



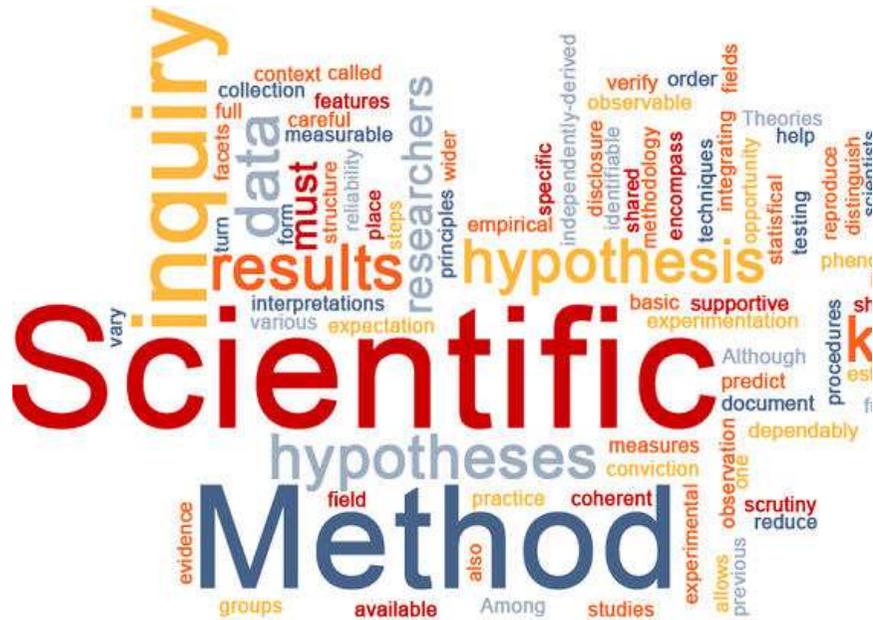
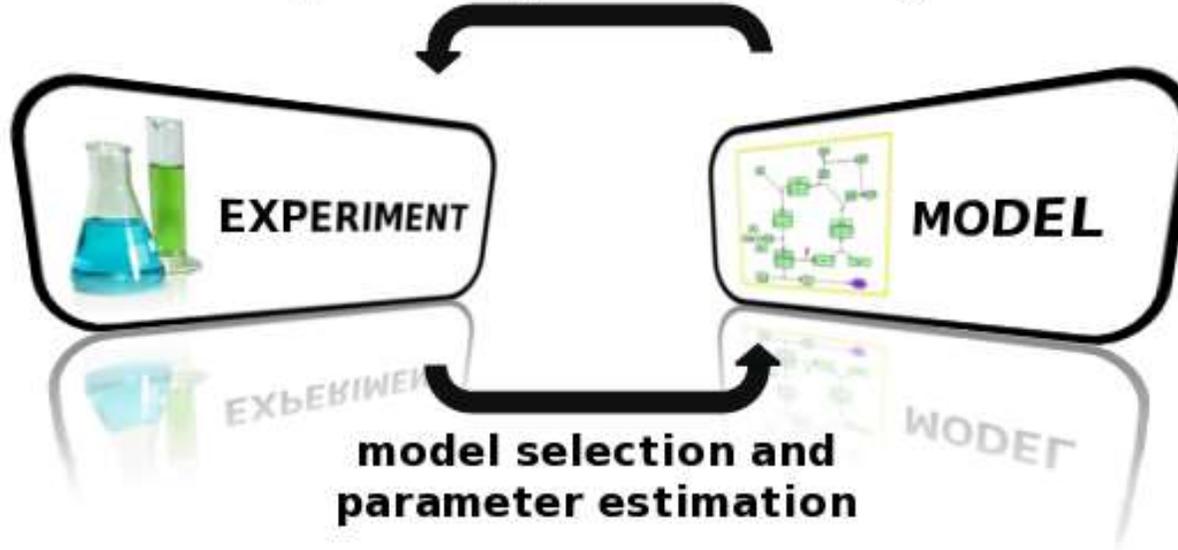
LA SCELTA DI SPECIE A MINOR IMPATTO

VITTORIA RAFFA
DIPARTIMENTO DI BIOLOGIA
VITTORIA.RAFFA@UNIFI.IT



26/11/2020

optimal experimental design



DIRECTIVE 2010/63/EU OF THE
EUROPEAN PARLIAMENT AND OF THE
COUNCIL OF 22 SEPTEMBER 2010 ON THE
PROTECTION OF ANIMALS USED FOR SCIENTIFIC
PURPOSES

- (10) While it is desirable to replace the use of live animals in procedures by other methods not entailing the use of live animals, **the use of live animals continues to be necessary** to protect human and animal health and the environment.
- (12) **Animals have an intrinsic value** which must be respected.



DIRECTIVE 2010/63/EU OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL OF 22 SEPTEMBER 2010 ON THE PROTECTION OF ANIMALS USED FOR SCIENTIFIC PURPOSES

- (13) The choice of methods should therefore ensure the selection of the **method** that is able to provide **the most satisfactory results and is likely to cause the minimum pain, suffering or distress.** The methods selected should use the minimum number of animals that would provide reliable results and require **the use of species with the lowest capacity to experience pain, suffering, distress or lasting harm** that are optimal for extrapolation into target species.

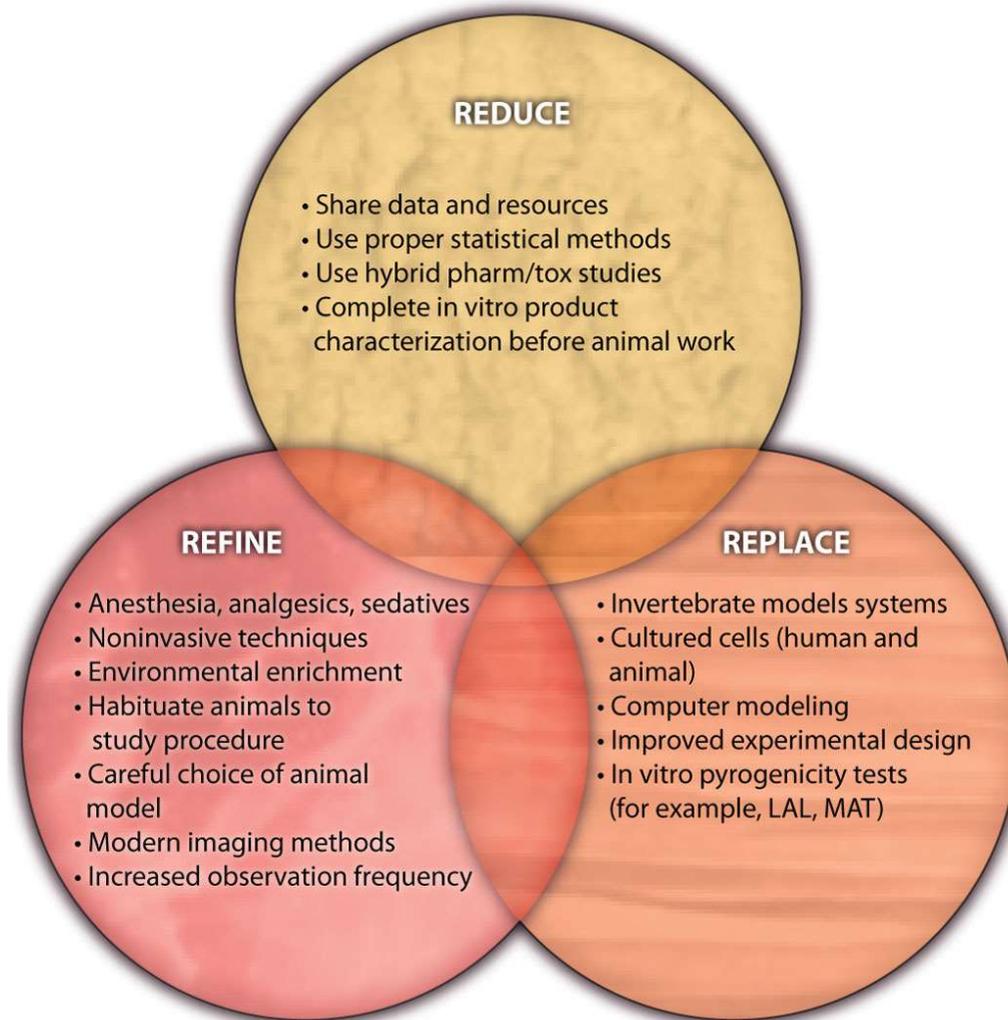


DIRECTIVE 2010/63/EU OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL OF 22 SEPTEMBER 2010 ON THE PROTECTION OF ANIMALS USED FOR SCIENTIFIC PURPOSES

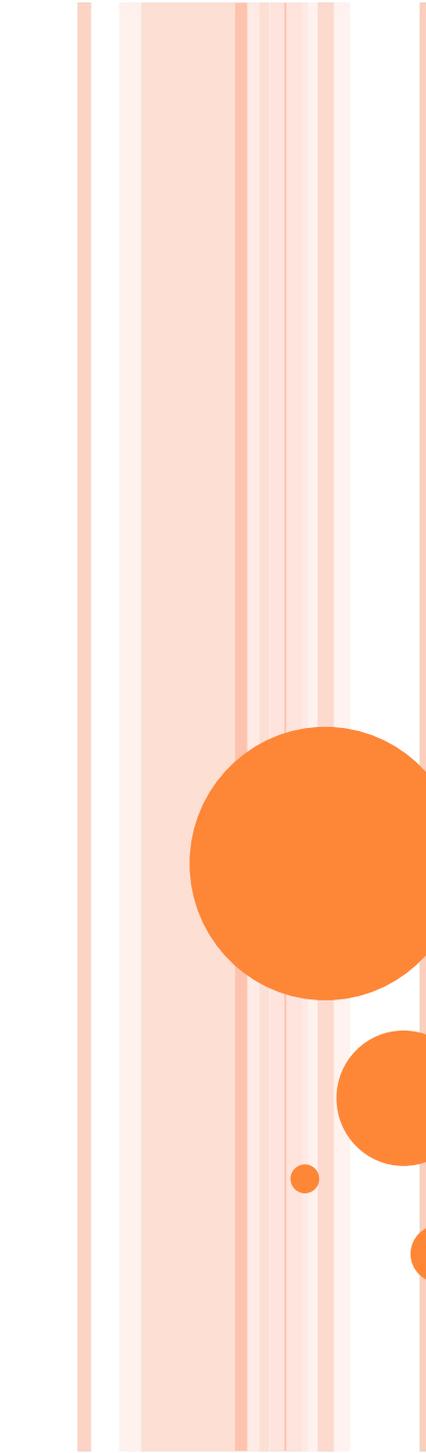
- (11) When choosing methods, **the principles of replacement, reduction and refinement** should be implemented through a strict hierarchy of the requirement to use alternative methods. Where no alternative method is recognised by the legislation of the Union, the numbers of animals used may be reduced by resorting to other methods and by implementing testing strategies, such as the use of in vitro and other methods that would reduce and refine the use of animals.



Fig. 1. Regenerative regulation.



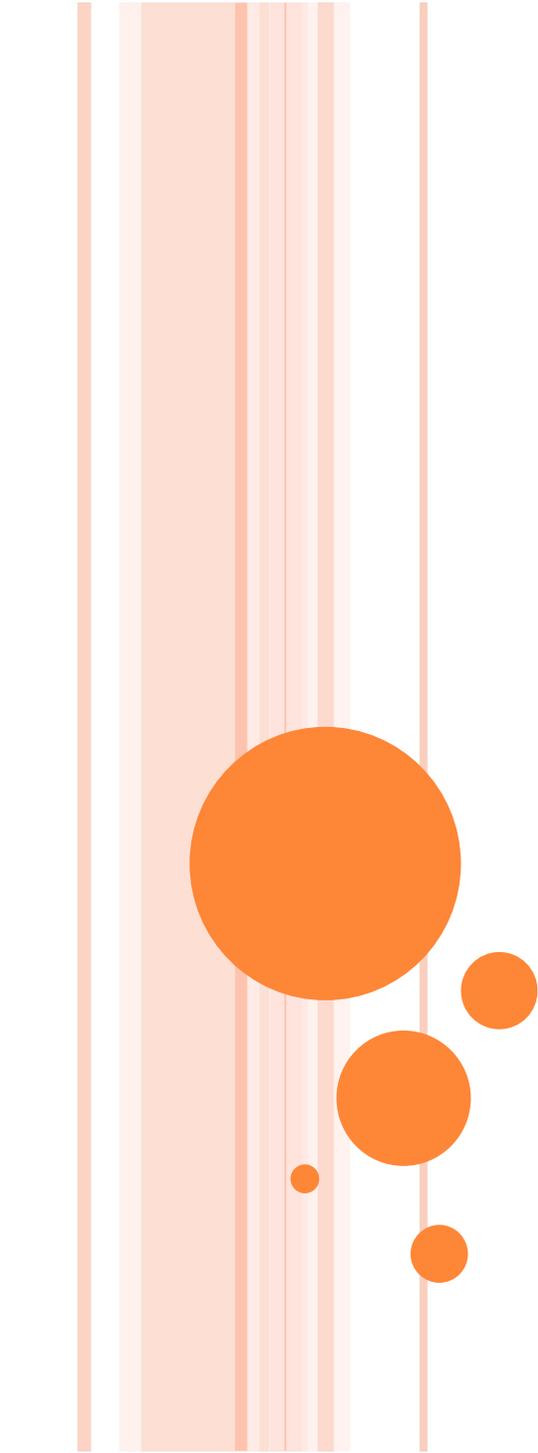
Rebecca Robinson Sci Transl Med 2011;3:112fs11



**IT IS IN SCIENTISTS' INTEREST TO
ADOPT AN ETHICAL AND HUMANE
APPROACH TO HUSBANDRY AND
EXPERIMENTAL DESIGN, AS HEALTHY
ANIMALS PRODUCE ROBUST,
RELIABLE RESULTS, UNDERLYING
VALID SCIENTIFIC OUTPUTS**

**For example, improved husbandry and handling
of rodents reduces stress and this leads to less-
variable data and more meaningful results**

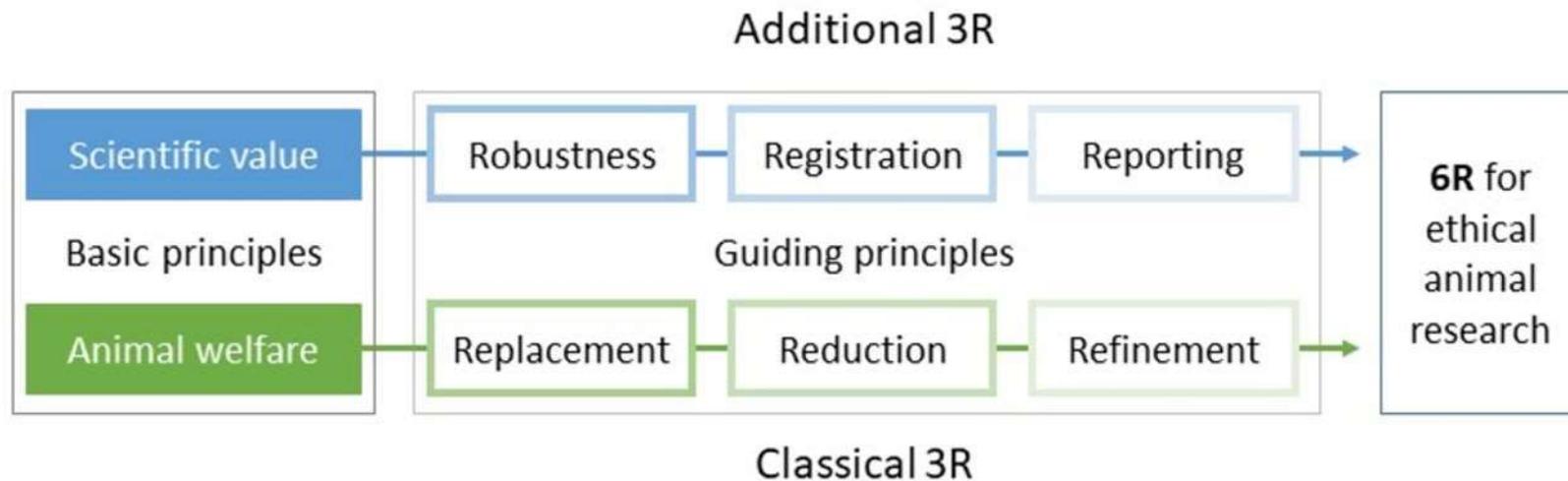
10.1136/bmjos-2018-000048



**RESEARCH ON ANIMALS IS ONLY
ETHICAL IF IT GENERATES VALUE
FOR SCIENCE AND SOCIETY**

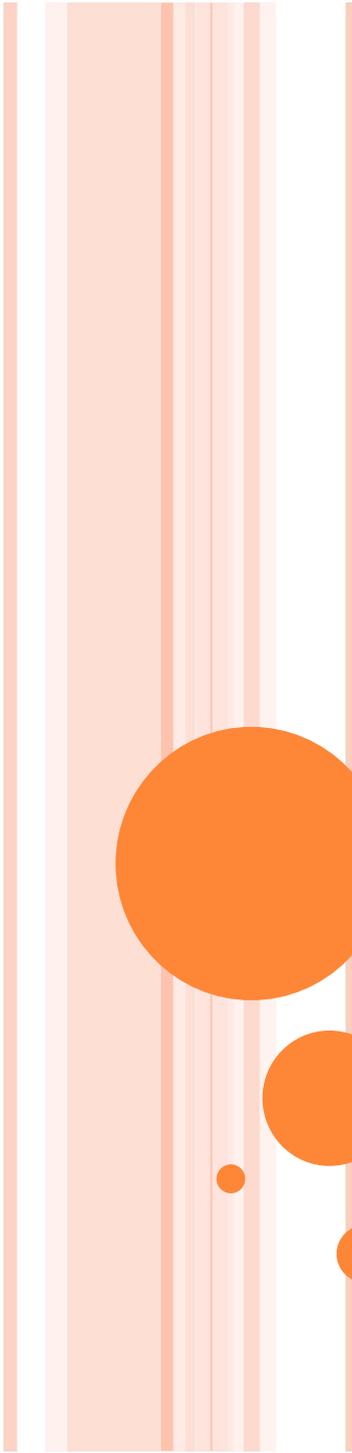
10.1136/bmjos-2018-000048

Two basic principles for animal research ethics translate into six practice-guiding principles (6R).



Daniel Strech, and Ulrich Dirnagl *BMJ Open Science*
2019;3:bmjos-2018-000048





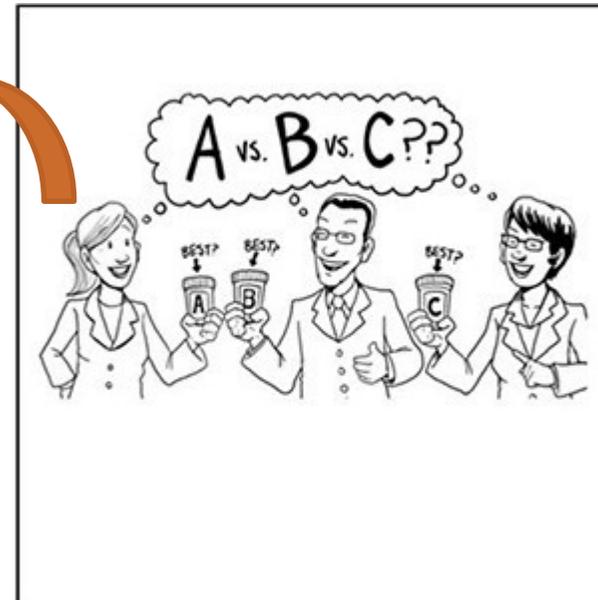
REPLACING PROTECTED ANIMALS WITH

- COMPUTER MODELLING APPROACHES**
- CELL LINES**
- PRIMARY CELLS**
- TISSUES**
- LESS SENTIENT FORMS OR SPECIES**

Robustness, scientific validity



Publical acceptability

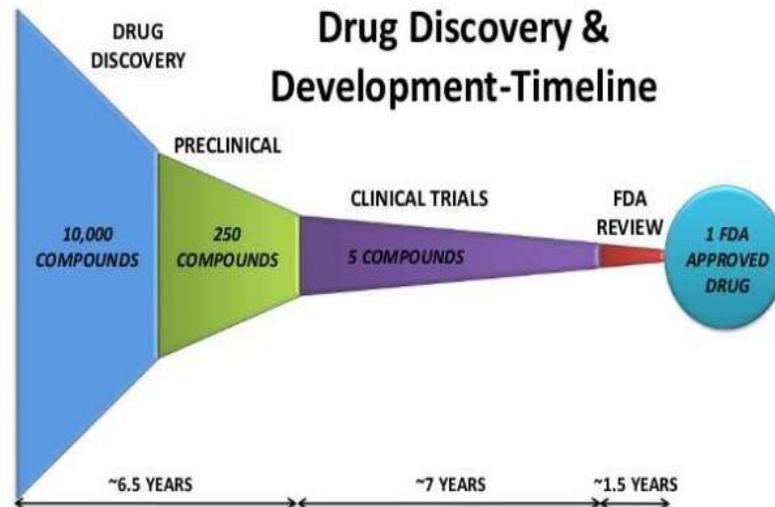
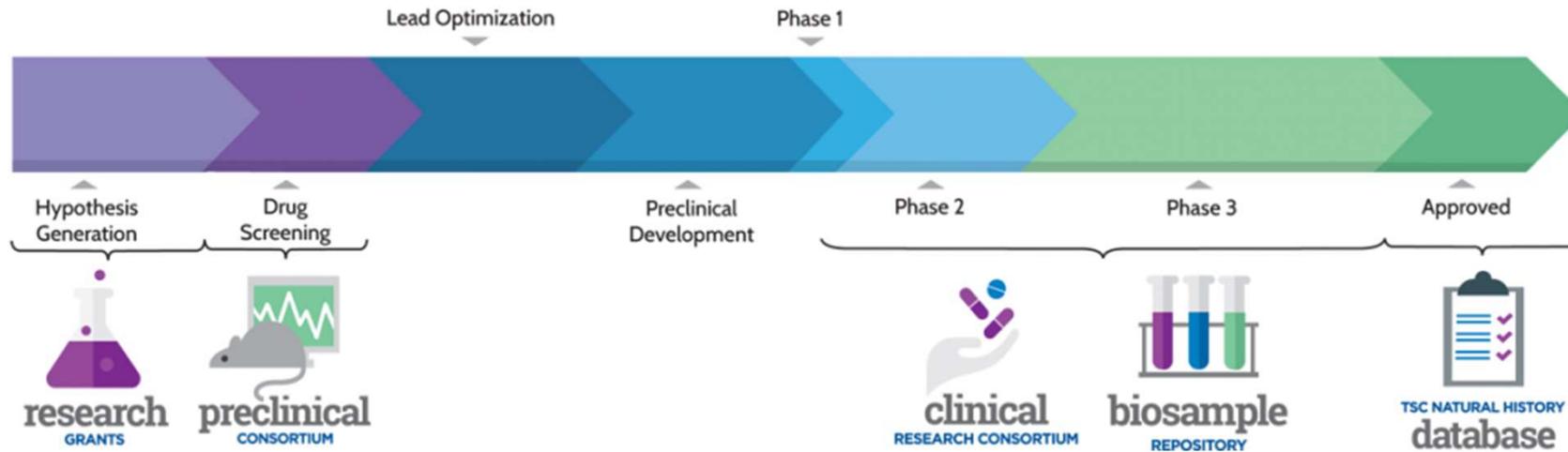


No stress, no distress

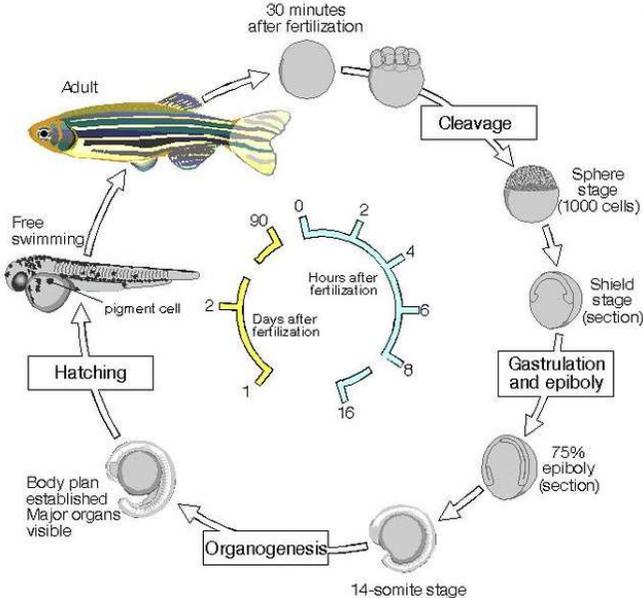


Knowledge, information, education

PRE-CLINICAL RESEARCH



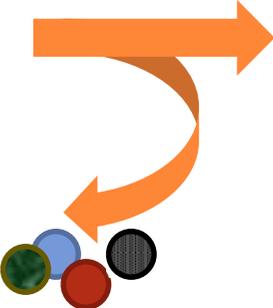
ZEBRAFISH PRE-SCREENING



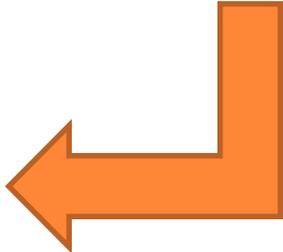
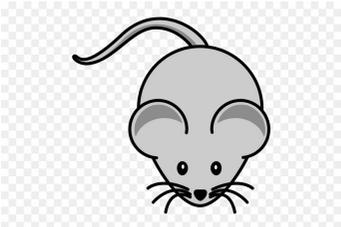
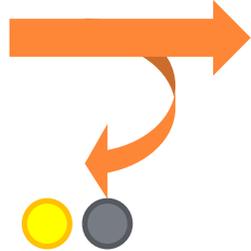
Preliminary Toxicity



Preliminary Efficacy



Preliminary biodistribution





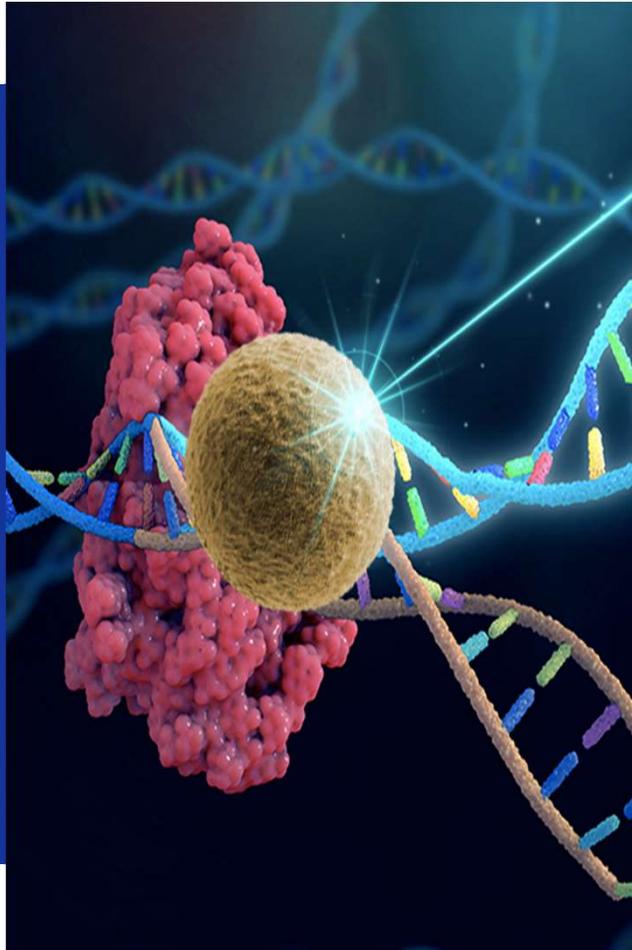
This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 862714.

I-GENE

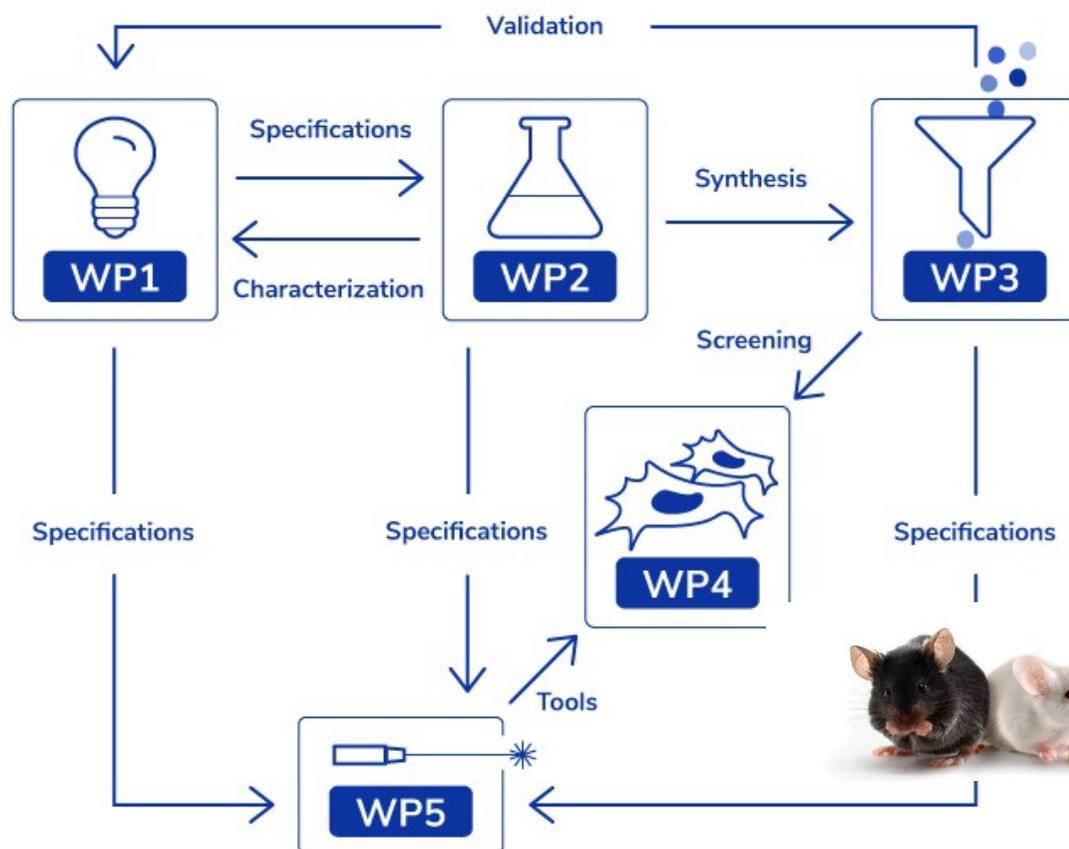
IN-VIVO GENE EDITING BY NANOTRANSDUCERS



**A light-activable
nanoformulation
of Cas9
(PCT/IB2020/050432)**



WORKPLAN



MiO
MELANOMA
ITALIA ONLUS

CAPIRE IL MELANOMA ✓

COSA FACCIAMO



Scrivi a
CLAUDIA PIAZZA CIDONIO

Contatta i nostri PATIENT ADVOCATES

Siamo qui per te, nel momento in cui hai bisogno di risposte.
È gratuito e 100% condizionale.

ADVANTAGES OF ZEBRAFISH AS *IN VIVO* ANIMAL MODEL

Easy maintenance

Reduced adult size
Cheap maintenance

Easy manipulation of adult and embryos

Rapid development

embryo pigmentation is already evident after 2 days post-fertilization, mainly due to the activity of tyrosinase enzyme

Easy check of gene-editing activity

Selecting *tyrosinase* gene as the target, the successful gene-editing may be evidenced by phenotypical observation of embryo depigmentation
(Jao et al., PNAS 2013)

High fecundity

Crossing a male and a female,
100-200 embryos may be obtained

External fertilization

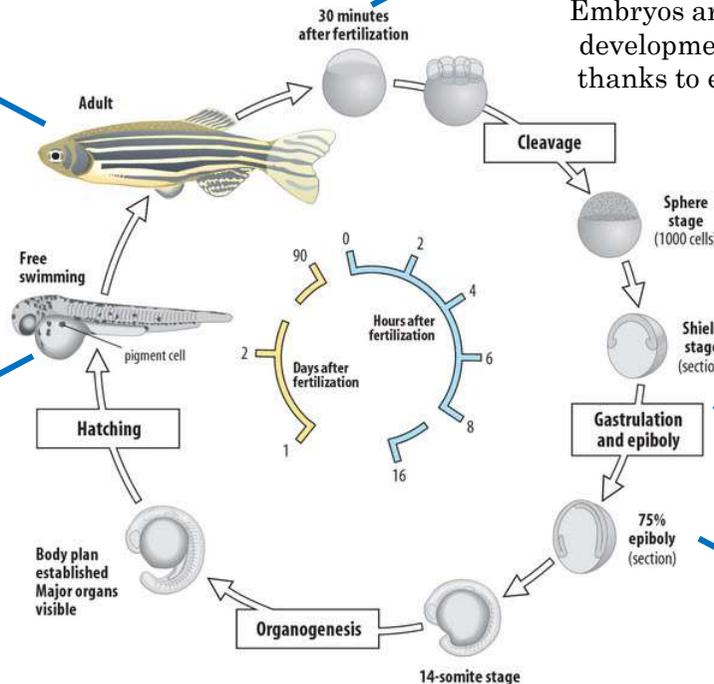
Embryos are easily collected and development may be visualized thanks to embryo transparency

Efficient delivery of CRISPR/Cas9 system

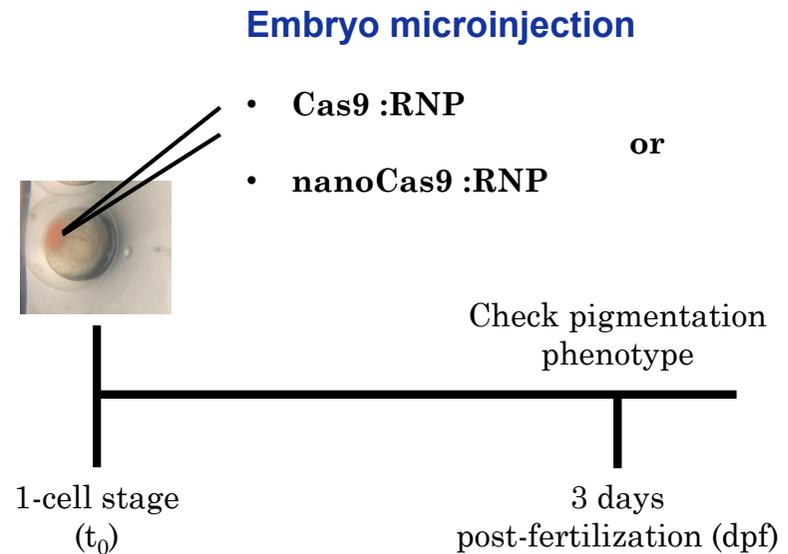
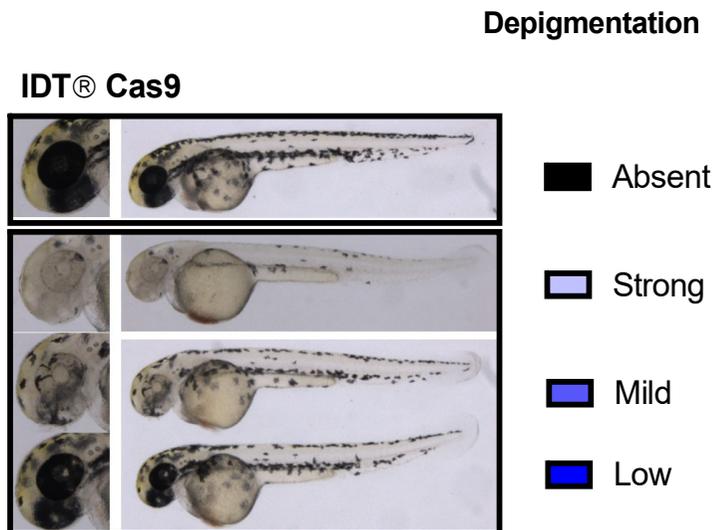
Cas9/gRNA system can be efficiently transferred to 1-cell embryo by microinjection

Low ethical impact

Up to 5 days post-fertilization, zebrafish embryos are classified as not sentient, according to the Directive 2010/63/EU.



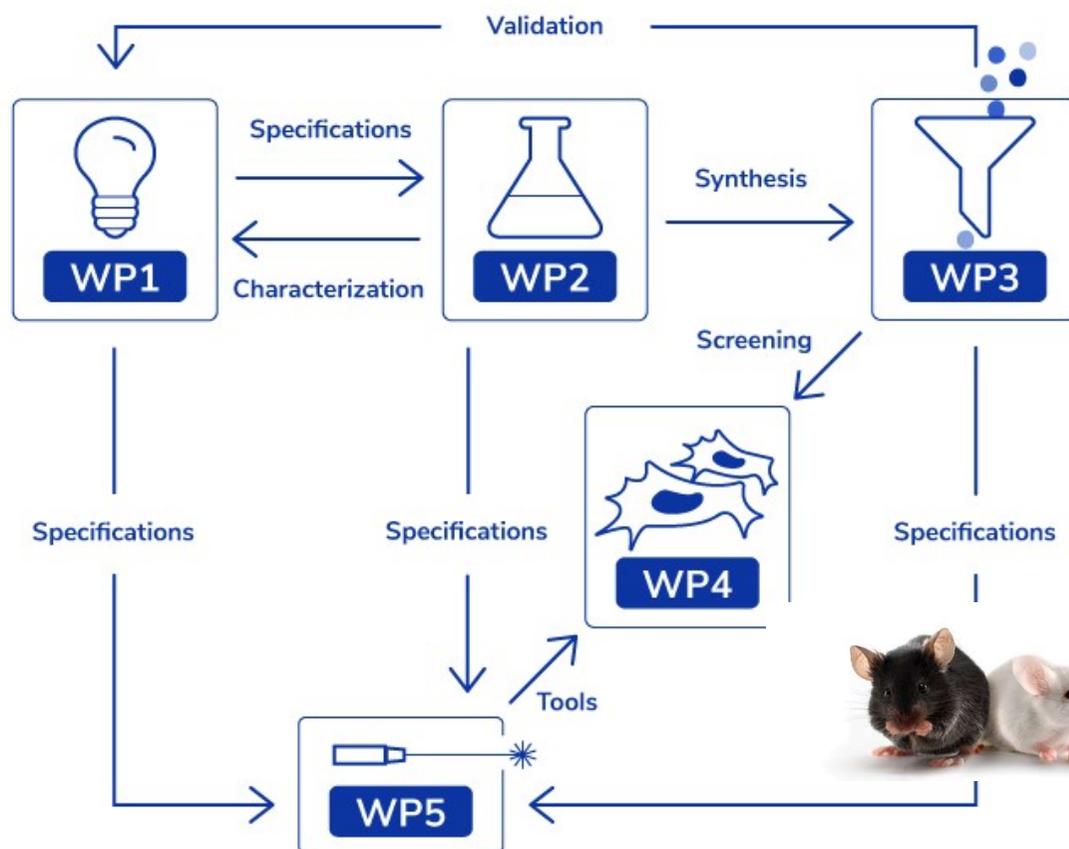
Zebrafish for gene-editing validation



Images of depigmentation phenotypes of 3dpf zebrafish embryos after editing.



WORKPLAN



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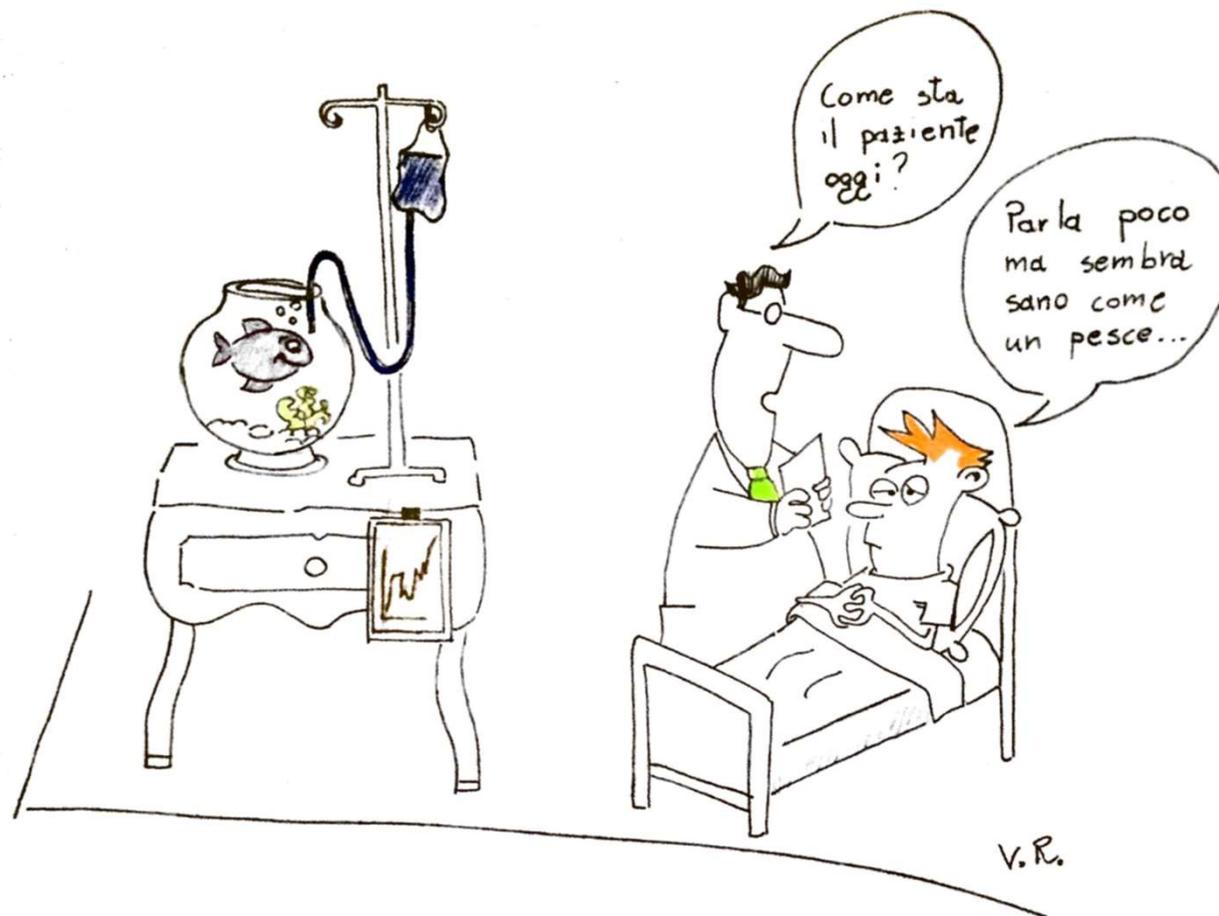


Scrivi a
CLAUDIA PIAZZA CIDONIO

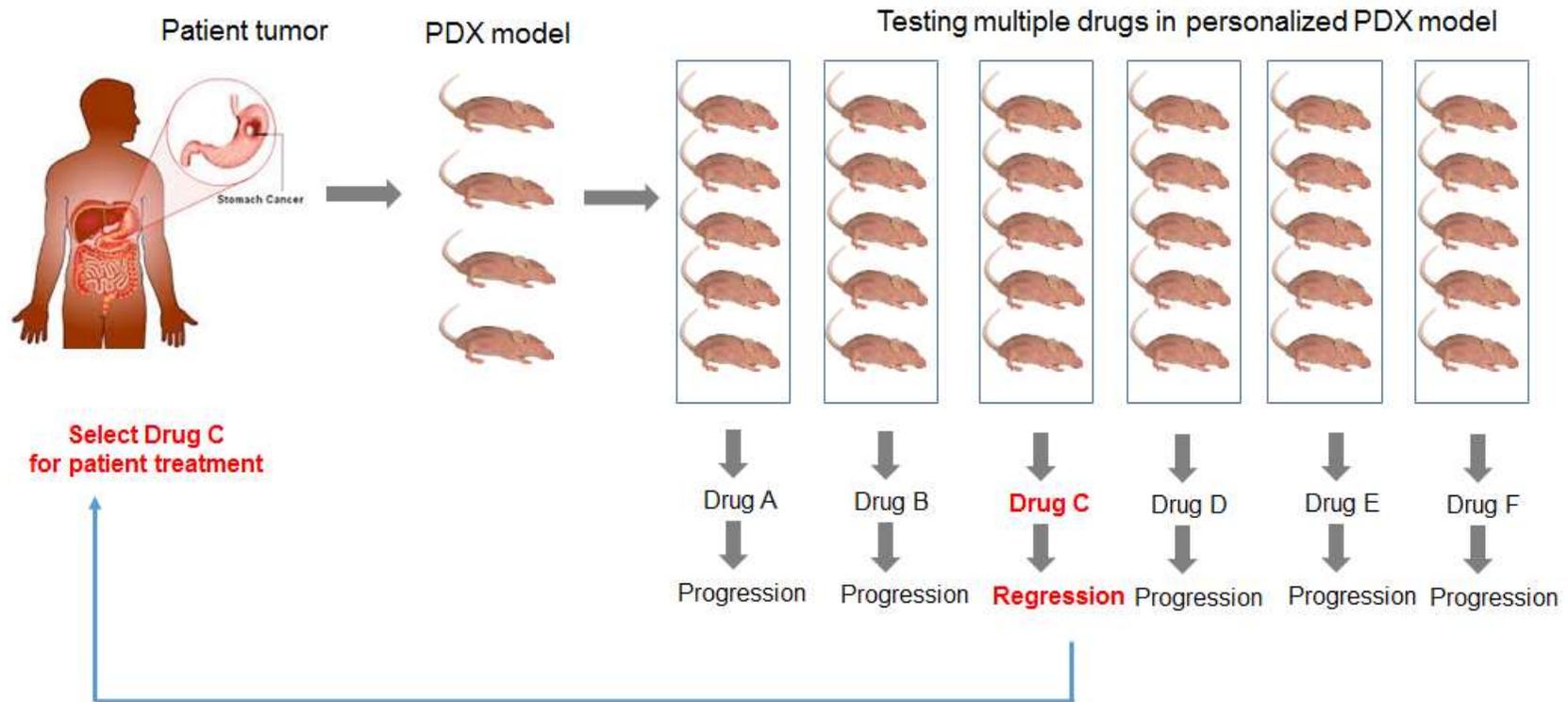
Contatta i nostri PATIENT ADVOCATES

Siamo qui per te, nel momento in cui hai bisogno di risposte.
È gratuito e 100% condizionale.

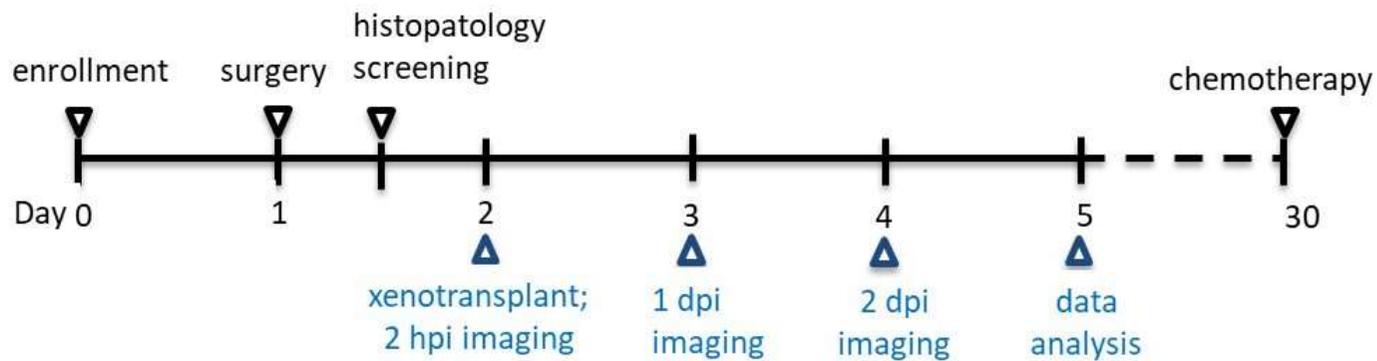
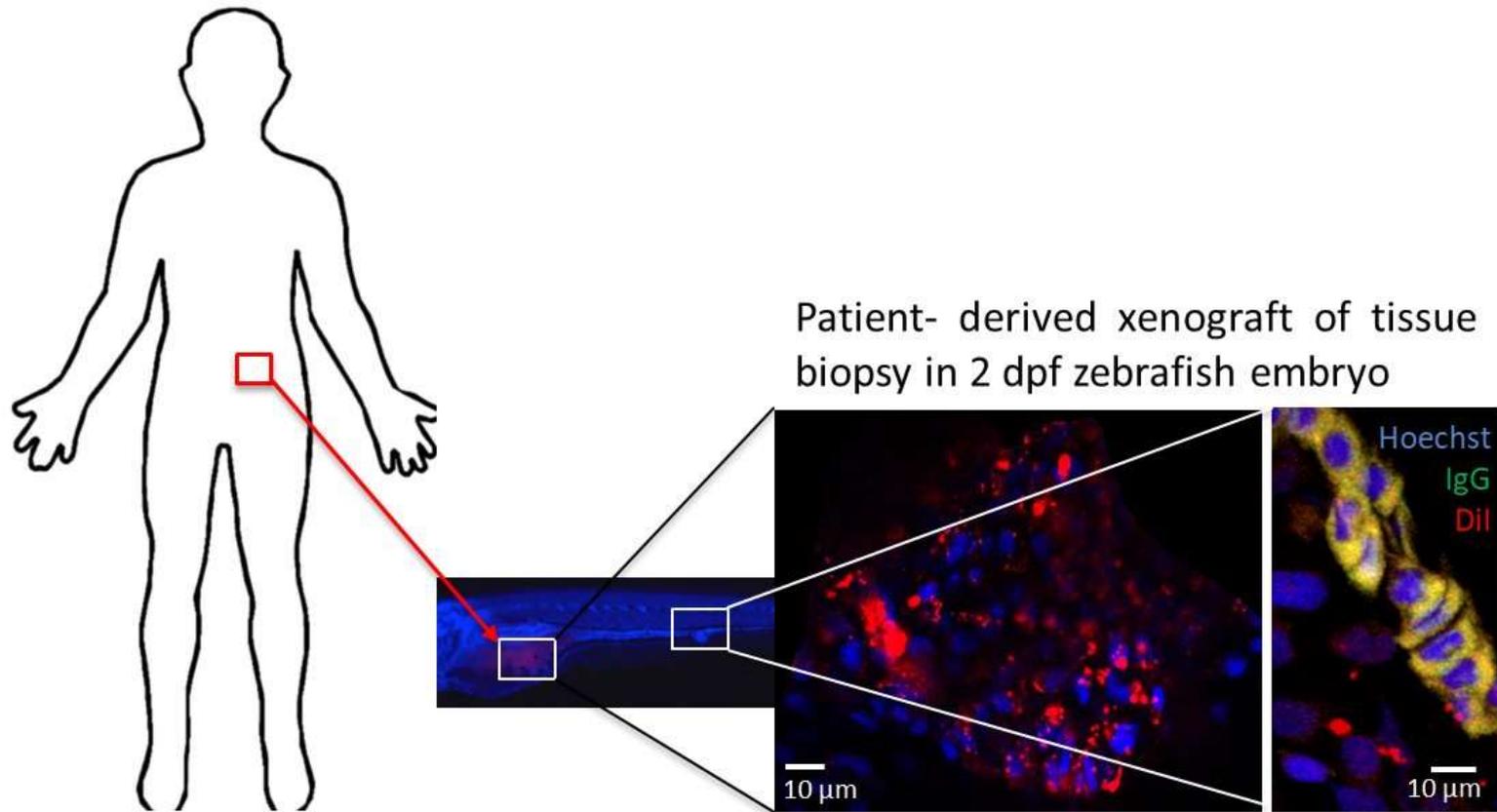
XENOTRAPIANTO DI CELLULE TUMORALI PRIMARIE IN EMBRIONI DI ZEBRAFISH: NUOVO APPROCCIO DI MEDICINA PERSONALIZZATA



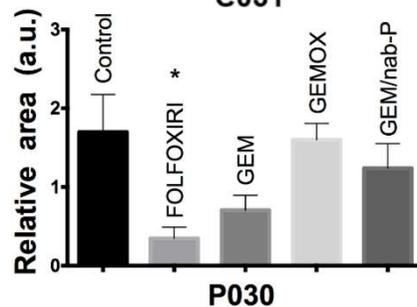
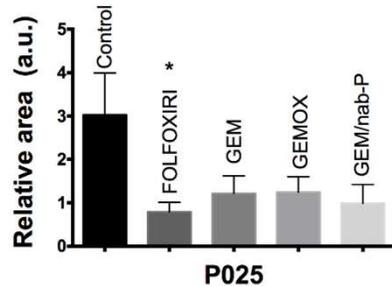
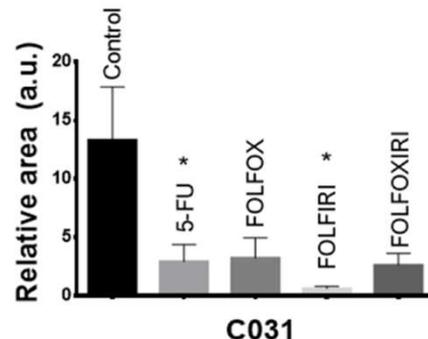
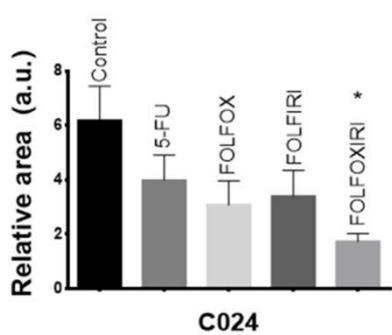
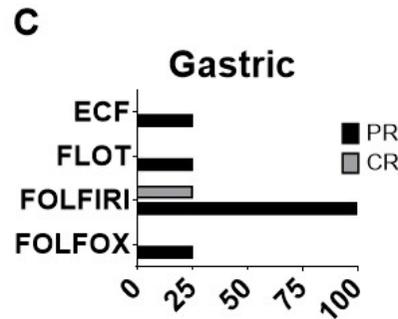
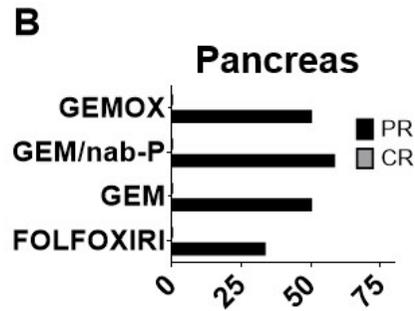
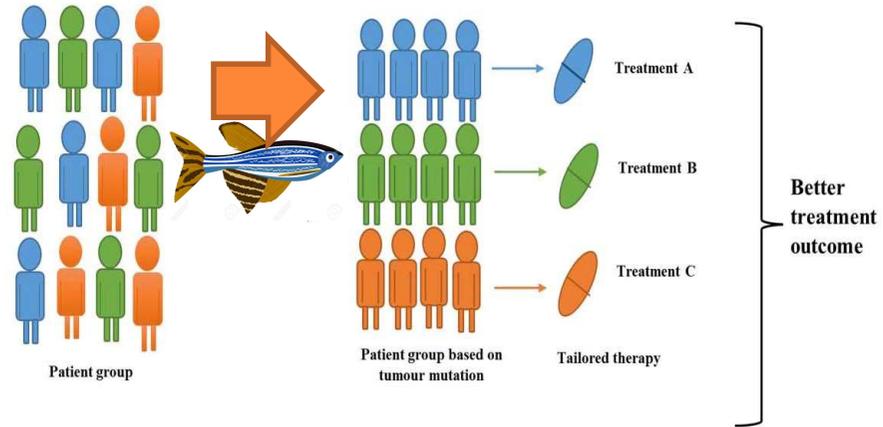
MOUSE AVATAR



ZEBRAFISH & HUMAN CO-TRIAL



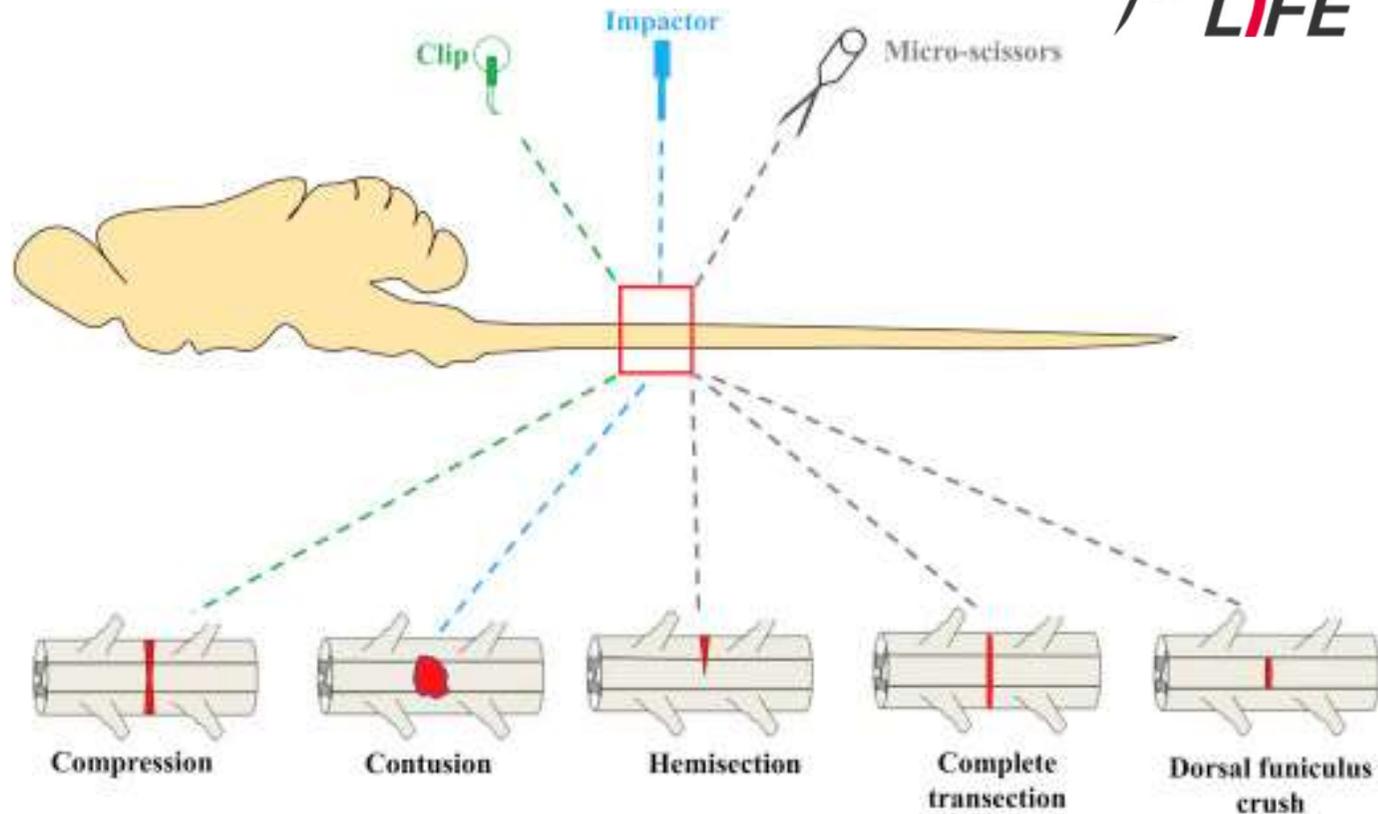
A MODEL OF A ZEBRAFISH AVATAR FOR CO-CLINICAL TRIALS



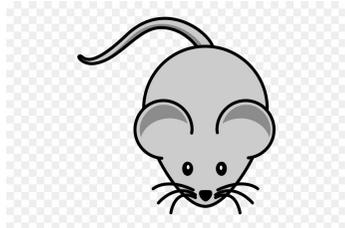
TOP: percentage of partial response (PR) and complete response (CR). Zebrafish avatars xenotransplanted with colon tumor (A); pancreas (B); gastric tumor (C).

BOTTOM: chemosensitivity assay. Two dpf embryos were injected with fragments of patient tumor tissue and incubated for 48 h with chemotherapy compounds. Representative cases of colon cancer (C024 and C031 patient-derived xenograft (PDX)) and pancreatic cancer (P025 and P030 PDX) with quantitative analysis of the relative tumor area (2 dpi/2 hpi for colon and 2 dpi/1 dpi for pancreas). Mean \pm SEM and analyzed by 1-way ANOVA followed by Dunnett's multiple comparisons test. * $p < 0.05$.

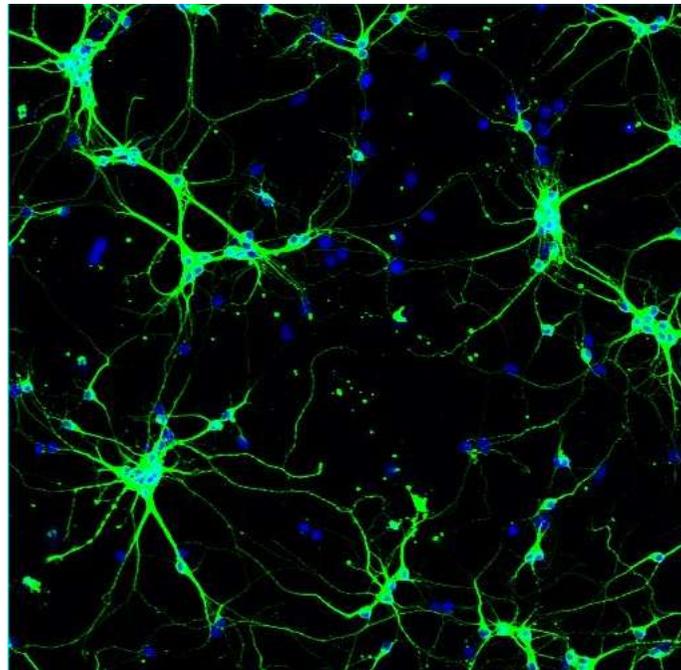
Mechanotransduction of neurons: a future strategy for the regeneration of spinal cord lesions?



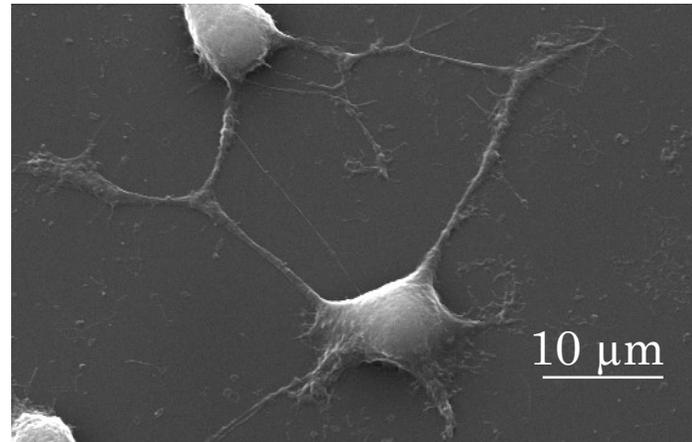
IN VITRO REGENERATION MODEL



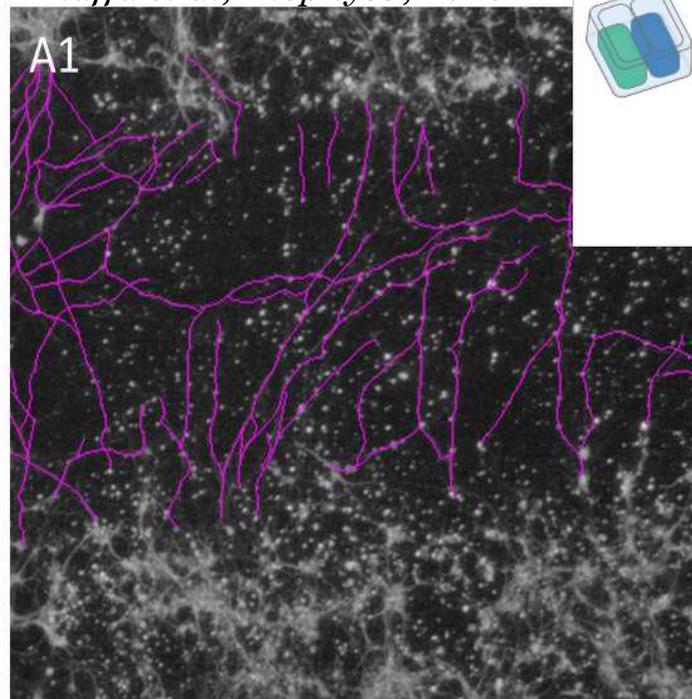
PC12 cell line



Hippocampal neurons



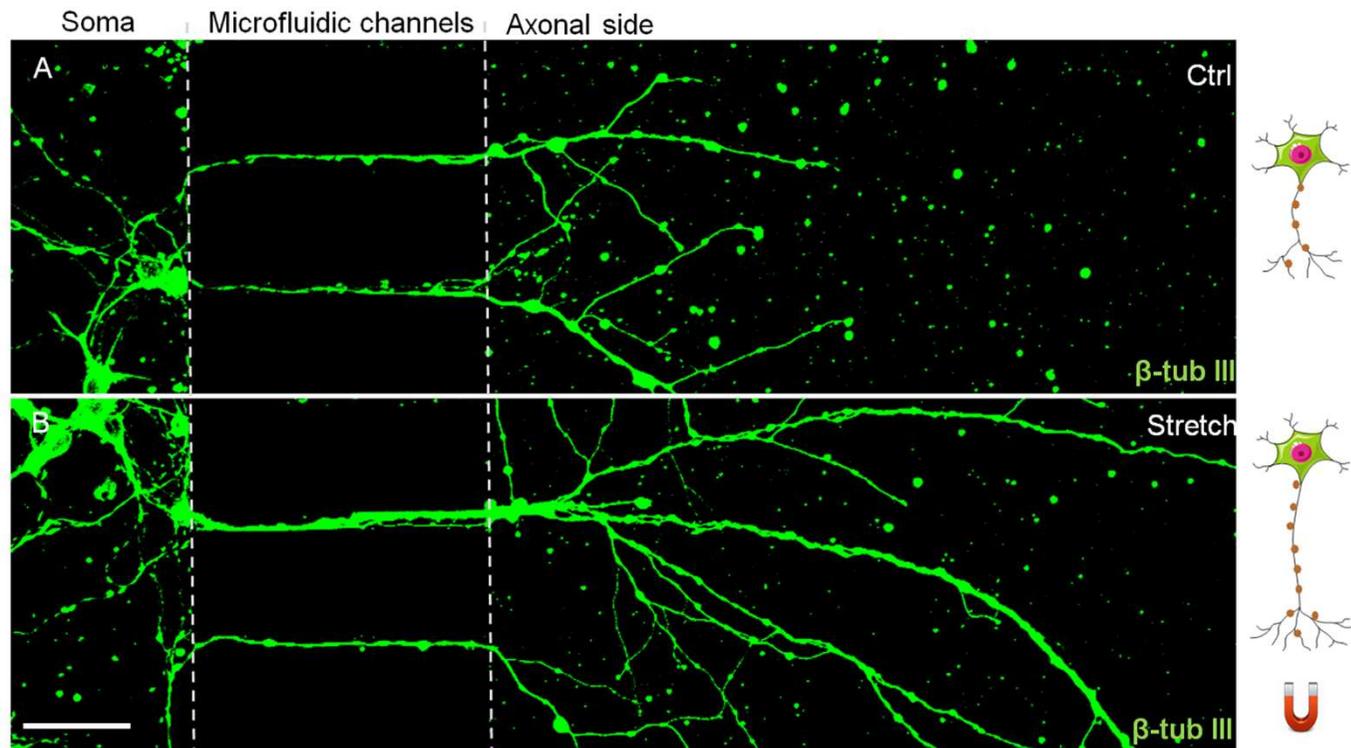
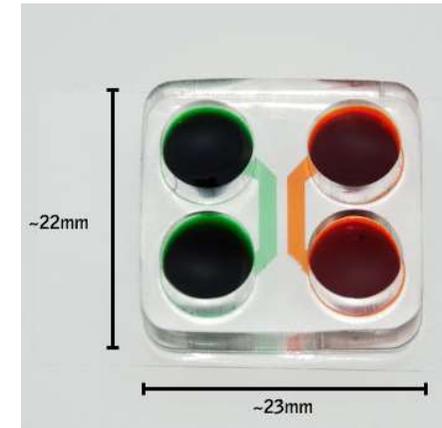
Raffa et al, Nanomedicine 2014
Raffa et al, BiophysJ, 2018



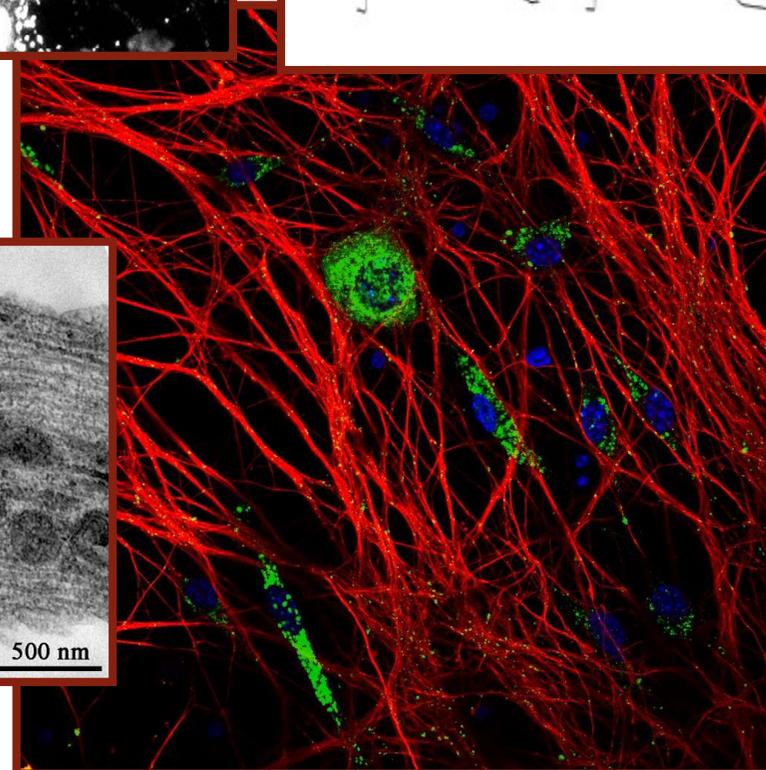
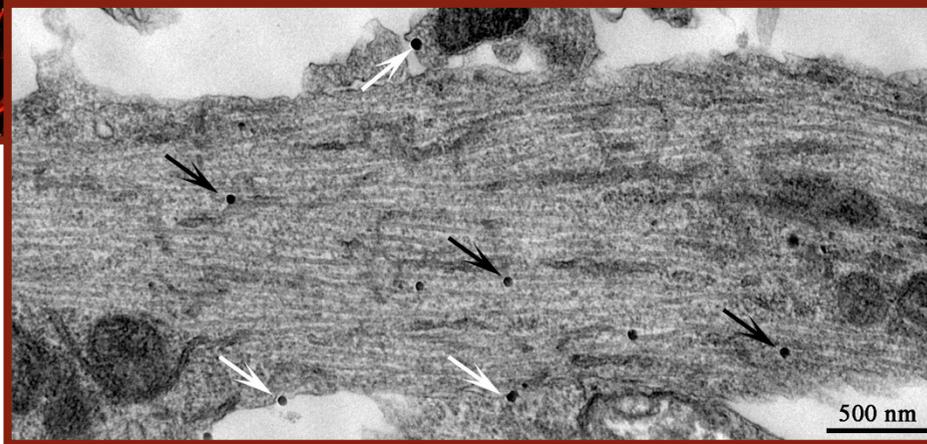
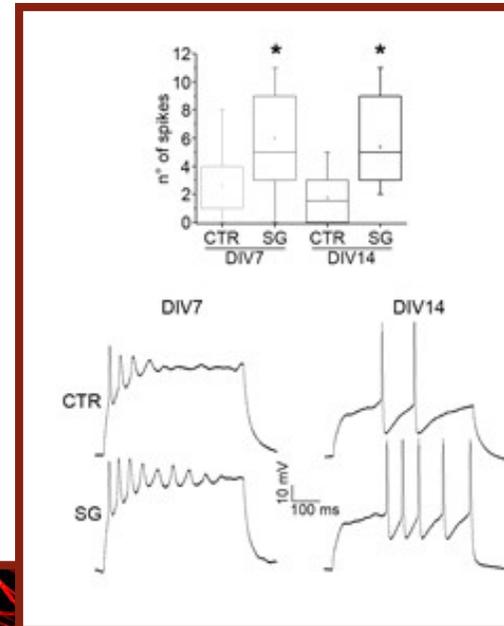
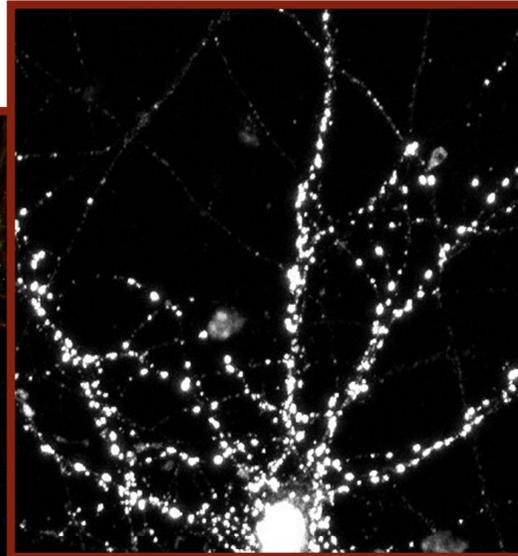
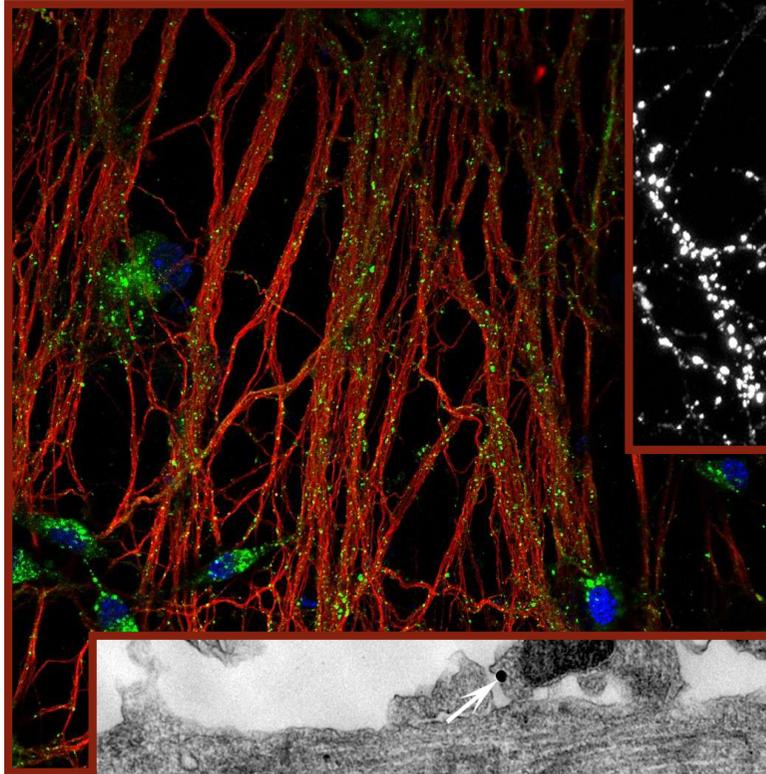
*In vitro regeneration model
created with IBIDI cell insert*



IN VITRO REGENERATION MODEL

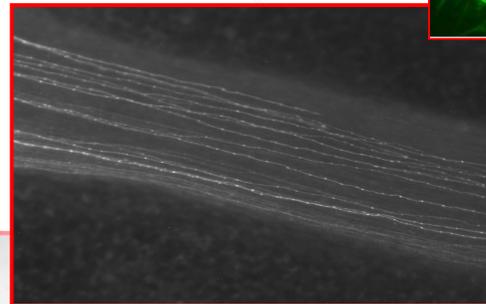
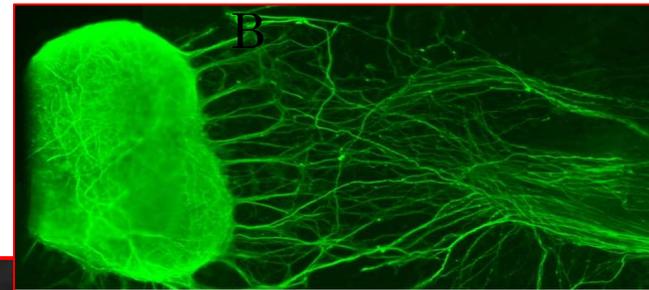
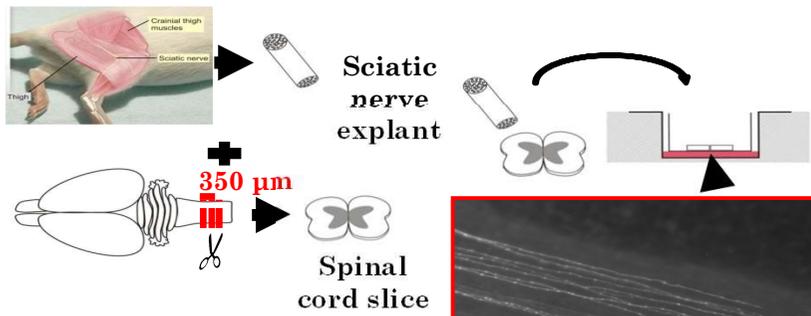


IN VITRO REGENERATION MODEL

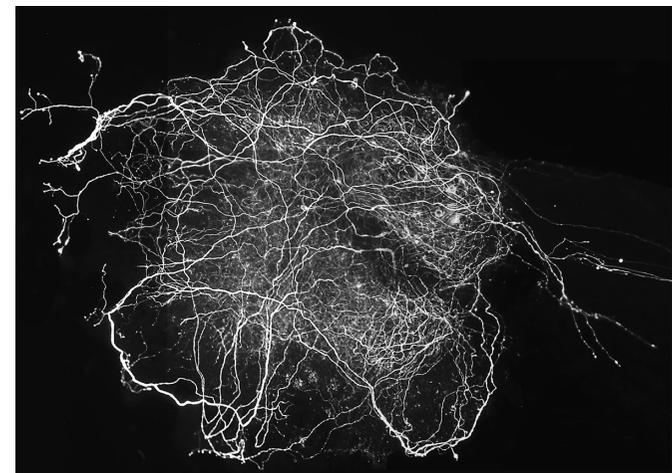
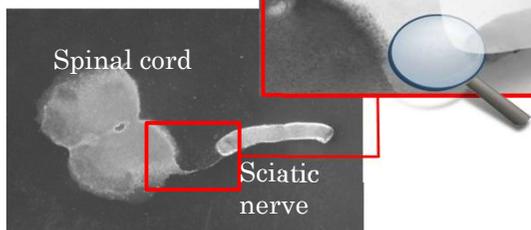


EX-VIVO REGENERATION MODEL

A



A1



WE MUST NEVER LOSE SIGHT OF THE OBJECTIVE



Posta in arrivo | GRAZIE - Posta in arrivo

Scarica messaggi | Scrivi | Chat | Rubrica | Etichetta | Filtro veloce

Cerca <Ctrl+K>

Rispondi | Inoltra | Archivia | Indesiderata | Elimina | Altro

Oggetto **GRAZIE** 06/09/2020, 12.16

A Me ★

Salve Dott.ssa Raffa,

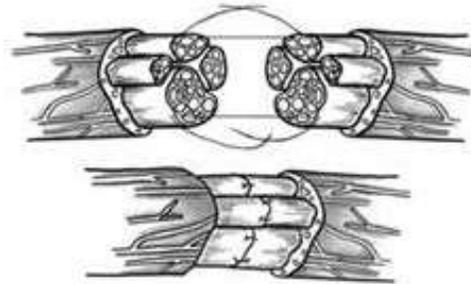
Mi chiamo [redacted], sono un ragazzo di 35 anni che a causa di un incidente stradale lo scorso anno, sono stato costretto a su una sedia a rotelle.

Ho letto l'articolo della ricerca effettuata presso il suo laboratorio e ci tenevo a ringraziarla insieme a tutto il suo staff per il lavoro svolto..



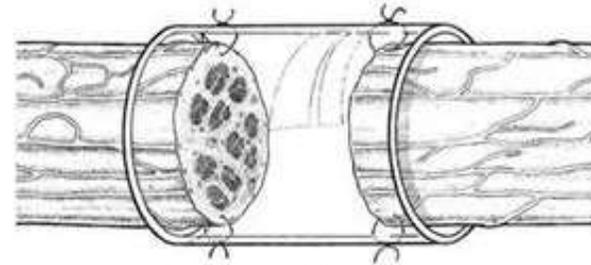
PERIPHERAL NERVE REGENERATION

Nerve lesion without gap



Suture

Nerve lesion with gap



Collagene conduit

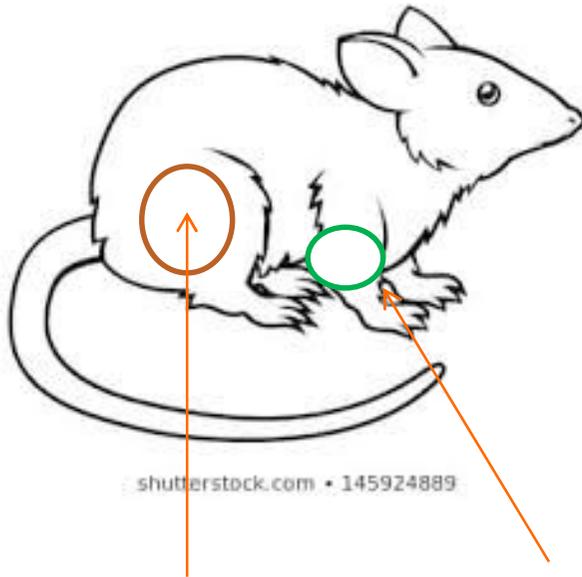


ADVANCED HEALTHCARE MATERIALS

Communication | [Free Access](#)

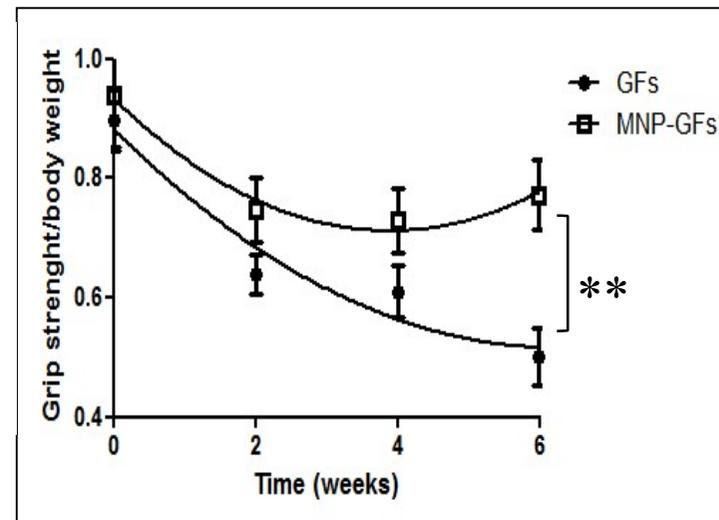
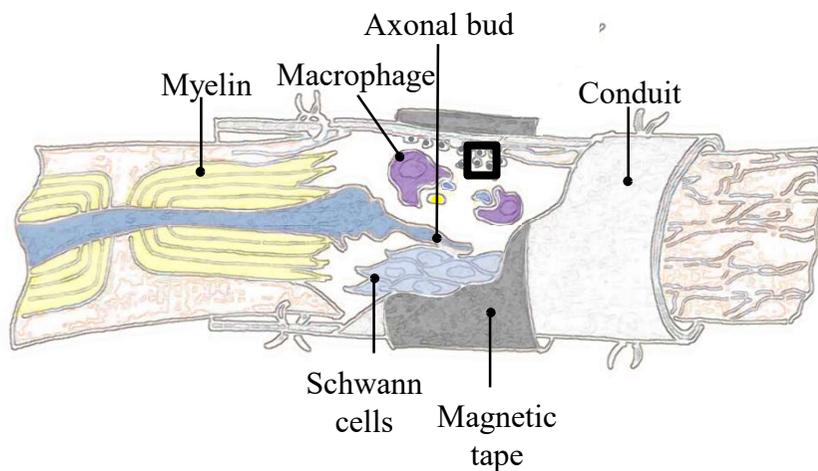
Magnetic Nanoparticles for Efficient Delivery of Growth Factors: Stimulation of Peripheral Nerve Regeneration

Martina Giannaccini, M. Pilar Calatayud, Andrea Poggetti, Silvia Corbianco, Michela Novelli, Melania Paoli, Pietro Battistini, Maura Castagna, Luciana Dente, Paolo Parchi, Michele Lisanti, Gabriella Cavallini, Concepción Junquera, Gerardo F. Goya, Vittoria Raffa ✉



Sciatic nerve

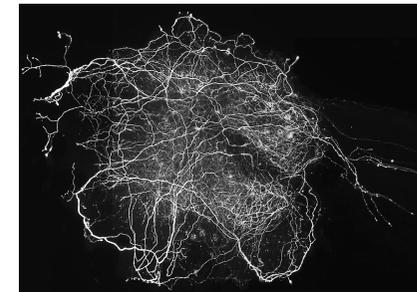
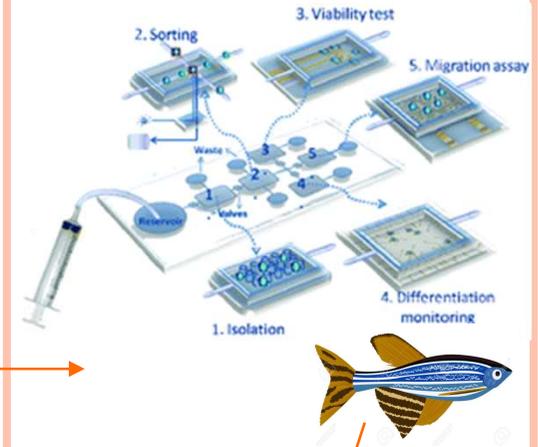
Median nerve



Studio della letteratura

Esistono alternative alla sperimentazione animale che rispondono alla domanda biologica?

si



no

OPBA

E' la mia tecnologia sufficientemente matura?

no

si

