Supplementary

Nano-targeting of Thyrointegrin αvβ3 Receptor in Solid Tumors and Impact of Radiosensitization

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Tumor target radiation methodology

We used Faxitron CP-160 Rodent Irradiator as the X-ray source for irradiation purposes. This machine is housed in a steel cabinet of dimensions 85×85×110 cm^3 with a shelf for placing the samples. The shelf has different adjustable positions and can be rotated or kept stationary depending upon the requirement of irradiation. The shelf distance from the source is called Source to Surface Distance (SSD), and it has ten different adjustable SSD positions. Various shelf positions along with their SSD and the field sizes are given in **Table S1**. The machine comes with an inherent filtration of 0.5 mm Al to the beam. To get uniform dose to the target, in addition to the inherent filtration, 0.5 mm Cu filtration was also used. A special plastic container was designed to keep the animal under normal temperature and pressure conditions such that the housing requirements of IACUC could be met. Wood chips were provided as bedding for the mouse to maintain the body temperature and a HEPA filter at the face of the lid to maintain normal air flow. The cage and carrier for movement of the mouse cage under IACUC protocols is shown in **Figure S1**.

The X-ray beam field is circular and the field size is expressed in terms of area of the beam at various shelf positions (**Table S1**). At shelf position number 7, the field size is 560 cm^2. After placing the carrying cage on shelf position number 7, the net distance of the tumor from the X-ray source was 15.5 cm. The field size at this location was calculated to be 123 cm^2. Shielding was designed to cover an area of 123 cm^2.

Table S1: Field sizes at various shelf positions

Shelf Number	SSD	Diameter (cm)	Field Size (cm^2)
Floor	99.1	72	4072
1	94.0	72	4072
2	83.8	72	4072
3	73.7	59.5	2781
4	63.5	51.3	2067
5	53.4	43.1	1459
6	43.2	34.9	957
7	33	26.7	560
8	22.9	18.5	269
9	12.7	10.3	83

SSD = Source to Surface Distance

Lipowitz alloy, commonly known as Cerrobend, is a readily available alloy at medical centers that is used for shielding purposes. This alloy has a density of 9.4 g/cm^3 at 20 degrees, which is 83%

of the density of the lead and much lower melting point as compared to lead. We used Cerrobend to customize a square sheet of dimensions 21 x 21 cm^2. Thickness of 1 cm was tested to block all the radiation from the X-ray source at maximum voltage and current settings of the machine. A hole of 0.6 mm diameter at the center of the shield was created to allow passage of X-ray beam as shown in Figure 1B in the main manuscript. This shielding sheet was placed on the top of the container and the hole in the shielding was aligned with the target location.

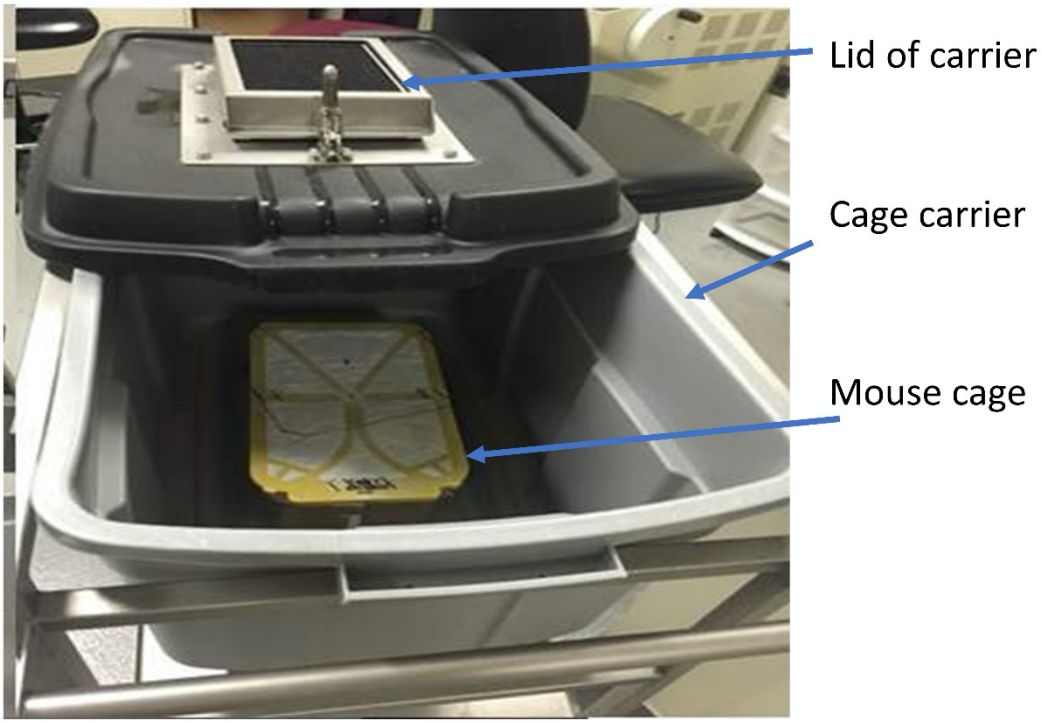

Figure S1: Mouse cage and carrier container for movement of the cage as required by the animal control facility when irradiating a mouse outside of the facility.

Alignment of this setup was performed with the help of a hand-held laser pointer having a magnetic base and mounted vertically on an L-shape stand designed for this purpose. A

horizontal and vertical bubble leveler was used to align the laser beam with the X-ray source. The laser beam was quite visible on the target after passing through the white HEPA filter cage lid. The center of the X-ray beam was aligned with the center of the target tumor located specifically at the skin of the mouse (Figure 1F in the main manuscript).

A mouse phantom (Figure 1C in the main manuscript) was fabricated by cutting a Styrofoam piece of suitable thickness in an elliptical shape to test the proper dose delivery at the target. Because the center of the beam is not coincident with the geometrical center of the tray, we pasted a metallic bead as a marker of the tumor location on the mouse phantom and also to confirm the beam center. The test exposure of the bead is shown in Figure 1D in the main manuscript and shows that the beam center is approximately aligned with the center of the target location (mouse phantom in this case). After test exposure, the location of the mouse phantom was marked, and an anesthetized mouse was placed at the same location for irradiation.

Machine output for different kV_p and mA setting for shelf position number 7 is provided in the units of R/min as shown in **Table S2** and was converted into Gy/min for the maximum kV_p and mA setting. The time for a test dose of 30 Gy was calculated and is given in **Table S3.**

Table S2: Machine output at shelf position Number 7

Technique Factors		Max. mA	1 mA	2 mA	4 mA
kV_p	Max. mA	(R/min)	(R/min)	(R/min)	(R/min)
100	10	29	2	5	10
110	9.1	35	3	7	14
120	8.3	42	4	9	18
130	7.7	50	5	11	22
140	7.1	56	6	14	27
150	6.7	60	8	17	32
160	6.2	66	9	19	38

Table S3: Time calculation for delivering 1 Gy, 5 Gy, and 30 Gy doses, based on distance of mice tumors from source (23.5 cm)

Filter	Machine Output (R/min)	Dose Rate (cGy/min)	Time for 30 Gy (min)	Time for 5 Gy (min)	Time for 1 Gy (min)
0.5 mm Cu	66	57.882	26.28	4.38	0.88

Radiation safety requirements

Before the start of the irradiation study, a detailed radiation survey was performed at different voltage and current settings of the machine with the help of a hand-held portable radiation survey meter. There was a background level radiation close to the machine cabinet in all voltage and current settings of the machine. Therefore, there was no radiation hazard outsize of the machine while the machine was in operation.

Integrin αvβ3 expression in pancreatic and lung cancer cells

Flow cytometry analysis showed that the expression level of integrin αvβ3 in SUIT-2 and H1299 cells was more than 30%, compared to unstained cells. When these cells were exposed to 1 Gy, no change in the expression of αvβ3 was observed compared to non-irradiated cells (**Figure S2**). Expression of αvβ3 increased by 4-5% after 5 Gy radiation in both cell lines, compared to non-irradiated cells (**Figure S2**). NDAT administration (10 μg/ml) in SUIT-2 or H1299 cells after 24 hrs resulted in no difference of expression of αvβ3 compared to untreated cells (**Figure S3**).

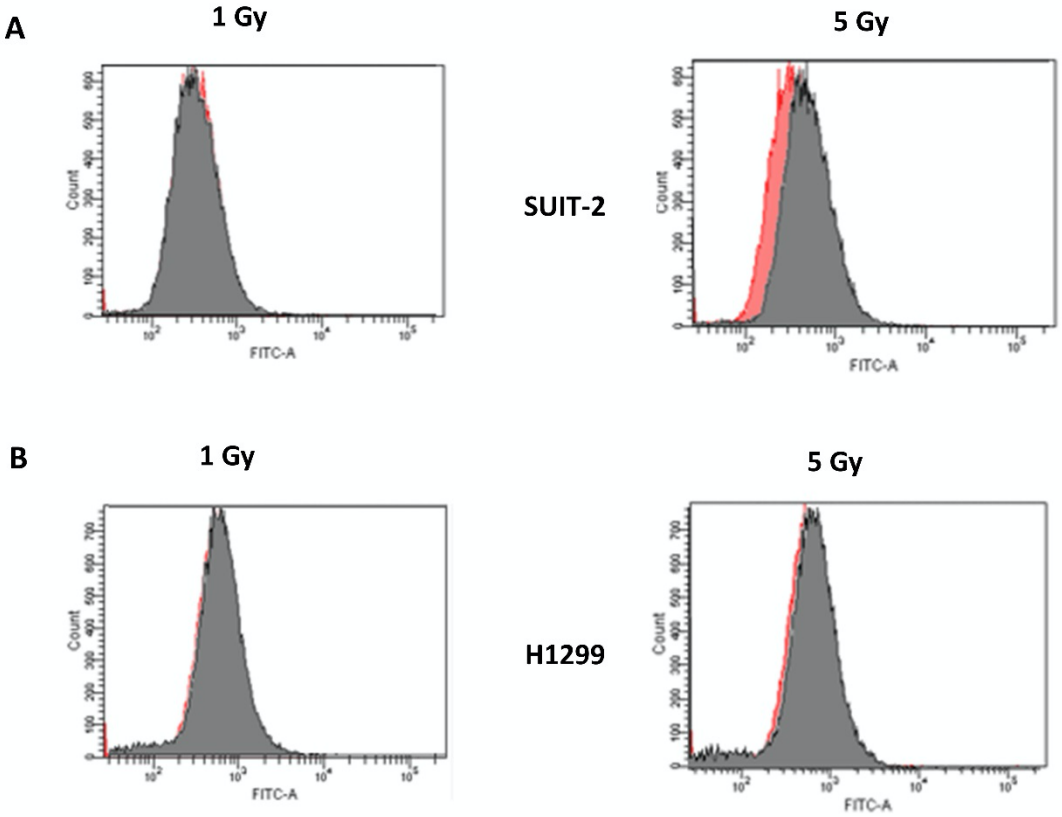

Figure S2. Flow cytometric data of αvβ3 expression in SUIT-2 pancreatic cancer and H1299 non-small cell lung carcinoma cells 24 hrs after radiation. **A)** SUIT-2 cells exposed to 1 Gy or 5 Gy. **B)** H1299 cells exposed to 1 Gy or 5 Gy. After 24 hrs radiation, expression of αvβ3 was analyzed with

flow cytometry; grey-colored histogram is of the irradiated cells and red-colored histogram is of the non-irradiated cells. There was less than 2-5% increase in the expression of αvβ3 at 5 Gy but no effect at 1 Gy.

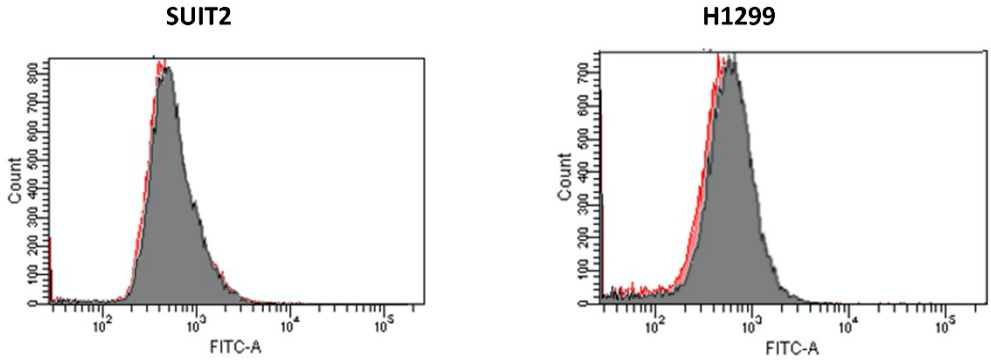

Figure S3. Flow cytometric data of αvβ3 expression after 24 hrs treatment with NDAT (10 µg/ml) in SUIT-2 and H1299 cells. There was no change in the expression of αvβ3.