Induction of gamma-H2AX Foci in Lymphocytes, Fibroblasts and Tumor Cells by Single and Pulsed High Dose Rate X-rays

N. Franken[a], B. Pieters[a], R. Ten Cate[a], A. Güçlü[a], L. Stalpers[a], B. Klein[b], M. Giphart-Gassler[b], J.P. Medema[a], J. Haveman[a], C. Van Bree[a] and H. Vrieling[b]

[a] *Academic Medical Center, University of Amsterdam, LEXOR, CEMM, Department of radiation Oncology, PO Box 22700, 1100 DE Amsterdam, Netherlands;* [b] *Leiden University Medical Center, Department of Toxicogenetics, Einthovenweg 20, PO Box 9600, 2300 RC Leiden, Netherlands*

n.a.franken@amc.uva.nl

Purpose and background: Variation in radiosensitivity of normal tissues is related to genetic susceptibility (Svensson et al. 2006). Persistence of gamma-H2AX foci after ionizing radiation is higher in DNA-DSB repair deficient cells than in normal cells. We investigated whether differences in number of ?-H2AX foci may be useful as a predictor of radiosensitivity.

Materials and methods: Induction of gamma-H2AX foci was measured in various cell types: freshly isolated lymphocytes, fibroblast cells (AT+/+, AT+/-, AT-/-), DNA-DSB repair deficient and normal hamster cells and human tumour cells (lung carcinoma, SW-1573 and prostate cancer cell lines with different TP53 status: LNCaP: TP53 wt, PC3: TP53 null and DU145: TP53 mutant). A radiation dose of 2.4 Gy was given as a single dose or as pulsed doses of 100 mGy in 24 h (PDR: 240 pulses of 100mGy, 1 pulse every 6 min). The number of gamma-H2AX foci was determined 30 min and 24 h after single dose and directly after PDR.

Results: Maximal number of γ-H2AX foci occurred after single dose at 30 min after irradiation without significant differences in numbers of foci between different cell lines. The number of gamma-H2AX foci was radiation dose dependent and linearly correlated with clonogenic survival. Both 24 h after a single dose and after PDR more remaining foci are observed in repair deficient cells than in normal cells. In addition the TP53 null cells have more remaining foci than the mutant and wt TP53 cells.

Conclusions: The detection gamma-H2AX after in vitro irradiation of lymphocytes and cell lines is simple, reproducible and sensitive. The number of residual gamma-H2AX foci after single dose as well as after PDR enables us to distinguish cells with differences in DNA-DSB repair capacities. However, it remains to be established whether gamma-H2AX assay applied in lymphocytes can be used to predict for late normal tissue toxicity after radiotherapy. Sponsored by the Dutch Cancer Foundation UVA2008-4019