Threshold Dose in Spatially Fractionated Radiation Therapy for Preclinical Triple Negative Breast Cancer

Hye Ri Han[1], Vyshnavi Pachipulusu[2], Hualin Zhang[1], Yu Zhu[2], Alan Epstein[2], Lauren Lukas[1]

[1]Department of Radiation Oncology, Norris Comprehensive Cancer Center, University of Southern California

[2]Department of Pathology, University of Southern California

Abstract

Objectives: Clinical studies of spatially fractionated radiation therapy (SFRT) followed by conventional radiotherapy have shown promising results for treating bulky tumors. However, the specific dosimetric parameters of SFRT that are critical for optimizing tumor control and reducing toxicity remain unclear. This study aimed to evaluate the contributions of peak dose, valley dose, and equivalent uniform dose (EUD) in a preclinical syngeneic murine model of triple-negative breast cancer.

Methods: 4T1 murine carcinoma cells were injected subcutaneously into the bilateral hindlimbs of adult BALB/c mice. Tumors measuring 5–10 mm in diameter were irradiated using either whole-tumor radiation or GRID collimators. The GRID collimators consisted of brass plates (3 mm and 5 mm thick) with three equally spaced, 3 mm-diameter holes. The holes were separated by 2 mm, resulting in a center-to-center distance of 5 mm. Irradiation was performed using an XRD-320 small animal irradiator at 250 kV, 16 mA, and 50 FSD, with a field size of 10 x 10 cm. The remainder of the mouse was shielded with a lead shield. Mice were randomized into groups of three, and ipsilateral hindlimb tumors were irradiated using a 3-hole GRID collimator with a thickness of 3 mm (peak-to-valley dose ratio (PVDR) of 3), a 5 mm-thick GRID collimator (PVDR of 7), or a whole-tumor open field at escalating doses of 10 Gy, 15 Gy, 20 Gy, 22 Gy, and 25 Gy. A control group remained unirradiated, and contralateral tumors were not treated. Tumor growth and survival were monitored.

Results: Doses of 10, 15, and 20 Gy resulted in greater tumor volume reduction in both irradiated and untreated tumors in mice treated with open-field radiation compared to those treated with either GRID collimator. At 22 and 25 Gy, similar decreases in tumor volume were observed in both irradiated and untreated tumors for mice in the open-field and GRID-treated groups. There were no significant differences in median survival times between the groups.

Conclusions: This study is hypothesis-generating, suggesting that a threshold peak dose may be required to trigger immune effects from SFRT in a preclinical syngeneic murine model of triple-negative breast cancer. The results were similar between the mouse cohorts with different PVDRs, and therefore different valley doses. Both the whole-tumor treated and GRID-treated mice exhibited comparable abscopal effects in the contralateral untreated tumors at 22 Gy and 25 Gy. Survival rates were also similar between the GRID-treated and whole-tumor treated groups, indicating that both cytotoxicity and immune responses may have contributed to overall survival in the GRID-treated mice.

Introduction

Clinical Studies of SFRT:

- Clinical studies of spatially fractionated radiation therapy (SFRT) have shown promising results for treating bulky tumors[1].
- SFRT involves delivering high doses of radiation to specific areas within the tumor, creating regions of high and low radiation doses.

Dosimetric Parameters:

- Despite the promising results, the specific dosimetric parameters of SFRT that are critical for optimizing tumor control and reducing toxicity remain unclear[2].
- Key dosimetric parameters include peak dose, valley dose, peak-to-valley dose ratio, and the percentage of tumor volume directly irradiated.
- Further research is needed to identify the optimal dosimetric parameters for SFRT to maximize its therapeutic benefits.

Immune Effects in Triple Negative Breast Cancer:

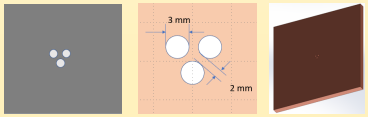
- TNBC is a subtype of breast cancer that lacks estrogen, progesterone, and HER2 receptors, making it more challenging to treat.
- Radiation can enhance the infiltration of immune cells, such as tumor-infiltrating lymphocytes (TILs), into the tumor microenvironment.
- Increased levels of TILs have been associated with better prognosis and reduced risk of recurrence in TNBC patients[3].
- Understanding the immune-modulatory effects of radiation in TNBC could lead to improved treatment strategies and better patient outcomes.

Methodology

Cell culture: 4T1 murine breast carcinoma cells were cultured in DMEM with 10% fetal bovine serum and 1% penn/strep, kept in a humidified incubator at 37°C and 5% CO2, and passaged twice weekly.

GRID design and characterization: GRID collimators were designed with 3 mm and 5 mm thick brass plates featuring holes precisely drilled and equally spaced. The plates had 3 holes with 3 mm diameter and 2 mm spacing (Figure 1). Whole-tumor radiation was delivered using brass plates with single holes of 6-10 mm diameter. Irradiation was performed at 250 kV, 1 mA, 50 FSD, 3 Gy/min. Dose profiles were measured using Gafchromic film, and dosimetric parameters were calculated.

Figure 1. Schema of the GRID collimators. Dotdecimal™ GRID collimators (5mm, 10mm thicknesses); PVDR: Peak valley dose ratio



Tumor inoculation: Experiments followed IACUC-approved protocols. Adult BALB/c mice were injected subcutaneously with 3.5 million 4T1 luciferase-transfected cells in the hindlimbs. Tumor size was measured every 2-3 days using calipers, and volume was estimated with the formula (a^2b)/2.

Irradiation procedure: Tumors of 5-10 mm diameter were irradiated using whole-tumor radiation or GRID collimators. GRID collimators provided dose modulation with high dose peaks and low dose valleys. Irradiation was performed at 250 kV, 16 mA, 50 FSD. Mice were positioned to target only the primary tumor, shielding the rest of the body with lead.

Animal experiments: Groups of 3 mice were irradiated with a 3-hole GRID collimator (PVDR 3 or 7) or whole-tumor radiation at escalating doses, compared to a control group and followed for tumor measurements and survival

Table 2. Cohorts of mice treated with escalating doses from 10-25 Gy using a 3 mm or 5 mm thick GRID collimator or an open field

Dose (Gy)	PVDR=7 3 mm GRID	PVDR=3 5 mm GRID	open	untreated	
10	3	3	3		
15	3	3	3		
20	3	3	3		
22	3	3	3		
25	3	3	3		
Control				3	
					Total
	15	15	15	3	48

Tumor measurements: Mice were monitored thrice weekly for weight and tumor measurements. Euthanasia endpoints were tumor volume, ulceration, or day 7 for tumor collection.

Bioluminescence Imaging: In vivo bioluminescence imaging was performed three weeks post-radiation to assess tumor growth. Mice were sedated and injected with D-luciferin before imaging, analyzed using M3 vision software.

Results

Threshold dose for isoeffective tumor control and abscopal effects

- At 10, 15 and 20 Gy doses, open-field RT resulted in a greater reduction in tumor burden in the mice compared to the GRID cohorts
- At 22 and 25Gy doses, GRID therapy and open-field RT result in comparable decreases in tumor burden in mice (Figure 3A)
- The abscopal effect is also seen in the groups with a similar pattern (Figure 3B)

Figure 3. A: 15 Gy ipsilateral irradiated hindlimb tumor volume B: Contralateral unirradiated hindlimb tumor volume

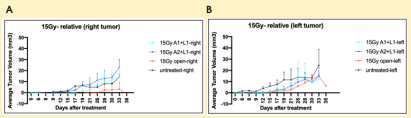
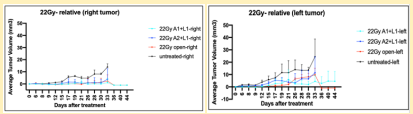
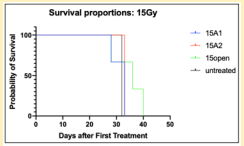


Figure 4. A: 22 Gy ipsilateral irradiated hindlimb tumor volume B: Contralateral unirradiated hindlimb tumor volume



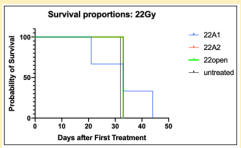
- At 15 Gy, open field-treated mice had similar survival compared to A1/A2 GRID therapy cohorts

Figure 5. 15 Gy survival proportion for GRID field, open field treated mice and controls



- At 22 Gy, GRID treated mice had similar survival compared to the open field-treated mice

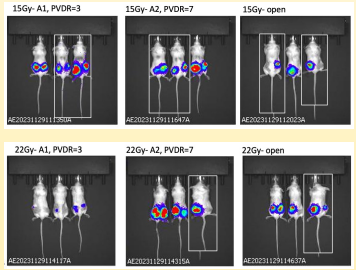
Figure 6. 22 Gy survival proportion for GRID field, open field treated mice and controls



GRID radiation results in development of necrosis

- Bioluminescence imaging was used to assess for development of necrosis
- The GRID treated mice developed increased tumor necrosis compared to the whole-tumor treated mice

Figure 7. Bioluminescence imaging of GRID treated and open field treated mice at 15 and 22 Gy levels



Conclusions

- At 10, 15 and 20 Gy doses, open-field RT results in a greater reduction in tumor burden in the mice compared to the GRID cohorts.
- At 22 and 25Gy doses, GRID therapy and open-field RT result in comparable decreases in tumor burden in mice.
- The abscopal effect is also seen in the groups with a similar pattern.
- A threshold peak dose may be required to trigger immune effects from SFRT in a preclinical syngeneic murine model of triple-negative breast cancer.
- The results were similar between the mouse cohorts with different PVDRs, and therefore different valley doses.
- Survival rates were also similar between the GRID-treated and whole-tumor treated groups, indicating that both cytotoxicity and immune responses may have contributed to overall survival in the GRID-treated mice.
- Survival comparisons were limited due to mice being near end-point at the time of irradiation due to tumor size requirements for the GRID collimator.
- The mice had better health in the GRID therapy compared to the whole tumor therapy in terms of weight and physical activeness. The whole tumor treated mice had ruffled ungroomed fur, had diarrhea and were not as active.
- It may be necessary to administer a threshold dose of GRID radiation in this 4T1 murine model to achieve equivalent tumor control while minimizing adverse effects.
- Future directions include modification of the GRID collimator and immune effects assessment.

References

1. Zhang H, Wu X. Which Modality of SFRT Should be Considered First for Bulky Tumor Radiation Therapy, GRID or LATTICE? Semin Radiat Oncol. 2024 Jul;34(3):302-309
2. Rivera JN, Kierski TM, Kasoji SK, Abrantes AS, Dayton PA, Chang SX. Conventional dose rate spatially-fractionated radiation therapy (SFRT) treatment response and its association with dosimetric parameters-A preclinical study in a Fischer 344 rat model. PLoS One. 2020 Jun 22;15(6):e0229053
3. Leon-Ferre RA, Jonas SF, Salgado R, Loi S, de Jong V, Carter JM, Nielsen TO, Leung S, Riaz N, Chia S, Jules-Clément G, Curigliano G, Criscitiello C, Cockenpot V, Lambertini M, Suman VJ, Linderholm B, Martens JWM, van Deurzen CHM, Timmermans AM, Shimoi T, Yazaki S, Yoshida M, Kim SB, Lee HJ, Dieci MV, Bataillon G, Vincent-Salomon A, André F, Kok M, Linn SC, Goetz MP, Michiels S; International Immuno-Oncology Biomarker Working Group. Tumor-Infiltrating Lymphocytes in Triple-Negative Breast Cancer. JAMA. 2024 Apr 2;331(13):1135-1144