Modelos experimentales en biomedicina: De la mesa del laboratorio a la clínica

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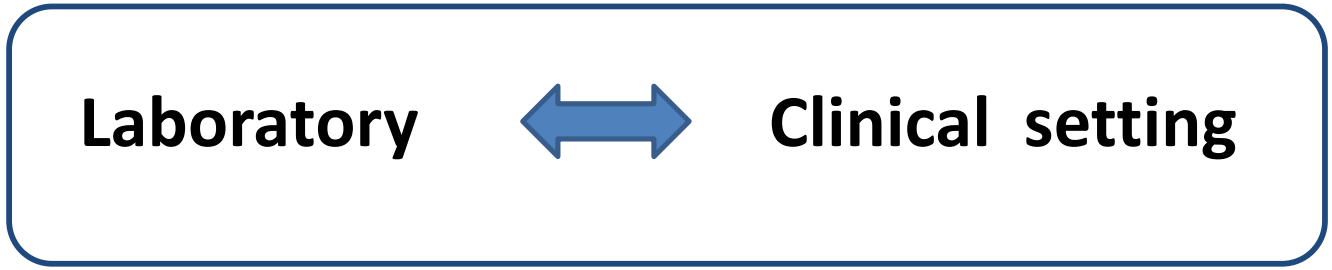
Real Academia de Medicina de la C. Valenciana.

What type of research?: High quality and competitive

Basic research

Scientific and technical applications

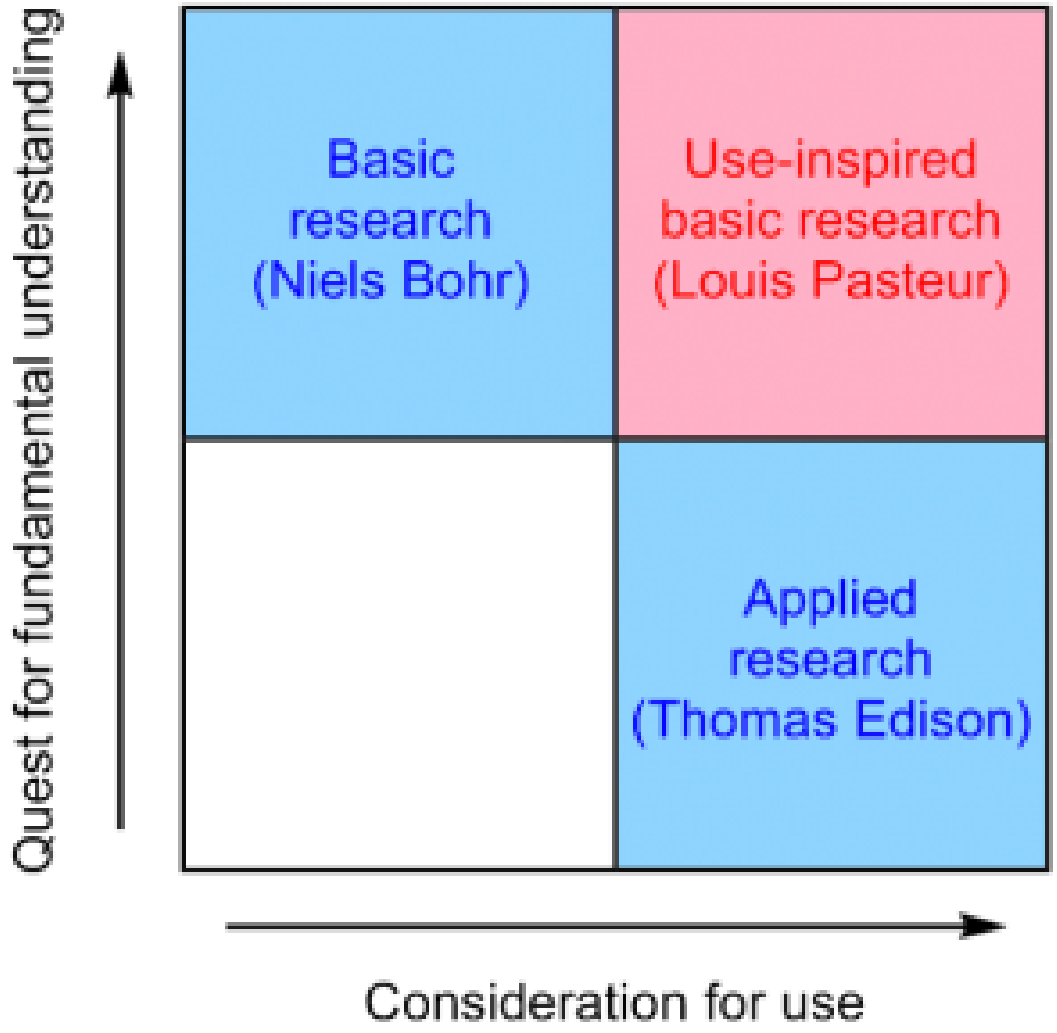
Traslational medicine



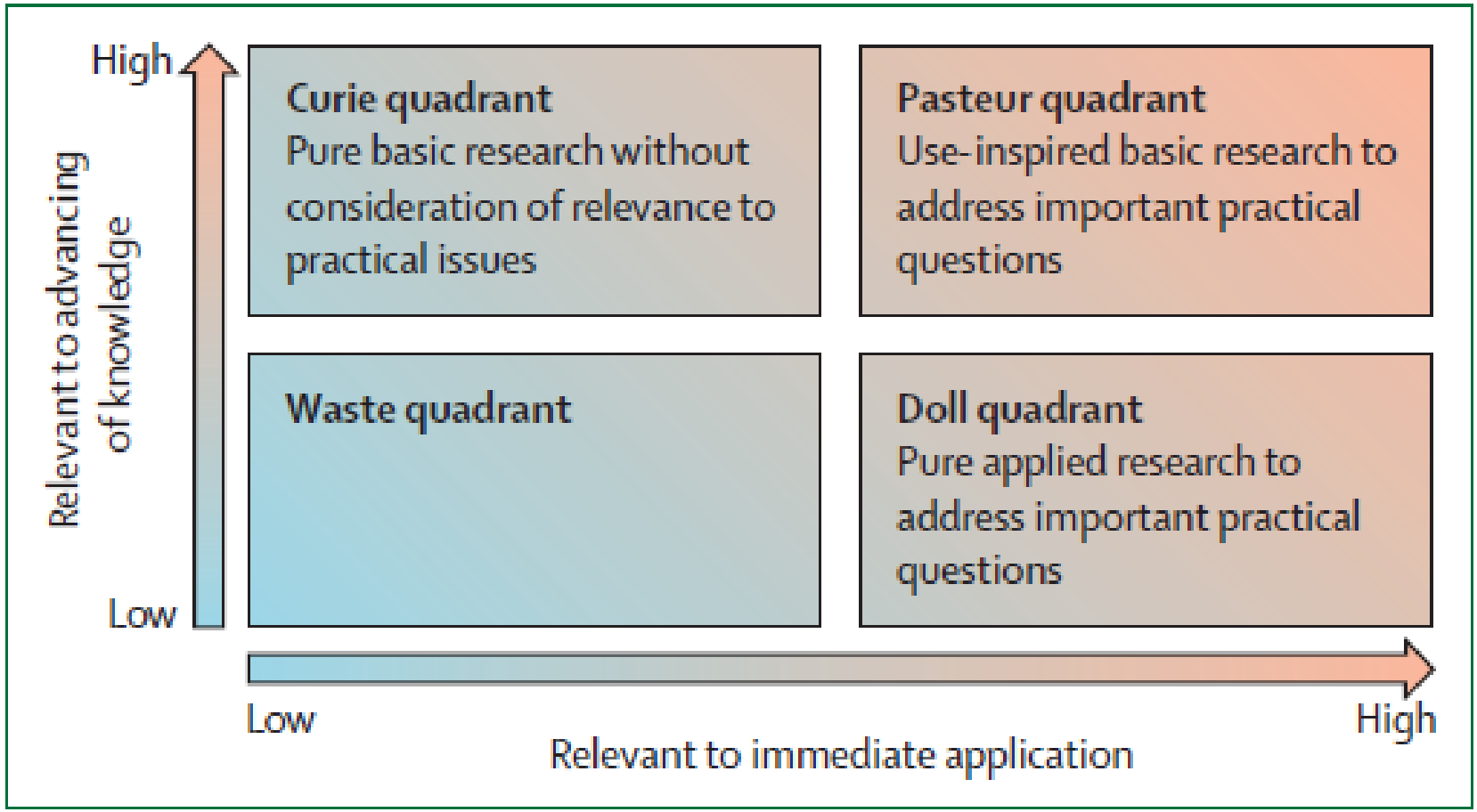
“Bench to bedside”: A round trip to improve precision medicine

Pasteur´s quadrant-basic science and technological innovation

Stroke DE 1997



Classification of different categories of research



Chalmers, I et al (2014) Lancet 383:145

Best advices

- Ask the right question. (Claude Bernard)
- Select the right experimental model. (August Krogh)
- Select the right mentor. (Hans A. Krebs)
- Be passionate about solving a problem. (Peter Medawar)
- Be audacious and take risks. (Richard Feynman)
- Avoid boring people. (James Watson)
- Blending existing knowledge with imaginative thinking . (Joseph L. Goldstein)
- Importance of non-standard models. (Sydney Brenner)

“On asking the right kind of question in biological research”
Hans A. Krebs (1979)

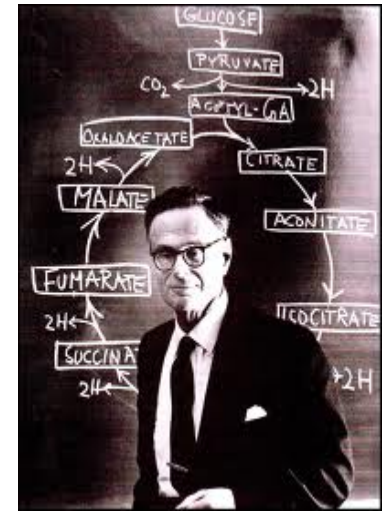
- Choose the model to be studied
- Ask the right question.
- Obtain data of strategic value.
- The answer of good question opens the field to new and interesting question
-”Do not fall in love” with your own ideas

The August Krogh Principle

"For a large number of problems there will be some animal of choice, or a few such animals, on which it can be most conveniently studied"

Krogh A. (1929) Progresss in Physiology. Am. J. Physiol 90, 243-260.

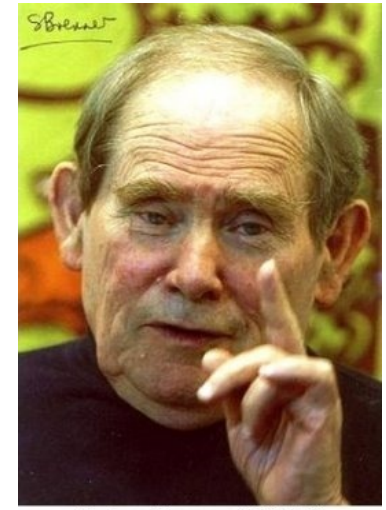
August Krogh Principle



While this is true at the molecular level of biological organization, generalizations from one species to another must necessarily be more restricted at higher and more complex levels such as those of ecology and behavior where specialized functional adoptions have evolved

Krebs HA & Krebs JR (1980) Comp. Biochem. Physiol. 67B 379-380.

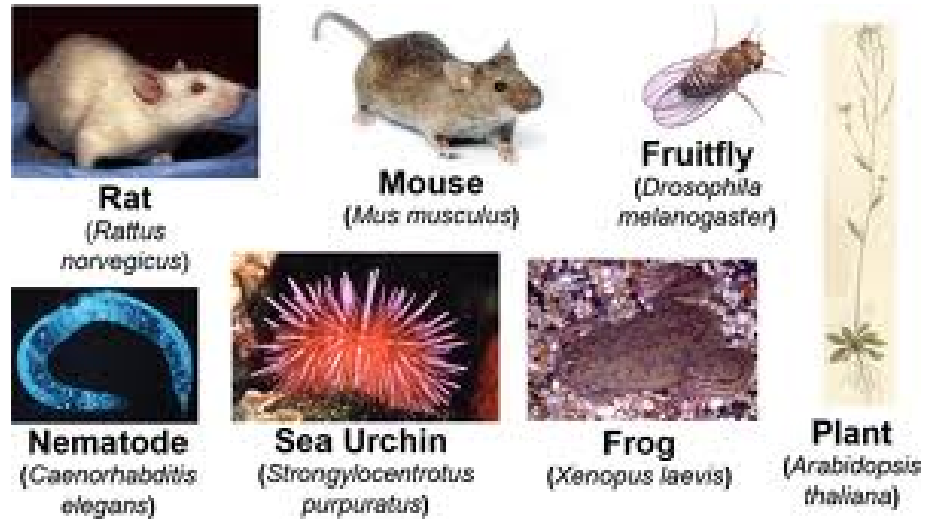
It is important to work on “nonstandard” organisms. We will miss a lot if we focus on a few well worked-out model systems. We can learn so much from diversity of life


Sydney Brenner (1927 -)

The importance of the experimental model

- **Unicellular eukaryotic :**
  - Yeast (*S. cerevisiae*)
  - Tetrahymena thermophila

- **Pluricellular eukaryotic :**
  - Invertebrates
    - *Caenorhabditis elegans.*
    - *Daphnia magna.*
    - *Drosophila*
  - Vertebrates
    - Fugu, the japanese pufferfish, Zebrafish
    - The lactating mammary gland
    - Pigeon breast muscle.
    - Genetically manipulated mice: Knock out and knock in.
    - Genetically manipulated mice by RNA interference



http://www.nih.gov/science/models/

Model Organisms
for Biomedical Research

Mammalian Models:


➤ Mouse


➤ Rat

Non-Mammalian Models:

➤ *S. cerevisiae* (budding yeast)


➤ *S. pombe (fission yeast)*


➤ Neurospora (filamentous fungus)


➤ *D. discoideum* (social amoebae)


➤ *C. elegans* (round worm)


➤ *Daphnia (water flea)*


➤ *D. melanogaster* (fruit fly)


➤ *D. rerio* (zebrafish)


➤ *Xenopus* (frog)


➤ *Gallus (chicken)*

Other Model Organisms:


➤ *Arabidopsis*

Other:

➤ Reports

➤ Funding Opportunities

➤ Process for Considering Support

➤ NIH Policy on Sharing of Model Organisms for Biomedical Research

➤ A User's Guide to the Human Genome

➤ Opportunity to Propose New Organisms for Sequencing

➤ Bacterial Artificial Chromosome (BAC) Resource Network

➤ Rate Setting Manual *for Animal Research Facilities*

➤ Final NIH Statement on Sharing Research Data

➤ Resource Sharing Guidelines

➤ What's New

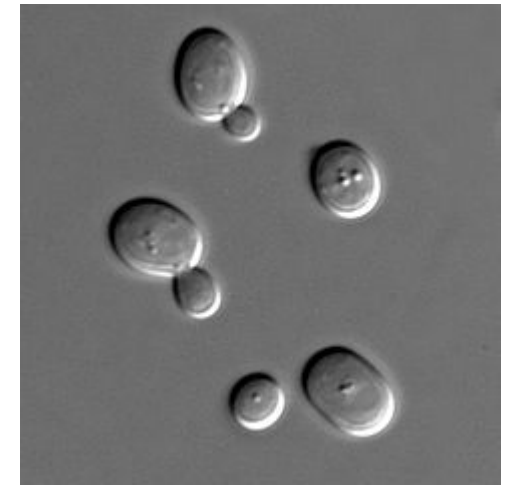
We hope this web site provides you with information about national and international activities and major resources that are being developed to facilitate biomedical research using the animal models listed here. For organisms not listed, web pages may be developed in the future.

If you have suggestions as to how we can enhance the information provided, please send a message to Bettie Graham at bettie_graham@nih.gov.

Thank you for visiting our web site.

Francis S. Collins, MD, Ph.D.
Director, National Institutes of Health

Yeast *Saccharomyces cerevisiae*

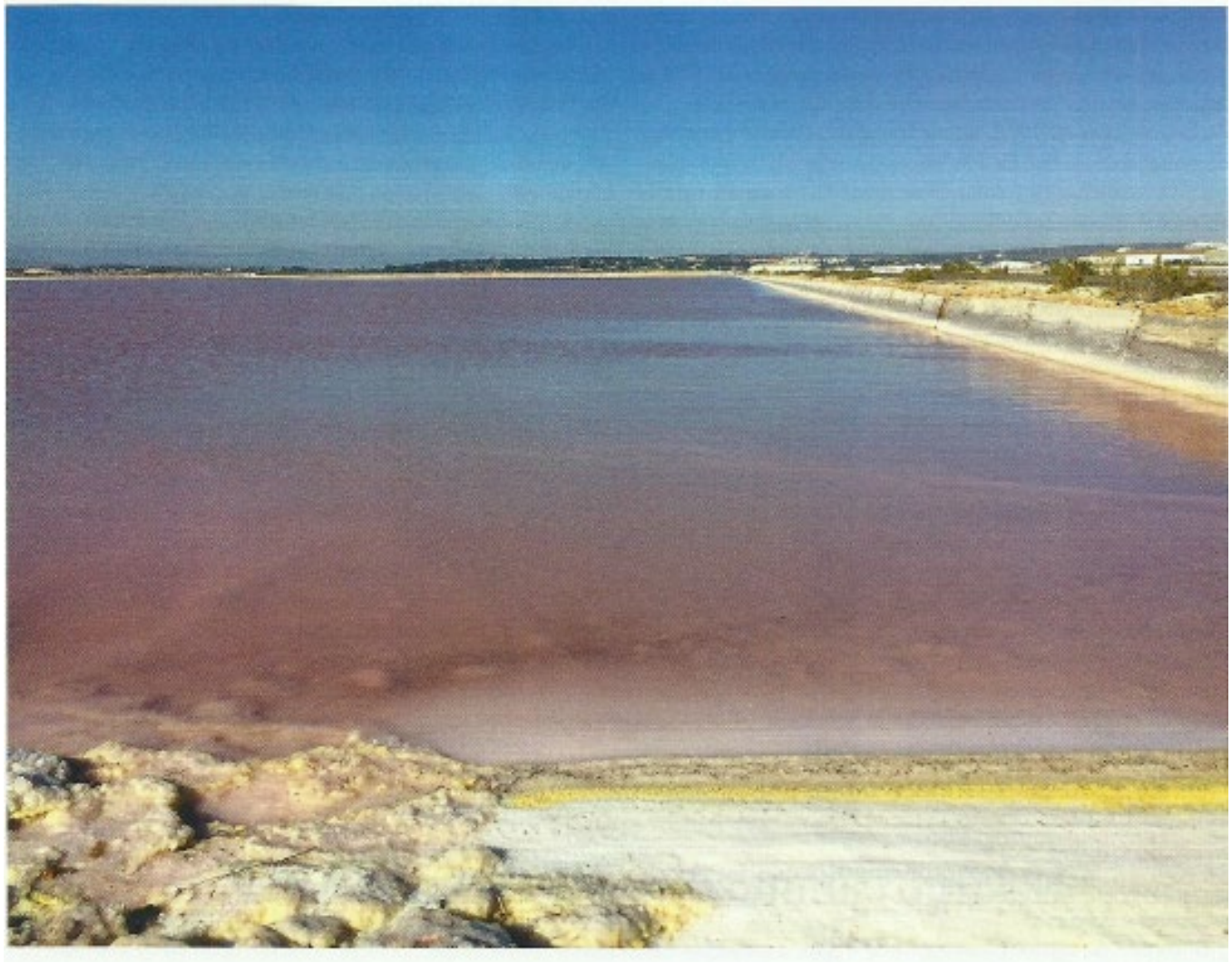


Many proteins important in human biology were first discovered by studying their homologs in yeast.

As a single celled organism S. cerevisiae is small with a **short generation time** (doubling time 1.25–2 hours[3] at 30 °C or 86 °F) and can be easily cultured.

S. cerevisiae can be transformed allowing for either the addition of new genes or deletion through homologous recombination.

As a eukaryote, S. cerevisiae shares the complex internal cell structure of plants and animals **without the high percentage of non-coding DNA** that can confound research in higher eukaryotes.



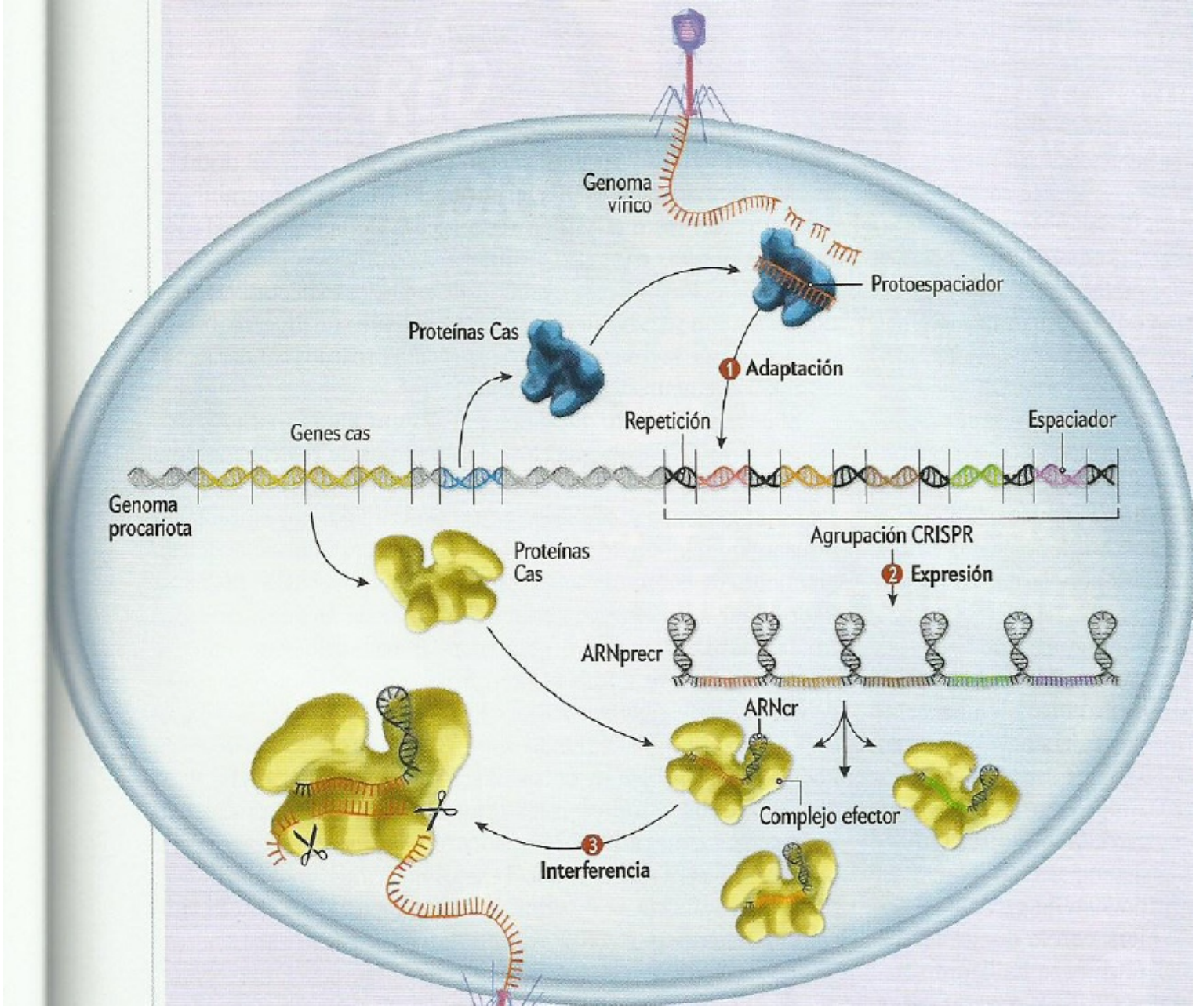
Prof. FJM Mójica

Salinas de Santa Pola: Aarquea Haloferax Mediterranei

From Gram-negative bacterias and archaea Haloferax mediterrani to human cells

Clustered regularly-interspaced short palindromic repeat (CRISPR) sequences cooperate with CRISPR-associated (Cas) proteins to form the basis of CRISPR-Cas adaptive immune systems in prokaryotes. In 2012, the potential of CRISPR-Cas systems was uncovered and these were presented as genome-editing tools with an outstanding capacity to trigger targeted genetic modifications that can be applied to virtually any organism including human cells. **Shortly thereafter, in early 2013, these tool were shown to efficiently drive specific modification of mammalian genomes.**

On the Origin of CRISPR-Cas Technology: From Prokaryotes to Mammals. Mojica FJM & Montoliu L (2016)



Así funciona CRISPR-Cas Mojica & Almendralejos Investigación y Ciencia (2017)

Caenorhabditis elegans

Male C elegans worms form 1179 somatic cells, of which 148 are condemned to die by apoptosis. This simple worm has 13 apoptosis genes, all but two of them acting within every somatic cell.

Daphnia Magna

Photo credit:
Joachim.mergeay@bio.kuleuven.be
University of Leuven, Belgium

Before they were used as "canaries of water", Mechnikov studied Daphnia infection by a fungus and confirmed what was happening in the embryos of the starfish: the accumulation of motile cells, equivalent to our white blood cells around spores, after being swallowed, pass through the intestine to the body cavity.

Drosophila Melanogaster

DM is a powerful genetic model organism, which has been instrumental in the determination of essential developmental and neurological pathways conserved from invertebrates to humans.

A life span measured in weeks rather than years, the fruit fly is easy to experiment with.

Researchers can track a gene's effects throughout the animal's life span. At least 80 genes implicated in cancer have homologs in flies.

Fugu, the japanese pufferfish.

The Fugu has the smallest vertebrate genome but has a similar gene repertoire to other vertebrates.

Its genes are densely packed with short intergenic and intronic sequences devoid of repetitive elements.

It is probably close to a “minimal” vertebrate genome.

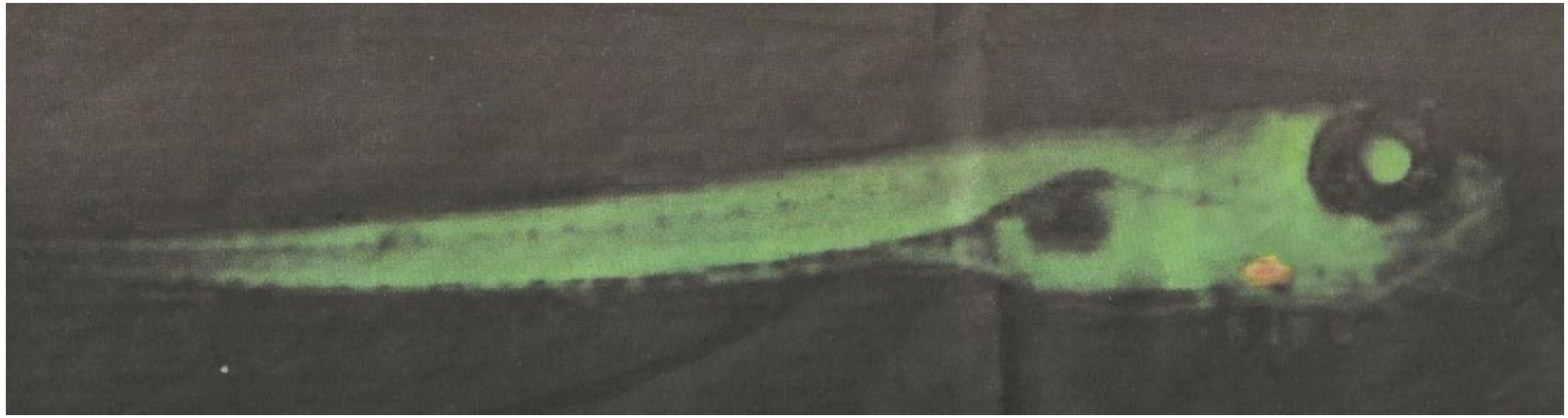
Zebrafish (Danio reiro)

Zebrafish embryos develop externally and can be viewed and manipulated at all stages.

A female zebrafish can **lay up to 200 eggs per week**, while a mouse may produce a litter of up to 10 embryos in 21 days.

It is easy to induce new mutations in zebrafish and large-scale screens have been carried out to identify mutations causing defects in particular biological processes, such as the developing nervous system.

The technology for gene transfer to zebrafish is highly advanced (also Transgenic mice).



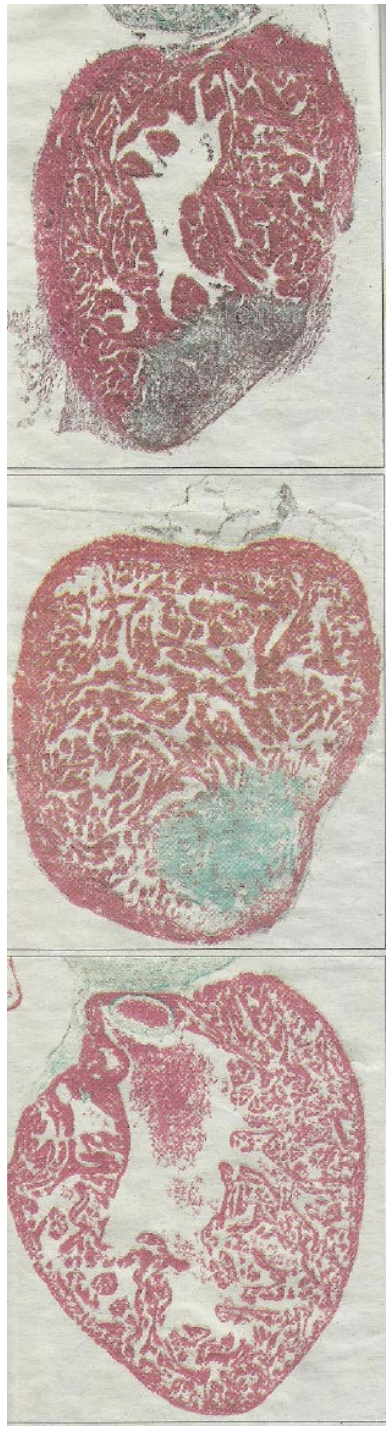
A small zebrafish where heart can be seen in orange.
Zebrafish and humans share 70% of their genes

Can the heart be regenerated ?

Healthy mammalian heart tissue has a measurable but limited ability to regenerate. Over a normal human lifespan, around 45% of heart-muscle cells (cardiomyocytes) are renewed, with the remaining 55% persisting from birth.

This rate is not sufficient to repair the injury caused by myocardial infarction, or heart attack as it is commonly known. Instead, the infarcted area becomes populated by fibroblast cells, which form a non-contractile collagenous scar — a quick fix that progressively decreases the heart's pumping capacity.

Vunjak-Novakkovic (2015) Nature 525: 461



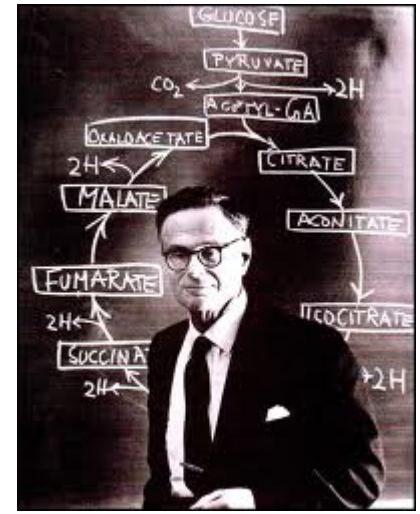
Picture of the infracted heart 3 days after the injection of liquid nitrogen

Picture of the infracted heart 21 days after the injection of liquid nitrogen

Picture of the infracted heart 131 days after the injection of liquid nitrogen

Bednarek D et (2015) Cell Rep 12:1691.

Pigeon breast muscle.



It played a key role in the discovery of the Krebs cycle.

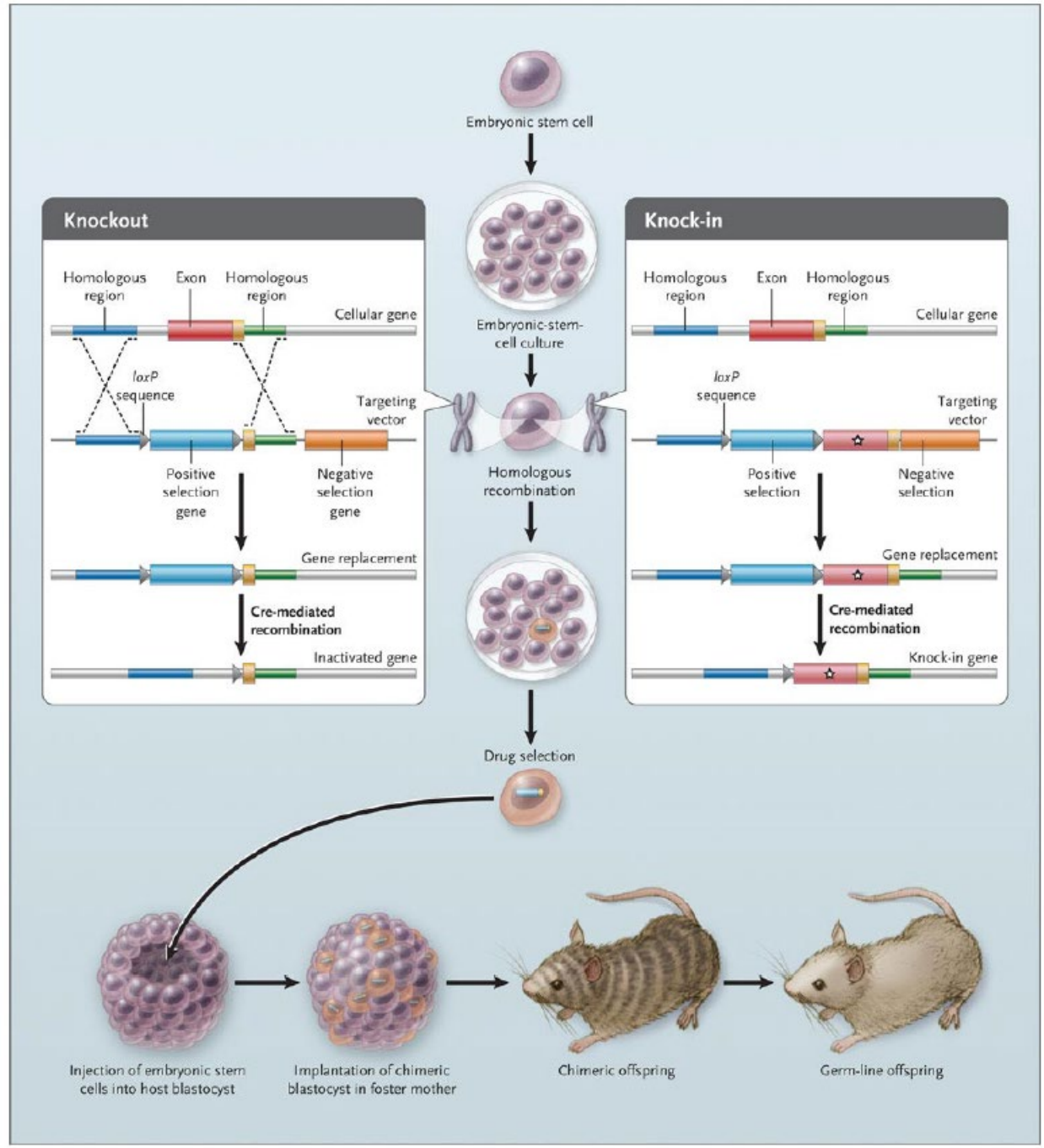
Estructura del asta de Ammon: Trabajo de Lorente de Nó y consejo de Ramón y Cajal sobre el correcto modelo experimental que es aconsejable usar

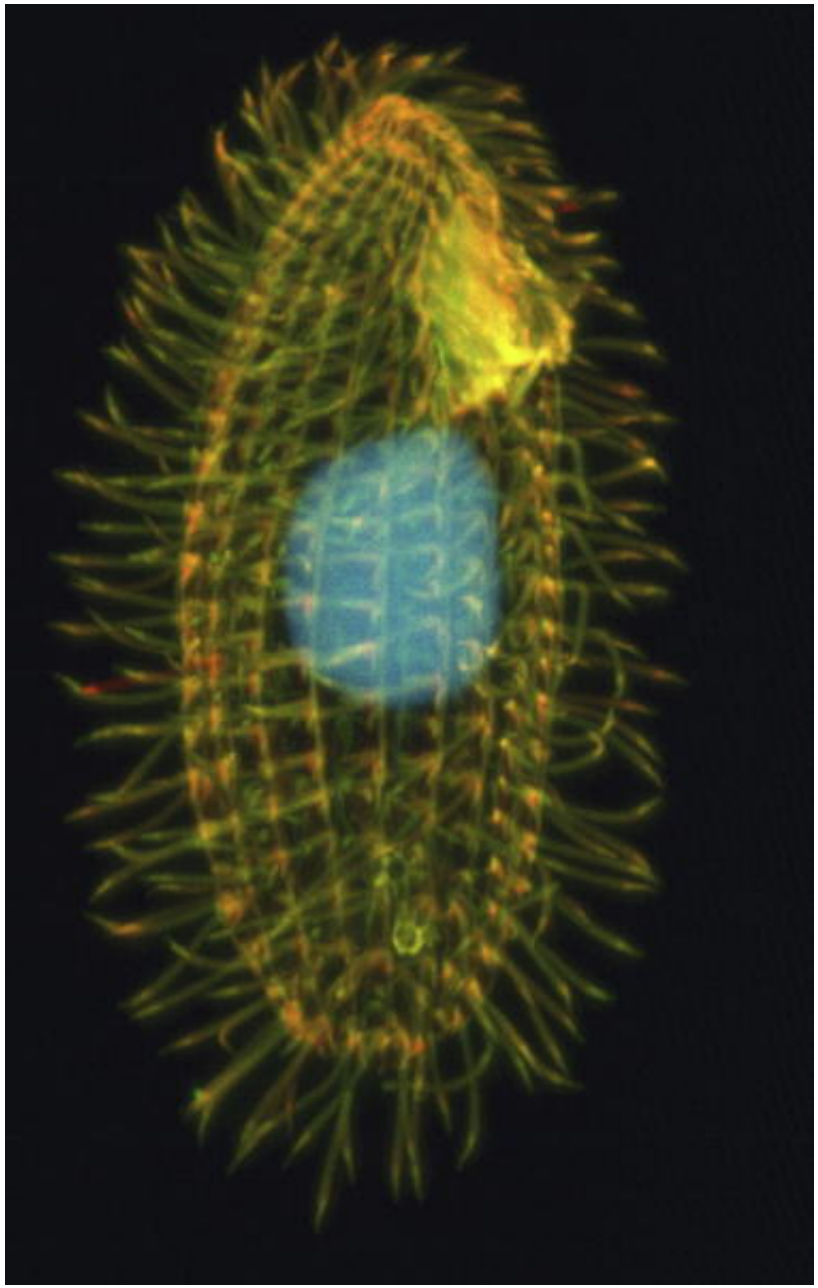
"El ratón es poco favorable para un estudio estructural. Es difícil descubrir las células de axón corto y ofrece una tendencia excesiva a dar macizos de fibras sin detalle de origen ni terminación. ¿Por qué no ha trabajado usted en el conejo de 20 a 40 días?"

El Cox (un método de tinción) me proporcionó magnifica arborización suelta de células de axón corto y multitud de detalles que no siempre se ven con el método de Golgi

Carta de Cajal a Rafael Lorente de Nó. Madrid 15 de Octubre de 1934

Santiago Ramón y Cajal (2006) José Mª López Piñero. Publicaciones Universidad de Valencia


Embryonic stem cell
Embryonic-stem-cell culture
Homologous recombination
Drug selection
Knockout
Homologous region
Exon
Homologous region
Cellular gene
loxP sequence
Targeting vector
Positive selection gene
Negative selection gene
Gene replacement
Cre-mediated recombination
Inactivated gene
Knock-in
Homologous region
Exon
Homologous region
Cellular gene
loxP sequence
Targeting vector
Positive selection
Negative selection
Gene replacement
Cre-mediated recombination
Knock-in gene
Injection of embryonic stem cells into host blastocyst
Implantation of chimeric blastocyst in foster mother
Chimeric offspring
Germ-line offspring



Importance of basic research

Telomeres and telomerase: the path from maize, Tetrahymena and yeast to human cancer and aging

Múller (1938) Fruit fly as an experimental model
McClintock (1939) Maize as an experimental model

Blackburn, Greider & Szostack (2006)
Nature Medicina 12:1333

Standards and Ethics

Proper compliance with ethical principles and animal welfare

In 1959 William Russell and Rex Burch, in his book "The Principles of Humane Animal experimental techniques", made the statement for the first time, that scientific excellence is strongly linked to the humanitarian use of laboratory animals. Define the rules under which ethical principles are based on animal research: the three "Rs": **Reduce, Replace and Refine**.

These are the foundations for a successful strategy to minimize animal use and everything that causes pain and suffering. Kramer M& Font E (2017) Biological Reviews 92:431-445.

European legislation: 2010/63/UE
Spanish legislation Real decreto 53/2013.

Animals used in experimental research in Spain

2009…….1.400.000 animals

2013…….920.000 animals

2014…….794.275 animals: 475.000 mice, 190.000 fish, 61.000 rats 35000 fowl, 24.000 rabbits, 765 dogs, 38 horses, etc..

2017…….. 793.000 animals

Pearls:

-These numbers do not include animals used in animal nutrition research

-These numbers are anecdotic when compared with the numbers of the food industry (30 millions of pigs in 2017)

Techniques to study transport

IN VIVO

Indicator dilution technique

Brain uptake index

Perfusion *In situ*

Infusion and autoradiography

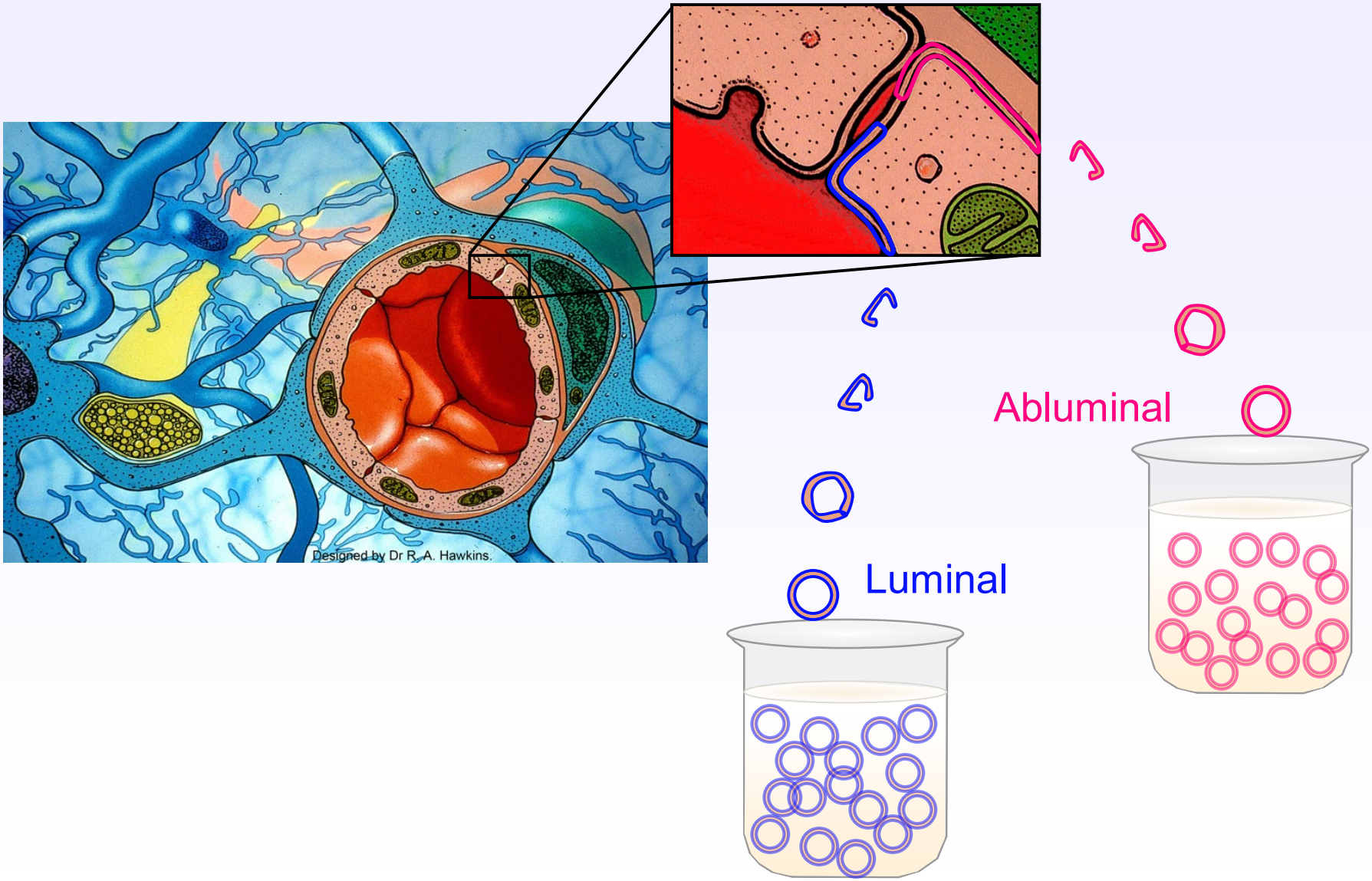
Imaging technology

IN VITRO

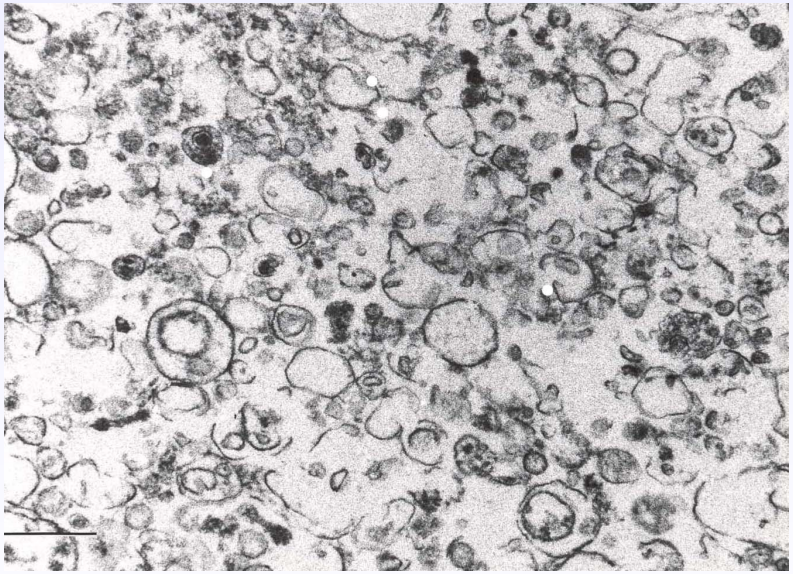
Endothelial cell co-culure with astrocytes

Isolated bovine brain microvessels

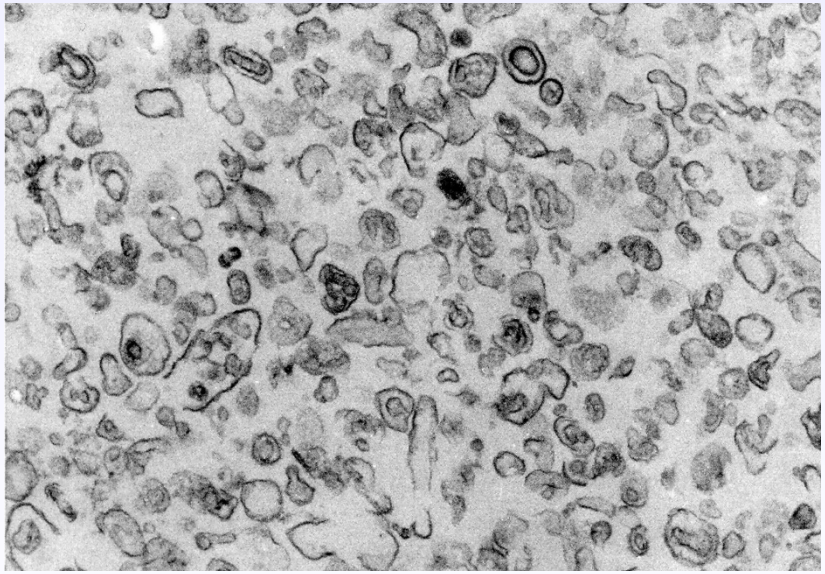
Isolated membrane vesicles


Designed by Dr R. A. Hawkins.
Abluminal
Luminal

Membrane vesicles after separation



Abluminal vesicles
Mean diameter = 125 ± 12 nm



Luminal vesicles
Mean diameter = 164 ± 15 nm

Rhesus monkeys as an experimental models. Beware of the small details

Ageing: Effect of caloric restriction (CR).

Restricting the intake of calories as a way to increase longevity and quality of life has been carried out for more than 500 years. The Venetian Luigi Cornaro, who was born in the 15th century, wrote “La vita sorba” where he described the importance of eating little to prolong life.

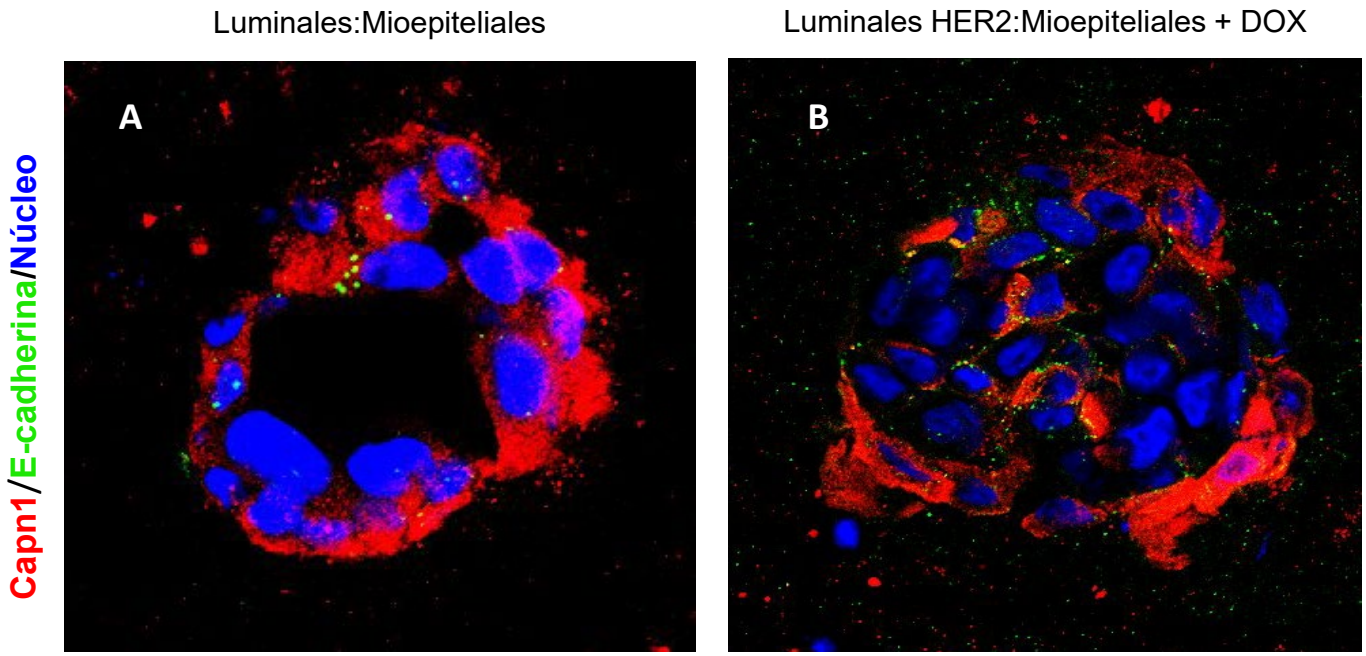
Experimental work has confirmed the success of this approach which was first investigated in a systematic way almost 100 years ago (McCay y colaboradores, 1935). It has been shown in different animals that continuous CR prolongs life by almost 40%. The impact is less the later CR is started.

Controversial results: Studies performed in rhesus monkeys. The Wisconsin National Prime Research Center (2009) CR prolongs life. The National Institute of Aging in Bethesda (2012) CR does not prolongs life., but improves health. Genetic and dietary composition and origin of the food matters.

Data from both UW and NIA studies support the concept that lower food intake in adult or advanced age is associated with improved survival in nonhuman primates (Mattisson et al 2017).

Alternative models to animals experiments

- Informatics
- Cell cultures
- 3D cell cultures
- Organ-on-chips
- Animal models and virtual reality



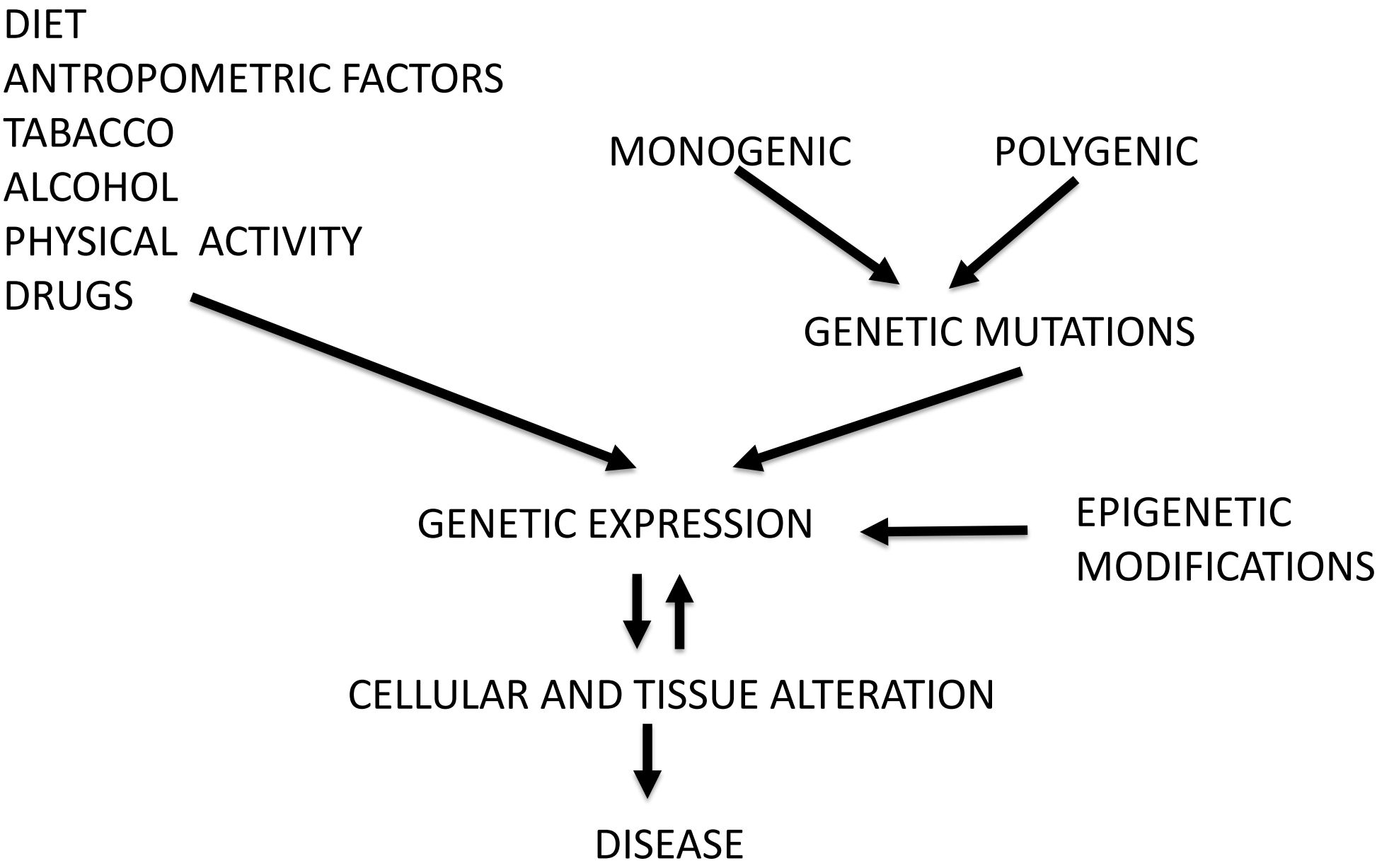
Estructuras de ductos de glándula mamaria obtenidos en cultivos 3D de colágeno.

A) Ductos de glándula mamaria control formados por la bicapa de células luminales y mioepiteliales. Los ductos fueron marcados con anticuerpos anti- calpaína 1 (rojo) y E-Cadherina (verde)
B) Ductos de glándula mamaria formados por células luminales en las cuales se ha inducido la sobreexpresión de HER2 por doxiciclina y células mioepiteliales control.

L. Rodríguez-Fernández L, Zaragozá R, Viña JR, resultados sin publicar.

Human studies

Genes and environment interrelationship



Evidence-based medicine → Precision medicine (PM)

Basic/Clinical studies
Clinical trials
Clinical guides
Protocols

Needs of individual patients on the basis of genetic, biomarker, phenotypic, or psychosocial characteristics that distinguish a given patient from other patients with similar clinical presentations.

The development of therapeutic agents that target molecular mechanisms is driving innovation in clinical-trial strategies

Evidence-based medicine: clinical trials


Sir Austin Bradford Hill

In 1946, Sir Austin Bradford Hill, from the Medial Research Council designed a controlled clinical trial for *Streptomycin in Tuberculosis Trials Committee*. Sixty years latter, the structure, the conditions and the analyses of the trials are basically similar.

Clinical trials

Phase I trials are the first stage of testing in human subjects. These trials are designed to assess **the safety, tolerability, pharmacokinetics, and pharmaco-dynamics of a drug**

Small group of **healthy volunteers** are included. In some circumstances real patients are used, such as patients who have cancer and lack treatment options.

Phase II are designed **to assess how the drug works, as well as to continue phase I safety assessments. This study designed to know dosing requirements and efficacy**. Some phase II are designed as case series and other are designed as randomized clinical trials, where a group of patients receive the drug and others receive placebo/standard treatment.

These trials are performed on larger groups (a few hundreds)

Clinical trials

Phase III studies are **randomized controlled multicenter trials**. Large patient groups and are used. This trial is aimed at being the **definitive assessment of how effective the drug is in com**parison with current gold standard treatment.

Large patient groups are used in these studies

Phase IV trials are designed as post marketing surveillance

Are changes needed in clinical trials?

There is a need of reform of all the aspects of clinical trials

Richard Smith (Ex-Director del BMJ)

Do clinical trials work?
The New YorK Times /Sunday Review July 2013

A Cancer Conundrum: Too Many Drug Trials, Too Few Patients. The New York Times. By Gina Kolata. August 12, 2017

Are changes needed in clinical trials?

Do clinical trials work? Absolutely

Can they be done better? Yes

- For subtypes of diseases that are already known, it may be feasible to design small clinical trials and enroll only those who have the appropriate genetic or molecular signature.

Example: Herceptin (Trastuzumab) useful to tumors that express HER2.

Dr. Dennis Slamon. 1998

Time for increased emphasis on gender medicine.

Biankin AV et al. (2015) Nature 526: 361-370.

Treatment for oncologic diseases: Role of biochemistry, molecular biology and immunology

- Surgery
- Radiotherapy
- Conventional Chemotherapy
- Precision medicine:
  - Problems: Cancer can affect different pathways, suppression genes are inhibited, it can block essential pathways, resistance, etc..
- Immunotherapy:
  - No selective, but I does not mean no efficient, and independent of the mutation

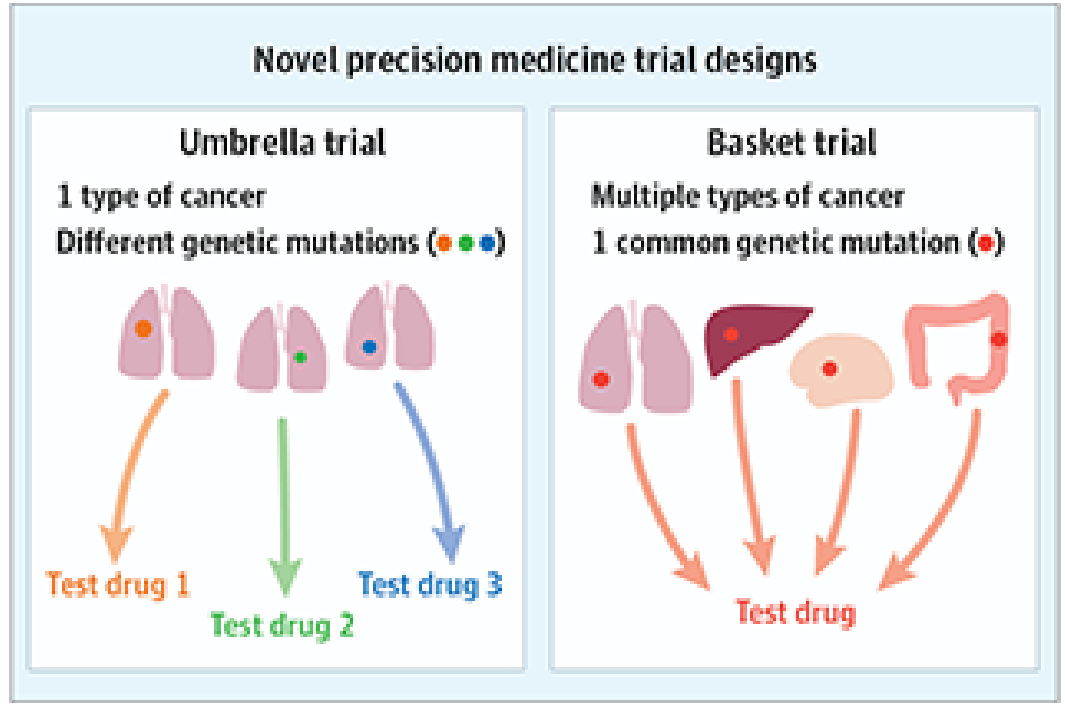
Baskets and Umbrellas: Trial Design for Precision Oncology

December 9, 2016 | An article published online on *JAMA Oncology's* Patient Page yesterday proposes two types of clinical trial for testing precision medicine treatments of oncology.

As we shift from categorizing cancers by their tissue of origin to their driver mutations, new types of trials are required. These subgroups of patients are small, though, making clinical trials challenging.

"Two new and very different trial designs for molecular targets in cancer care are **umbrella trials** and **basket trials**," West writes. Umbrella trials take patients with the same type of cancer and assign them to different arms of a study based on their mutations. Basket trials group patients by mutation, regardless of which organs are involved.. JAMA Oncology

Baskets and Umbrellas: Trial Design for Precision Oncology



JAMA Oncology: doi:10.1001/jamaoncol.2016.5299

Precision medicine: Time for increased emphasis on gender medicine

- In 2014, Francis Collins, Director of the National Institutes of Health (NIH), and Janine Clayton, Director of the Office of Research on Women Health (ORWH) at NIH, announced that NIH would begin requiring all preclinical grant proposals to address sex a biological variable. (Clayton & Collins (2014) Nature 509; 282-283).

- Similar guidelines are imposed by The European Commission and Canadian Institutes of Health Research

Historia de un viaje de ida y vuelta

Multicenter, randomized, double-blind, placebo-controlled primary prevention trial (CARET 1996).

18314 smokers, former smokers, and workers exposed to asbestos. The effects of a combination of 30 mg of beta carotene per day and 25,000 IU of retinol on the primary end point, the incidence of lung cancer, were compared with those of placebo.

After 4 years, the supplementation had no benefit and may have had an adverse effect on the incidence of lung cancer and on the risk of death from lung cancer, cardiovascular disease and any cause in these subjects.

In January 11, 1996, the trial was stopped.

INCLIVA: En el 2109 activos 432 ensayos clínicos

Phase I	45
Phase II	84
Phase III	176
Phase IV	21
Observational	100
Other	6

INCLIVA: En el 2109 activos 432 ensayos clínicos

Allergy	1
Anesthesia and Reanimation	14
Cardiology	40
Dermatology	4
Digestive Medicine	29
Endocrinology	5
General Surgery	2
Gynecology and Obstetrics	6
Haematology	77
Infectious Diseases unit	6
Internal Medicine	13
Medical Emergency Unit	1
Medical Oncology	171
Nefrology	8
Neurology	27
Otorhinolaryngology	2
Pediatrics	5
Pharmacy	2
Primary Health Care	10
Psychiatry	1
Respiratory Diseases	4
Radiotherapy	4
TOTAL	432

INCLIVA: Iniciados por año

	2016	2017	2018	2019
FASE 0	0	0	2	0
FASE I	17	13	20	8
FASE II	27	31	25	34
FASE III	49	46	58	27
FASE IV	7	6	4	4
OBSERVACIONALES	42	43	46	26
OTROS DISEÑOS	4	4	2	8

Modern science needs plans to enhance reproductibility

- Reproducibility, rigour, transparency and independent verification are cornerstone of the scientific methods

Manrai et al (2016) Pac Symp Biocomput 21: 180
Collins & Tabak (2014) Nature 505:612
McNutt (2014) Science 343:229
Dominguez N (2017) 10 de Enero EL PAÍS

La mitad de los ensayos clínicos de la UE incumple la ley de publicar sus resultados. EL PAIS 13 Septiembre 2018

- El Registro de ensayos Clínicos de la UE tiene identificados más de 33.000 ensayos clínicos en todo el territorio, la segunda mayor base de datos del mundo en este campo. De los 7.274 que ya deberían haber publicado sus resultados, solo el 49% lo ha hecho

- Goldacre B et al (2018) BMJ 362:K3218.

La AEMPS (Agencia Española de Medicamentos y Productos Sanitarios) durante el periodo 2005-2017 ha autorizado 9267 ensayos clínicos. Más del 75% de los ensayos autorizados fueron financiados por al industria farmacéutica. El resto fueron ensayos no comerciales

Future: The research units

The half-life of a good research team

Starzl T. (1994) El hombre puzzle. Memorias de un cirujano de transplantes

Future: The practice of medicine

In the future, medicine is of those that are well trained in molecular medicine, understand the basis of human behavior and know how to keep the right balance along hesitancies of life