

Report on the outcomes of a Short-Term Scientific Mission¹

Action number: CA20129

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Details of the STSM

Title: Synergistic effect between hyperthermia and proton therapy

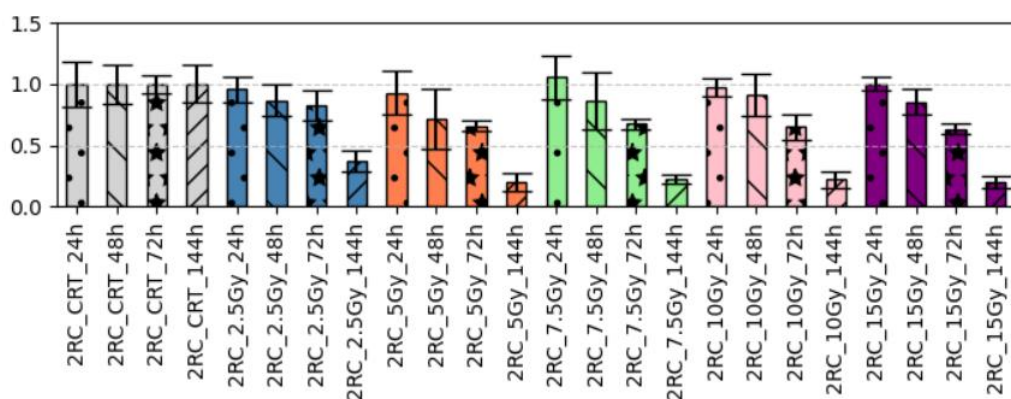
Start and end date: 18/08/2025 to 05/09/2025

Description of the work carried out during the STSM

The Short-Term Scientific Mission (STSM) was carried out at the Universidad Autónoma de Madrid. The activities were aligned with the initial working plan, focusing on the evaluation of the combined effects of proton therapy and hyperthermia on colorectal cancer cells (HCT116).

1. Proton Therapy Studies

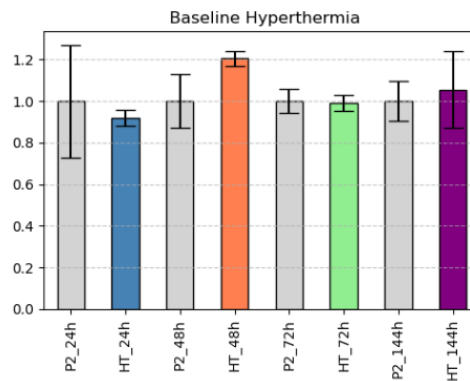
Dose–Response Characterization: HCT116 cell cultures (2500 cells/well) were irradiated with clinical proton beams at doses ranging from 2.5 to 15 Gy. Cell viability was monitored at 24, 48, 72 and 144 hours post-irradiation using Alamar Blue assays. A reproducible dose-dependent reduction in cell viability was observed, confirming the baseline sensitivity of the cell line to proton irradiation.



2. Baseline Hyperthermia: Cells were exposed to controlled hyperthermia at 42–45 °C for 30 minutes. Again, cell viability was measured from 24 to 144 hours post-treatment. The results indicated that hyperthermia alone did not produce significant alterations in cell viability under the tested conditions (without AuNRs). This finding is

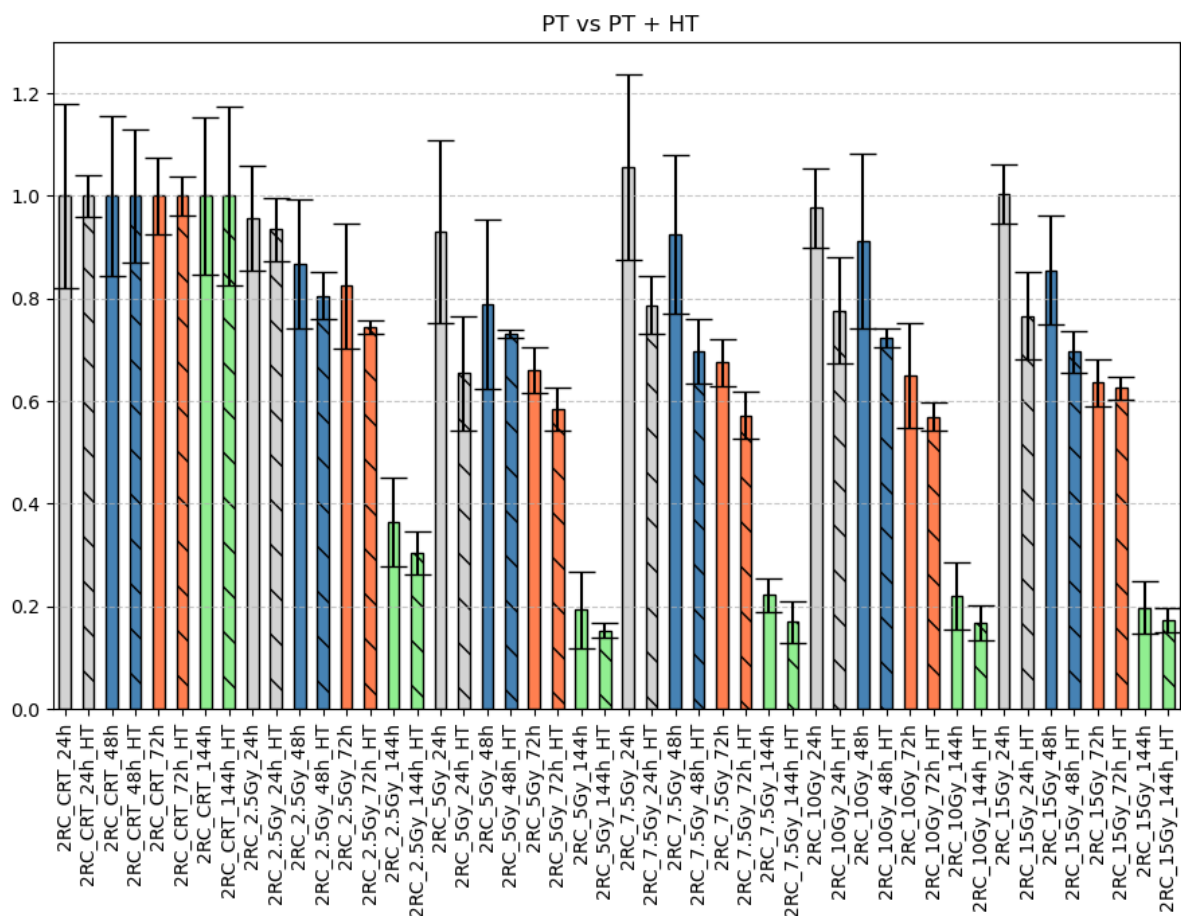
¹ This report is submitted by the grantee to the Action MC for approval and for claiming payment of the awarded grant. The Grant Awarding Coordinator coordinates the evaluation of this report on behalf of the Action MC and instructs the GH for payment of the Grant.

valuable, as it establishes a clear baseline, confirming that any observed cytotoxic effects in subsequent combination studies can be attributed primarily to the interaction between hyperthermia and proton irradiation (with or without AuNRs).



3. Sequential Proton Therapy + Hyperthermia

Combination Studies: When hyperthermia was applied following proton irradiation (in the same experimental conditions), a reduction in cellular metabolic activity was observed compared to proton treatment alone. This indicates that, although mild hyperthermia by itself did not produce significant effects, its sequential application after proton therapy enhanced the overall cytotoxic outcome. These results highlight the potential of combining proton therapy with hyperthermia as a promising strategy to sensitize colorectal cancer cells.



4.Future Work: Integration of AuNRs in Sequential Treatments

To fully assess the therapeutic potential of the proposed strategy, the sequential proton therapy + hyperthermia experiments will be repeated with the inclusion of AuNRs. This step will allow us to evaluate the full extent of the synergistic effects and to determine whether AuNRs can significantly amplify the benefits of sequential proton therapy and hyperthermia in colorectal cancer cells.

5. Deviations from the Initial Plan: Gold Nanorods Integration

One deviation from the original working plan was the integration of functionalized AuNRs into the sequential proton therapy + hyperthermia studies. Due to technical limitations encountered during the STSM, it was not possible to incorporate the nanorods into this phase of the experiments. Nevertheless, significant progress was achieved in developing and refining the synthesis process of AuNRs. Particular attention was given to optimizing their morphology, ensuring reproducibility and stability, which will facilitate their effective use in follow-up studies.

Description of the STSM main achievements and planned follow-up activities

Main Achievements

The STSM established the baseline dose–response of HCT116 colorectal cancer cells to proton irradiation, confirming a reproducible, dose-dependent reduction in viability. Controlled hyperthermia (42–45 °C, 30min) was also tested and did not produce significant effects when applied alone. However, when applied sequentially after proton therapy, hyperthermia enhanced cytotoxicity, demonstrating the synergistic potential of the combined treatment.

Planned Follow-up Activities

Building on the progress achieved, the following activities are planned:

- Repetition of the sequential proton therapy + hyperthermia studies with the inclusion of AuNRs to fully evaluate their radiosensitizing and thermosensitizing potential.
- Expansion of cell viability assays to include complementary endpoints (e.g., clonogenic survival and apoptosis markers) to provide deeper mechanistic insights.

This STSM directly contributes to the Action’s objectives by advancing knowledge on innovative combined therapies for cancer treatment, specifically integrating nanotechnology with advanced ionizing radiation therapy modalities. The work provides experimental evidence for the synergistic potential of proton therapy and hyperthermia, while laying the foundation for future studies involving AuNRs