EORTC-PAMM one-day educational course

(Pre)-clinical pharmacology of anticancer drugs made (amusingly) simple

A hands-on course to think, test and learn!

Image kindly provided by OggettiAmo by Floriana Fioravanti

FEBRUARY 6, 2019, Verona, Italy

Student Early bird Fee (12/10): Euro 50 (Standard Fee: Euro 60)

Including participation to the EORTC-PAMM Meeting, FEBRUARY 7-9, 2019

With practical sessions on data analysis & OMICS data

PAMM
PHARMACOLOGY & MOLECULAR MECHANISMS GROUP
40th EORTC-PAMM WINTER MEETING
6th - 9th February 2019
Verona, Italy

Neighbourhood matters: new tools and practical approaches for co-culturing cancer cells

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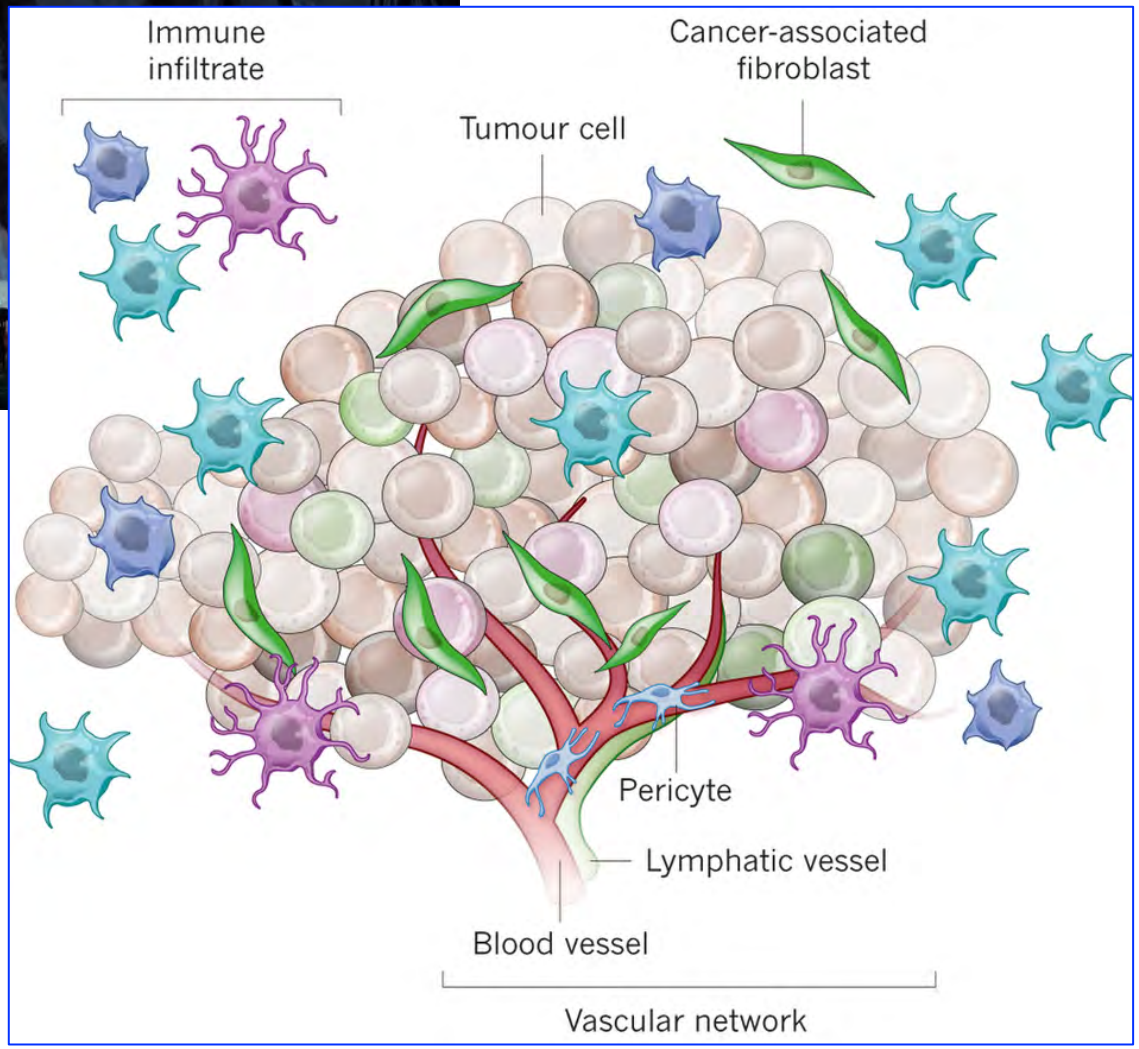
Amsterdam UMC, Cancer Center Amsterdam
Cancer Pharmacology Lab, AIRC Start-Up Unit, Pisa

February 6th 2019

http://conceptartworld.com/news/avatar-concept-art-by-seth-engstrom/

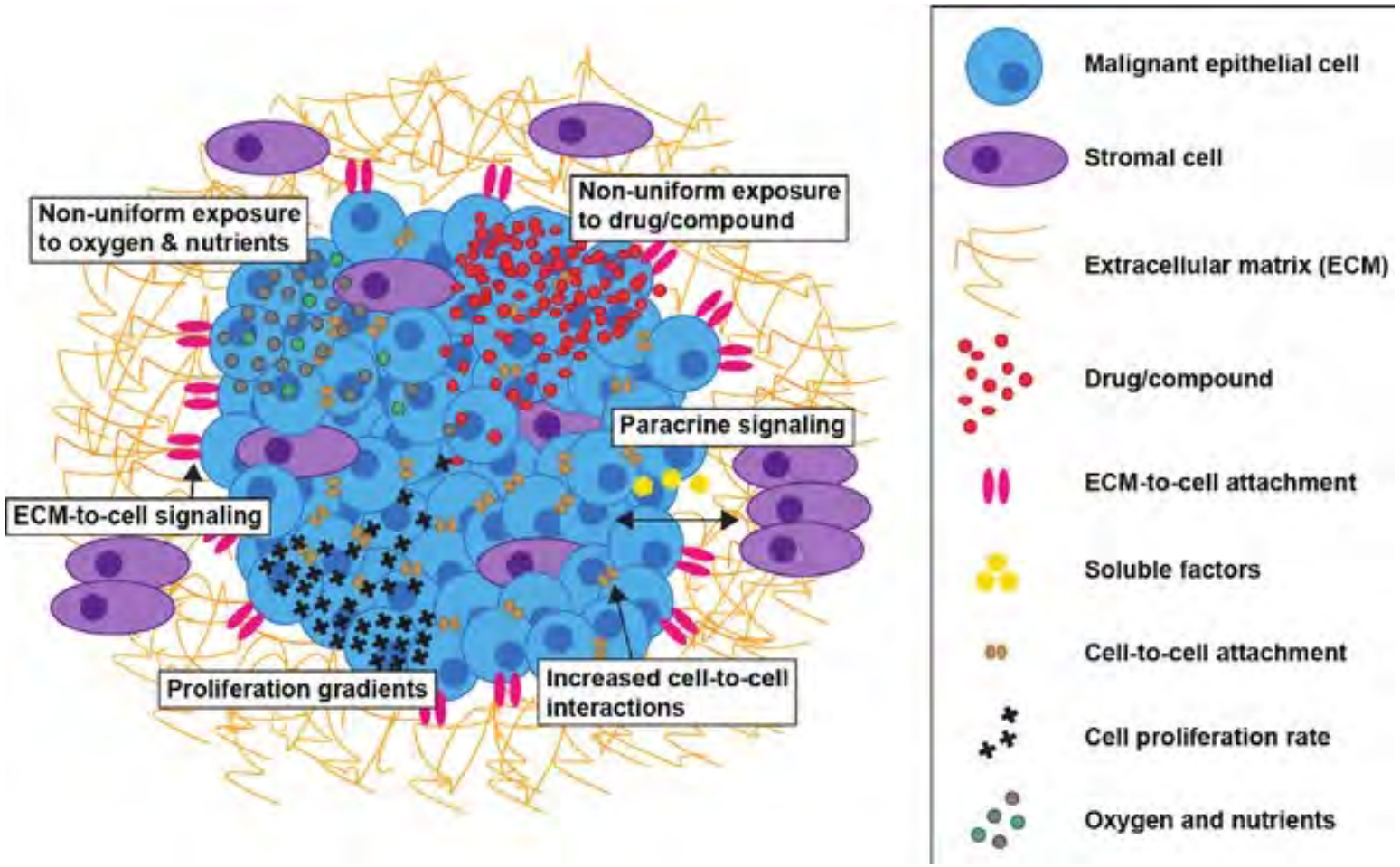
Cancer tissue is a "**complex organ**"

The TME is composed of a variety of cells, including tumor cells, cancer stem cells, inflammatory cells, and cancer associated fibroblasts along with blood vessels



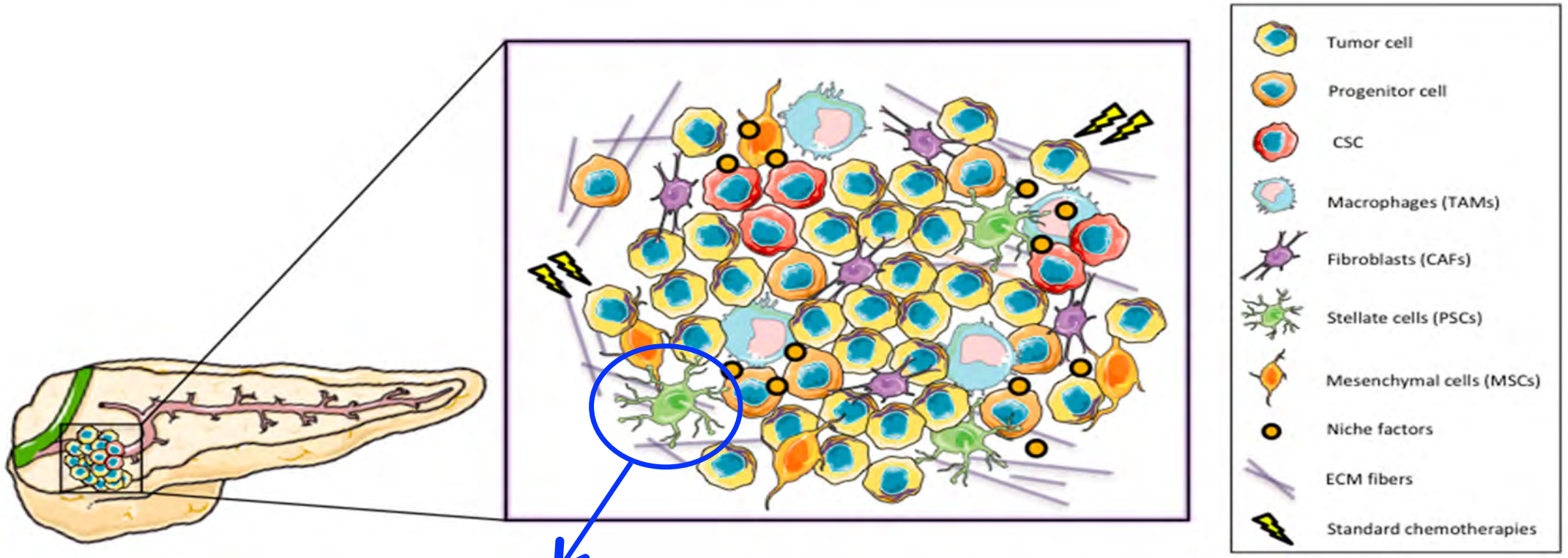
Influence of tumour micro-environment heterogeneity on therapeutic response
Junttila and de Sauvage, Nature 501, 346–354 (19 September 2013)

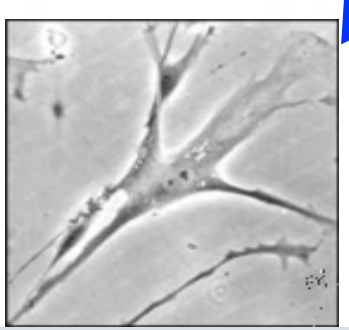
Potential advantages of incorporating elements of the tumor microenvironment in drug discovery programs



Lovitt et al., *Biology,* **2014**

A practical example: how to study the role of pancreatic stellate cells (PSCs) in pancreatic cancer (PC) microenvironment

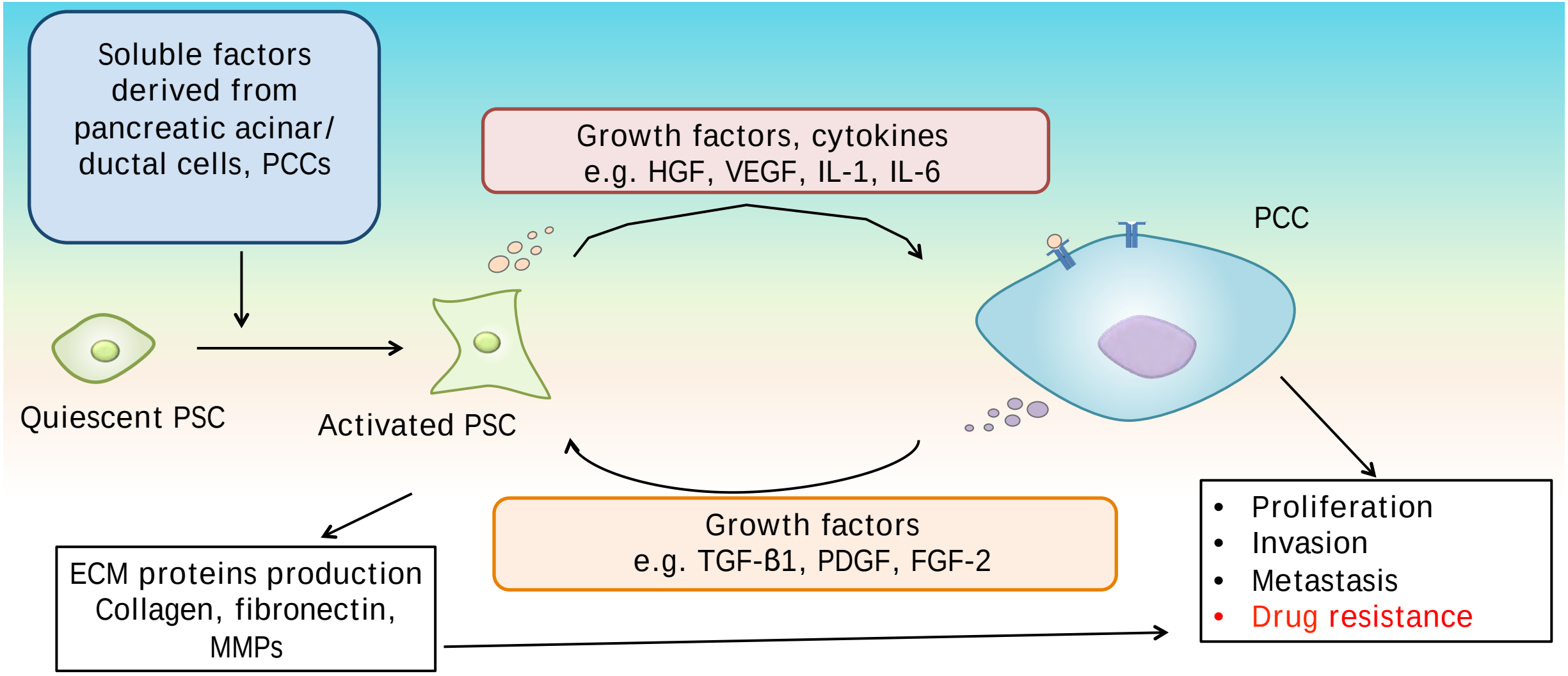




PSCs identified as the cause of desmoplasia

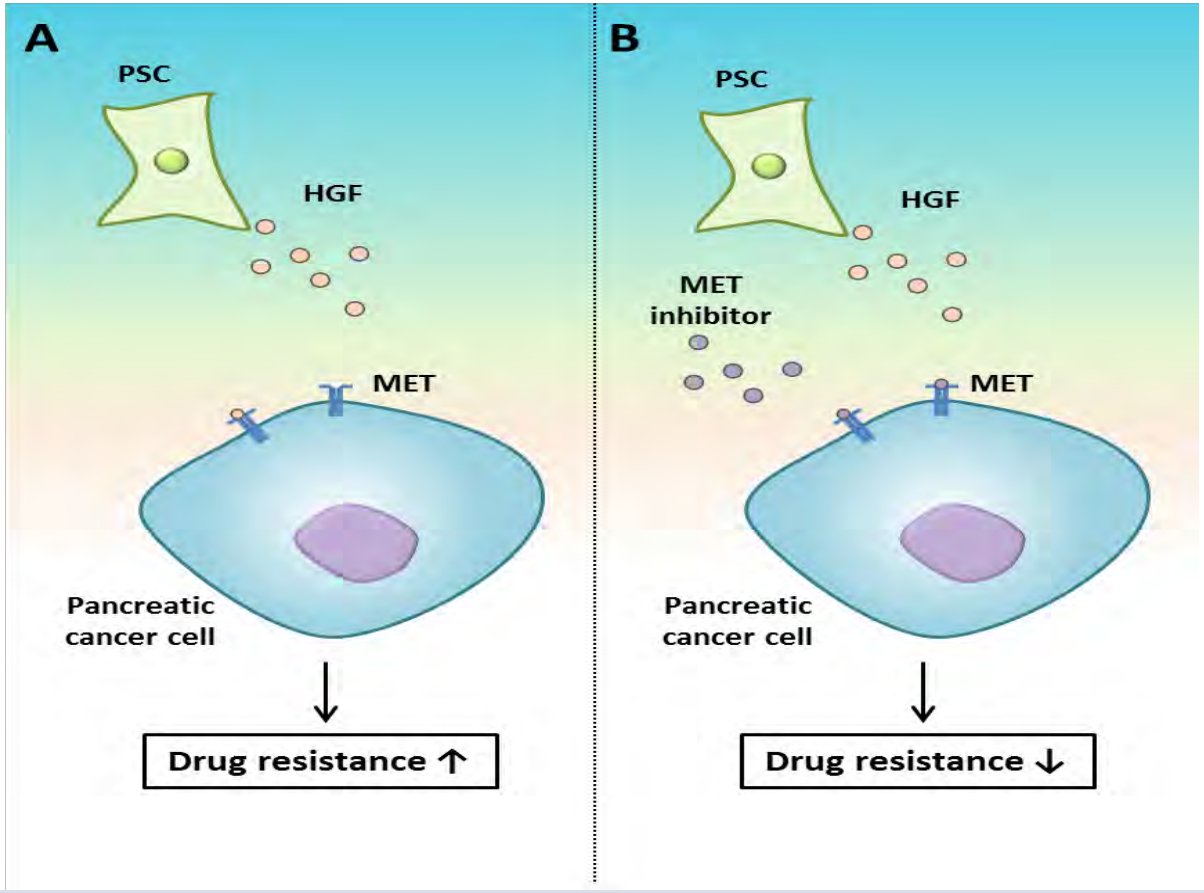
Firuzi et al., *Cancer,* **2019**

Paracrine signaling between PSC and PCC results in tumor progression

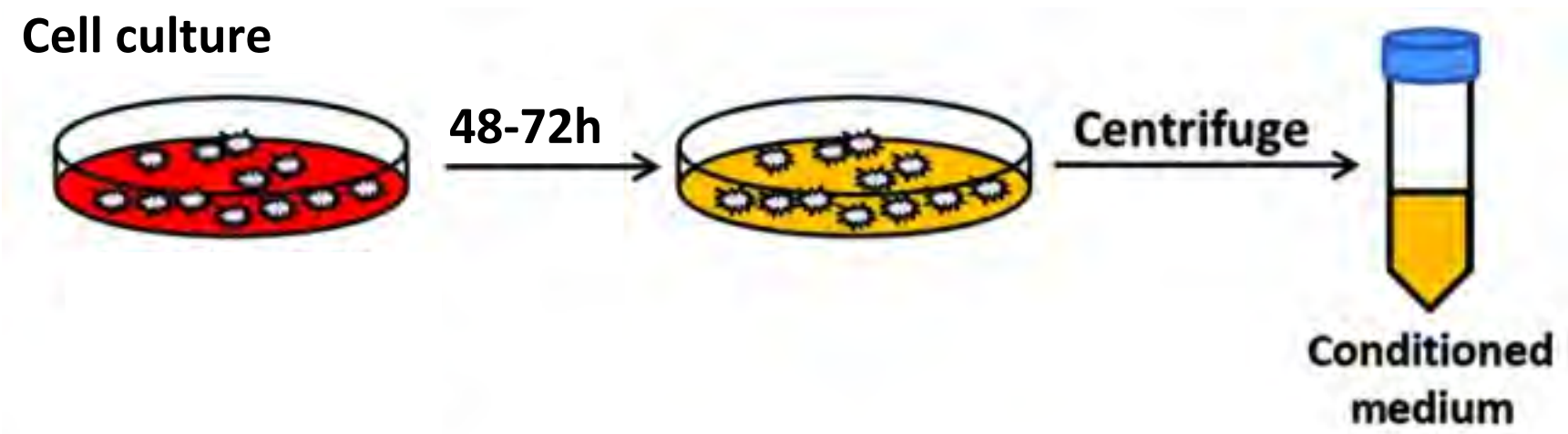


PCC = pancreatic cancer cells, ECM = extracellular matrix, MMP = matrix metalloproteinase

How we can study if the PSCs in the tumor microenvironment play a role also in PDAC drug resistance ? (mediated by HGF-c-MET pathway)

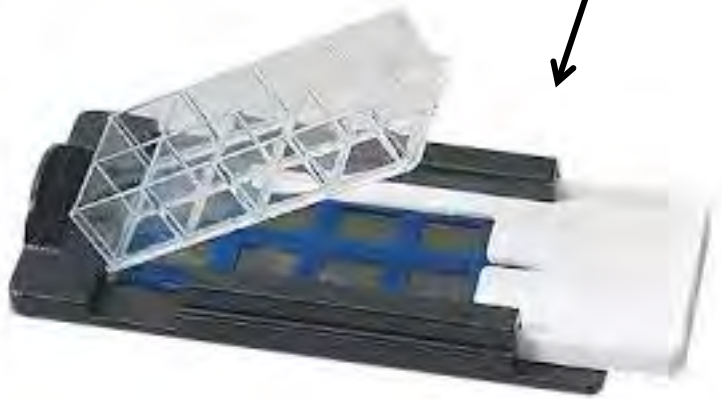


Use of conditioned medium & chamber slides

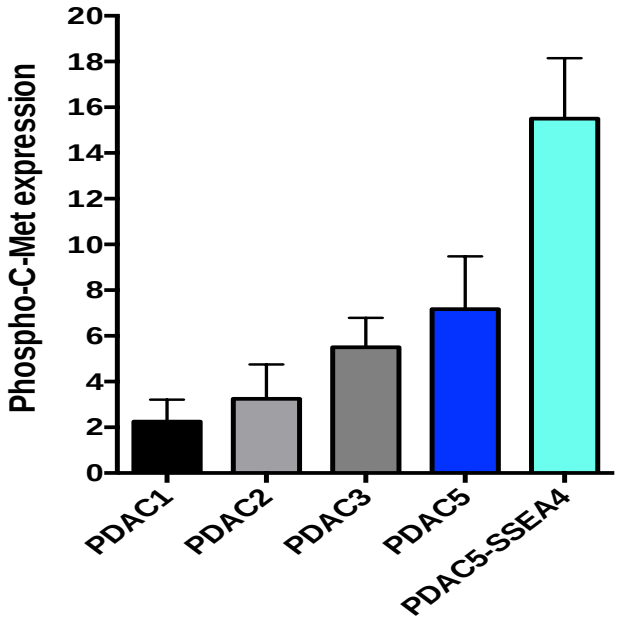


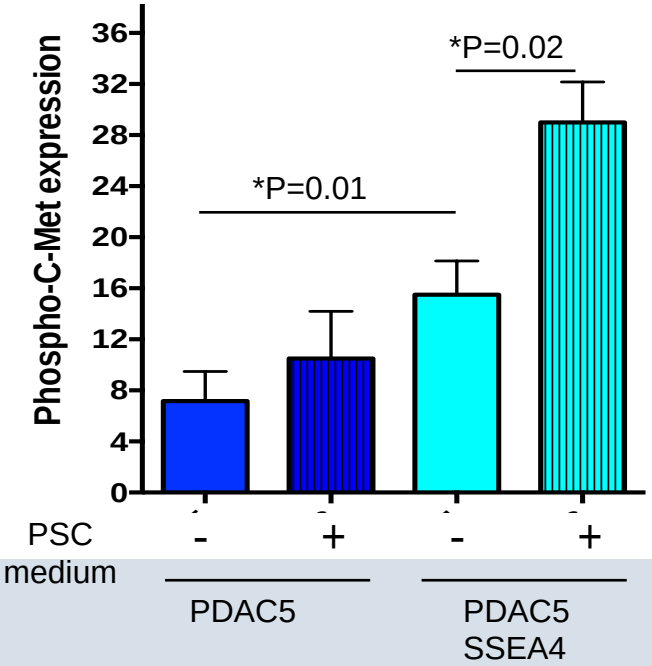
Conditioned medium is spent media harvested from cultured cells.
It contains metabolites, growth factors, and extracellular matrix proteins secreted into the medium by the cultured cells

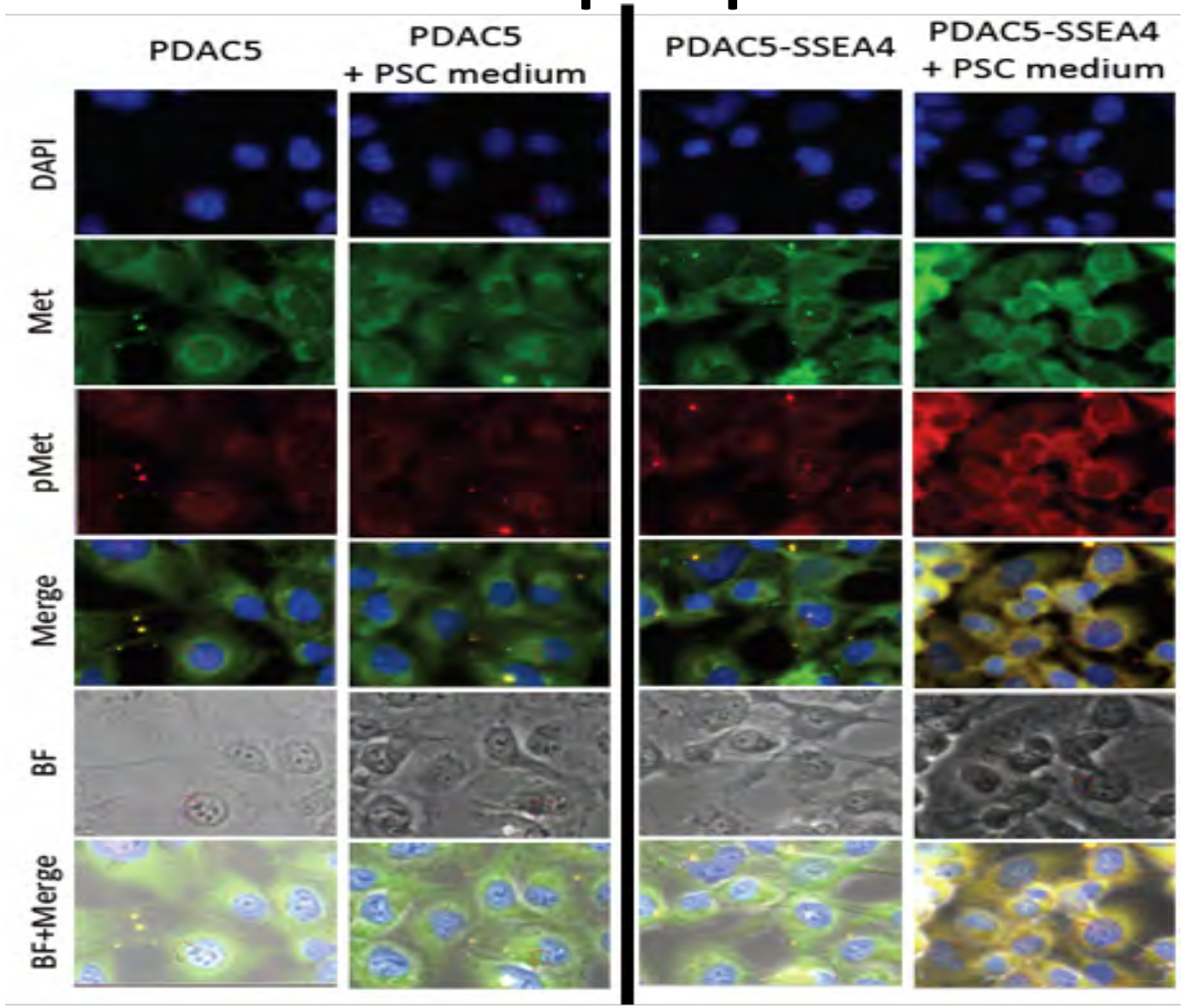
Chamber slides consist of removable polystyrene media chambers attached to a standard glass slide adapted for adherent cell culture.
They are designed for growth, fixation, staining and microscopic examination of cultured cells



PSC conditioned medium increases phospho-c-MET

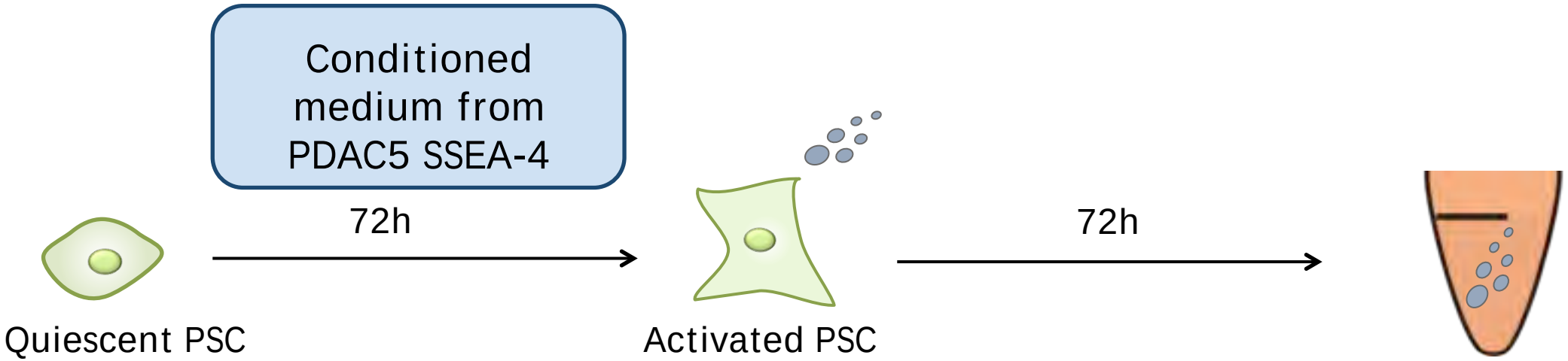






Firuzi et al., *Cancer,* **2019**

Increased human HGF level in stimulated PCM

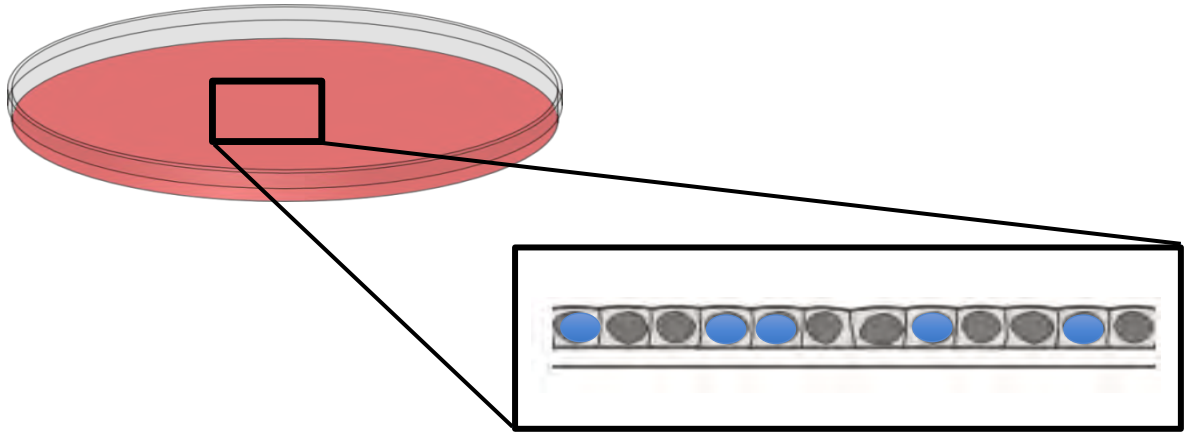


Condition	Human HGF (pg/mL) after 72h of conditioning
Control medium	6.5
Stimulated PSC medium	39.9

Firuzi et al., *Cancer,* **2019**

Co-cultures

Monolayer 2D (co-)culture



Spheroid 3D (co-)culture

Forcing cells to grow in 2D induces **alterations in cell morphology** that in turn translates in changes of the **gene/protein expression**, as well as **cell behavior** compared to the tissue of origin

These limitations are partially overcome by 3D cell cultures that represent the donor-tissues' architecture including cell–cell (and cell–matrix) interactions and are thus valuable tools for investigating the influence of the microenvironment and gradients of nutrients and oxygen on the interplay of cells within a tumor and **their response to drug treatment**

Hickman et al., *Biotech J,* **2014**

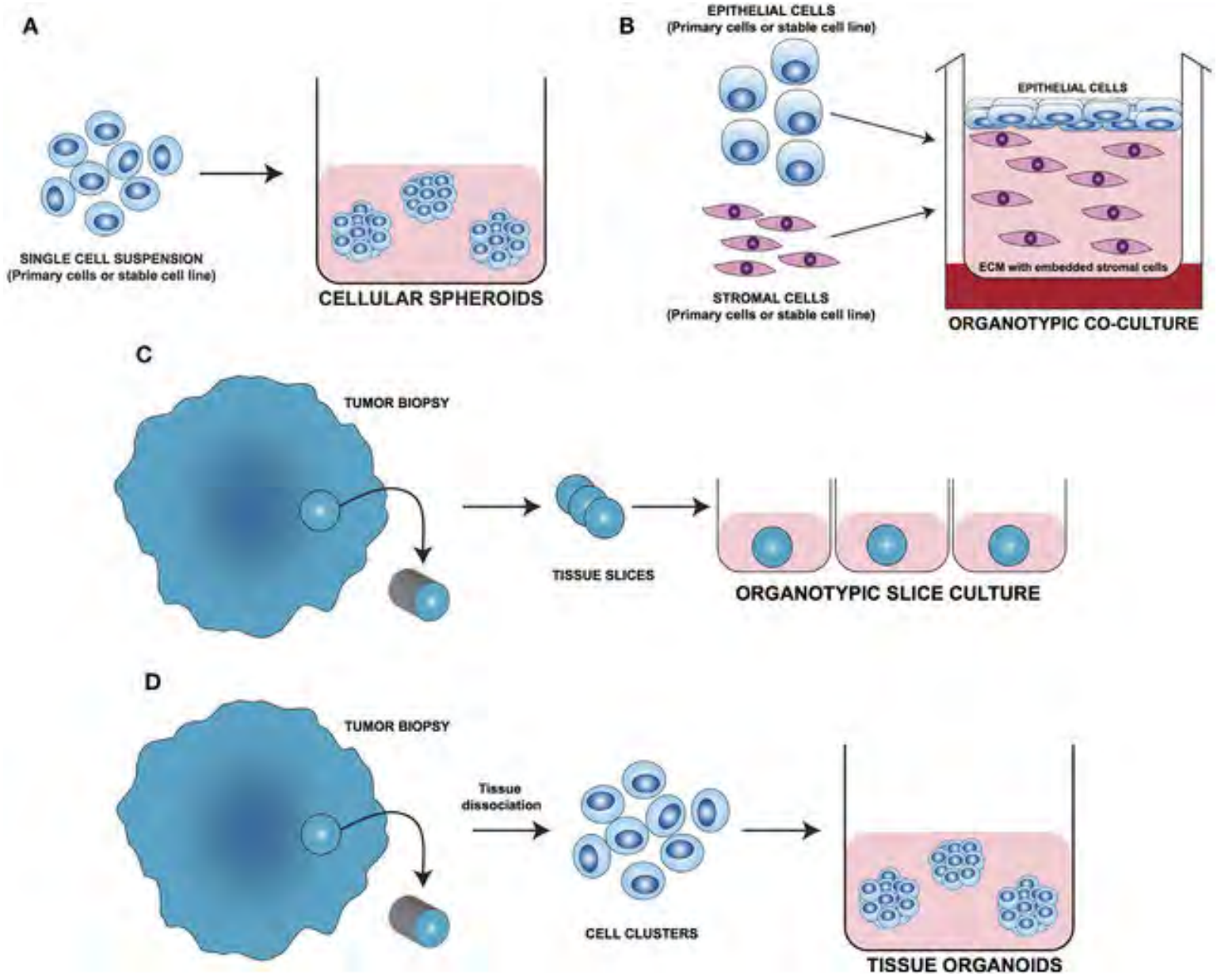
Different approaches for 3D cell culture model development

(A) **Cellular spheroids**: single cells from primary or stable cell lines aggregate together forming 3D structures

(B) **Organotypic coculture**: epithelial cells are cocultured with stroma cells (embedded in a supporting matrix)

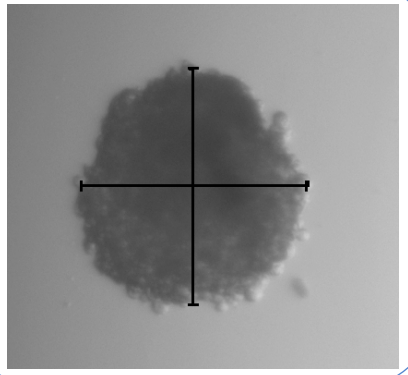
(C) **Organotypic slice culture**: tissue slices obtained from the whole organ or from fragments of it are directly cultivated ex vivo

(D) **Tissue organoids**: primary cells isolated from fresh tissue without prior cell enrichment are grown as 3D multicellular structures

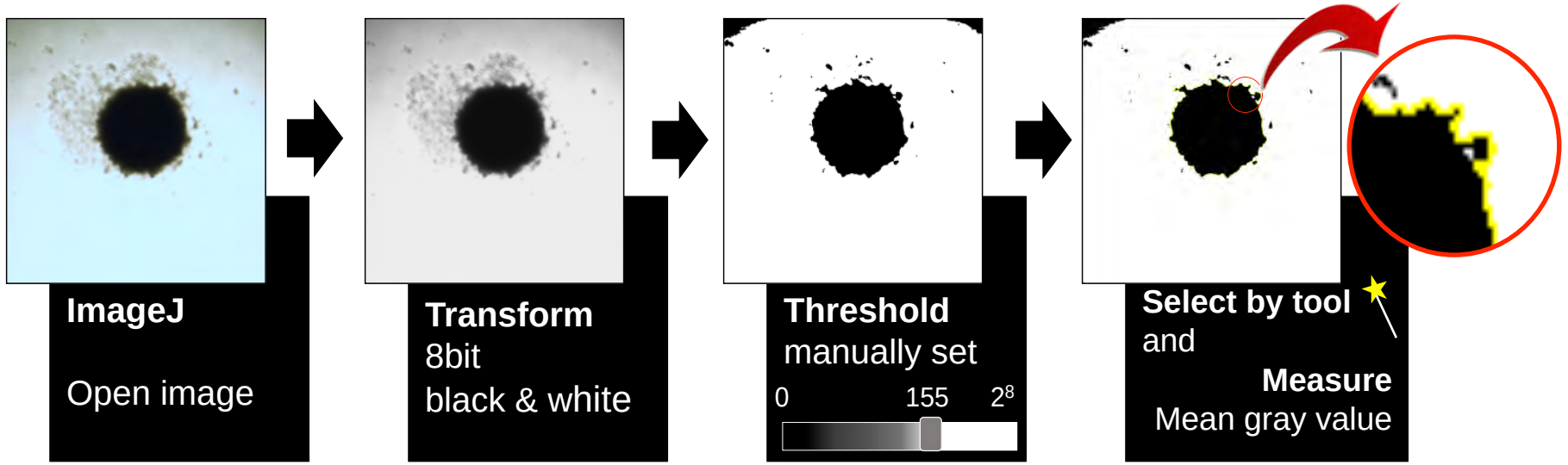


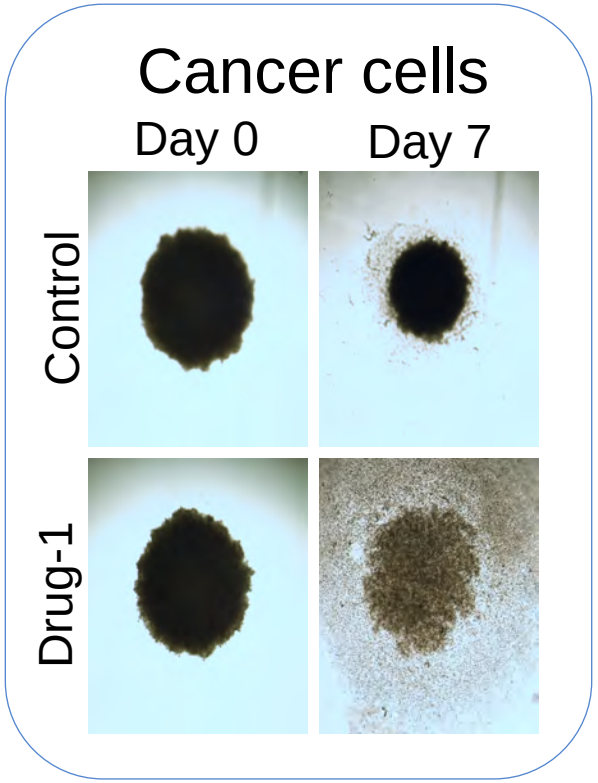
Gaebler et al., *Front Oncol,* **2017**

Image analysis of spheroids, based on 1) dimension and/or 2) density



Spheroid volume (V) can be calculated from the geometric mean of the perpendicular diameters
D = (Dmax + Dmin)/2, ($V = 4/3\pi (D/2)3$)



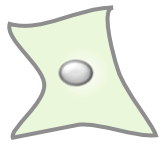


$$\text{Mean gray value} = \frac{\text{Sum gray value of all the pixels in the selection}}{\text{Number of pixels within the selection}}$$

Avan et al., *Mol Cancer Ther,* **2012**

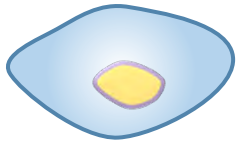
Sciarrillo et al., *EBiomedicine,* **2019**

Establishment of (co-cultured) bioluminescent spheroid models



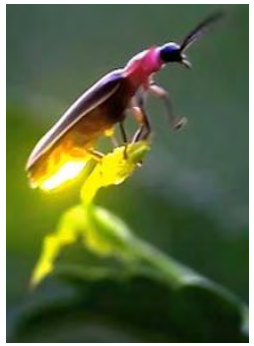
PSC
+ GFP

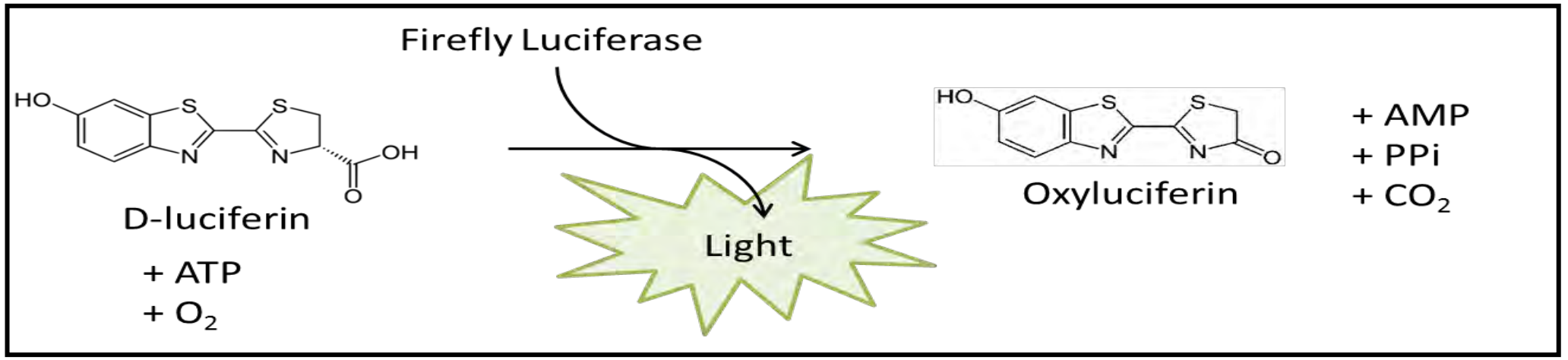
+



PDAC5 SSEA-4
+ CFP
+ Firefly Luciferase (FLuc)

Serum-free
adaptation
(0% FBS)

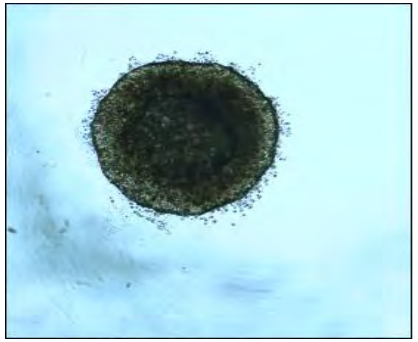




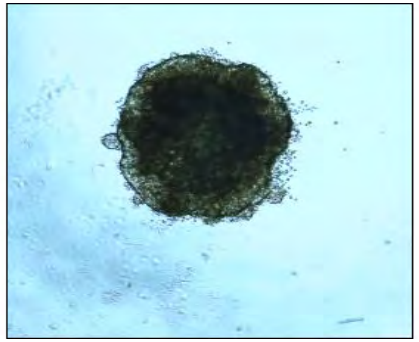
Avan et al., *Cancer Res,* **2013**

Confocal imaging of co-cultured spheroids shows increased density and structural reorganization

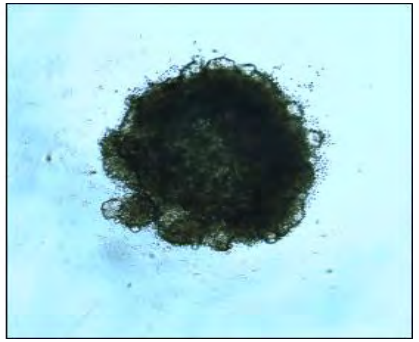
PDAC5 SSEA-4 20K

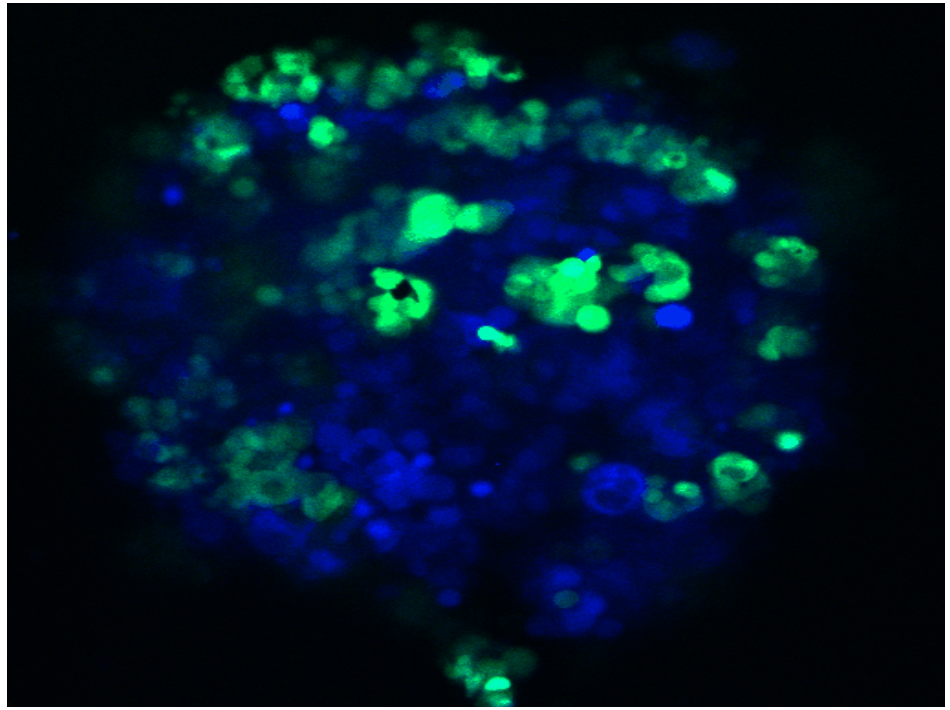


PSC 10K/PDAC5 SSEA-4 20K



PSC 20K/PDAC5 SSEA-4 20K

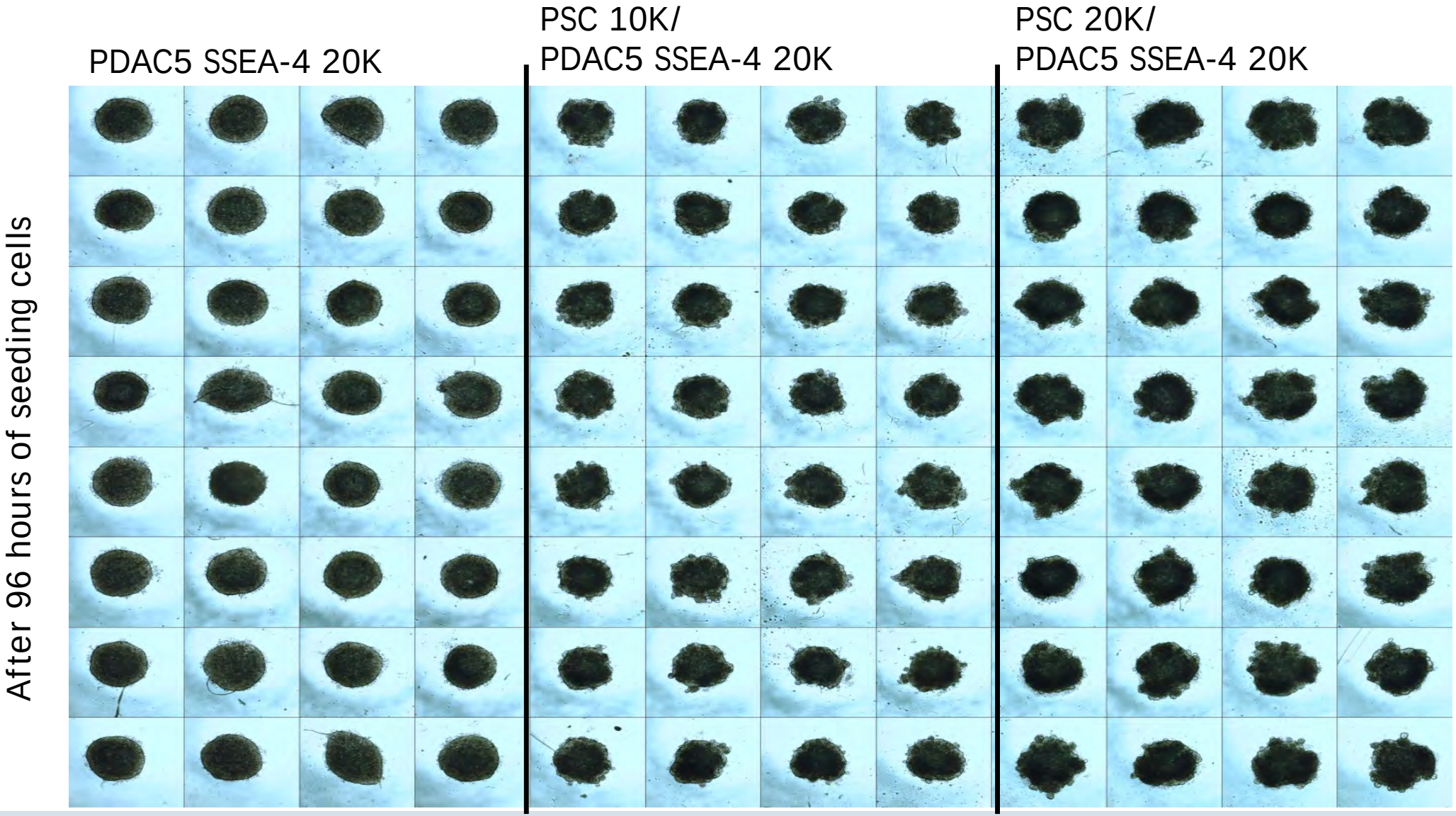




PDAC5 SSEA-4
PSC

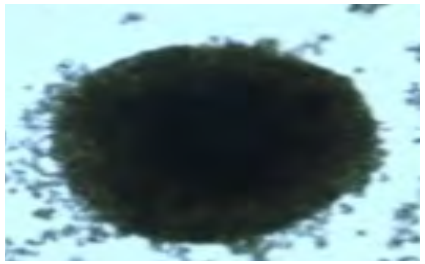
Firuzi et al., *Cancer,* **2019**

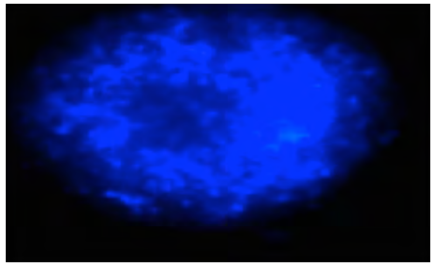
96-well plate overview of spheroids



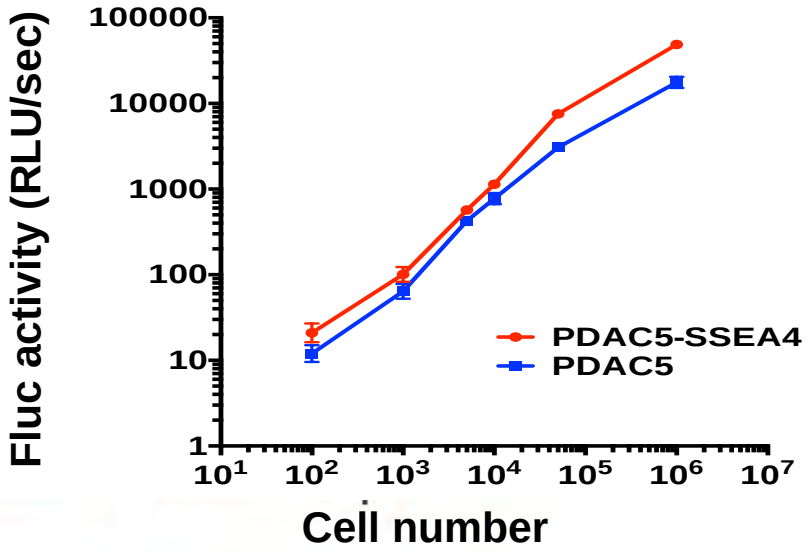
Firuzi et al., *Cancer,* **2019**

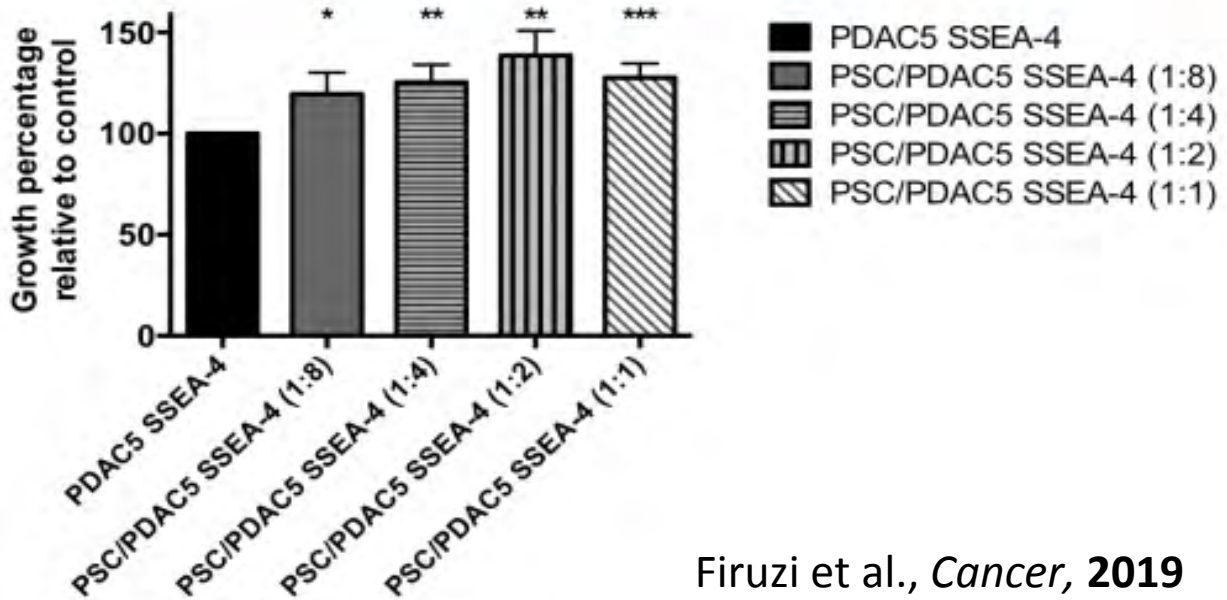
Evaluation of homo- and hetero-spheroids by luciferase assay shows increased PC cells growth in hetero-spheroids





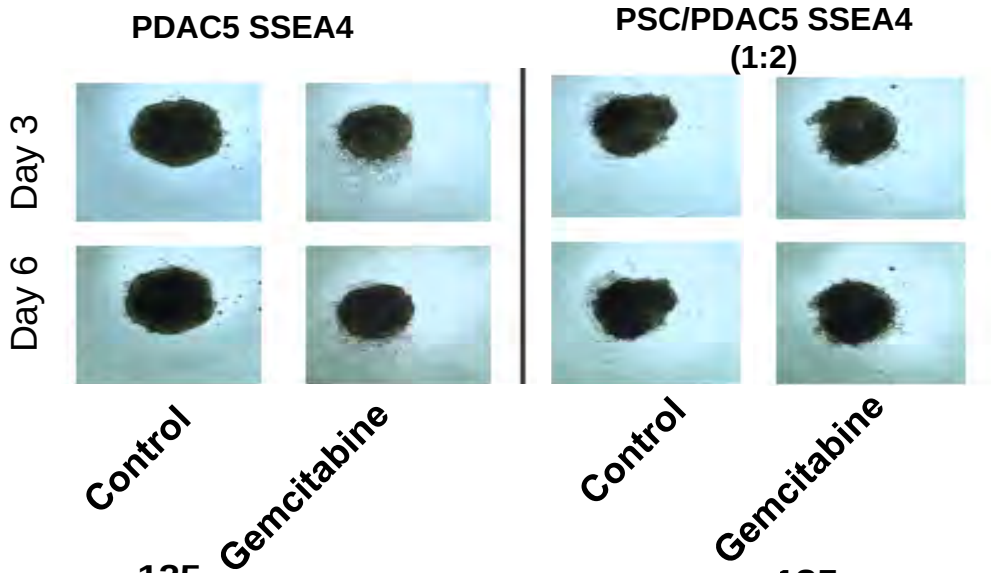
PDAC5 SSEA-4 (CFP)

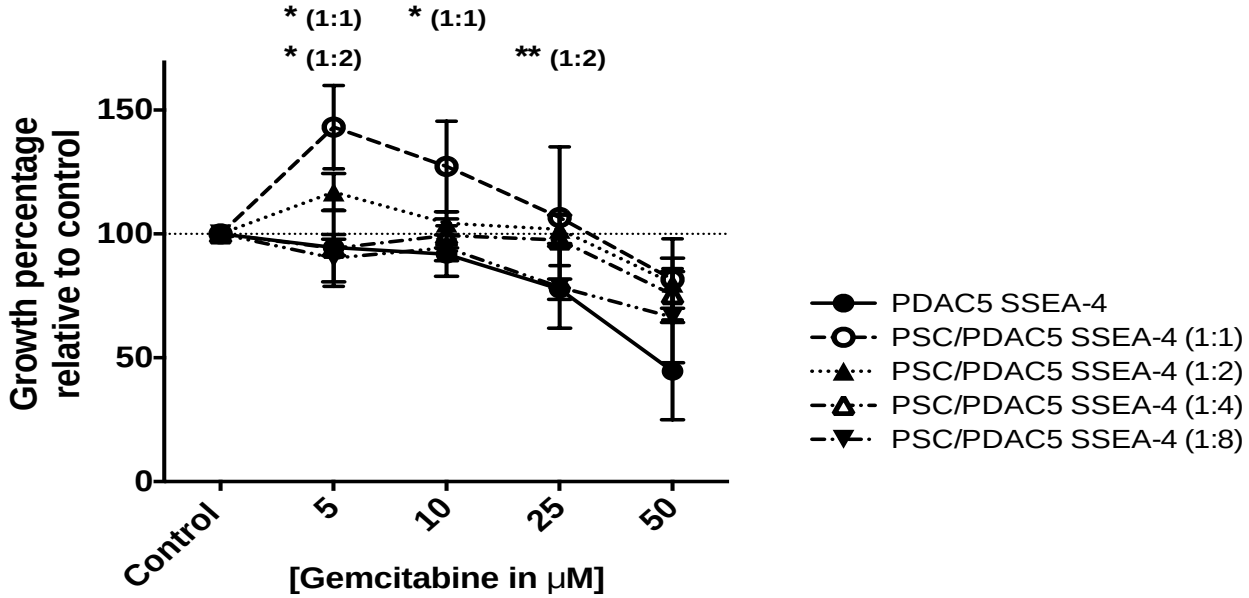


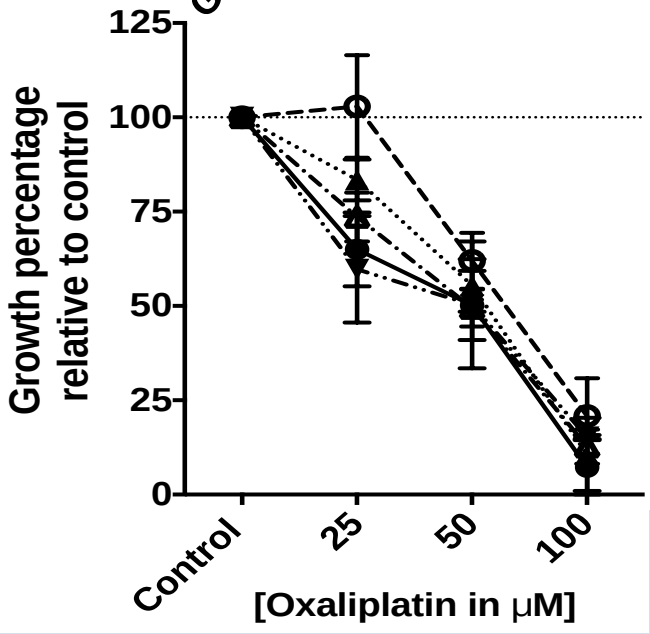


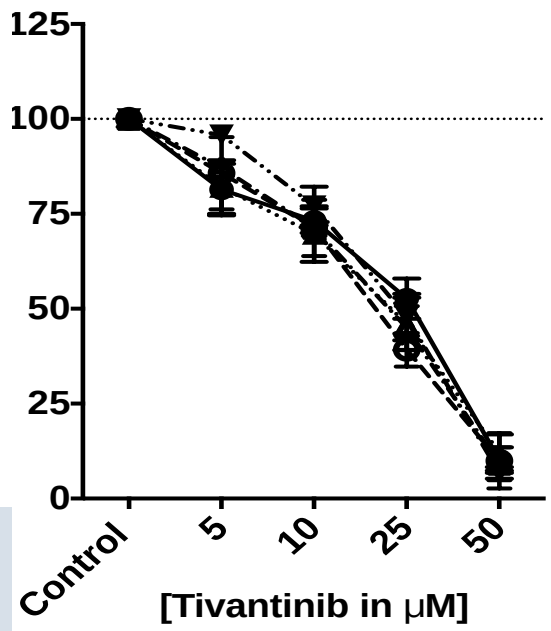
Firuzi et al., *Cancer,* **2019**

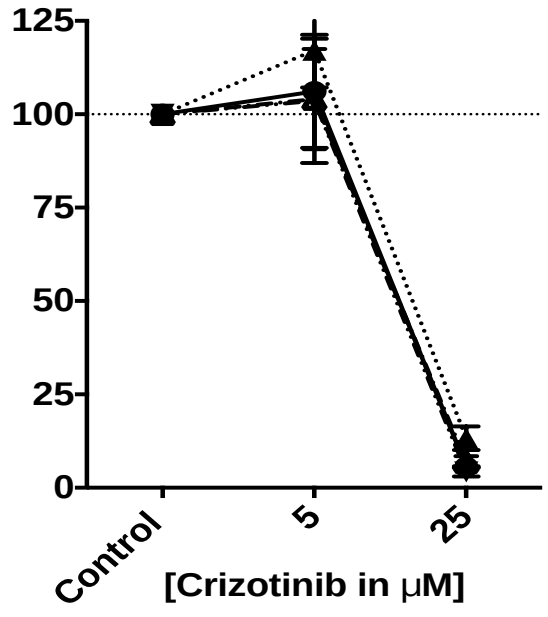
Hetero-spheroids show increased resistance to gemcitabine, but not to c-MET inhibitors

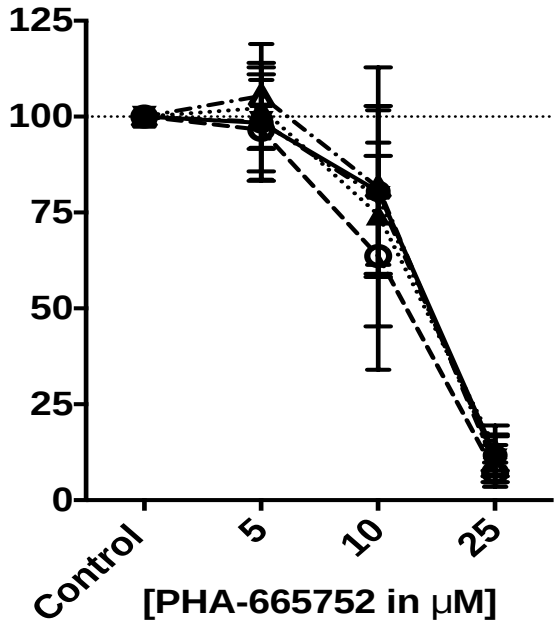




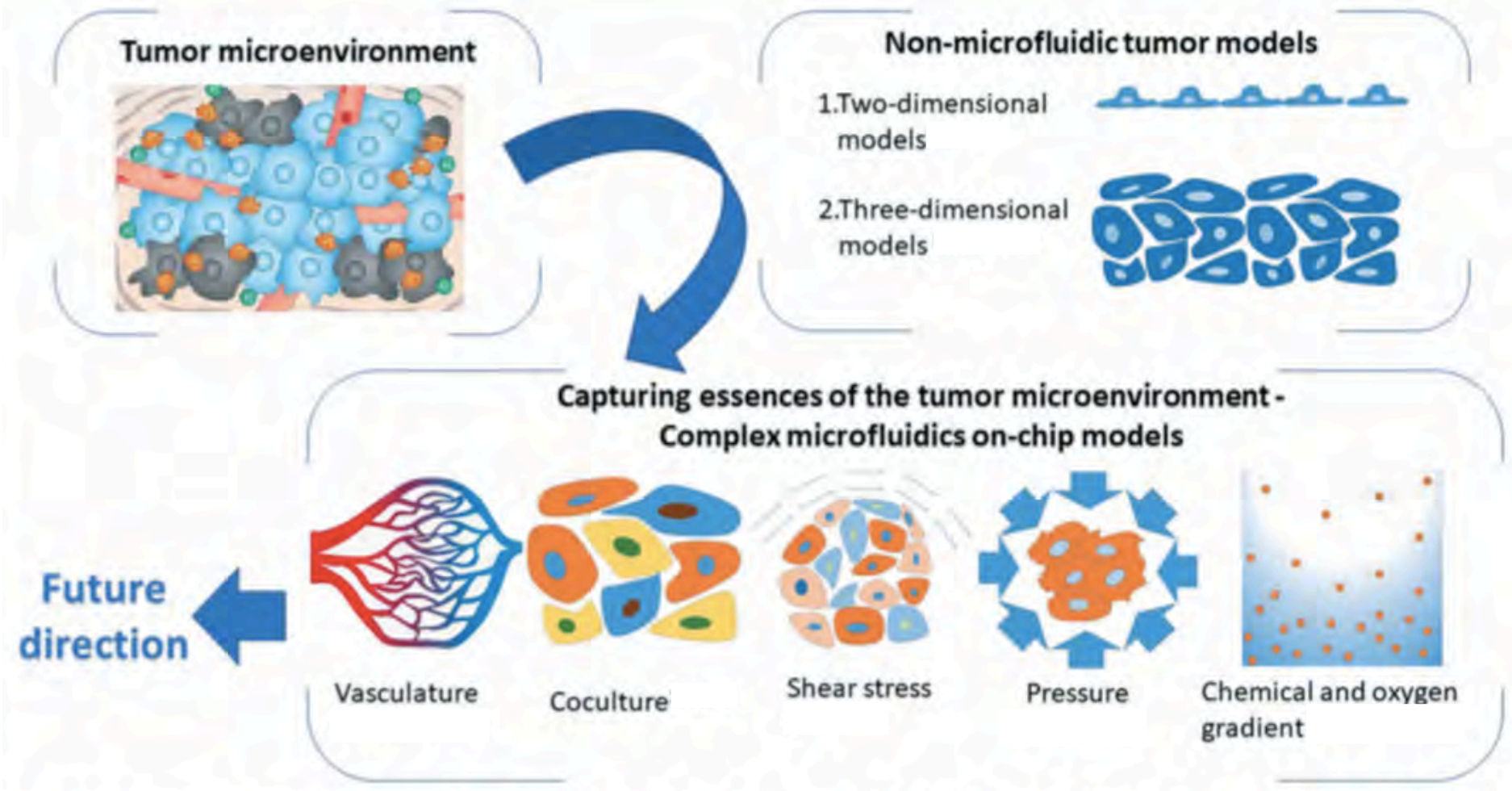






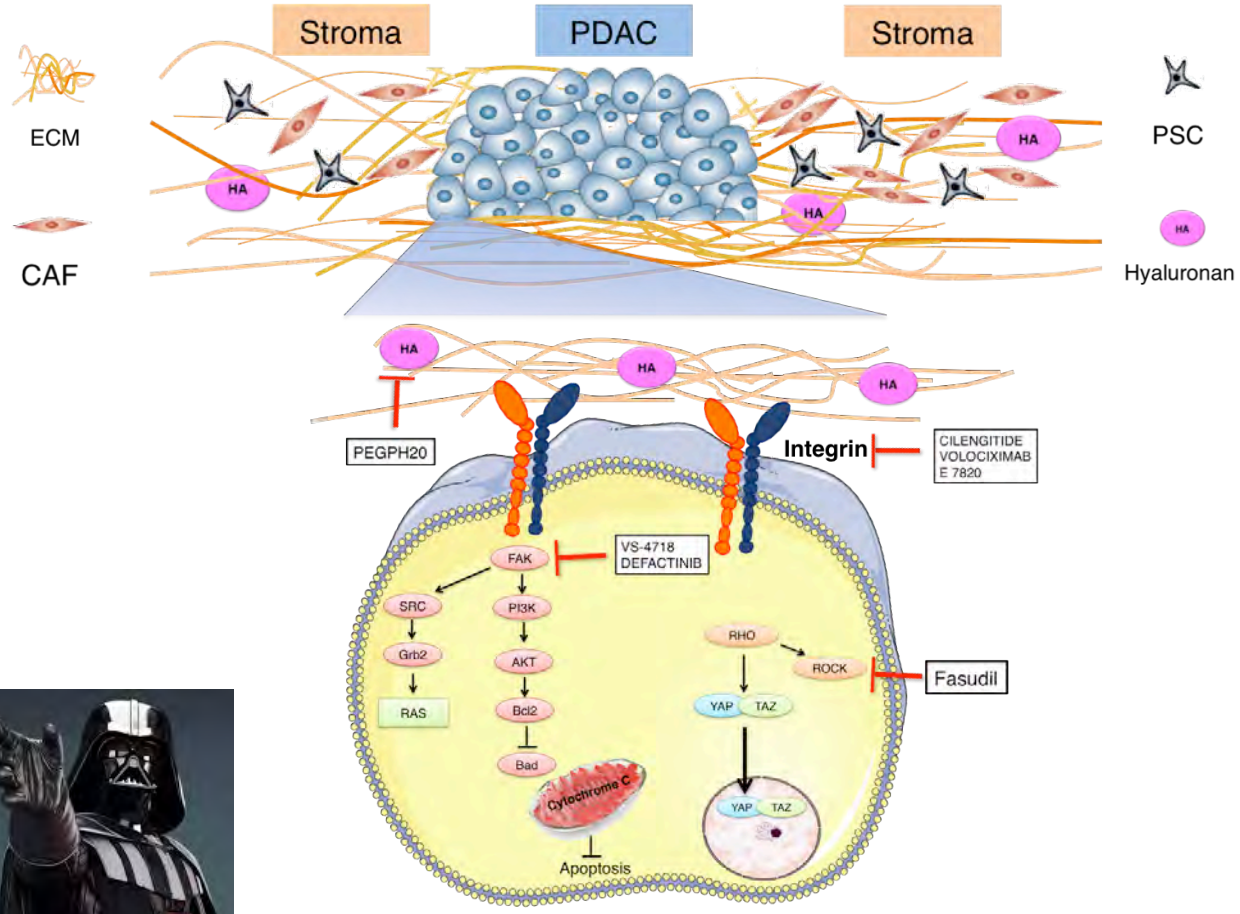


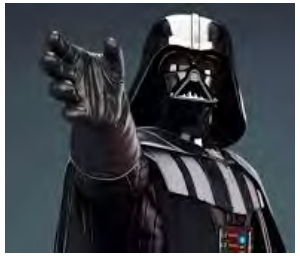
Future models



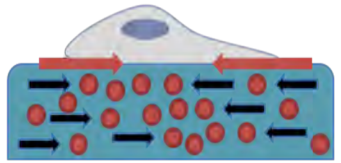
Shang et al., *Lab Chip,* **2019**

Last tools: “sensing the force”

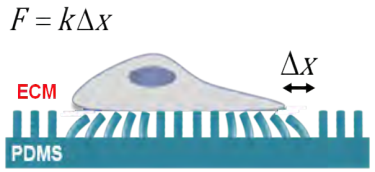




a. Traction Force Microscopy

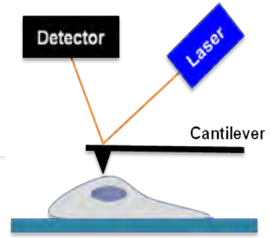


b. Elastic micropillar arrays

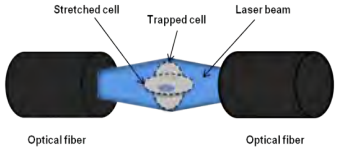


c. Micropipette aspiration

d. Atomic Force Microscopy

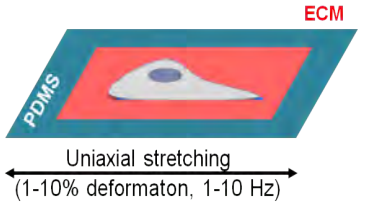


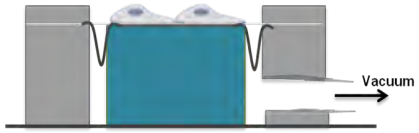
e. Optical stretcher



f. RT-deformability cytometry

g. Cell stretcher





Coppola et al., *Drug Res Updates,* **2017**

Capula et al., EORTC-PAMM 2019

Take-home messages

- TME is emerging as a key determinant of anticancer drug activity/resistance
- To perform optimal pharmacological studies in vitro, new models reproducing the structure, interactions and different cell types of the TME are warranted
- 3D models and co-cultures are valuable tools for investigating the interplay of cells within a tumor and their response to drug treatment
- Future microfluidic models might further help in the evaluation of chemical gradient and physical stress

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- Tom Würdinger
- Stefano Coppola
- Mjriam Capula
- Niccola Funel
- Godefridus J Peters
- Rainer Heuchel
- Mathias Lohr

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Karolinska Institutet

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Cancer Pharmacology Lab
AIRC Start-up Unit

"Human beings are members of a whole
In creation of one essence and soul
If one member is afflicted with pain
Other members uneasy will remain
If you have no sympathy for human pain
The name of human you cannot retain"

Saadi, from Shiraz

DUTCH CANCER SOCIETY

ITT Istituto Toscano Tumori

AIRC

Cancer Center Amsterdam Foundation

NWO Nederlandse Organisatie voor Wetenschappelijk Onderzoek

EORTC European Organization for Research and Treatment of Cancer

PAMM Pharmacology & Molecular Mechanisms Group

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