Dartmouth Cancer Center

Comparison of UHDR and CONV RT in mouse flank tumor and normal skin tissue model

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Introduction

Although radiation therapy (RT) has been a staple of cancer treatment for over a century, normal tissue damage (both early and late) remains the primary radiation dose and cure-limiting factor. The key consideration of every radiation treatment is achieving adequate dose to the tumor volume, while sparing normal tissue as much as possible. Recently, ultra-high dose rate radiation (UHDR-RT) has shown the ability to spare normal tissue, in what has been called the FLASH effect, as compared to conventional dose rate radiation therapy (CONV-RT), at the same total dose and without compromising tumor control. The overall goal of this murine study was to determine if UHDR-RT can improve tumor control and /or reduce normal tissue toxicity when compared to CONV-RT.

Methods

Mouse Tumor Model

- Female and male 6-week-old C57BL6 mice
- 1.5 x 10^6 B16F10 or GL261 tumor cells injected in the right flank (Figure 1)
- Tumors grown to at least 100 mm^3 before radiation treatment
- Prescribed doses: 11, 3x6*, 15, 3x8**, 25 Gy
- Mice assessed daily for tumor size and skin damage post-radiation

Mouse Normal Tissue Model

- Left flanks of mice were selected as healthy skin models for UHDR-RT or CONV-RT
- Prescribed doses: 25 and 30 Gy
- Mice assessed daily for skin damage post-radiation using grading system (Table 1)

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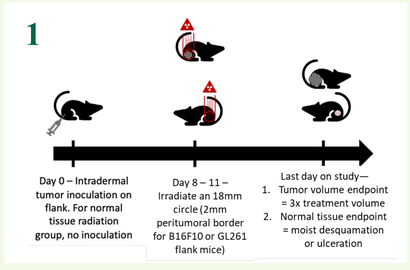

Table 1. Clinical damage scoring

Damage Grade	Gross observation
0	Normal, shaved; skin is slightly variable in color (grey to light or dark brown)
1	Dry/pre-moist desquamation. Variable skin wrinkling, erythema, pale coloration and/or flaking of the epidermal cells. Skin is not moist
2/3	Partial/full thickness epidermal lysis (ulceration)/desquamation with moisture.

Table 2. Radiation Delivery

Parameter	CONV-RT	UHDR-RT
Machine	Varian Clinac 2100 C/D	
Beam energy	9 MeV	10 MeV
Circular cutout	ø=18 mm	ø=18 mm
Dose rate	0.1220 Gy/s	270 Gy/s
SSD	100 cm	100 cm

Endpoints

- Tumor control: time to reach 3X pre-radiation volume
- Normal tissue damage: time for skin to reach lesion grade of 2 or greater

Acknowledgements

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Results

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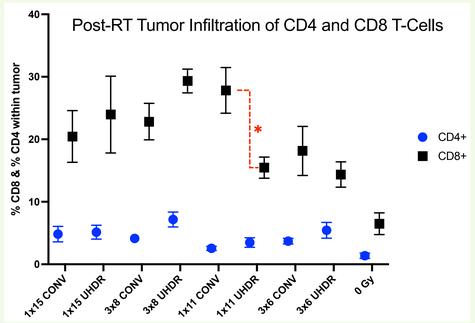

- Figure 2. No significant difference found in the tumor infiltration of CD4+ cells for both UHDR and CONV radiation. 1x11 Gy dose group showed a statistical difference in CD8+ cells between UHDR and CONV RT (CONV demonstrating a higher CD8+ infiltration). There was not a corresponding difference in tumor control (tripling time) at 1x11 Gy.
- Four dose groups in mRNA analysis did not demonstrate consistent pathway-level expression differences between UHDR RT and CONV RT treatment across the 28 tested pathways (Table 3)

Genetic Expression Analysis Data

Table 3. Top 5 highest undirected GSA scores for each dose group and direction of expression change

11 Gy	15 Gy	3x6 Gy	3x8 Gy
Mast Cell Functions (1.94, ↓)	TLR (3.17, ↑)	TLR (2.21, ↓)	MHC (2.61, ↓)
NK Cell Functions (1.84, ↓)	Dendritic Cell Functions (2.94, ↓)	Mast Cell Functions (1.9, ↑)	Antigen Processing (2.49, ↓)
Basic Cell Functions (1.81, ↑)	Antigen Processing (2.64, ↓)	Macrophage Functions (1.83, ↑)	NK Cell Functions (2.36, ↓)
Adhesion (1.76, ↑)	TNF Superfamily (2.48, ↓)	Adhesion (1.72, ↑)	T-Cell Functions (1.89, ↓)
Humoral (1.75, ↓)	Pathogen Response (2.48, ↓)	Complement Pathway (1.68, ↑)	Interferon (1.84, ↓)

Normal Tissue Damage and Tumor Control Data

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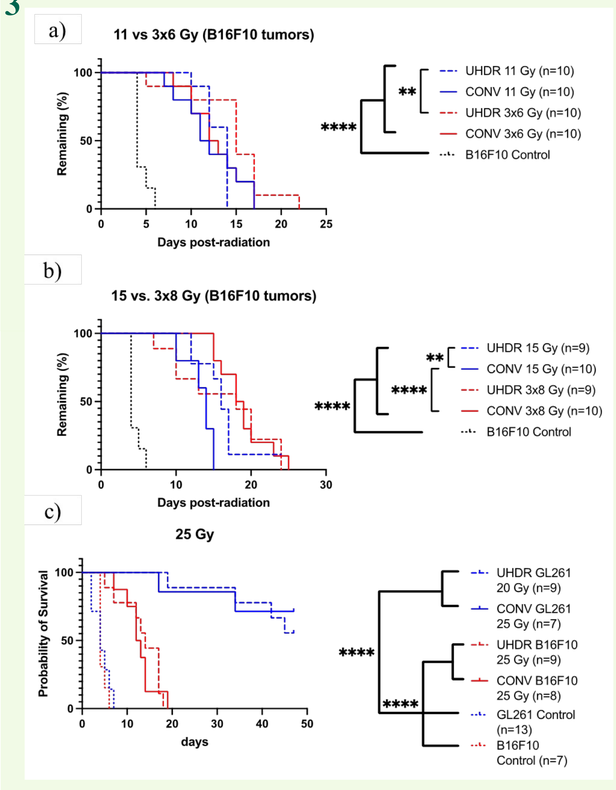

- Figure 3: Kaplan – Meier curves demonstrate the days post treatment required to reach a 3-fold increase in tumor volume for different UHDR and CONV fractionation schemes. Tumor tripling time for irradiated tumors was significantly different from non-irradiated tumors for all doses and radiation type (UHDR or CONV). (a) Significant difference in B16F10 tumor growth response ($p < 0.01$) between 1x11 Gy and 3x6 Gy UHDR RT, but not between equivalent doses of UHDR and CONV RT. (b) Small but significant difference in B16F10 tumor growth response ($p < 0.01$) between UHDR and CONV RT at 15 Gy and between 15 Gy and 3x8 Gy CONV RT. (c) No difference in tumor tripling time at 1x25 Gy, for either tumor type, following UHDR or CONV RT ($p < 0.05$).

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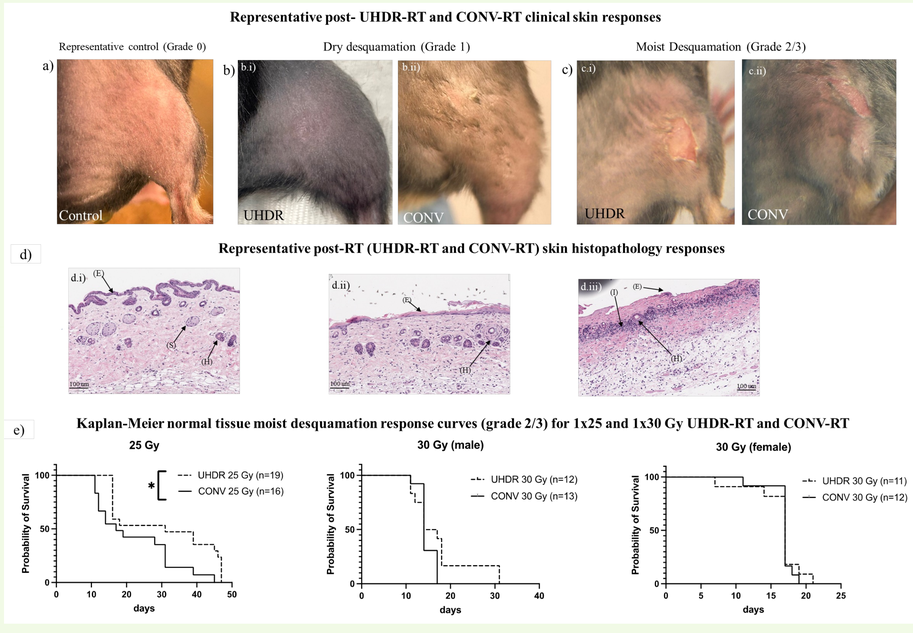

- Figure 4: (a-c) Gross photographs demonstrating skin damage grading. Shaved normal mouse skin (4a, grade 0), dry desquamation for UHDR-RT and CONV-RT (4b.i-ii, grade 1, 25 Gy), and moist desquamation/ulceration (4c.i-ii, grade 2/3 25 Gy). (d) 10X photomicrographs of skin damage grading. (e) Kaplan–Meier curves demonstrate a longer post-irradiation latent period (time to lesioning) for moist desquamation onset following UHDR-RT at 1x25 Gy, however there was no significant difference in the post-irradiation time to moist desquamation (UHDR vs CONV RT) at 1x30 Gy. (* indicates $p < 0.05$ (log-rank test))

Discussion

- None of the four dose groups studies demonstrate consistent, statistically significant pathway-level expression differences between UHDR-RT and CONV-RT across the 28 tested cancer immune and cell death pathways
- Histomorphometric assessment of the infiltration of CD8+ and CD4+ T-cells in the parenchyma of B16F10 tumors, 96 hours post-irradiation, showed only minor differences which did not translate to improved tumor control
- With the possible exception of 1x15 Gy (challenged by an outlier), tumor control was similar for UHDR-RT and CONV-RT at these doses, fractionation regimens, and tumor models
- Statistically significant UHDR sparing at 25 Gy: 7-day difference in post-irradiation time to moist desquamation/ulceration lesion formation
- Contrary to previous published data suggesting higher-dose UHDR-RT may be more effective at reducing the incidence of severe toxicity, this study found at 30 Gy the UHDR skin sparing benefit was reduced.