Targeting Metabolic Vulnerabilities with L-Asparaginase as a Radiosensitizer in Solid Tumors: Synergy Assessment and Cell Cycle Modeling

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Background

Combination therapies targeting cancer metabolic vulnerabilities and enhancing radiosensitivity offer promising strategies to improve treatment efficacy.

L-Asparaginase (L-ASNase/**EcAII** from E. Coli Asparaginase type II [1]) is a clinically established chemotherapy agent used to treat Acute Lymphoblastic Leukemia (ALL), it exploits asparagine auxotrophy of cancer cells by depriving them of asparagine (Asn) and glutamine (Gln).

However, its possible application in solid tumors remains underexplored. Building on preliminary findings which demonstrated EcAII's efficacy in reducing proliferation in adenocarcinomas and triple-negative breast cancer (TBNC) cell lines [2], we Investigated the combined effects of EcAII and **ionizing radiation (IR)** across selected solid tumor *in vitro* models.

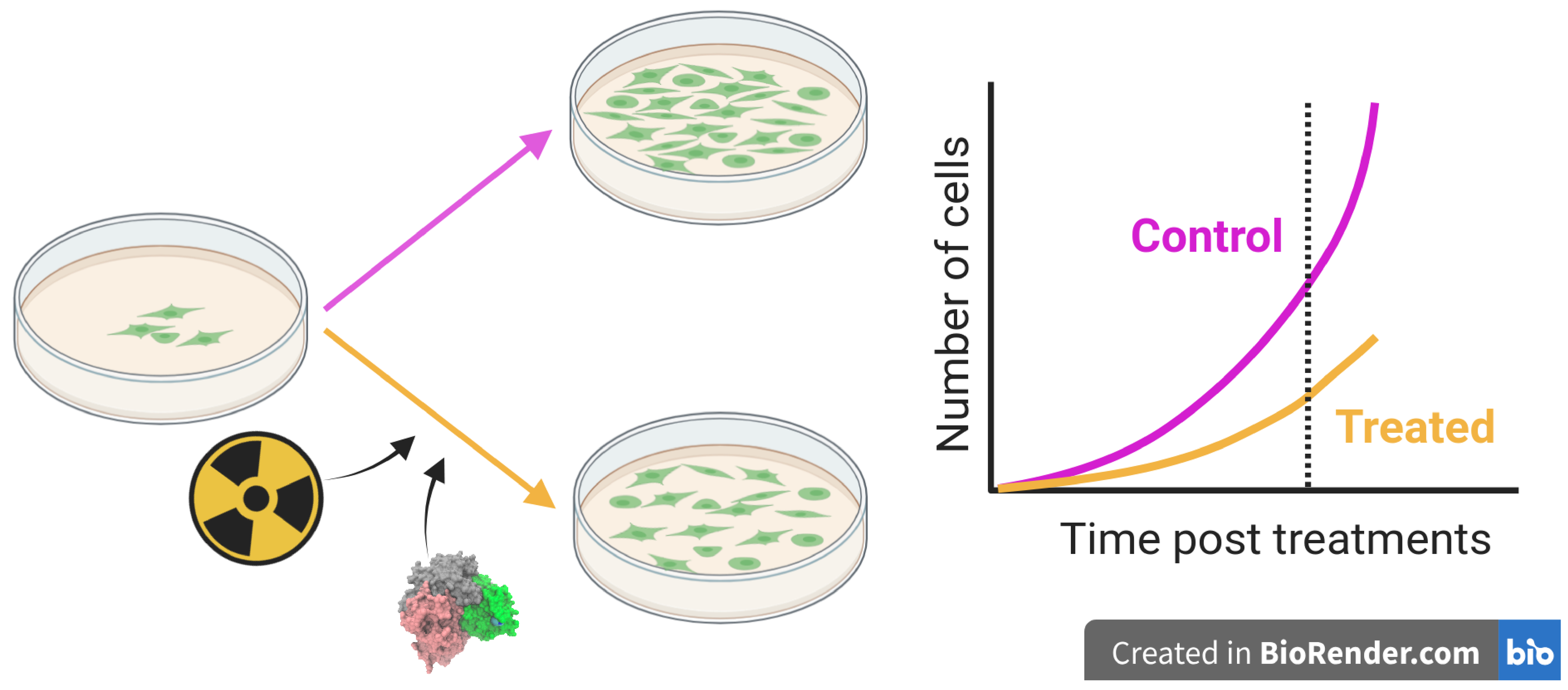

Fig 1. Diagram of proiliferation scoring, EcAII was added 24h post seeding at the same time of irradiation. Cell numbers were scored 72h post treatments.

Experimental Materials and Methods

In vitro models included **adenocarcinoma** (A549, lung, 786-O, renal) and **papillary**, invasive **ductal tumor** (BT549, MDA-MB-231) cell lines, treated with **EcAII (0.05-3 U/ml)** and/or **X-rays (1 Gy-5 Gy)**. Effects on the single and combined treatments were quantified via short-term **proliferation (72-h growth** assays, Fig. 1), in at least 3 biological replicates for each cell line.

Dose-response curves for monotherapies (EcAII or X-rays only) were generated investigating clinically relevant doses centered on EC50 values for each agent; data were fitted via the Hill's equation (Fig. 2).

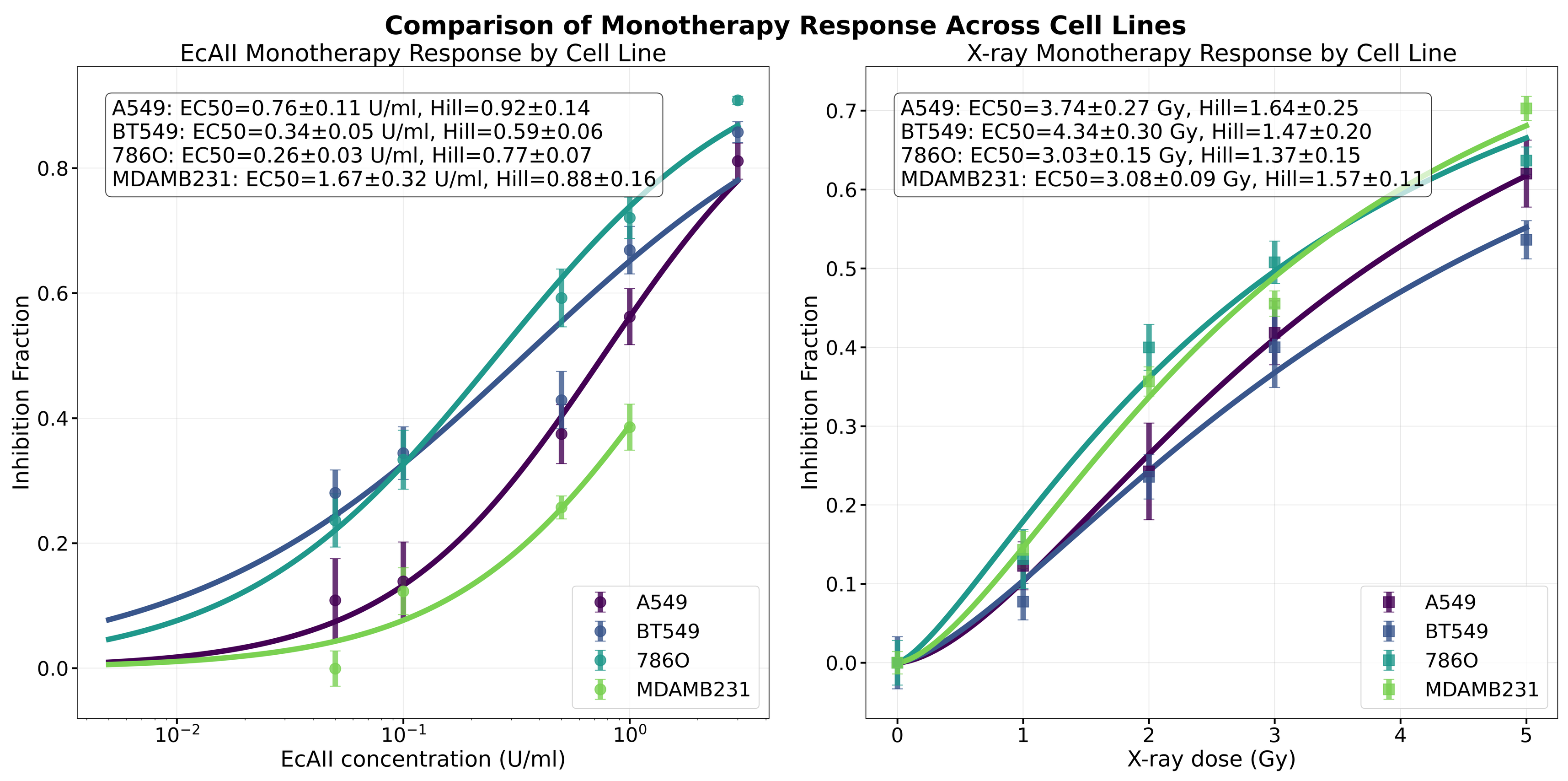

Fig 2. Mono-therapies response for A549, BT549, 786O, MDA-MB-231 cell ines, as functions of EcAII concentrations and X-rays doses. Best fit parameters are also reported with errors as 1 standard deviation.

Computational Methods

Quantification of synergistic effects between EcAII and X-ray radiation were assessed using the ZIP (**Zero-interaction Potency** [4]) model with a Python implementation. The ZIP model measures **synergy** by comparing observed combination effects to expected effects under the assumption of no interaction.

Similarly to fitting dose-response with no interaction, we fitted the observed combination effects using the projected potency and shape parameters of one agent when adding a fixed dose of the other agent.

We calculated delta scores (δ) across effect levels as **deviations of the observed combined effects from the expected additive effects**: where δ=0, >0 or <0 corresponds to zero interaction, synergy or antagonism.

We implemented bootstrap analysis (n=1000) to estimate confidence intervals and determine statistical significance of synergistic interactions. This approach allowed us to identify regions of dose combinations with significant synergy across multiple cell line.

Results

ZIP model analysis revealed distinct response patterns across four solid tumor cell lines. While all cell lines demonstrated sensitivity to EcAII monotherapy (a novel finding for solid tumors [2]), synergistic interactions with radiation were cell line-dependent.

MDA-MB-231 cells exhibited **significant synergy** (δ>22%, p<0.01) at low dose combinations (0.05-0.01 U/ml EcAII with 2-3 Gy radiation).

Also **A549** cells exhibited significant **synergy** (δ≈16%, p<0.01) in the combination region of the lowest dose of EcAII and 1-2 Gy radiation.

Whereas **BT549 and 786-O** displayed a strong **antagonism** (δ<-15%, p<0.01) at high doses of EcAII, with less sensitivity when increasing the radiation dose. Interestingly, the synergy was most pronounced at clinically relevant low doses, suggesting potential therapeutic advantages with reduced side effects.

These cell line-specific responses highlight the importance of screening approaches when considering EcAII as a radiation sensitizer in treatment for various types of cancer.

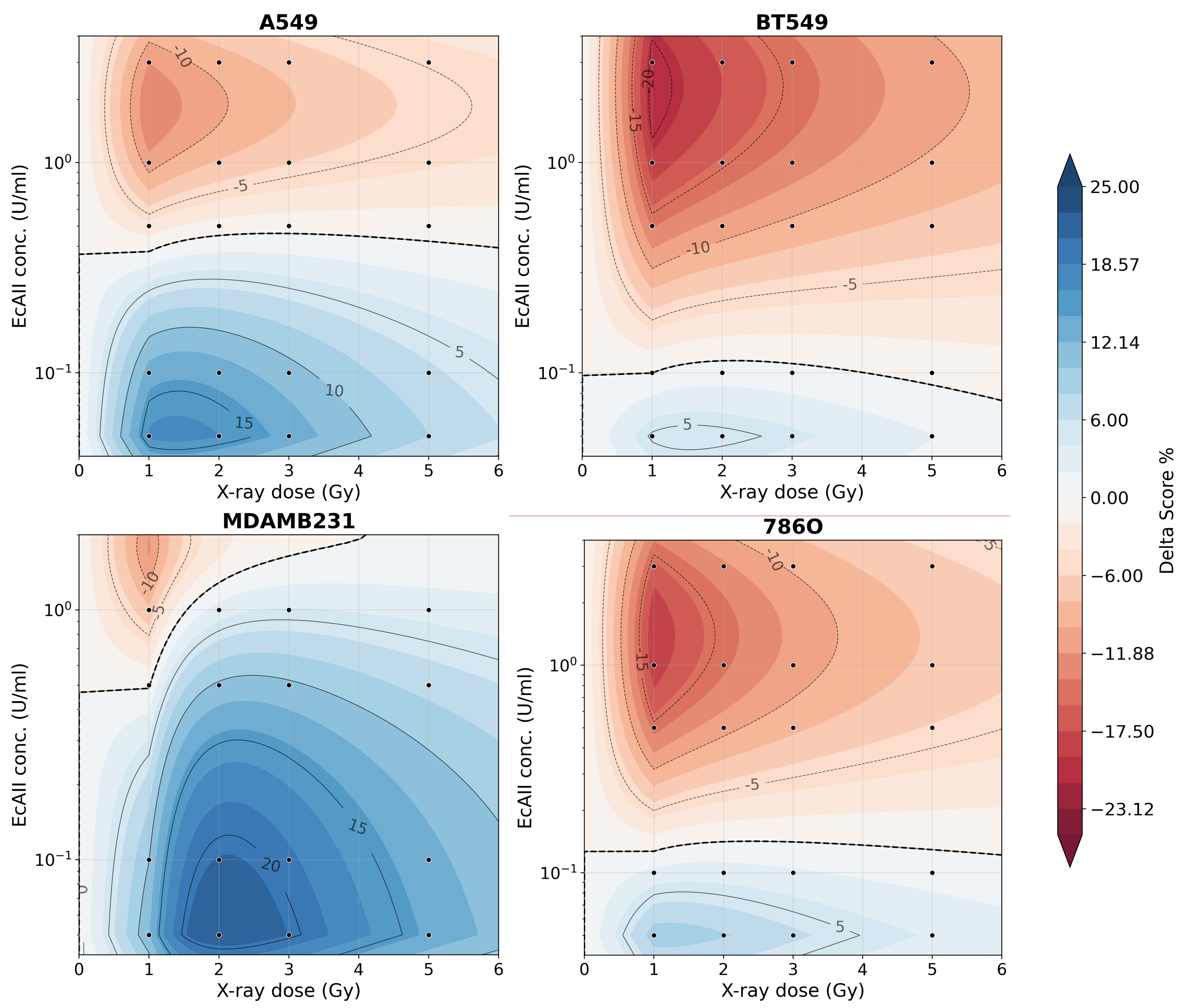

Fig 3. Contour plots of synergy scoring calculated with ZIP model, δ-scores (as percentages) represent variation from pure additivity model (dark blue regions highlight synergy, dark red highlight antagonism between the two tested agents).

Conclusion

This quantitative framework evaluated the possible synergy for combined EcAII-IR treatments, identifying low doses of EcalI as interesting for metabolic radiosensitization. Experimental data were integrated with parametrized compartmental models to predict phase-delay cell cycle perturbations induced by treatments, enhancing mechanistic understanding. Limitations include *in vitro* model constraints; ongoing work employs 3D/organoid models to refine this predictive framework. These insights advance quantitative synergy evaluation in combined chemo-radiotherapy, offering strategies for optimizing combination therapies in solid tumors.

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