

Carolina TESTA

Expected PhD starting date: November 2020  
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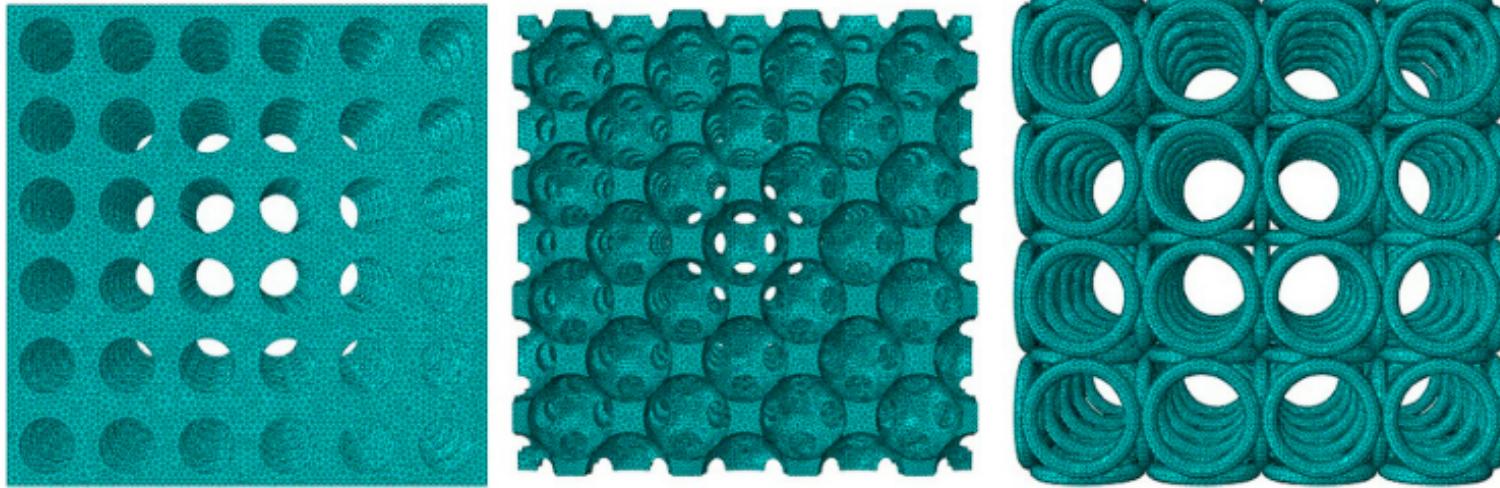


# **VALIDATION OF A THREE-DIMENSIONAL TUMOR NICHE-ON-A-CHIP MODEL OF MALIGNANT PLEURAL MESOTHELIOMA**



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# Three-dimensional substrates for *in vitro* cell culture



[Kantaros et al., 2016]

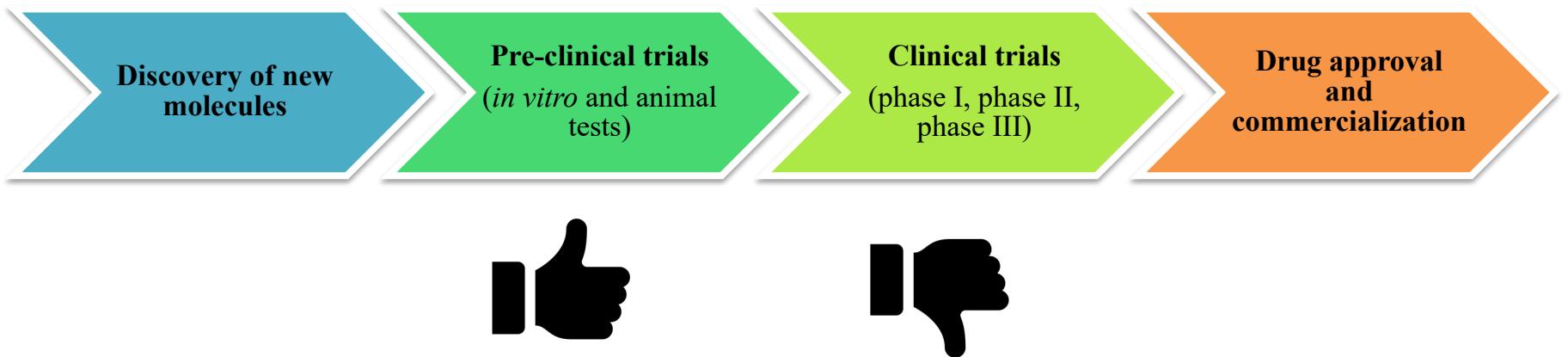
Three-dimensional (3D) cell culture systems *in vitro* provides a **more realistic model for drug discovery, physiopathology and tissue engineering** compared to conventional bidimensional (2D) approaches.

3D culture condition promotes a cell response **more comparable with the *in vivo* one** by replicating the **native biological environment**.

# Three-dimensional culture of cancer cells

3D cell culture seems to be a promising tool for a **better identification of tumor features**, in particular in **response to drug administration**.

Cells in 3D culture generally display a **diminished sensitivity to certain chemotherapeutic agents** compared to monolayers.

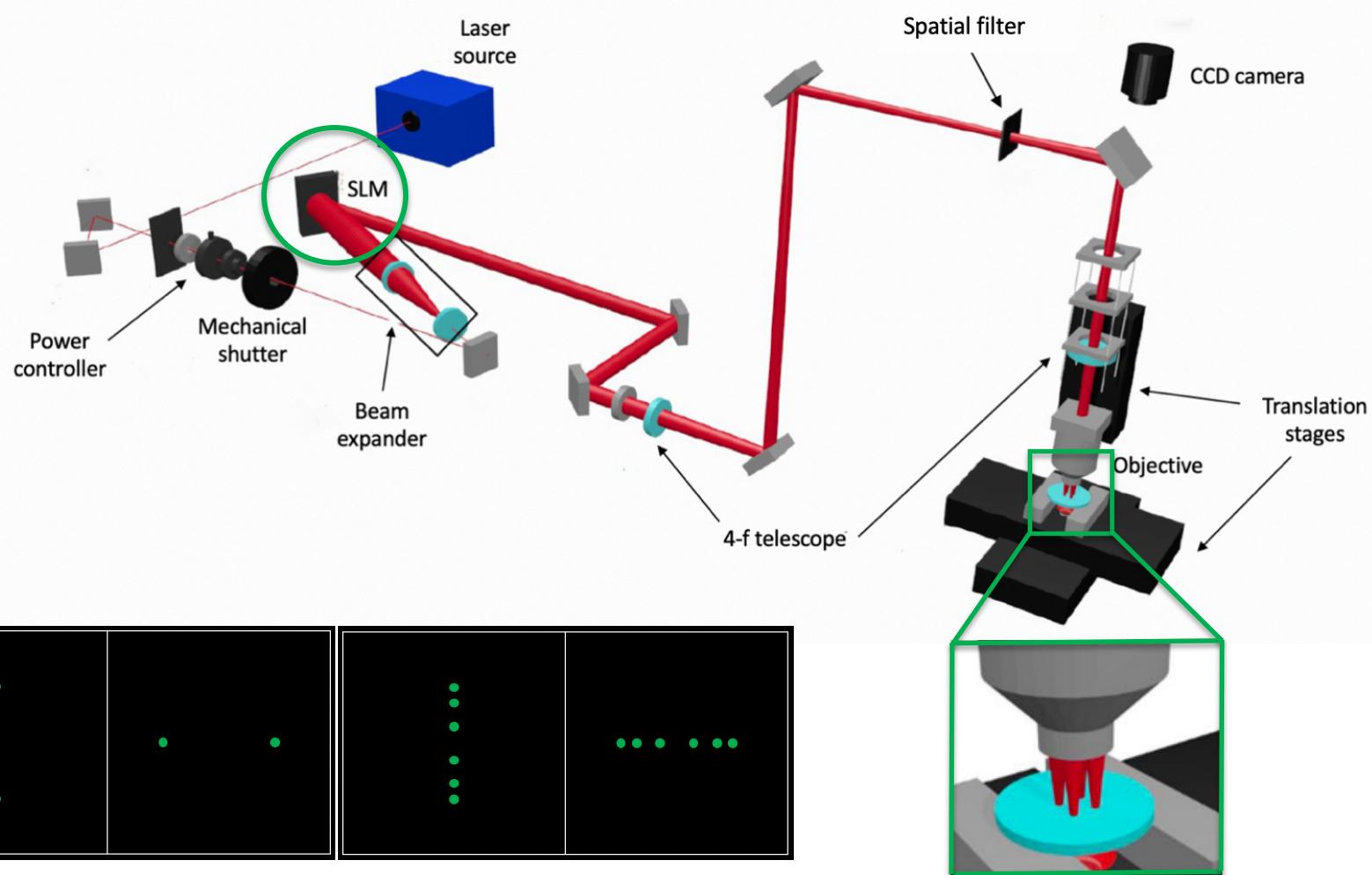


# Aim of the work



Recreate the mechanical environment of the tumor niche in its native configuration, thus producing an ***in vitro* cell-based tumor model** of Malignant Pleural Mesothelioma using the Nichoid as the designed culture substrate.

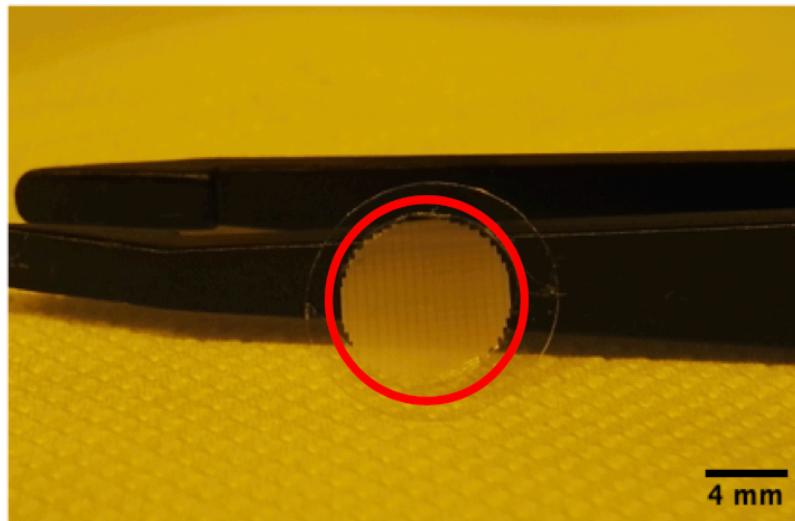
# The laser setup



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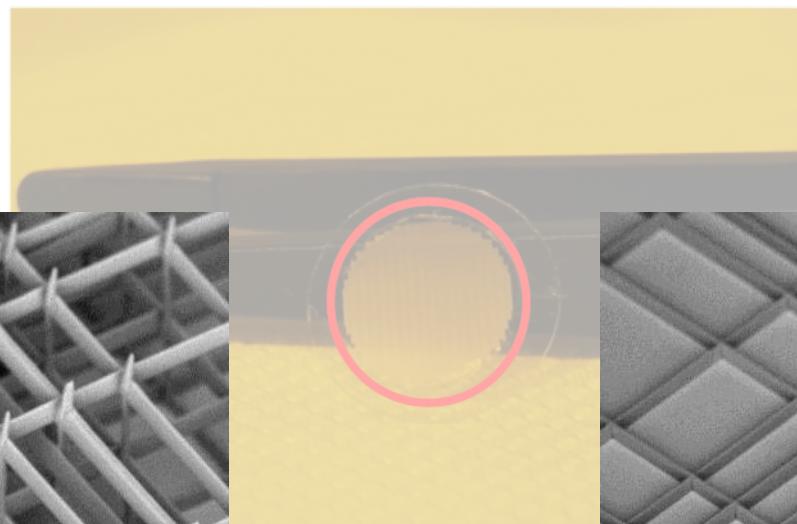
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ISTITUTO NAZIONALE GENETICA MOLECOLARE  
"Romeo ed Enrica Invernizzi"

# Nichoid microfabrication

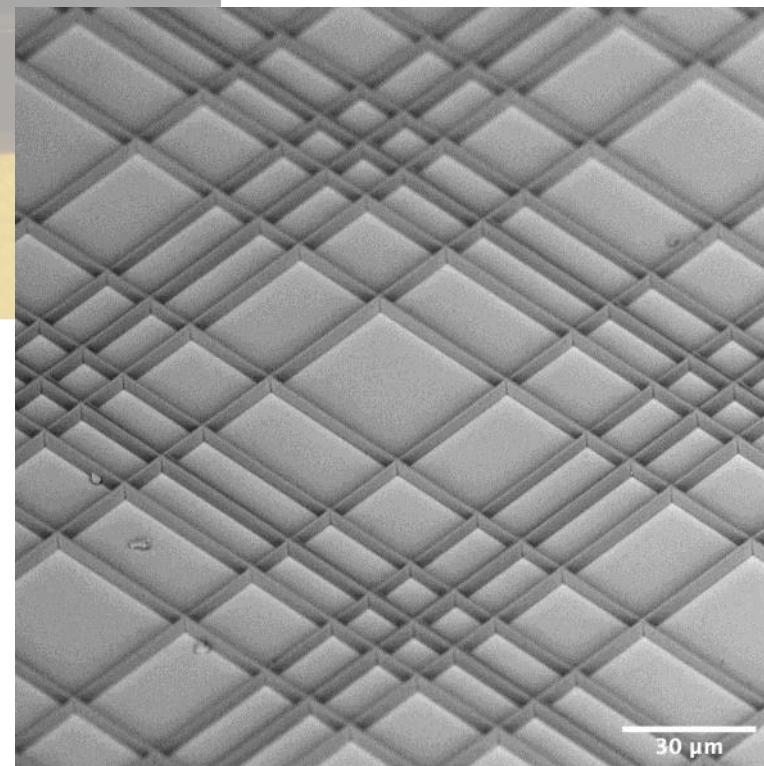


# Nichoid microfabrication

**3D Nichoids**



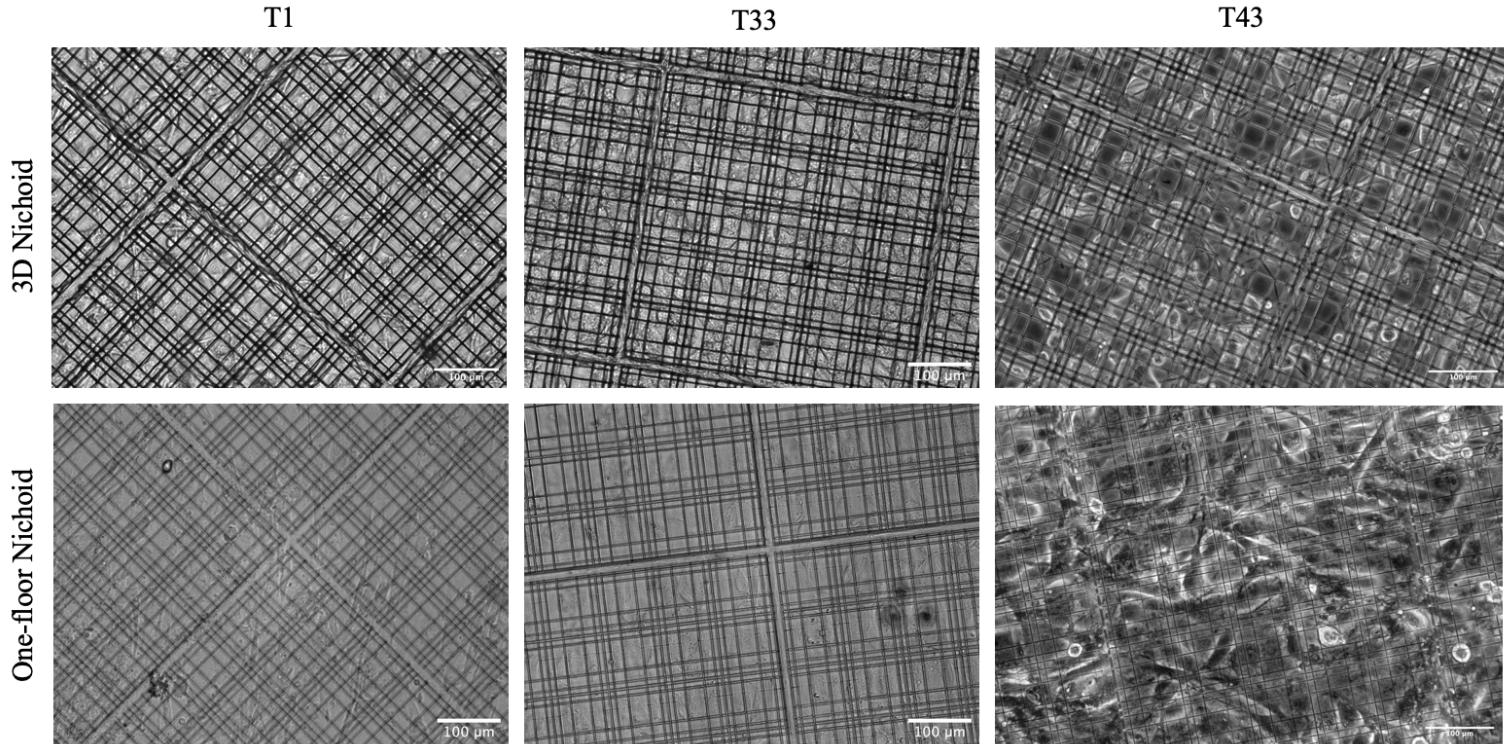
**2D controls**



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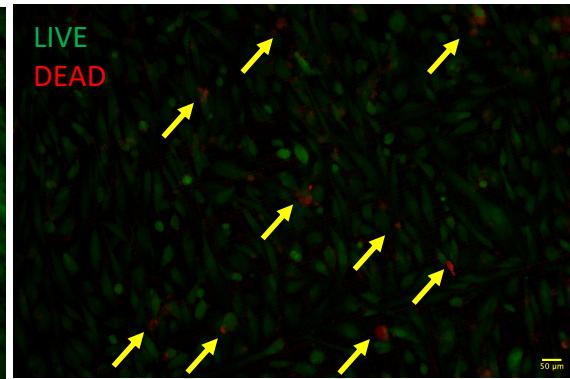
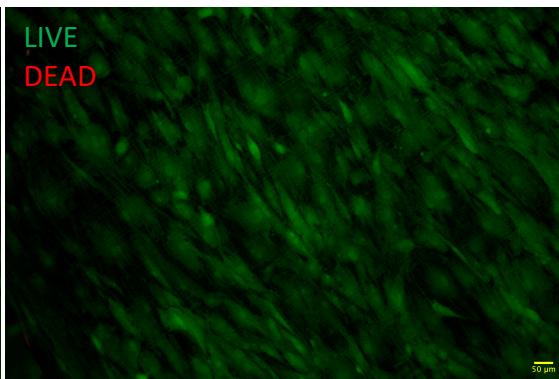
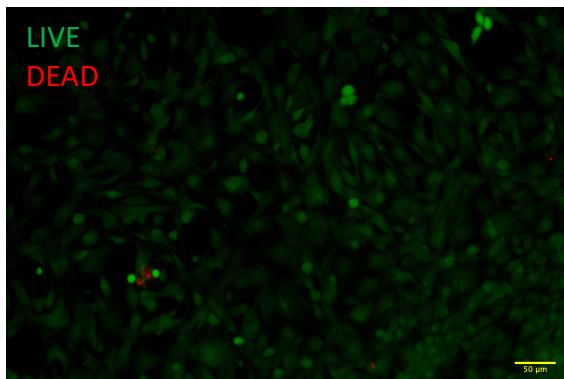
# Nichoid culture of MPM primary derived cell lines



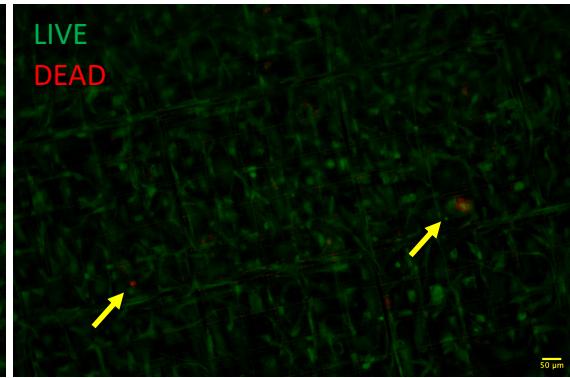
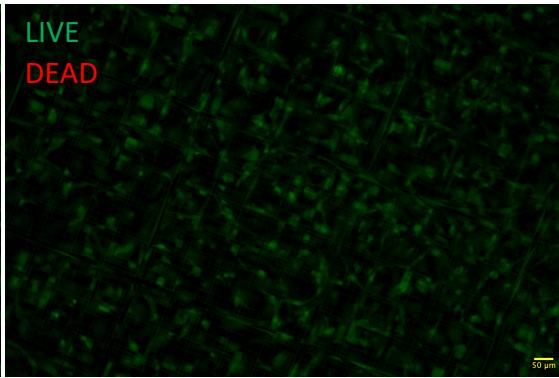
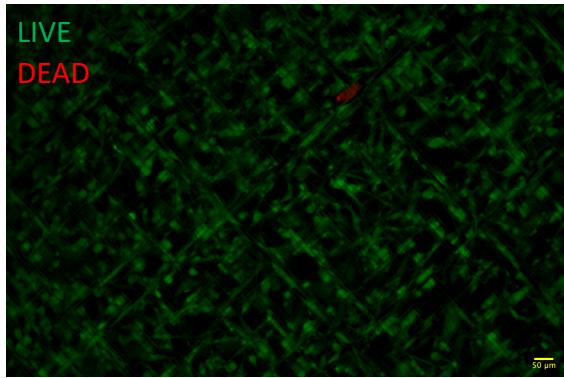
Cells grown in 2D controls display to be **more elongated** and to have a morphology more similar to the one of cells cultured in traditional petri dishes.

# Viability assay

2D Control



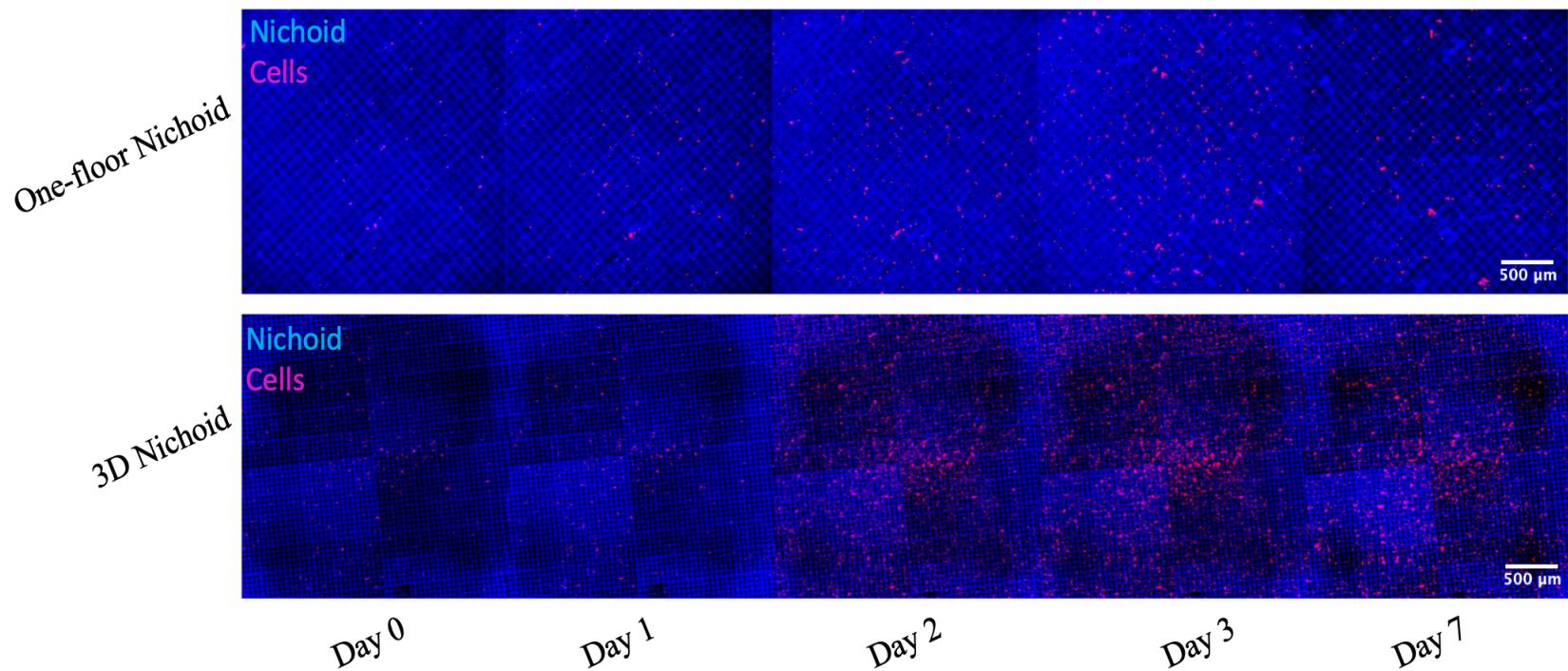
3D Nichoid



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# Proliferation assay

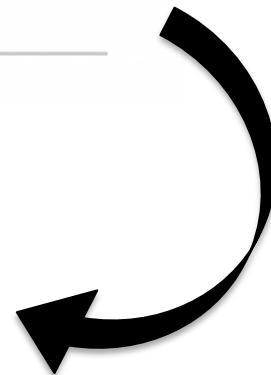


# RNA extraction

	<b>Cell line</b>	<b>RIN</b>
<b>T1</b>	Nichoid	8.9
	Control	9.4
<b>T33</b>	Nichoid	9.9
	Control	9.4
<b>T43</b>	Nichoid	10
	Control	9.6

**Next Generation  
Sequencing (NGS)**

**illumina®**

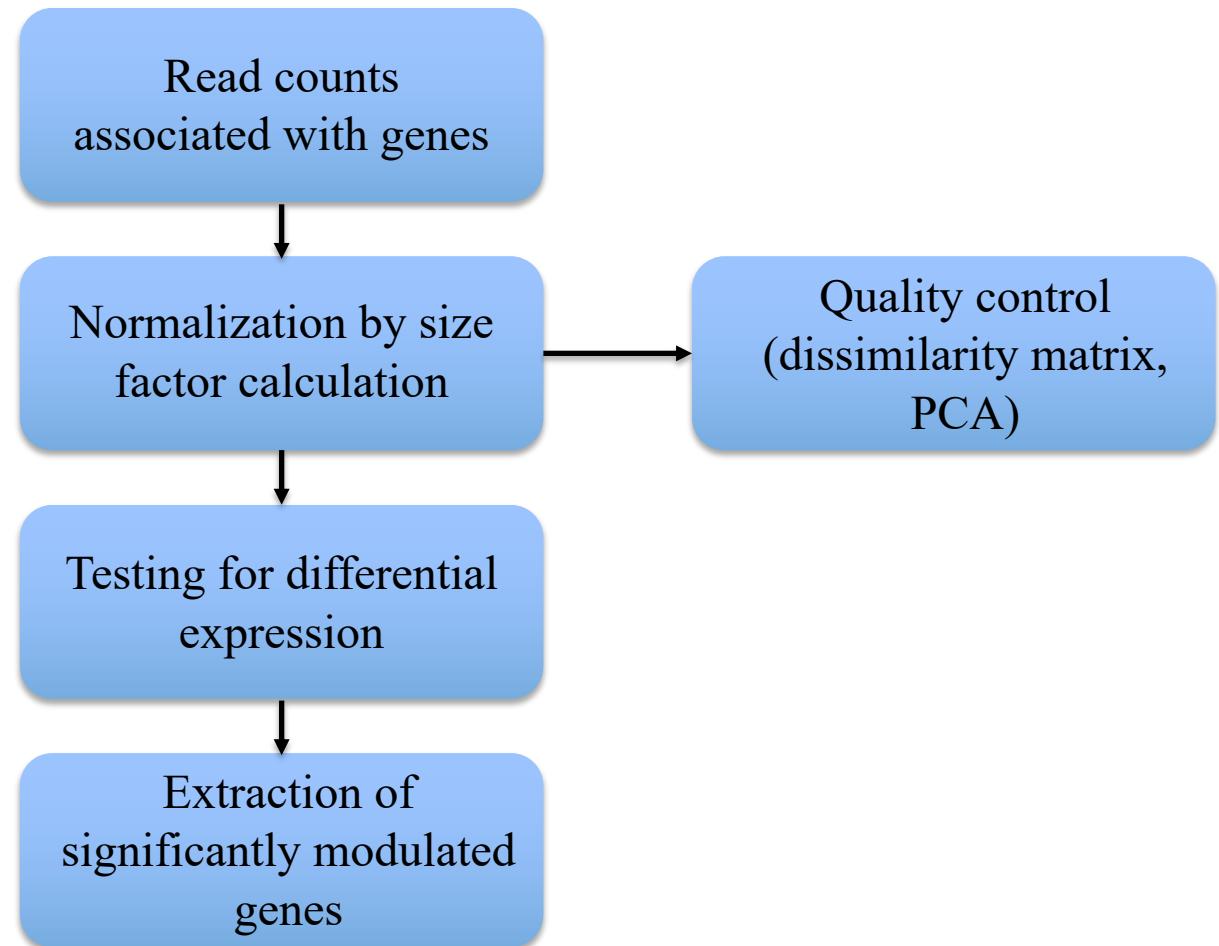


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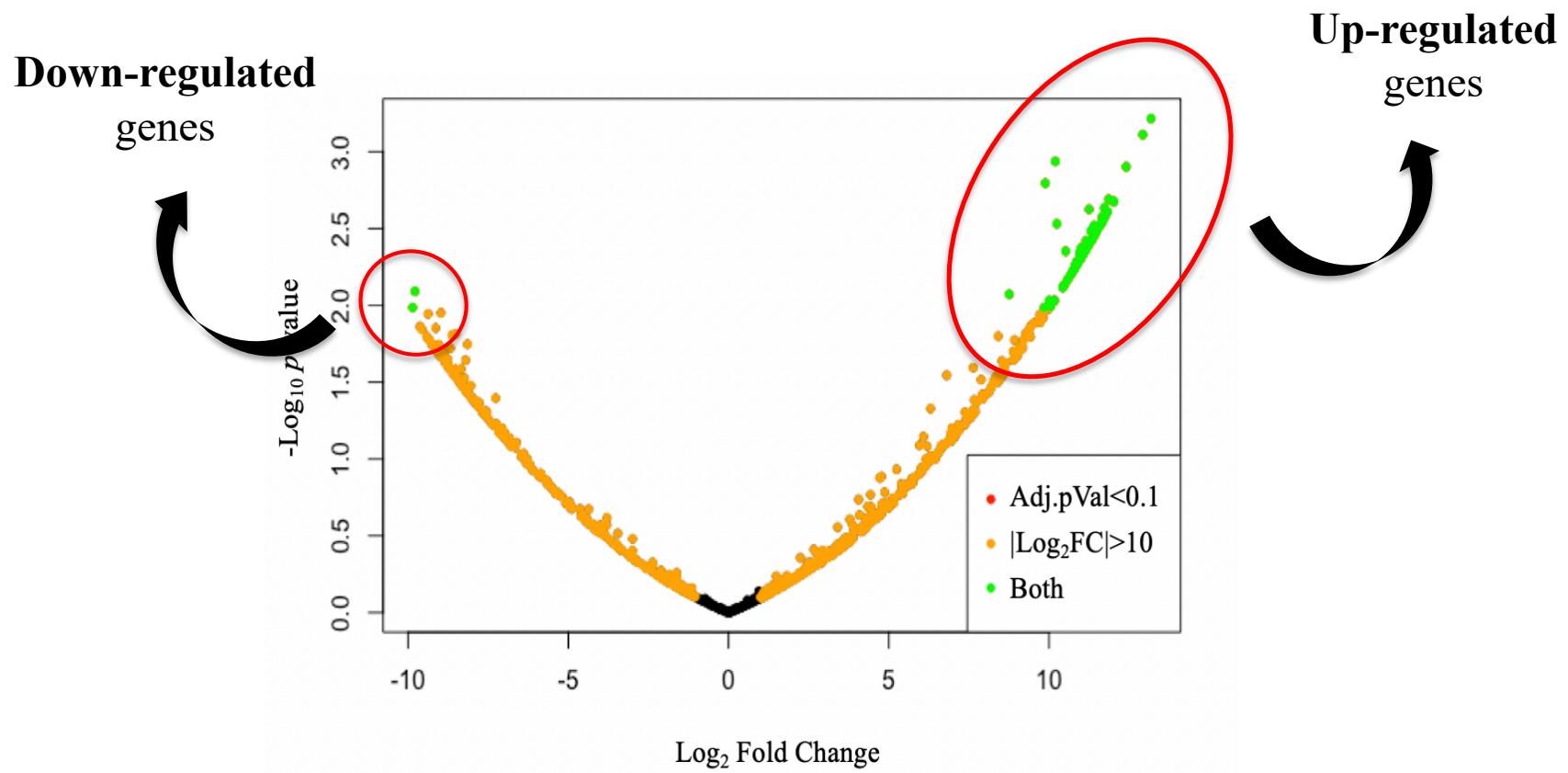
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# Bioinformatics analysis

DEseq2 package

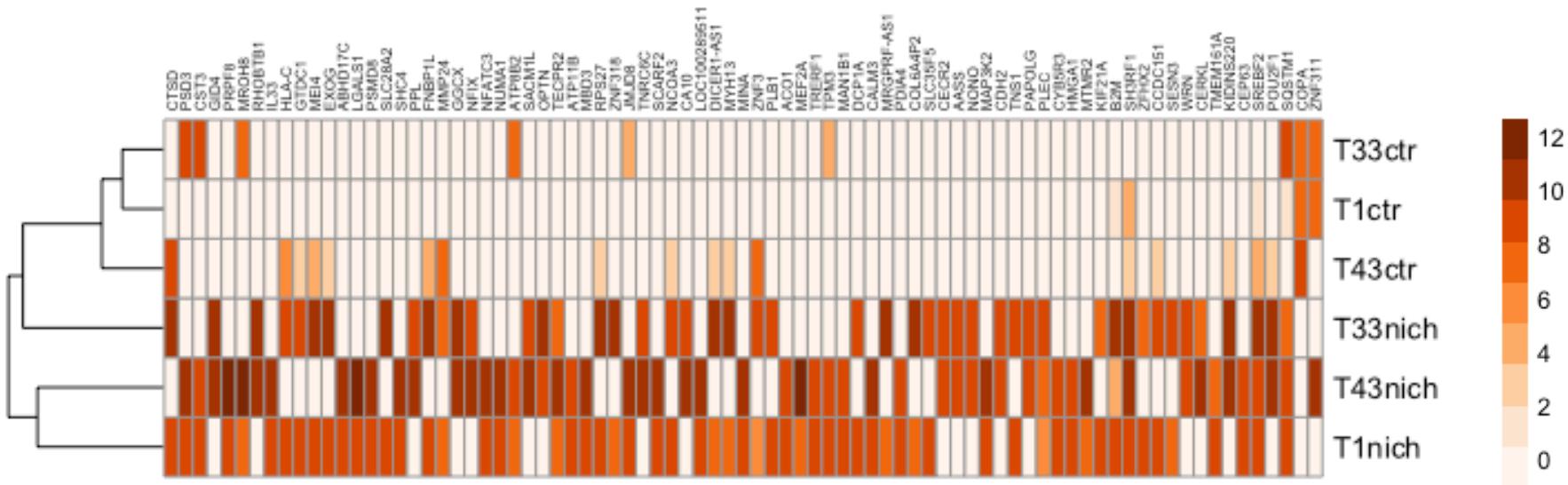


# Differential expression analysis - Volcano plot

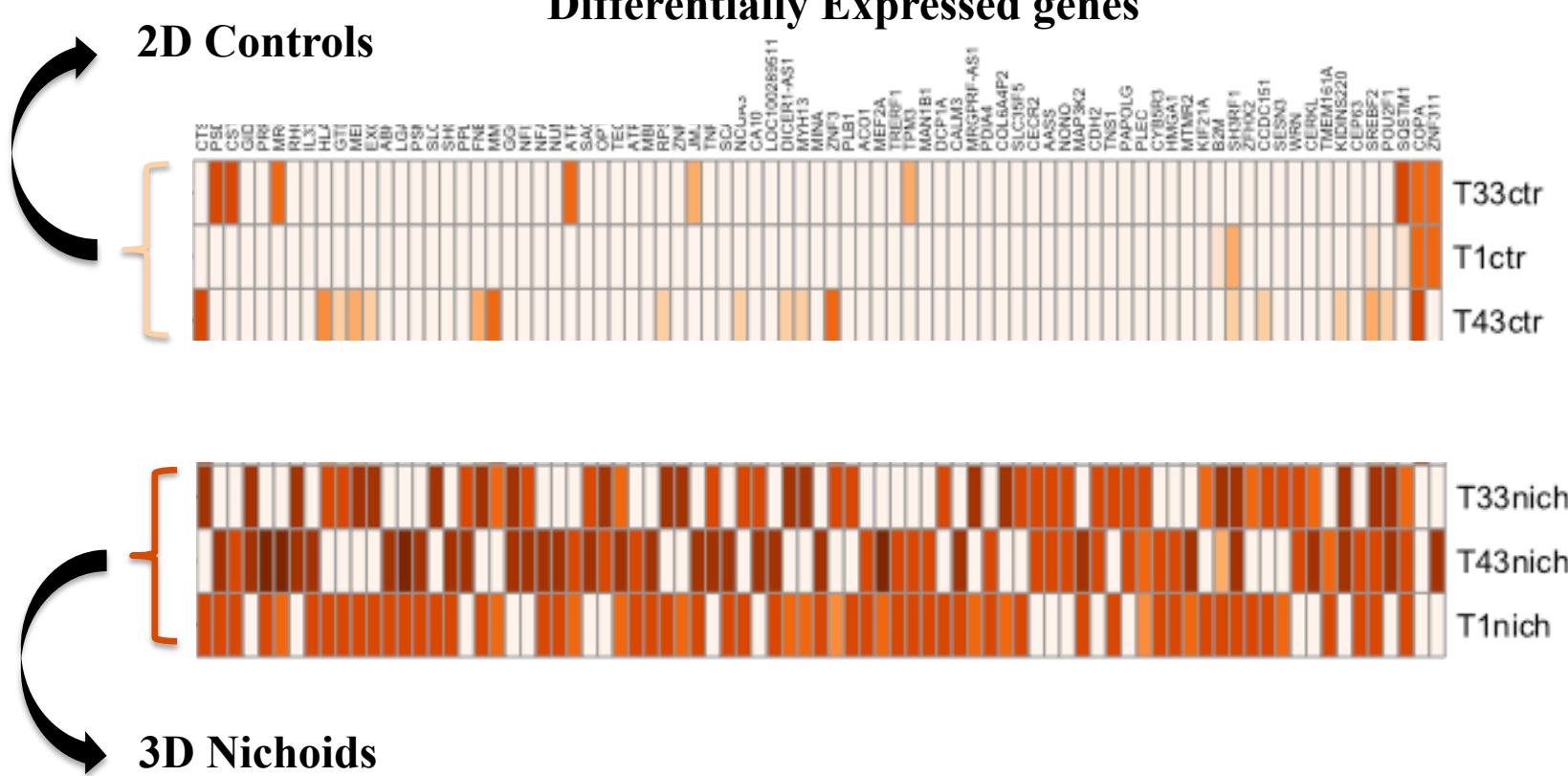


# Differential expression analysis - Heatmap

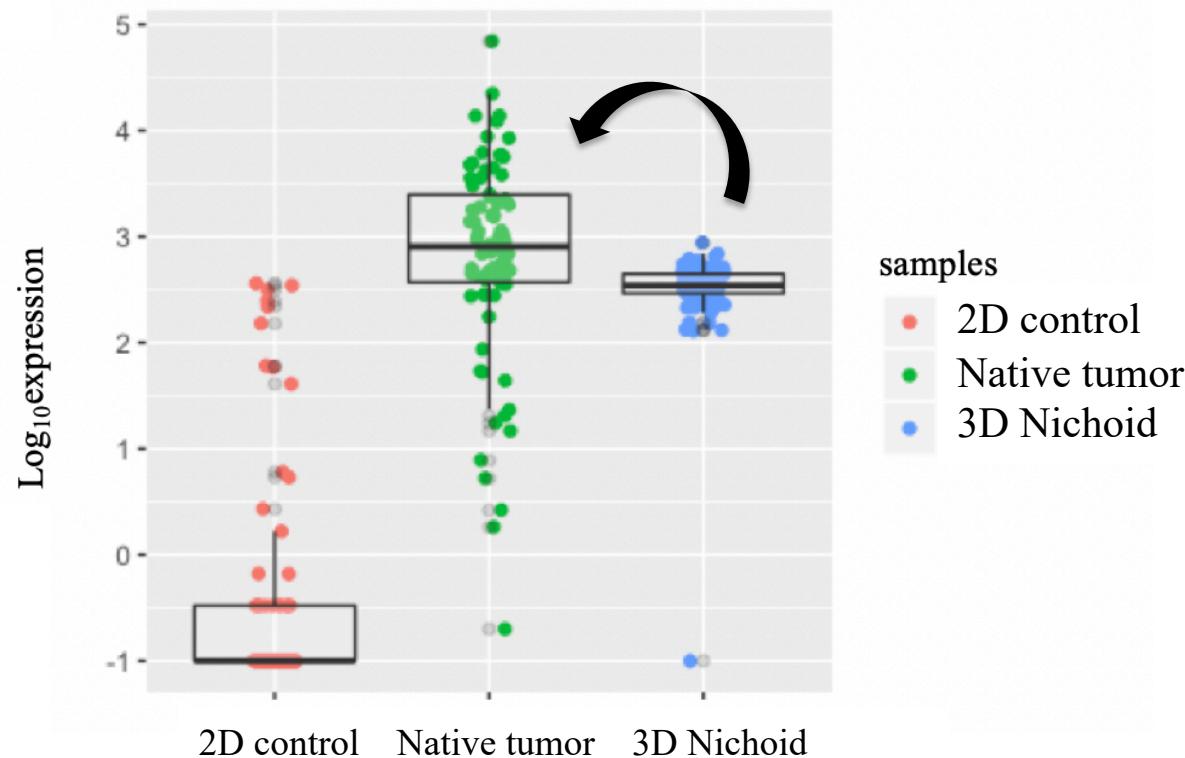
## Differentially Expressed genes



# Differential expression analysis - Heatmap



# Comparative analysis with native tumor

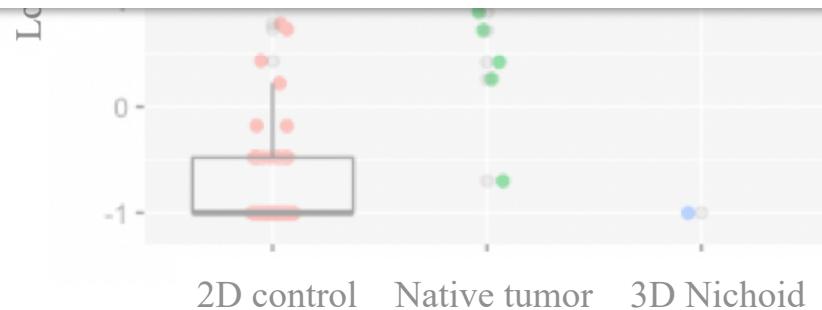


# Comparative analysis with native tumor



**Changes in Global Gene Expression Associated with 3D Structure of Tumors: An *Ex Vivo* Matrix-Free Mesothelioma Spheroid Model**

Heungnam Kim, Yen Phung, Mitchell Ho\*



# What's next?

- ❖ Re-sequencing of MPM samples in order to **increase the number of MPM cell lines** and thus the statistical significance
- ❖ **Functional genomics analysis** to investigate in which pathways differentially expressed genes are involved
- ❖ Use of Genomic Computing to deepen this behavior and to find **possible molecules (drugs)** that can be tested *in-vitro*
- ❖ Extend the analysis to **other types of cancer**