Preparation of C57-Mice with B16-Melanoma for Proton Irradiation ◇

L. Weissfloch [a], J. Besserer, J. de Boer, M. Moosburger, S. Porciani [b], M. Brückner [a], K. Greulich [a], M. Peller [c], H. Bruckbauer [d], R. Senekowitsch-Schmidtke [e], K. Tempel [f], A. Becciolini [b], and M. Molls [a]

[a] Department of Radiation Oncology, Klinikum rechts der Isar, Technical University of Munich (TUM)
[b] Laboratory of Radiation Biology, Department of Clinical Physiopathology, University of Florence, Italy
[c] Dept. of Nuclear Magnetic Resonance, Klinikum Grosshadern, LMU, Germany
[d] Dept. of Dermatology and Allergology, Klinik am Biederstein, TUM, Germany
[e] Dept. of Nuclear Medicine, Klinikum rechts der Isar, TUM, Germany
[f] Institute for Pharmacology, Toxology and Pharmacy, Faculty of Veterinary Medicine, LMU, Germany

Dry-ice stored, murine B16-melanoma cells from "Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH" (DSMZ), Braunschweig, were cultured in D-MEM-medium. The female C57/BL6-mice, aged six weeks, were obtained from Charles River GmbH, Germany.

For shaving the right flank and inocculation of the melanoma cells the animals received a short-term anesthesia as follows: 0.1 ml / 10 g b.w. of a solution of 6 ml Fentanyl-Janssen ^{TM} [0.157 mg Fentanylhydrogen-citrate / 2 ml] and 6 ml Hypnodil^{TM} [50 mg Metomidathydrochloride / ml] diluted in 38 ml 0.9% NaCl were injected intraperitoneally.

The shavings were done with a Moser^{TM} animal shaving machine and a common dry shaver for the fluff. Two days later the melanoma cell suspension was injected subcutaneously with a Hamilton^{TM} syringe. Each mouse received 200.000 cells in a volume of 25 μl. Ten days after this inocculation tumor growth could be seen.

For determining the tumor size, especially its thickness, measurements with nuclear magnetic resonance and ultrasound were carried out. For this purpose and for the proton irradiation itself the tumor-bearing mice received longterm anesthesia: To 35 ml 0.9% NaCl 2.5 ml Tileat 500^{TM} [250 mg Tileataminhydrochloride and 250 mg Zolazepamhydrochloride/ml] and 4 ml Rompun^{TM} [20 mg Xylazine / ml] were added and 0.15 ml of this solution intraperitoneally injected. The tumors and healthy skin were exposed to 10, 20, 30 or 40 Gy of protons in a single fraction.

After various intervals up to three weeks after proton exposure the animals received an intraperitoneal injection of ^{3}H-Thymidine [10 μCu / 10 μl]. One hour later the animals were sacrificed in Halothane^{TM} atmosphere. The tumors and samples of unirradiated and irradiated skin were taken and stored in 10% buffered formaline as well as in dry-ice for transportation and further investigation at the University of Florence.

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