Glutaminase Inhibition Radiosensitizes Non-Small Cell Lung Cancer Cells to X-rays and Protons

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Objective:

The purpose of this study is to investigate the effect of glutaminase inhibition on the radiosensitivity of lung carcinoma cells to x-rays and protons. We hypothesize that IACS-6274, a GLS1i inhibitor, is an effective radiosensitizer of non-small cell lung cancer cells to x-rays and even more so to protons, likely due to more complex DNA damage and increased ROS production induced by protons.

Background:

Glutamine is an essential amino acid required for cell proliferation. Cancer cells demonstrate increased glutamine metabolism, controlled in part by oncogene-induced expression of Glutaminase-1 (GLS1).[1] GLS1 converts glutamine to glutamate. Glutamate can synthesized via ɣ-glutamylcysteine (GGC) to form glutathione (GSH), an important cellular antioxidant, or nucleotides needed for DNA replication. Low GSH levels impair a cell's ability to prevent damage caused by reactive oxygen species (ROS),[1] and failure to produce nucleotides can induce replication fork stalling, both of which may intensify the effects of radiotherapy (RT). Here we examine the impact of a GLS1 inhibitor (GLS1i) that is being used in a clinical trial (NCT03894540), on the radiosensitization of non-small cell lung cancer cells to two forms of radiation, X-rays, and protons, which vary in their ability to produce clustered DNA damage.

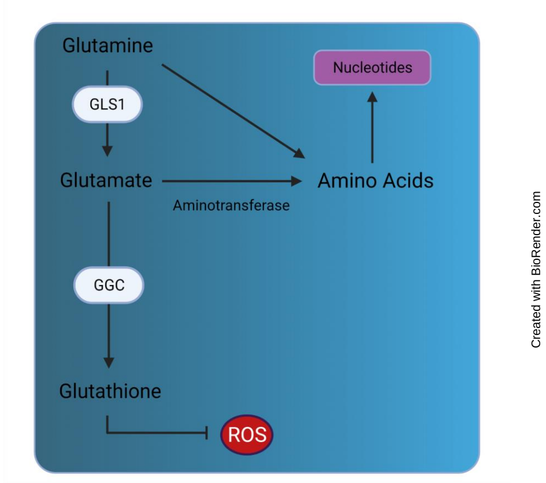
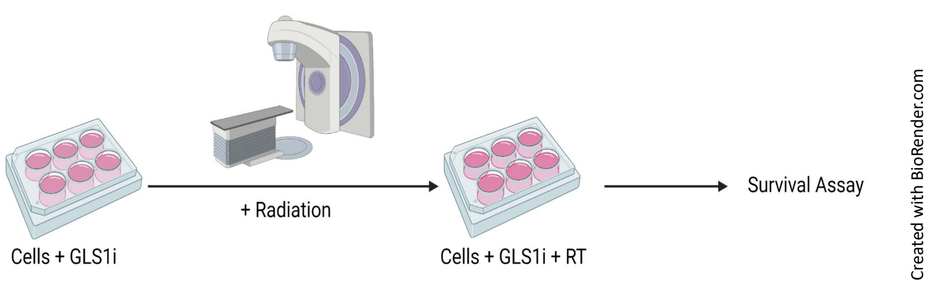


Figure 1. Glutamine uptake in cancer cells promotes cell proliferation. GLS1, an isoform of glutaminase, regulates the conversion of glutamine to glutamate, which is used to synthesize nucleotides and GSH. GLS1 inhibition leads to a limited supply of nucleotides which are needed for replication to continue. Stalled replication forks will be unable to progress without available nucleotides potentially causing more cell death. GSH acts as an antioxidant and inhibits cellular levels of ROS. Radiation-induced ROS production coupled with GLS1 inhibition may elevate ROS levels resulting in oxidative stress and DNA damage.

Materials and Methods:

We performed clonogenic assays with H460 lung carcinoma cells exposed to 6 MV X-rays or 9.9 keV/µm protons with and without a GLS1i (IACS-6274; 0.1 and 1 µM). The cells were fixed 7-10 days post-irradiation, and ImageJ macros were used to count the colonies. Radiosensitivity was represented using SF2Gy (surviving fraction at 2 Gy). We also quantified the efficacy of GLS1i-+-protons by comparing the relative biological effectiveness (RBE, the ratio of SF2Gy for X-rays divided by SF2Gy for protons) of vehicle vs GLS1i.



Results:

H460 cells exposed to X-rays demonstrated a SF2Gy of 0.51±0.03 with vehicle. GLS1i minimally sensitized cells with 0.1 µM (0.50±0.06) but strongly sensitized with 1 µM (0.41±0.07) concentration. H460 cells exposed to protons exhibited a SF2Gy of 0.42±0.03 with vehicle. GLS1i showed strong sensitization at both 0.1 µM (0.164±0.016) and 1 µM (0.10±0.02) concentrations. Protons combined with vehicle had an RBE of 1.21±0.11, whereas the RBE for protons combined with GLS1i had RBE values of 3.0±0.5 for 0.1 µM, and 4.0±1.0 for 1 µM GLS1i.

Results: Effect of Drug

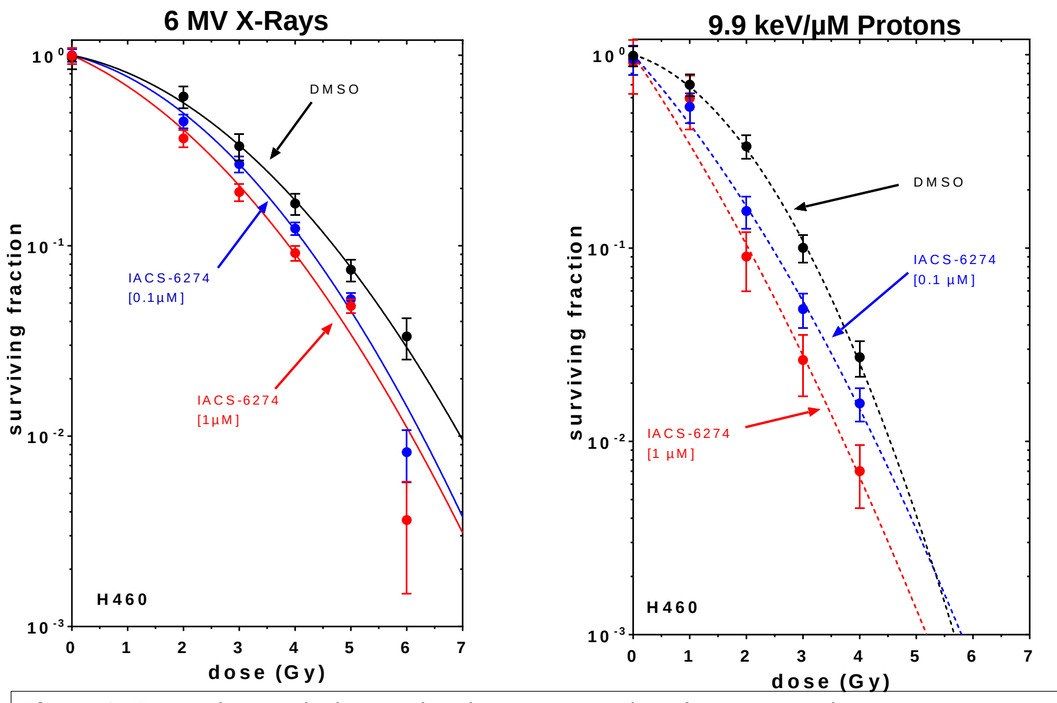


Figure 2. Comparing survival at varying drug concentrations for X-rays and protons.

Results: Effect of Radiation Type

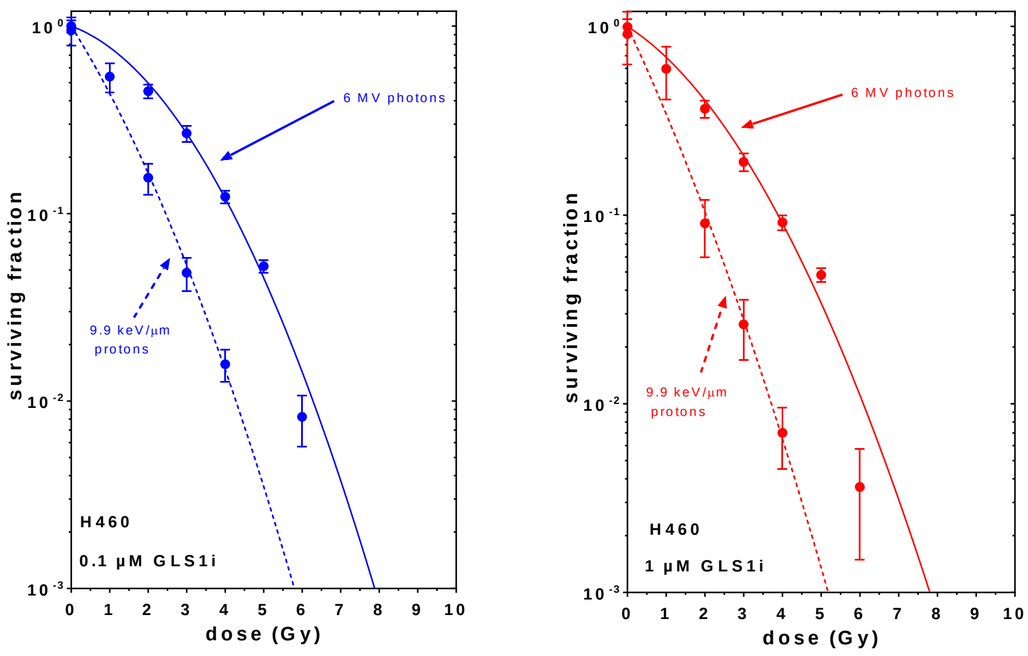


Figure 3. Comparing survival for cells treated with X-rays or protons at set drug concentrations.

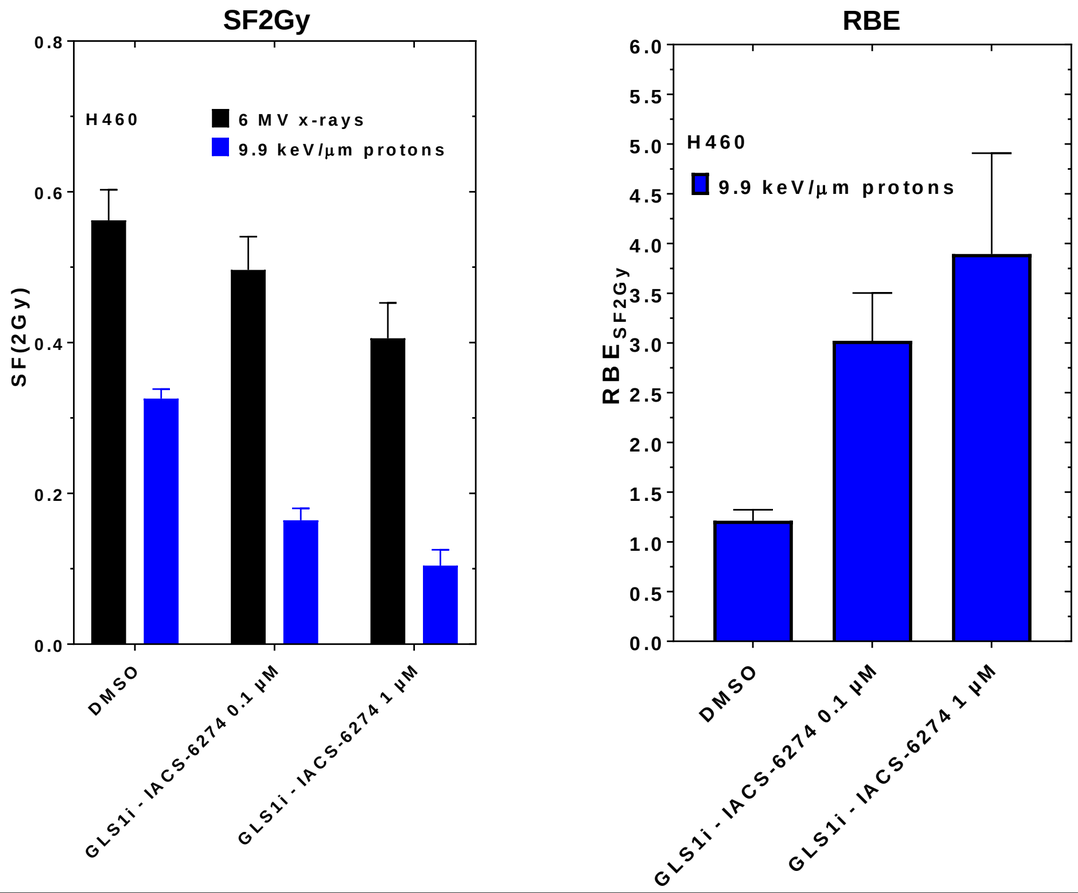


Figure 4. Survival fraction at 2 Gy dose and RBE at SF(2Gy) for H460 cells treated with vehicle, 0.1µM GLS1i, and 1µM GLS1i.

Conclusions:

- GLS1 inhibition via IACS-6274 effectively radiosensitized H460 cells to X-ray and proton RT.
- GLS1i appeared to be a more effective radiosensitizer for protons than X-rays, possibly due to increased ROS production by protons combined with oxidative stress induced by GLS1i.
- Future analysis will investigate the mechanism of radiosensitization and increased RBE, focusing on DNA damage response and cell cycle progression.

References:

1. Wang, Z., Liu, F., Fan, N., Zhou, C., Li, D., Macvicar, T., ... & Zhao, Y. (2020). Targeting Glutaminolysis: New Perspectives to Understand Cancer Development and Novel Strategies for Potential Target Therapies. *Frontiers in Oncology*, *10*, 2321..