Τετάρτη 11 Δεκεμβρίου 2024

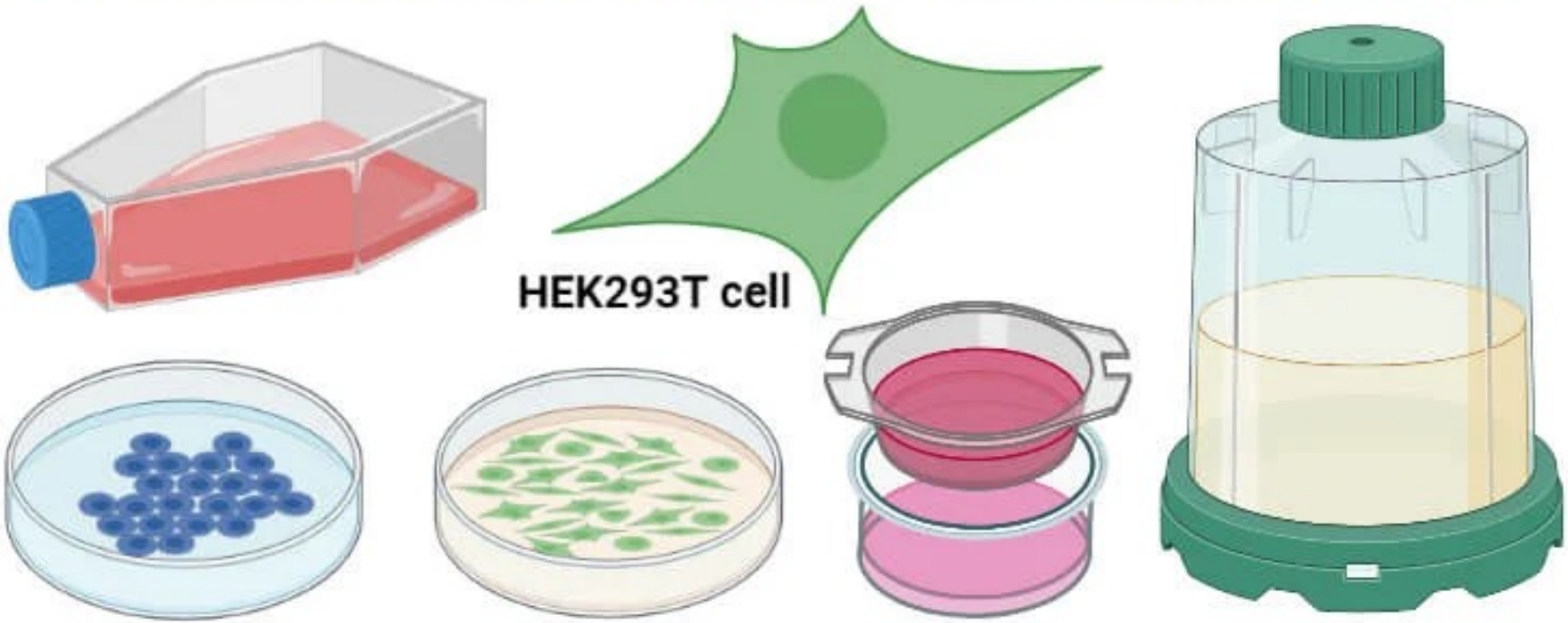
Μεταπτυχιακό Μάθημα:

Κυτταροκαλλιέργειες

Κουρεπίνη Ευαγγελία, PhD.
Εθνικό Ίδρυμα Ερευνών

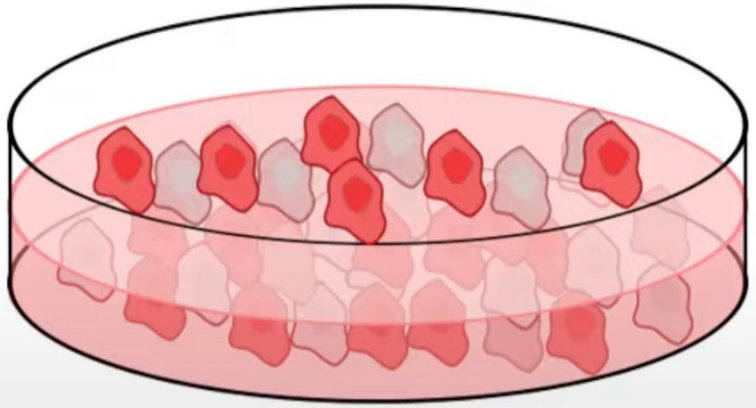
Animal Cell Culture

Definition, Types, Cell Lines, Procedure, Applications

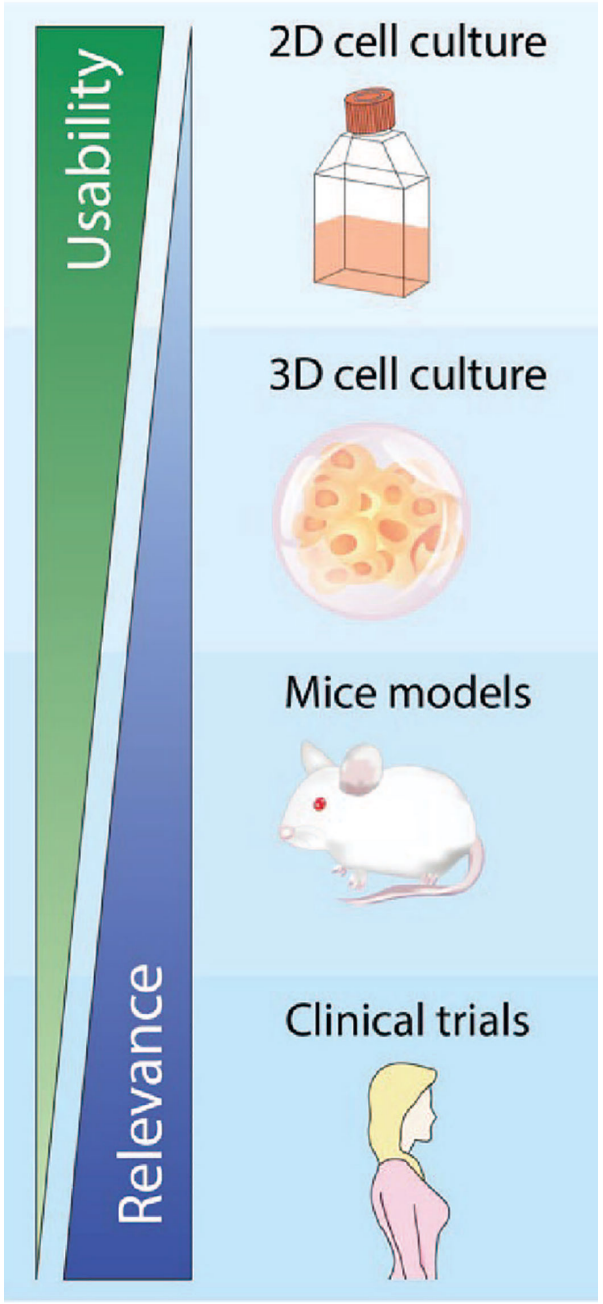


Cell Culture

Allows researchers to grow cells outside the body



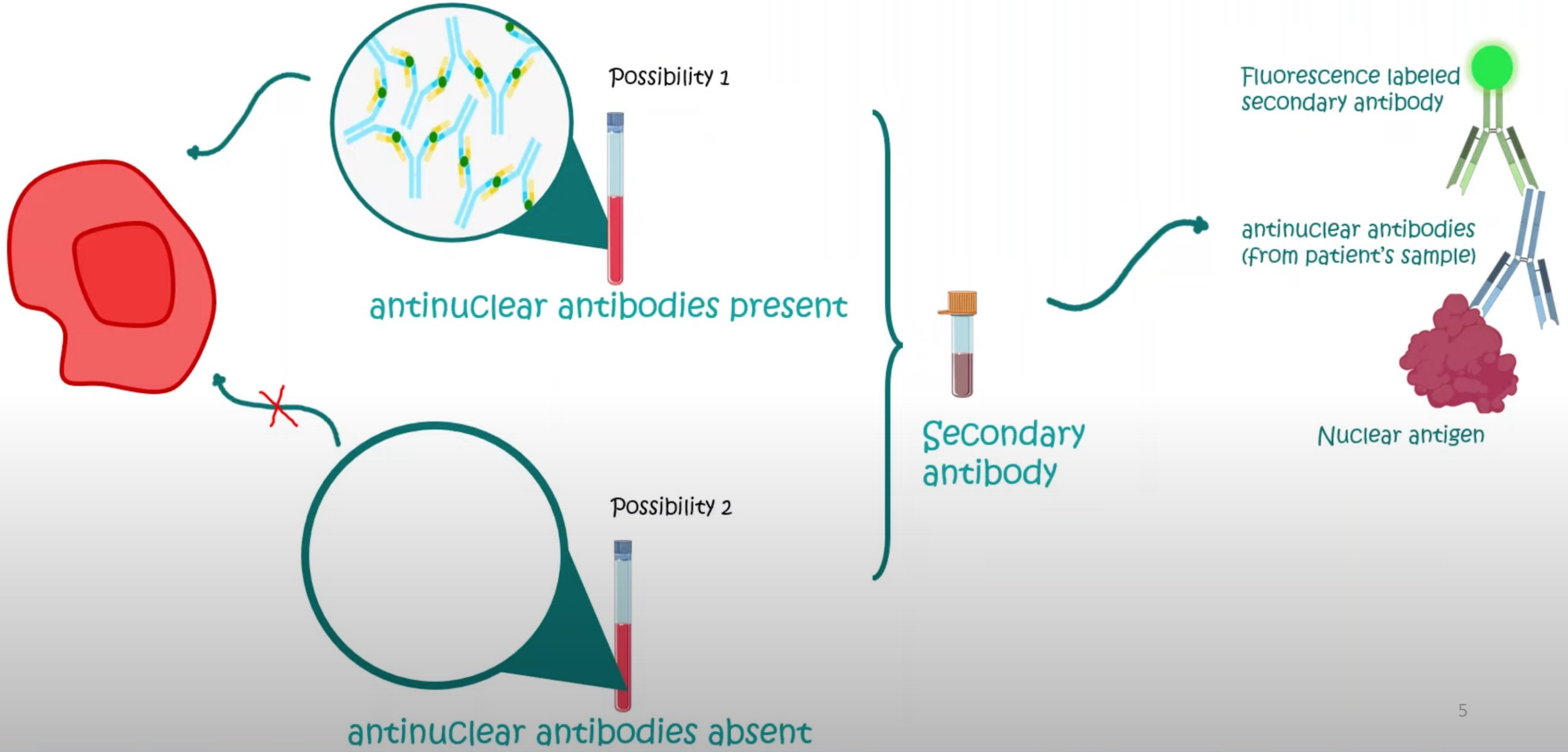
Research tools



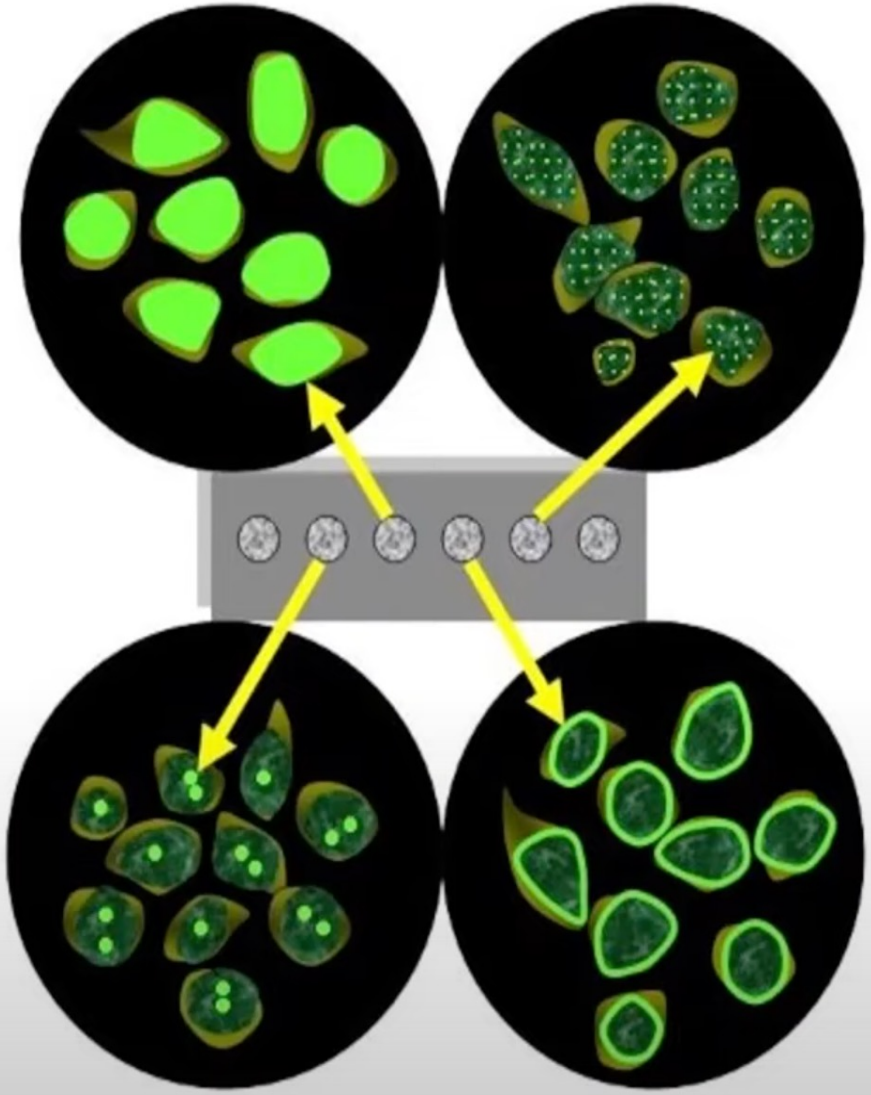
Law et al. Frontiers Oncology, 2021.

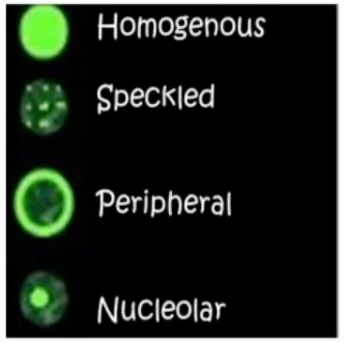
Diagnostic tools

The HEp-2 cell line, which is a native protein array with hundreds of antigens, provides an ideal substrate for the detection of ANA



An ANA (anti nuclear antigen) test looks for antinuclear antibodies in your blood. If the test finds antinuclear antibodies in your blood, it may mean you have an autoimmune disorder.

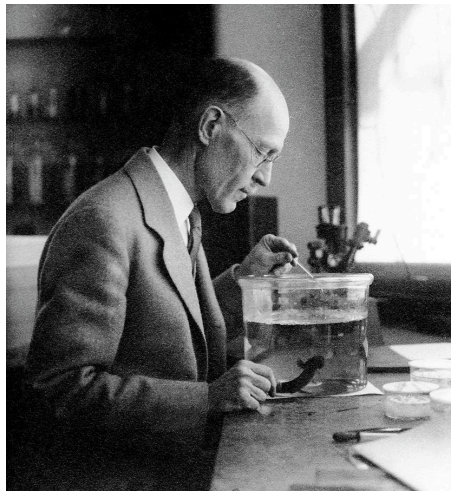




Systemic lupus erythematosus (SLE)

History

-1907 Ross Harrison: Showed development of nerve fibers from frog embryo tissue *in vitro.*



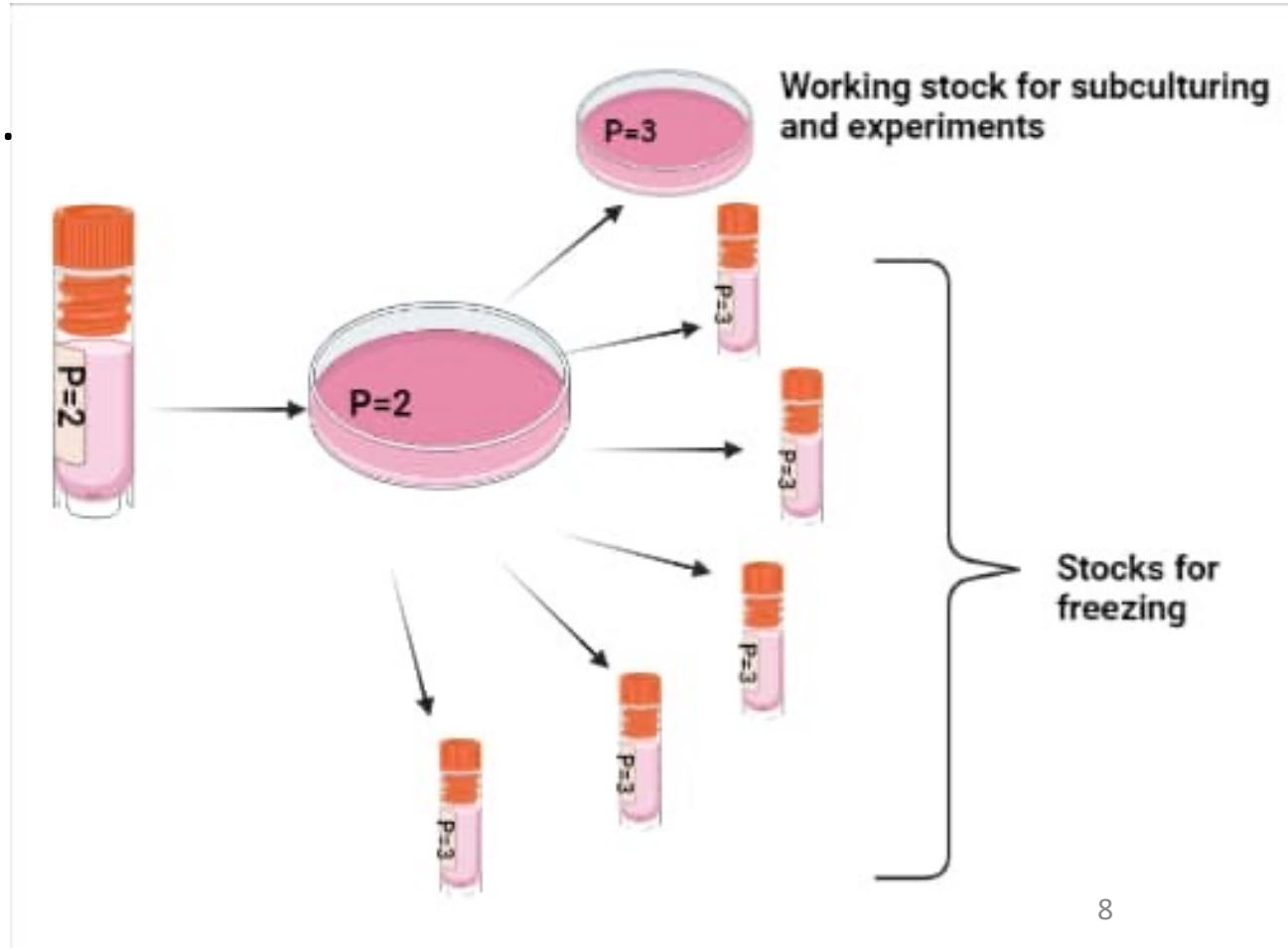
- 1912 Alexis Carrel: Kept fragments of chicken embryo heart alive and discovered cell passaging/sub-culturing.

- Till 1950 mainly tissue explants were used for culture techniques. After this year dispersed cells in cell culture media were used.

Cell culture

Removal of cells of an animal or plant and their subsequent growth in a favorable artificial environment.

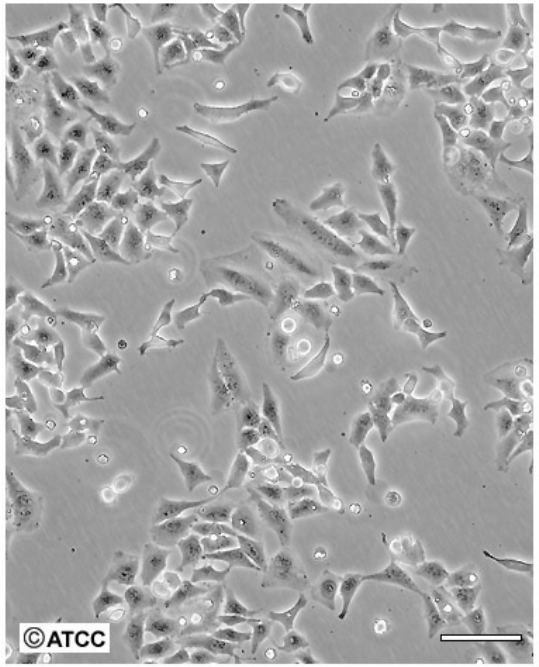
A) Primary cells: Passage by sub-culturing.
B) Cell line: Infinite, continuous.



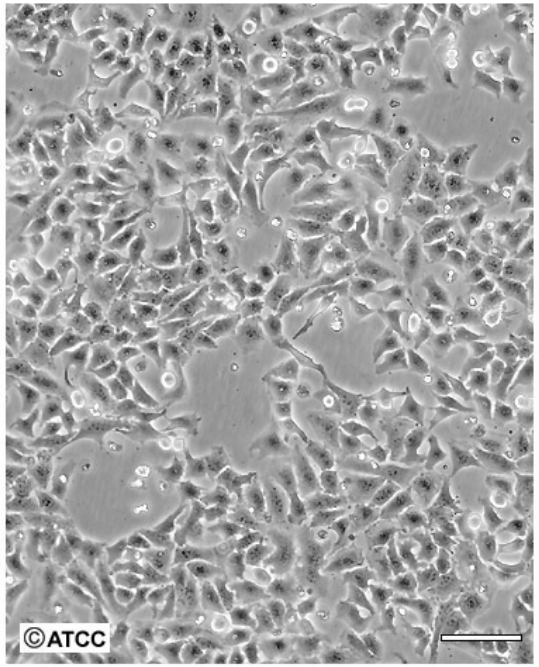
HeLa: The most commonly used cell line

In the early 1980s, German virologist Harald zur Hausen found that HeLa cells contained multiple copies of human papillomavirus 18 (HPV-18), a strain of HPV later found to cause the type of cervical cancer that killed Lacks.

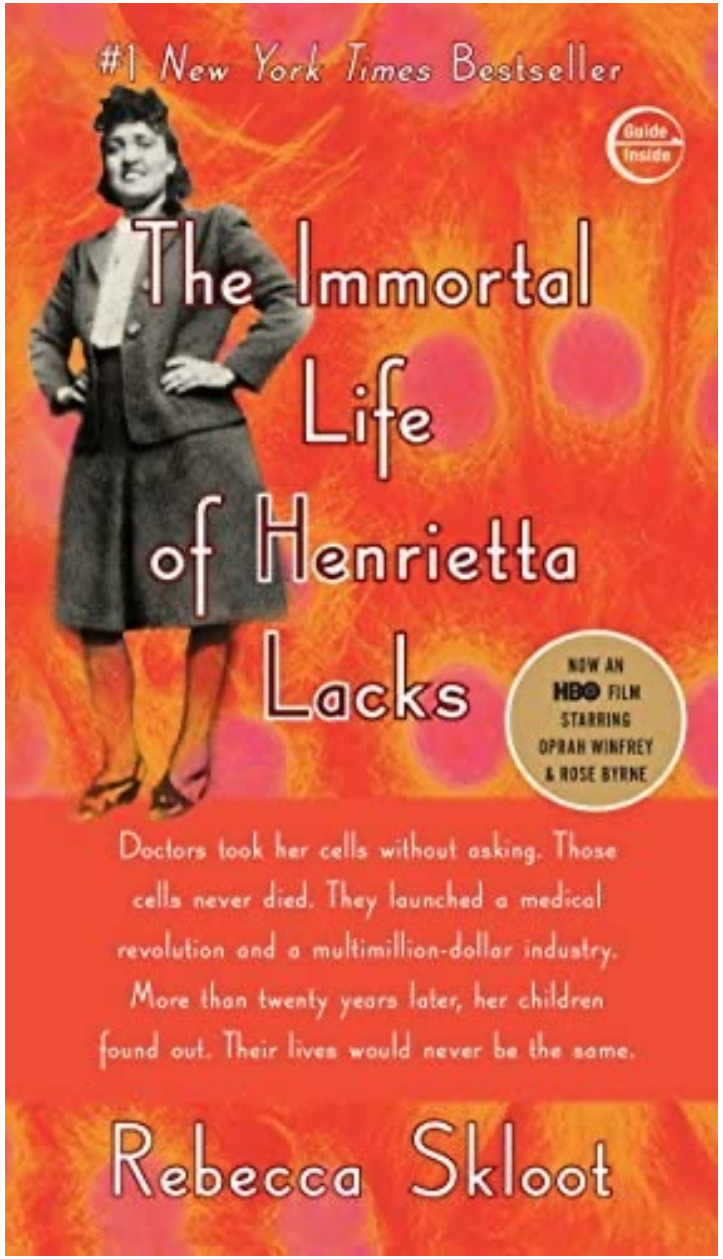
ATCC Number: **CCL-2**
Designation: **HeLa**

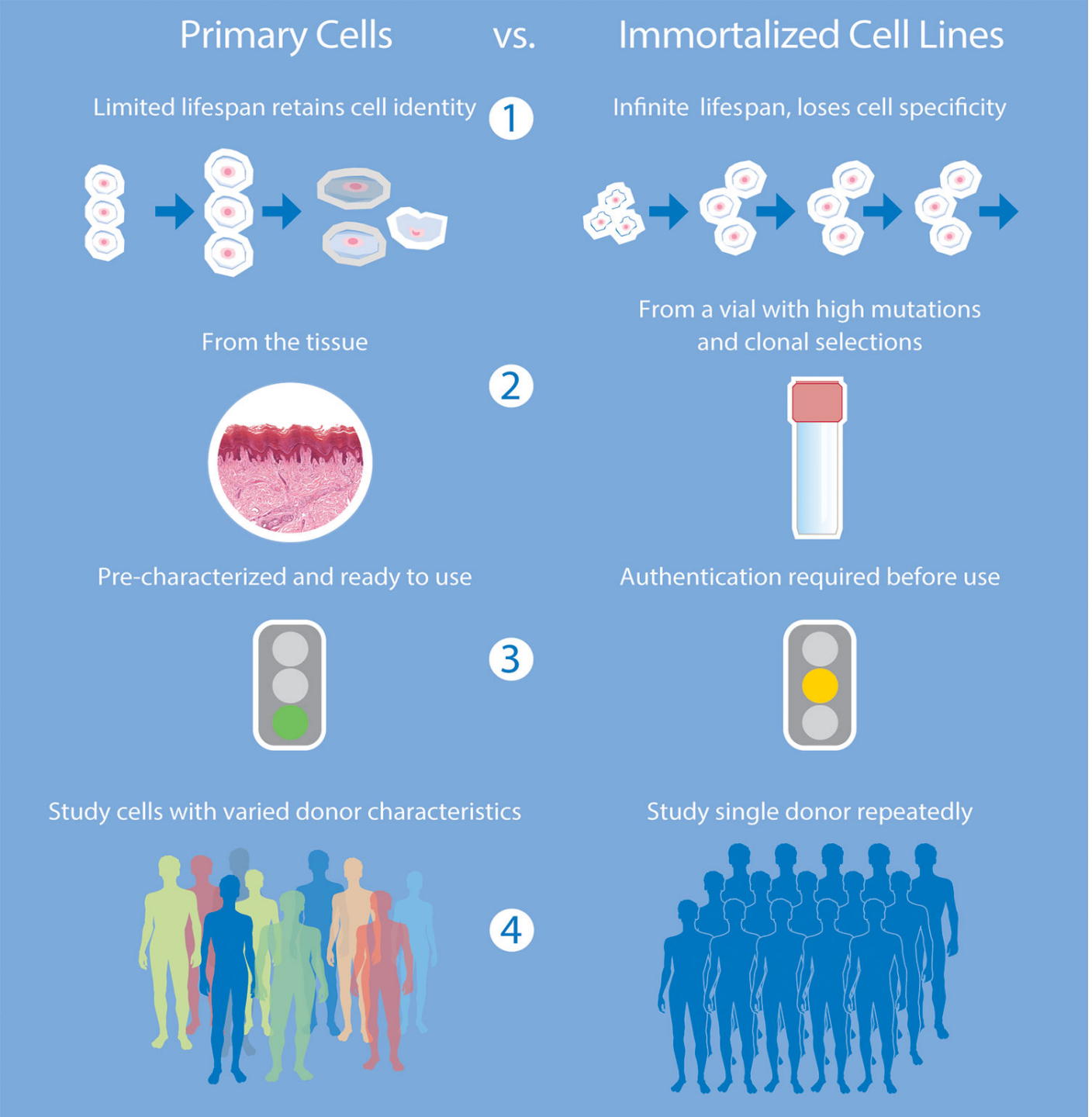


Low Density Scale Bar = 100µm



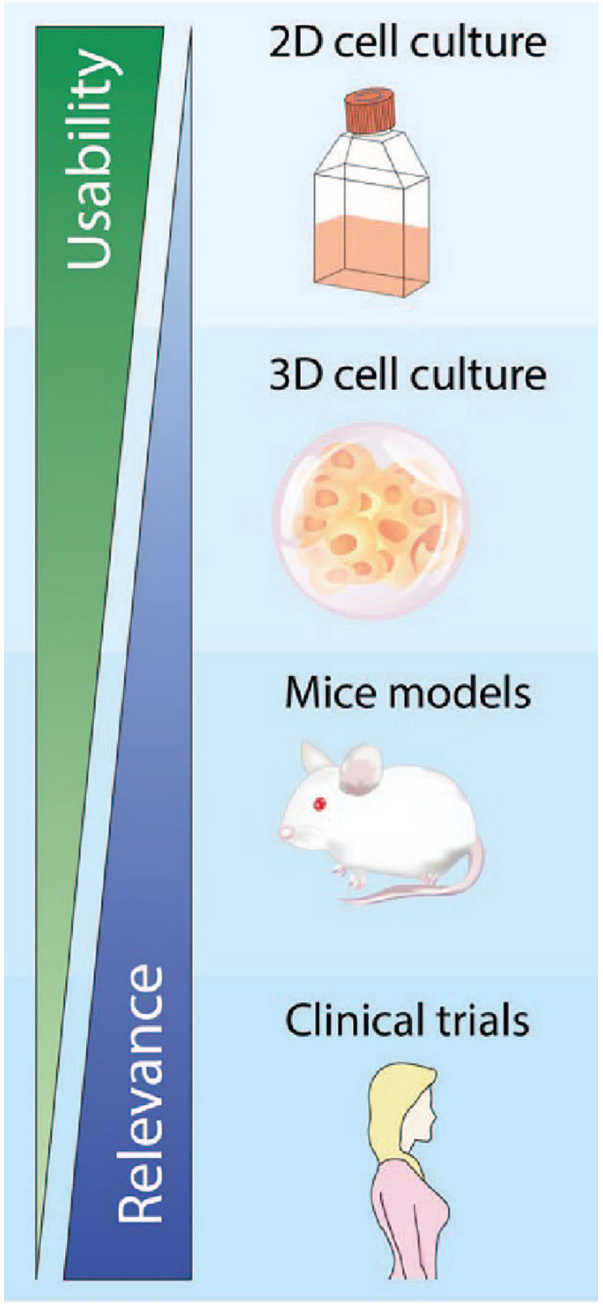
High Density Scale Bar = 100µm





Cancer research initiatives, such as the Cancer Genome Atlas, prefer to use primary cells rather than cell lines to sequence cancer genomes because they are more biologically relevant.

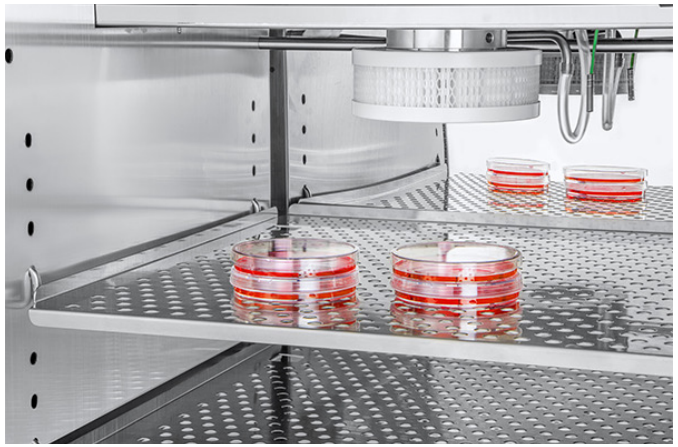
Research tools



Law et al. Frontiers Oncology, 2021.

Equipment

Particular conditions of treatment and culture: temperature, air in a sterile incubator, sterile laminar flow hood, water bath, centrifuge etc.



Cell Culture Media

There are six main ingredients found in cell culture media:

1. Carbon source (e.g., glucose).
2. Buffering system (e.g., HEPES).
3. pH Indicator (e.g., phenol red).
4. Serum.
5. Metabolites, vitamins, minerals and secrets (e.g. 2-me).
6. Antibiotics.

Standard Operating Procedures (SOP) for Cell Culture Room

November 2023

The policies outlined here are to be used for common routine maintenance and cleaning of the cell culture facilities in National Hellenic Research Foundation.

General

- Wear clean laboratory coats, not contaminated with blood or any other animal tissue.
- Wear gloves. Dispose of gloves when overtly contaminated or when the integrity of the glove is compromised. This is crucial as specific assays will utilize immune cells which are sensitive to activation even with remnants of dermal cells, dust, etc. Do not wear disposable gloves for touching "clean" surfaces (keyboards, telephones, etc.), and do not wear them outside the lab.

Important: gloves should be passed with 70% ethanol when they come in contact with surfaces outside of the hood.

- All bench and work areas should be kept clean with as few items and pieces of equipment as possible. Keep the access door for the culture rooms closed when the incubator door is open and/or the hoods are operating.

- No items should be stored inside the hoods unless the supplies are off the surface of the hood (Exception: sterile pipettes, tips).

- Wipe down with 70% ethanol for **every** item placed in the hood such as pipetting devices, bottles of pre-warmed media, aliquots, ice buckets, tip boxes etc.

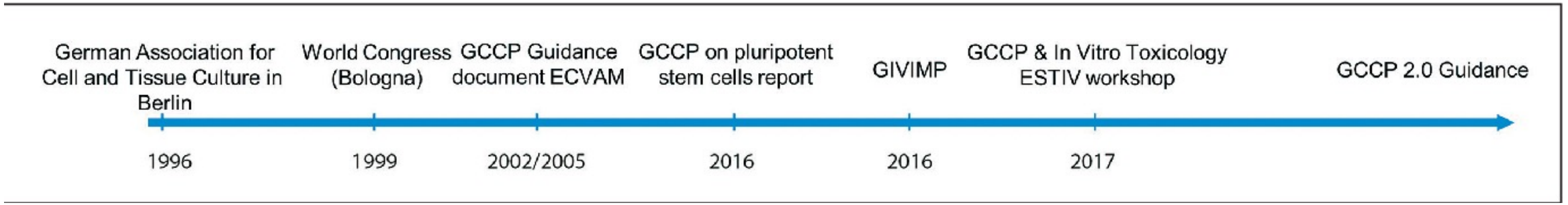
To be done daily

- Hood and bench work surfaces (including microscope working areas) must be cleaned and wiped down with water and then 70% ethanol before and after every use.
- If full, biohazard bags and other trash receptacles must be sealed and removed from the lab at the end of the workday and a new empty bag placed in the receptacle. When a bag is full, specific cleaning personnel (Anastasia) should be aware to sent it or sterilization.
- Important: Glass pasteur pipettes must be sent for sterilization by the person that finishes the box. After sterilization, the box must be dried in the oven of Chemical Carcinogenesis lab.
- The incubator's temperature and CO2 readings should be confirmed in the morning and before placing any cell culture dishes.
- The most important for our experiments: please be extremely careful with the microscope as the lamp is very easily overheated, thus be sure that when you leave the microscope (even for some minutes) the microscope is closed, in order to avoid the destruction of the lamp that is very expensive/ time-consuming to be replaced.
- Do not forget to turn off the vacuum pump motor when it is not used (e.g. during trypsinization).

Good Cell Culture Practice (GCCP), an effort to develop minimum quality standards applicable in academia.

A major reason for the current reproducibility crisis in life sciences is the poor implementation of quality control measures and reporting standards ➔
Improvement is needed, especially regarding increasingly complex in vitro methods.

- Development of induced pluripotent stem cells (iPSCs) and gene-edited cells.
- Human stem-cell-derived models and bioengineering of organo-typic cell cultures.
- Including organoids, organ-on-chip and human-on-chip approaches.

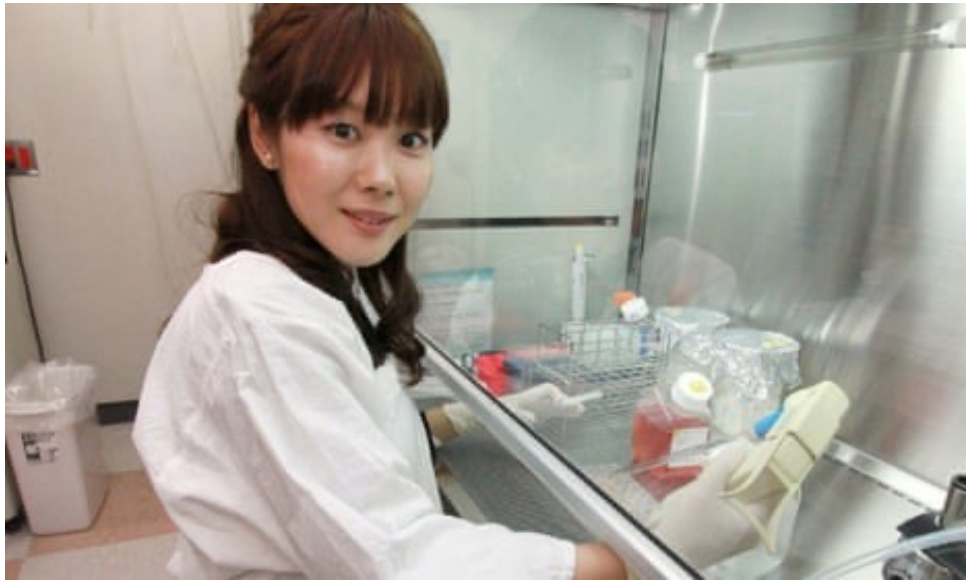


➔ Not only are most experiments not reproduced, most are probably not reproducible.

The culprit: carelessness and hubris in the drive to make a historic discovery.

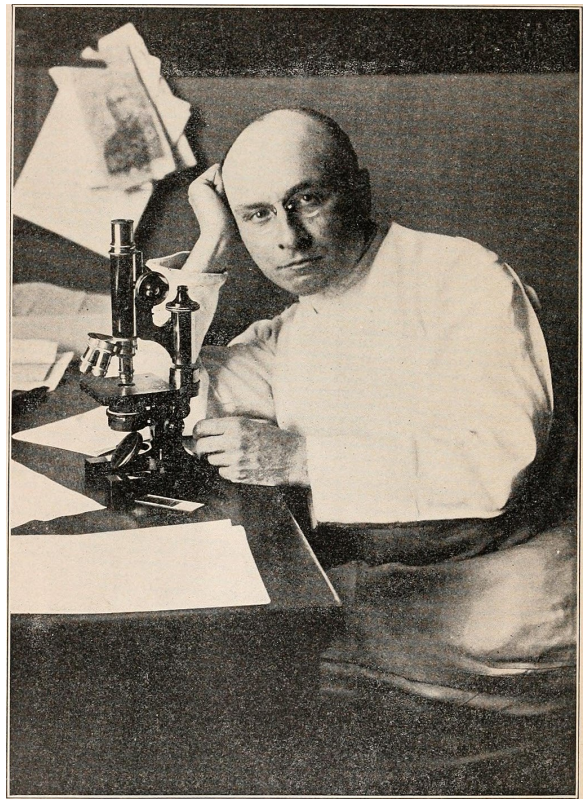
Haruko Obokata

The spectacular fall of the scientist who claimed to have triggered stem cell abilities in regular body cells is not uncommon in the scientific community. Stap cells.



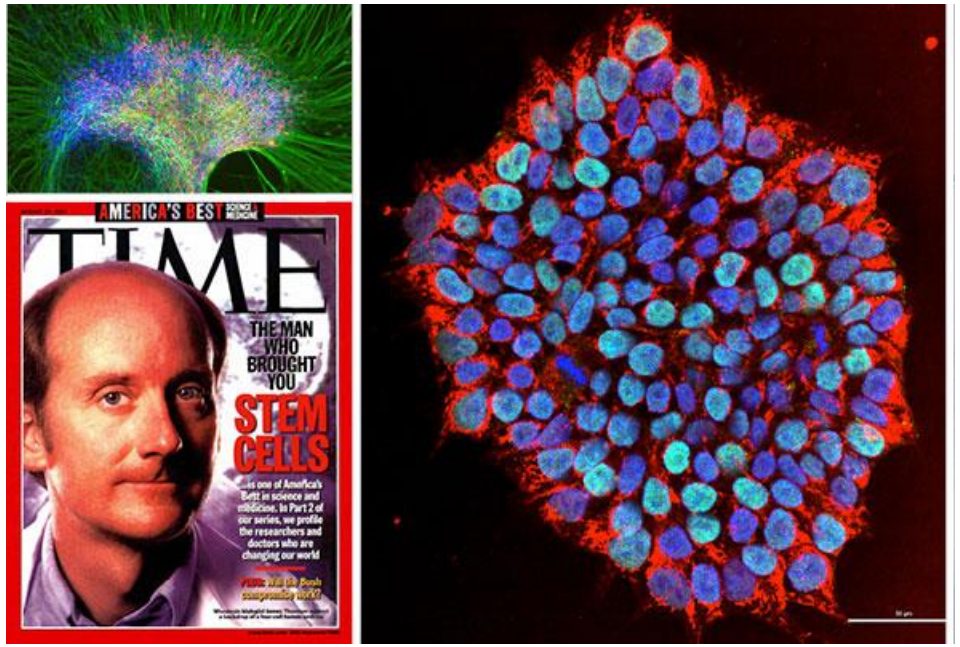
Alexis Carrel

Surgeon and biologist who was awarded the Nobel Prize in Physiology of Medicine in 1912 for pioneering vascular suturing techniques. He invented the first perfusion pump with Charles A. Lindberghn opening the way to organ transplantation.



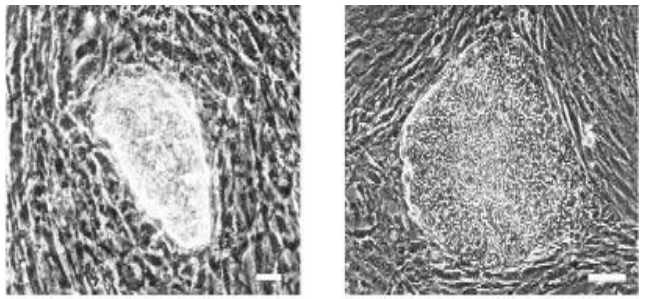
Stem cell cultures

Thomson JA et al, **“Embryonic stem cell lines derived from human blastocysts.”** Science **1998** Nov 6;282(5391):1145-7.



H9 cell line: XX, normal karyotype

- Origin: ‘Fetus after IVF, informed consent
- Number of passages: 32, 8 months



ES colonies (3 days in vitro culture)

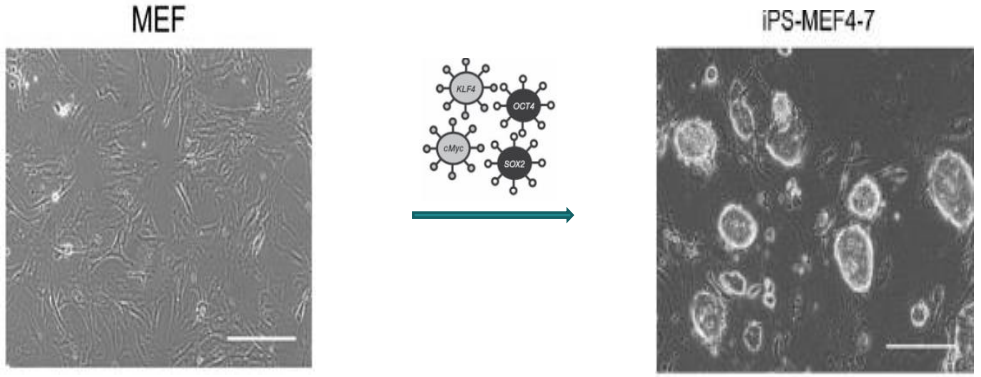
Dr. Shinya Yamanaka, PhD

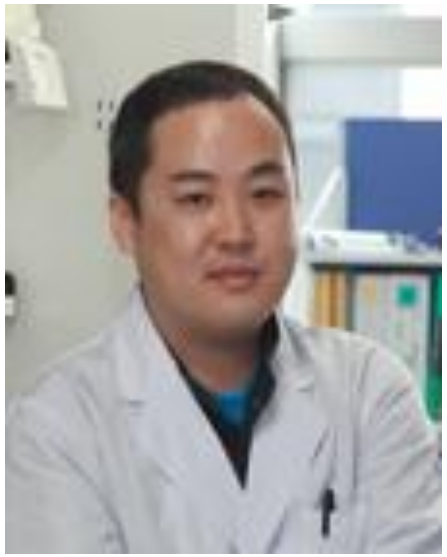
Induction of Pluripotent Stem Cells from Mouse Embryonic and Adult Fibroblast Cultures by Defined Factors

Cell

Kazutoshi Takahashi[1] and Shinya Yamanaka[1,2,*]

Cell *126*, 663–676, August 25, 2006



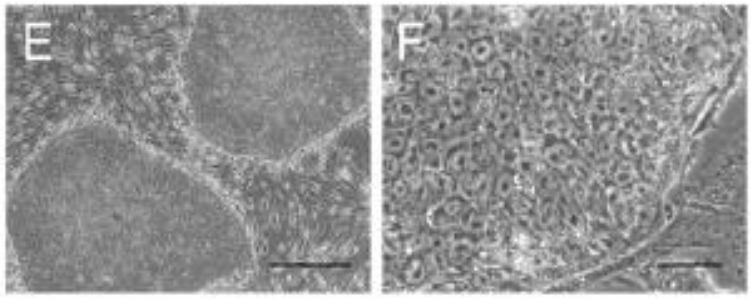


Dr. Kazutoshi Takahashi, PhD

Induction of Pluripotent Stem Cells from Adult Human Fibroblasts by Defined Factors

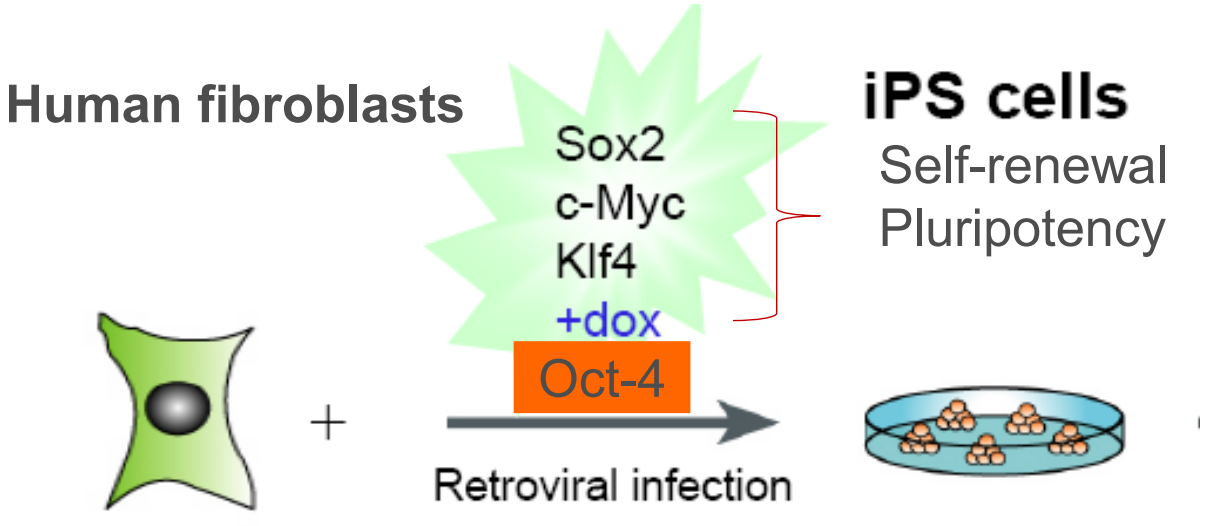
Cell

Kazutoshi Takahashi,[1] Koji Tanabe,[1] Mari Ohnuki,[1] Megumi Narita,[1,2] Tomoko Ichisaka,[1,2] Kiichiro Tomoda,[3] and Shinya Yamanaka[1,2,3,4,*]



Ανθρώπινα iPSCs

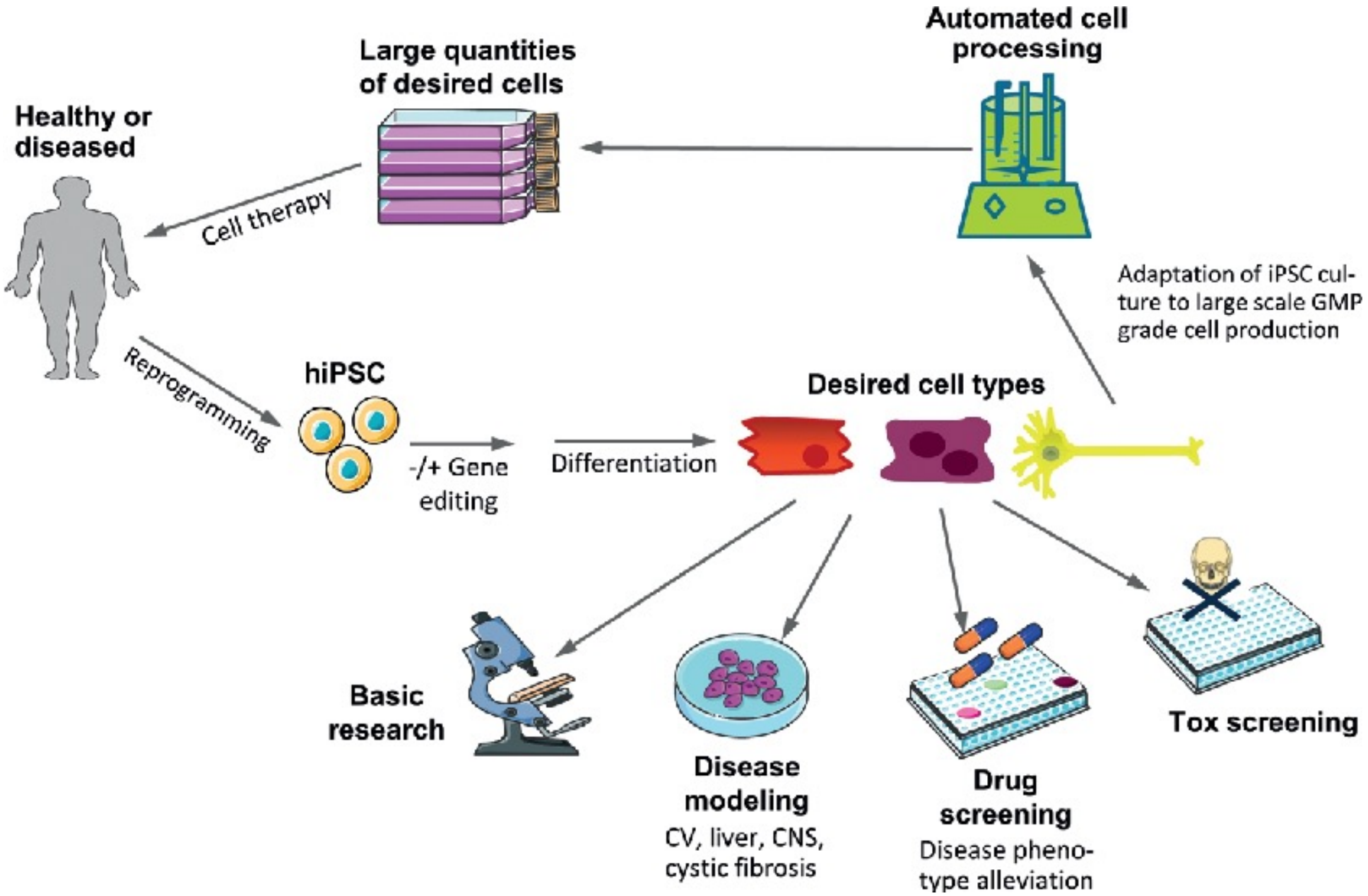
Stem cell cultures for restoration/regeneration



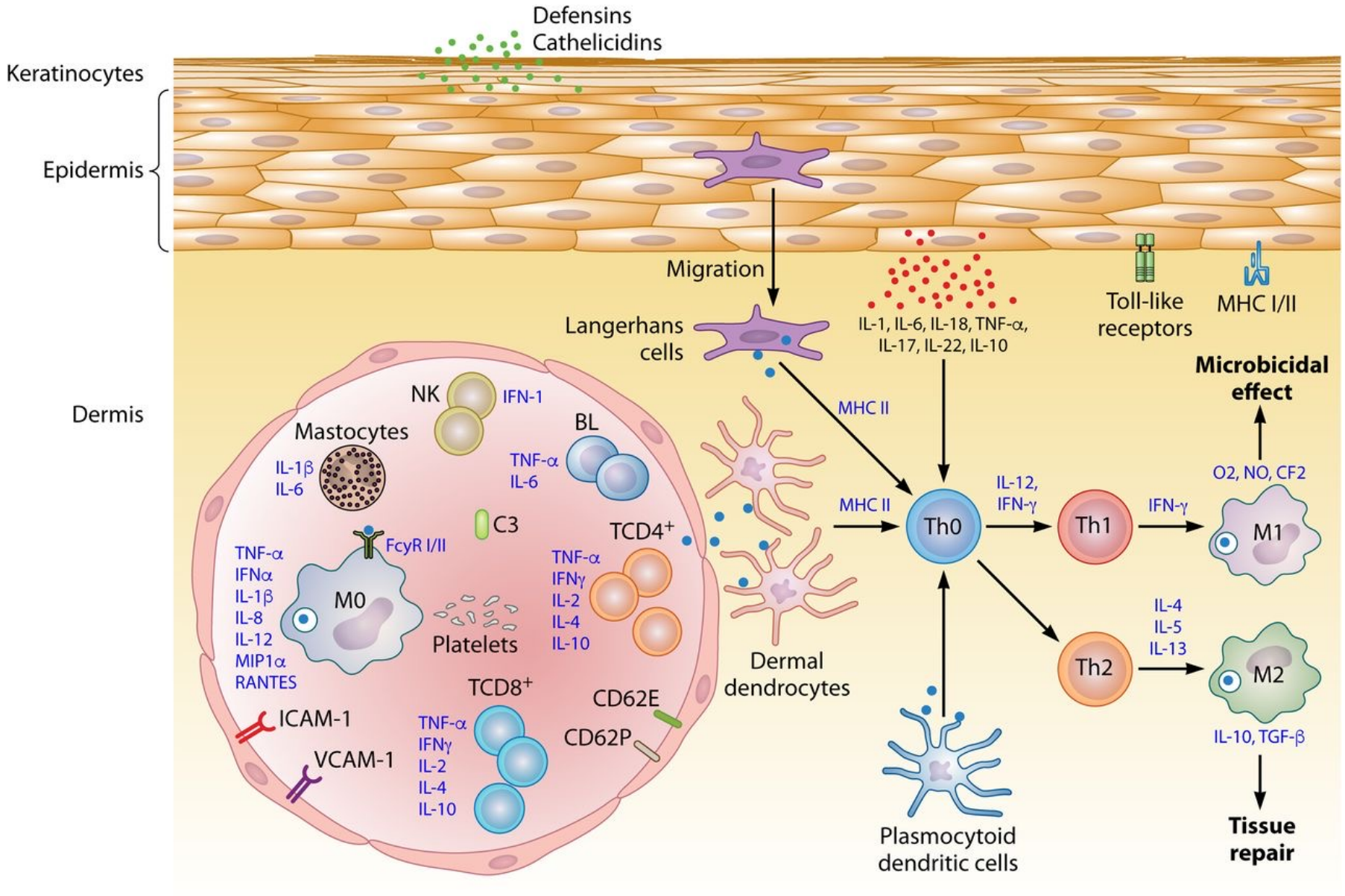
IPS (Induced Pluripotent Stem cells)

IPS:
"Adult cells that have been genetically reprogrammed to an embryonic stem cell–like state by being forced to express genes and factors important for maintaining the defining properties of embryonic stem cells."

Stem cell cultures for research and therapies

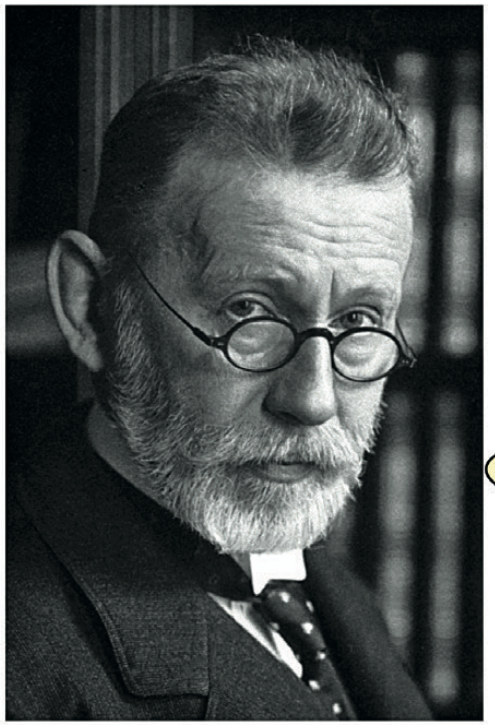


Immune cell isolation and culture

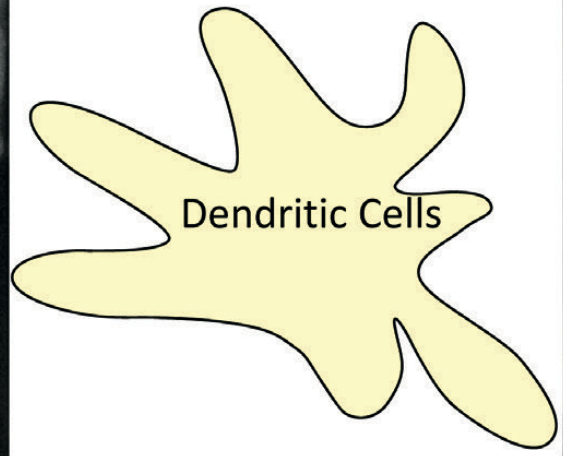


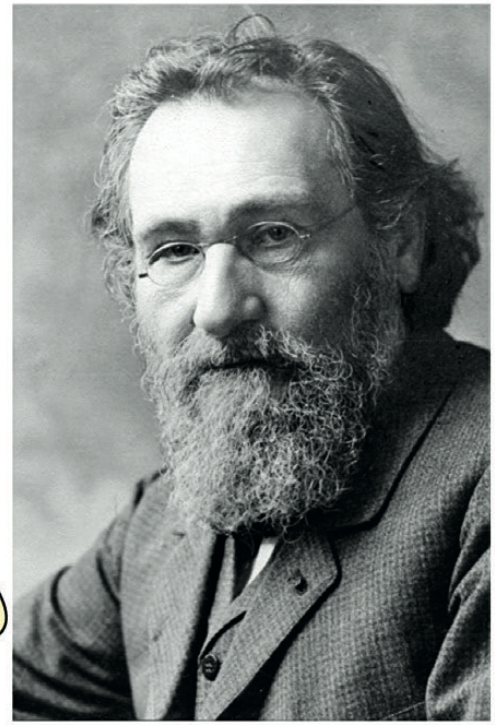
Quaresma et al. Clinical microbiology 2019

Immune cell culture



Paul Ehrlich
Adaptive Immunity



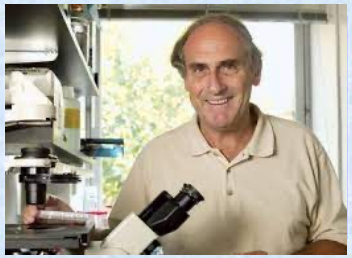


Ilya Ilyich Metchnikov
Innate Immunity

Adaptive and innate immune systems featured in the work of Paul Ehrlich and Ilya Metchnikov, who were awarded the Nobel Prize in Physiology or Medicine in 1908.

1973:

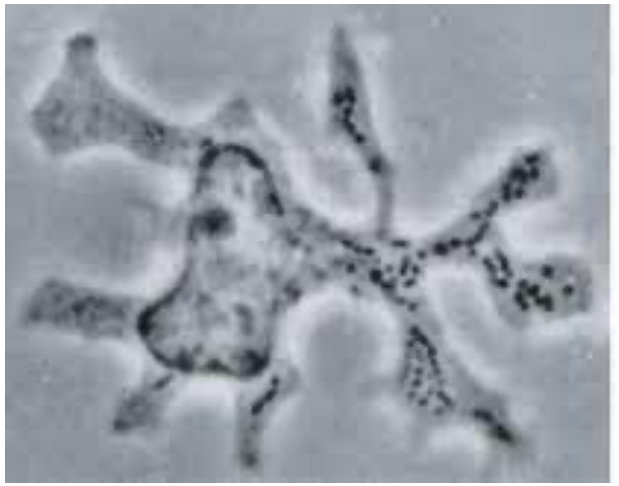
Dendritic Cells: Linking Innate to Different Forms of Adaptive Immunity



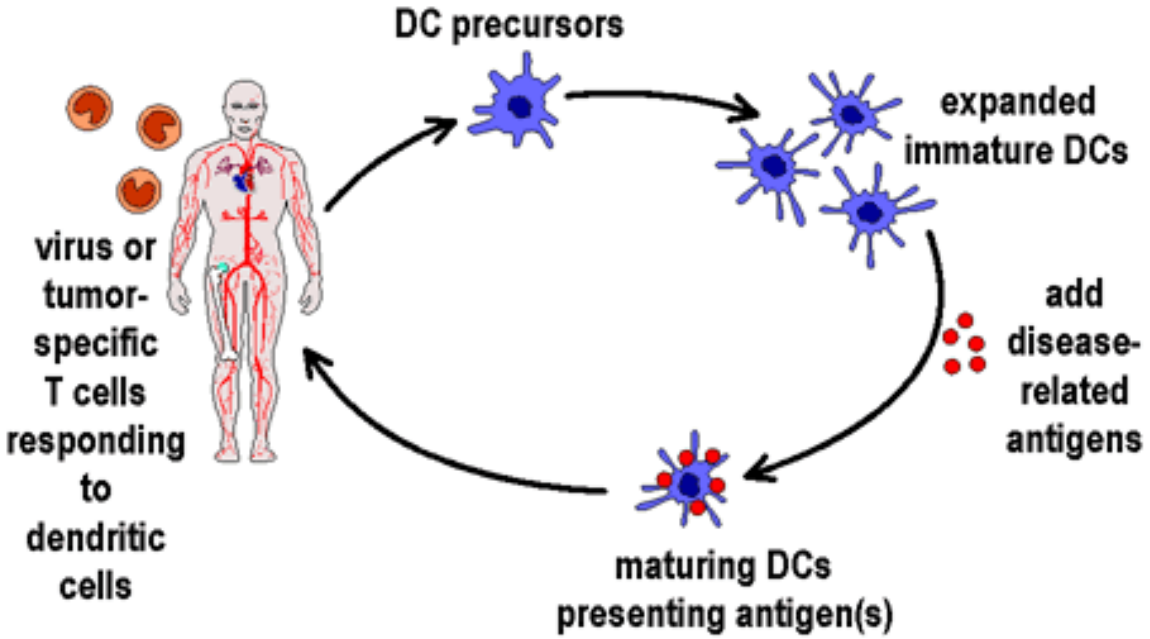
Prof. Ralph M Steinman

Laboratory of Cellular Physiology and Immunology

The Rockefeller University



- Infection
- Cancer
- Transplantation
- Autoimmunity and chronic inflammation
- Allergy
- Vaccines

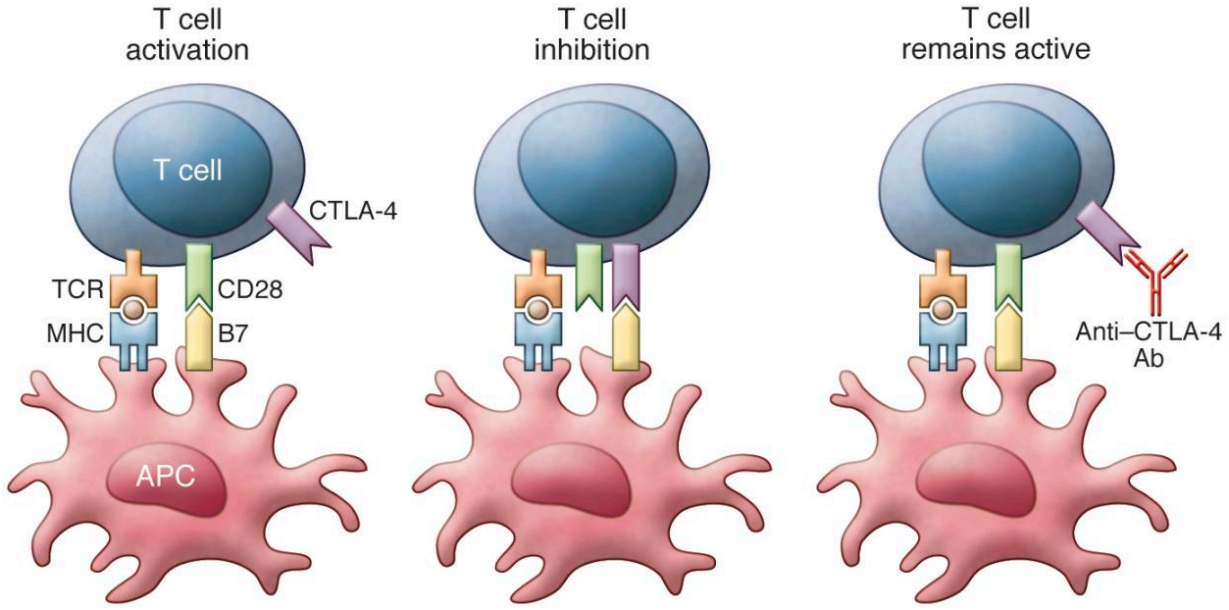


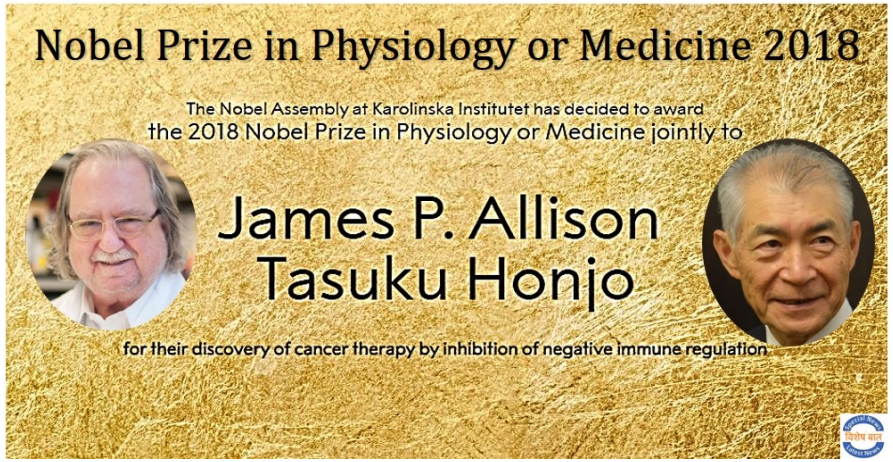
Banchereau and Steinman, Nature 392: 245-252 (1998)
Steinman, Ann.Rev.Immunol. 9: 271-296 (1991)

How tumors evade the host immune response?

➔ Expression of programmed death ligand 1 (PD-L1). The interaction of PD-L1 with PD-1 receptors on T cells inhibits their antitumor activity.
Pembrolizumab and nivolumab are monoclonal antibodies (mAbs) that block the PD-1 interaction with PD-L1 can restore antitumor responses.
Ipilimumab, which blocks cytotoxic T lymphocyte antigen-4 (CTLA-4), another immune checkpoint inhibitor, is also effective against tumors.

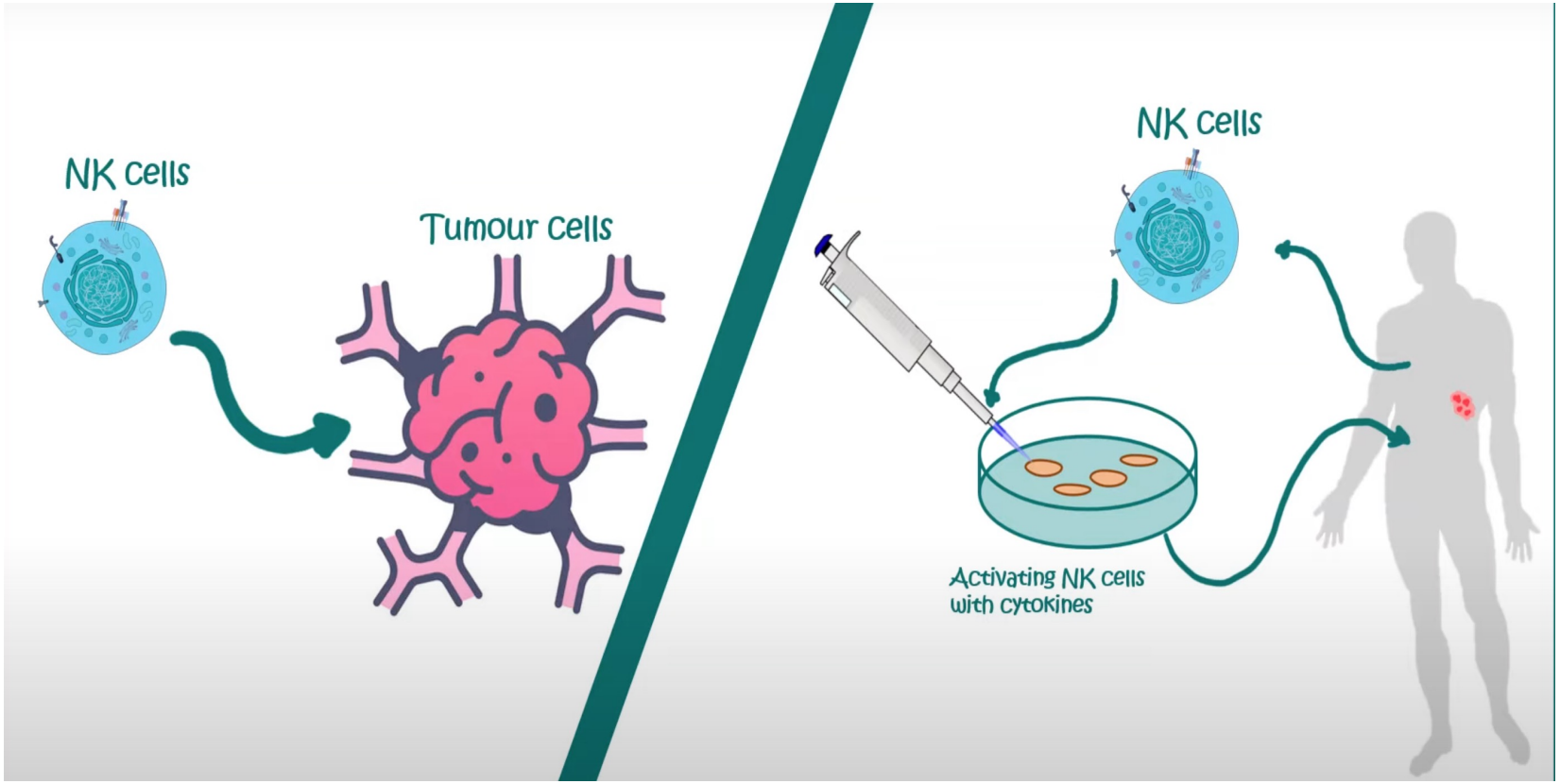
➔ Tumor immunotherapy with these antibodies results in objective responses and prolonged survival in patients e.g. with metastatic melanoma.





Jillian H. Hurst *J Clin Invest.* 2015

Immune cell cultures for cancer treatment



- NK cell culture with cytokines for activation.

- DC vaccination: Dendritic cell culture for encounter and maturation with neoantigens derived from patient's tumor.

- T regulatory (Treg) cell culture to "ease" their suppressive phenotype and expand them.

- CART cell therapy:
T cell engineering and expansion to induce their anti-tumor effective phenotype- tumor related TCR specificity.

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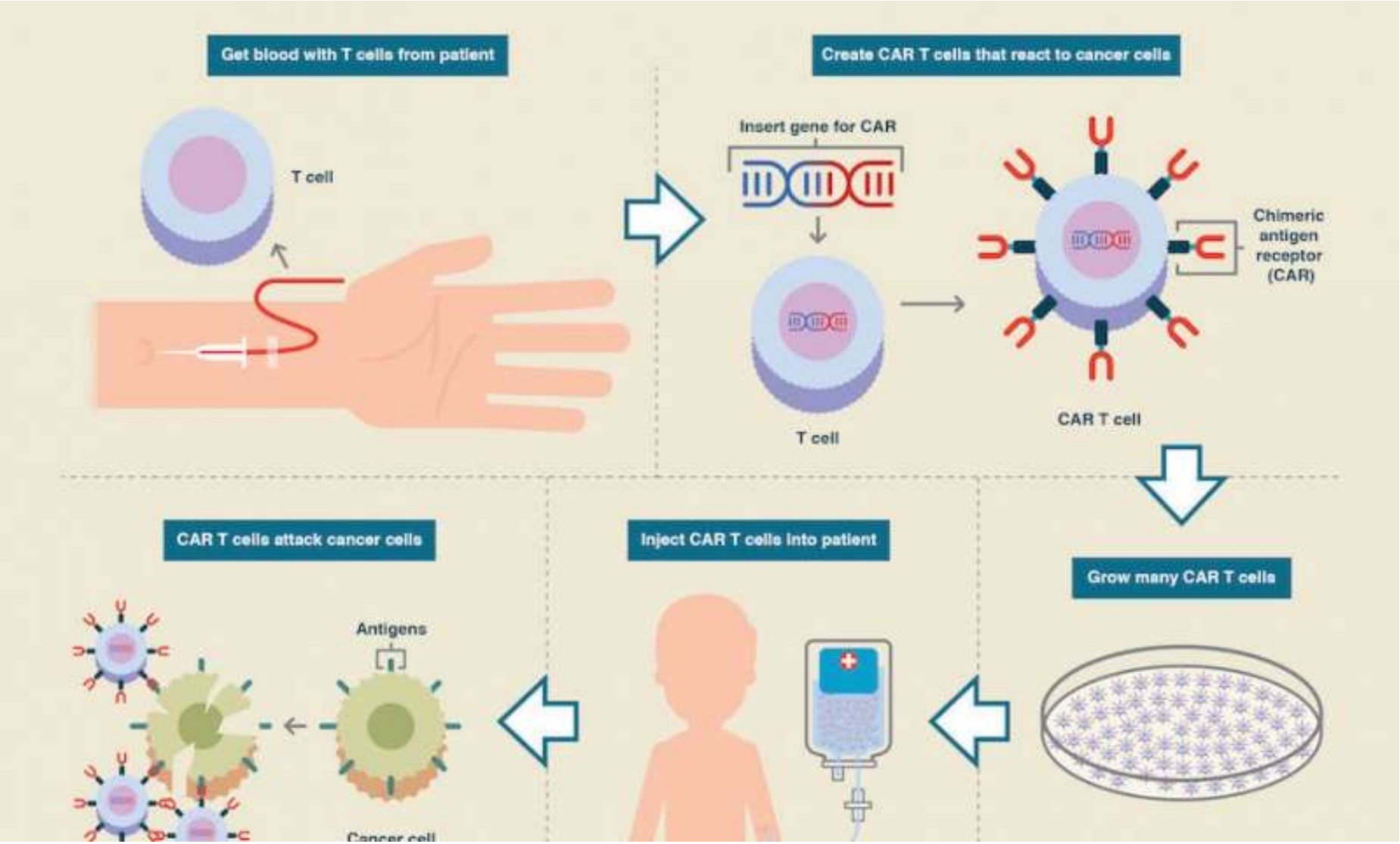
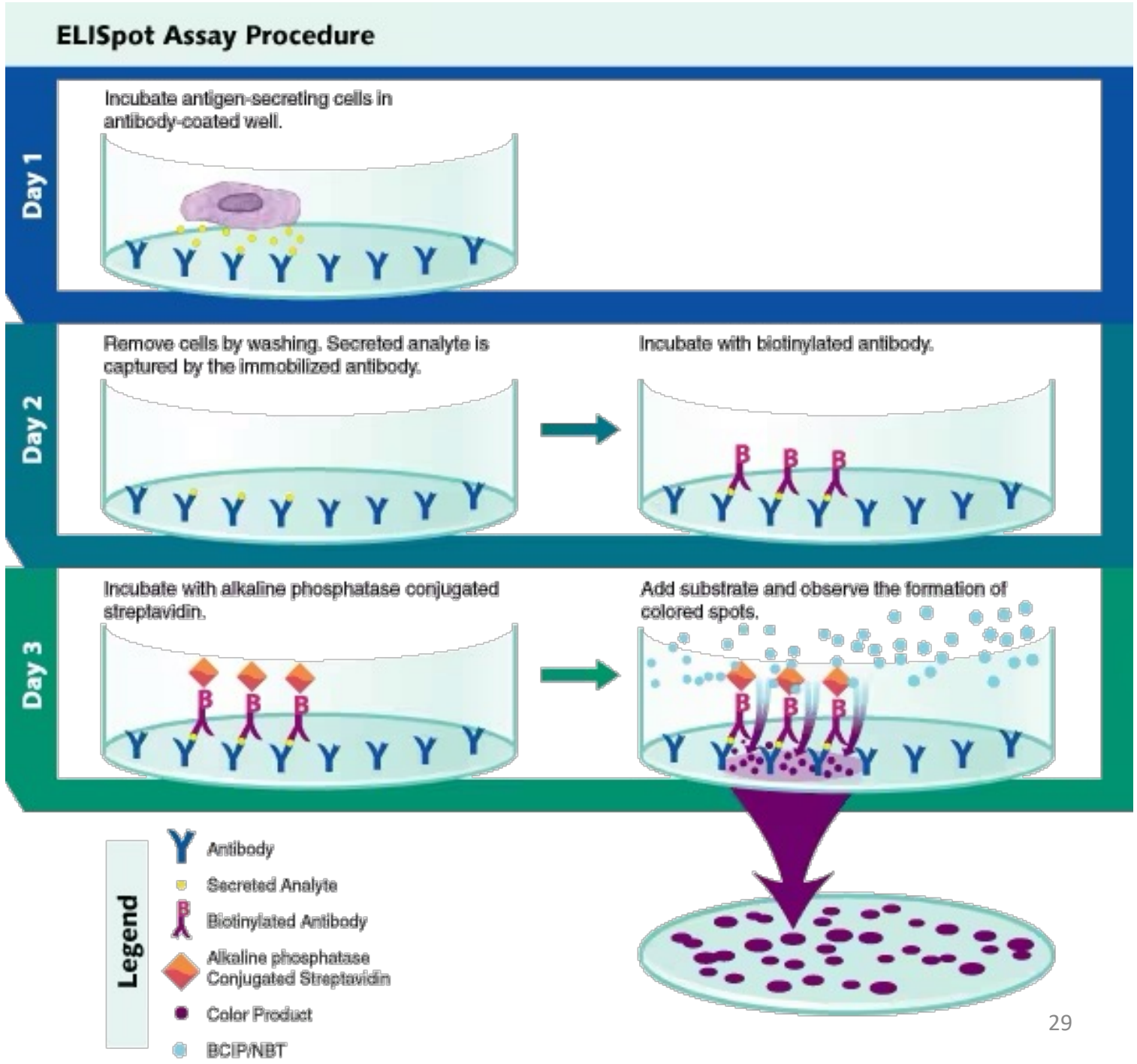


Immune cell cultures for vaccine development

ELISPOT assay:

assay to determine cytokine producing cells



Example of an experiment of vaccine/cancer immunotherapy development:

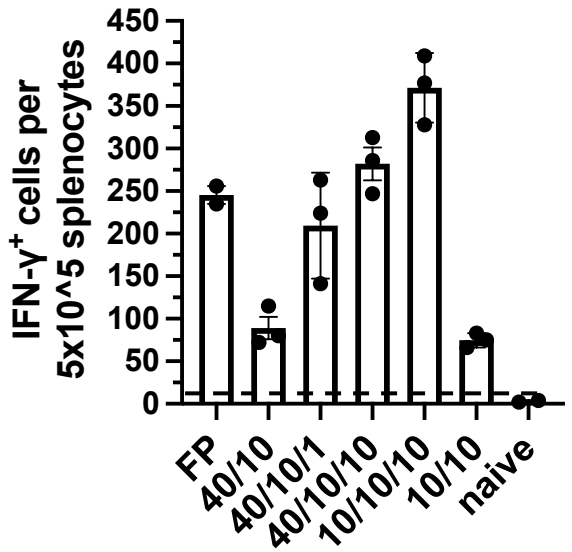
Group	N*	Treatment
Mice:	18	
1	3 (F)	**40ug** SVLP/PADRE(Cha) + **10ug** E7-flanked
2	3 (F)	**40ug** SVLP/PADRE(Cha) + **10ug** E7-flanked + **1ug** poly(I:C)
3	3 (F)	**40ug** SVLP/PADRE(Cha) + **10ug** E7-flanked + **10ug** poly(I:C)
4	3 (F)	**10ug** SVLP/PADRE(Cha) + **10ug** E7-flanked + **10ug** poly(I:C)
5	3(F)	**10ug** SVLP/PADRE(Cha) + **10ug** E7-flanked
6	2 (F)	FP 10µg + poly(I:C) 10µg + IFA (positive ctrl)
7	1 (F)	Naïve

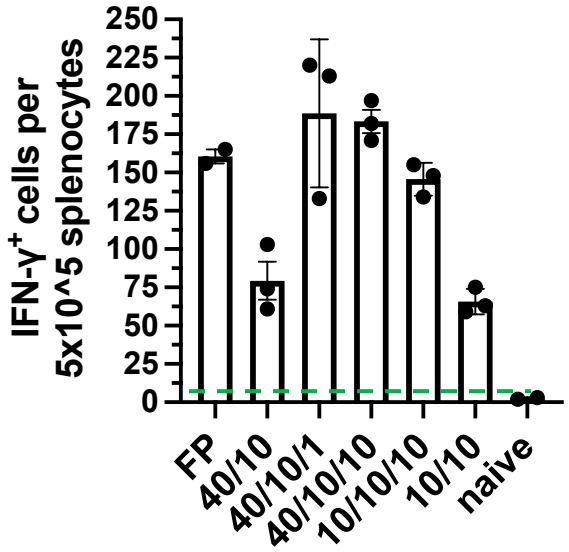
ELISPOT at 9 days after 3 weekly immunizations (45h).

T cell responses: **E7-flanked-specific**

PADRE-specific

IFN-γ

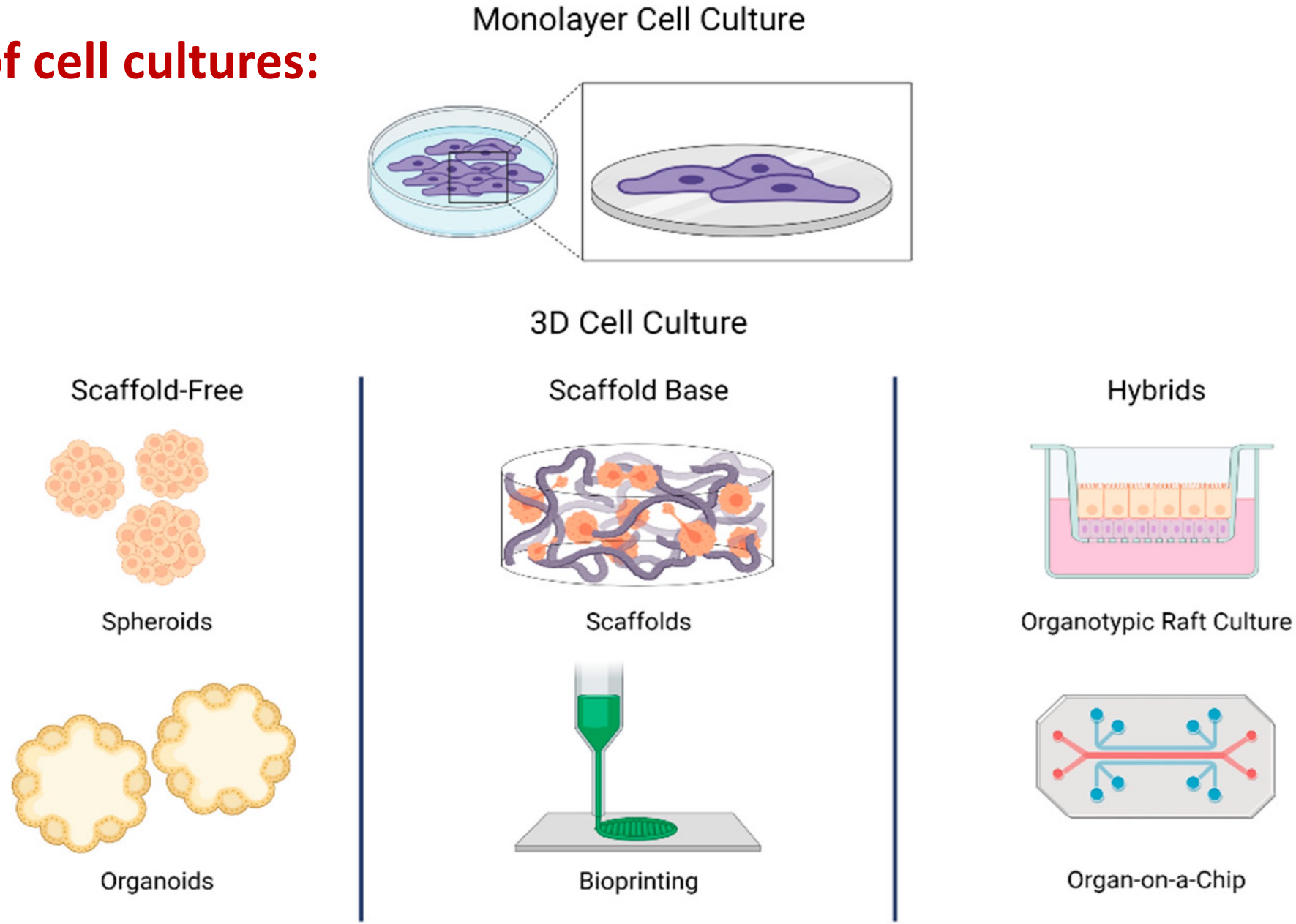




-Bigger spleens with 1ug/mouse of polyI:C.
-Bigger spleens/LNs with 10ug/mouse of polyI:C.

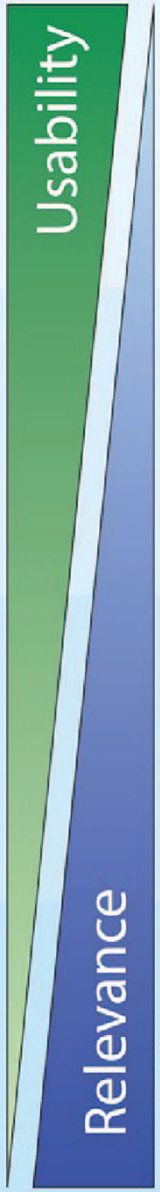
Cutoff value:	Confidence:
12,21	99.9%

Types of cell cultures:



de Dios-Figueroa et al. Biomedicines, 2021.

e.g. cancer cell cultures:



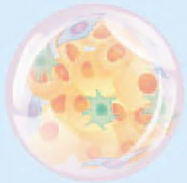
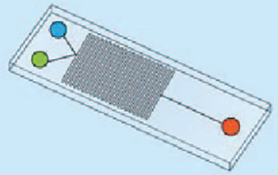
	Advantages	Disadvantages
2D cell culture 	• Standardised protocol • Cheap and simple • Can be automated • Compatible with high-throughput • Easily expandable • Compatible with various cell types	• Static conditions • No ECM and TME • No concentration gradient • Homogenous populations • Low physiological relevance • Not clinically predictive
3D cell culture 	• Efficacy • Drug resistance • Cell-cell and cell-ECM interactions • Sensitivity similar to in vivo • Co-culturing • Heterogenous	• Static environment • Low TME mimicry • Challenges to automate for high content screening • Inefficient waste and nutrient diffusion
Mice models 	• Efficacy • Drug resistance • Whole-body pharmokinetics • Side effects • TME mimicry • Genetically modifiable	• Immunodeficient (PDX) • Unable to upscale • Engraftment failures • Different pathophysiology to humans • Long tumour latency • Murine microenvironment
Clinical trials 	• Efficacy • Drug resistance • Whole-body pharmokinetics • Adverse reactions • Immune response • Route of administration • Highly clinically relevant	• Programs require collaborations between numerous professionals and experts • Long-term follow-ups • Variable patient retention • Challenges in patient recruitment processes • Difficulties in setting trials for rare cancers • Logistical and financial constraints

Law et al. Frontiers Oncology, 2021.

	2D cell culture	3D cell culture
Cell-Cell	Limited cell-cell interaction	Surrounding cell-cell interaction
Cell-ECM	No cell-ECM interaction	Cell-ECM interaction
Cell adhesion	Restricted on 2D plane	Dispersed in 3D
Mobility	Uninhibited dispersion and migration	Sterically hindered dispersion and migration
Scaffold	Glass or polystyrene	Physical structure with matrix
Modification	Non-modifiable sites	Modifiable sites

	2D cell culture	3D cell culture
Stiffness	Untunable - very high (GPa)	Tunable - low (kPa)
Soluble gradient	Absent	Present
Drug resistance	Non-representative	Sensitivity similar to *in vivo*
Cell cycle stage	Cells all at same stage	Cells at various stages
Phenotypic diversity	Conforming	Diverse

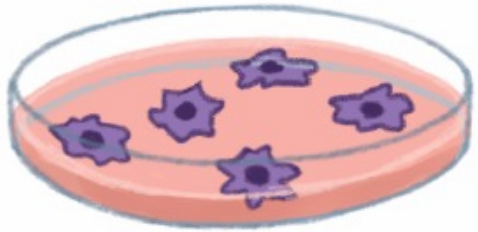
Law et al. Frontiers Oncology, 2021.

3D Culture Techniques	 Suspension Culture	 Hydrogel Scaffold	 Organ-on-chip	 3D bioprinting
Advantages	• Standardised protocols • Cheap and simple • Can be automated • Adaptable for high-throughput • Replicable	• Mimics TME • Relatively cheap and simple • Can be automated • Adaptable for high-throughput • Versatile application and hydrogel availability • Tunable properties	• Dynamic fluidics and perfusion • Simulate physiological processes (eg heartbeat) • Engineered vascularisation • Precise control over microenvironment • Model drug delivery systems	• High precision and resolution (100um) • Construct highly complex tissue structure • Can be automated • Fine tuning of tissue architecture and size • Various bioinks available
Disadvantages	• Simple TME • Static • Inefficient nutrient and waste diffusion • Spheroid sizes can vary depending on technique	• Simple architecture • Batch-to-batch variability (Natural hydrogels) • Require biofunctionalisation (Synthetic hydrogels) • Static • Inefficient nutrient and waste diffusion	• High expertise barrier • Difficult to upscale • Requires specialised equipments • Not suitable for long-term experiments	• High expertise barrier • Difficult to upscale • Expensive • Requires specialised equipments • Cell viability can vary depending on technique

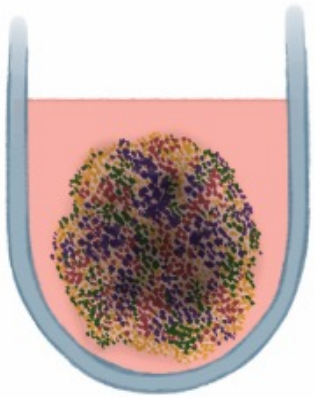
Law et al. Frontiers Oncology, 2021.

Three-Dimensional (3D) Cell Culture (Microphysiological-MPS) Platforms as Drug Development Tools

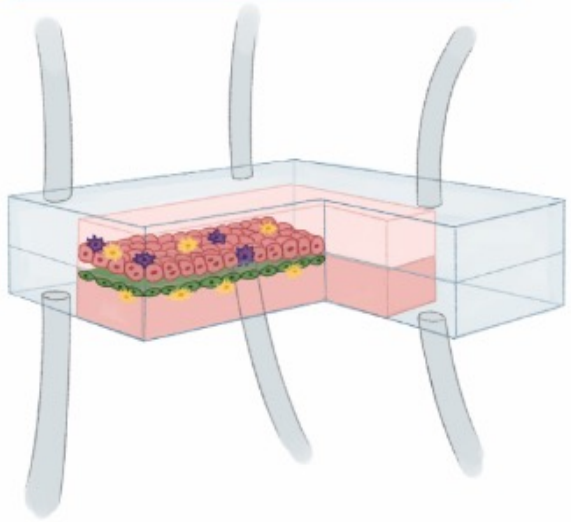
2D cultures → **3D organoids** → **microphysiological systems (MPS)** → … → **model multi-organ physiology**



- cells
- culture media, dishes, plates, wells
- extracellular adhesive proteins

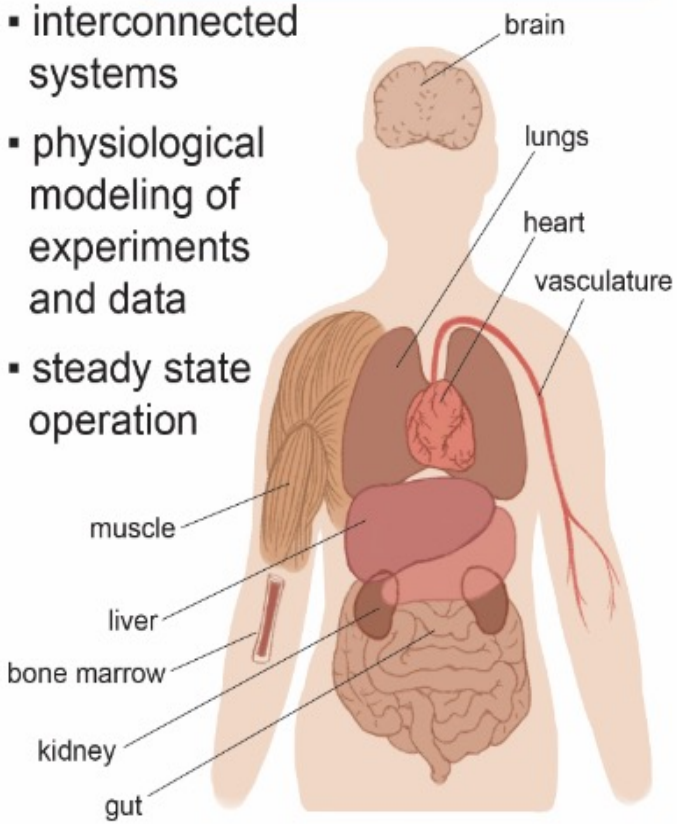


- multiple cell types
- cell-cell interactions
- extracellular proteins
- cell-induced extracellular remodeling

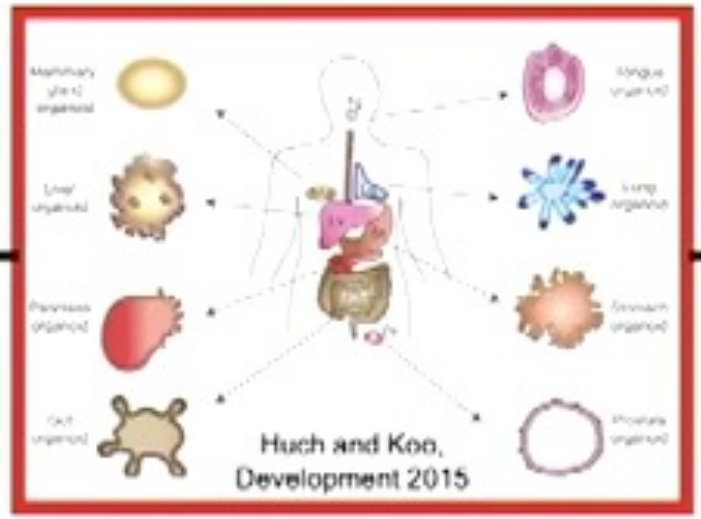


- organoid technology
- microfabrication of dedicated compartments
- microfluidic circulation of media

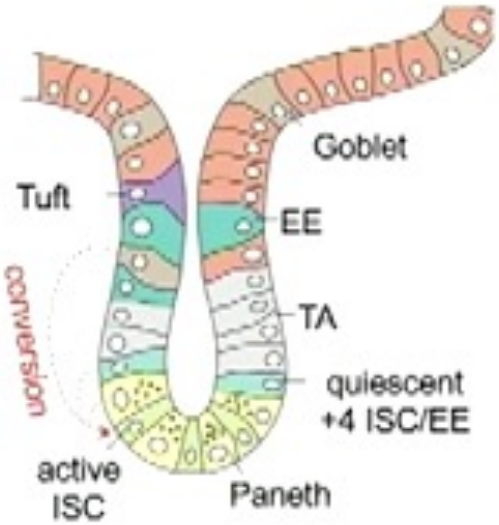
- interconnected systems
- physiological modeling of experiments and data
- steady state operation



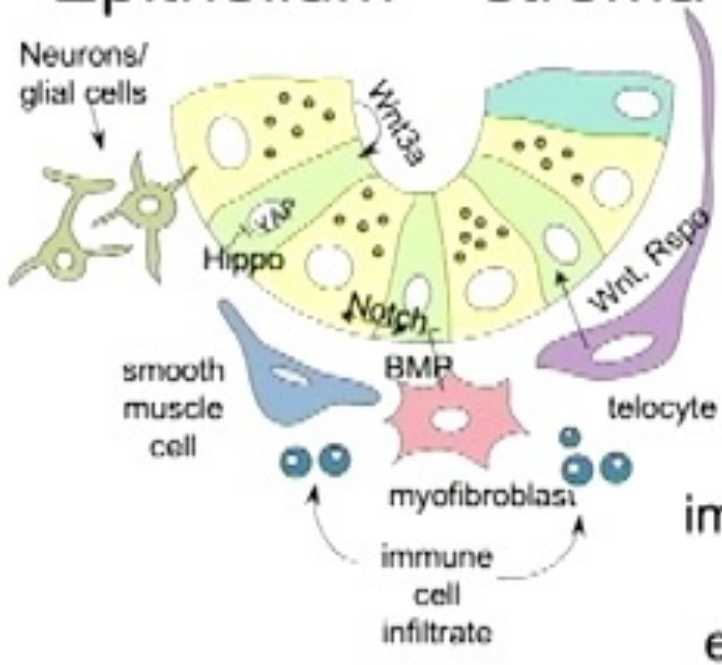
Adult human organoids



Epithelial cells only

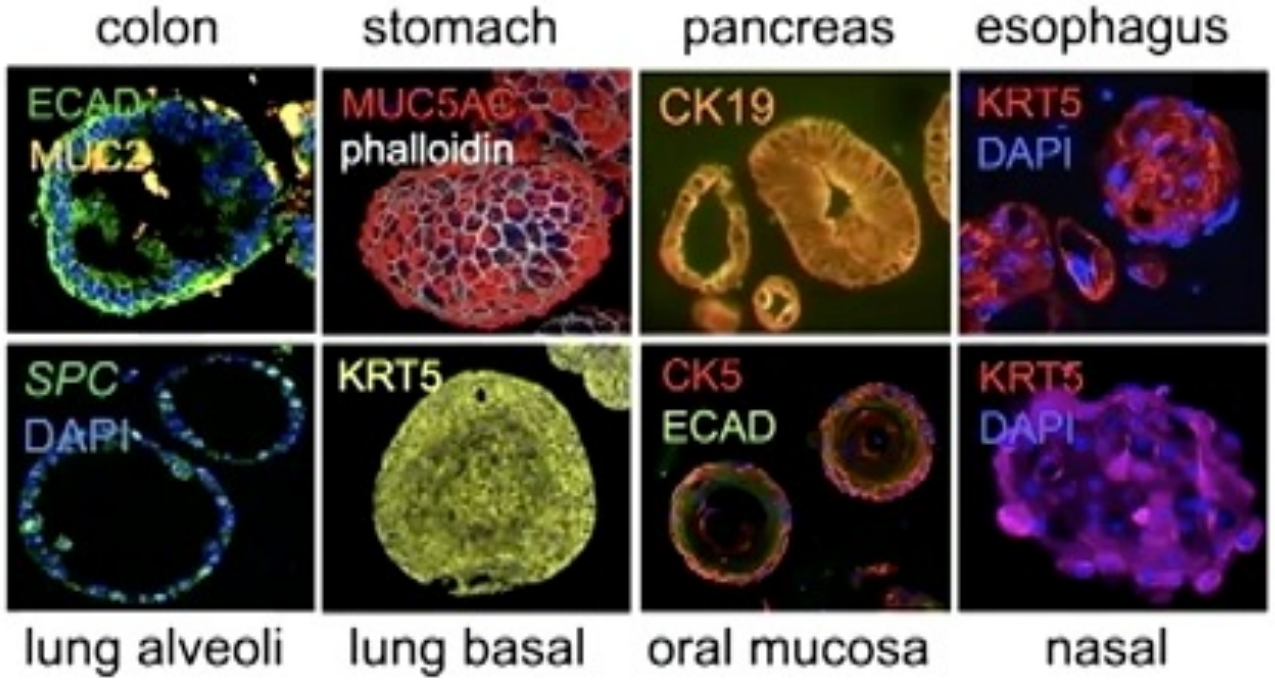


Epithelium + stroma



fibroblasts
immune cells
neurons
endothelium

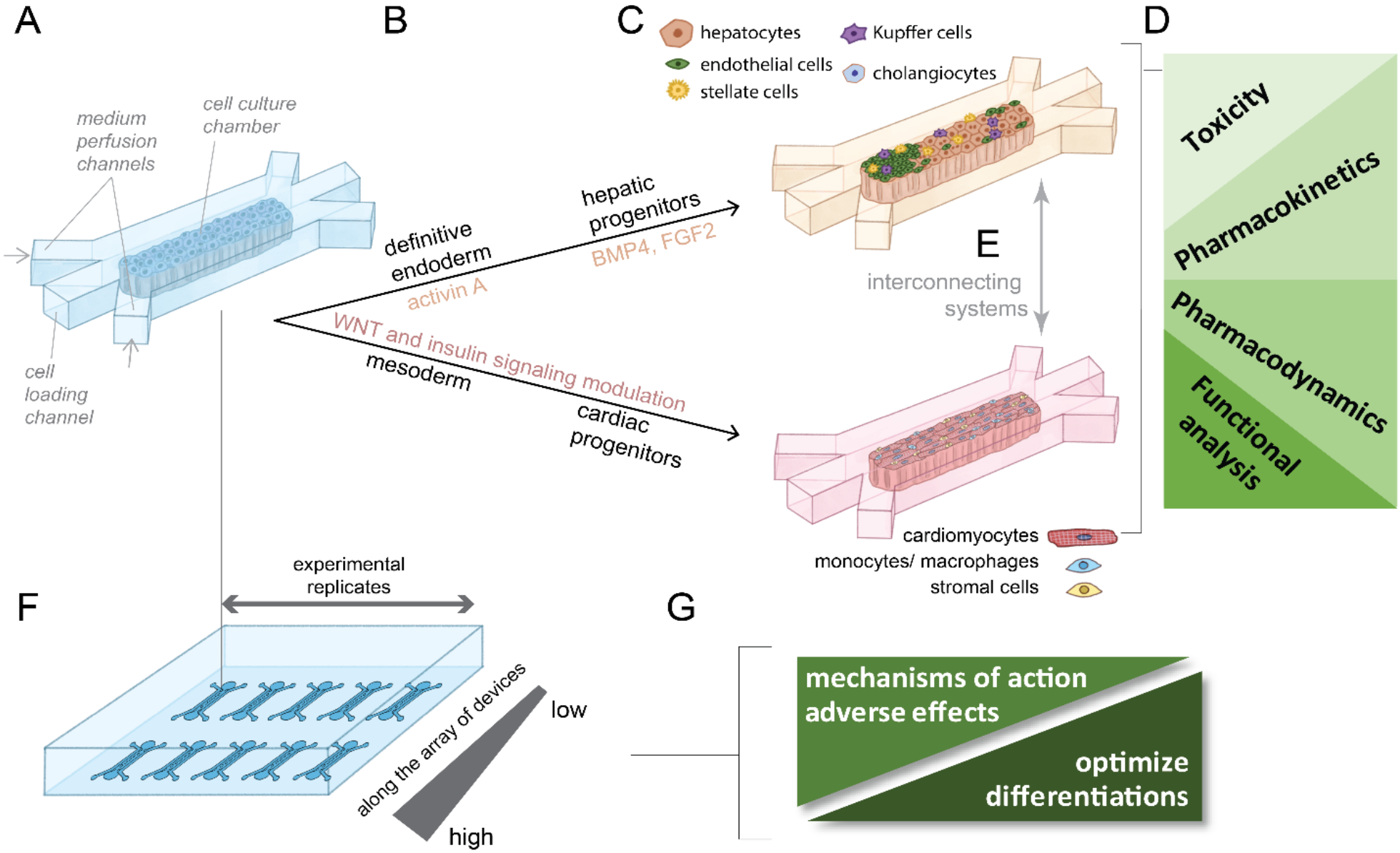
“Epithelial-only” organoid culture of diverse human tissues



Many others

A Focus on Liver and Cardiac Platforms

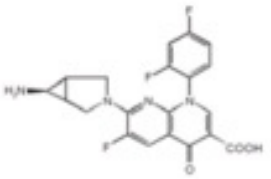
maintenance of iPSCs | differentiation & maturation methods | use in drug evaluation



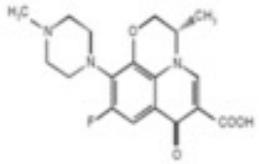
high throughput arrays | testing applications

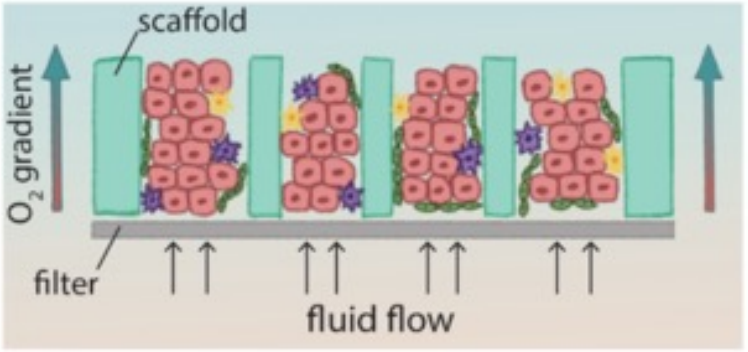
Quality Control Criteria to Ensure the Reproducibility of Liver Microphysiological Systems and Engineered Heart Tissues

Trovafloxacin
(Hepatotoxic)



Levofloxacin
(Not Hepatotoxic)





Liver system

- Similar Results Between Two Sites

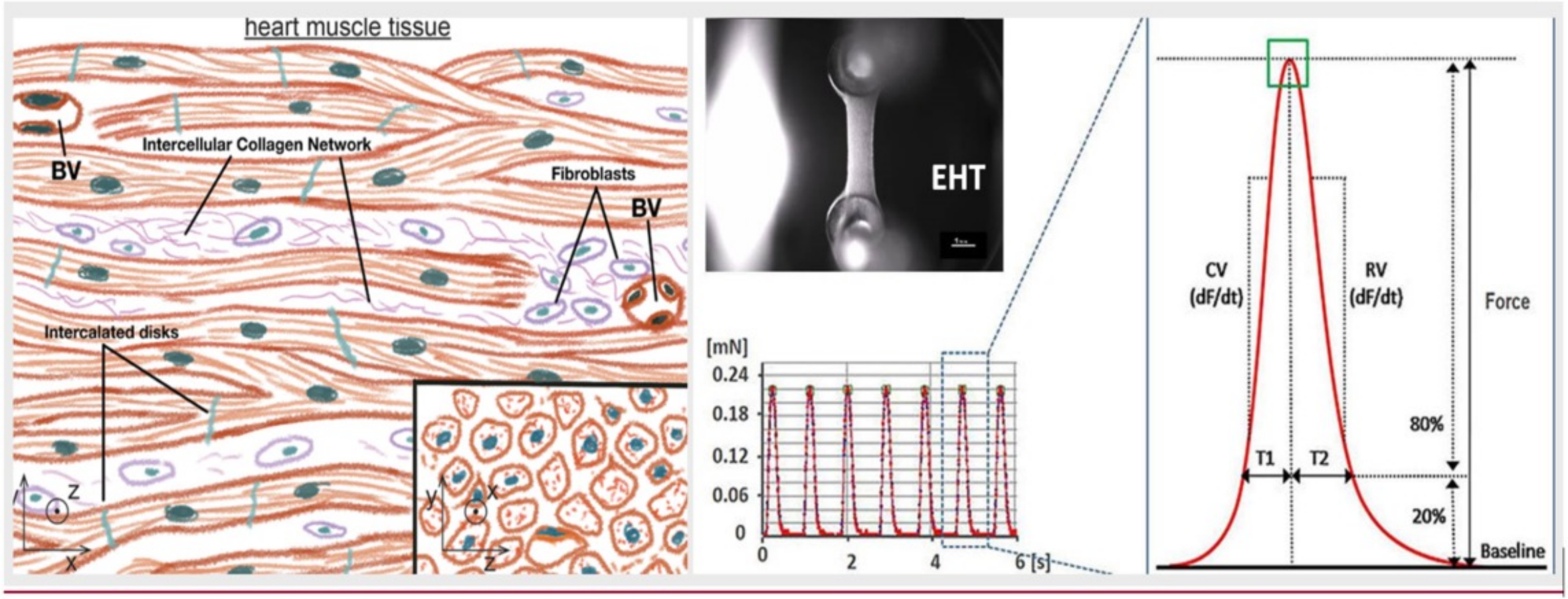
- Similar Results Within a Site When Using Different Batches of Kuppfer Cells

Batch 1

Batch 2

- Identified Quality Control Criteria for Kuppfer Cells

Engineered heart tissues (EHTs) recapitulate the 3D nature of heart muscle tissue (e.g., contractile alignment), as shown on the blood vessels, (BVs). By immobilizing these tissues between force sensors, researchers analyzed drug-induced variations in the contractility of these platforms and derived functional parameters.

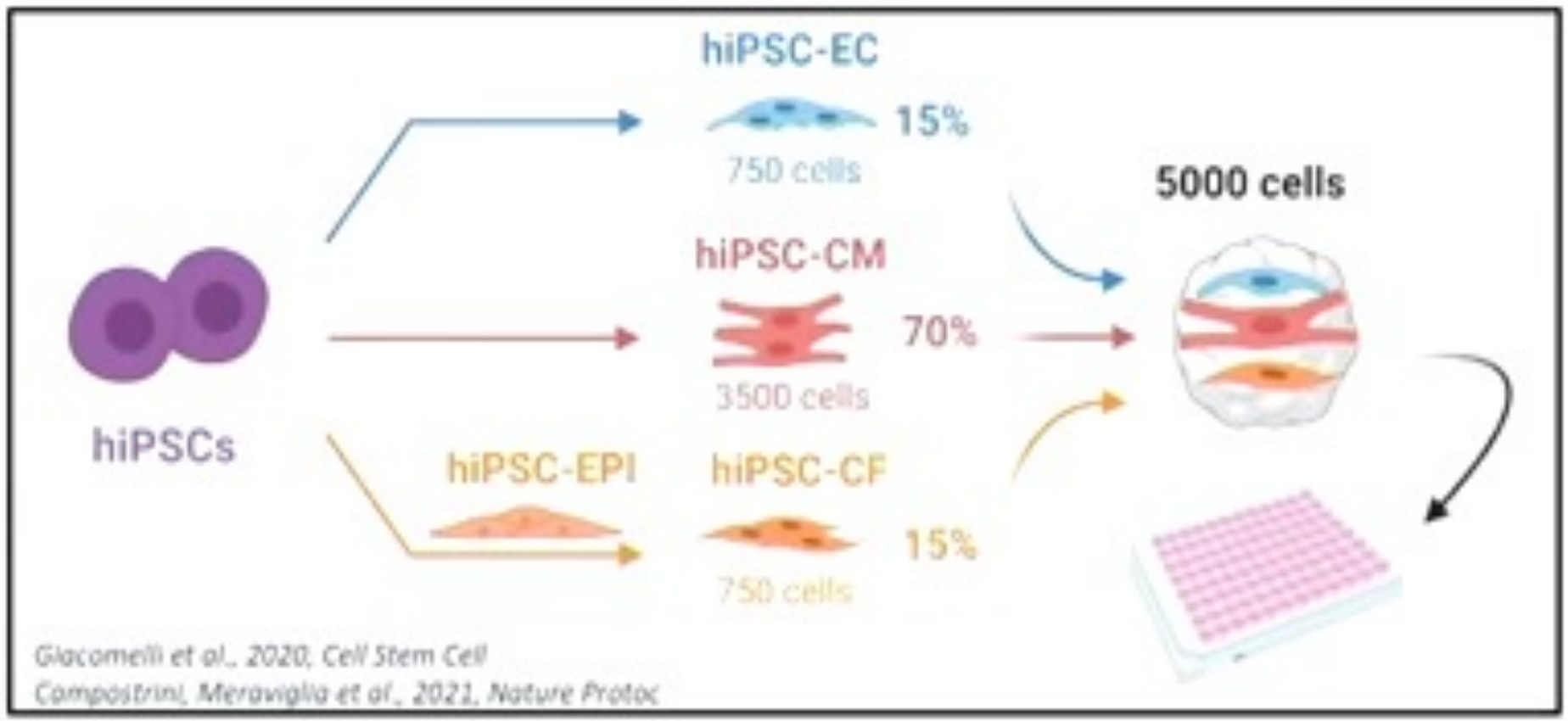


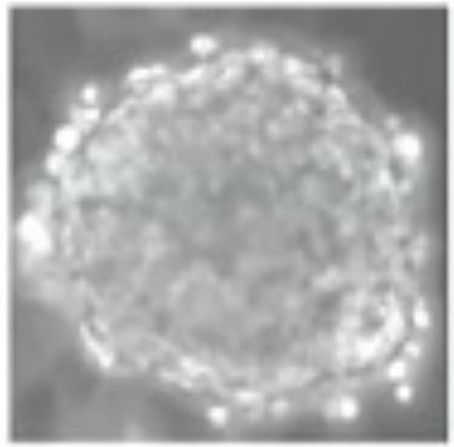
Cell Stem Cell

CellPress
OPEN ACCESS

Article

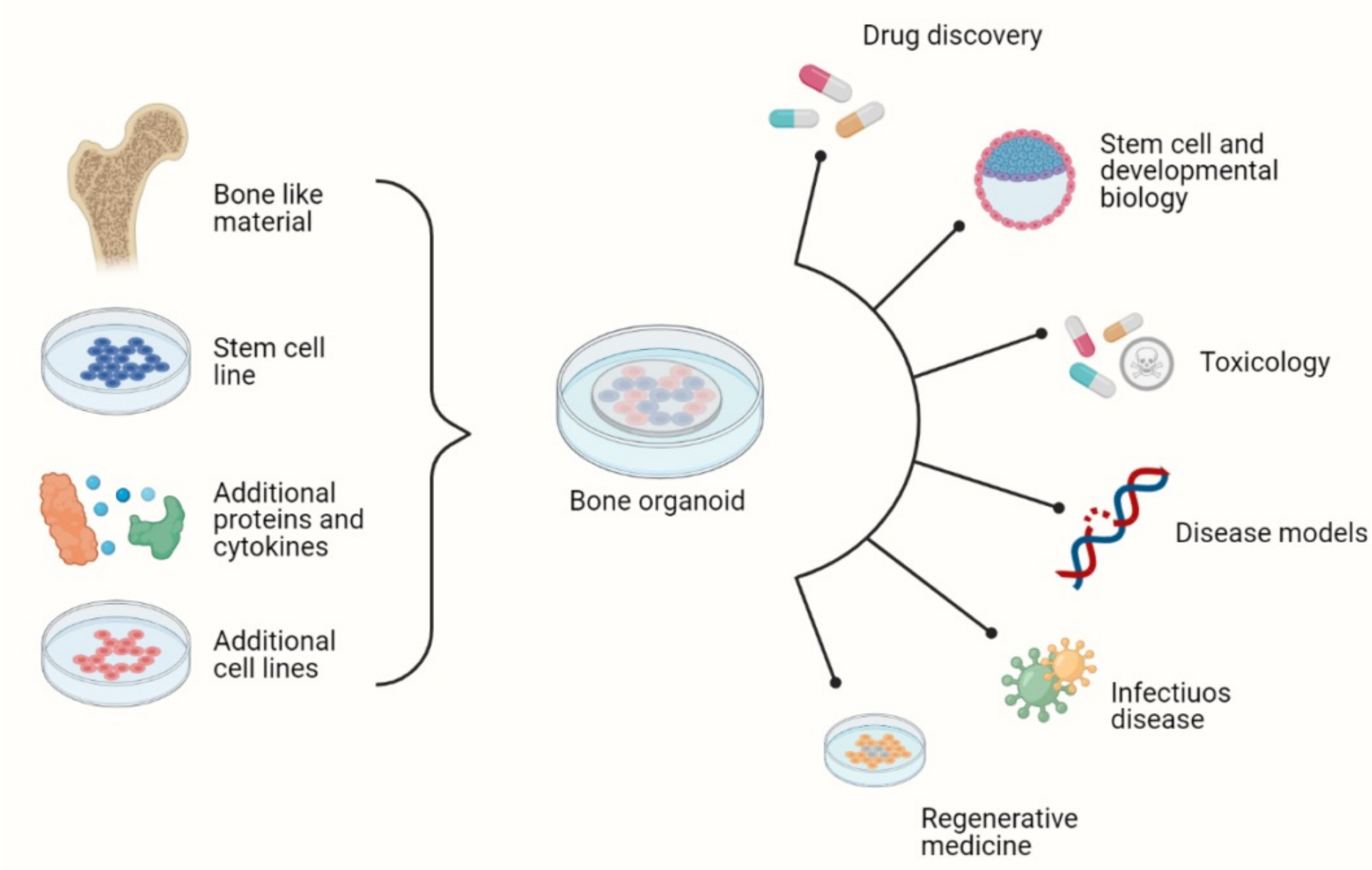
Human-iPSC-Derived Cardiac Stromal Cells Enhance Maturation in 3D Cardiac Microtissues and Reveal Non-cardiomyocyte Contributions to Heart Disease





- Simple
- Works using cryopreserved cells
- Low tech

Bone organoids



Growing organoids from clinical cancer specimens

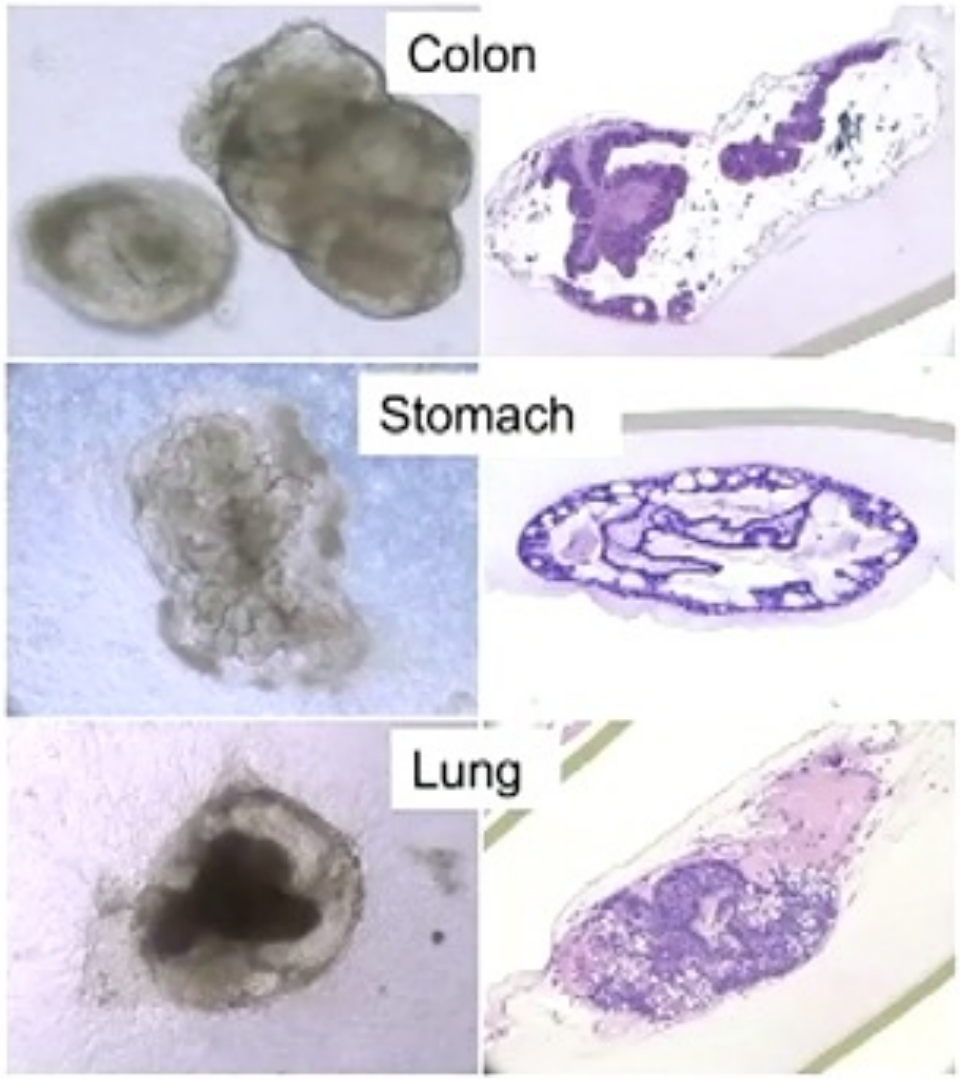
Law Andrew M. K., Rodriguez de la Fuente Laura, Grundy Thomas J., Fang Guocheng, Valdes-Mora Fatima, Gallego-Ortega David

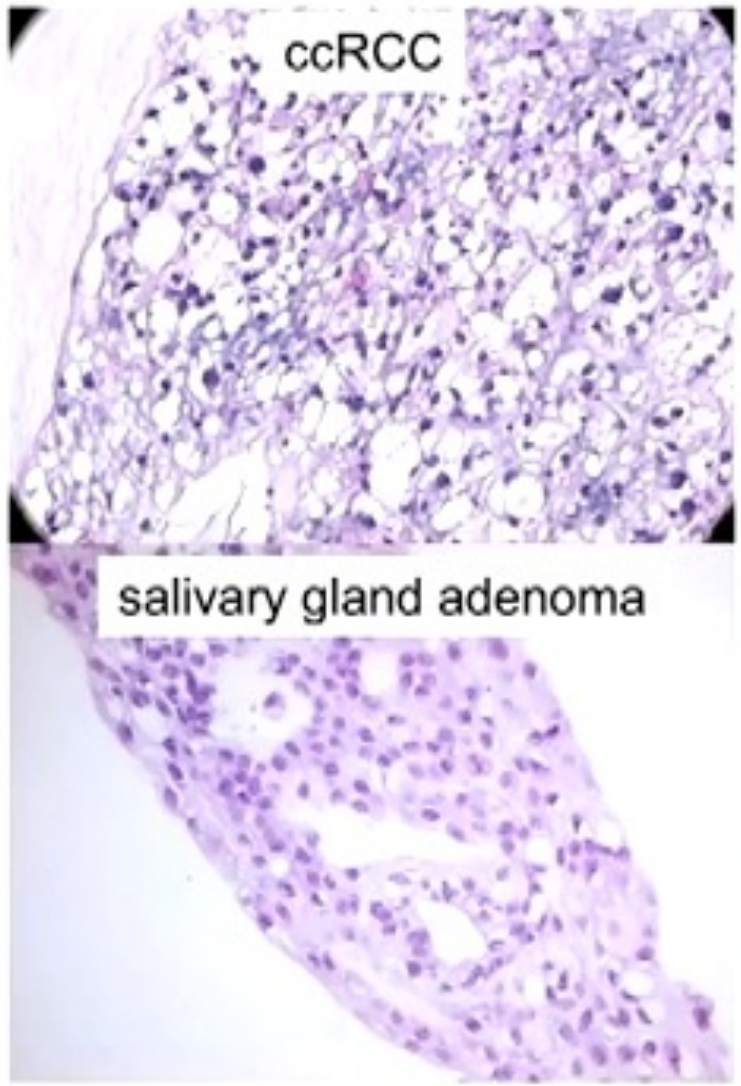
Advancements in 3D Cell Culture Systems for Personalizing Anti-Cancer Therapies

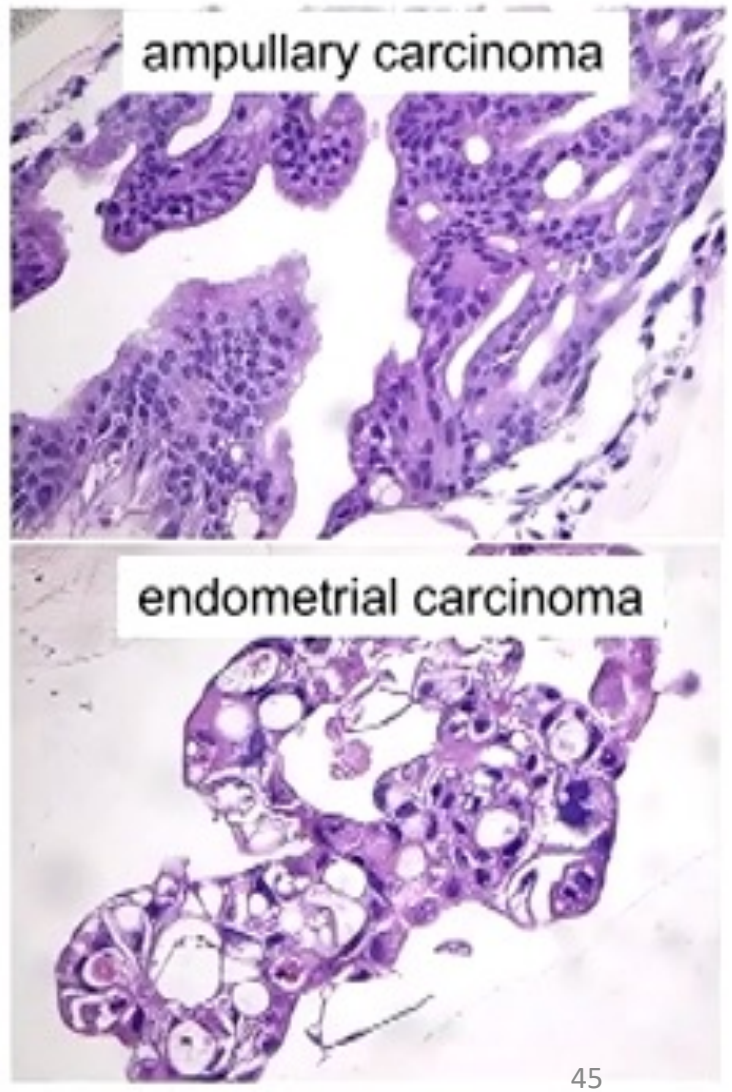
Frontiers in Oncology 2021

Over 90% of potential anti-cancer drug candidates results in translational failures in clinical trials. The main reason for this failure can be attributed to the non-accurate pre-clinical models that are being currently used for drug development and in personalised therapies. To ensure that the assessment of drug efficacy and their mechanism of action have clinical translatability, the complexity of the tumor microenvironment needs to be properly modelled. 3D culture models are emerging as a powerful research tool that recapitulates in vivo characteristics. Technological advancements in this field show promising application in improving drug discovery, pre-clinical validation, and precision medicine. In this review, we discuss the significance of the tumor microenvironment and its impact on therapy success, the current developments of 3D culture, and the opportunities that advancements that in vitro technologies can provide to improve cancer therapeutics.

Patient-Derived tumor Organoids (PDOs) from diverse neoplasms

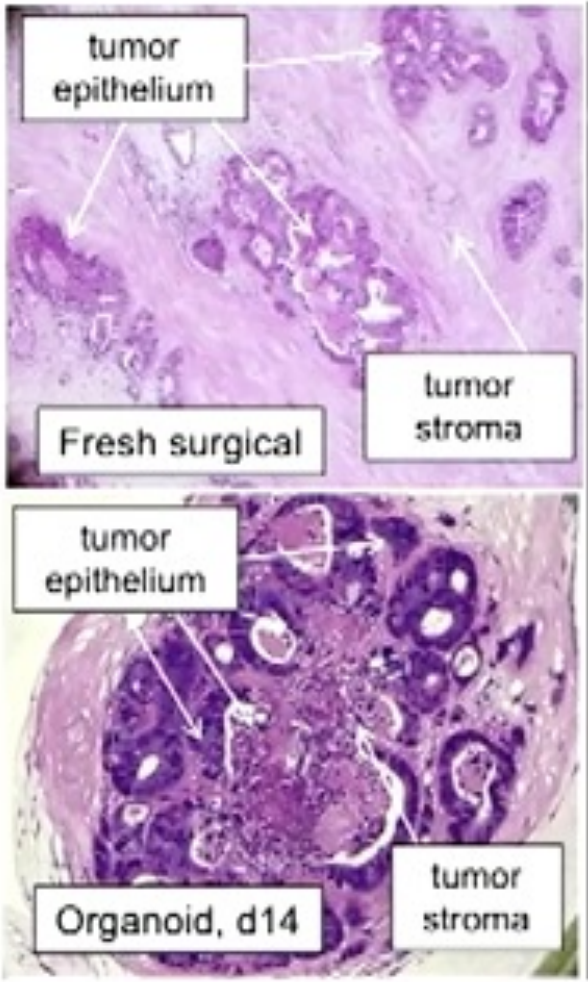






PDO tumor organoids recapitulate fibroblast stroma

Colon adenoCa PDO

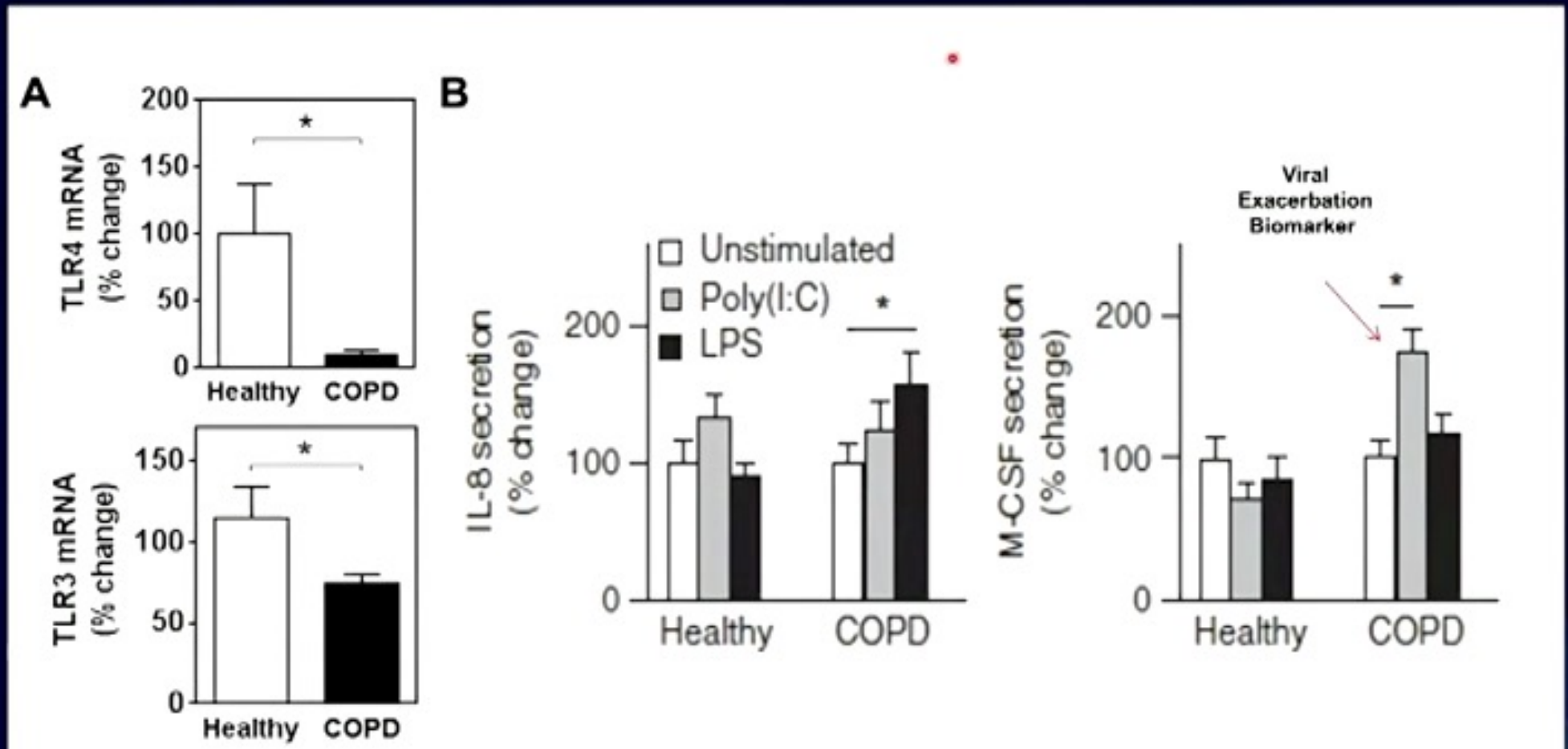


Lung adenoCa PDO

Chips Lined by Cells from COPD Patients

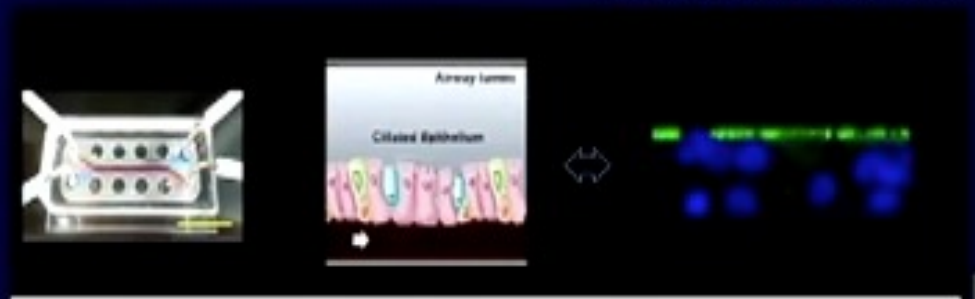
(COPD = Chronic Obstructive Pulmonary Disease)

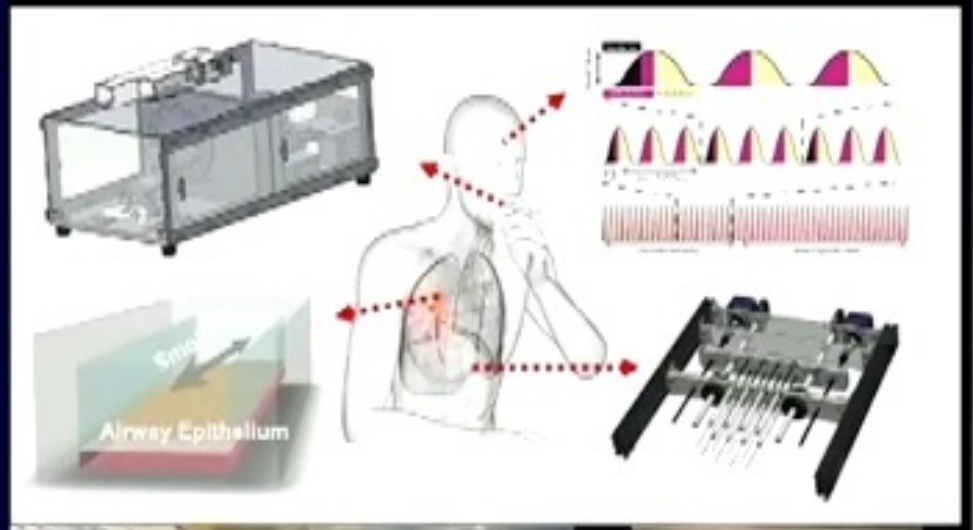
Human COPD *Exacerbations* Recapitulated On-Chip

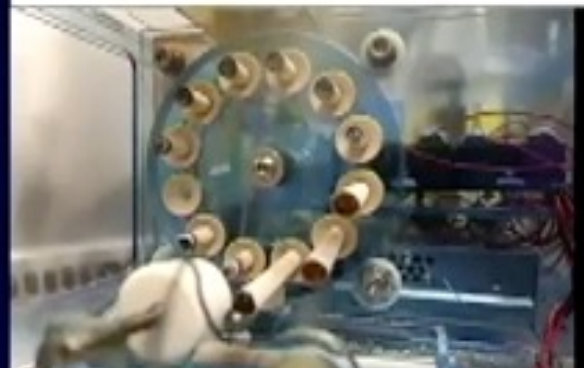


Cigarette Smoke Exposure in Airway Chips

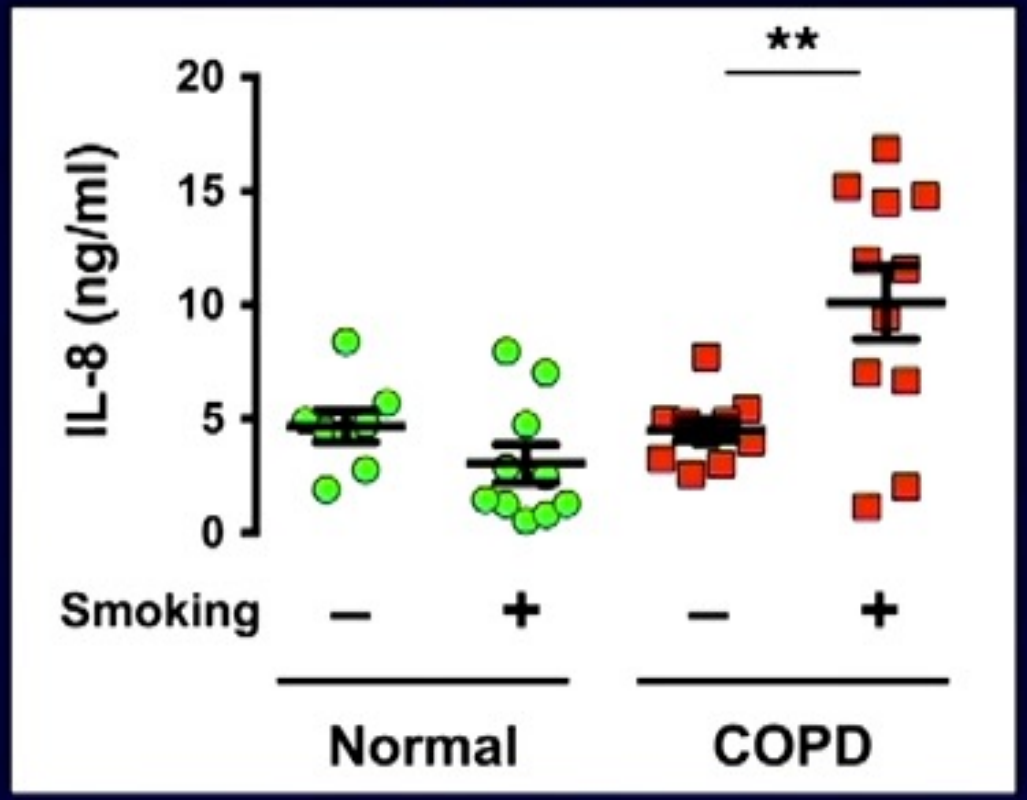
(KAMBEZ BENAM et al. *Cell Syst.* 2016)

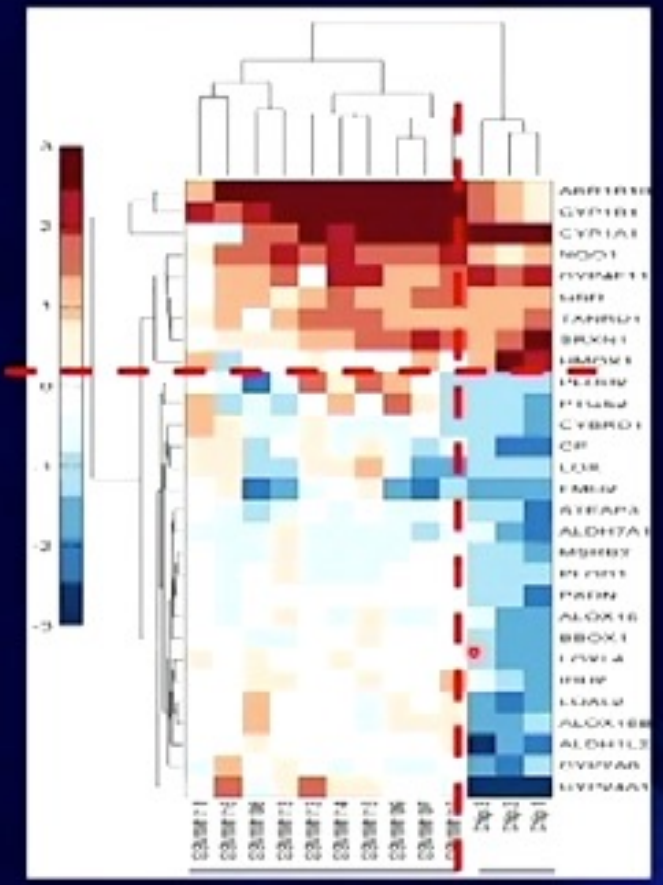






Cigarette Smoke: Matched Comparative Modeling in Lung Chips

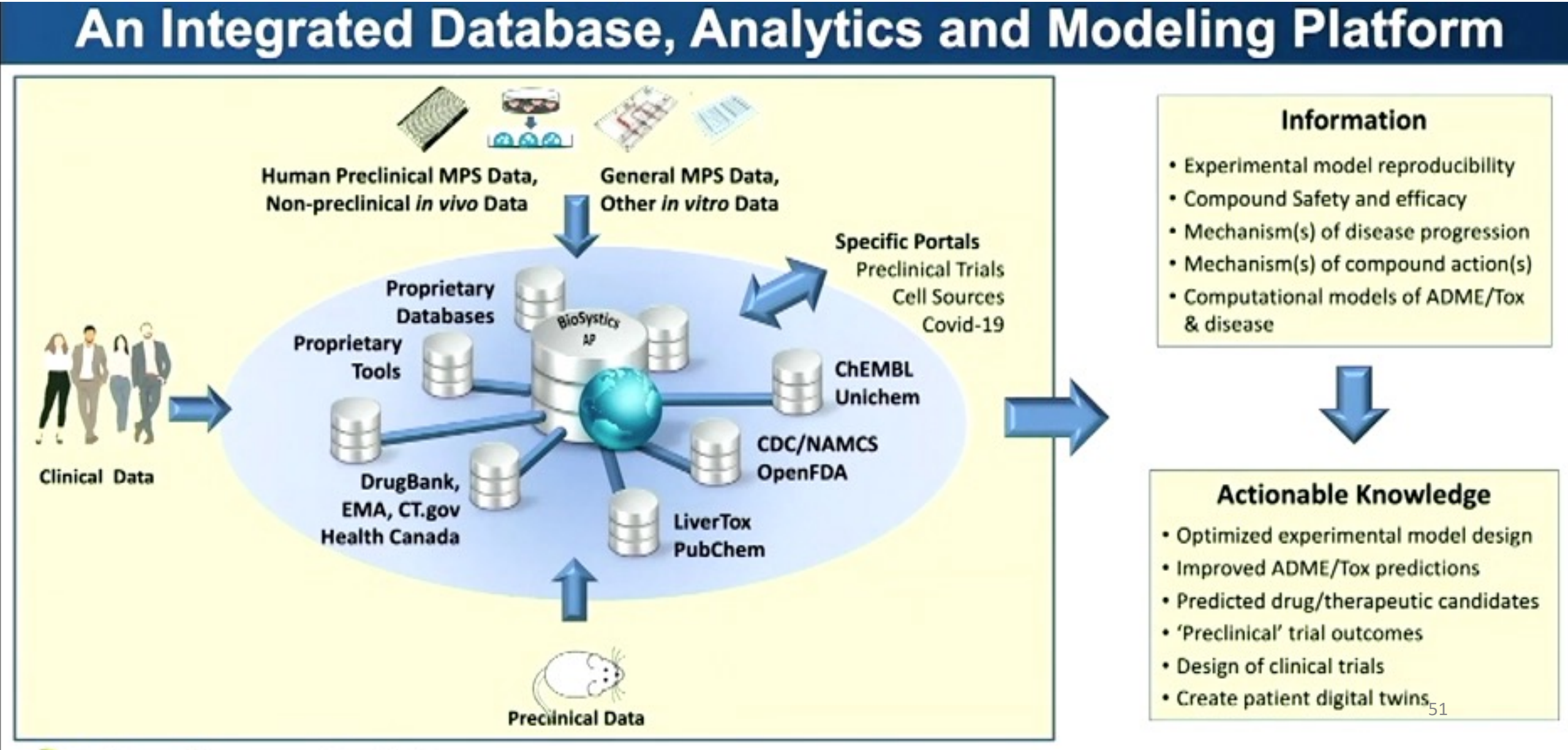




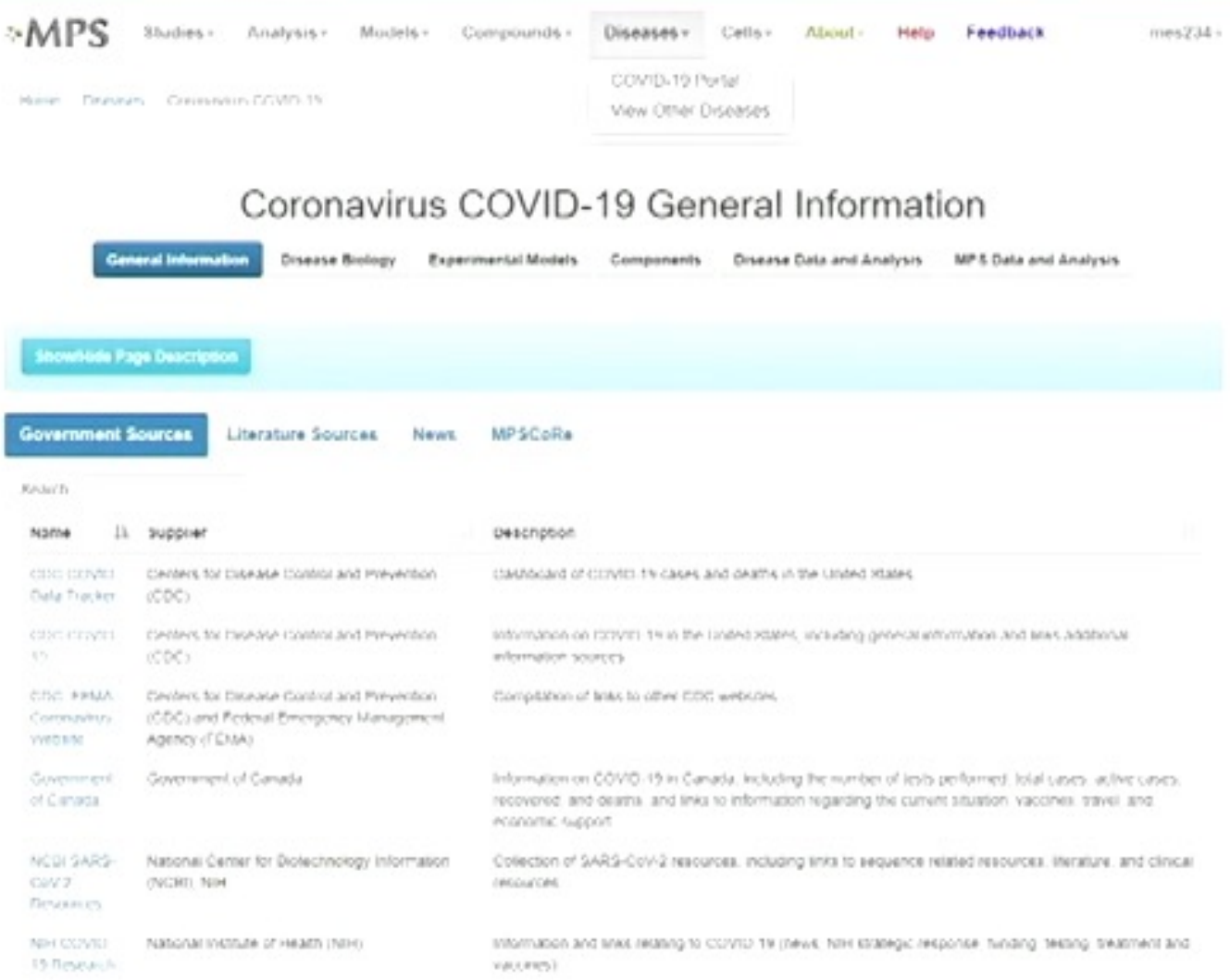
Take Home Message

- *Human Organ Chips are more than potential animal replacements*
- *They are also Mechanistic Drug Discovery Tools that*
  - *can provide new insight into human pathophysiology*
  - *can be combined with advanced analytical approaches*
  - *enable rapid drug repurposing*
  - *accelerate discovery of novel therapeutics*

MPS DB: The BioSystics Analytics Platform



COVID-19 Disease Model Portal Provides Easy and Rapid Access to a Wide Range of Disease Resources



Extensive links to information on:

- General COVID-19 information
  - Including literature hubs
- COVID-19 disease biology
- Experimental models
  - Both *in vitro* and *in vivo*
- Components to implement COVID-19 studies
  - Direct links to vendor sources
- Preclinical and clinical data
- Computational modeling resources
- COVID-19 data in the MPS-Db

Designed to be a central repository for sharing all COVID-19 experimental data

iPSC Portal

- Resource that allows users to share information about available iPSC-derived cells
- Resource for finding available iPCS and iPSC derived cells that can be used for specific applications such as the development of COVID-19 experimental models
- Provide information about the characteristics of the cells
  - Mutation status
  - Maturity level
  - Engineered features
  - Etc.
- Provide information and links to obtain the cell samples
- Provide information on how to use the cells
  - Differentiation protocols
  - Culturing protocols

Provide information needed to quickly identify and decide on an appropriate iPSC sample or iPSC derived cell for your model, where to obtain the cells, and how to use them.

ach iPS-cell instance include:

Characteristics of the iPSCs
- Differentiated cell type(s)
- Differentiated phenotypes
- Differentiated maturity level
- Functional profiles

Patient profile
- Demographic
- Known disease conditions
- Genomic abnormalities

Source of cells
- Vendor
- Collaborator
- Others

Protocols
- Isolation of patient cells (e.g. skin, blood, other cells)
- Differentiation to target cell type
- Preparation for model

Established applications
- Healthy tissue/organ model(s)
- Disease tissue/organ model(s)

Neutralization Assays

Advantages *versus* Drawbacks

- Long history, many options
- Can be automated for routine use
- Derive activity-concentration relationships
- No animal use

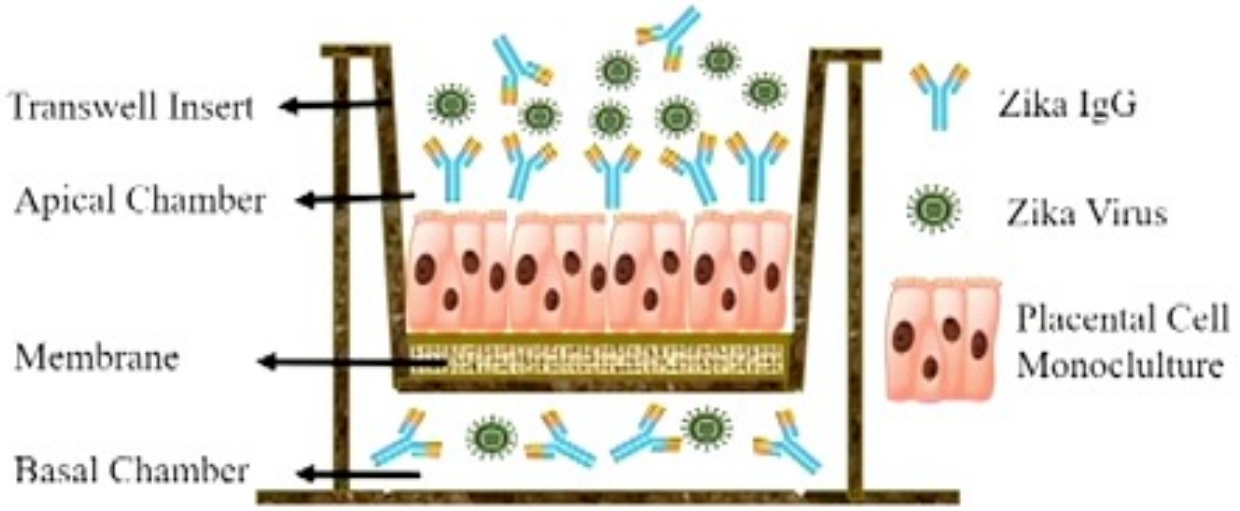
- Simplified models (one cell line)
- No spatial/temporal information
- Converting active concentration(s) to dose can be a challenge
  - i.e. pregnancy

MPS as a bridge between neutralization and *in vivo* assays

In vitro Models of Placenta Barrier: Stepwise Approach

Monocultures

- Choriocarcinoma cells (trophoblast origin):
  - BeWo, JEG-3
  - Form confluent polarized layers suitable for transport studies



Co-cultures

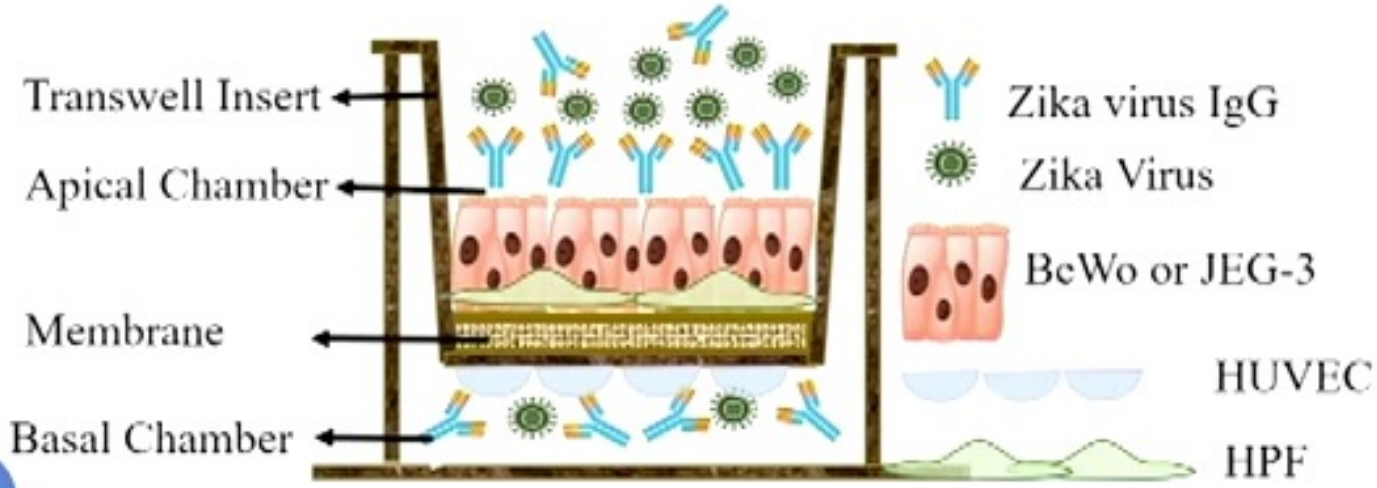
Three-dimensional cultures

In vitro Models of Placenta Barrier: Co-Cultures of Placental Cells

Monocultures

Co-cultures

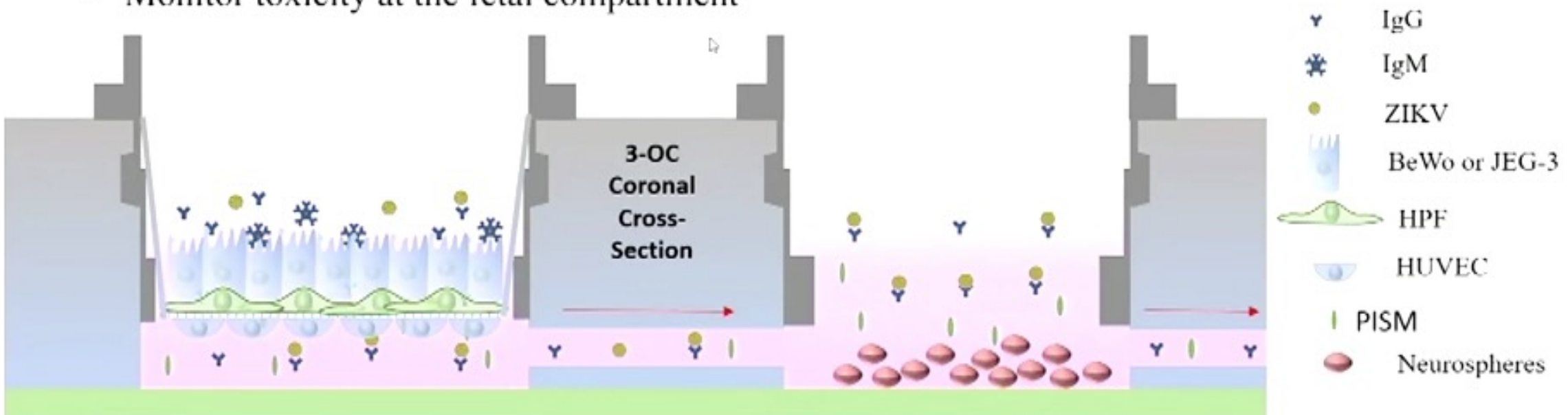
Three-dimensional cultures



- **Choriocarcinoma Cells (BeWo, JEG-3)**
- **Human Fetal Endothelial Cells (HUVEC)**
- **Human Placenta Fibroblasts (HPF, Hs 795.PI)**

Human-Based Placental Barrier MPS for ZIKV Infection

- Compartment 1: Replicate placental barrier
- Compartment 2: Fetal compartment containing tissues susceptible to ZIKV infection (neurospheres)
- Treat placental barrier with ZIKV with and without antiviral Ig
  - Monitor placental cytotoxicity, metabolic function, transcytosis and pro-inflammatory signaling molecules
  - Monitor toxicity at the fetal compartment



ZIKV infected placental barrier MPS

Kidney Spheroids or Neurospheres

19

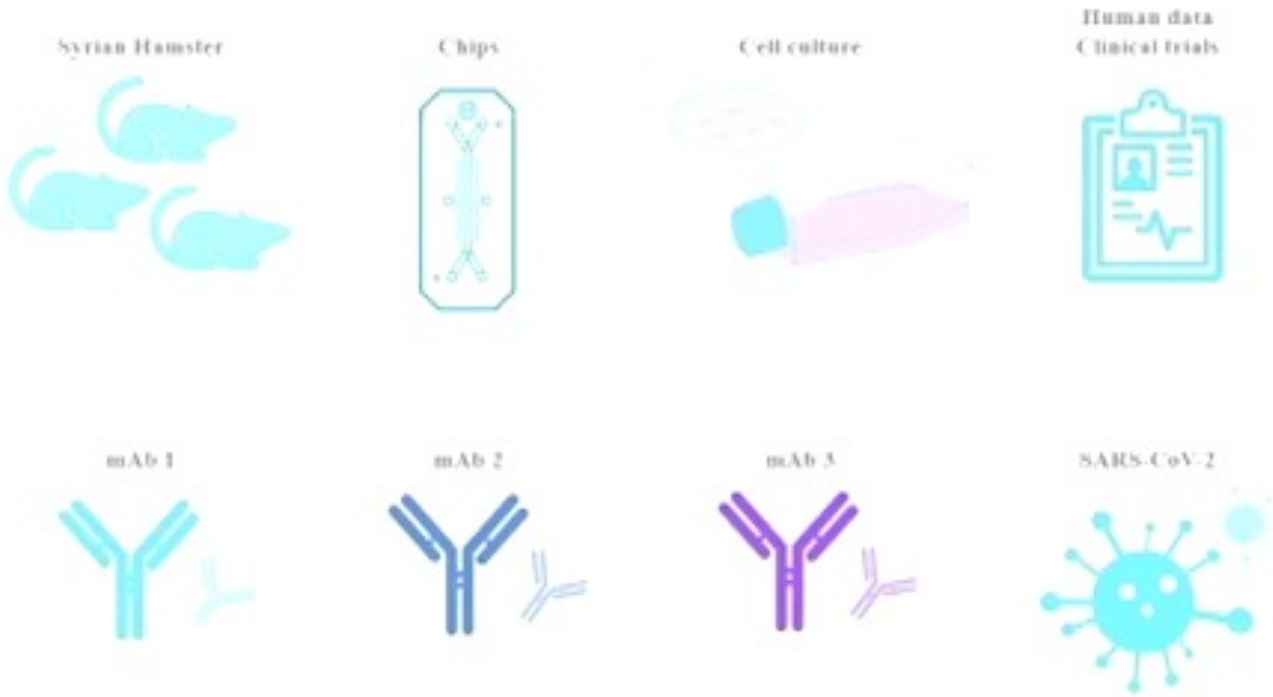
Considerations when Submitting MPS Derived Data

Tab. 1: Aspects to consider when evaluating a new technology

- Does an assay provide data that can be used to answer fundamental drug development questions?
- Is the assay mature enough?
  - Is the platform stable (e.g. biologic function, timeframe)?
  - Are cells available and characterized?
- What endpoints are being measured?
  - Are they predictive of *in vivo* effects?
  - Are they translatable to human?
- Has scientific validity been shown?
  - Is the method reproducible?
  - What test compounds have been assessed?
    - Has data been compared with *in vivo* data?
    - What positives and negatives were used?
- Has the applicability domain been defined?
  - Have the compounds that the assay can assess/not assess been defined?
- Have criteria for success been defined and met?
  - What is the accuracy, sensitivity, and specificity?

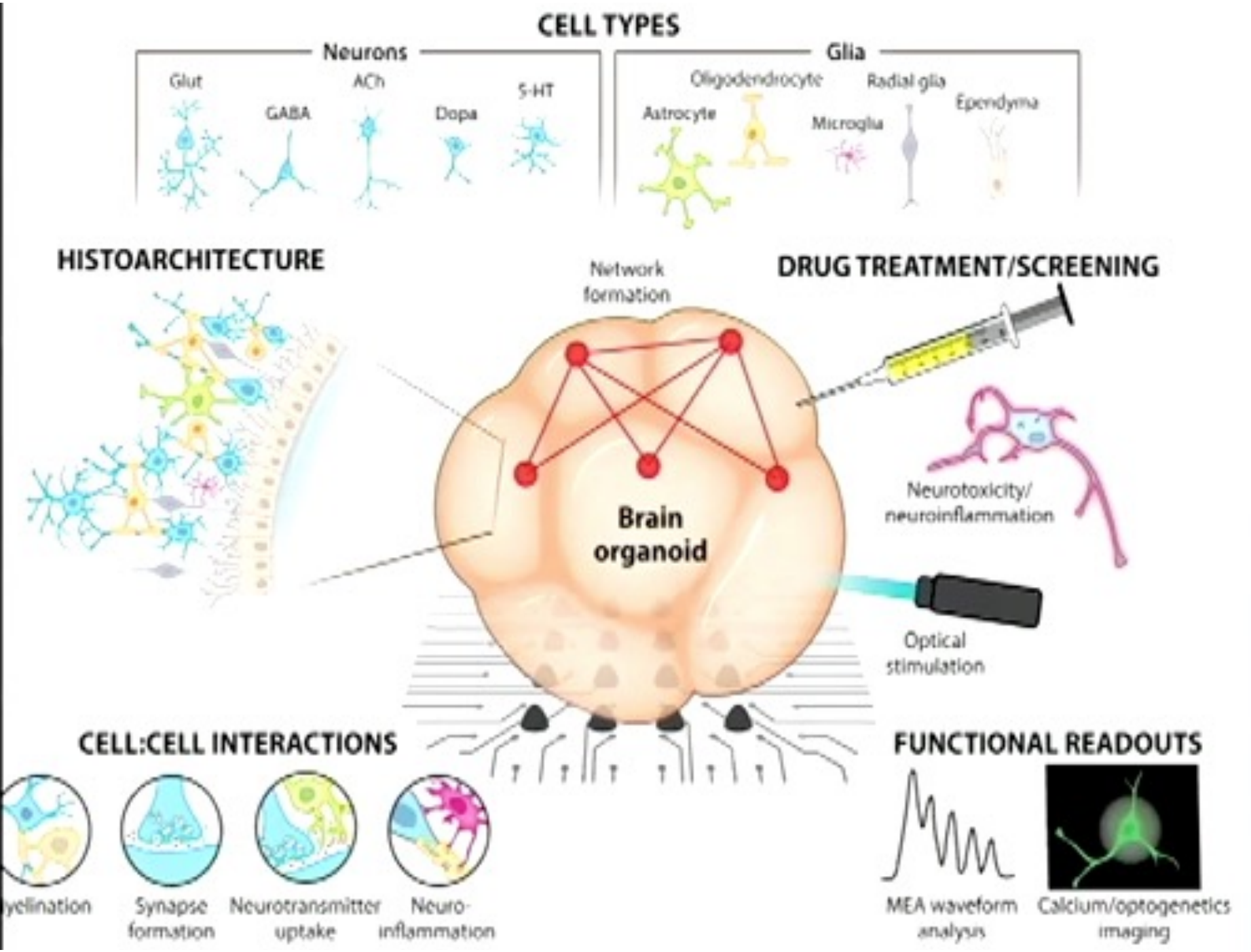
Baran et al. ALTEX, 2022, doi:10.14573/altex.2112203.

Proof-of-concept System Comparison

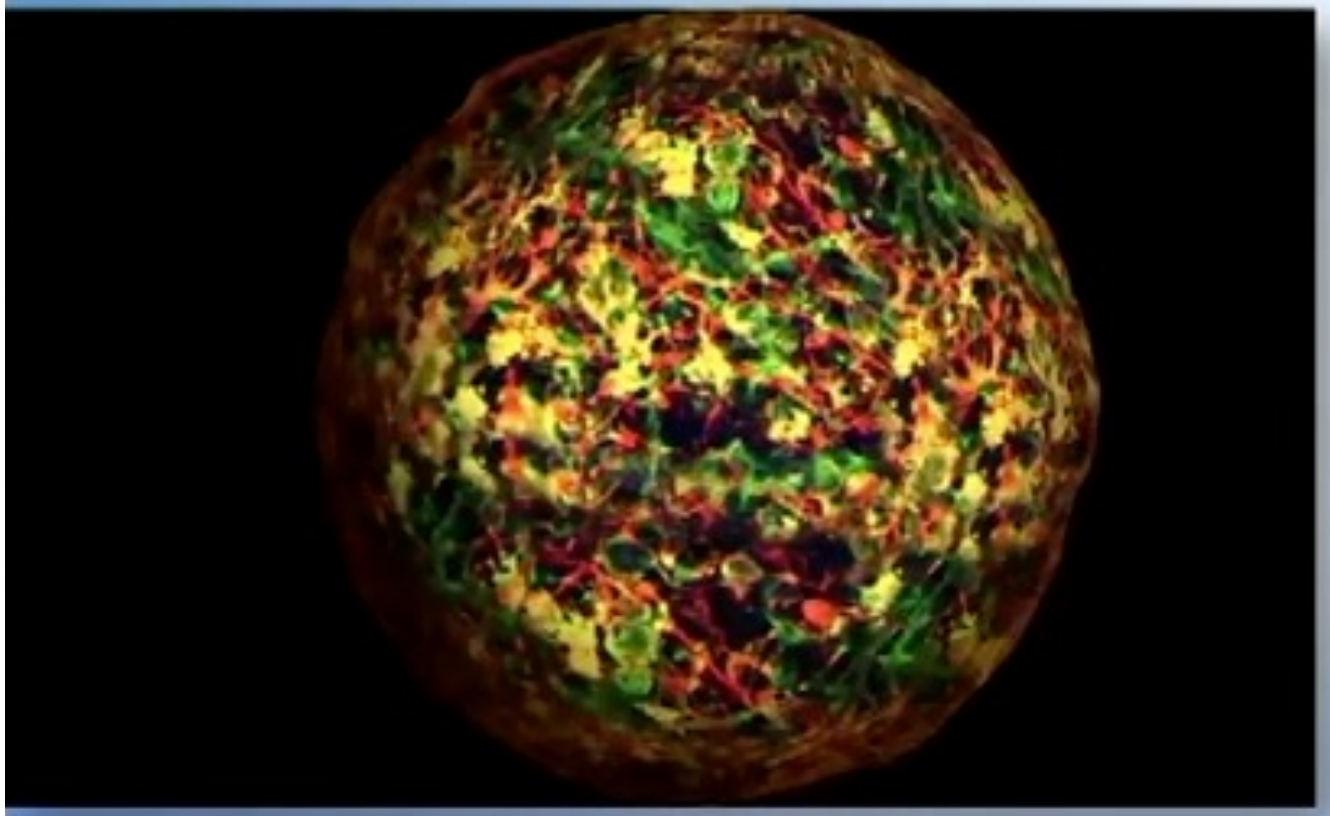


- Test novel therapeutics (e.g. human-derived synthetic Ab) in MPS models and compare with pre/clinical data.
- Side-by-side evaluation of low and high complexity models through comparison of the following data:
  - In vitro cell culture systems
  - Organ-Chip technology (lung airway models)
  - Low-level animal model (golden hamster)
  - Human data from clinical trials
- Led by NIAID/IRF, partially supported by DoD (training) and NICEATM (chips, study design assistance)

Brain organoid applications



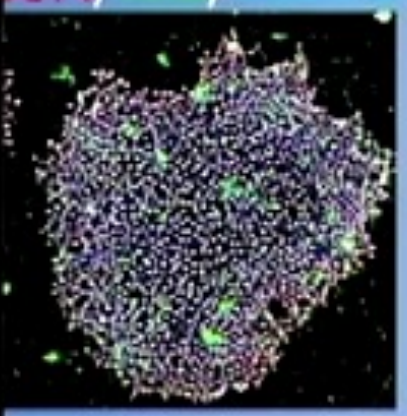
BrainSpheres: iPSC-derived human organotypic brain cultures



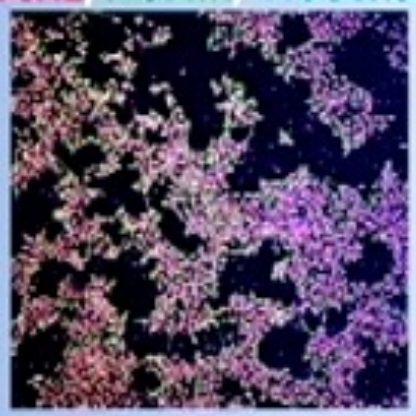
- All cell types but micro-glia
- 350 um diameter
- Reproducible in size and composition
- Myelination
- Genetic background from patient iPSC

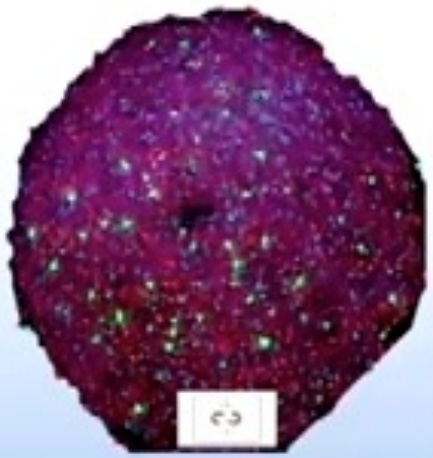
iPSC-derived human organotypic brain cultures

iPSCs → NPCs →



Sox2/Nestin/ Hoechst





iPPC expansion

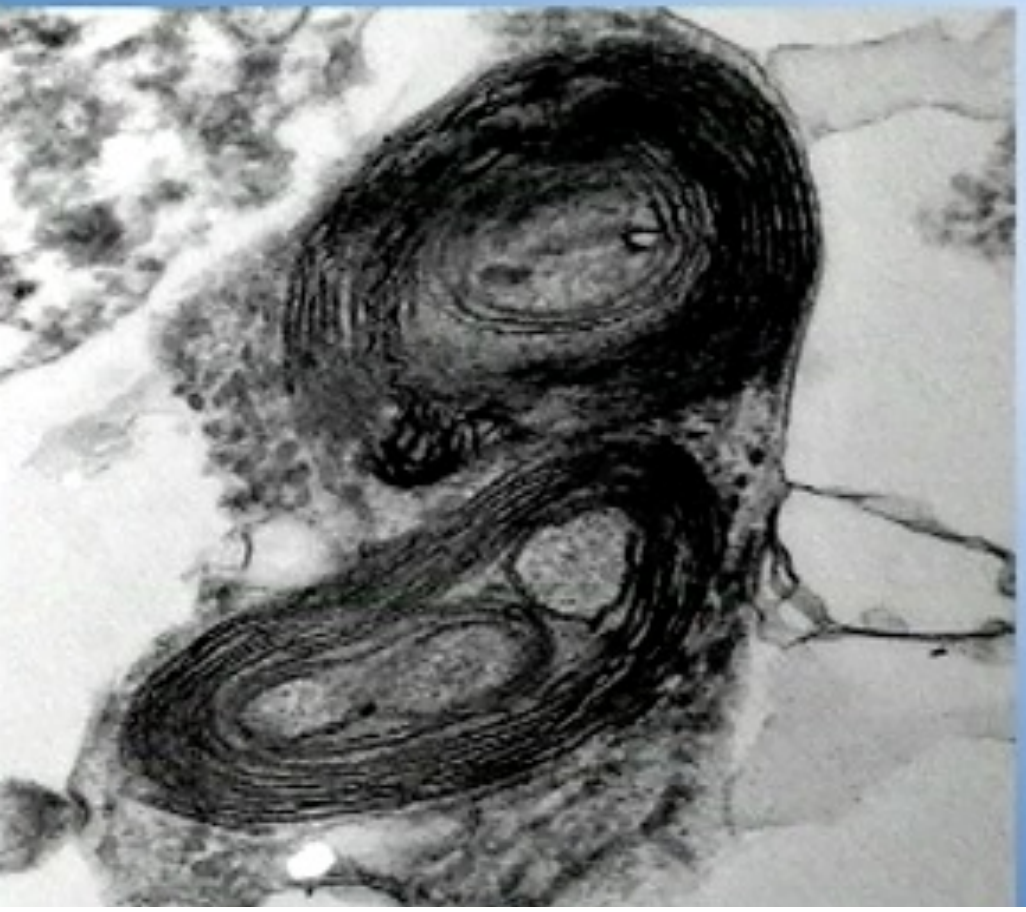
NPC expansion

NPC single cell plating for 3D differentiation

Gyratory shaking 8 weeks

Myelination

- 40% axons myelinated
- Allows studying de- and re-myelination



International Journal of Molecular Sciences

Int. J. Mol. Sci. 2021, 22, 9473

Article

Human IPSC-Derived Model to Study Myelin Disruption

Megan Chesnut [1], Hélène Paschoud [2], Cendrine Repond [2], Lena Smirnova [1], Thomas Hartung [1,3], Marie-Gabrielle Zurich [2,4], Helena T. Hogberg [1,*] and David Pamies [1,2,4,*]

International Journal of Molecular Sciences

Int. J. Mol. Sci. 2021, 22, 7929

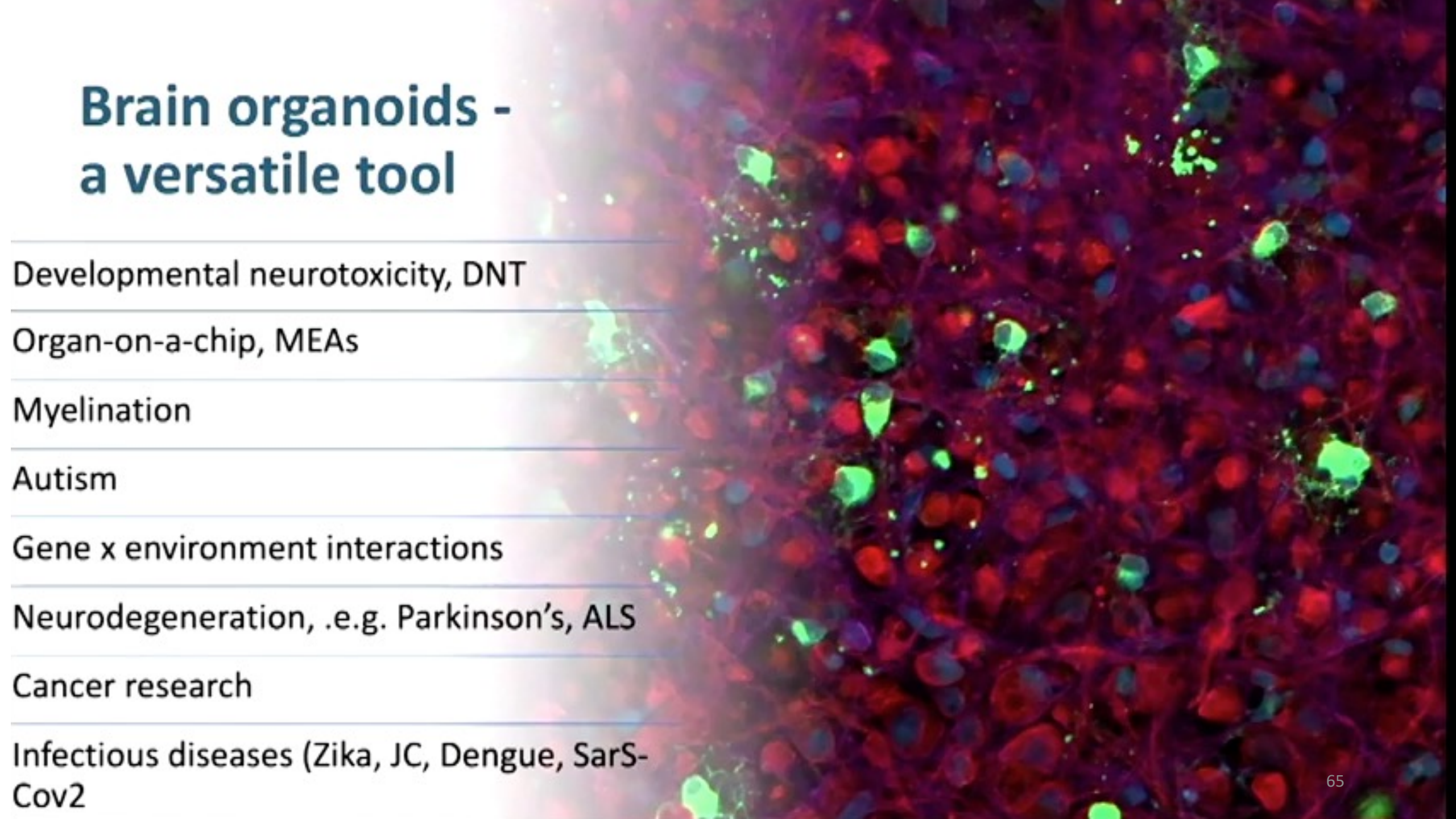
Review

Human Oligodendrocytes and Myelin In Vitro to Evaluate Developmental Neurotoxicity

Megan Chesnut [1], Thomas Hartung [1,2], Helena Hogberg [1,*] and David Pamies [1,3,4,*]

Brain organoids - a versatile tool

- Developmental neurotoxicity, DNT
- Organ-on-a-chip, MEAs
- Myelination
- Autism
- Gene x environment interactions
- Neurodegeneration, .e.g. Parkinson's, ALS
- Cancer research
- Infectious diseases (Zika, JC, Dengue, SarS-Cov2

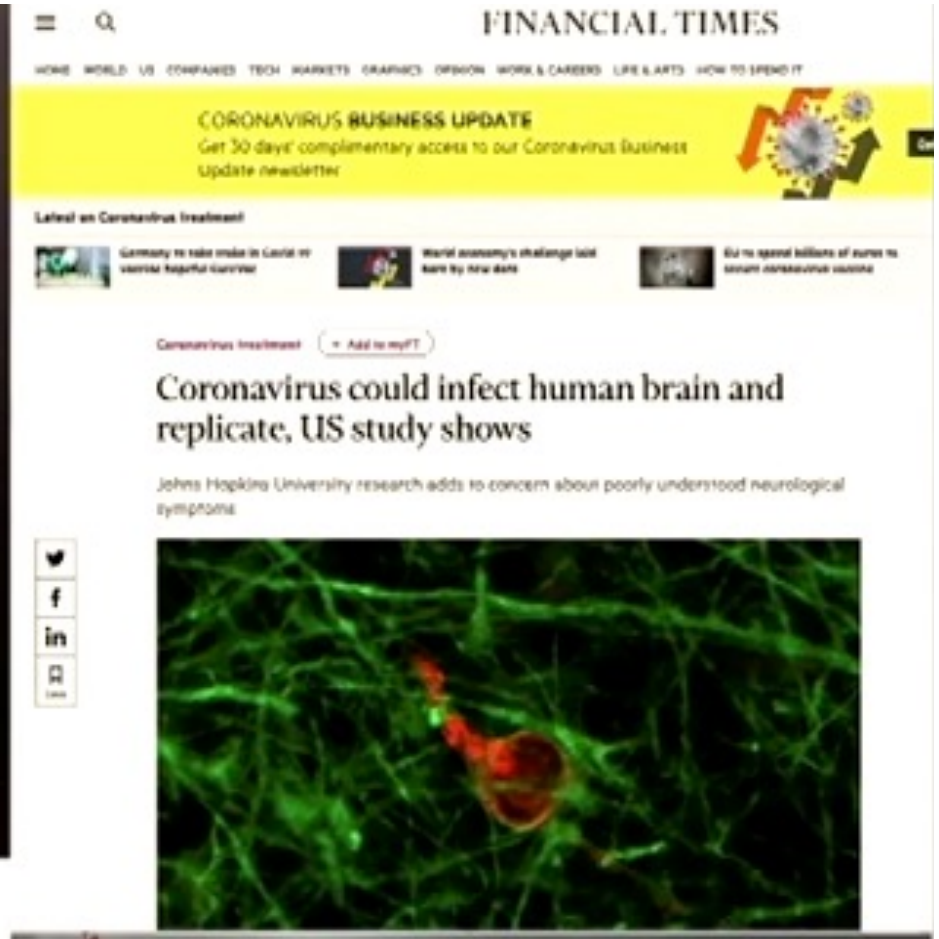


ALTEX preprint
published June 26, 2020
doi:10.14573/altex.2006111

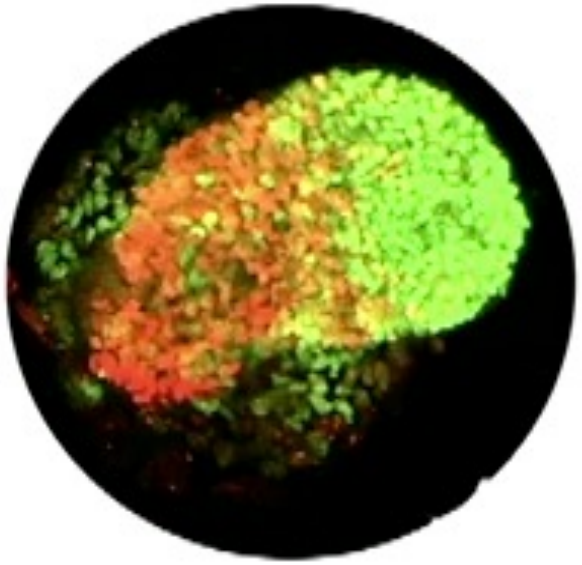
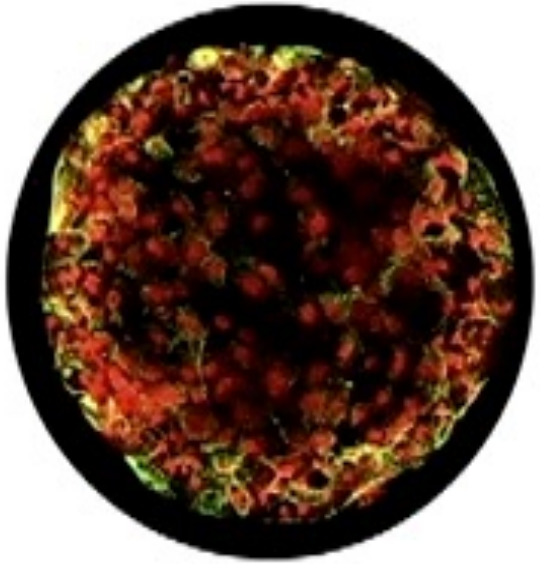
hort communication

nfectability of Human BrainSphere Neurons Suggests eurotropism of SARS-CoV-2

Korin Bullen[1,*], Helena Therese Hogberg[2,*], Asli Bahadirli-Talbott[1], William R. Bishai[1], Thomas rtung[2,3,4], Casey Keuthan[5], Monika M. Looney[1], Andrew Pekosz[4], July Carolina Romero[2], Fenna C. M.



RUMI'S HUMAN MINI-ORGAN MODELS

	Mini-lungs	Mini-livers	Mini-kidneys	Mini-brains
				
Tissues	Alveolar cells Airway cells	Bile duct cells Hepatocytes	Distal Tubules Proximal tubules Glomerulus	Neurons
	Respiratory viruses	Flaviviruses, adenoviruses and hepatitis viruses	Polyomaviruses, HIV, Flaviviruses, Parvoviruses	Zika, SARS-CoV-2, HSV

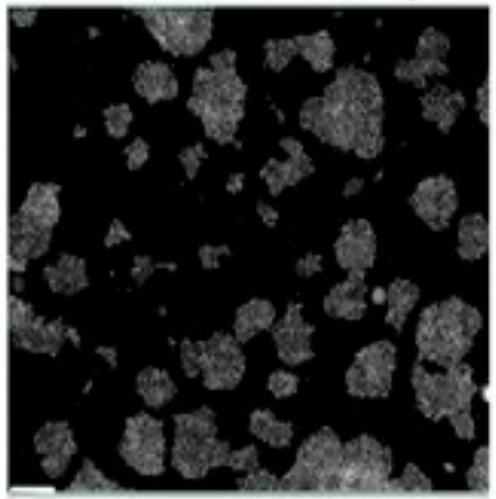
RumiViro's platform provides fast and scalable access to reproducible physiological human tissues to model viral disease.

BIOENGINEERING: BUILDING THE RIGHT TISSUE

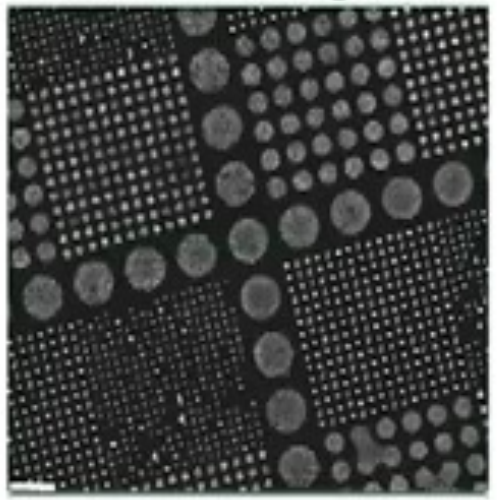
Micro-patterning technologies for exquisite control of tissue organization

Breakthrough#1: Standardization

Regular stem cell cultures are messy

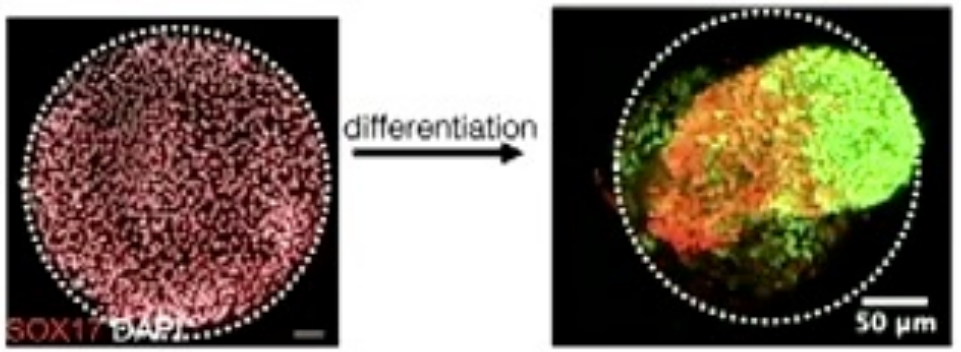


RUMI's Micropatterned stem cells are organized



Breakthrough#2: Physiological Organization

Differentiating stem cells self-organize under confinement to create complex yet reproducible tissues



Standardized geometrical confinement induces robust self-organization of physiological human mini-organs.

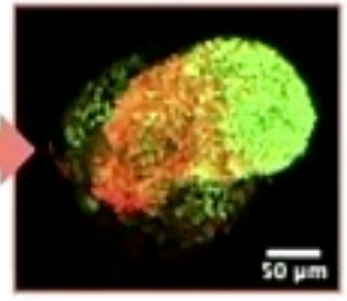
High impact publications from the founder's labs: Nature Methods, 2014; Dev Cell, 2016; *Nature Protocols*, 2016; *Nature Biotech*, 2019

RUMI'S SOLUTION: WELL-DEFINED, SCALABLE, REPRODUCIBLE

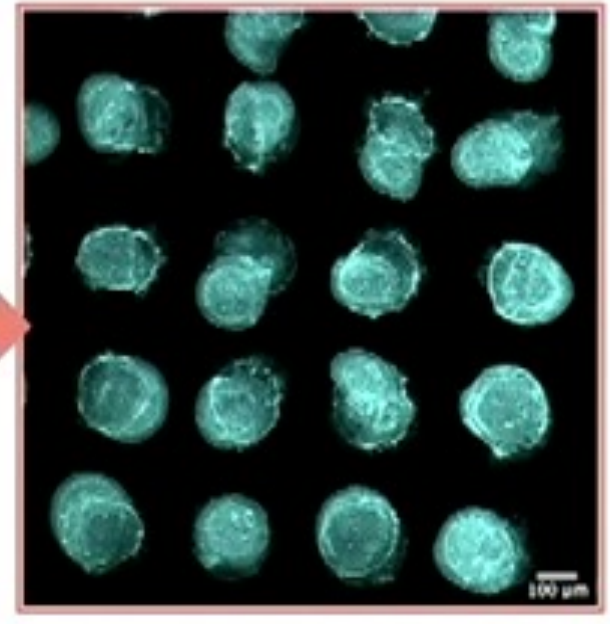
Current 3D organoids are heterogeneous and variable → Not well-adapted to drug discovery

Ex: lung organoids

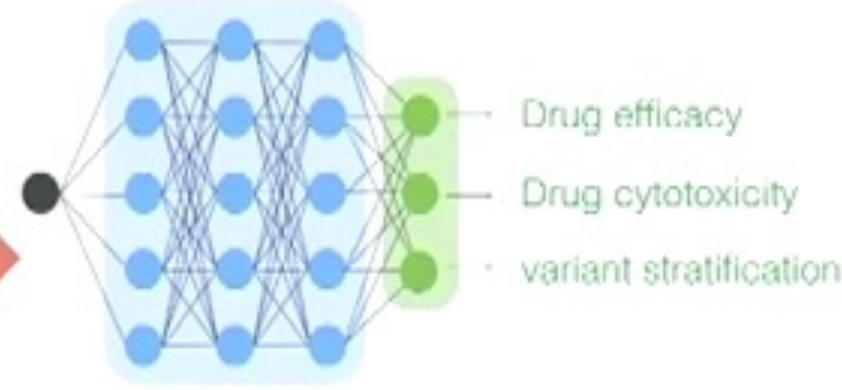
RUMI's Targeted reconstruction



Large image data banks



Deep learning of quantitative disease features, and drug's potential



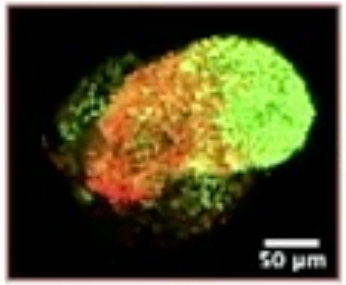
Breakthrough#3: Quantitative phenotypic analysis using AI

Core technology protected by a suite of 3 patents

Unique opportunity for quantitative analysis, at scale, of healthy and infected human tissues + screening of large chemical libraries

MINI-LUNGS RECAPITULATE COVID19 DISEASE

RumiViro's mini-lungs



SARS-COV2 infection

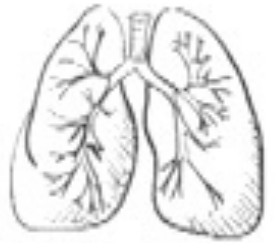
mock

Direct comparison with patient lungs

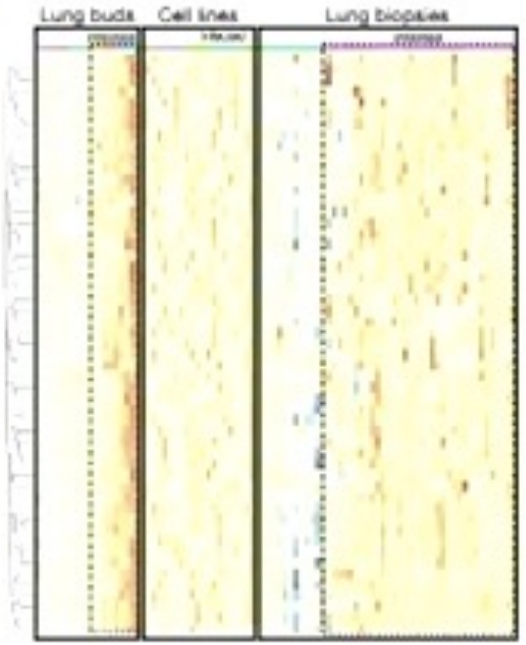
Post-mortem tissues

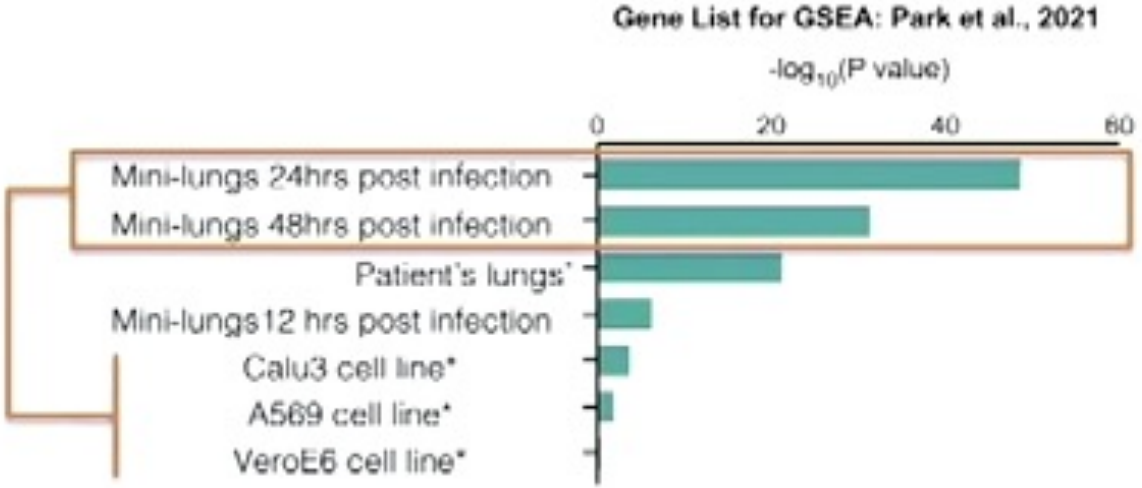
36 COVID19 patients

4 unaffected controls



Park et al. (2021) Cell Reports Medicine

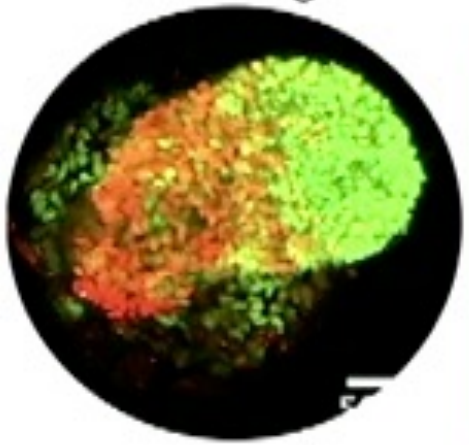




n collaboration with Charles Rice and Ali Brivanlou at The Rockefeller University; Rosado-Olivieri et al. (2021) bioRxiv

DEVELOPING BROAD-SPECTRUM HOST-BASED THERAPEUTICS

mini-lungs



Broad-spectrum antivirals for respiratory viral infection

- Coronaviruses, paramyxoviruses, orthomyxoviruses, rhinoviruses, and pneumoviruses

mini-kidneys

Broad-spectrum antivirals for acute viral kidney injury

- Polyomaviruses, HIV, flaviviruses, CMV, and parvoviruses

Mini-organs allow for the identification of tissue-targeted host-based therapeutics at scale

The end.

Thank you!

For further questions or lab visit:
6972212037