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Lung - tumorspheres as an effective *in vitro* and *in vivo* platform for screening anti-cancer stem cell drugs

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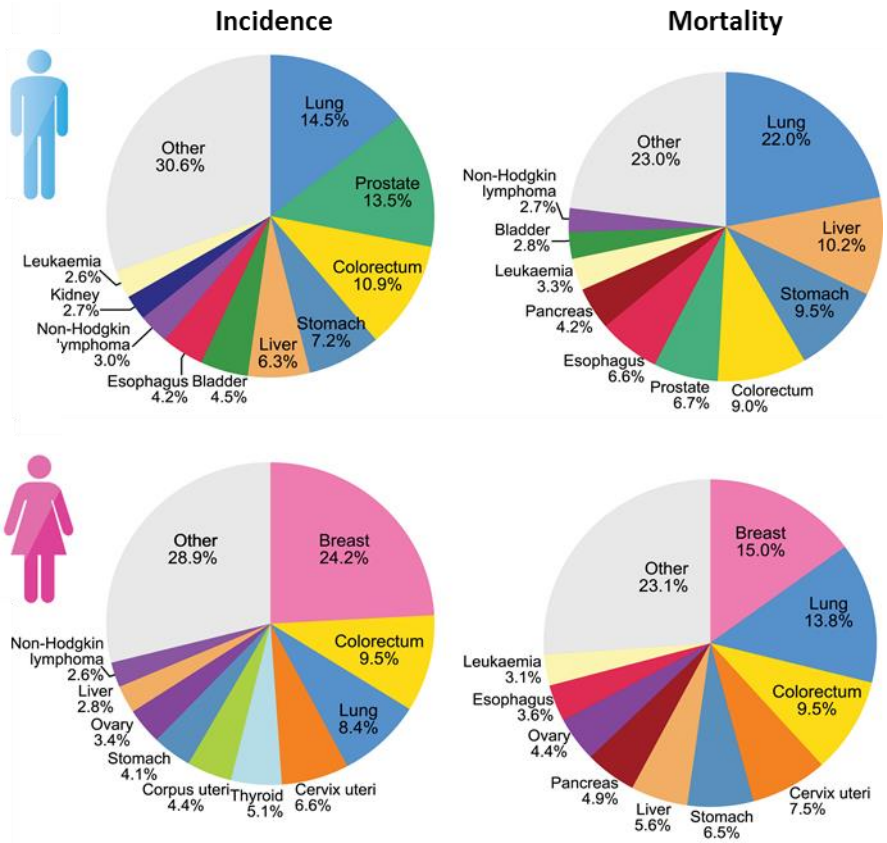
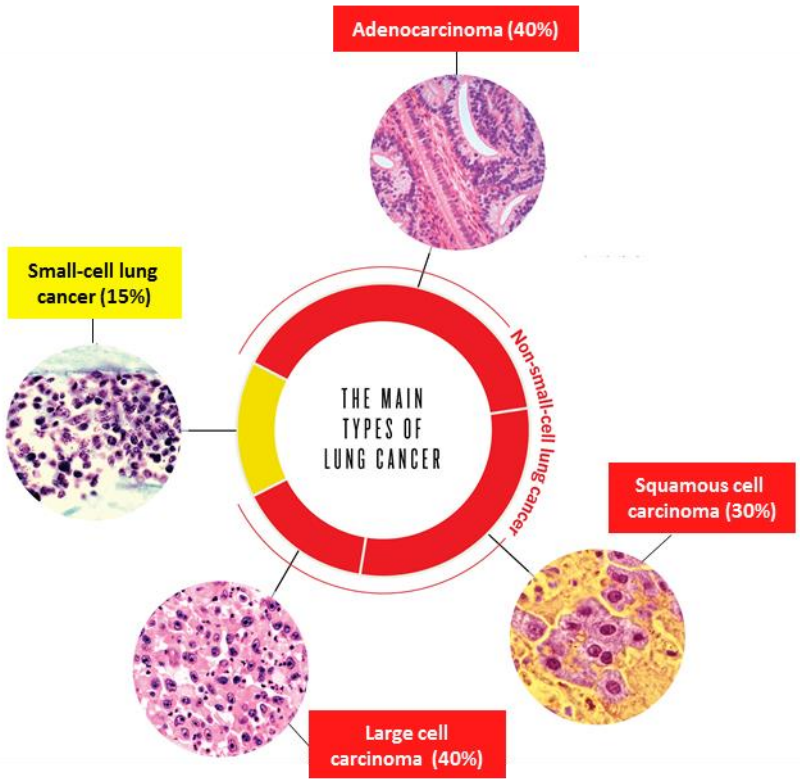
Lung cancer

Statistics worldwide

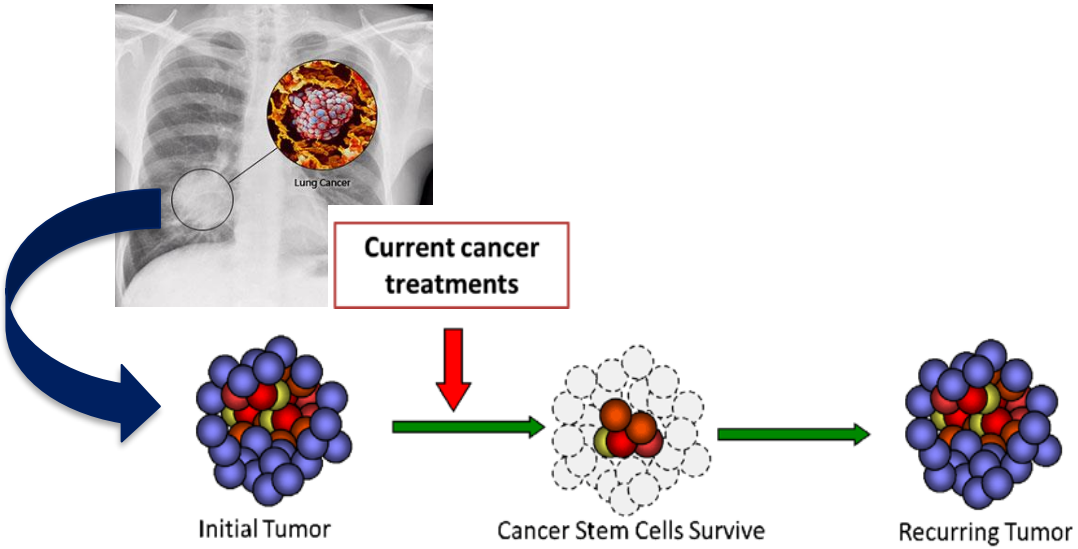


Histological subtypes

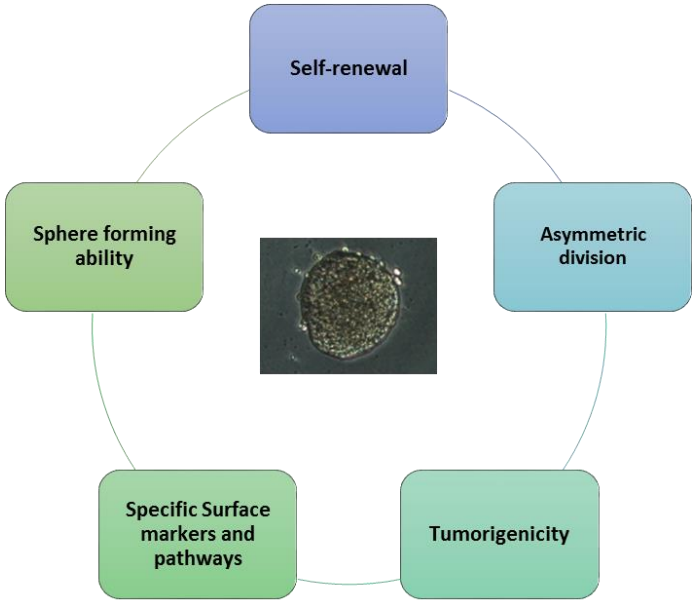


Bray F *et al., CA Cancer J Clin*, 2018.

Lung Cancer Stem Cells (Lung-CSCs)



Resistance to conventional treatment

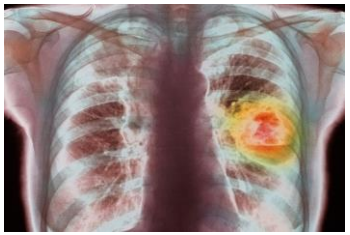


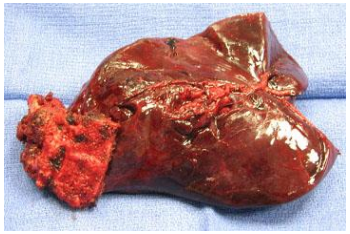
Objective: To discover new therapeutic strategies against lung-CSCs through screening platforms.

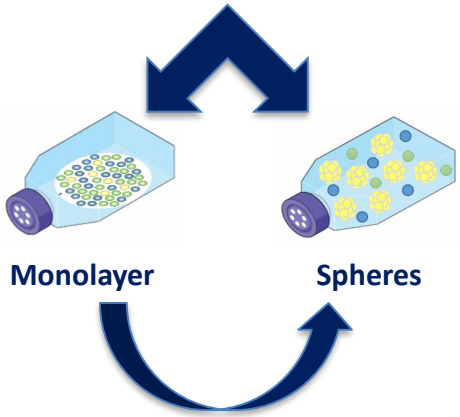
Zhang DEG *et al., Oncol Lett*, 2014.

Ghani FI *et al., Biochem Biophys Res Commun*, 2011.

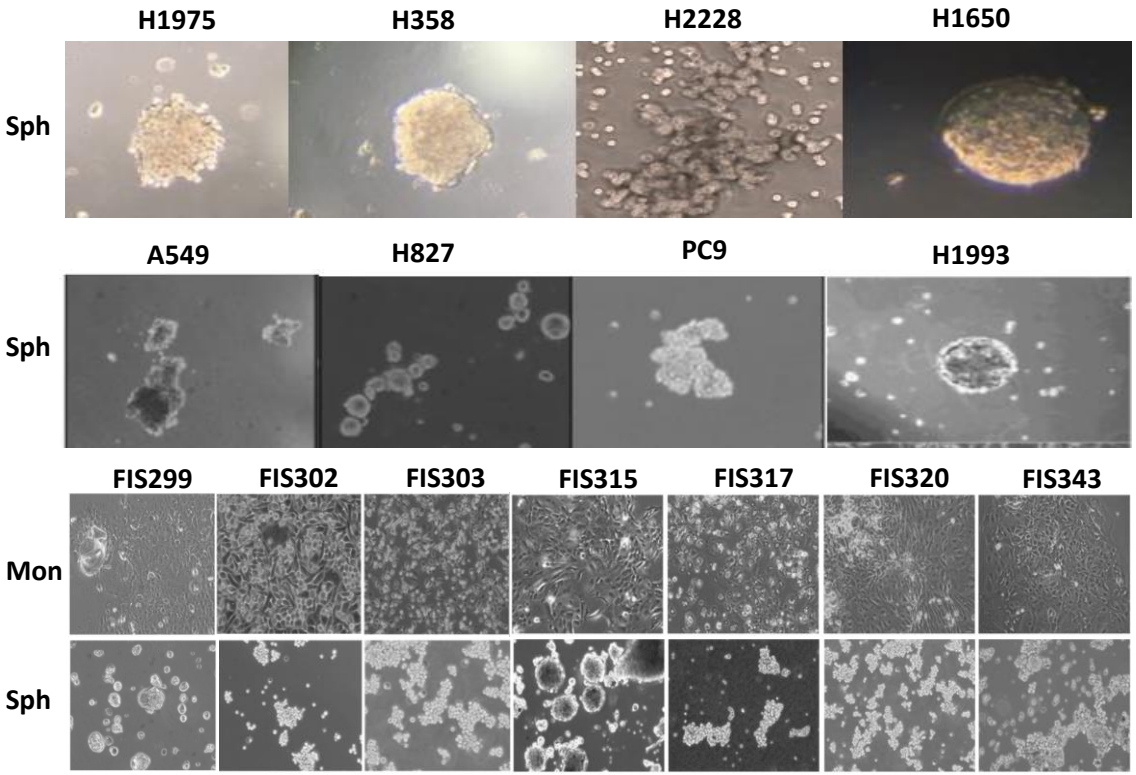
Formation spheres assay



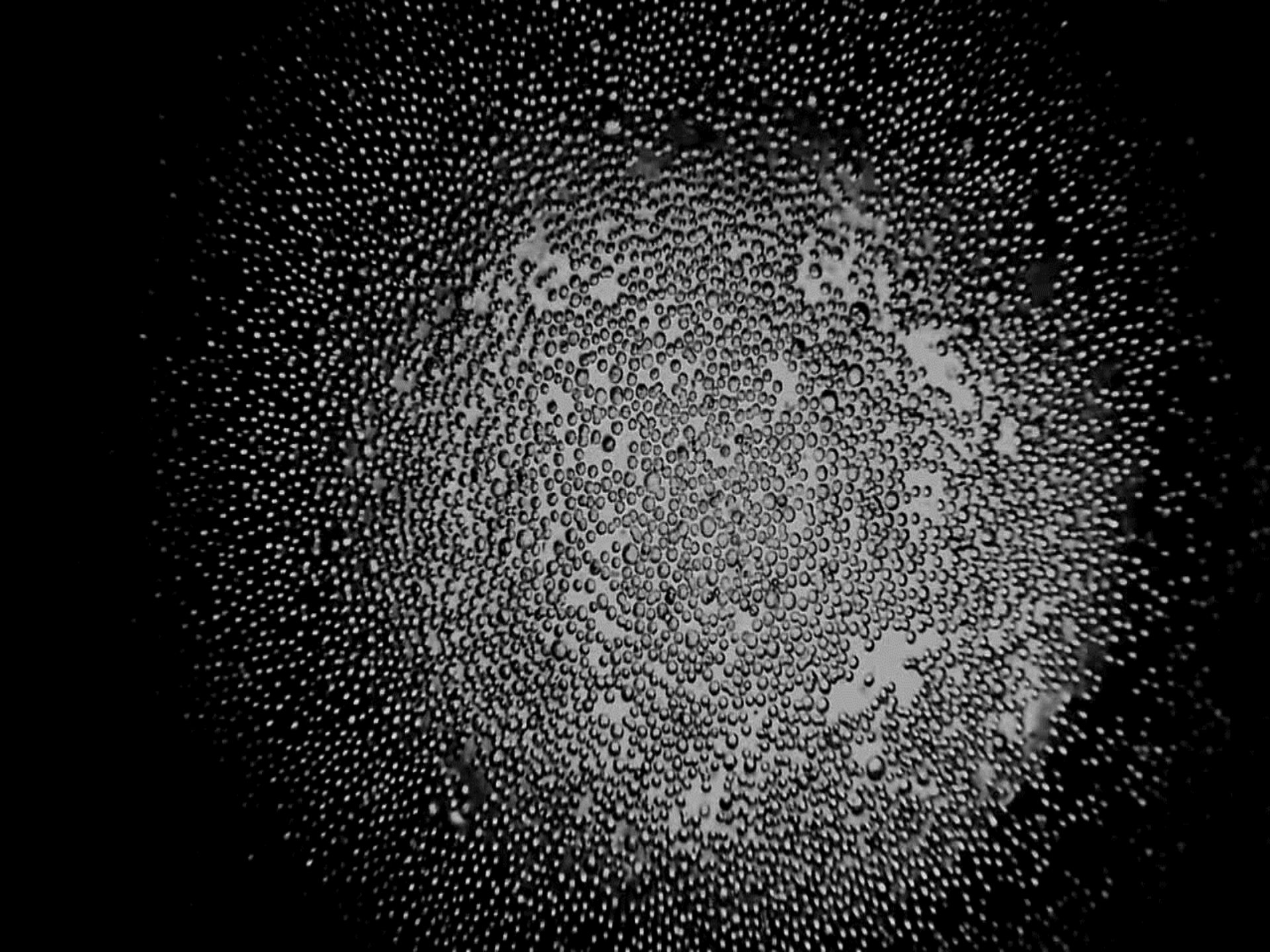




11 cell lines / 7 patients (NSCLC)



Ester Munera – Maravilla *et al., RTICC*, 2015.



Methods

High-throughput screening for drug discovery

H1650

Prestwick Library
1280 approved compounds at 1 µM

18 positive compounds
(Toxicity > 50% in spheres)

Myria Library
10 000 compounds not approved at 1 µM

48 positive compounds
(Toxicity > 50% in spheres)

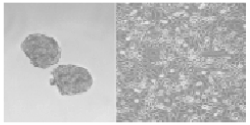
A549, H23, H1650, H1993, PC9

Confirmation of 13 compounds with Toxicity > 60% in spheres (0.1 µM - 1 µM)

Selection of 5 compounds (PW2B8, PW3D11, PW6C9, PW8A11 and PW10C7) with greater antitumor potential

Selection of 15 compounds (Toxicity > 60% in spheres)

Confirmation of 14 compounds in A549 and H1650 (0.1 µM - 1 µM)



in vitro

A549, H23, H358, H460, H1650, H1395, H1975, H1993, H2228, PC9, FIS343

Validation (0.1 µM - 5 µM)

Selection of 3 compounds (PW2B8, PW6C9 and PW10C7) with more anti-CSCs potential

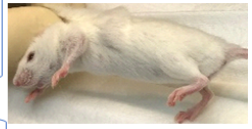
Selection of 3 compounds (SG124F6, SG124G6 and SG125A2) with more anti-CSCs potential

Validation in A549, H23, H358, H460, H1650, H1395, H1993, H2228 and PC9 (0.1 µM - 5 µM)

Selection of PW2B8 (DSF), SG124F6 and SG124G6 with similar chemical structure

A549, H1650, H1975, SW900, FIS299, FIS302, FIS303, FIS315, FIS317, FIS320

Validation of **DSF** and synthesized drugs **[SG124F6 (JMM 1-3) and SG124G6 (JMM 1-4)]** (0.01 µM - 50 µM)



In vivo validation of DSF, JMM 1-3 and JMM 1-4
(tested on xenografts with tumorspheres of H1650 and FIS343 in NOD / SCID mice)

in vivo

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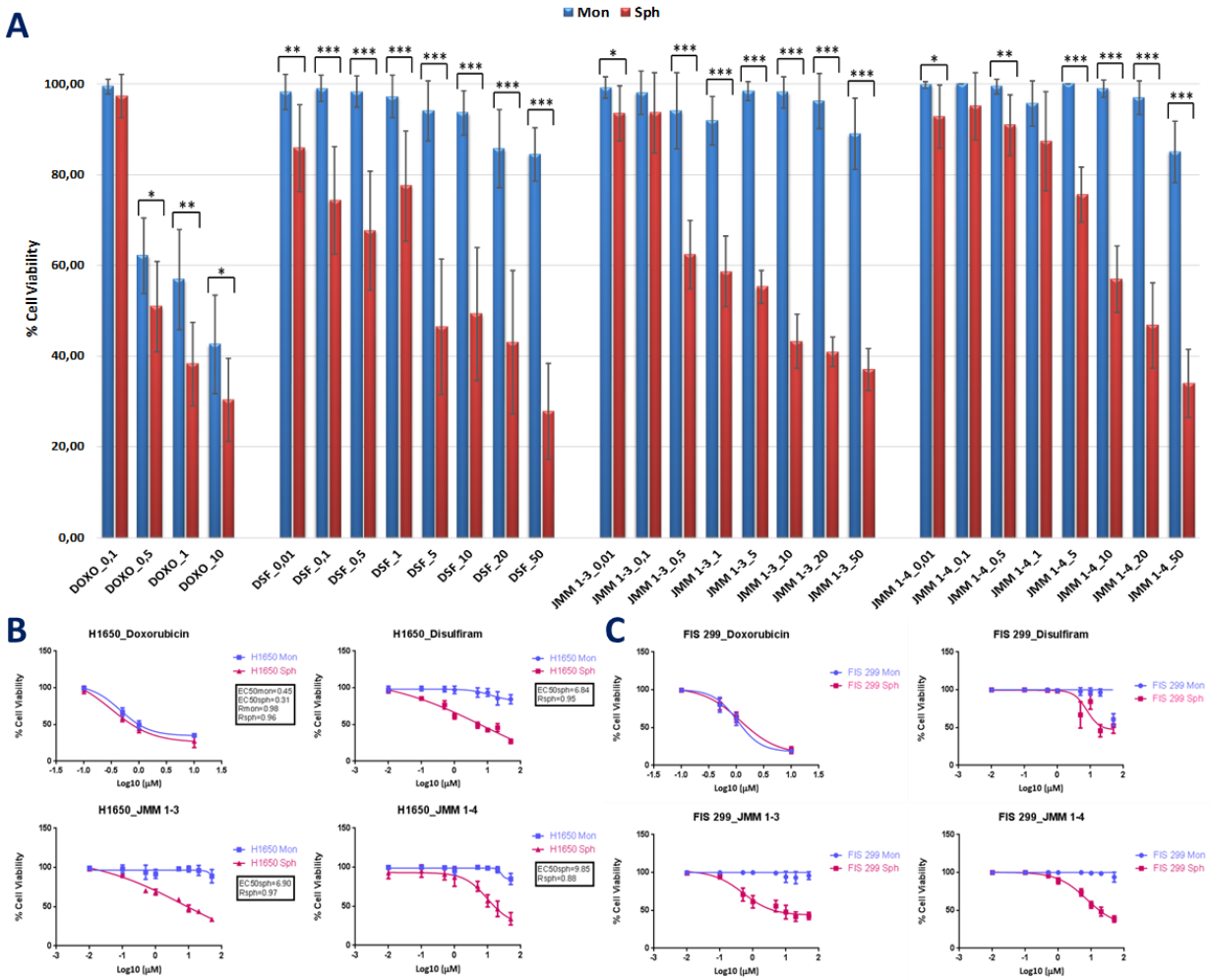
Results

In vitro



A) Percentage of cell viability of the H1650 cell line in monolayer and in spheres at 48 hours of treatment with Doxorubicin, DSF, JMM 1-3 and JMM 1-4. The concentrations of the drugs used were: 0.01 µM, 0.1 µM, 0.5 µM, 1 µM, 5 µM, 10 µM, 20 µM and 50 µM. The bars represent the mean of three independent determinations in triplicate with the standard deviation indicated by error bars. (*) <0.05, (**) <0.01, (***) <0.001. **B) EC50 of Doxorubicin, DSF, JMM 1-3 and JMM 1-4 in H1650 cell line. C) EC50 of Doxorubicin, DSF, JMM 1-3 and JMM 1-4 in FIS299 patient.**

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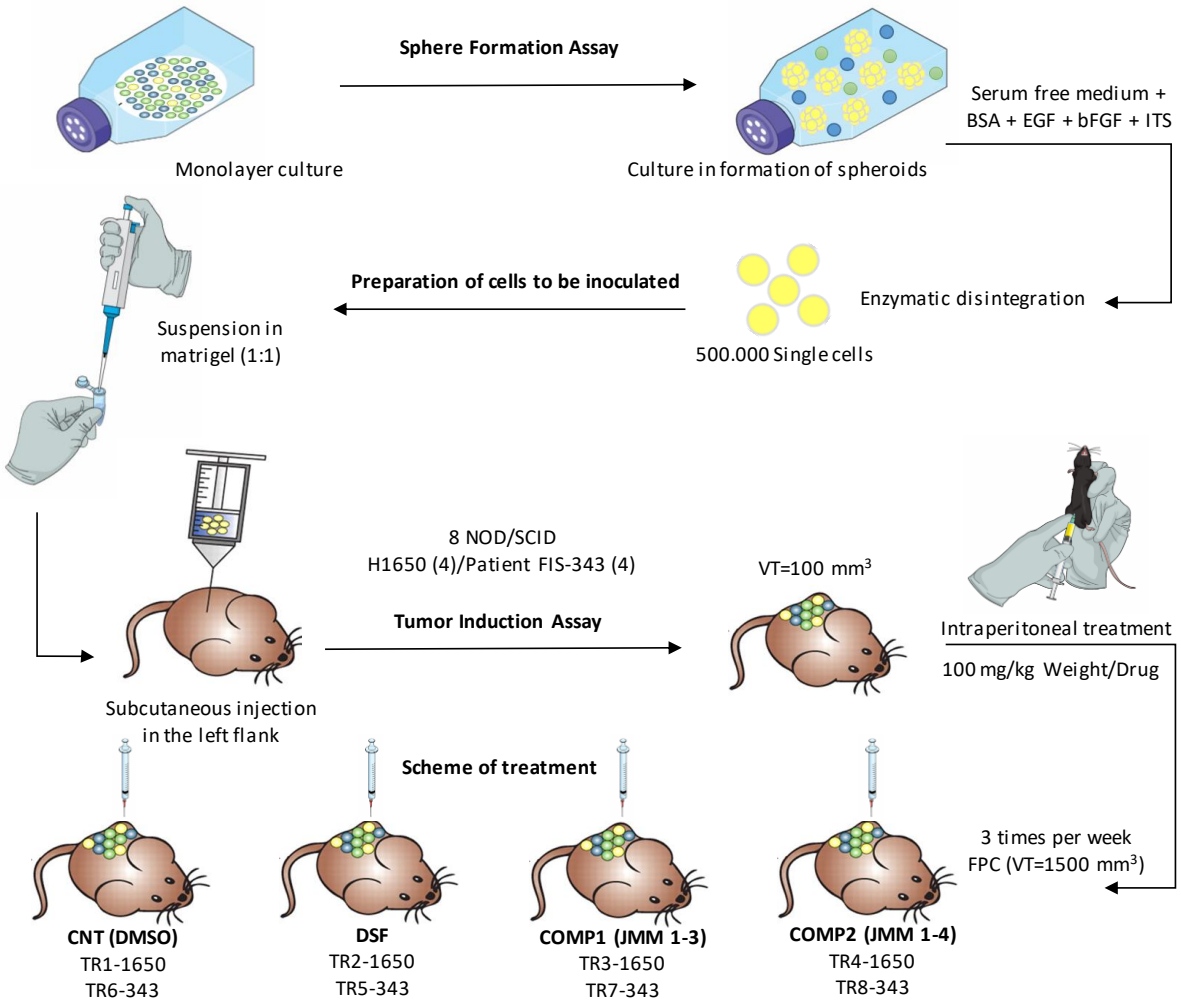
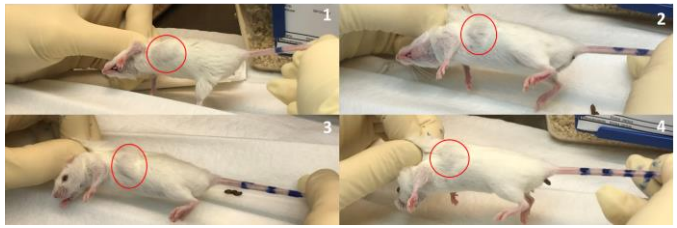
Methods

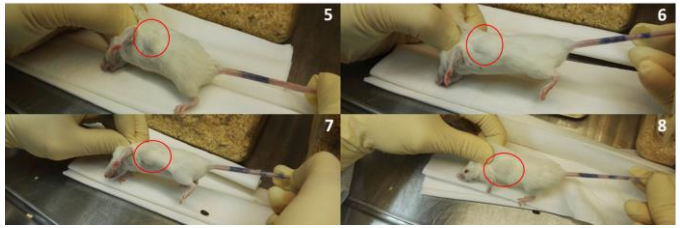
In vivo model



Xenografts with H1650 cell line



Xenografts with FIS343 patient



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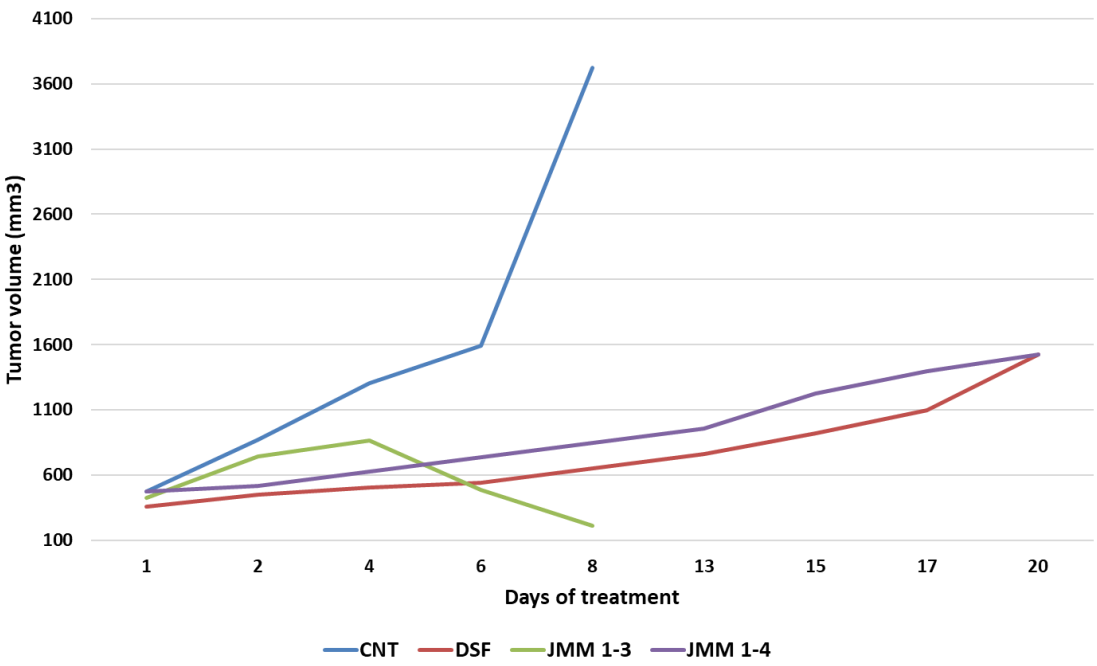
Results

In vivo model

H1650 cell line



Mice (Line/Patient)	Tumor Volume (mm3)	Tumor Reduction (%)
H1650_CNT	1593.11	
H1650_DSF	541.44	66.02
H1650_JMM 1-3	483.72	69.64
H1650_JMM 1-4	276.98	82.62
FIS343_CNT	1645.68	
FIS343_DSF	490.00	70.23
FIS343_JMM 1-3	721.56	56.16
FIS343_JMM 1-4	1020.00	38.02

At this time, we are characterizing the possible inhibition pathways involved in the mechanisms of action of these drugs.

Conclusions:

- Lung-tumorspheres are an effective *in vitro* and *in vivo* platform for screening anti-CSCs drugs.
- The selected compounds **(DSF, JMM 1-3 and JMM 1-4)** have the ability to inhibit tumorspheres with characteristics of lung-CSCs and the capacity to slow and inhibit tumor growth in xenografts, being identified as possible therapeutic strategies against lung-CSCs.

View Posters:

- **"An expression signature characterizes cancer stem cells from lung adenocarcinoma patients".**
  Alejandro Herreros-Pomares et al; PhD Student UPV (FIHGUV).
- **"Interactions of cancer stem cell and immune microenvironment in non- small cell lung cancer (NSCLC)".**
  Feiyu Zhang et al; PhD Student UV (FIHGUV).

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