In vitro and *in vivo* characterization of the combinatorial effect of Axitinib and N-acetyl cysteine treatment in glioblastoma.

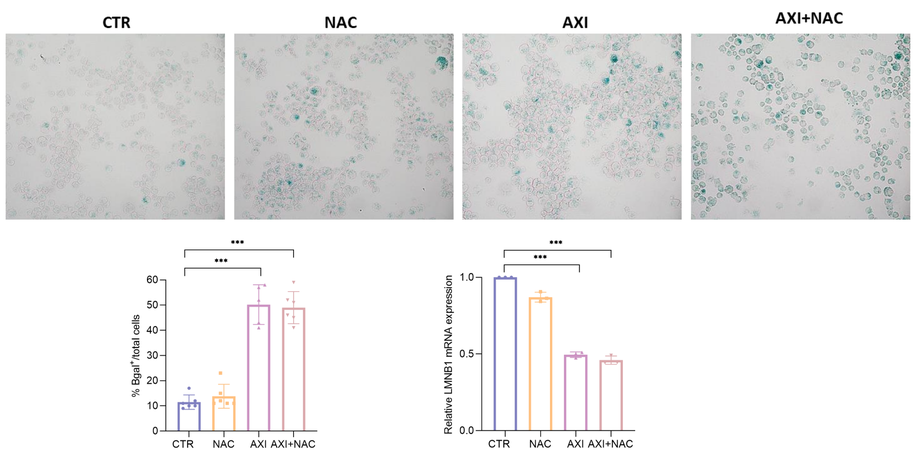
[1]***Alessia Formato***, [1] Elisa Orecchini, [1]Maria Salbini, [1]Maria Laura Falchetti, [2]Quintino Giorgio d' Alessandris, [2]Roberto Pallini and [1]Maria Patrizia Mongiardi

[1]Institute of Biochemistry and Cell Biology, IBBC-CNR, Campus Adriano Buzzati Traverso, Via Ercole Ramarini 32, Monterotondo Scalo, 00015 Rome, Italy;
[2] Institute of Neurosurgery, Fondazione IRCCS Policlinico Universitario A. Gemelli, 00168 Rome, Italy

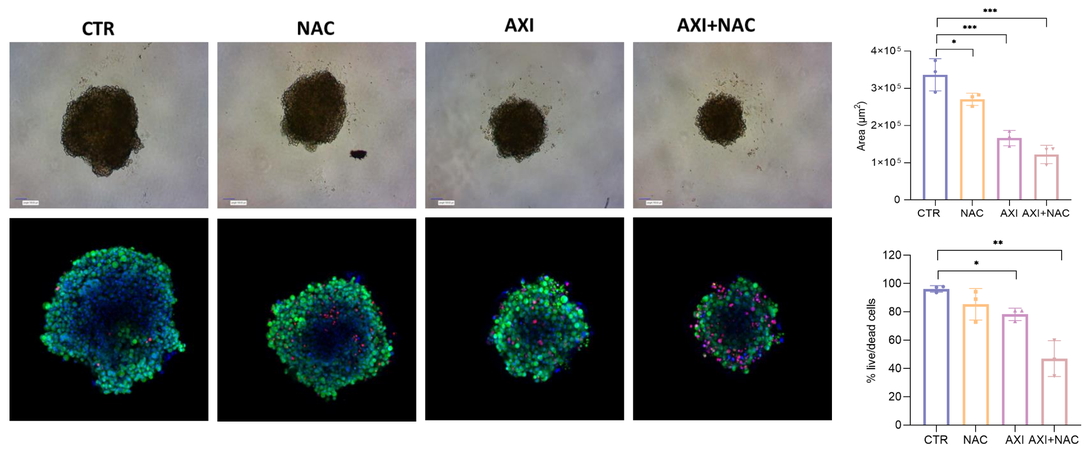
Introduction and aim

Glioblastoma IDH-wildtype (GBM) is the most aggressive brain tumor, with a peculiar ability to induce neoangiogenesis and cause relapses after surgical resection, due to the presence of tumor stem cells, which are resistant to chemotherapy. Axitinib (Axi) is an orally available kinase inhibitor, with high specificity for vascular endothelial growth factor receptors 1,2, and 3 (VEGFR-1, -2, -3) and it is already in use for several types of cancer. It has been previously shown that following Axi treatment, both GBM and normal cells undergo senescence. It has also been demonstrated that co-treatment with the antioxidant N-acetylcysteine (NAC) inhibits the induction of the senescent phenotype selectively in normal cellular contexts, without altering the induction of senescence in tumorous ones [1]. The aim of the present study is to verify if the co-administration of NAC with Axi leads to a reduction of the Axi-dependent damage of normal cells, without influencing the efficacy of the drug on tumor cells, in both glioblastoma stem cells (GSCs) and GBM in vivo model.

In vitro experiments

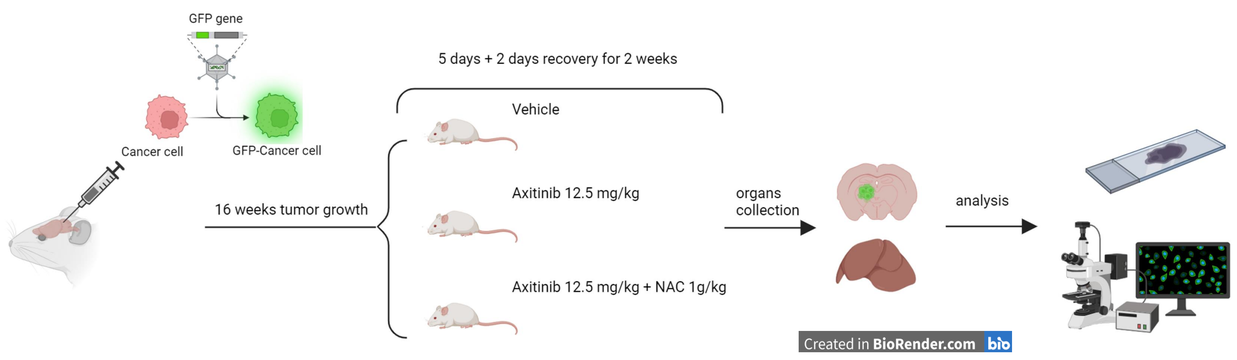


Senescence analysis on patient-derived GSCs (GSC#1). Cells were treated with Axi, NAC or Axi+NAC. Four days later we performed SA-β-Gal assay and we analyzed gene expression of the senescence marker LaminB1. The percentage of SA-β-gal positive cells increases in Axi-treated cells and NAC co-administration does not prevent senescence-induction. Coherently, LaminB1 gene expression decreases, as addressed by qRT-PCR, following Axi and Axi+NAC treatments.

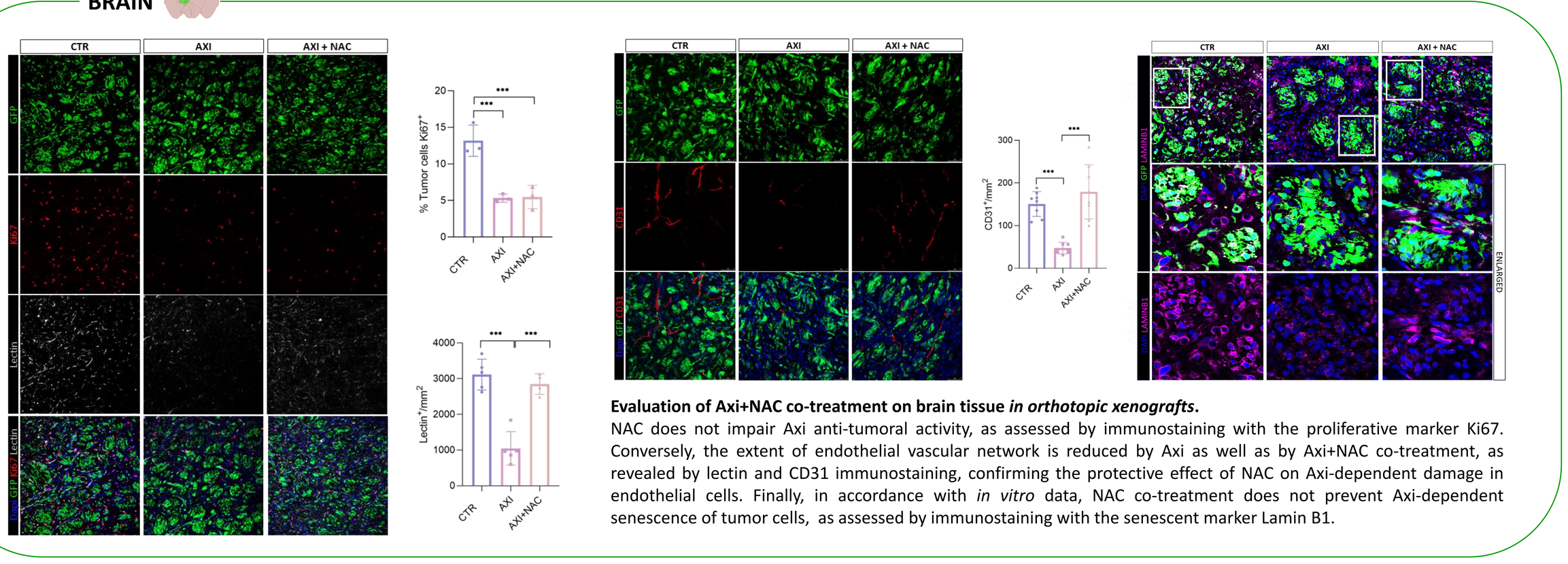


3D tumor spheroids from GSC#1. Cells were seeded in 96-well ultra-low attachment (ULA) plates and spheroid formation was induced by plating cells in 20% methylcellulose in culture medium. We allowed spheroids to grow for 48 h before starting treatment. After 1 week, spheroids were stained with calcein AM, propidium iodide and Hoechst, for staining metabolically active cells, dead cells, and cell nuclei, respectively, and analyzed by confocal microscopy. We assessed that Axi is able to reduce spheroids growth and NAC does not impair Axi anti-tumoral activity.

In vivo experiments

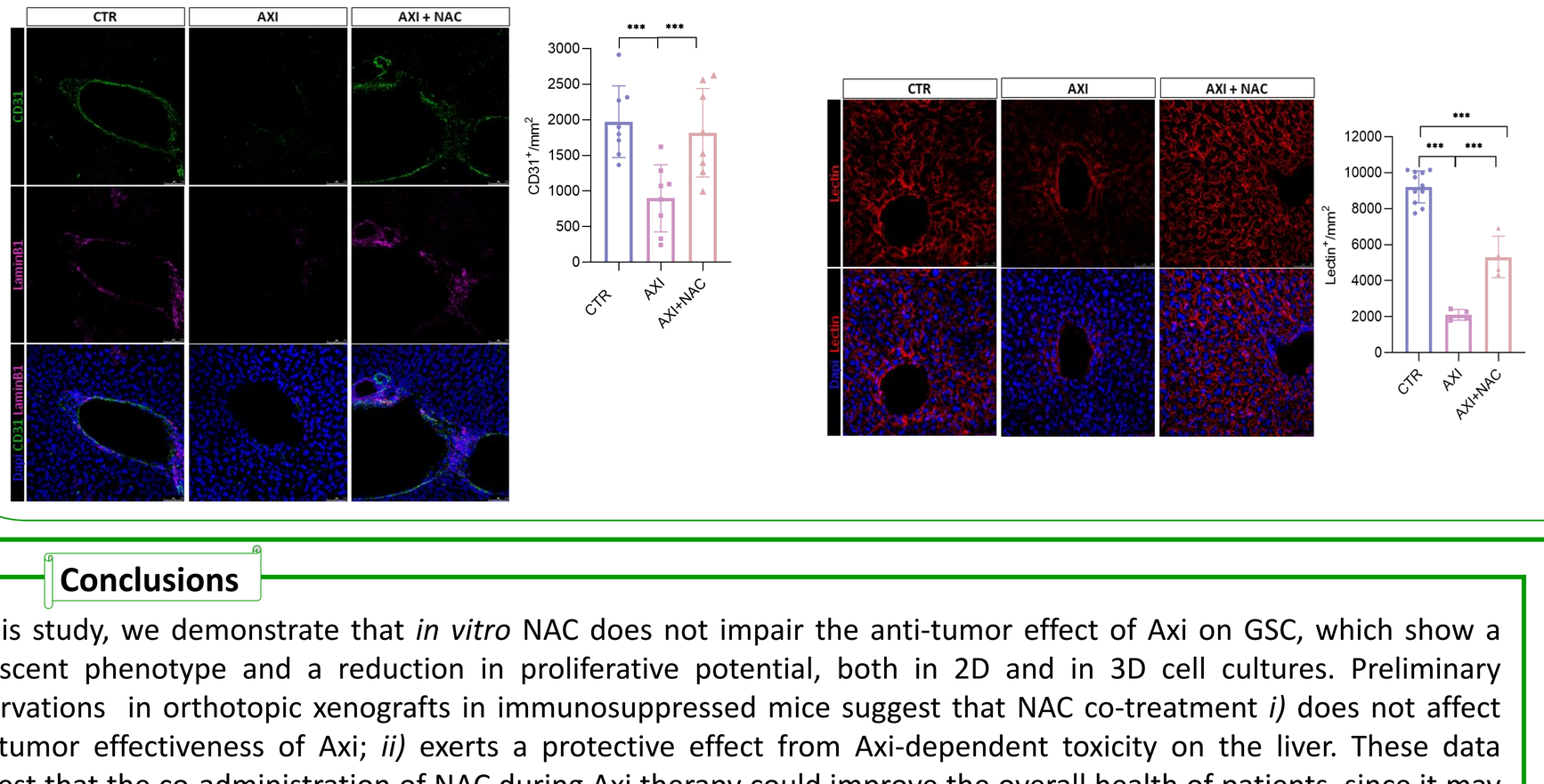


BRAIN



Evaluation of Axi+NAC co-treatment on brain tissue *in orthotopic xenografts*.
NAC does not impair Axi anti-tumoral activity, as assessed by immunostaining with the proliferative marker Ki67. Conversely, the extent of endothelial vascular network is reduced by Axi as well as by Axi+NAC co-treatment, as revealed by lectin and CD31 immunostaining, confirming the protective effect of NAC on Axi-dependent damage in endothelial cells. Finally, in accordance with *in vitro* data, NAC co-treatment does not prevent Axi-dependent senescence of tumor cells, as assessed by immunostaining with the senescent marker Lamin B1.

LIVER



Evaluation of Axi+NAC treatment on liver tissue of xenografetd mice.
Axi induces senescence and reduces the microvessel density in liver, as demonstrated by decreased LaminB1 and CD31/Lectin immunostaining, respectively. Coherently with *in vitro* data, NAC protects endothelial cells from Axi-induced senescence, restoring vessels integrity.

Conclusions

In this study, we demonstrate that *in vitro* NAC does not impair the anti-tumor effect of Axi on GSC, which show a senescent phenotype and a reduction in proliferative potential, both in 2D and in 3D cell cultures. Preliminary observations in orthotopic xenografts in immunosuppressed mice suggest that NAC co-treatment *i)* does not affect anti-tumor effectiveness of Axi; *ii)* exerts a protective effect from Axi-dependent toxicity on the liver. These data suggest that the co-administration of NAC during Axi therapy could improve the overall health of patients, since it may limit Axi-related side effects.

References

1. Mongiardi MP et al. Axitinib exposure triggers endothelial cells senescence through ROS accumulation and ATM activation. Oncogene. 2019 Jul;38(27):5413-5424.

This research was funded by: FESR Fondo Europeo di Sviluppo Regionale–Programma Operativo regionale del Lazio–"Progetti di Gruppi di ricerca 2020", grant N. A0375-2020-36524

Alessia Formato, PhD
alessia.formato@ibbc.cnr.it

SCAN ME

EACR Conference
CELLULAR BASES for Patient Response to Cancer Therapies
14 - 16 November 2023, Lyon

All data are confidential. DO NOT POST.