ORGANOIDI DI GLIOMI DI BASSO GRADO PEDIATRICI: UNA PIATTAFORMA INNOVATIVA PER LO SVILUPPO DI APPROCCI DI IMMUNOTERAPIA AVANZATI

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Il sottoscritto Gabriele Canciani

ai sensi dell'art. 3.3 sul Conflitto di Interessi, pag. 17 del Reg. Applicativo dell'Accordo Stato-Regione del 5 novembre 2009,

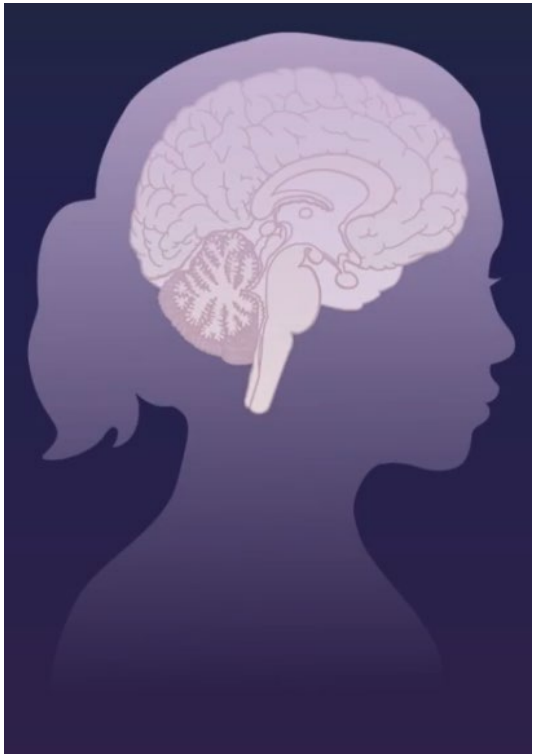
dichiara

☒ *che negli ultimi due anni NON ha avuto rapporti diretti di finanziamento con soggetti portatori di interessi commerciali in campo sanitario*

❑ *che negli ultimi due anni ha avuto rapporti diretti di finanziamento con i seguenti soggetti portatori di interessi commerciali in campo sanitario: - ……*

- *……*
- *……..*

Pediatric Low-Grade Glioma (pLGG)



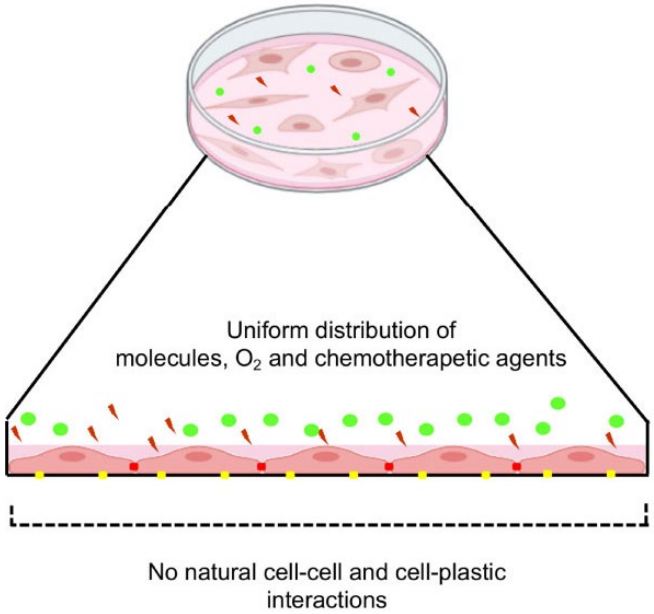
- Most common Central Nervous System (CNS) tumors of childhood
- Gross total resection (GTR) represents the main therapeutical option
- Chemotherapy and, in selected cases, radiotherapy or target therapy
- Slow proliferation and senescence limit the *in vitro* establishment of cell lines

In need of innovative culture systems for preclinical evaluation of antitumoral therapies

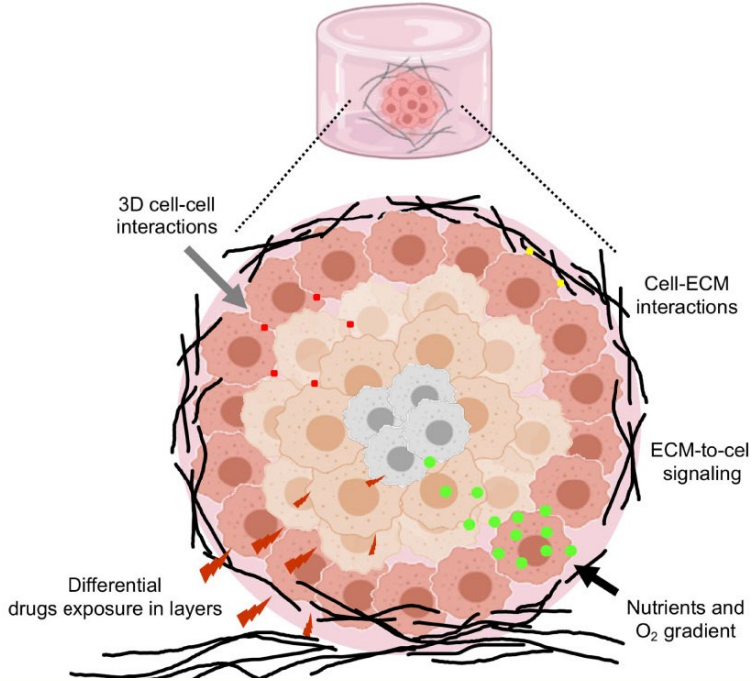
Cohen AR, NEJM 2022; Bouffet E. et al., NEJM 2023
Manhoarhan N. et al., Neoplasia 2023

Culture systems for primary tumor cells

2D conventional cultures

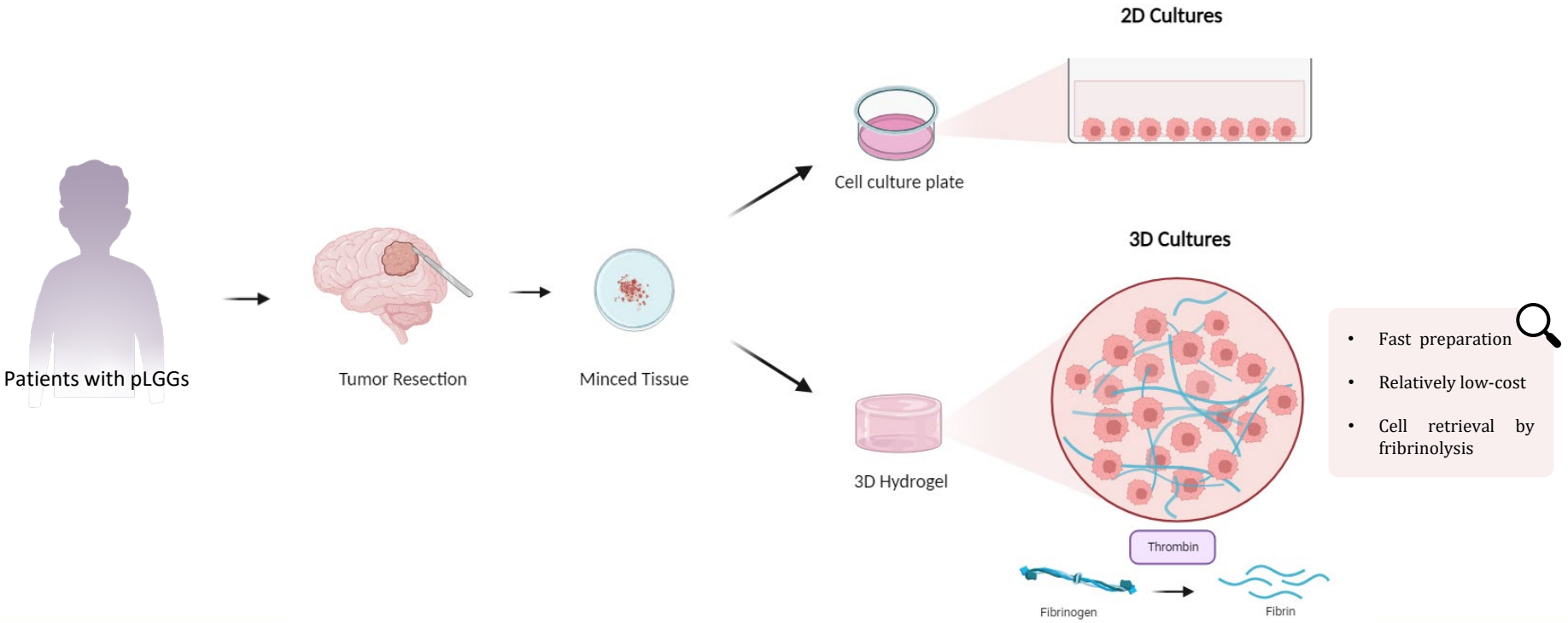


3D hydrogel cultures



Salinas-Vera Y et al., Frontiers in Oncology 2022
Veninvga V et al., Cancer Cell, 2021

Primary pLGGs cultures derived from tumor samples

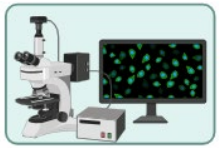


- Fast preparation
- Relatively low-cost
- Cell retrieval by fribrinolysis

Illustration created with Biorender
Del Bufalo et al., Biomaterials, 2016

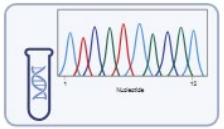
CELL LINES' CHARACTERIZATION

Immunofluorescence (IF)



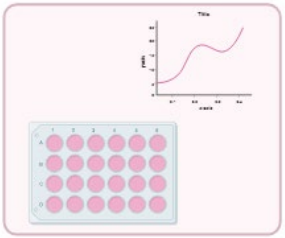
Immunoistochemistry (IHC)

STR analysis

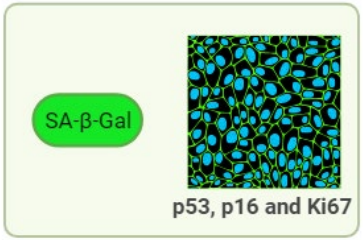


BIOLOGICAL FEATURES

Growth monitoring



Senescence quantification

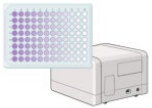


RESPONSE TO TREATMENT

Chemotherapy

Radiation

MTT Assay

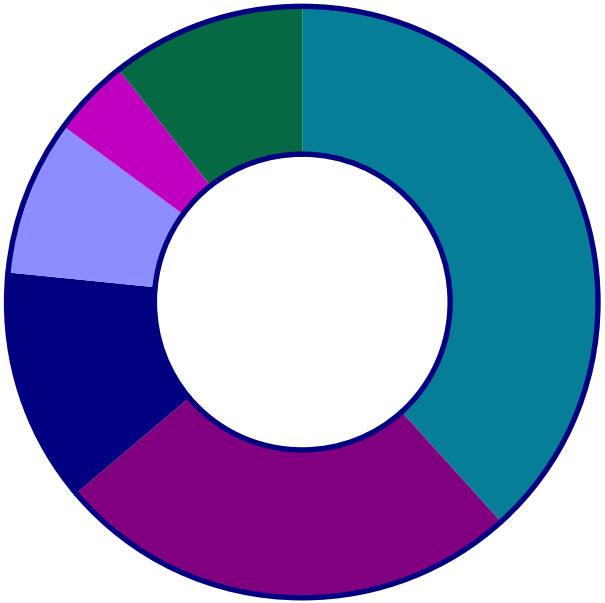


Oncolytic adenovirus (OA)

Illustration created with BioRender

STR: Short Tandem Repeats; SA-B-Gal: Senescence-associated Beta-Galactosidase; MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyl tetrazolium bromide

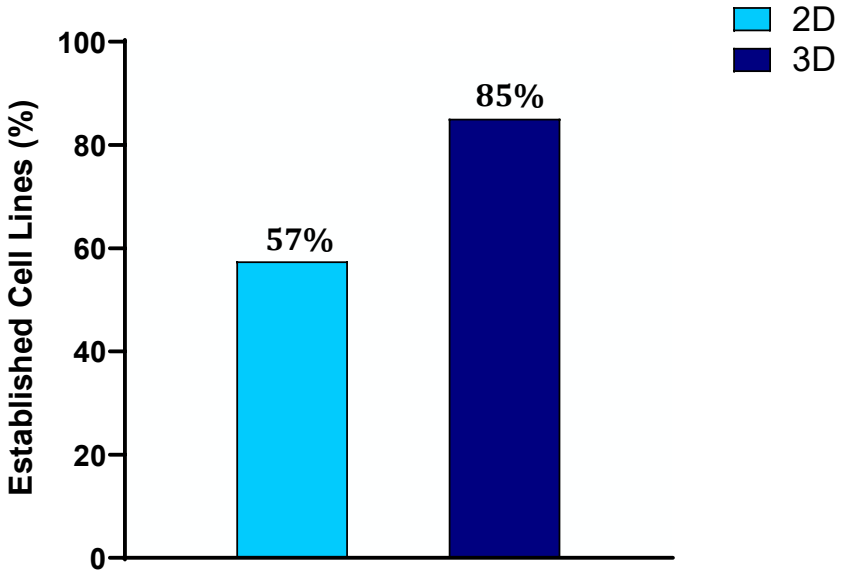
Histological diagnosis of pLGGs included in the study



n= 47 (2-17 years old)

- Pilocytic Astrocytoma (n= 18)
- Ganglioglioma (n=12)
- Dysembrioblastic Neuroepithelial Tumor (n=6)
- Polymorphous low-grade neuroepithelial tumor of the young (n=4)
- Angiocentric Glioma (n=2)
- Others (Subependymal Giant Cell Astrocytoma, Dysplastic Cerebellar Gangliocytoma, Undefined Glioneuronal Tumors; n=5)

Establishment of primary pLGGs cultures in 2D and 3D

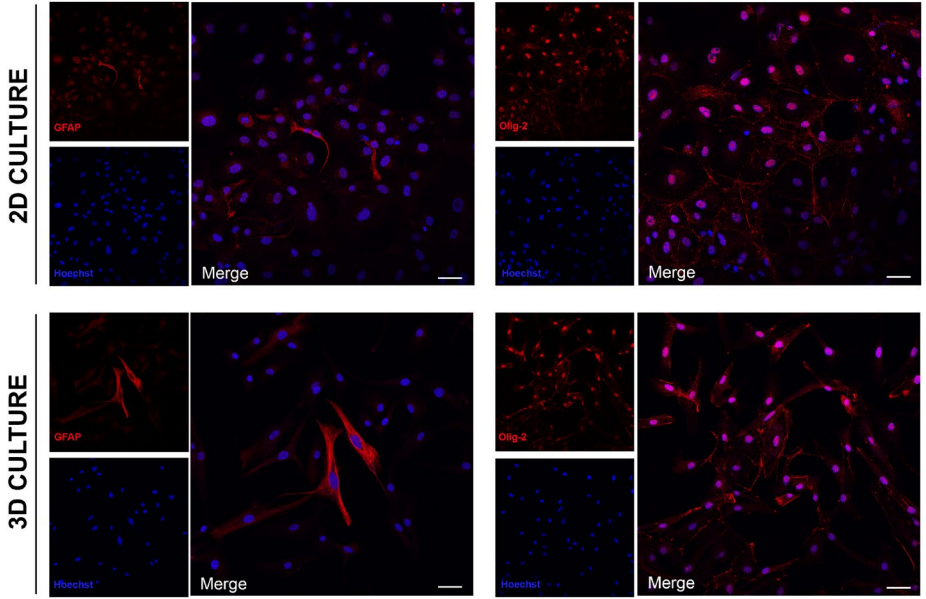


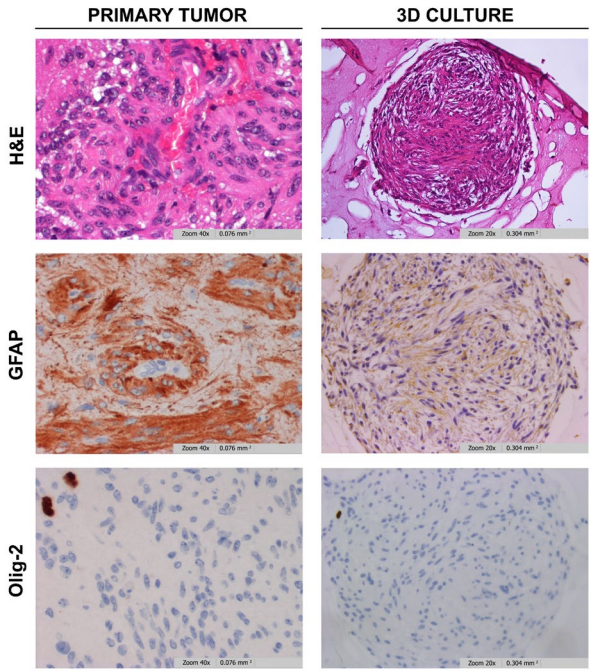
STR profile

Name	2D	3D
AM	X, X	X, X
D3S1358	15, 18	15, 18
D1S1656	14, 15	14, 15
D6S1043	19, 19	19, 19
D13S317	11, 13	11, 13
Penta E	11, 12	11, 12
D18S51	12, 15	12, 15
D2S1338	18, 18	18, 18
CSF1PO	12, 13	12, 13
Penta D	10, 11	10, 11
TH01	6, 10	6, 10
vWA	16, 17	16, 17
D21S11	28, 31.2	28, 31.2
D7S820	8, 9	8, 9
D5S818	11, 12	11, 12
TPOX	8, 11	8, 11
D8S1179	11, 11	11, 11
D12S391	20, 23	20, 23
D19S433	13.2, 14	13.2, 14
FGA	24, 27	24, 27

3D cultures achieve a superior success rate of cell lines establishment compared to 2D

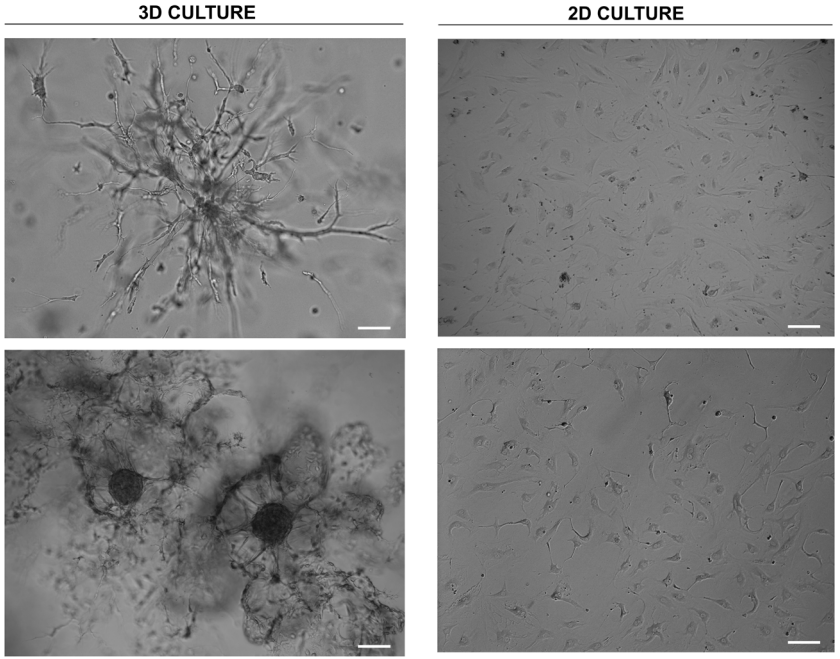
pLGGs: Pediatric Low-Grade Gliomas
STR: Short Tandem Repeats

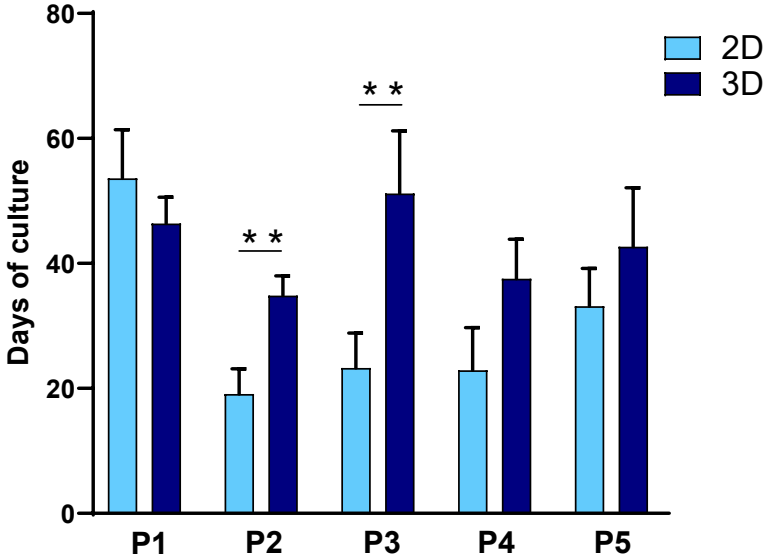




IF and IHC confirm the preservation of key pLGGs histopathological features in 2D and 3D cell lines

IF: Immunofluorescence; IHC: Immunoistochemistry
pLGGs: Pediatric Low-Grade Gliomas

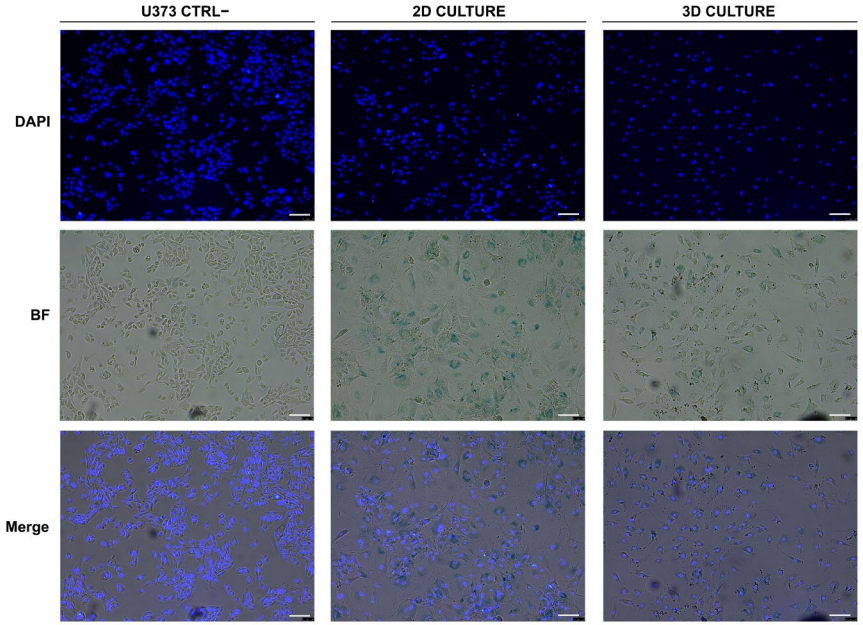


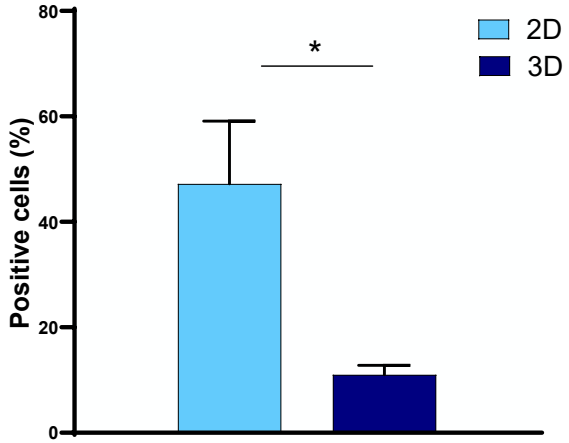


Organoid self-organize in complex 3D structures and show more regular growth trend compared to 2D cultures

Data are expressed as mean ± standard error for the mean (SEM)
* is used for p<0.05; ** is used for p<0.01

Senescence-Associated Beta Galactosidase Activity

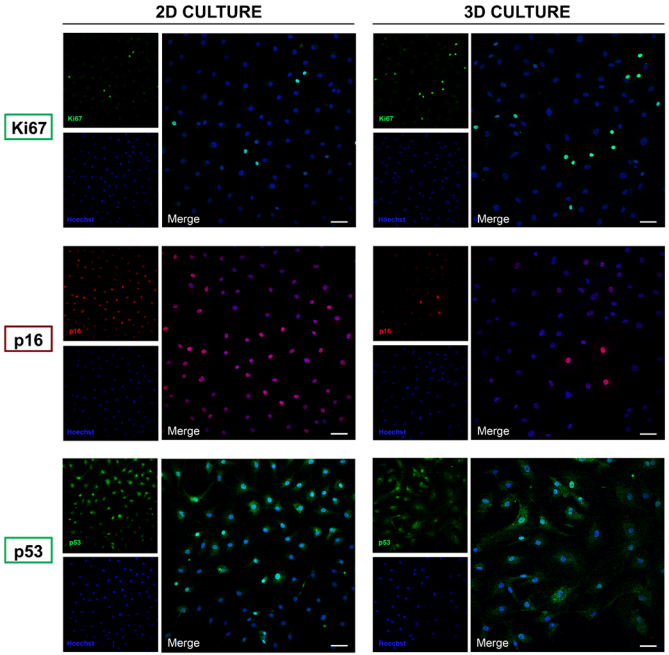


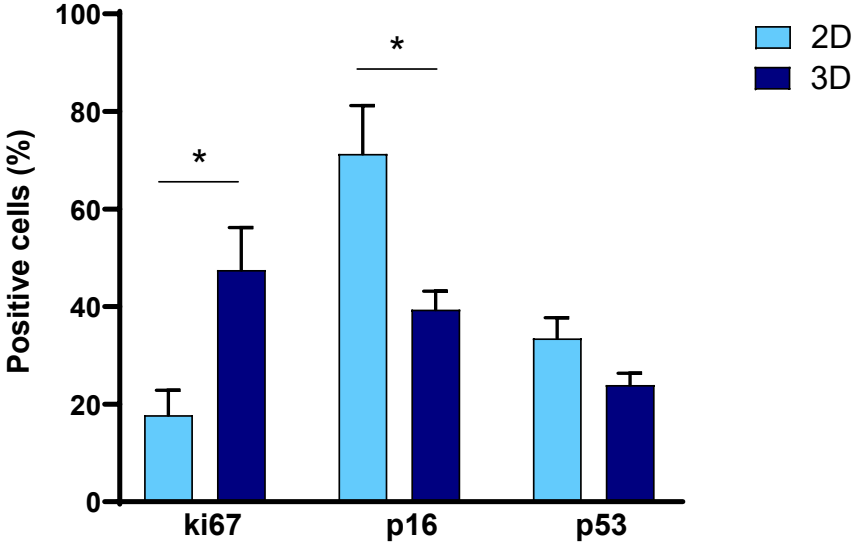


2D systems show a larger proportion of senescent cells compared to 3D hydrogels

Data are expressed as mean ± standard error for the mean (SEM); * is used for $p<0.05$
DAPI: 4',6-diamidino-2-phenylindole; BF: Bright Field

Senescence-Associated Immunofluorescence Markers

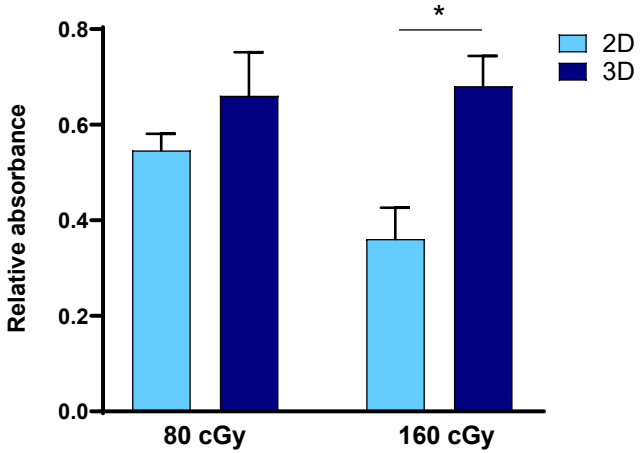




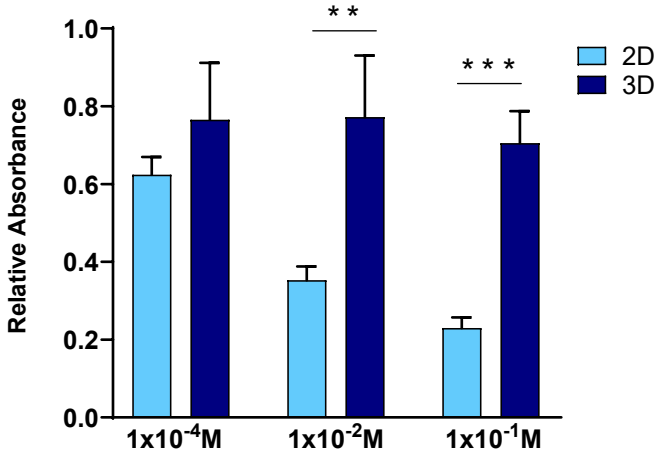
IF analysis confirms higher levels of senescence in 2D compared to 3D cultures

Data are expressed as mean ± standard error for the mean (SEM);
* is used for $p<0.05$

Radiotherapy



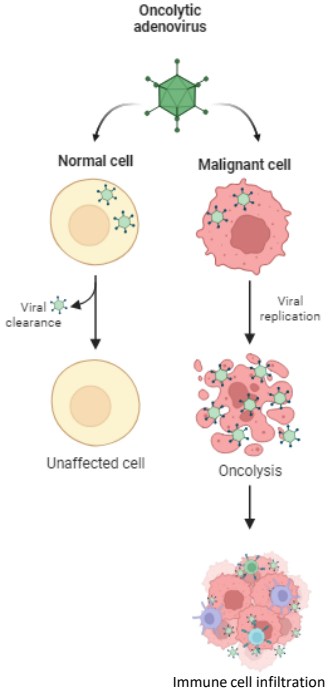
Vincristine



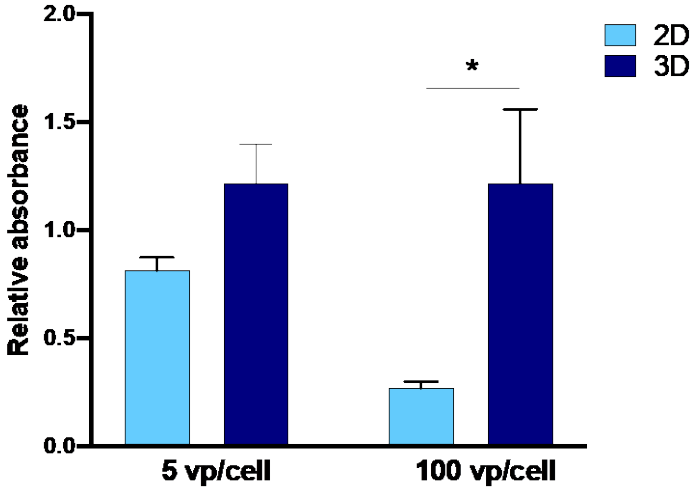
2D cultured cells are more sensitive to conventional treatments than 3D organoids

Data are expressed as mean ± standard error for the mean (SEM);
* is used for $p<0.05$; ** is used for $p<0.01$; *** is used for $p<0.001$; cGy: centiGray;

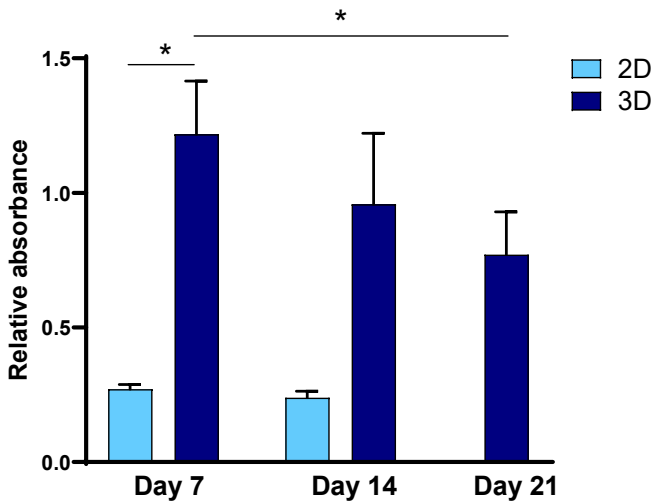
Oncolytic Adenovirus (OA)



OA at Day 7



OA 100 vp/cell



OA induces superior anti-tumoral effects in 2D than in 3D system

Data are expressed as mean ± standard error for the mean (SEM); * is used for $p<0.05$;
OA: Oncolytic Adenovirus; Illustration created with BioRender

Conclusions

- 3D organoids display morphological features and growth kinetic resembling *in vivo* pLGGs
- Lower senescence levels in 3D organoids facilitate the *in vitro* establishment of primary cell lines
- 2D systems overestimate the efficacy of therapies because of the the lack of structural complexity

Future perspectives

- Characterization of the tumor microenvironment *in vitro*
- Intermediate platform between 2D cultures and murine models
- Development of sophisticated immunotherapeutic strategies for pediatric solid tumors

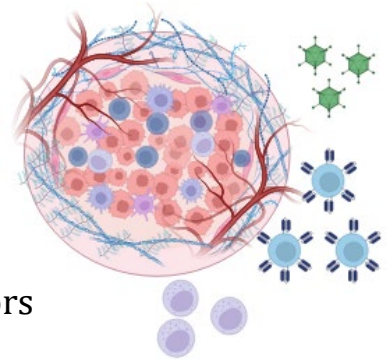


Illustration created with BioRender

Acknowledgments

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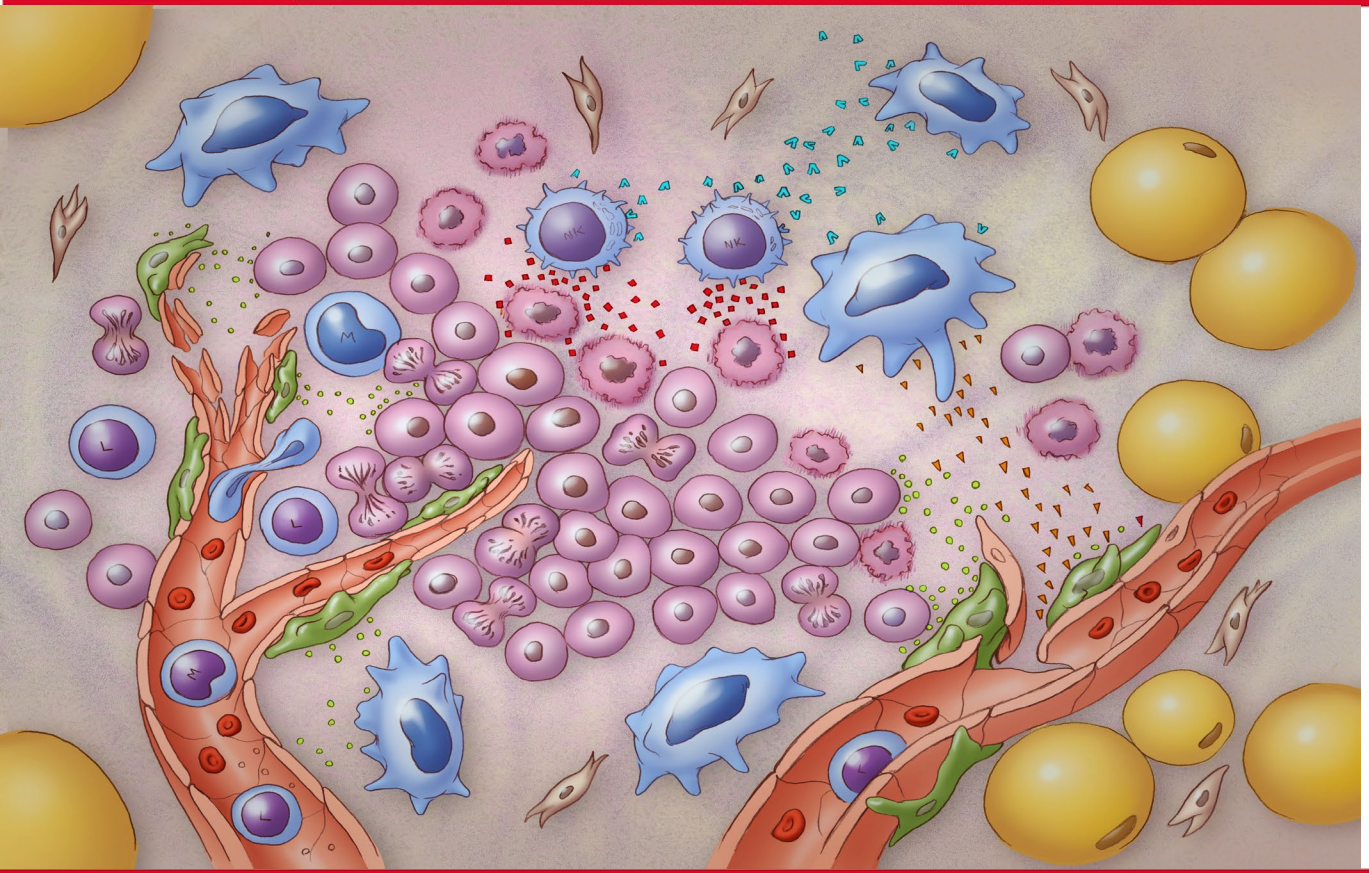

Thanks for your attention!