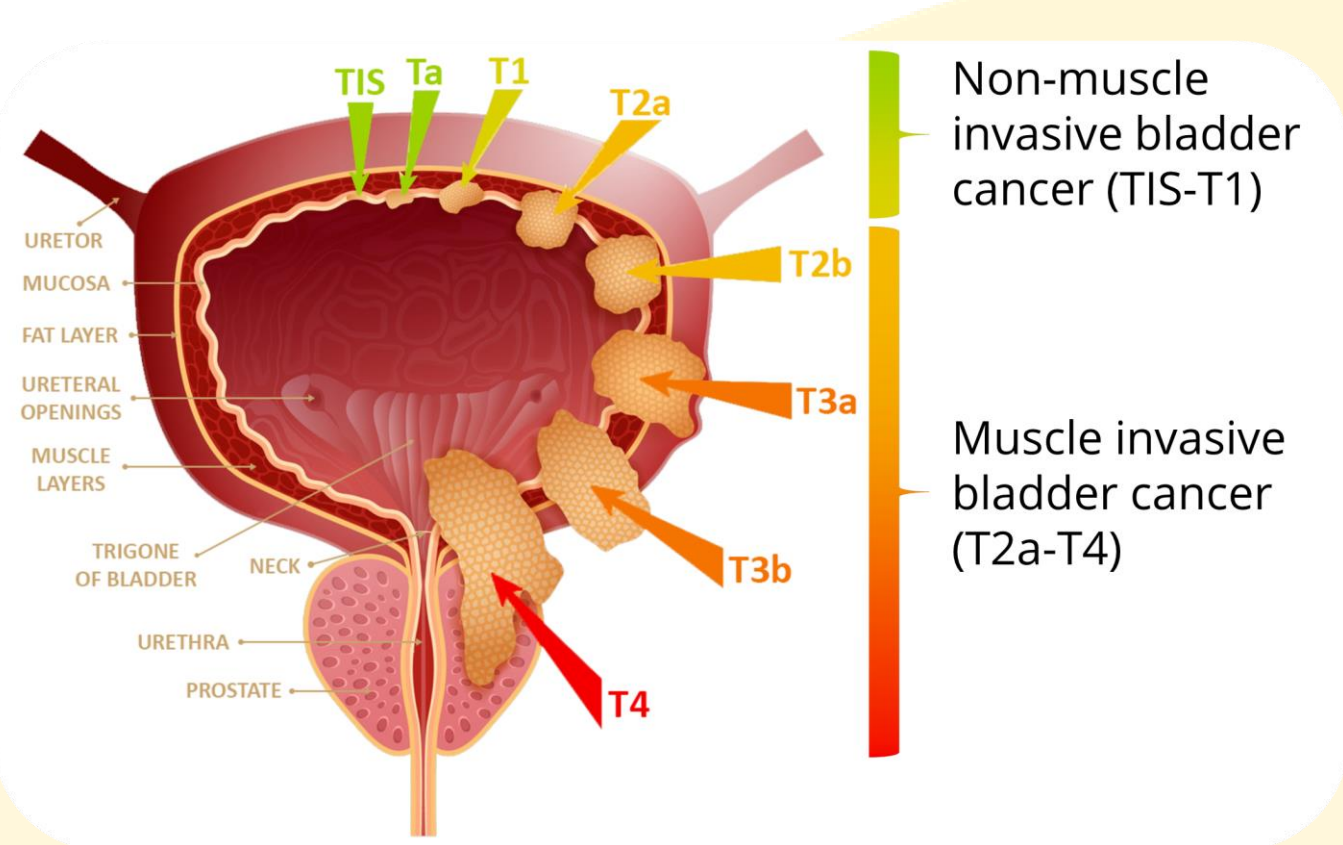


Giacomo Ducci*^{1,2}, Valentina Pasquale^{1,2}, Stefano Rota¹, Edoardo Arrigoni¹, Gloria Campioni^{1,2}, Marco Vanoni^{1,2} and Elena Sacco^{1,2}

*g.ducci@campus.unimib.it

¹Department of Biotechnology and Bioscience, University of Milano-Bicocca, Milan 20126, Italy
²SYSBIO, Centre of Systems Biology, Milan 20126, Italy

Background



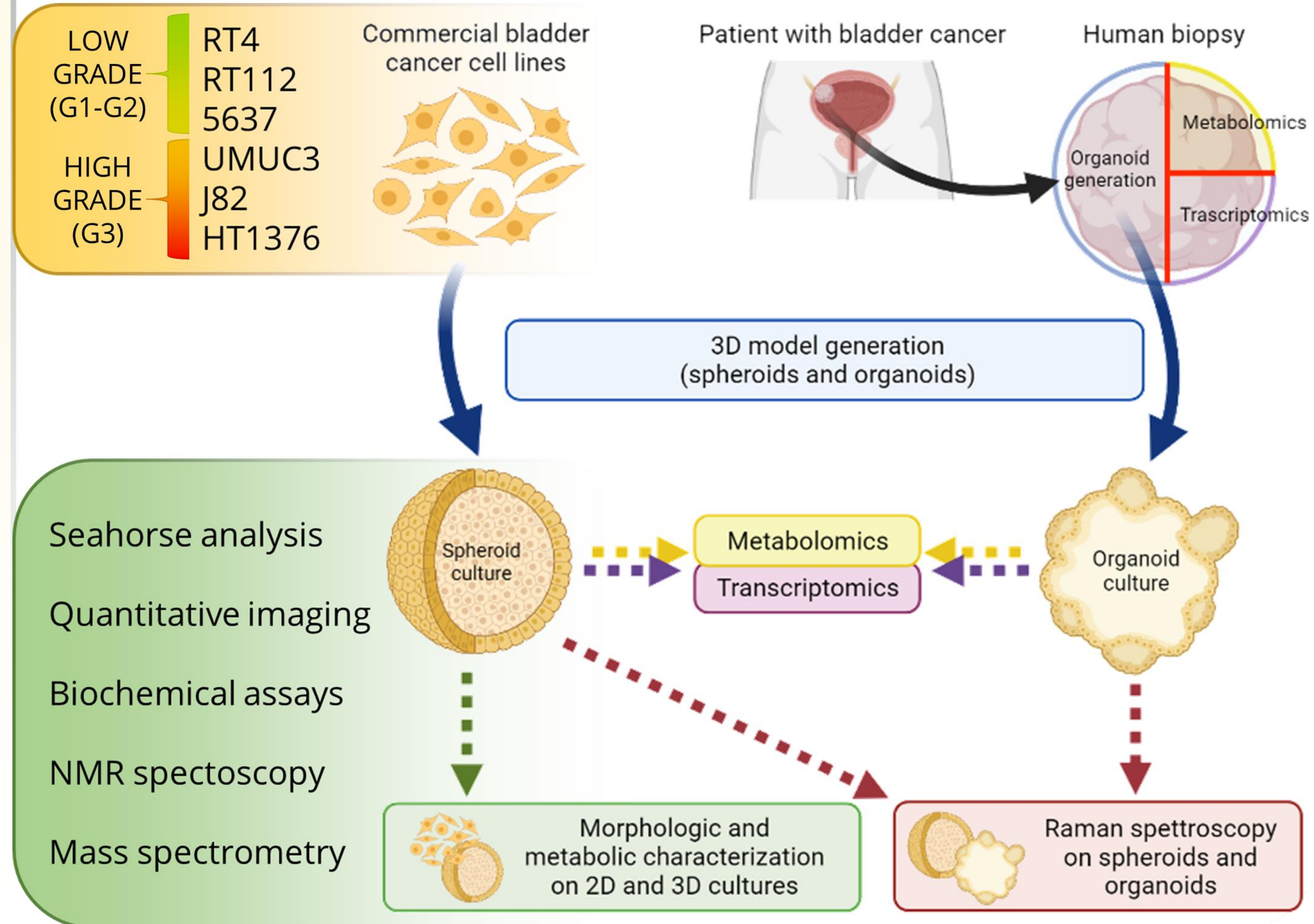
Most bladder cancer (BC) patients are diagnosed with non-muscle invasive BC with frequent recurrences leading to invasive tumors, reducing survival expectations.

3D cultures constitute a more clinically relevant model for studying cancer.

Aim

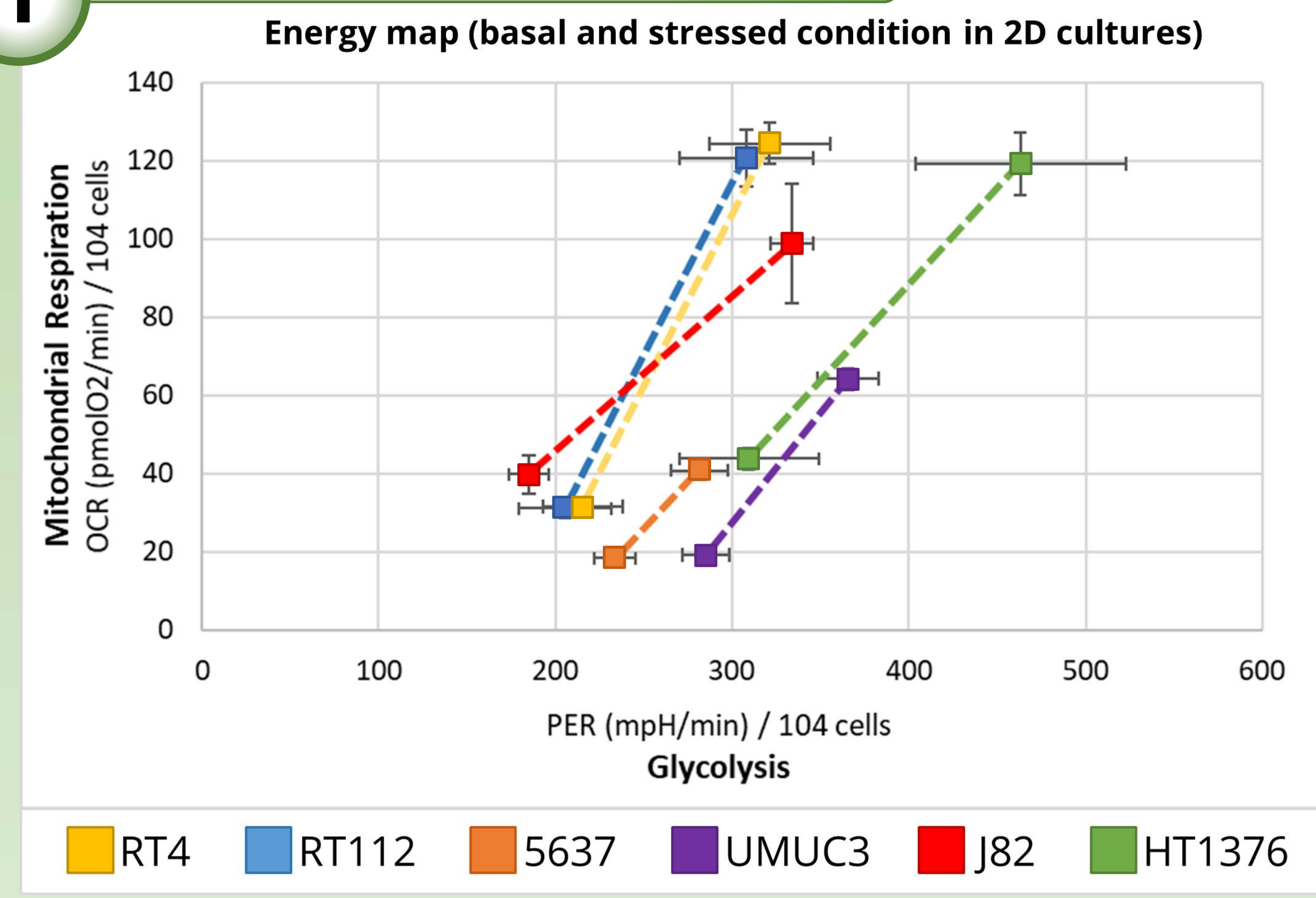
To **characterize spheroid formation** capacity and **energetic metabolism** in a panel of 6 BC cell lines, comparing 2D and 3D cultures.

Project workflow



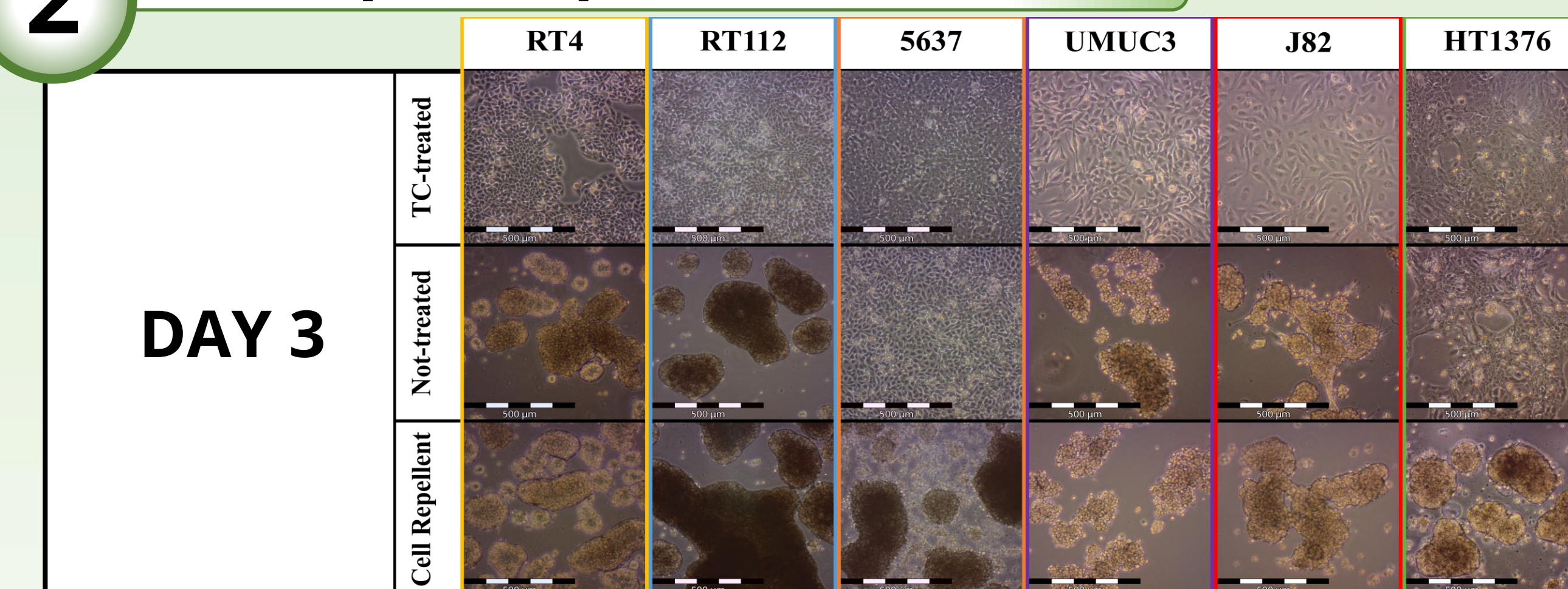
Results

1 2D cultures characterization



2D cultures from high grade cell lines show an enhanced maximal glycolytic capacity compared to low grade. This analysis is not possible on 3D cultures.

2 6-well plate spheroid formation



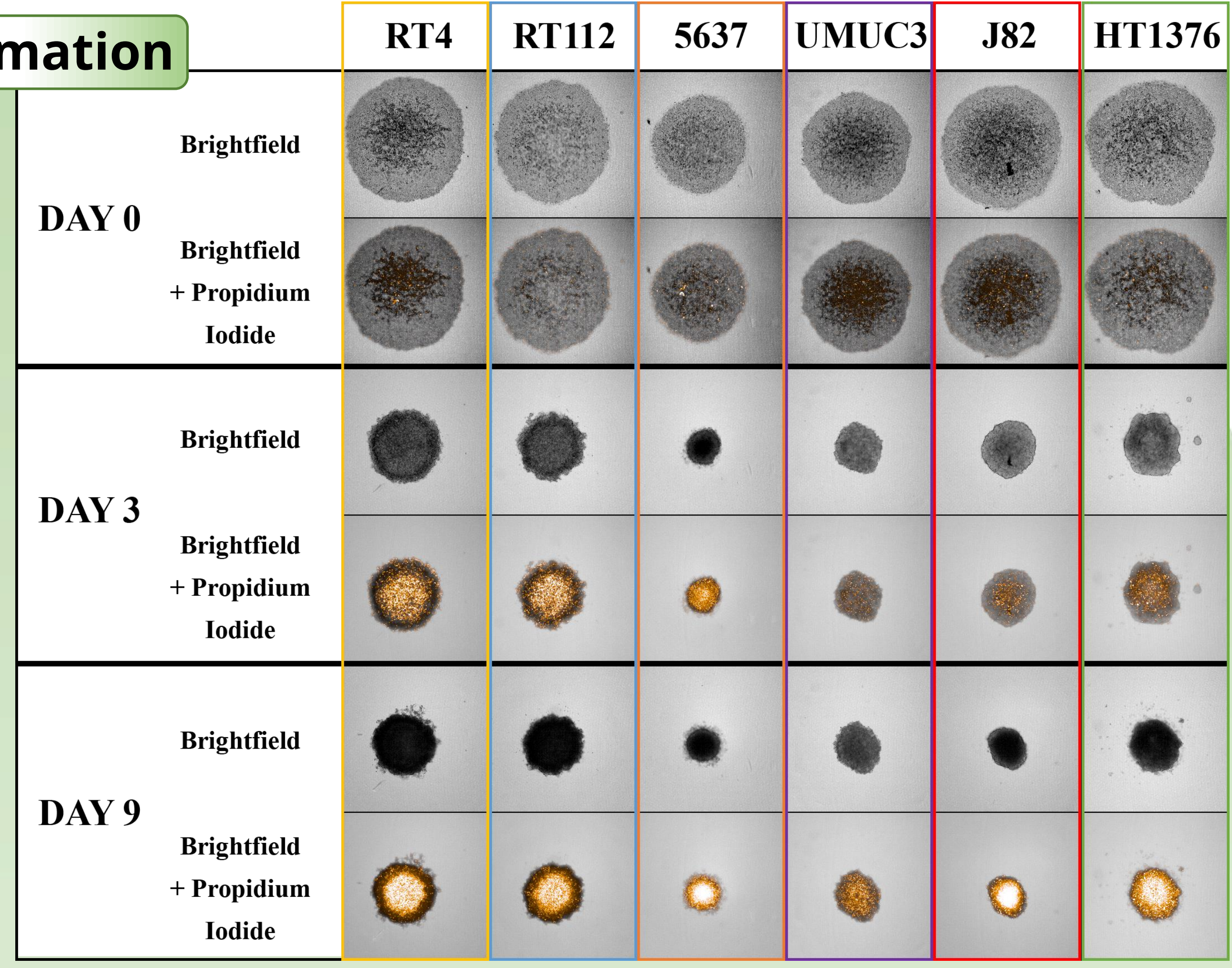
All cell lines are able to form spheroids in cell repellent condition.

Conclusion: high grade cell lines show an increased capacity to form vital spheroids. Cells obtained from 3D cultures in these lines show ATP production comparable to 2D condition.

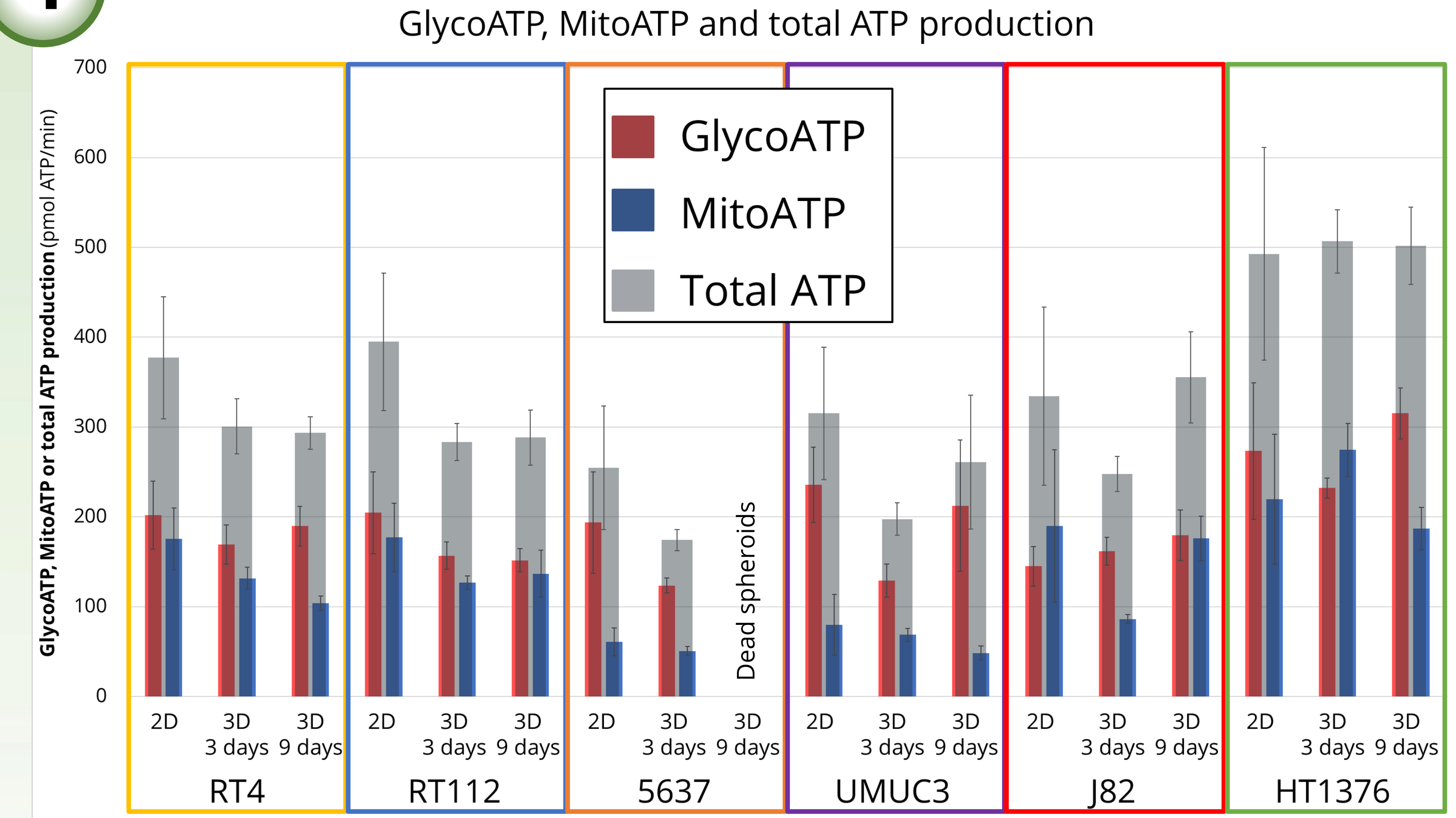
Future perspectives: protocols for metabolic analysis on whole spheroids will be set-up. Preliminary analysis on organoids from patient biopsies will be performed. During a six-month stay in Philadelphia, US, effects of perturbation of signaling pathways correlated to cancer progression will be deepened.

3 96-well plate spheroid formation

After 3 days of growth in U-bottom cell repellent 96well plates, low grade cell lines, respect to high grade cells, form spheroids with a wider core of dead cells (propidium iodide, orange to white color) in relation to the spheroid total area (brightfield). After 9 days all cell lines show a dense core of dead cells.



4 ATP production in 2D and 3D cultures



Assays on cells obtained from disaggregated spheroids show that the total ATP production (gray bars) decreases in 9-days spheroids formed only from low grade cell lines, compared to 2D condition.

Acknowledgements We acknowledge financial support of the H2020 Amplitude n. 871277 Grant to MV and of the Italian Ministry of University and Research (MIUR) through grant 'Dipartimenti di Eccellenza 2017' to University of Milano Bicocca, Dept. Biotechnology and Biosciences.