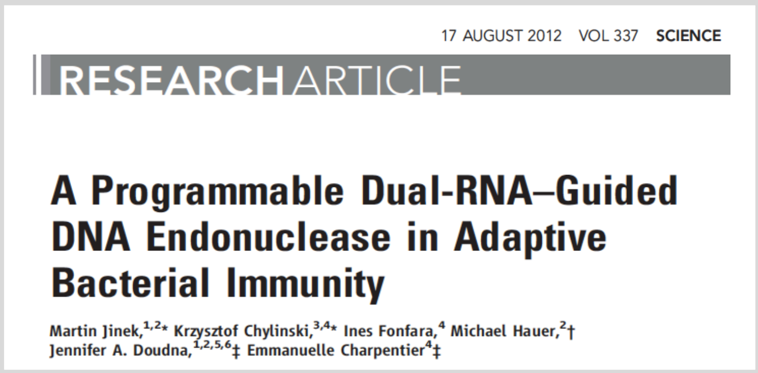
Discovery
- Novel pathogenetic players
- Novel therapeutic targets
- Novel diagnostic/prognostic factors

Modeling
- Gene/protein function
- Drug testing
- Resistance screening

Diagnostics & Therapy
- Diagnosis
- Risk stratification
- Tailored treatment approaches
- Monitoring of therapeutic response



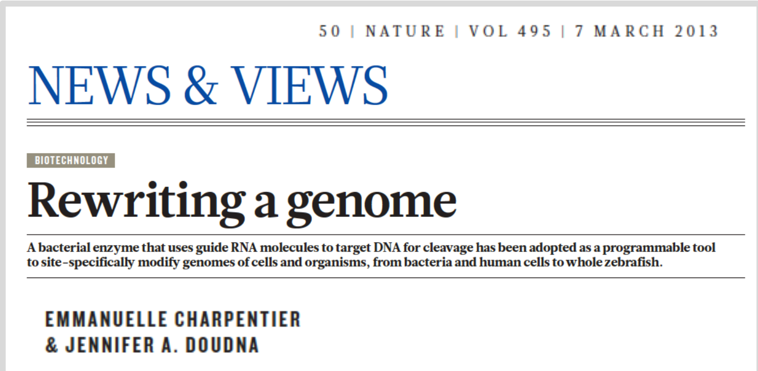
Genome editing: the (short) path from discovery to the Nobel prize


17 AUGUST 2012 VOL 337 SCIENCE

RESEARCH ARTICLE

A Programmable Dual-RNA–Guided DNA Endonuclease in Adaptive Bacterial Immunity

Martin Jinek,[1,2]* Krzysztof Chylinski,[3,4]* Ines Fonfara,[4] Michael Hauer,[2]† Jennifer A. Doudna,[1,2,5,6]‡ Emmanuelle Charpentier[4]‡


50 | NATURE | VOL 495 | 7 MARCH 2013

NEWS & VIEWS

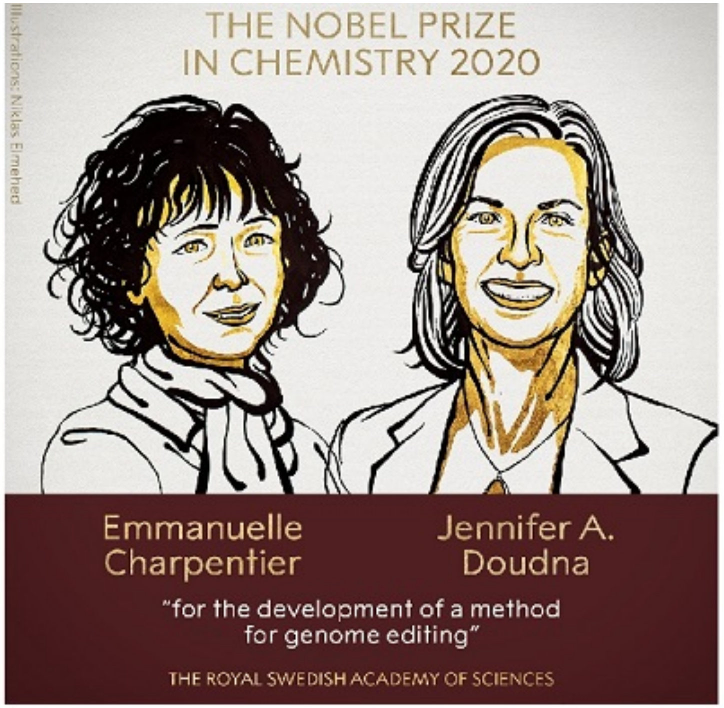
BIOTECHNOLOGY

Rewriting a genome

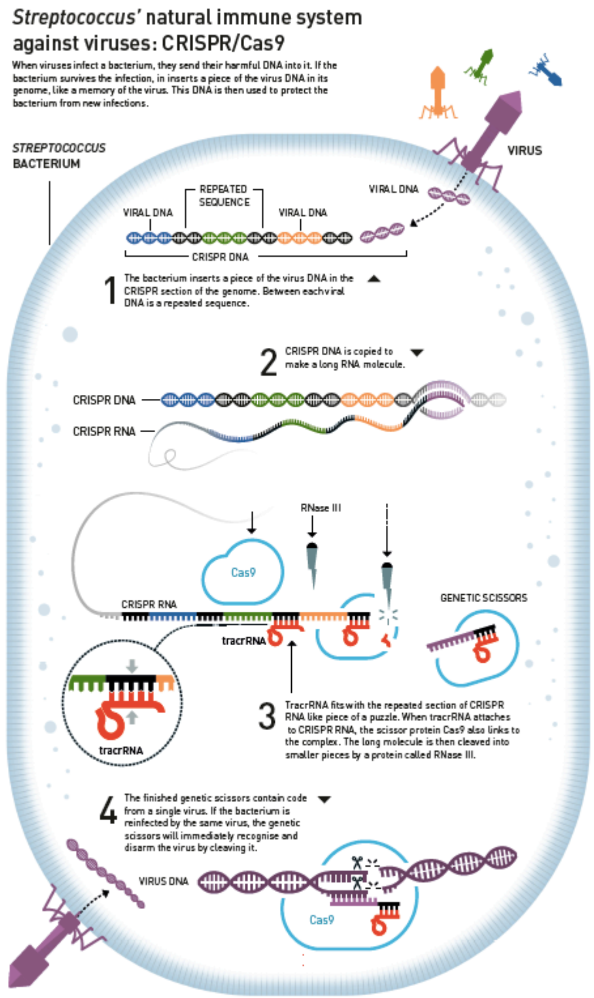
A bacterial enzyme that uses guide RNA molecules to target DNA for cleavage has been adopted as a programmable tool to site-specifically modify genomes of cells and organisms, from bacteria and human cells to whole zebrafish.

EMMANUELLE CHARPENTIER & JENNIFER A. DOUDNA

12/18/2015, Vol 350 Issue 6267



CRISPR/CAS9 genome editing: a lesson learned from bacteria

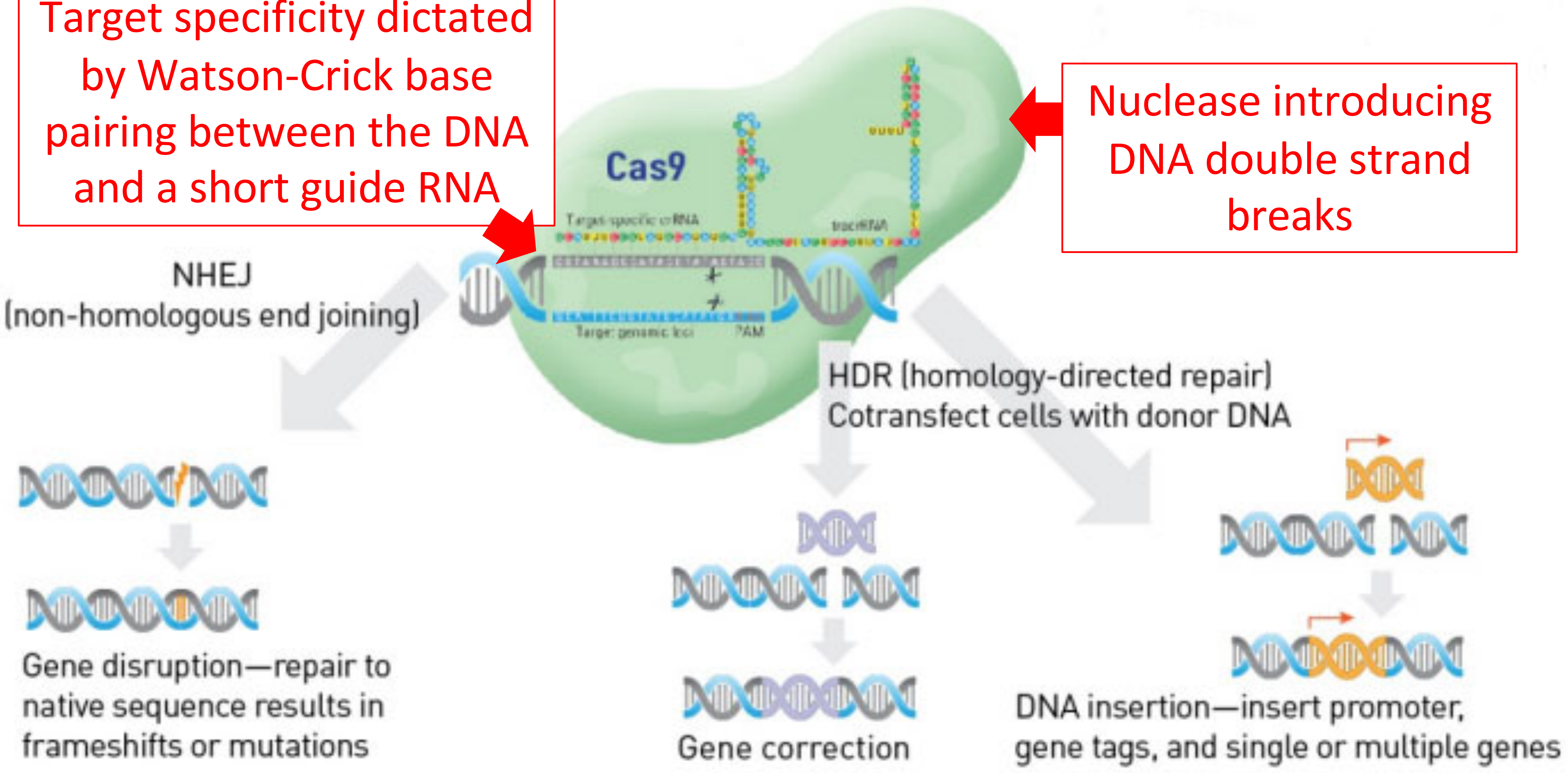


- The CRISPR/CAS9 tool was derived from a naturally occurring genome editing system in bacteria serving as an adaptive immune system
- Bacteria capture snippets of DNA from invading viruses and use them to create DNA segments known as CRISPR ('Clustered Regularly Interspaced Short Palindromic Repeats') arrays which serve as a sort of 'immune memory'
- If the virus attacks again, bacteria produce RNA segments from the CRISPR arrays to target the virus' DNA. Bacteria then use the Cas9 endonuclease to cut the DNA apart, disabling the virus

How the CRISPR/CAS9 genome editing toolbox works

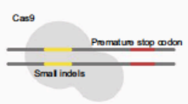
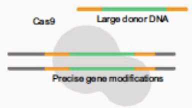
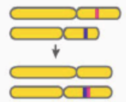
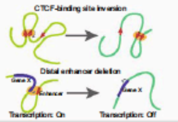
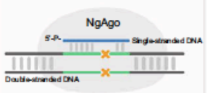
Target specificity dictated by Watson-Crick base pairing between the DNA and a short guide RNA

Nuclease introducing DNA double strand breaks

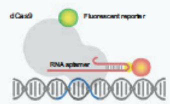
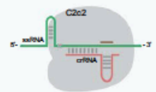


CRISPR/CAS9 applications in basic and translational research

Gene editing applications of CRISPR technology

	Schematic	Applications	Reference
Generation of small indels		• Gene inactivation • Modeling loss-of-function mutation	Cong et al., 2013 Wang et al. 2013
Point mutations		• Precise mutagenesis • Modeling gain-of-function mutation	Mali et al., 2013 Xue et al., 2014
Tag/large fragment insertions		• Protein/lncRNA tag labeling • Fluorescence reporter insertion • Recombination signal (LoxP/Frt) insertion • Insertion of transcriptional STOP signal	Yang et al., 2013 Yang et al., 2014 Lee et al., 2016
Large fragment deletions		• Gene Deletion • Investigating higher-order chromatin structure	Yang et al., 2014 Gröschel et al., 2014
Chromosomal rearrangements		• Chromosomal rearrangement	Choi et al., 2014 Maddalo et al., 2014 Blasco et al., 2014
Manipulation of *cis*-regulatory elements		• Regulation of gene expression by *cis*-regulatory element	Guo et al., 2015 Gröschel et al., 2014 Canver et al., 2015
DNA-guided genome editing		• DNA-guided DNA editing • Multiplexed gene targeting	Gao et al., 2016

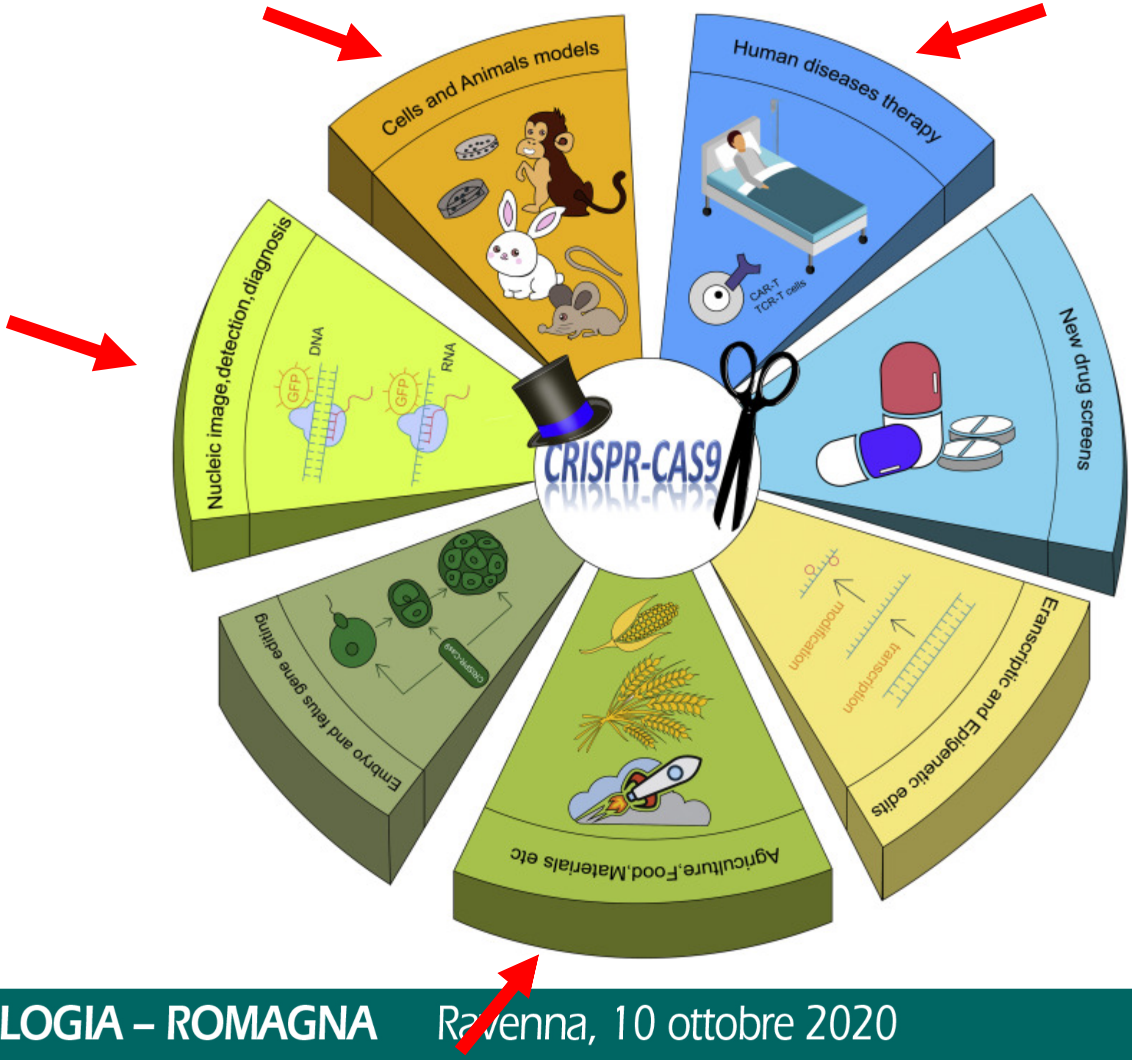
Non-editing applications of CRISPR technologogy

	Schematic	Applications	Reference
Transcriptional regulation		• Precise regulation of gene expression • Genome-wide gene activation/repression screens	Gilbert et al., 2013 Gilbert et al., 2014 Konermann et al., 2015 Dominguez et al., 2016
Chromatin modification		• Epigenetic regulation on *cis*-regulatory elements • DNA methylation	Kearns et al., 2015 Hilton et al., 2015 Dominguez et al., 2016 Vojta et al., 2016
Visualizing genomic loci		• Visualizing repetitive and non-repetitive genetic elements • Revealing chromatin dynamics by live-cell imaging	Chen et al., 2013 Fu et al., 2016
Targeting ssRNA		• Targeting specific RNA • Modulating non-coding RNAs	Abudayyeh et al., 2016

+ CRISPRa: **transcriptional activation** complex using a nuclease-dead version of Cas9 (dCas9) combined with a transactivation protein

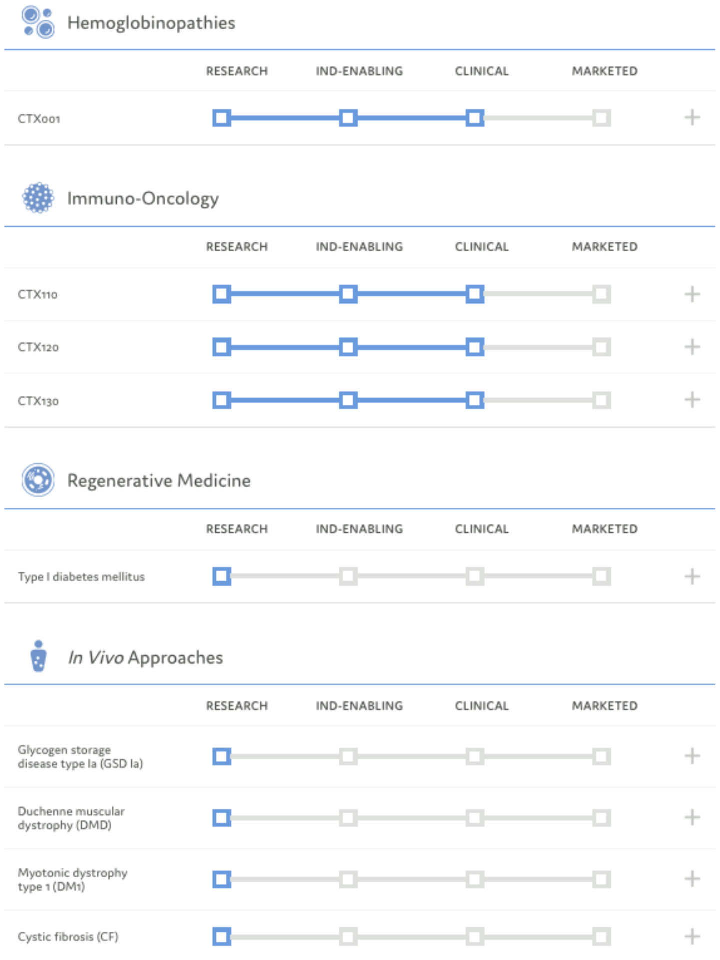
CRISPRi: **transcriptional silencing** complex using a nuclease-dead version of Cas9 (dCas9) combined with a repressor

The manifold CRISPR/CAS9 applications



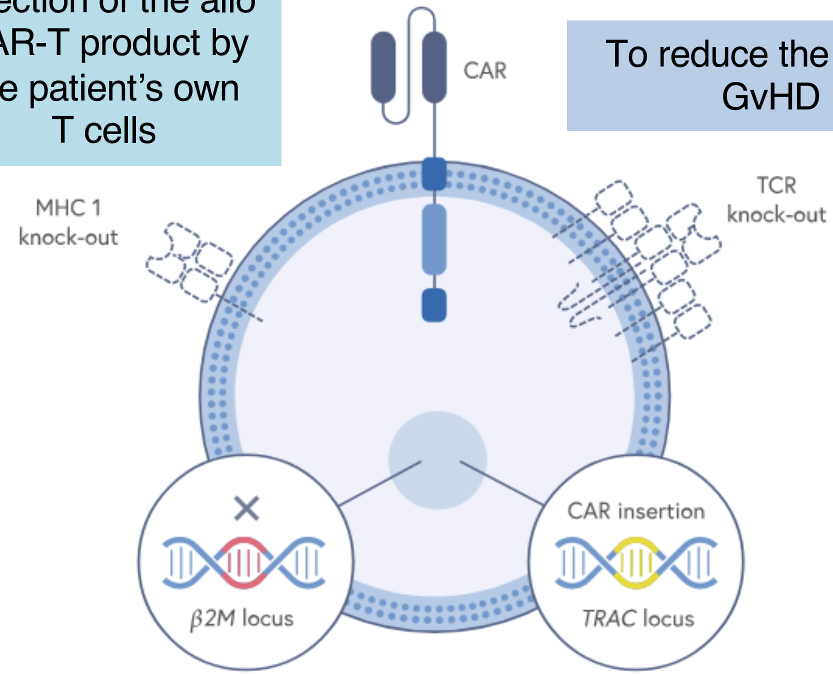
The CRISPR/CAS9 revolution: first clinical applications

CRISPR THERAPEUTICS



To mitigate the rejection of the allo CAR-T product by the patient's own T cells

To reduce the risk of GvHD

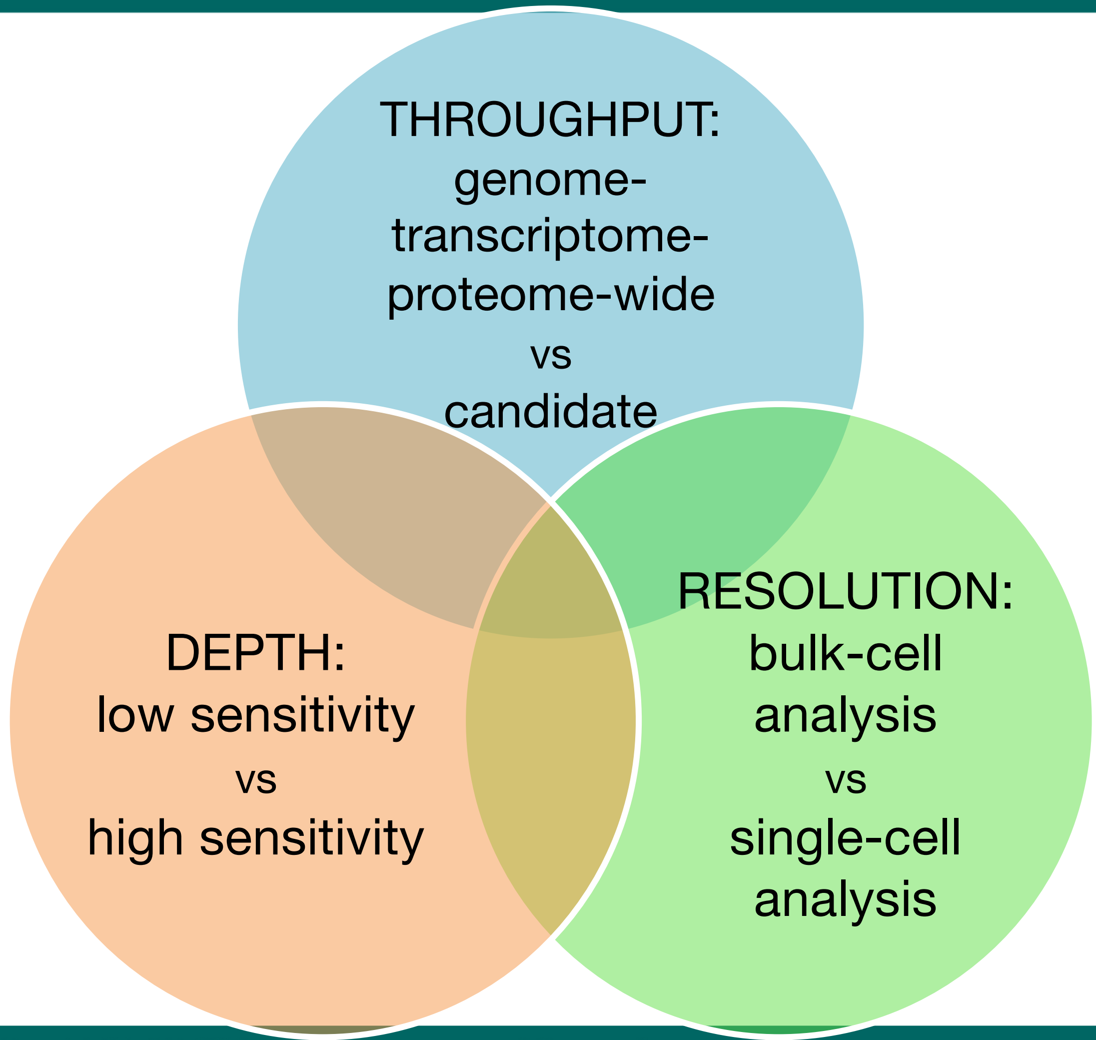


To insert the CAR construct more precisely, resulting in a safer and more consistent product

CRISPR/CAS9: remaining obstacles and open issues

- Improve delivery systems
- Increase the rate of gene correction
- Short- and long term effects and safety (e.g., ‘genotoxic’ off-target effects; immunogenicity of the Cas9 nuclease; carcinogenic effect of CRISPR complex components….)
- Ethical issues: e.g., genetically modified embryos

How novel technologies make discoveries easier



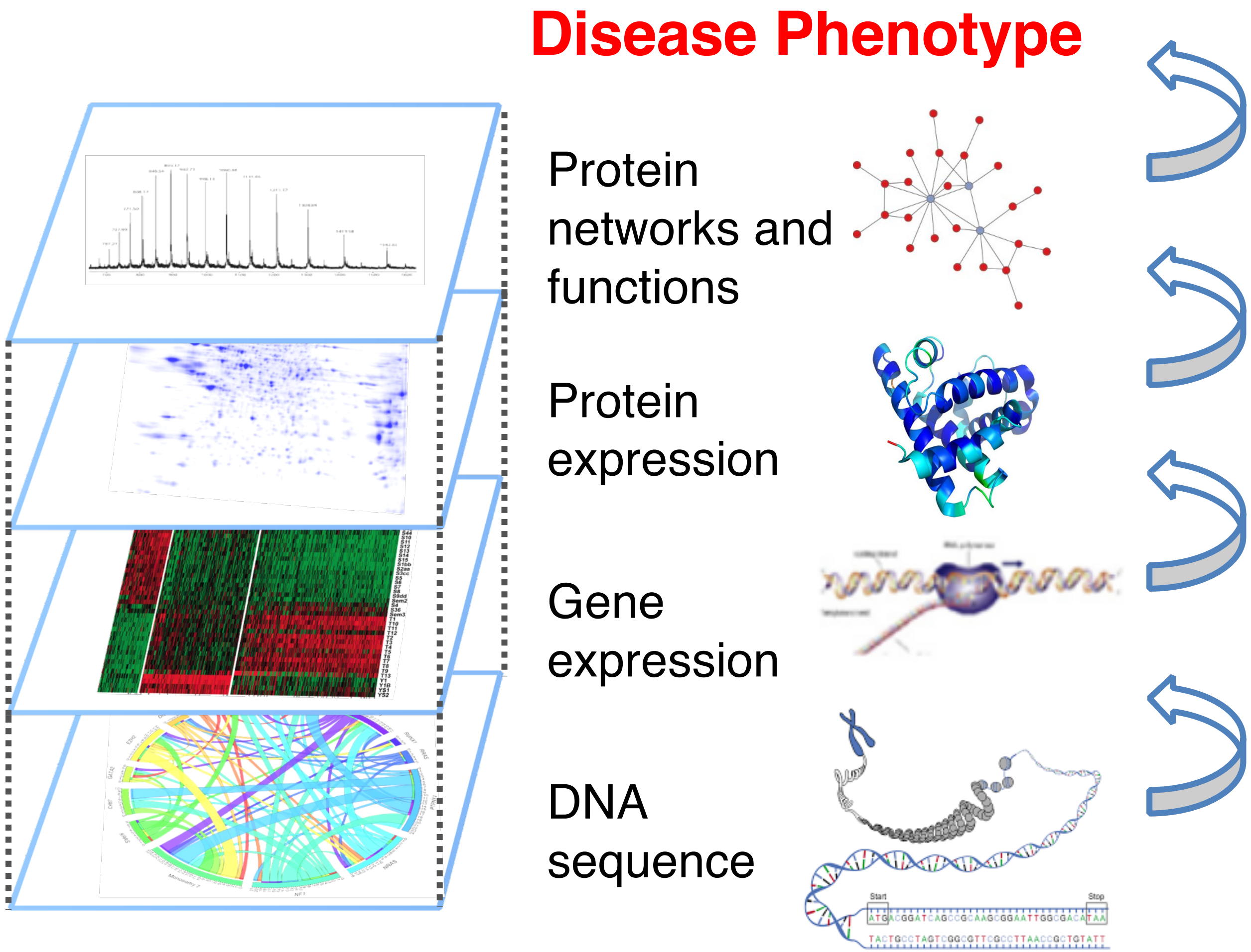
High-throughput technologies enable detailed shapshots at multiple levels

Metabolomics: Mass Spec, NMR

Proteomics: Mass Spec

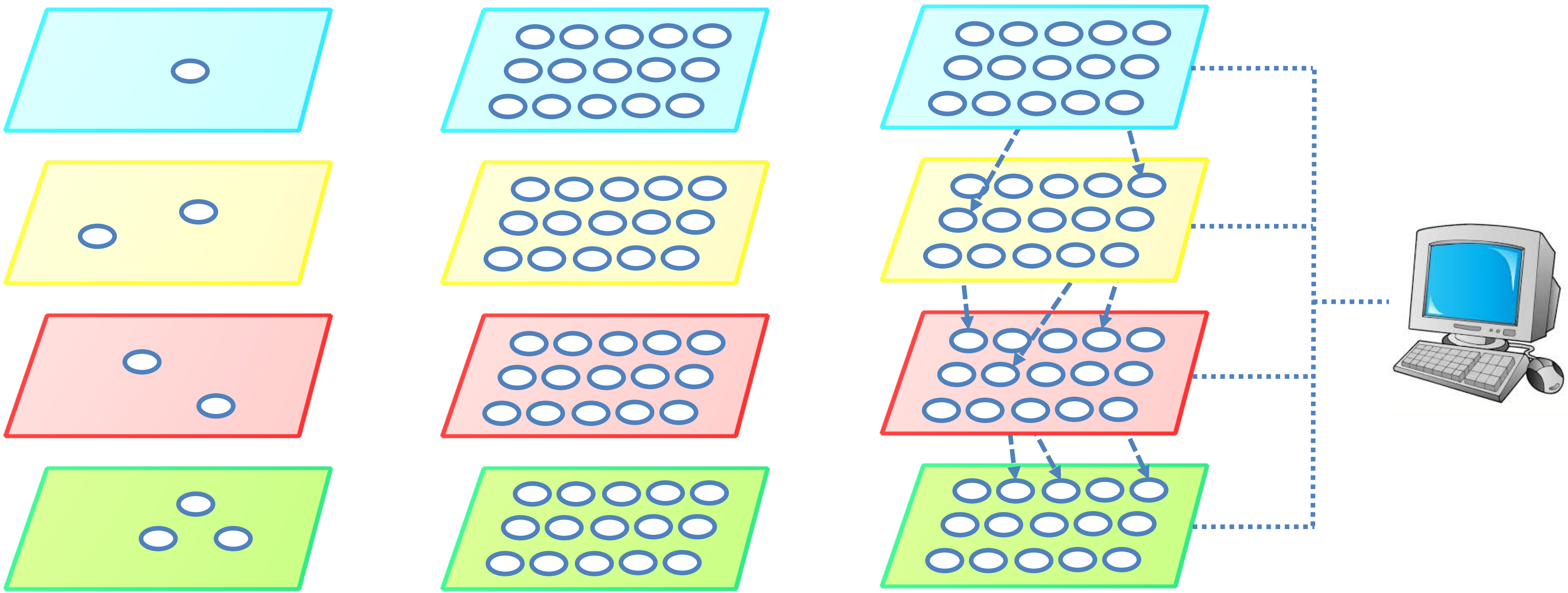
Transcriptomics: RNA-seq, microarrays

Genomics: WES, WGS, SNP-arrays, array-CGH



Multi-omics integration of single omics layers

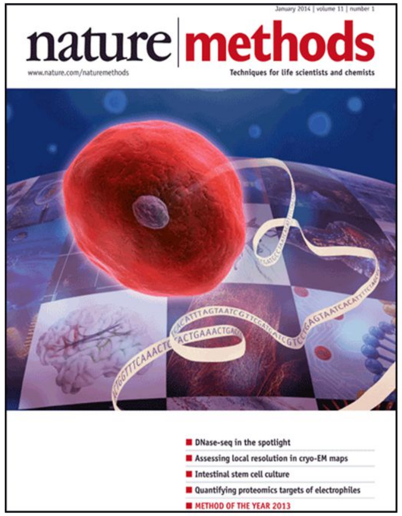
Old ('candidate') approach | Single Omics | Multi-Omics

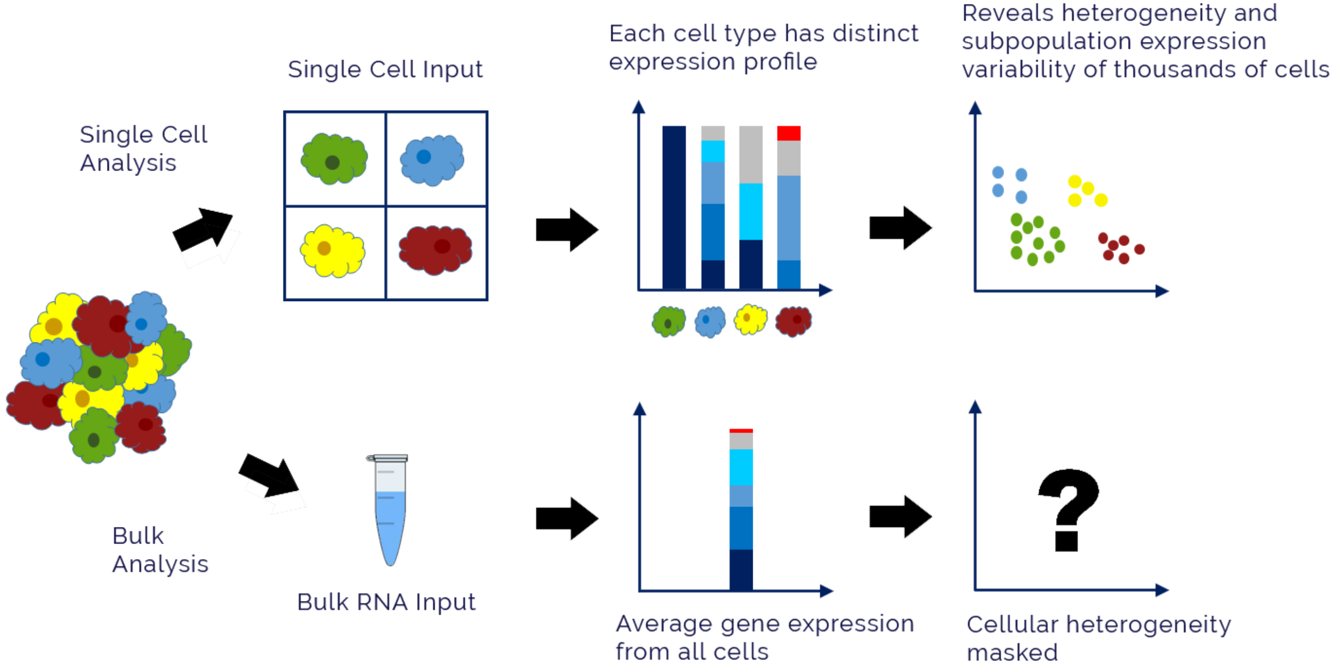


Bulk cell vs single-cell complexity

- Single-cell trascriptomics has been the forerunner of single cell analyses
- It enables to dissect transcriptional heterogeity, identifying distinct and/or rare cell subtypes, functional or evolutionary states

Methods to sequence the DNA and RNA of single cells are poised to transform many areas of biology and medicine.
--- Nature Methods

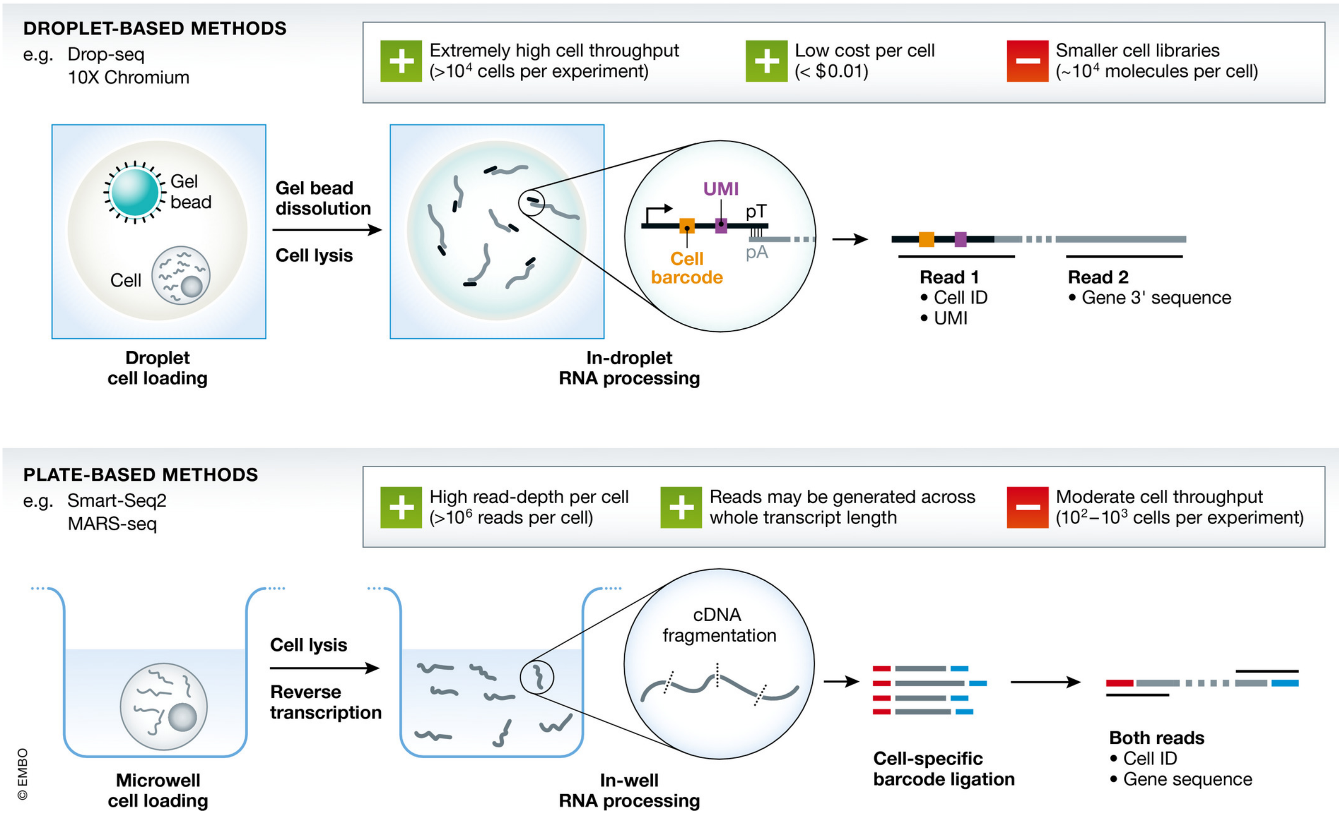




How can we isolate and study individual cells

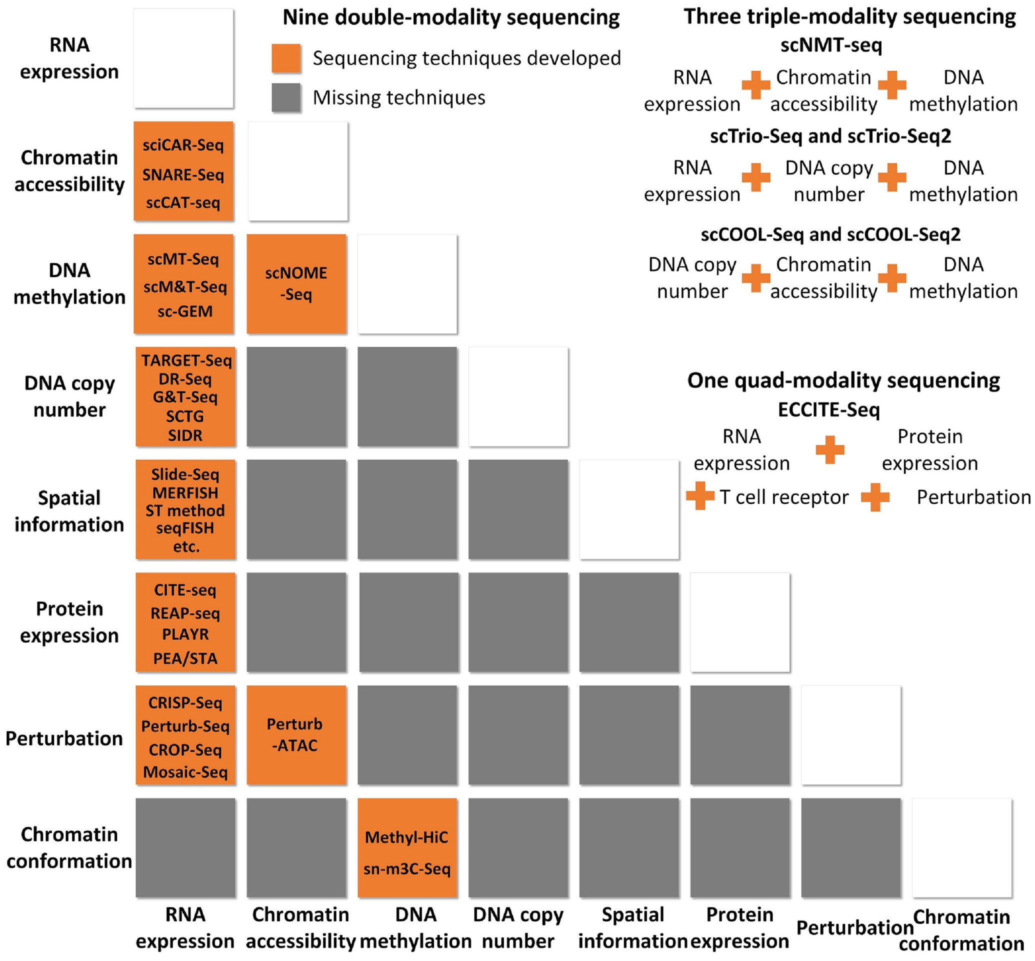
Remaining challenges are technological, statistical and computational:

- how to ensure adequate quantity and purity
- how to analyze very small amounts of nucleic acids
- How to validate and benchmark analysis tools



The ultimate frontier: single cell multimodal omics

work is ongoing to develop more and more methods to profile multiple sources of information in the same cell



Trends in Biotechnology

Today's program

10:30 – 12:00 **LE CONOSCENZE IN BIOLOGIA CORRONO!**

Introduzione *S. Soverini*

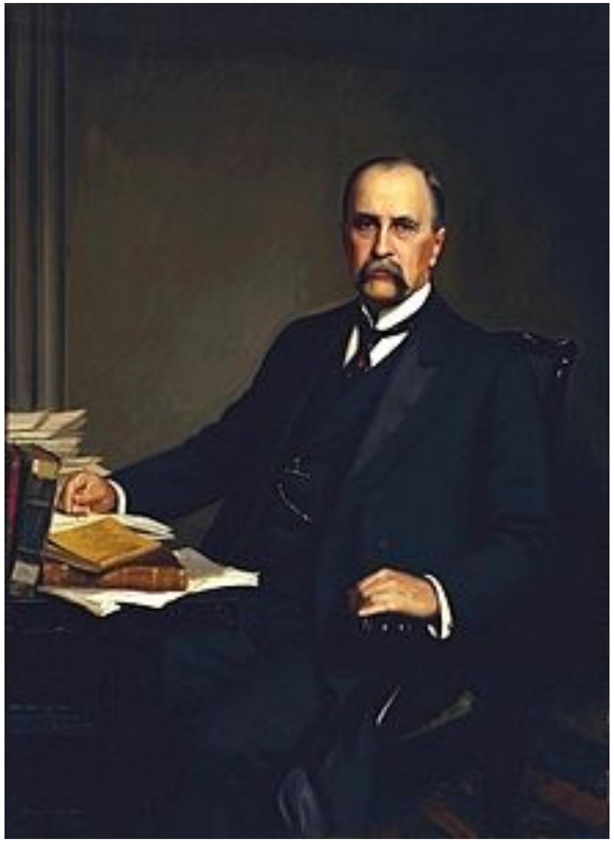
L'impatto delle nuove tecnologie nella diagnostica e terapia personalizzata delle leucemie *S. Bruno*

Dalla biologia alla terapia: una storia a lieto fine per la leucemia mieloide cronica e una storia ancora tutta da scrivere per la mastocitosi sistemica *M. Mancini*

Towards the goal of precision medicine, from early '900 to today

"Ask not what disease the person has, but rather what person the disease has"

"The good physician treats the disease; the great physician treats the patient who has the disease"



Sir William Osler