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Role of KRAS mutation on NSCLC resistance to X-rays and protons

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Lung cancer is one of the most common cancers and one of the leading cause of cancer-related death worldwide. **Non-Small Cell Lung Cancer (NSCLC)** represents ~85% of all lung cancer and the prognosis remains low with a 5-years survival rate <15% despite treatments with chemotherapy, radiotherapy and immunotherapy. This poor survival rate is due to development of resistance mechanisms by the cancer cells. Several resistance mechanisms to treatment have been highlighted including the **KRAS gene mutations**. The KRAS protein is a signaling GTPase that act as a molecular switch in the growth factor signaling pathway. Most frequent KRAS mutation occurs on codon 12 (G12C mutations), leading to the constitutive activation of KRAS and resulting in tumor growth. A recently approved KRAS G12C inhibitor, Sotorasib, has revealed promising results in early phase clinical trials for NSCLC patients and could have an interest in combined treatment with radiotherapy. However, the radiosensitizing effect of this molecule remains unknown.

Irradiation by protons compared to X-ray irradiations could be beneficial for these patients. **Cancer stem cells (CSCs)**, a subpopulation within the tumors that plays an important role in treatment resistance, might be related to KRAS mutation resistance mechanisms.

Here, we evaluated the responses to irradiations (X-ray and protons) combined with Sotorasib and cisplatin (chemotherapy) on KRAS G12C mutated cell lines (vs. non-mutated).

Following a complete characterization of the cell lines and drug toxicity tests, the clonogenic survival of the cells and CSCs population by tumorsphere formation assay were analyzed.

Aim of the study :

- Investigate the effect of irradiations (X-ray and protons) on KRAS G12C mutated cells in combination with Sotorasib and Cisplatin
 - Determine the drugs concentrations to use by toxicity assay (MTT test)
 - Study the response to irradiation with and without the drugs
- Investigate the CSCs proportion following the treatment with irradiation and drugs (sphere formation assay)

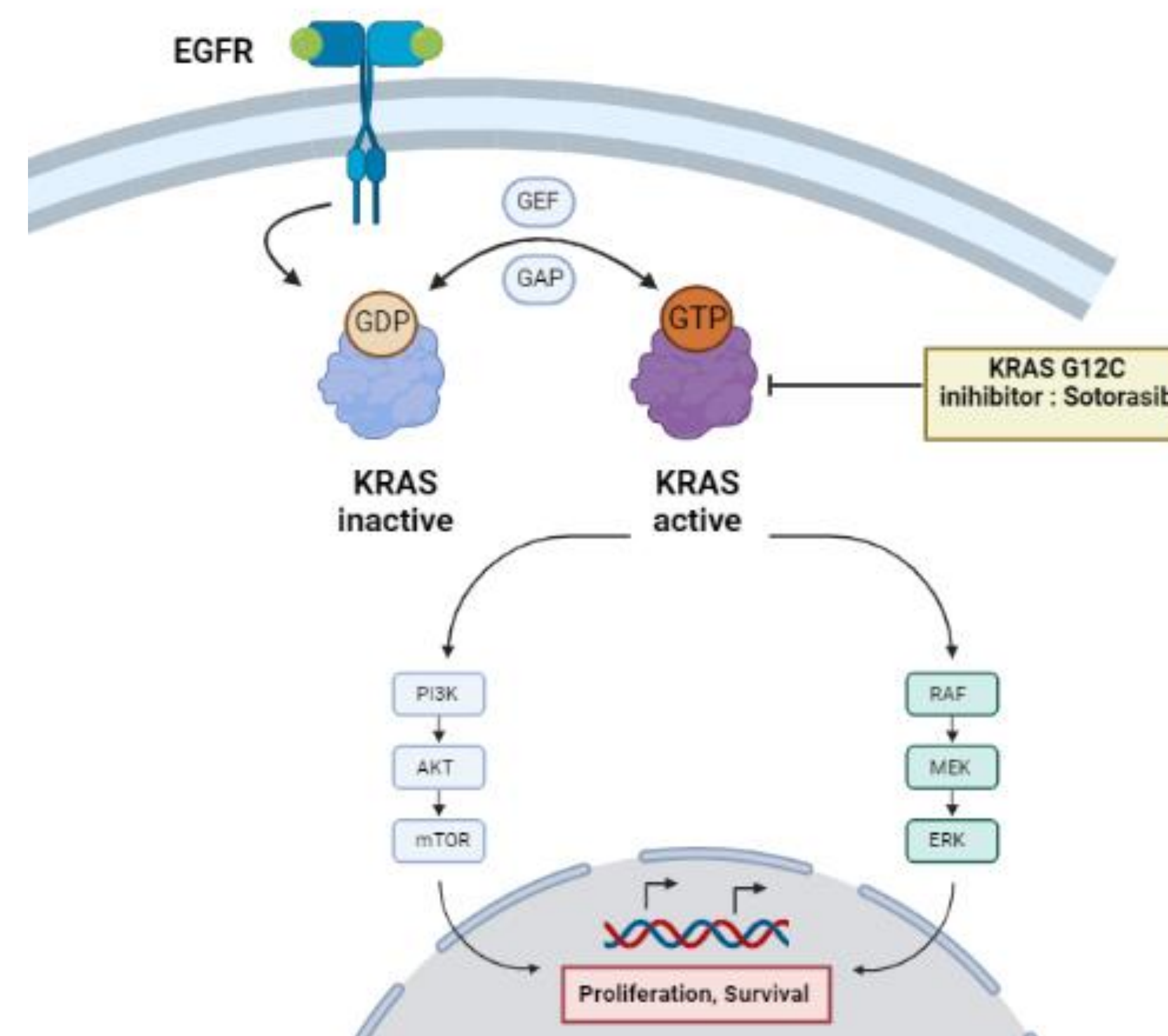
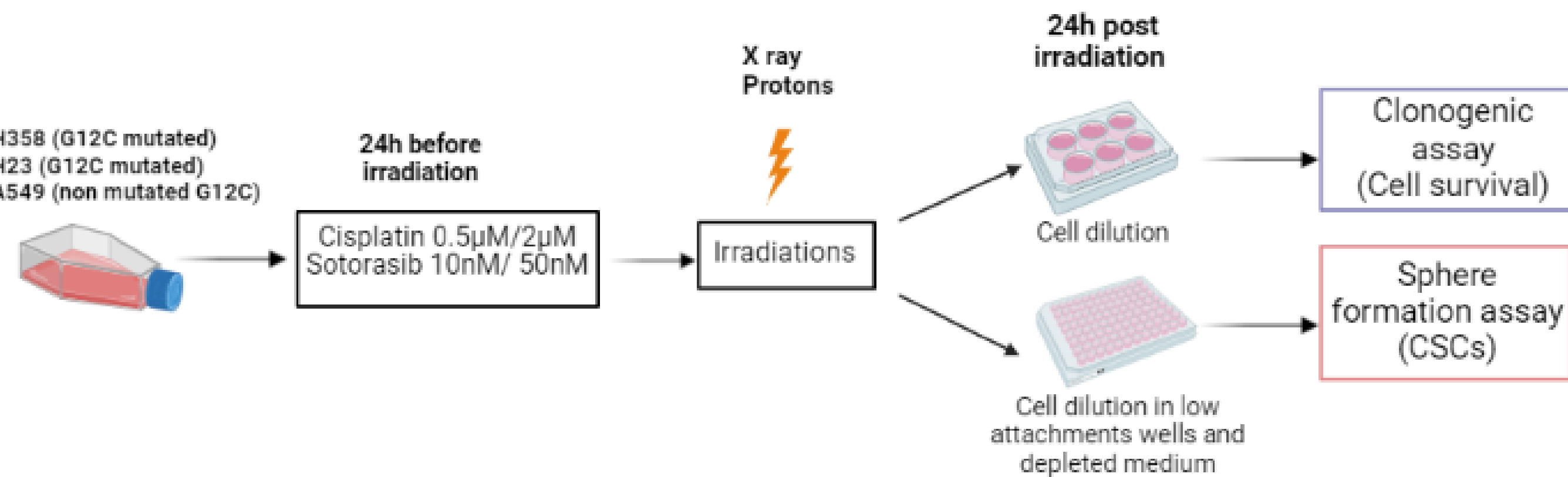


Fig.1 : KRAS signaling pathways and mechanism of action of KRAS G12C inhibitor (Sotorasib). KRAS protein acts as a molecular switch of the growth factor (EGFR) signaling pathway. KRAS has a GTPase activity and convert GDP into GTP (inactive phase to active phase). Activated KRAS induce the activation of downstream proteins pathways leading to cell proliferation and survival. KRAS G12C mutations locks KRAS in its activated form. KRAS G12C inhibitor blocks KRAS in its inactive phase.

Experimentations

- Drugs concentrations were determined by an MTT assay in order to limit cell mortality
- Cells response to drugs and irradiations :



Cell viability of NSCLC cell lines exposed to KRAS G12C inhibitor (Sotorasib) or Cisplatin at different concentrations

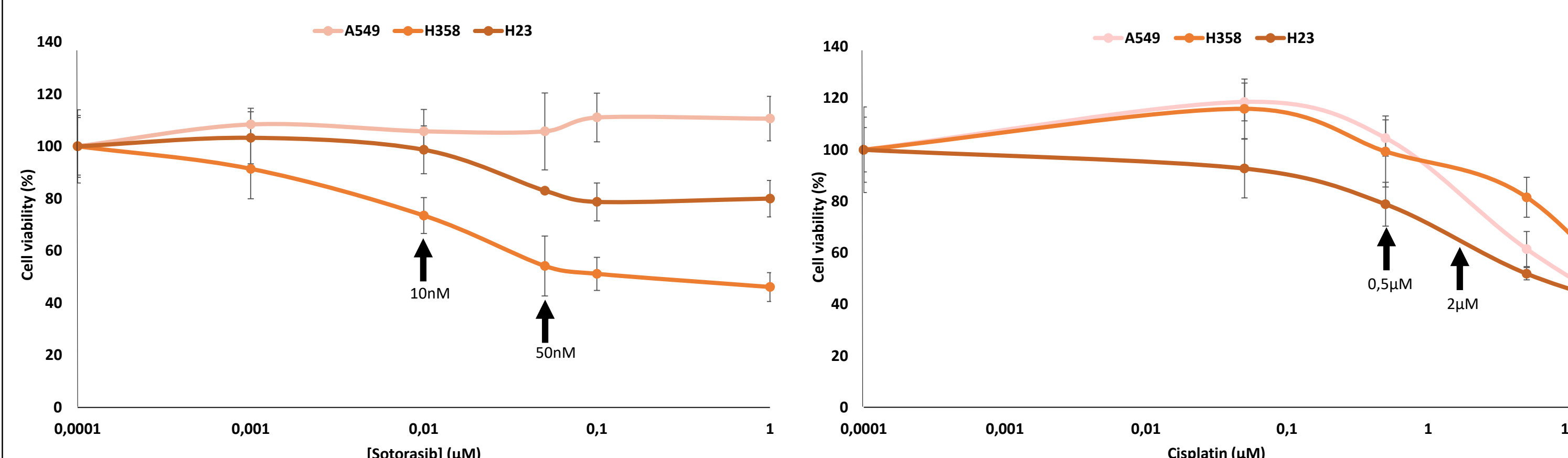


Fig. 2 Cell viability (MTT test) of KRAS G12C mutated and non mutated cell lines after 72h exposure to sotorasib and cisplatin at different concentrations. **Sotorasib** did not induce any effect on A549 cell line. Cell mortality was observed on H23 and H358 cell lines treated with Sotorasib from 1nM and 10nM respectively. **Cisplatin** induced cell mortality from 1µM on both A549 and H358 and from 0,5µM on H23.

KRAS G12C mutated (vs. non mutated) NSCLC cells survival exposed to X-rays irradiation in combination with KRAS G12C inhibitors (Sotorasib) or Cisplatin

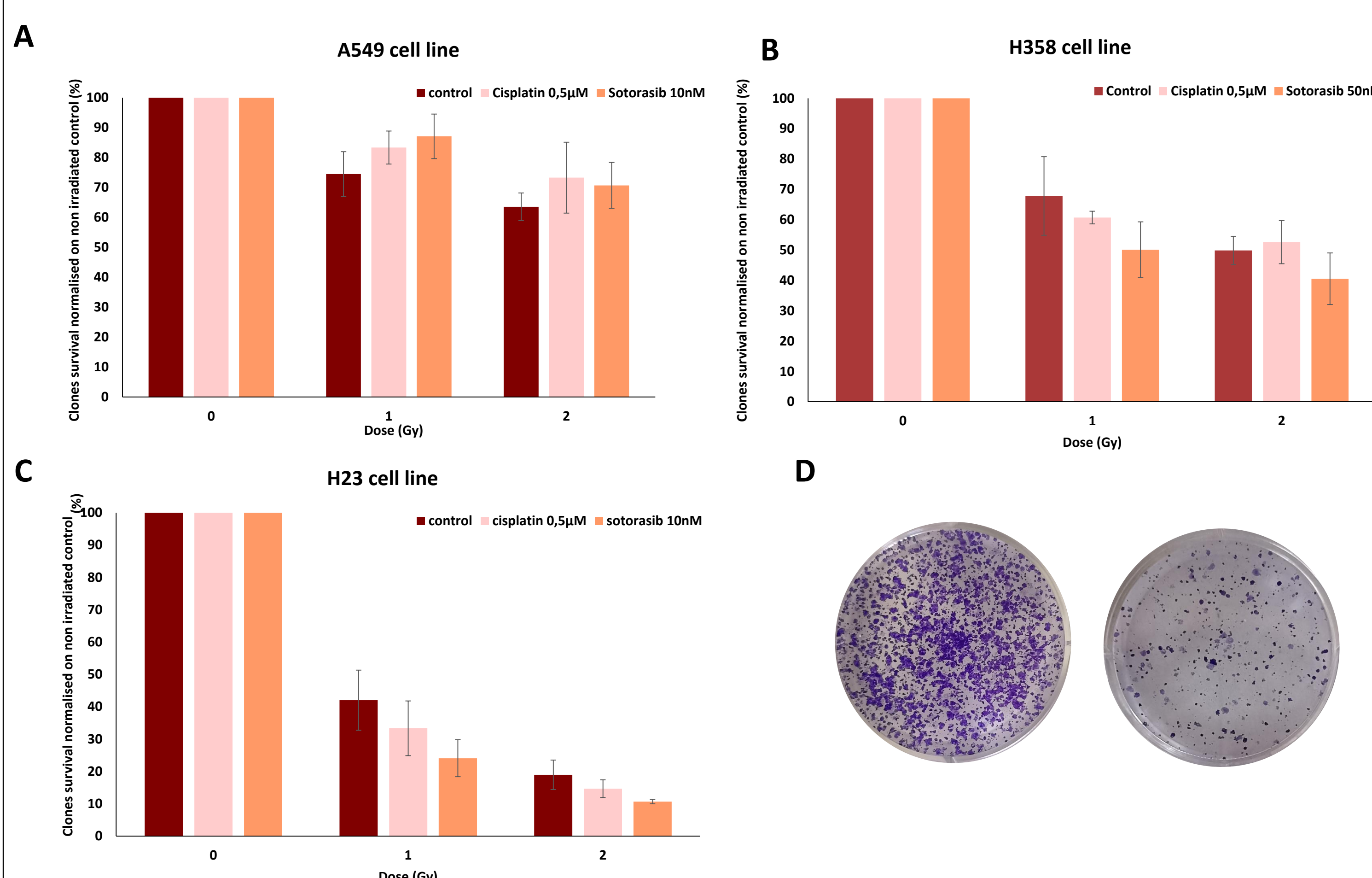


Fig.3 : Cell survival of KRAS G12C non mutated (A) and mutated (B,C) cell lines by clonogenic assay. All conditions were normalized on their non-irradiated control. None toxic effect of Sotorasib and cisplatin was observed for all non irradiated cell lines.

Sotorasib in combination with X-rays irradiation decreased clonogenic survival compared with X-rays irradiation alone in both **H358 (B)** and **H23 (C)** cell lines (KRAS G12C mutated cell lines).

A549 cell line was used as a control and **no effect** of Sotorasib and Cisplatin in combination with X-rays irradiation was observed compared to X-rays irradiation alone (A).

(D) Representative images of the clonogenic assay after crystal violet staining on H358 cell line, control (left) and irradiated (right).

KRAS G12C mutated (vs. non mutated) NSCLC cells spheres formation exposed to proton irradiation in combination with KRAS G12C inhibitor (Sotorasib) or Cisplatin

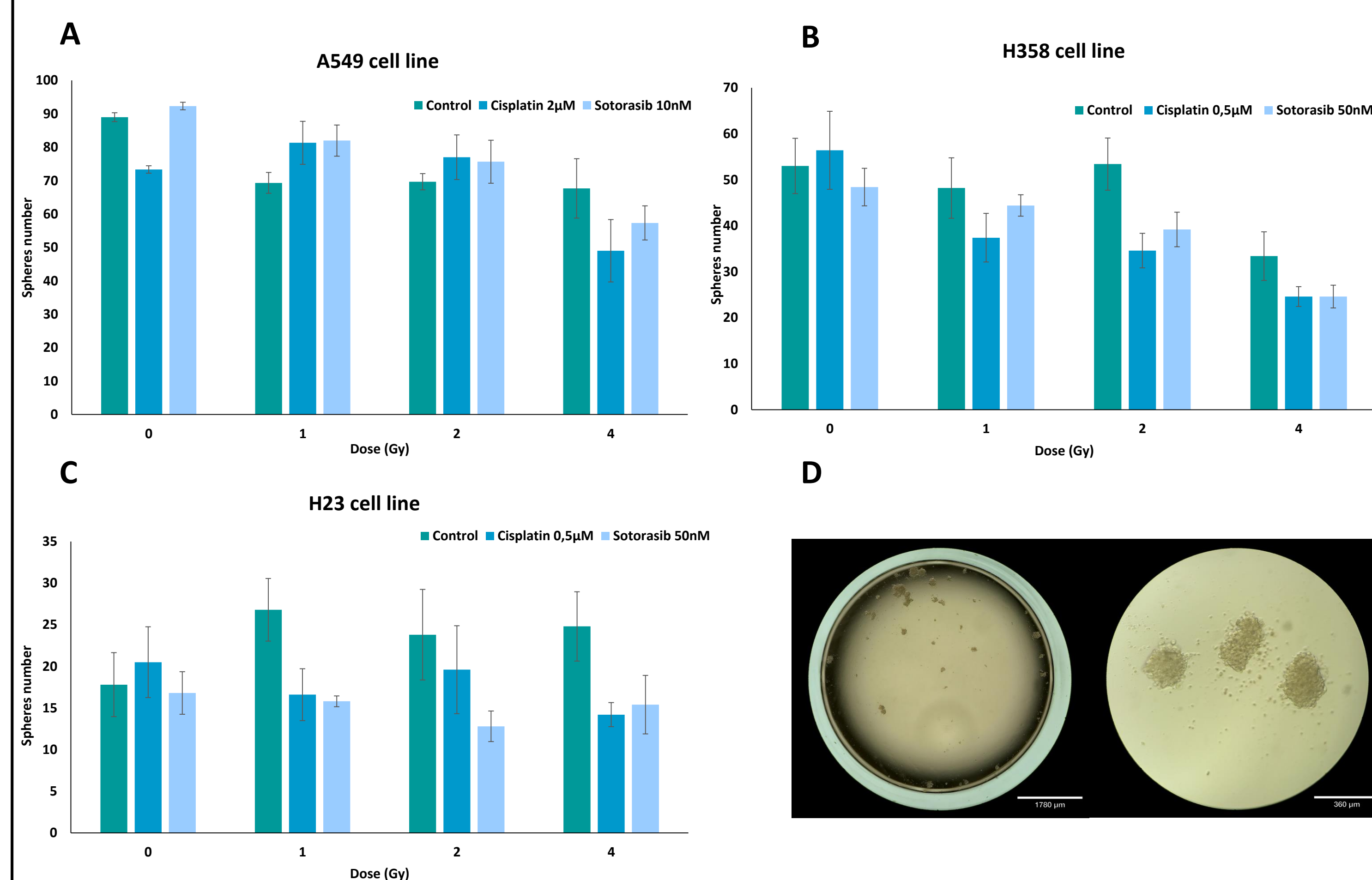


Fig. 4 : Sphere formation assay of KRAS G12C non mutated (A) and mutated (B,C) cell lines.

Sotorasib and **Cisplatin** in combination with protons irradiation decreased ability to form tumorspheres compared with protons irradiation alone in both **H358 (B)** and **H23 (C)** cell lines,

A549 cell line was used as a control and **no effect** of Sotorasib and cisplatin in combination with protons irradiation was observed compared to protons irradiation alone (A).

(D) Representative images of the tumorspheres of H23 cell line.

Conclusion

- Preliminary results showed that **Sotorasib** (KRAS G12C inhibitor) **decreased the clonogenic survival** in combination with X-rays irradiations in **KRAS G12C mutated NSCLC cell lines** *in vitro*. Further explorations are needed to confirm the efficacy of this combination treatment.
- Sphere formation assays** revealed putative **cancer stem cells (CSCs)** within the three cell lines after **protons** irradiation. KRAS G12C inhibitor in combination with protons irradiation **inhibit the formation of spheres** and might have an effect on CSCs.
- Proliferation inhibition of KRAS mutated cell lines by Sotorasib should be tested by western blot to study the inhibition of the downstream proteins pathway of KRAS (p-ERK signal)
- CSCs markers should be studied (RT-qPCR and flow cytometry) in order to further investigate the CSCs population and the effect of the combination treatment of KRAS G12C inhibitor and proton irradiation on CSCs.