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**Sex-specific differences in radiation sensitivity –
epidemiological, clinical and biological studies**

Statement by the German Commission on Radiological Protection

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**Geschlechtsspezifische Unterschiede der Strahlenempfindlichkeit –
epidemiologische, klinische und biologische Studien**

Stellungnahme der Strahlenschutzkommission

This translation is for informational purposes only, and is not a substitute for the official statement. The original version of the statement, published on www.ssk.de, is the only definitive and official version.

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1 Introduction

The International Commission on Radiological Protection (ICRP) has reiterated, in its latest Recommendations, that in radiological protection, it is useful to determine a single value of effective dose and that there is no reason to distinguish between the sexes, despite the possible existence of sex-specific differences in radiation-related risks [ICRP 07]. Whereas the ICRP states that it is appropriate to use sex- and even age-specific data for the purposes of *retrospective individual* evaluation of radiation-related risks following internal or external exposure to ionising radiation, for the purposes of *radiological protection* it remains the policy of the Commission to determine a single value of effective dose. Accordingly, the tissue weighting factors (w_T) introduced by the ICRP are age- and sex-averaged. This also applies to the male and female breast and the gonads. The ICRP justifies its approach *inter alia* on the grounds that there are great uncertainties surrounding the values of tissue weighting factors and that there could be possibly discriminatory effects if sex-specific differences are made in the calculation of risks.

Other national and international institutions emphasise possible sex-specific differences, however. For example, the latest report by the National Research Council (NRC) in the USA calculates different excess cases of both solid cancer and leukaemia for males and females after exposure to ionising radiation, assuming an age distribution similar to that of the entire US population [NRC 06]. The United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR) also assumes that for all solid cancers, the excess absolute risk and the excess relative risk are higher in women than in men by a factor of around 2 [UNSC 08].

Epidemiological studies provide evidence of a significantly lower **spontaneous** cancer incidence for women than for men. The figures provided by SEER^{*)} (1975-2005) for all types of cancer, age-corrected, are 403 cases (females) and 519 cases (males) per 100,000 persons [Rie 08]. Looking at individual cancers, some tumour entities are more common among men than women (e. g. liver cancer, squamous cell carcinoma and lymphoma), whereas meningeal sarcomas and thyroid cancer are more common in women. It is unclear whether the causes of sex-specific differences in spontaneous cancer incidence are due to genetic and/or hormonal factors, and what influence occupational factors and lifestyle have.

There are allusions to differences in the radiation sensitivity of male and female tissue in clinical studies in which the early or late side-effects of radiotherapy and combined radio-/chemotherapy are broken down by sex [Bor 09]. However, very few studies to date have *systematically* analysed possible sex-specific differences in sensitivity to ionising radiation. By contrast, a number of epidemiological studies have analysed sex-specific differences in radiation-induced cancer incidence and mortality. The Life Span Study (LSS) carried out among atomic bomb survivors, studies among survivors of Chernobyl and studies among cohorts exposed to therapeutic radiation are highly significant in this context (see Chapter 2 and Annexes 1 and 2). However, in relation to sex-specific differences, the findings of these studies are often close to the limits of significance, with the method of calculation having an important influence, and in some cases the findings of the various studies conflict. Significant differences are observed, however, in the solar radiation-induced incidence of non-melanoma skin cancers and in the age-standardised mortality from malignant skin melanomas, which are significantly higher for men than women [SSK 08].

In order to provide sound evidence of sex-specific differences in radiation sensitivity, studies

^{*)} Surveillance, Epidemiology and End Results

on suitable cell models and animal models are very important. Models which are proven to be suitable can then be used in order to investigate the biological and molecular bases of the postulated sex-specific radioreactions (see overview in [Bor 09]). There are hardly any systematic studies on the potential molecular and cellular bases for sex-specific radioreactions. The long-term objective should be to demonstrate whether there are statistically significant sex-specific differences in radiation sensitivity, not only at cellular level but also at the level of organisms, i. e. in humans and in suitable animal models. Based on clearly proven sex-specific differences in the animal model, analyses of the possible cellular and molecular bases of the postulated sex-specific differences in radiation sensitivity in humans should then be carried out.

2 Epidemiological Studies

In the UNSCEAR 2000 Report [UNSC 00], UNSCEAR concludes, after a review of the literature, that the excess absolute risk (EAR) of solid cancer occurring after exposure to ionising radiation is higher for women than men. In the new UNSCEAR 2006 Report, this issue is only touched upon peripherally [UNSC 08]. However, here too, the authors of the report conclude that for all solid cancers as a group, the excess relative risk and excess absolute risk are higher in women than in men by a factor of around 2.

These estimates are largely based on analyses of data relating to the Hiroshima and Nagasaki atomic bomb survivors. In a recently published report on solid cancer incidence in atomic bomb survivors [Pre 07], which includes a follow-up from 1958 to 1998, the authors find that for all solid cancers as a group excluding gender-specific cancers, women had somewhat higher rates of excess relative risk (ERR¹) than men. There is no significant sex difference in the values for excess absolute risk (EAR). However, sex-specific differences can be observed in some cases in the ERR and EAR values if cancer frequency is analysed separately for specific organs: for example, for women, the ERR values for cancer of the stomach (significant), lung (significant), bladder and thyroid are higher than for men, whereas the values for cancer of the colon and liver are lower. Similarly, in women, the EAR values for lung and thyroid are (significantly) higher than in men, whereas the values for colon (significant), liver (significant) and bladder are lower. For other types of cancer, no ERR or EAR values could be obtained due to the small number of cases in the atomic bomb survivor cohort.

The most recent report on mortality among atomic bomb survivors, which includes a follow-up from 1950 to 1997 [Pre 03], shows that the excess relative risk (ERR) of cancer is far higher for females than males. However, there are no significant differences in excess absolute risk (EAR). Preston et al. explain this in terms of the different spontaneous rates of cancer in males and females.

In a study on the occurrence of thyroid cancer, Ron et al. [Ron 95], based on a pooled analysis of seven studies, conclude that the ERR is greater for females than males by a factor of around 2, but that this difference is not significant: due to the spontaneous rate of the occurrence of thyroid cancer in females, the EAR is higher for women as well. This finding is consistent with that of Preston et al. [Pre 07]. However, Ron et al. also emphasise that in some studies, the opposite effect has been noted, with the ERR being higher for males than for females. In a study of children and adolescents in areas of Ukraine and Belarus affected by the

¹ For an explanation of ERR and EAR, see Annex 1.

Chernobyl accident, undertaken by Jacob et al. [Jac 06], the ERR was found to be smaller for females than for males by a factor of 3.8, which also appears to conflict with the LSS data on atomic bomb survivors. However, if the ERR values for the occurrence of thyroid cancer in the LSS for those survivors who were under 15 years of age at the time of the atomic bomb explosion are included, a male:female (M/F) ratio of 2.9 is obtained (see Table A17, Annex 1), which is consistent with the findings of Jacob et al. Ivanov et al. also found lower values for excess relative risk for girls who were exposed as children before 9 years of age after the Chernobyl accident than for boys, but this difference was not significant [Iva 06].

In another study, Gilbert et al. evaluated aspects of radiation-induced lung cancer in patients with Hodgkin's disease. They found that the ERR for males was about four times that for females, although the difference was not statistically significant [Gil 03]. This finding conflicts with that of the LSS (which found higher ERR values for women than for men, see above). Gilbert et al. attribute this to the different spontaneous rates of lung cancer (e. g. the low spontaneous rates in Japan), different dosages, fractionation, dose rates, and the possible failure to take adequate account of the effects of smoking and chemotherapy.

In a study of lung cancer mortality in Mayak workers, for internal dose, the ERR for females was significantly, i. e. about four times, higher than for males [Gil 04], which is consistent with the findings of the LSS. However, the EAR for females was less than that for males by a factor of 0.43.

Travis et al. investigated the carcinogenic effects of long-term internal exposure to alpha-particle-emitting radionuclides among cohorts from Sweden, Denmark and the USA [Tra 03]. No significant differences between men and women were found in relation to relative risk in any of the cohorts studied if all solid cancers were analysed as a group. However, they found a not significantly increased risk of non-Hodgkin's lymphoma and cancer of the rectum among men, and an increased risk of basal cell and squamous cell carcinoma, multiple myeloma, and cancer of the colon, lung and thyroid among women. None of these sex-specific differences was statistically significant. However, a clear difference emerged when, in the Danish/Swedish data, female (cervix, uterus, ovaries) and male (prostate, testicles) sex organs were analysed separately. This produced an RR for women of 1.1, compared with a value of 4.7 for men [Tra. 03].

In relation to leukaemia, the most reliable information on possible sex-specific differences is based on the data relating to atomic bomb survivors. Based on the excess absolute risk, it has been found that women have a significantly lower risk than men (female/male (F/M) ratio: 0.6 (90% CI: 0.4; 0.8)). The latest UNSCEAR Report [UNSC 08] confirms this finding. In the BEIR VII report, lower relative and absolute risks are assumed for women than for men. However, the confidence intervals of these estimates are large. In a study among workers at four US nuclear weapons facilities and a nuclear naval shipyard, the authors find a similar significantly lower excess relative risk for women (F/M ratio: 0.42 (95% CI: 0.23; 0.71)) [Sch 07]. This conflicts with the latest analysis by UNSCEAR which, based on data from the atomic bomb survivors, does not find any sex-specific differences in ERR [UNSC 08]. By contrast, studies carried out among people living on the Techa River in the Southern Urals [Kre 05] and among Mayak workers [Shi 03] point to higher excess relative risks for women (Techa River: ERR for women is around 70% higher than for men; Mayak workers: F/M ratio 1.7 (90% CI: 0.23; 11.2)). However, these findings are not statistically significant. In all other studies on leukaemia risk after exposure to ionising radiation (see Annex 1) considered within the framework of this recommendation, no information could be found about possible sex-specific differences.

3 Clinical Findings after Radiation Therapy

Men's and women's different radiation sensitivity could have an effect on tumour response and local control (i. e. prevention of recurrence at the site of the primary cancer) during radiation therapy, but also on adverse early and late side-effects, including the induction of secondary malignancies. Despite the importance of this issue, also with regard to therapeutic decisions, there are very few systematic studies on the specific influence of sex on these endpoints. With regard to local control and survival, some types of cancer consistently show increased response rates in women compared to men (for overview, see [Bor 09]), which can be attributed at least in part to the greater effectiveness of chemotherapy in women [Sin 05]. However, systematic comparisons which take account of the modalities of radiation therapy are not available. What's more, very little data are available on the adverse side-effects of radiation therapy among males and females. The few available findings do not allow any conclusions to be drawn about sex-specific differences in radiation sensitivity. As regards the occurrence of secondary malignancies in cancer survivors, there is increasing availability of findings of major studies based on clinical and epidemiological cancer registers with tens of thousands of patients. Although these studies routinely calculate secondary malignancy risks separately for men and women and, in normal cases – as far as possible – compare the risks between patients who have undergone radiation treatment for the first cancer, and those who have not, very few of the studies investigate possible sex-specific differences in radiation effect. An increased risk of breast cancer in female children and adolescents following irradiation of the thorax, e. g. during therapy for Hodgkin's disease [Bha 02] or non-Hodgkin's lymphoma [Blu 08] has consistently been observed. In these studies, the risk of secondary malignancies remained higher for females even if breast cancer was excluded.

4 Radiobiological Studies

Epidemiological studies have produced many indications of possible sex-specific radiation sensitivity in humans. However, further biological studies are required to produce reliable evidence of this. In these biological studies, suitable experimental models must be used which, assuming sufficient scope of the experiment, can provide clear evidence of the postulated sex-specific radiation sensitivity. Apart from regulation through genetic and epigenetic mechanisms, other factors, especially hormones and lifestyle differences, may be influential. Animal models in which cancer incidence and, if appropriate, other endpoints can be analysed and quantified under physiological conditions as indicators of radiation sensitivity therefore appear to be more suitable than cell models as a method of providing evidence of sex-specific radiation sensitivity.

Experiments carried out on animal models to date confirm and expand the evidence obtained from epidemiological studies for the existence of sex-specific differences in radiation sensitivity. For example, female mice show a much higher incidence of thymic lymphoma and a higher incidence of osteosarcoma after treatment with ^{227}Th than male mice [Mül 78]. By contrast, after chronic exposure to doses of UVB or to chemical carcinogens, male mice had more tumours than female mice [Tho 07, Huf 91]. In order to obtain information about the mechanisms underlying the postulated sex-specific radiation sensitivity, cell models were used alongside the animal models. They included human peripheral blood cells from healthy subjects, and cell lines or primary cells from selected organs of the mouse models, which were used to demonstrate sex-specific radiation sensitivity. Besides functional assays to study cellular responses to radiation, e. g. DNA damage and repair, mutations and cell death (apoptosis) (for an overview, see [Bor 09]), whole genome screening processes have been

used, such as microarrays to analyse gene expression (quantification of transcript levels) [Mea 08, Whi 03], and various techniques to analyse epigenetic regulatory mechanisms (DNA methylation changes, histone modification patterns, expression of microRNAs) [Kov 04, Pog 04, Sil 04].

In the microarray analyses of radiation-induced gene expression, a number of sex-specific expressed genes were found, but no correlations could be established with corresponding cellular phenotypes which are valid indicators of radiation sensitivity, such as apoptosis or DNA damage and repair. There was also little evidence of correlations with the sex-specific radiation-induced cancers postulated in the epidemiological studies, which is probably also due to the fact that a wide variety of testing systems were used in the experiments. For that reason, all these approaches are of only limited informative value.

In recent years, new experimental approaches have been developed, primarily through the extensive work carried out by the research group headed by O. Kovalchuk. This group has provided a substantial body of evidence that the bystander effect (a phenomenon previously described by other authors as the “abscopal effect”), which could be demonstrated *in vivo* in the unirradiated spleen of mice and rats after local irradiation of the head, shows sex-specific differences. These sex-specific differences manifested in parameters such as DNA breaks and apoptosis, but above all in epigenetic parameters such as DNA methylation, histone modifications and levels and patterns of expression of microRNAs [Il'n 09, Kot 06, Kot 07, Kot 08]. Using mice whose gonads had been removed after birth, it was possible to produce compelling evidence that hormones have a clear influence on sex-specific radiation sensitivity [Kot 08].

Using the established animal models, major interdisciplinary studies can thus be carried out in future. These should allow sex-specific differences – from molecular and cellular processes to cancer incidence and mortality – to be analysed in comprehensive experiments using male and female animals, controlling for/regulating important hormonal differences.

5 Summary

The epidemiological studies in which risk coefficients are given separately for females and males provide conflicting results about sex-specific radiation sensitivity. The studies carried out among atomic bomb survivors show – both for mortality and for incidence – significantly higher ERR values for women than for men for all solid cancers. However, as women have lower spontaneous rates than men, the corresponding EAR values for women and men are similar, but this only applies as long as sex-specific organs are not considered. If all organs, including sex-specific organs, are analysed as a group, a significantly higher EAR value remains for women in the incidence data, whereas the EAR values are comparable in relation to mortality. It must be emphasised, however, that the few epidemiological studies which deal with radiation-induced occurrence of the same cancer type in different cohorts sometimes yield conflicting results. For example, the data collected among atomic bomb survivors yield a higher excess absolute risk for women than for men for thyroid cancer, whereas other studies produce the opposite result.

In calculating risk coefficients and tissue weighting factors, it is necessary to transfer the risk estimates obtained from studies of atomic bomb survivors to other populations. This is possible both on the basis of an ERR model (multiplicative) and an EAR model (additive). As it is often not clear which model is better, the ICRP uses the mean value of the risks from ERR and EAR for most types of cancer. For bone-marrow and the female breast, however, only the EAR values are used; for thyroid and skin, only the ERR values are used; and for the

lung, the EAR value is weighted by a factor of 0.3 and the ERR value by a factor of 0.7. Due to the uncertainties associated with the derived detriment values, the ICRP does not consider it necessary to take account of possible sex-specific differences.

With regard to radiobiological experiments, the situation can be summed up as follows: for various endpoints (e. g. cancer development in experimental models, changes in gene expression and epigenetic patterns after irradiation), sex-specific differences have been described but very few of the observations could be reproduced so far. Moreover, for the majority of observations, it is quite unclear whether differences in these endpoints have an impact on cancer risk. Using modern screening processes, a large number of genes have been identified which show sex-specific differences in their expression in response to radiation, but their influence on radiation sensitivity is largely unknown. Furthermore, a great many results obtained in experiments on mice indicate that besides direct radiation-induced effects, persistent bystander effects also play a role, which are influenced by sex hormones and caused by epigenetic processes. Here too, the molecular mechanisms underlying these effects are largely unknown. Based on the data currently available, targeted experimental approaches could be developed in future in order to gain an understanding of sex-specific molecular and cellular reactions to radiation exposure in terms of their molecular mechanisms. In the long term, establishing a correlation with cancer incidences determined in the same animal model should be the aim.

Opinion of the Commission on Radiological Protection (SSK):

1. Epidemiological, clinical and biological studies have produced indications but no clear evidence of possible sex-specific differences in radiation sensitivity (endpoints: mortality and cancer). There is therefore a need for further research to provide conclusive evidence and an understanding of possible sex-specific differences in the radiation-induced incidence of cancer in individual organs and in the radiation sensitivity of entire organisms based on molecular, cellular and tissue reactions to irradiation. Future studies should, from the conceptual stage onwards, be optimised with a view to subsequent evaluation of sex-specific differences in radiation risk.
2. Due to the limited data availability described above, both with regard to clinical studies and biological experiments, and the sometimes conflicting findings of epidemiological studies, the Commission on Radiological Protection takes the view that it is not possible, at present, to make unequivocal statements about sex-specific differences in radiation sensitivity. Having analysed the individual studies, the Commission cannot identify any basis for the certainty expressed in some of the statements made by other national and international institutions regarding generally higher radiation sensitivity in females.
3. After careful analysis, the Commission therefore concludes that it is not necessary, at present, to consider possible sex-specific differences in radiation sensitivity in the context of radiological protection.

References

- [Bha 02] Bhatia, S.; Sklar, C.: Second cancers in survivors of childhood cancer. *Nat Rev Cancer* 2(2):124-32, 2002
- [Blu 08] Bluhm, E. C.; Ronckers, C.; Hayashi, R. J.; Neglia, J. P.; Mertens, A. C.; Stovall, M.; Meadows, A. T.; Mitby, P. A.; Whitton, J. A.; Hammond, S.; Barker, J. D.; Donaldson, S. S.; Robison, L. L.; Inskip, P. D.: Cause-specific mortality and second cancer incidence after non-Hodgkin lymphoma: a report from the Childhood Cancer Survivor Study. *Blood* 111(8):4014-4021, 2008
- [Bor 09] Borgmann, K. et al.: Sex-specific aspects of tumor therapy. *Radiat Environ Biophys* 48(2):115-24, 2009
- [Gil 03] Gilbert, E. S.; Stovall, M.; Gospodarowicz, M.; van Leeuwen, F. E.; Andersson, M.; Glimelius, B.; Joensuu, T.; Lynch, C. F.; Curtis, R. E.; Holowaty, E.; Storm, H.; Pukkala, E.; van't Veer, M. B.; Fraumeni, J. F. Jr; Boice, J. D. Jr; Clarke, E. A.; Travis, L. B.: Lung Cancer after Treatment for Hodgkin's Disease: Focus on Radiation Effects. *Radiat Res* 159:161-173, 2003
- [Gil 04] Gilbert, E. S.; Koshurnikova, N. A.; Sokolnikov, M. E.; Shilnikova, N. S.; Preston, D. L.; Ron, E.; Okatenko, P. V.; Khokhryakov, V. F.; Vasilenko, E. K.; Miller, S.; Eckerman, K.; Romanov, S. A.: Lung Cancer in Mayak Workers. *Radiat Res* 162:505-516, 2004
- [Huf 91] Huff, J. et al.: Chemicals associated with site-specific neoplasia in 1394 long-term carcinogenesis experiments in laboratory rodents. *Environ Health Perspect* 93:247-270, 1991
- [Iln 09] Ilnytsky, Y.; Koturbash, I.; Kovalchuk, O.: Radiation-induced bystander effects in vivo are epigenetically regulated in a tissue-specific manner. *Environ Mol Mutagen* 50(2):105-113, 2009
- [ICRP 07] International Commission on Radiological Protection ICRP: The 2007 Recommendations of the International Commission on Radiological Protection. ICRP Publication 103. *Ann. ICRP* 37(2-4), Oxford: Pergamon Press, 2007
- [Iva 06] Ivanov, V. K.; Gorski, A. K.; Tsyb, A. F.; Maksimov, M. A.; Tumanov, K. A.; Vlasov, O. K.: Radiation-epidemiological studies of thyroid cancer incidence among children and adolescents in the Bryansk oblast of Russia after the Chernobyl accident (1991-2001 follow-up period). *Radiat Environ Biophys* 45:9-16, 2006

- [Jac 06] Jacob, P.; Bogdanova, T. I.; Buglova, E.; Chepurniy, M.; Demidchik, Y.; Gavrillin, Y.; Kenigsberg, J.; Meckbach, R.; Schotola, C.; Shinkarev, S.; Tronko, M. D.; Ulanovsky, A.; Vavilov, S.; Walsh, L.: Thyroid cancer risk in areas of Ukraine and Belarus affected by the Chernobyl accident. *Radiat Res* 165:1-8, 2006
- [Kot 06] Koturbash, I. et al.: Irradiation induces DNA damage and modulates epigenetic effectors in distant bystander tissue in vivo. *Oncogene*, 25(31):4267-4275, 2006
- [Kot 07] Koturbash, I. et al.: Role of epigenetic effectors in maintenance of the long-term persistent bystander effect in spleen in vivo. *Carcinogenesis*, 28(8):1831-1838, 2007
- [Kot 08] Koturbash, I. et al.: Radiation-induced bystander effects in vivo are sex specific. *Mutat Res* 642(1-2):28-36, 2008
- [Kov 04] Kovalchuk, O. et al.: Dissimilar genome response to acute and chronic low-dose radiation in male and female mice. *Mutat Res.* 550(1-2):59-72, 2004
- [Kre 05] Krestinina, L. Yu.; Preston, D. L.; Ostroumova, E. V.; Degteva, M. O.; Ron, E.; Vyushkova, O. V.; Startsev, N. V.; Kossenko, M. M.; Akleyev, A. V.: Protracted radiation exposure and cancer mortality in the Techa River Cohort. *Radiat Res* 164:602-611, 2005
- [Mea 08] Meadows, S. K. et al.: Gene expression signatures of radiation response are specific, durable and accurate in mice and humans. *PLoS ONE* 3(4): e1912, 2008
- [Mül 78] Müller, W. A. et al.: Late effects after incorporation of the short-lived alpha-emitters ^{224}Ra and ^{227}Th in mice. *Health Phys* 35(1):33-55, 1978
- [NCR 06] National Research Council (NRC) of the National Academies: Health Risks from exposure to low levels of ionizing radiation – BEIR VII Phase 2. Board on Radiation Effects Research, Division on Earth and Life Studies, National Research Council of the National Academies, The National Academies Press, Washington, D.C., 2006
- [Pog 04] Pogribny, I. et al.: Dose-dependence, sex- and tissue-specificity, and persistence of radiation-induced genomic DNA methylation changes. *Biochem Biophys Res Commun* 320(4):1253-1261, 2004
- [Pre 03] Preston, D. L.; Shimizu, Y.; Pierce, D. A.; Suyama, A.; Mabuchi, K.: Studies of mortality of atomic bomb survivors. Report 13: Solid cancer and noncancer disease mortality: 1950-1997. *Radiat Res* 160:381-407, 2003

- [Pre 07] Preston, D. L.; Ron, E.; Tokuoka, S.; Funamoto, S.; Nishi, N.; Soda, M.; Mabuchi, K.; Kodama, K.: Solid cancer incidence in atomic bomb survivors: 1958-1998. *Radiat Res* 168:1-64, 2007
- [Rie 08] Ries, L. A. G. et al.: *SEER Cancer Statistics Review, 1975 - 2005*. National Cancer Institute: Bethesda, MD. 2008
- [Ron 95] Ron, E.; Lubin, J. H.; Shore, R. E.; Mabuchi, K.; Modan, B.; Pottern, L. M.; Schneider, A. B.; Tucker, M. A.; Boice, J. D. Jr: Thyroid cancer after exposure to external radiation: a pooled analysis of seven studies, *Radiat Res* 141(3):259-277, 1995
- [Sch 07] Schubauer-Berigan, M. K.; Daniels, R. D.; Fleming, D. A.; Markey, A. M.; Couch, J. R.; Ahrenholtz, S. H.; Burphy, J. S.; Anderson, J. L.; Tseng, C.: Risk of chronic myeloid and acute leukemia mortality after exposure to ionizing radiation among workers at four U.S. nuclear weapons facilities and a nuclear naval shipyard. *Radiat Res* 167:222-232, 2007
- [Shi 03] Shilnikova, N. S.; Preston, D. L.; Ron, E.; Gilbert, E. S.; Vassilenko, E. K.; Romanov, S. A.; Kuznetsova, I. S.; Sokolnikov, M. E.; Okatenko, P. V.; Kreslov, V. V.; Koshurnikova, N. A.: Cancer mortality risk among workers at the Mayak Nuclear Complex. *Radiat Res* 159:787-798, 2003
- [Sil 04] Silasi, G. et al.: Selective brain responses to acute and chronic low-dose X-ray irradiation in males and females. *Biochem Biophys Res Commun* 325(4):1223-1235, 2004
- [Sin 05] Singh, S.; Parulekar, W.; Murray, N.; Feld, R.; Evans, W. K.; Tu, D. and Shepherd, F. A.: Influence of sex on toxicity and treatment outcome in small-cell lung cancer. *J Clin Oncol* 23:850-856, 2005
- [SSK 08] Strahlenschutzkommission (SSK): Einfluss der natürlichen Strahlenexposition auf die Krebsentstehung in Deutschland. Veröffentlichungen der Strahlenschutzkommission, Band 62, 215-313. Stellungnahme der Strahlenschutzkommission mit wissenschaftlicher Begründung. H. Hoffmann GmbH - Fachverlag, Berlin, 2008
- [Tho 07] Thomas-Ahner, J. M. et al.: Gender differences in UVB-induced skin carcinogenesis, inflammation, and DNA damage. *Cancer Res* 67(7):3468-3474, 2007
- [Tra 03] Travis, L. B.; Hauptmann, M.; Knudson Gaul, L.; Storm, H. H.; Goldman, M. B.; Nyberg, U.; Berger, E.; Janower, M. L.; Hall, P.; Monson, R. R.; Holm, L. E.; Land, C. E.; Schottenfeld, D.; Boice, J. D. Jr; Andersson, M.: Site-specific cancer incidence and mortality after cerebral angiography with radioactive thorotrast. *Radiat Res* 160:691-706, 2003

- [UNSC 00] United Nations Scientific Committee on the Effects of Atomic Radiation: Sources and Effects of ionizing radiation: UNSCEAR 2000 Report to the General Assembly, United Nations, New York, 2000
- [UNSC 08] United Nations Scientific Committee on the Effects of Atomic Radiation: Effects of ionizing radiation – UNSCEAR 2006 Report, Vol I. Report to the General Assembly, with scientific annexes A and B. United Nations sales publications. United Nations, New York, 2008
- [Whi 03] Whitney, A. R. et al.: Individuality and variation in gene expression patterns in human blood. Proc Natl Acad Sci USA 100(4):1896-1901, 2003

ANNEX 1: Summary of Radioepidemiological Findings

1 Assessments of Other National and International Institutions

In recent years, a number of pre-eminent scientific institutions have produced reports on the risks from exposure to ionising radiation. They include the BEIR VII report by the National Research Council (NRC) in the USA [NRC 06], the UNSCEAR 2000 and UNSCEAR 2006 reports [UNSC 00, 08] and the new ICRP Recommendations [ICRP 07]. One of the topics discussed in these reports is possible sex-specific differences in the radiation sensitivity of human tissue.

1.1 BEIR VII

The latest report, entitled *Biological Effects of Ionizing Radiation (BEIR VII)*, published by the National Research Council (NRC) in the USA calculates different excess cases of both solid cancer and leukaemia for males and females after exposure to ionising radiation, assuming an age distribution similar to that of the entire US population (Table A1) [NRC 06].

Table A1: Estimated excess cases (incidence / mortality) expected to result in 100 000 persons in the US population exposed to 0.1 Gy (“Life-time attributable risk”); 95% confidence intervals are shown in parentheses [NRC 06].

	All Solid Cancer		Leukaemia	
	Males	Females	Males	Females
Excess cases (incidence)	800 (400, 1 600)	1 300 (690, 2 500)	100 (30, 300)	70 (20, 250)
Excess deaths (mortality)	410 (200, 830)	610 (300, 1 200)	70 (20, 220)	50 (10, 190)

Table A1 shows that according to the NCR, out of 100 000 American women exposed to 0.1 Gy, around 1 300 will develop a solid cancer, compared with only 800 for every 100 000 American men. While these differences are not statistically significant (the corresponding 95% confidence intervals overlap), they can be seen as an expression of possible sex-specific differences in radiation sensitivity in humans. In contrast to solid cancer, however, the leukaemia risk appears to be somewhat higher for males than for females.

These figures are based on an analysis and evaluation of various cohorts whose members were exposed to ionising radiation, with the LSS cohort (LSS: Life Span Study), comprising the atomic bomb survivors from Hiroshima and Nagasaki, making the most important contribution. The health data from these cohorts are calculated using two different models: Excess Relative Risk: ERR, equation 1, and Excess Absolute Risk: EAR, equation 2.

$$\lambda(c,s,a,b,d) = \lambda(c,s,a,b)[1 + \beta_s \text{ERR}(e,a) \times d] \quad (1)$$

$$\lambda(c,s,a,b,d) = \lambda(c,s,a,b) + \beta_s \text{EAR}(e,a) \times d \quad (2)$$

$\lambda(c,s,a,b)$ is the spontaneous rate which occurs without an additional dose of ionising radiation; c = city (Hiroshima or Nagasaki), which is required to evaluate the LSS data, s = sex (male, female), a = age at onset of disease/death, b = year of birth, e = age at exposure, and d = dose. The function β_s in each model signifies the increase in a linear dose-response model, while $\text{ERR}(e,a)$ and $\text{EAR}(e,a)$ are weighting factors for the influence of age at exposure, e , and age at onset of disease, a .

ERR(s,d) and EAR(s,d) are dose-dependent functions. If a linear dose-response relationship is assumed without a threshold (LNT hypothesis), the (sex-dependent) risk coefficient β_s yields the increase in absolute risk per dose:

$$\beta_s = \frac{EAR(s,d)}{d} \text{ or } \beta_s = \frac{ERR(s,d)}{d} \cdot \lambda(c,s,a,b) \text{ with } [\beta_s] = PY^{-1}Sv^{-1}$$

PY: person-years

In order to quantify radiation sensitivity in relation to an investigated endpoint, the following functions are often used in epidemiological studies:

$$\beta = \frac{EAR}{d} \text{ or } \frac{ERR}{d}$$

(i. e. the slope in the dose-risk ratio). In many of these studies (including the LSS studies), the expressions EAR/Sv (or EAR/Gy) or ERR/Sv (or ERR/Gy), respectively, are used here; these are somewhat misleading and, strictly speaking, are not correct. However, in order to facilitate direct comparison with the studies cited, this notation will be retained below.

The BEIR VII report, based on data collected among atomic bomb survivors, gives the values listed in Table A2 for all solid cancers (excluding thyroid cancer and non-melanoma skin cancer), adjusted for age at exposure = 30 years and attained age = 60 years. Table A2 shows that according to the NRC, based on the data from the atomic bomb survivors, clear differences in male and female radiation sensitivity can be inferred. The BEIR VII report also mentions these differences in relation to the various organs with the exception of breast and thyroid, also based on analysis of data from the LSS cohort (Table A3).

Table A2: Values derived from the atomic bomb survivors for ERR/Sv and EAR/Sv (incidence/mortality) for all solid cancers, age at exposure = 30 years and attained age = 60; CI: confidence interval [NRC 06].

	ERR per Sv (95% CI)		EAR per Sv and 10 ⁴ Person-Years (95% CI)	
	Males	Females	Males	Females
Incidence	0.33 (0.24; 0.47)	0.57 (0.44; 0.74)	22 (15; 30)	28 (22; 36)
Mortality	0.23 (0.15; 0.36)	0.47 (0.34; 0.65)	11 (7.5; 17)	13 (9.8; 18)

Table A3: Site-specific values derived from the LSS cohort for ERR and EAR (incidence and mortality), age at exposure = 30 years, attained age = 60 years; CI: confidence interval [NRC 06]; breast: based on [Pre 02]; thyroid: based on [Ron 95] and [NIH 2003].

Site of solid cancers	ERR per Sv (95% CI)		EAR per Sv and 10 ⁴ Person-Years (95% CI)	
	Males	Females	Males	Females
Stomach	0.21 (0.11; 0.40)	0.48 (0.31; 0.73)	4.9 (2.7; 8.9)	4.9 (3.2; 7.3)
Colon	0.63 (0.37; 1.1)	0.43 (0.19; 0.96)	3.2 (1.8; 5.6)	1.6 (0.8; 3.2)
Liver	0.32 (0.16; 0.64)	0.32 (0.10; 1.0)	2.2 (1.9; 5.3)	1.0 (0.4; 2.5)
Lung	0.32 (0.15; 0.70)	1.40 (0.94; 2.1)	2.3 (1.1; 5.0)	3.4 (2.3; 4.9)
Breast	-	0.51 (0.28; 0.83)		9.4 (6.7; 13.3)
Prostate	0.12 (< 0; 0.69)	-	0.11 (<0; 1.0)	-
Uterus	-	0.055 (< 0; 0.22)	-	1.2 (<0; 2.6)
Ovary	-	0.38 (0.10; 1.4)	-	0.7 (0.2; 2.1)
Bladder	0.50 (0.18; 1.4)	1.65 (0.69; 4.0)	1.2 (0.4; 3.7)	0.75 (0.3; 1.7)
Other solid	0.27 (0.15; 0.50)	0.45 (0.27; 0.75)	6.2 (3.8; 7.3)	4.8 (3.2; 7.3)
Thyroid	0.53 (0.14; 2.0)	1.05 (0.28; 3.9)		

With the exception of colon and liver, the ERR values for the organs of females are generally higher than for the same organs of males. However, the BEIR VII report emphasises that the statistical uncertainties surrounding the values are often high. Interestingly, the trend towards higher values for females than males appears to be less pronounced for the EAR values (see discussion below).

1.2 UNSCEAR

Based on a literature review, UNSCEAR concludes that the excess absolute risk of solid tumour induction after exposure to ionising radiation is higher in women than in men: “Although differences in the absolute risk of tumour induction with sex are not large and vary with site, for most solid cancers the absolute risk is higher in women than in men” (paragraph 74, p. 11 [UNSC 00]). UNSCEAR suggests that with an acute dose of 1 Sv, lifetime risk estimates for solid cancer mortality might be taken as 9% for men and 13% for women. The uncertainties in the estimates may be a factor of about 2, higher or lower. For leukaemia, by contrast, no sex-specific difference is observed, and the lifetime risk of death from leukaemia may be taken as 1%, for either gender, following an acute dose of 1 Sv (paragraphs 84 and 85, page 13 [UNSC 00]).

Based on the analysis of the atomic bomb survivors, sex-specific differences are also found for various organs (Table A4), which indicate that the corresponding excess relative risk is higher by a factor of about 2 for women than for men.

It should be noted, however, that the female/male (F/M) sex ratio for the colon (2.1) does not accord with the value of 0.5 given by Preston et al. [Pre 07] for atomic bomb survivors (see Section 2.2.3). The same applies to the value for the bladder (0.8 in Table A4, as compared with 3.1 in Section 2.2.6).

Table A4: Values for ERR/Sv derived from the atomic bomb survivor data for age at exposure = 30 years (age-at-exposure model; incidence data) (Table 31 in [UNSC 00]).

Cancer type	Males	Females	female/male
All solid cancer	0.38	0.79	2.1
Oesophagus	0.41	0.84	2.1
Stomach	0.29	0.60	2.1
Colon	0.46	0.95	2.1
Liver	0.58	0.58	1.0
Lung	0.50	2.18	4.3
Breast	0	1.55	-
Bladder	1.18	0.98	0.8
Other cancer	0.47	0.28	0.6

The new UNSCEAR 2006 Report, recently published, presents the findings of a range of studies on radiation-induced cancer risk in various organs, including leukaemia (Tables 19–44 in [UNSC 08]). The findings of the LSS are cited separately for males and females with regard to both mortality and incidence (mostly based on [Pre 03] and [Pre 07]. The authors of [UNSC 08] conclude that “... both the ERR and the EAR for total solid cancers are somewhat higher (by about a factor of 2) for women than for men.” ([UNSC 08], para. 136, page 56). For lung cancer, they explicitly state that “... the ERR per unit dose (Sv-1) was larger for females than for males, but the EARs were similar for both sexes.” ([UNSC 08], para. 273, p. 80) (cf. also Section 2.2.5). Finally, the UNSCEAR Report points out that thyroid cancer naturally occurs more frequently in women than in men, and specifically mentions that the BEIR VII report also postulates a higher rate of radiation-induced thyroid cancer in women. Despite that, UNSCEAR concludes that: “... the role of sex in determining radiation risk is unclear. ...” ([UNSC 08], para. 467, p. 108), one probable reason for this being that: “... The effect of sex is not consistent in all studies.” ([UNSC 08], para. 467, p. 108) (see also Section 2.3, Table A17).

1.3 ICRP

The International Commission on Radiological Protection (ICRP) has reiterated, in its most recent Recommendations (ICRP Publication 103), that in radiological protection, it is useful to determine a single value of effective dose and that there is no reason to distinguish between the sexes, despite the possible existence of sex-specific differences in radiation-related risks [ICRP 07]. Although the ICRP advocates the use of sex- and even age-specific approaches for certain purposes (for example, the use of reference voxel phantoms for male and female are

soon to be introduced to compute individual dose after external or internal exposure to ionising radiation, and voxel phantoms are also being developed for children of various ages), the ICRP nonetheless states that : “... for the purpose of radiological protection it is useful to apply a single value of effective dose for both sexes.” ([ICRP 07]¹, para. 132, p. 69). Accordingly, the tissue weighting factors (w_T) introduced by the ICRP are age- and sex-averaged. This also applies to the male and female breast and the gonads. However, this weighting is only intended for use in radiological protection and is not suitable to assess individual risk after exposure to ionising radiation. (“This averaging implies that the application of this approach is restricted to the determination of effective dose E in radiological protection and, in particular, cannot be used for the assessment of individual risk.” ([ICRP 07], para. 132, p. 69). The ICRP emphasises that: “However, for the purposes of retrospective evaluation of radiation-related risks ... it is appropriate to use sex- and age-specific data and calculate sex- and age-specific risks.” ([ICRP 07], para. 33, p. 42). The ICRP justifies its approach inter alia by the greater uncertainties surrounding the calculation of effective dose: “In view of the uncertainties ... the Commission considers it appropriate for radiological protection purposes to use age- and sex-averaged tissue weighting factors and numerical risk estimates.” ([ICRP 07], para. 33, p. 42) and possible discriminatory elements if sex-specific differences are made in the calculation of risk: “Moreover, this obviates the requirement for sex- and age-specific radiological protection criteria which could prove unnecessarily discriminatory.” ([ICRP 07], para. 33, p. 42).

Despite these reservations, the ICRP’s new Recommendations include sex-specific organ-dependent values for detriment ([ICRP 07], Table A.4.18, p. 209), although the ICRP reiterates that they do not have a role in the Commission’s current system of radiological protection: “... These sex-specific values for detriment do not have specific functions in the Commission’s system of radiological protection ...” ([ICRP 07], Table A.4.18, p. 209). The relative sex-specific values derived by the ICRP for the population are shown in Table A5 for various organs alongside the mean relative values for detriment calculated by the ICRP ([ICRP 07], Table A.4.1, p. 179) and the w_T values actually used to calculate effective dose.

What is striking, for example, is the higher value for males than for females, by a factor of around 3, for the colon, which can be attributed to the EAR model (see Section 2.2.3). The w_T value actually used is then 0.12. A similar situation applies for the liver (see Section 2.2.4), although here, the ICRP has opted for a w_T value of 0.04, which corresponds approximately to the sex-specific mean. In the case of the thyroid, the relative detriment for females is far higher than for males. Here, the ICRP bases its estimates largely on the findings of the study by Ron et al. (Section 2.3). Compared with the sex-specific mean of 0.022, the ICRP increases the weighting somewhat arbitrarily and uses a value that is twice as high for w_T , i. e. 0.04. The ICRP justifies this on the grounds that this is intended to take account of the particularly high radiation sensitivity of the thyroid in childhood. For all other organs with the exception of the lung, the relative detriment values for males are higher than for females. It should be noted that for females, 27% of the detriment derives from the breast and ovaries. The risks of cancer of the salivary glands and brain – although not separately derivable from the data – are estimated by the ICRP to be higher than for cancer in the 14 remainder tissues. The w_T values for the salivary glands and brain were therefore each assigned a separate value of 0.01.

¹ A German translation is available at: www.bfs.de/de/ion/ICRP.html

Table A5: Sex-dependent values for detriment for the population (age at exposure 0 – 85 years) (Table A.4.18 from [ICRP 07]), corresponding mean values (Table A.4.1. from [ICRP 07]) and actual tissue weighting factors adopted by the ICRP (Table 3 from [ICRP 07]).

Organ	Males	Females	ICRP mean	Actual ICRP tissue weighting factor
Oesophagus	0.026	0.021	0.023	0.04
Stomach	0.12	0.117	0.118	0.12
Colon	0.138	0.044	0.083	0.12
Liver	0.075	0.026	0.046	0.04
Lung	0.124	0.182	0.157	0.12
Bone	0.011	0.008	0.009	0.01 ^{a)}
Skin	0.008	0.006	0.007	0.01
Breast	-	0.240	0.139	0.12
Ovary	-	0.030	0.017	-
Bladder	0.036	0.024	0.029	0.04
Thyroid	0.010	0.031	0.022	0.04
Bone-marrow	0.144	0.080	0.107	0.12
Remainder Tissues (14)	0.256	0.155	0.198	0.12
Gonads	0.053 ^{b)}	0.038 ^{b)}	0.044 ^{b)}	0.08 ^{c)}
Brain ^{d)}	-	-	-	0.01
Salivary glands ^{d)}	-	-	-	0.01
Total	1.00	1.00	1.00	1.00

^{a)} Bone surface ^{b)} Genetic damage ^{c)} Genetic damage and cancer ^{d)} Previously listed in ICRP 60 under “Remainder Tissues” category

2 Individual Studies

2.1 Report on mortality of atomic bomb survivors: solid cancers (Preston et al. 2003)

In a report on mortality of atomic bomb survivors published by Preston et al., which includes follow-up for the period from 1950 to 1997 [Pre 03], possible variations in the radiation-associated excess risks with sex are discussed. For example, Table A6 shows that based on a model that allows the ERR to vary with age at exposure while also varying with attained age,

the ERR values are clearly greater for women than for men. However, there is little sex difference in the EAR values for absolute risk.

*Table A6: ERR and EAR values for all solid cancers for age at exposure = 30 years and attained age = 70 years; CI = confidence interval; produced by Preston et al. from atomic bomb survivor data [Pre 03]. * presumably a typographical error in [Pre 03].*

	ERR/Sv (90% CI)		EAR per Sv and 10 ⁴ Person-Years (90% CI)	
	Males	Females	Males	Females
Mortality	0.35 (0.24; 0.46)	0.59 (0.45; 0.74)	29 (30*; 39)	30 (24; 37)

According to Preston et al., this has been known for some time (“... it has long been reported that the estimated EAR/Sv depends little on sex despite the significant sex effect on the ERR ...”). They attribute this apparent contradiction to the fact that the background (spontaneous) rates of mortality from solid cancers in Japan are far lower for women than for men, which primarily affects the excess relative risk (“Clearly the relative risks are greater for women than for men ($p < 0.003$), but this largely serves to offset a reciprocal ratio in background rates, and there is little sex difference in the EAR”). Table A7 shows that this interpretation is valid for selected organs other than the liver.

In a further study published by Preston et al. soon afterwards, which mainly investigates the effect of the new dosimetry system, DS02, on atomic bomb survivor risk estimates and has a extended follow-up from 1950 to 2000, similar results are reported [Pre 04]: for the ERR, an F/M ratio of 1.9 (90% CI: 1.4; 2.7) was reported, which is significantly different from 1, whereas for EAR an F/M ratio of 1.1 (90% CI: 0.8; 1.6) was reported, which is not significantly different from 1.

Table A7: *ERR values for all solid cancers (cf. Table A6) and corresponding values for specific organs for age of exposure 30 years and attained age 70. The p values in column 4 apply to the null hypothesis of an F/M value of 1 (no sex-specific differences). For the purpose of comparison, the M/F ratio for the spontaneous rates is given in column 5; where similar values are given in columns 4 and 5, it can be assumed that the increased ERR values for females are attributable to the lower spontaneous incidences and that with the EAR, only minor differences between the sexes exist [Pre 03].*

Site of solid cancers	ERR/Sv			Background (spontaneous) rate M/F
	Males (M)	Females (F)	F/M	
All solid cancers	0.347	0.588	1.7 (p = 0.01)	1.8
Stomach	0.196	0.636	3.2 (p = 0.01)	2.3
Lung	0.472	1.05	2.2 (p = 0.02)	2.4
Colon	0.370	0.414	1.1 (p > 0.5)	1.3
Liver	0.402	0.400	1.0 (p > 0.5)	2.1
Other organs	0.351	0.326	0.93 (p > 0.5)	1.3

2.2 Report on solid cancer incidence in atomic bomb survivors (Preston et al. 2007)

The recently published report on solid cancer incidence in atomic bomb survivors covers an observation period from 1958 to 1998. This report considers excess relative and absolute risks for all solid cancers as a group and for individual organs separately where there is a sufficient number of radiation-associated cases. Alongside other parameters, sex dependence of risks is also analysed [Pre 07].

2.2.1 All solid cancers

Table A8 shows the findings of this analysis for all solid cancers as a group and for non-sex-specific cancers excluding cancer of the breast, prostate and other sex organs.

Table A8: *Excess Relative Risk and Excess Absolute Risk for all cancers, age at exposure 30 years and attained age 70; the uncertainties indicated correspond to 90% confidence intervals; all cancers with the exception of breast, prostate and other sex organs are included under the heading “non-sex-specific solid cancers” [Pre 07].*

All cancers	Males (M)	Females (F)	Mean	F/M
All solid cancers				
ERR / Gy	0.35 (0.28; 0.43)	0.58 (0.43; 0.69)	0.47 (0.40; 0.54)	1.6 (1.31; 2.09)
EAR / 10 ⁴ PY Gy	43 (33; 55)	60 (51; 69)	52 (43; 60)	1.4 (1.10; 1.79)
Non-sex-specific solid cancers				
ERR / Gy	0.34 (0.27; 0.42)	0.61 (0.50; 0.43)	0.48 (0.39; 0.56)	1.8 (1.31; 2.09)
EAR / 10 ⁴ PY Gy	48 (36; 61)	44 (37; 52)	46 (38; 55)	0.9 (0.72; 1.20)

For all solid cancers as a group, females were found to have somewhat higher ERR values than males, as is also the case with the mortality data (see Table A6). In contrast to the mortality data, however, women had somewhat higher excess absolute rates (EAR) than men here, albeit to a slightly lesser extent than the ERR values. Preston et al. note: “While apparent gender effects on the ERR may reflect differences in background cancer rates and/or possible gender differences in radiosensitivity, gender differences in the EAR which is not influenced by background rates, might be thought to be a more direct indication of gender differences in radiosensitivity”. If sex-specific organs (female breast, prostate, other sex organs) are excluded from the analysis, the difference in the ERR values for males and females remains, presumably due to the differences in the spontaneous rates (“These results suggest that the greater ERR for women than men largely reflects gender differences in the background rates”). The EAR values for men and women no longer differ significantly, however (F/M = 0.9), a finding which accords with that obtained from the mortality data.

2.2.2 Stomach

Stomach cancer is the most common form of cancer in Japan. The spontaneous incidence rates of stomach cancer in Japan are around 10 times higher than the global average. In the Life Span Study (LSS), stomach cancer cases (4 730 cases in total) account for 27% of all cancer cases. Using a linear dose-response relationship, the analyses carried out by Preston et al. reveal 151 radiation-induced cases of stomach cancer, with around one third of the survivor cases having received stomach doses greater than 1 Gy. As regards the spontaneous rates of stomach cancer, both the global data and the data from the LSS show a far higher value for males than for females (see Table A9).

Table A9 illustrates the pattern already discussed above: the ERR value for females is higher by a factor of around 2 than the value for males. One explanation may be that with the spontaneous incidence, the corresponding value for females is smaller by a factor of around 2 than for males. Accordingly, there is no significant difference in the EAR values for males and females.

Table A9: Excess Relative Risk and Excess Absolute Risk for stomach cancer, age at exposure 30 years and attained age 70; the uncertainties indicated correspond to 90% confidence intervals [Pre 07].

Stomach	Males (M)	Females (F)	Mean	F/M
ERR / Gy	0.21 (0.10; 0.34)	0.47 (0.29; 0.68)	0.34 (0.22; 0.47)	2.3 (1.2; 4.5)
EAR / 10 ⁴ PY Gy	9.4 (4.4; 16)	9.7 (6.4; 14)	9.5 (6.1; 14)	1.0 (0.5; 2.1)
Spontaneous rate (Japan)	-	-	-	0.39
Spontaneous rate (LSS)	-	-	-	0.29 – 0.40

2.2.3 Colon

In Japan, the spontaneous incidence of colon cancer has increased considerably over the last 25 years, with a stronger increase among men than among women. In terms of the global mean, the age-standardised rates for men are around twice as high as for women. Colon cancer is the third most common type of cancer in Japan after stomach and lung cancer. The spontaneous incidence rates obtained from analysis of the LSS cohort reflect these trends (increase over time; values for men roughly twice as high as for women for birth year 1900). The use of a linear dose-response relationship yields 78 radiation-induced cases of colon cancer out of a total of 1 156 cases.

Table A10: Excess Relative Risk and Excess Absolute Risk for colon cancer, age at exposure 30 years and attained age 70; the uncertainties indicated correspond to 90% confidence intervals; [Pre 07].

Colon	Males (M)	Females (F)	Mean	F/M
ERR / Gy	0.73 (0.38; 1.17)	0.34 (0.13; 0.63)	0.54 (0.30; 0.81)	0.5 (0.17; 1.01)
EAR / 10 ⁴ PY Gy	13.0 (4.4; 16)	3.0 (6.4; 14)	8.0 (4.4; 12)	0.2 (0.06; 0.52)
Spontaneous rate (Japan)	-	-	-	0.59
Spontaneous rate (LSS)	-	-	-	0.5 (year of birth 1900)

For colon cancer, the ERR value for females is only around half the value for males (Table A10). The EAR value for females is lower by a factor of 5 than that of males. This is probably due to the fact that the spontaneous incidence for females is also only half that of males. **Colon cancer is thus the only type of cancer for which the ERR value is considerably lower for females than for males.**

2.2.4 Liver

Japan has one of the highest spontaneous rates of liver cancer in the world, largely as a result of infection with either the hepatitis B or C virus. For Japanese men, the age-standardised

spontaneous rate is around three times higher than for women. This is reflected in the LSS cohort as well, where men have spontaneous rates three to four times higher than those of women. The use of a linear dose-response relationship yields 54 radiation-induced cases out of a total of 1 494 cases.

Table A11: Excess Relative Risk and Excess Absolute Risk for liver cancer, age at exposure 30 years and attained age 70; the uncertainties indicated correspond to 90% confidence intervals [Pre 07].

Liver	Males (M)	Females (F)	Mean	F/M
ERR / Gy	0.32 (0.12; 0.60)	0.28 (0.05; 0.63)	0.30 (0.11; 0.55)	0.9 (0.16; 2.4)
EAR / 10 ⁴ PY Gy	6.4 (0.2; 12)	2.1 (0.6; 4.3)	4.3 (0.2; 7.2)	0.3 (0.10; 0.32)
Spontaneous rate (Japan)	-	-	-	0.32
Spontaneous rate (LSS)	-	-	-	0.25 – 0.33

Table A11 shows that for liver cancer, the ERR values for males and females are similar. The far lower EAR value for females than for males largely reflects the corresponding differences in the spontaneous rates.

2.2.5 Lung

Lung cancer is the most frequent type of cancer worldwide. In Japan, it is the second most common type of cancer among men and the fifth most common type among women. The rise in the incidence rates observed over time in Japan is mainly due to changes in smoking behaviour. The use of a linear dose-response relationship yields 117 radiation-induced cases out of a total of 1 759 cases.

Table A12: Excess Relative Risk and Excess Absolute Risk for lung cancer, age at exposure 30 years and attained age 70; the uncertainties indicated correspond to 90% confidence intervals [Pre 07].

Lung	Males (M)	Females (F)	Mean	F/M
ERR / Gy	0.28 (0.12; 0.49)	1.33 (0.91; 1.8)	0.81 (0.56; 1.1)	4.8 (2.6; 12)
EAR / 10 ⁴ PY Gy	6.0 (2.3; 11)	9.1 (6.4; 12)	7.5 (5.1; 10)	1.5 (0.82; 3.9)
Spontaneous rate (Japan)	-	-	-	0.31
Spontaneous rate (LSS)	-	-	-	0.26 – 0.27*

* according to year of birth

Table A12 shows that the ERR value for lung cancer is far higher for females than for males, whereas the corresponding EAR values are similar. The much lower EAR value for females than for males generally reflects the corresponding differences in the spontaneous rates. **The**

lung is the organ with the largest F/M ratio for ERR values.

However, it is important to point out at this juncture that in the study by Preston et al., no adjustment was made for the smoking behaviour of survivors. In an earlier study, however, such an adjustment was made for survivors for whom information about personal smoking behaviour was available [Pie 03]. **The authors report an original F/M ratio of 5.8 for the ERR value which, after adjustment for smoking behaviour, is reduced to 1.6.**

2.2.6 Bladder

In Japan, bladder cancer accounts for less than 3% of all types of cancer. In 1999, the age-standardised spontaneous rate in Japan was 4.4 times higher for males than for females. In the LSS, using a linear dose-response relationship, 35 cases of bladder cancer were attributed to ionising radiation out of a total of 469 observed cases. With a mean ERR value of 1.23/Gy, the bladder is the organ with the highest ERR.

Table A13: Excess Relative Risk and Excess Absolute Risk for bladder cancer, age at exposure 30 years and attained age 70; the uncertainties indicated correspond to 90% confidence intervals [Pre 07].

Bladder	Males (M)	Females (F)	Mean	F/M
ERR / Gy	0.61 (0.11; 1.2)	1.9 (0.79; 3.4)	1.23 (0.59; 2.1)	3.1 (0.17; 10)
EAR / 10 ⁴ PY Gy	3.8 (0.2; 8.0)	2.6 (1.1; 4.4)	3.2 (1.1; 5.4)	0.7 (0.21; 10)
Spontaneous rate (Japan)	-	-	-	0.23

Table A13 shows that for bladder cancer, the ERR value for females is around three times higher than for males, with the associated uncertainty being high due to the low number of radiation-induced cases. This ratio is exceeded only by lung cancer in the LSS cohort, where the corresponding value is 4.8 (Table A12). Much of the difference in ERR between males and females can be explained by the fact that males have a much higher spontaneous risk of bladder cancer. Accordingly, the sex difference in the EAR values is much lower (F/M ratio: 0.7; Table A13).

2.2.7 Thyroid

Worldwide, thyroid cancer is far more common in women than in men. This is apparent from the relevant data for Japan for 1999, which yield an F/M ratio of 4.1. **The thyroid is therefore an exception – for all other organs investigated, the corresponding spontaneous incidence rates of cancer are higher for males than females.** In the LSS cohort, for the period 1958-1998, 471 cases of thyroid cancer were observed: 381 in women and 90 in men. Using a linear dose-response relationship, 63 cases of thyroid cancer were attributed to ionising radiation.

Table A14: Excess Relative Risk and Excess Absolute Risk for thyroid cancer, age at exposure 30 years and attained age 70; the uncertainties indicated correspond to 90% confidence intervals [Pre 07].

Thyroid	Males (M)	Females (F)	Mean	F/M
ERR / Gy	0.49 (0.15; 1.15)	0.65 (0.27; 1.25)	0.57 (0.24; 1.1)	1.3 (0.56; 3.9)
EAR / 10 ⁴ PY Gy	0.5 (0.3; 1.5)	1.9 (1.3; 4.2)	1.2 (0.48; 2.2)	3.6 (1.78; 9.5)
Spontaneous rate (Japan)	-	-	-	4.1

Table A14 shows that for thyroid cancer, the ERR for females is around 30% higher than for males. Due to the clear differences between males and females in the spontaneous induction of thyroid cancer (see above), the EAR for women is higher than for men by a factor of 3.6. **This is the highest F/M ratio observed for EAR in the study.**

2.2.8 Other organs

For the other organs investigated (oral cavity and salivary glands, oesophagus, rectum, gall bladder, pancreas, brain), the low number of radiation-induced cancer cases made it impossible to determine any sex dependency of relative and absolute risk.

2.3 Thyroid cancer after exposure to external radiation: a pooled analysis (Ron et al. 1995)

In a detailed study, Ron et al. investigated the occurrence of radiation-induced thyroid cancer based on a pooled analysis of seven studies. These studies include five cohort studies and two case-control studies (see Table A15, which describes some of the features of these studies).

Table A15: Cohorts used in the pooled analysis [Ron 95]; LSS: Life Span Study; MRH: Michael Reese Hospital; CHMC: Children's Hospital Medical Center.

Cohort Studies						
Study/Place	Number of exposed	Number of non-exposed	Percentage female (%)	Years of exposure	Mean follow-up years	Mean age at exposure
LSS (Japan)	41 234	38 738	60	1945	24	27
Thymus (USA)	2 475	4 991	42	1926-1957	35	0.1
Tinea Capitis (Israel)	10 834	16 226	51	1948-1960	30	7
Tonsils (MRH, USA)	2 634	0	40	1939-1962	33	4
Tonsils (CHMC, USA)	1 192	1 063	40	1938-1969	29	6
Case-Control Studies						
Study/Place	Cases	Controls	Percentage female (%)	Years of exposure	Mean follow-up years	Mean age at exposure
Cervical cancer (international)	43	81	100	1926-1971	-	53
Childhood cancer (international)	22	82	45	1936-1979	-	7

Table A15 shows that the studies chosen by Ron et al. for the pooled analysis are extremely heterogeneous; in particular, there is considerable variation in the exposure conditions among the studies (as regards dose range, field size, energy of radiation, fractionation etc.). It is therefore not surprising that there is sometimes considerable variation in the risk estimates derived from these studies (Table A16).

Table A16: Excess Relative Risk (ERR) and Excess Absolute Risk (EAR) values for thyroid cancer derived by Ron et al. from the data from various studies; CI: confidence interval [Ron 95].

Age at Exposure < 15 years old	ERR/Gy (95% CI)	EAR/10⁴ PY Gy (95% CI)
Thymus	9.1 (3.6; 28.8)	2.6 (1.7; 3.6)
LSS	4.7 (1.7; 10.9)	2.7 (1.2; 4.6)
Tinea Capitis	32.5 (14.0; 57.1)	7.6 (2.7; 13.0)
Tonsils (MRH)	2.5 (0.6; 26.0)	3.0 (0.5; 17.1)
Childhood cancer	1.1 (0.4; 29.4)	-
Age at Exposure ≥ 15 years old	ERR/Gy (95% CI)	EAR/10⁴ PY Gy (95% CI)
Cervical cancer	34.9 (-2.2; ∞)	-
LSS	0.4 (-0.1; 1.2)	0.4 (-0.1; 1.4)

Accordingly, the differences in the risk coefficients for males and females obtained from these studies also vary widely (Table A17). **Ron et al. conclude that the ERR is nearly twice as high for females than males when the findings of all studies are analysed, but that this difference is not significant.** (“Results on gender differences in sensitivity for developing radiation-induced thyroid cancer have been inconsistent. Higher ERRs/Gy for women than men have been reported from some studies, but the opposite effect has also been noted. In the joint analysis, the ERR was higher for females than for males, but the difference was not statistically significant”). Because of the greater risk of spontaneous thyroid cancer in females, the EAR value is also larger for females than males. This finding is consistent with that of [Pre 07] (Section 2.2.7).

Table A17: Values stated in [Ron 95] for the M/F ratio (based on the ERR values in Table A16).

Study	M/F (ERR)
Thymus	∞
LSS (< 15 years old)	2.9
LSS ($\geq$ 15 years old)	0.2
Tinea Capitis	0.2
Tonsils (MRH)	1.8
Childhood cancer	0.6
Total	0.5

2.4 Analysis of thyroid cancer incidence risk after the Chernobyl accident (Jacob et al. 2006, SSK 2006)

In a detailed study, Jacob et al. investigated the thyroid cancer incidence risk among children and adolescents in the birth-year cohort 1968–1985 from areas of Ukraine and Belarus. Data were analysed for a total of 1 034 settlements in Ukraine and Belarus, in which more than 10 measurements of the ^{131}I content in human thyroids had been performed in May/June 1986 after the Chernobyl accident (Ukraine: 608 settlements with 75 313 measurements; Belarus: 426 settlements with 90 699 measurements). Based on the measurements, individual thyroid doses were assessed. Thyroid doses of more than 0.3 Gy were detected in around 100 000 persons studied. In some settlements, individual thyroid doses varied by a factor of up to 50. From the individual dose values, sex- and age-specific thyroid doses in the individual settlements were estimated. In more than 30 settlements, thyroid doses of more than 3 Gy were estimated. At the time of the accident, 997 000 children and adolescents were living in the Ukrainian settlements studied, while 623 000 children and adolescents were living in the settlements in Belarus.

The surgical operations to remove thyroid cancers in children and adolescents in the birth years 1968–1985 during the period 1990–2001 in both countries were taken as the basis for determining the incidence of thyroid cancer. For the Ukrainian settlements studied, 512 cases were reported (378 in females and 134 in males). Similarly, 577 cases were identified for the settlements studied in Belarus (368 in females and 209 in males). For the spontaneous induction of thyroid cancer, the mean obtained for both countries was 14 cases per 10^6 person-years for 20-year-olds in the mid-1990s. In the age range studied (5–33 years), an increase in the spontaneous thyroid cancer incidence with age was observed which was proportional to $(\text{age})^{3.8}$. For females, a higher spontaneous rate was observed, by a factor of 5.9, than for males. A similar observation was also made in the LSS, for example, where an F/M ratio of 4.1 is reported for the spontaneous rates (see Section 2.2.7).

For the linear increase in the dose-response curve, an overall ERR/Gy value (increase of linear component of the dose-response curve) of 18.9 (95% CI: 11.1; 26.7) was obtained. **For the F/M ratio, the authors give a value of $1/3.8 = 0.26$.** As regards age dependence, the ERR is reduced by a factor of 6.5 when age is doubled. An F/M value of 0.26 for ERR

conflicts with the findings of the pooled analysis by Ron et al., which reports a (non-significant) F/M value of 2 (Section 2.3) and the findings of the LSS, which gives an F/M value of 1.3 (0.56; 3.9) (Section 2.2.7, Table A14). If, however, the ERR data for the incidence of thyroid cancer in the LSS are analysed separately for survivors who were under 15 years of age at the time of the atomic bomb explosion, an F/M value of 0.34 is obtained (see Table A17), which is consistent with the findings of Jacob et al.

For excess absolute risk, a best estimate (increase in the linear component of the dose-effect curve) of 2.66 cases per 10^4 person-years is obtained (95% CI: 2.19; 3.13). **This yields an F/M value of 1.5.** The EAR is therefore greater for females than for males, although the ERR is much lower for females. This difference can be explained, again, by the very different spontaneous rates (F/M = 5.9; see above). The fact that the spontaneous rate increases faster than the excess relative risk with attained age means that the excess absolute risk rises with attained age. The EAR for females, which is higher by a factor of 1.5, compares with an F/M value for EAR of 3.6 (1.78; 9.5) stated in the LSS for atomic bomb survivors (all ages at exposure) (see Section 2.2.7, Table A14).

2.5 Studies of thyroid cancer incidence among children and adolescents in the Bryansk oblast after the Chernobyl accident (Ivanov et al. 2006)

In a study of thyroid cancer incidence, Ivanov et al. [Iva 06] investigated persons who were exposed as children and adolescents at an age between 0 and 17 years and who were living in the Bryansk oblast, the worst contaminated area of Russia after the Chernobyl accident. The follow-up period was 1991–2001 and the study covered a total of 373 827 persons. Thyroid doses were determined based on the official methodology approved in Russia, in which place of residence and age at exposure are the key parameters for dose determination. A total of 199 thyroid cancer cases (144 in males and 55 in females) were diagnosed during the considered time period. The mean thyroid dose for the population studied was 0.08 Gy; for male cases, it was 0.22 Gy and for female cases 0.09 Gy.

A significant increase in thyroid cancer incidence rates was observed in the cohort studied compared with the spontaneous rate derived for Russia; a standardised incidence ratio (SIR) of 4.9 (95% CI: 4.2; 5.8) was obtained for girls and 8.8 (95% CI: 6.6; 11.5) for boys. According to the authors of the study, reasons for the increased SIR values could lie in local variations in spontaneous rates and reporting rates, and differences in radiation exposure.

With internal control, for girls whose age at exposure was 0–4 years, the excess relative risk per 1 Gy (ERR/Gy) was 45.3 (95% CI: 5.2; 9 953) and for girls whose age at exposure was 5–9 years, the ERR/Gy was 10.1 (95% CI: -0.1; 84.7). For boys whose age at exposure was 0–9, the corresponding ERR/Gy was higher, i. e. 68.8 (95% CI: 10.0; 4 520).

The authors conclude by emphasising that the findings obtained show very large confidence intervals. Furthermore, as the dose estimates contain great uncertainties and the influence of screening effects also cannot be ruled out, they recommend viewing the findings as provisional. In another study carried out two years earlier in Bryansk, slightly higher risks were reported for girls than for boys, but no evidence of a statistically significant difference was found [Dav 04].

Therefore no definitive conclusions regarding differences in the incidence of thyroid cancer in males and females can be drawn from these studies.

2.6 Lung cancer after treatment for Hodgkin's disease, and comparison with the findings of other studies (Gilbert et al. 2003)

Gilbert et al. describe the results of an international study on the induction of lung cancer after treatment for Hodgkin's disease [Gil 03]. Unique features of the study include the large number of persons studied (227 Hodgkin's patients with lung cancer and 455 matched controls), dose determinations to the specific location in the lung where each cancer developed, and the availability of detailed quantitative data on both chemotherapy and tobacco use. For the latter, the following categories were introduced: "never smoker", "current cigarette smoker", "former cigarette smoker", "cigar and pipe smoker only", "no information". The number of cigarettes smoked was stated in packets per day. The number of chemotherapy cycles administered was also taken into account in the analysis. It was assumed that the interaction of radiation and chemotherapy was additive, but that smoking had a multiplicative effect.

In relation to ionising radiation, Gilbert et al. found that: "The ERR/Gy for males was about four times that for females, although the difference was not statistically significant" (Table A18). Gilbert et al. compare this finding with those obtained in other studies (see Table A18) and draw attention to the sometimes substantial variations in ERR values. Gilbert et al. explain this partly in terms of the different spontaneous rates (e. g. the low spontaneous rates in Japan), different dosages, fractionation, dose rates, and the possible failure to take adequate account of the effects of smoking and chemotherapy. **The findings obtained by [Gil 03] thus conflict with those which can be derived from the LSS (Section 2.2.5), even after adjustment to take account of smoking.**

Table A18: *ERR values (ERR/Gy) for lung cancer, shown separately for males and females (taking account of chemotherapy cycles and smoking), and comparison with findings of other studies [Gil 03].*

Males (M)	Females (F)	Mean	F/M	Ref.
0.18 (0.063; 0.52)	0.044 (-0.009; 0.53)	0.15 (0.057; 0.39)	0.24	Hodgkin [Gil 03]
0.34 (0.06; 0.69)	0.89 (0.41; 1.51)	0.53 (0.28; 0.84)	2.6	LSS Mortality [Pie 96]
0.47 (0.14; 0.90)	1.97 (1.21; 2.95)	0.95 (0.60; 1.36)	4.2	LSS Incidence [Tho 94]
0.02 (-0.01; 0.11)	-0.06 (-0.10; 0.07)	0.00 (-0.06; 0.04)	-	[How 95]

2.7 Lung cancer in Mayak workers (Gilbert et al. 2004)

The cohort of Mayak workers enables studies to be carried out on the health effects of exposure to plutonium as well as the effects of protracted external dose. In a study published by Gilbert et al. [Gil 04], risks were evaluated in 21 790 Mayak workers (of whom 16 458 were male) who had been hired at one of the Mayak production plants (nuclear reactors, radiochemical plants, plutonium production) between 1948 and 1972. During the study period from 1955 to the end of 2000, 8 493 deaths were recorded in this cohort, with the cause of death being established in 97% of cases. They included 594 lung cancer deaths of men and 61 lung cancer deaths of women.

Tab. A19: *Excess Relative Risk and Excess Absolute Risk for lung cancer mortality at attained age 60 with 95% confidence intervals [Gil 04].*

Lung Cancer	Males (M)	Females (F)	F/M
Internal Lung Dose			
ERR / Gy	4.4 (3.6; 6.7)	19 (9.5; 39)	4.0 (1.9; 8.8)
EAR / 10 ⁴ PY Gy	115 (81; 156)	49 (29; 78)	0.43 (0.24; 0.72)
External Dose			
ERR / Gy	0.17 (0.052; 0.32)	0.32 (<0; 1.3)	1.9 (<0; 11)
EAR / 10 ⁴ PY Gy	2.4 (0.56; 4.4)	0.43 (<0; 1.6)	0.18 (<0; 1.1)

The external exposure of the cohort members was determined on the basis of film dosimeter monitoring, routinely carried out since 1948. These data were available for 80% of subjects. The mean external dose of the monitored persons in the cohort was 0.8 Gy. Systematic monitoring of internal doses received from exposure to plutonium took place from the late 1960s. Monitoring of excretions (urine) was carried out for this purpose, and dose values were calculated for the lung based on the most up-to-date models of the biokinetics of plutonium in humans. In this way, lung doses could be calculated for around 40% of the 14 715 persons employed in the radiochemical plants and in plutonium production. Mean lung doses were determined at 0.21 Gy for male and 0.38 Gy for female employees. For subjects for whom no experimental data were available, the lung dose was estimated, inter alia based on their place and type of work.

Table A19 shows the ERR and EAR values obtained for subjects monitored for plutonium exposure and for subjects who had not worked in the radiochemical plants or in plutonium production (external exposure only).

According to Gilbert et al., for internal dose, the ERR per gray for females was significantly, i. e. about four times, higher than that for males. The corresponding factor of 1.9 for external dose is not significant, however. The values for absolute risk are lower for females than for males, however, and there is a significant factor of 0.43 for internal exposure and a non-significant factor of 0.18 for external exposure. It is pointed out that the different values for internal and external exposure shown in Table 19 can be explained by relative biological effectiveness of internal exposure (alpha radiation) of 33 (CI: 14; 98), compared with external exposure (gamma radiation). Incidentally, similar ERR/Gy values are obtained for internal exposure if only those workers are considered who had been monitored for plutonium

exposure (males: 4.2 (CI: 2.8; 6.0); females: 22 (CI: 9.5; 56); cf. Table A19). If smoking behaviour is also considered (using the categories “ever smoker” and “never smoker”, 74% of males but only 3.4% of females were identified as smokers), no significant differences compared with Table A19 arose for the ERR/Gy values (males: 3.9 (CI: 2.6; 5.8); females: 19 (CI: 7.7; 51); cf. Table A19). As regards the M/F ratio with regard to the spontaneous rate of lung cancer, taking account of smoking behaviour resulted in a change in the ratio from 11 to 2.

Gilbert et al. compare their findings with what they would expect for a corresponding age structure in the LSS (age at exposure: 16-60 years; attained age: 60 years) and, for the ERR model, derive a similar value of 3.6 (95% CI: 1.2; 11) for the F/M ratio (cf. Table A19: 4.0 (95% CI: 1.9; 8.8); Table A18: 2.6 and 4.2, respectively; Table A12: 4.8 (90% CI: 2.6; 12)).

Summing up, Gilbert et al. [Gil 04] found that the ERR per gray for females was about four times higher than that for males, whereas the EAR for females was less than half that for males. Gilbert et al. attribute this to the very different spontaneous rates for females and males, which can largely be explained by different smoking behaviour: “The different patterns for ERR and EAR models reflect the very strong difference in baseline risks for the two sexes with baseline risks for males estimated to be about 11 times those for females; smoking differences explain much of this difference.”

2.8 Site-specific cancer incidence and mortality after cerebral angiography with radioactive thorotrast (Travis et al. 2003)

Travis et al. investigated the cancer risk from long-term internal exposure to alpha-particle-emitting radionuclides [Tra 03]. To that end, they studied a cohort of 3 042 patients injected during cerebral angiography with either thorotrast (Thorium-232) or a nonradioactive agent. The cohort consisted of 1 953 patients from Denmark, 431 from Sweden and 658 from the USA. Although the main focus of this study was to undertake a detailed analysis of the risks as a function of incidence or mortality for a specific type of cancer, the authors also investigated sex-specific differences for all cancers as a group.

In the Danish/Swedish data for the incidence of all types of cancer, the authors found a relative risk (RR) of 3.6 (95% CI: 2.8; 4.8) for males and 3.3 (95% CI: 2.6; 4.2) for females and concluded that they could find no evidence of sex-specific differences: (“... risks were similar for males and females”). They mention, however, that they find a non-significant increased risk of non-Hodgkin’s lymphoma (RR=2.5) and cancer of the rectum (RR=3.2) for males, and an increased risk of basal cell and squamous cell carcinoma (non-melanoma skin cancer) (RR=2.8), multiple myeloma (RR=3.7), colon cancer (RR=1.9), lung cancer (RR=1.6) and thyroid cancer (RR=1.8) for females. However, they conclude that: “... none of the differences between men and women were statistically significant”.

However, a clear difference emerged when, in the Danish/Swedish data, female (cervix, uterus, ovaries) and male (prostate, testicles) sex organs were analysed separately. This produced an RR for women of 1.1 (95% CI: 0.6; 2.1) compared with a value of 4.7 (95% CI: 1.8; 15.0) for men.

In the American data, for mortality from all types of cancer, the authors report a relative risk (RR) of 3.9 (95% CI: 2.0; 8.2) for males and 4.1 (95% CI: 2.1; 8.7) for females.

2.9 Study on mortality from leukaemia among atomic bomb survivors (Preston et al. 2004)

The most recent study providing data on mortality from leukaemia among atomic bomb survivors was published by Preston et al. [Pre 04]. These authors report on the follow-up of 86 955 survivors from 1950-2000, with a total of 296 deaths from leukaemia, including six cases attributable to chronic lymphocytic leukaemia (CLL). The authors emphasise that the extent to which the findings of their analyses are dependent on whether or not these cases are considered is limited. Using a linear-quadratic dose model, Preston et al. estimate that 93 of the observed 296 cases must be attributed to ionising radiation. **Based on the excess absolute risk (EAR), it is shown that females have a significantly lower risk than men;** the corresponding F/M ratio is given as 0.6 (90% CI: 0.4; 0.8).

In [UNSC 08, Annex A, Table 44], it is shown that using the earlier data described in [Pre 94] for absolute leukaemia risk (incidence) for the atomic bomb survivors, a similar F/M ratio is obtained. **However, these data also show that for excess relative risk, there is no significant difference between females and males.**

2.10 Study on leukaemia among workers at four US nuclear weapons facilities and a nuclear naval shipyard (Schubauer-Berigan et al. 2007)

In this study, a total of 94 517 subjects working at Hanford (36 384 persons), Oak Ridge National Laboratory (ORNL: 19 815 persons), the Savannah River Site (SRS: 12 886 persons), Los Alamos National Laboratory (LANL: 12 179 persons; Zia company: 5 686 persons) and the Portsmouth Naval Shipyard (9 662 persons) were investigated (Sch 07). Workers were eligible if they worked at the site for at least 30 days and were monitored for exposure to external ionising radiation. In these cohorts, during follow-up periods of 25 – 34 years, a total of 257 deaths from leukaemia (including 206 non-CLL cases) were observed. In addition, 823 age-matched controls were selected.

The bone-marrow doses given in the study include radiation from external sources (gamma and X-rays) and internal sources (tritium, plutonium and neutrons). If possible, the doses were corrected to take account of specific aspects relating to the dosimeters used (responsiveness, calibration). The mean cumulative bone-marrow dose amounted to around 30 mSv for the cases. Furthermore, numerical values were assigned to qualitative exposure level estimates (High, Medium, Low, Very Low, and Unexposed) to allow calculation of exposure scores for both benzene and carbon tetrachloride. Finally, workers were classified (as “never-smokers”, “ex-smokers”, “current smokers”) to take account of cigarette consumption. Where insufficient smoking history information was available, smoking status was imputed based on the socioeconomic status as a surrogate method to identify smoking behaviour.

Omitting cases in which the type of leukaemia could not be established, and taking account of solvent exposure, for example, a (non-significant) ERR was 2.60% (95% CI: -1.0; 10.3) Sv⁻¹ was obtained. The authors observe far fewer cases of leukaemia occurring in females than males (“Women had less than half the age-adjusted non-CLL leukemia risk of men”), and only 15 of the non-CLL cases occurred in women. Detailed analyses in which the role of potential confounders (smoking status, exposure to benzene and carbon tetrachloride, socioeconomic status, hire year, etc.) were studied show no significant influence of these parameters on the findings of the study. **Schubauer-Berigan et al. state the F/M ratio as having a value of 0.42 (95% CI: 0.23; 0.71), which is consistent with the EAR finding described in Section 2.9 and obtained from studies of the atomic bomb survivors. However, the analyses carried out by [UNSC 08] and also mentioned in Section 2.9 show no sex-specific difference for the ERR for leukaemia.**

2.11 Other studies on radiation-induced leukaemia

The literature includes various other epidemiological studies which investigate possible leukaemia induction after exposure to ionising radiation. Some of these studies are listed here, although they do not provide any statistically significant information about sex-specific differences.

For example, the study by Krestinina et al. carried out among people living on the Techa River found a excess relative risk per gray for leukaemia, excluding chronic lymphocytic leukaemia, of 6.5 (CI 95% 1.8; 24) [Kre 05]. **In contrast to the findings described in Sections 2.9 and 2.10, they found a higher risk for females than for males, but conclude that the difference is not statistically significant:** “While the ERR for women is estimated to be about 70% greater than that for men, the difference is not statistically significant.”

Shilnikova et al. also report a significantly increased risk of leukaemia (excluding CLL) [Shi 03]. These authors studied 21 557 Mayak workers (16 291 males and 5 266 females) and found a total of 77 leukaemia deaths. Excluding 11 CLL cases, 55 leukaemia deaths occurred in males and 11 in females. Assuming a 2-year lag and a linear dose-response model, the estimated ERR per gray was 0.99 (90% CI 0.45; 2.12) and was significant. More detailed analysis of the data showed a higher risk of leukaemia for females (in contrast to the F/M ratio described in Sections 2.9 and 2.10). **However, this finding is not statistically significant, and the authors conclude that:** “There was no evidence of a significant sex difference in the ERR ($P > 0.5$, female:male ratio 1.7 (90% CI 0.23; 11.2) ...). The result was therefore similar to that obtained by Krestinina et al. in their study carried out among people living on the Techa River.

The study carried out by Muirhead et al. among a cohort of 174 541 workers registered in the UK’s National Registry for Radiation Workers revealed that leukaemia excluding CLL increased to a statistically significant extent with increasing radiation dose [Mui 09]. These authors report values for excess relative risk per dose of 1.712 (90% CI: 0.06; 4.29) Sv^{-1} (mortality) and 1.782 (90% CI: 0.17; 4.36) Sv^{-1} (incidence). However, the study does not include a sex-specific analysis of data in relation to ERR/ Sv values.

In the international (15-country) study of cancer risk among workers in the nuclear industry, out of the main study population of 407 391, a total of 24 158 people were known to have died during the study period, including 196 from leukaemia. The excess relative risk for leukaemia was significant (ERR/Sv = 1.93 (95% CI: < 0; 8.47)). However, this mainly reflects risks to men, as there were few exposed women in the cohort [Car 05].

Boice et al. studied leukaemia mortality in 5 801 radiation workers engaged in nuclear technology development at facilities in the USA [Boi 06]. They found that radiation exposure has not caused a detectable increase in cancer deaths in this population. Accordingly, this study does not provide any information about possible sex-specific differences.

Studies carried out among workers at the Portsmouth Naval Shipyard revealed a non-significant increase in leukaemia mortality (ERR/10 mSv=10.88% (95% CI: -0.90%; 38.77%)) [Yii 05], but also a significant positive association between leukaemia mortality and external radiation exposure (OR = 1.08 at 10 mSv of exposure; 95% CI = 1.01, 1.16). [Kub 05]. However, in this cohort, more than 97% of subjects were male, so no conclusions about sex-specific differences in leukaemia risk could be drawn.

The study by Mohan et al. is based on a nationwide cohort of 146 022 persons in the US who were shown to have worked as radiologic technologists for two or more years during the period 1926-1982 [Moh 03]. For leukaemia (excluding CLL), a non-significant increase in mortality could be observed among persons first employed prior to 1940 compared to those

first employed later (RR = 1.64; 95% CI: 0.42; 6.31). Furthermore, there was a significantly increased risk of mortality with increasing duration of employment prior to 1950 (based on 15 leukaemia deaths observed during this period). This study does not include a sex-specific analysis of relative risk.

The study by Möhner et al. on leukaemia and exposure to ionising radiation among German uranium miners [Möh 06] investigated a male-only cohort, so no sex-specific analysis could be carried out.

A study by Wing et al. carried out among Hanford workers (19 684 males and 6 705 females) produced a non-significant negative correlation between leukaemia risk and exposure. No conclusions about sex-specific differences could be drawn [Win 05].

Travis et al. found a significantly increased leukaemia risk among patients injected during cerebral angiography with either thorotrast (Thorium-232) (relative risk, incidence: 15.2 (95% CI: 4.4; 149.6) in the Danish/Swedish cohort; relative risk, mortality 16.8 (95% CI: 0.6; 211.7) in the US cohort) (see also Section 2.8), but were unable to draw any conclusions regarding sex-specific differences [Tra 03].

The Portuguese thorotrast study by dos Santos Silva investigated a cohort of 2 360 subjects. Although it found a significantly increased relative risk of leukaemia mortality (excluding CLL) of 10.2 (95% CI: 1.24; 471), no sex-specific differences were reported [San 2003].

Wang et al. investigated cancer incidence (1950-1995) among medical X-ray workers, finding significantly elevated risks in leukaemia. However, the study does not present an explicit analysis of possible sex-specific differences [Wan 02].

References

- [Boi 06] Boice, J. D.; Cohen, S. S.; Mumma, M. T.; Ellis, E. D.; Eckerman, K. F.; Leggett, R. W.; Boecker, B. B.; Brill, A. B.; Henderson, B. E.: Mortality among radiation workers at Rocketdyne (Atomics International), 1948-1999. *Radiat Res* 166:98-115, 2006
- [Car 05] Cardis, E.; Vrijheid, M.; Blettner, M.; Gilbert, E.; Hakama, M.; Hill, C. et al.: Risk of cancer after low doses of ionising radiation: retrospective cohort study in 15 countries. *BMJ* 331(7508):77, 9 Jul 2005
- [Dav 04] Davis, S.; Stepanenko, V.; Rivkind, N.; Kopecky, K.; Voilleque, P.; Shakhtarin, V.; Parshkov, E.; Kulikov, S.; Lushnikov, E.; Abrosimov, A.; Troshin, V.; Romanova, G.; Doroschenko, V.; Proshin, A.; Tsyb, A.: Risk of thyroid cancer in the Bryansk oblast of the Russian Federation after the Chernobyl Power Station Accident. *Radiat Res* 162: 241-248, 2004
- [Gil 03] Gilbert, E. S.; Stovall, M.; Gospodarowicz, M.; van Leeuwen, F. E.; Andersson, M.; Glimelius, B.; Joensuu, T.; Lynch, C. F.; Curtis, R. E.; Holowaty, E.; Storm, H.; Pukkala, E.; van't Veer, M. B.; Fraumeni, J. F. Jr; Boice, J. D. Jr; Clarke, E. A.; Travis, L. B.: Lung Cancer after Treatment for Hodgkin's Disease: Focus on Radiation Effects. *Radiat Res* 159:161-173, 2003

- [Gil 04] Gilbert, E. S.; Koshurnikova, N. A.; Sokolnikov, M. E.; Shilnikova, N. S.; Preston, D. L.; Ron, E.; Okatenko, P. V.; Khokhryakov, V. F.; Vasilenko, E. K.; Miller, S.; Eckerman, K.; Romanov, S. A.: Lung Cancer in Mayak Workers. *Radiat Res* 162:505-516, 2004
- [How 95] Howe, G. R.: Lung cancer mortality between 1950 and 1987 after exposure to fractionated moderate-dose-rate ionizing radiation in the Canadian fluoroscopy cohort study and a comparison with lung cancer mortality in the atomic bomb survivors study. *Radiat Res* 142:295-304, 1995
- [ICRP 07] International Commission on Radiological Protection (ICRP): The 2007 Recommendations of the International Commission on Radiological Protection. ICRP Publication 103. Ann. ICRP 37(2-4), Oxford: Pergamon Press, 2007
- [Iva 06] Ivanov, V. K.; Gorski, A. K.; Tsyb, A. F.; Maksimov, M. A.; Tumanov, K. A.; Vlasov, O. K.: Radiation-epidemiological studies of thyroid cancer incidence among children and adolescents in the Bryansk oblast of Russia after the Chernobyl accident (1991-2001 follow-up period). *Radiat Environ Biophys* 45:9-16, 2006
- [Jac 06] Jacob, P.; Bogdanova, T. I.; Buglova, E.; Chepur, M.; Demidchik, Y.; Gavrulin, Y.; Kenigsberg, J.; Meckbach, R.; Schotola, C.; Shinkarev, S.; Tronko, M. D.; Ulanovsky, A.; Vavilov, S.; Walsh, L.: Thyroid cancer risk in areas of Ukraine and Belarus affected by the Chernobyl accident. *Radiat Res* 165:1-8, 2006
- [Kre 05] Krestinina, L. Yu.; Preston, D. L.; Ostroumova, E. V.; Degteva, M. O.; Ron, E.; Vyushkova, O. V.; Startsev, N. V.; Kossenko, M. M.; Akleyev, A. V.: Protracted radiation exposure and cancer mortality in the Techa River Cohort. *Radiat Res* 164:602-611, 2005
- [Kub 05] Kubale, T. L.; Daniels, R. D.; Yiin, J. H.; Couch, J.; Schubauer-Berigan, M. K.; Kinnes, G. M.; Silver, S. R.; Nowlin, S. J.; Chen, P. H.: A nested case-control study of leukemia mortality and ionizing radiation at the Portsmouth Naval Shipyard. *Radiat Res* 164:810-819, 2005
- [Moh 03] Mohan, A. K.; Hauptmann, M.; Freedman, D. M.; Ron, E.; Matanoski, G. M.; Lubin, J. H.; Alexander, B. H.; Boice, J. D. Jr; Doody, M. M.; Linet, M. S.: Cancer and other causes of mortality among radiologic technologists in the United States. *Int J Cancer* 103:259-267, 2003
- [Möh 06] Möhner, M.; Lindtner, M.; Otten, H.; Gille, H.-G.: Leukemia and exposure to ionizing radiation among German uranium miners. *Am J Ind Med* 49:238-248, 2006
- [Mui 09] Muirhead, C. R.; O'Hagan, J. A.; Haylock, R. G. E.; Phillipson, M. A.; Willcock, T.; Berridge, G. L. C.; Zhang, W.: Mortality and cancer incidence following occupational radiation exposure: third analysis of the National Registry for Radiation Workers. *British Journal of Cancer* 100:206-212, 2009
- [NIH 03] National Institutes of Health: Report of the NCI-CDC Working Group to revise the 1985 NIH radioepidemiological Tables. NIH Publication No. 03-5387. Bethesda, MD, 2003

- [NRC 06] National Research Council of the National Academies: Health Risks from exposure to low levels of ionizing radiation – BEIR VII Phase 2. Board on Radiation Effects Research, Division on Earth and Life Studies, National Research Council of the National Academies, The National Academies Press, Washington, D.C., 2006
- [Pie 03] Pierce, D. A.; Sharp, G. B.; Mabuchi, M.: Joint effects of radiation and smoking on lung cancer risk among atomic bomb survivors. *Radiat Res* 159:511-520, 2003
- [Pie 96] Pierce, D. A.; Shimiza, Y.; Preston, D. L.; Vaeth, M.; Mabuchi, K.: Studies of the mortality of atomic bomb survivors. Report 12, Part I, Cancer. *Radiat Res* 146:1-27, 1996
- [Pre 94] Preston, D. L.; Kusumi, S.; Tomonaga, M. et al.: Cancer incidence in atomic bomb survivors. Part III: Leukemia, lymphoma and multiple myeloma, 1950-1987. *Radiat Res* 137:68-97, 1994
- [Pre 02] Preston, D. L.; Mattsson, A.; Holmberg, E.; Shore, R.; Hildreth, N. G.; Boice, J. D. Jr: Radiation effects on breast cancer risk: a pooled analysis of eight cohorts. *Radiat Res* 158:220-235, 2002
- [Pre 03] Preston, D. L.; Shimizu, Y.; Pierce, D. A.; Suyama, A.; Mabuchi, K.: Studies of mortality of atomic bomb survivors. Report 13: Solid cancer and noncancer disease mortality: 1950-1997. *Radiat Res* 160:381-407, 2003
- [Pre 04] Preston, D. L.; Pierce, D. A.; Shimizu, Y.; Cullings, H. M.; Fujita, S.; Funamoto, S.; Kodama, K.: Effect of recent changes in atomic bomb survivor dosimetry on cancer mortality risk estimates. *Radiat Res* 162:377-389, 2004
- [Pre 07] Preston, D. L.; Ron, E.; Tokuoka, S.; Funamoto, S.; Nishi, N.; Soda, M.; Mabuchi, K.; Kodama, K.: Solid cancer incidence in atomic bomb survivors: 1958-1998. *Radiat Res* 168:1-64, 2007
- [Ron 95] Ron, E.; Lubin, J. H.; Shore, R. E.; Mabuchi, K.; Modan, B.; Pottern, L. M.; Schneider, A. B.; Tucker, M. A.; Boice, J. D. Jr: Thyroid cancer after exposure to external radiation: a pooled analysis of seven studies, *Radiat Res* 141(3):259-277, 1995
- [San 03] dos Santos Silva, I.; Malveiro, F.; Jones, M. E.; Swerdlow, A. J.: Mortality after radiological investigation with radioactive thorotrast: a follow-up study of up to fifty years in Portugal. *Radiat Res* 159:521-534, 2003
- [Schu 07] Schubauer-Berigan, M. K.; Daniels, R. D.; Fleming, D. A.; Markey, A. M.; Couch, J. R.; Ahrenholtz, S. H.; Burphy, J. S.; Anderson, J. L.; Tseng, C.: Risk of chronic myeloid and acute leukemia mortality after exposure to ionizing radiation among workers at four U.S. nuclear weapons facilities and a nuclear naval shipyard. *Radiat Res* 167:222-232, 2007

- [Shi 03] Shilnikova, N. S.; Preston, D. L.; Ron, E.; Gilbert, E. S.; Vassilenko, E. K.; Romanov, S. A.; Kuznetsova, I. S.; Sokolnikov, M. E.; Okatenko, P. V.; Kreslov, V. V.; Koshurnikova, N. A.: Cancer mortality risk among workers at the Mayak Nuclear Complex. *Radiat Res* 159:787-798, 2003
- [Tho 94] Thomson, D. E.; Mabuchi, K.; Ron, E.; Soca, M.; Tokunaga, M.; Ochikubo, S.; Sugimoto, S.; Ikeda, T.; Teraski, M.; Preston, D. L.: Cancer incidence in atomic bomb survivors. Part II: Solid tumors, 1958-1987. *Radiat Res* 137 (Suppl.):17-67, 1994
- [Tra 03] Travis, L. B.; Hauptmann, M.; Knudson Gaul, L.; Storm, H. H.; Goldman, M. B.; Nyberg, U.; Berger, E.; Janower, M. L.; Hall, P.; Monson, R. R.; Holm, L. E.; Land, C. E.; Schottenfeld, D.; Boice, J. D. Jr; Andersson, M.: Site-specific cancer incidence and mortality after cerebral angiography with radioactive thorotrast. *Radiat Res* 168:691-706, 2003
- [UNSC 00] United Nations Scientific Committee on the Effects of Atomic Radiation: Sources and Effects of ionizing radiation: UNSCEAR 2000 Report to the General Assembly, United Nations, New York, 2000
- [UNSC 08] United Nations Scientific Committee on the Effects of Atomic Radiation: Effects of ionizing radiation – UNSCEAR 2006 Report, Vol I. Report to the General Assembly, with scientific annexes A and B. United Nations sales publications. United Nations, New York, 2008
- [Wan 02] Wang, J. X.; Zhang, L. A.; Li, B. X.; Zhao, Y. C.; Wang, Z. Q.; Zhang, J. Y.; Aoyama, T.: Cancer incidence and risk estimation among medical x-ray workers in China, 1950-1995. *Health Phys* 82:455-466, 2002
- [Win 05] Wing, S.; Richardson, D. B.: Age at exposure to ionising radiation and cancer mortality among Hanford workers: follow up through 1994. *Occup Environ Med* 62:465-472, 2005
- [Yii 05] Yiin, J. H.; Schubauer-Berigan, M. K.; Silver, S. R.; Daniels, R. D.; Kinnes, G. M.; Zaebst, D. D.; Couch, J. R.; Kubale, T. L.; Chen, P. H.: Risk of lung cancer and leukemia from exposure to ionizing radiation and potential confounders among workers at the Portsmouth Naval Shipyard. *Radiat Res* 163:603-613, 2005

ANNEX 2 : Summary of Clinical Findings

1 Introduction

Unlike the situation in Anglo-American usage, the German language does not contain separate words indicating whether observed differences between men and women can be attributed to biological sex (e. g. different body size, hormonal influences etc.) or to sociocultural factors. For the latter, the term *gender* has been coined, which encompasses the cultural and social role and personal identity attributed to man- or womanhood. In the present context, *gender* is relevant primarily in relation to epidemiological issues, e. g. women's changed smoking behaviour and incidence of lung cancer, and to aspects of disease management (e. g. experience of pain, naming of symptoms) and accessing medical care.

Men's and women's different radiation sensitivity could affect tumour response and local control (i. e. prevention of recurrence at the site of the primary cancer) during radiation therapy, but also adverse early and late side-effects, including the induction of secondary malignancies. Despite the importance of this issue, also with regard to therapeutic decisions, there are very few systematic studies on the specific influence of sex on these endpoints. An analysis of 661 prospective clinical studies found that women were under-represented as participants in recently published, high-impact studies of non-sex-specific cancers [Jag 09].

2 Effectiveness of Cancer Therapy

The radiation sensitivity of cancers varies widely and leads to widely varying local control in radiation therapy. It is influenced by numerous factors: first and foremost, by tumour biology, but also by patient-specific factors such as age, comorbidity and sex, as well as the therapy modalities additionally used (surgery, chemo- and immunotherapy). Sex, like age, is always included in the analyses of local control and survival in Phase II and Phase III studies, but very few specific studies are available on the sex-dependent radiation sensitivity of cancers.

An influence of sex on local control is consistently observed in relation to Hodgkin's lymphoma, soft tissue sarcoma, and head and neck cancer, and at least partially in cancers of the lung and rectum, Ewing's sarcoma, low malignancy grade glioma and meningeal sarcomas [Bor 09.]. The observed sex-specific differences may be due to biological factors but could also be attributable to different types of therapy and compliance, psychosocial effects, etc. [Bor 09].

An important role in sex-specific differences in tumour response, which is increasingly well-researched, is played by sensitivity to chemotherapeutic drugs. For example, Singh et al. [Sin 05], in a study of small-cell lung cancer (SCLC), analysed 1 000 patients from Phase II studies and found that women experienced greater treatment toxicity but also had significantly better survival after chemo- and radiotherapy. However, women per se appear to have a better prognosis than men, also after one operation for non-small cell lung cancer (NSCLC) in stage I and II. It is unclear whether this still applies if women's longer life expectancy and lower frequency of comorbidities are taken into account [Ale 02, Bat 05, Bir 06, Cal 07, Chat 04, Fer 03, Foe 07, Moo 04, Oka 03, Rad 02, Vis 04]. Other factors are associated with female sex which may influence response to therapy and thus local control and survival: Asian women with adenocarcinoma of the lung more often have a specific mutation of the EGFR gene (epidermal growth factor receptor), which is associated with better response to Gefitinib, a EGFR inhibitor [Pao 04, Pae 04].

In a Phase III study with 1 700 patients on adjuvant radiochemotherapy in rectal cancer, female sex was an independent positive factor for overall survival [Tep 02]. As shown in a meta-analysis of several comparable studies, women may experience more severe chemotherapy-related toxicity, especially to 5-fluorouracil (5-FU), which may indicate increased biological effectiveness of the chemotherapy and possibly result in better overall

survival [Chan 05]. In the long-term analysis carried out by the Swedish Rectal Cancer Trial, women had a better 13-year cancer-specific survival rate (41% vs. 30%, respectively; $p = 0.001$) and better overall survival rate (74% vs. 62%, respectively; $p = 0.004$) irrespective of whether radiotherapy was administered or not [Fol 05]. The local recurrence rate and the incidence of distant metastases were identical for men and women with stages and sites evenly distributed. A German multi-centre study on pre- vs. post-operative adjuvant radiochemotherapy in rectal cancer found that men were more likely than women to receive radiotherapy in adequate time and with adequate dose [Fie 07]. The reasons for this could not be clarified retrospectively for all patients. Increased post-operative intestinal toxicity resulted in delays in the administering of radiochemotherapy in some cases. Younger age of patient, treatment centre and treatment schedule all influenced the delivery of adequate therapy. Inadequate therapy was associated with an increased local recurrence rate ($21.2\% \pm 5.6$ vs. $6.8\% \pm 1.4$, $p=0.0001$) and reduced disease-specific survival ($57.4\% \pm 6.3$ vs. $69.1\% \pm 2.3$, $p=0.02$) in this post hoc analysis. In the further Phase III studies on adjuvant radio- or radiochemotherapy, the influence of sex was not investigated/reported [MRC 96, SRCT 97, Bos 06, Buj 06, Dah 90, Ger 06, Kap 01, Sau 04, Seb 09] or could not be demonstrated [Tve 97].

3 Adverse Effects of Radiation Therapy

There are a few clinical indications of sex-specific differences in the radiation sensitivity of normal tissue. An overview is provided in [Bor 09]. For various methodological reasons, it is difficult to make definitive statements about the existence or non-existence of adverse sex-specific effects of radiation. The documentation on adverse effects of radiation is not optimal [Ben 07, Sch 06]. Clinical studies often report them as a crude incidence, often with non-validated classifications or classifications which are unsuitable for radiation therapy, and rarely cover a time period which is adequate to encapsulate late adverse effects. Due to the dosages used in radiation therapy, there is a $\leq 5\%$ likelihood of medium to severe adverse effects occurring, and as co-factors such as additional forms of therapy, patient's age, comorbidity etc. must be taken into account, possible sex-specific differences in radioreaction can only be determined in large cohorts undergoing standardised treatment.

Sex was the only risk factor for cataract formation after curative radiotherapy for primary orbital lymphoma, with a far higher risk for males [Bha 02a]. Sex dependence was also identified for the myelopathy of Cauda equina after treatment with combined proton and photon beams. For the tolerance dose TD 5/5, which determines the dose at which toxicities occur in 5% of patients within five years, the value for males was significantly lower ($p = 0.017$) [Pie 06]. A similar effect was observed by the same working group for the risk of leukoencephalopathy in a dose-finding study on radiation of chordomas and chondrosarcomas of the base of the skull. Even when taking account of the relationship between leukoencephalopathy and age, tumour volume, diagnostic procedure, and radiation dose, gender (sex) was found to be the single most significant risk factor (univariate, $p=0.0155$) [San 98].

In a prospective study which investigated the acute effects of radiotherapy for head and neck cancer in 149 patients, no differences were observed with regard to most adverse effects such as mucositis, dysphagia and salivary changes. Only in relation to pain perception were higher values obtained for women [Pal 08].

As mentioned, targeted studies of major cohorts have shown that sex-specific differences in the toxicity of some chemotherapeutic drugs exist, which can be attributed to different pharmacokinetics [Chan 05, Gan 04, Mar 06, Rou 02, Sin 05]. Women, for example, have stronger and more frequent reactions to 5-fluorouracil and Cisplatin, which are often used in

combined radiochemotherapy for rectal and lung cancer. This increased toxicity may have been a contributory factor when, in a group of 48 patients with anal cancer, leukopenia after radiochemotherapy was significantly associated with female gender, decreased body mass index, increased lumbosacral bone marrow and lymph node metastatic spread [Mel 07].

4 Second Cancers after Radiation Therapy

Cancer survivors have a greater risk of developing a new cancer compared with persons who have never had cancer. Besides genetic and lifestyle factors, the treatment received for the first cancer may be a cause of the increased risk. Due to the large patient numbers, studies on second cancers which are based on population-wide cancer registers are particularly informative in describing influential factors such as time lag, sex, age at onset of first cancer, and age on diagnosis of the second cancer [Tra 06]. The therapy-related information contained in these studies is generally limited, however. For patient cohorts from clinical studies, on the other hand, detailed therapy information is generally available, but the subject numbers are often limited in this case.

Survivors of childhood cancer are particularly well-researched in terms of the occurrence of second cancers (see overview in [Bha 02b, Rob 09]). Their risk is around five times higher than that of the general population. Female sex is generally associated with increased risk, particularly due to the increased incidence of secondary breast cancer after radiation therapy for Hodgkin's lymphoma [Bha 02b]. However, Constone et al. (Con 08), in a study of 930 persons who had received treatment for Hodgkin's lymphoma as children, not only found that the standardised incidence ratio (SIR) for female subjects was significantly greater than for males for all second cancer entities (SIR=19.93 vs. SIR=8.41), but also that even after excluding breast cancer, the SIR for female patients was still significantly greater than for male patients (SIR=15.4). Bluhm et al. [Blu 08] report similar findings in a study among 1082 survivors of non-Hodgkin's lymphoma (NHL) under 21 years of age; the standardised incidence ratio (SIR) for solid second cancers was far higher for females than for males (SIR=6.9 [95% CI: 3.9; 10.8] vs. SIR=2.3 [95% CI: 1.1; 4.3]) and remained high even if tumours in the breast were excluded (SIR=6.4 [95% CI: 3.3; 11.1]).

Inskip et al. [Ins 2007] observed a cohort of around 26 000 survivors of cancer in childhood and adolescence, diagnosed before the age of 18 during 1988-2002, and noted similar risks of second cancer for females (O/E=5.9) and males (O/E=6.0). The risk of subsequent solid cancers was higher among persons whose initial treatment for childhood cancer included radiotherapy (RR=1.9, 95% CI: 1.5; 2.5). However, the authors did not undertake a separate sex-specific analysis of risk following radiation therapy.

Many of the studies carried out on second cancer incidence among adults after radiation therapy relate to sex-specific cancers (e. g. testes, cervix, breast) and are therefore unsuitable for a comparison by sex. An evaluation of the secondary cancer risk based on the data of the clinical cancer registry of the German state of Brandenburg [Til 09] shows that the general second cancer rate is considerably higher for males than for females. However, as the risk of first cancer is also higher for males, this results in higher second cancer risks for females than for males (SIR=1.5 (95% CI: 1.45; 1.59) vs. SIR=1.3 (95% CI: 1.28; 1.39)). Among patients who underwent radiation therapy as part of their primary treatment, the risks are comparable with those of the cohort as a whole (SIR=1.6 [CI: 1.48; 1.72] for females and SIR=1.3 [CI: 1.13; 1.36] for males), so no influence of radiation therapy was apparent. However, the mean observation period in this study was four years, which is far too short.

One of the few studies which have investigated radiation-associated second cancer risks on a sex-specific basis was undertaken by Boukheris [Bou 08]. The authors studied the risk of radiation-related salivary gland carcinomas (SGC) among around 21 000 survivors of

Hodgkin's lymphoma (HL) (≥ 1 -year survival). Patients who received radiotherapy as part of their initial treatment for HL had a significantly elevated risk of subsequent SGC compared with patients who had not received radiotherapy (O/E = 16.9 [95% CI: 10.4; 25.8] vs. O/E = 4.8 [95% CI: 1.31; 12.29]). Among the irradiated patients who developed SGC, 2/3 were female, even though the spontaneous risk for males is far higher than for females, indicating an increased radiation-related risk for females. These findings conflict, however, with the findings of a multinational study of HL survivors [Dor 02], the findings of the Life Span Study [Pre 07] and the results of a study among children who underwent radiation treatment for benign conditions of the head and neck [Schn 98], where, in each case, no sex-specific differences in the incidence of SGC were observed.

Several studies provide information on second cancer risks in relation to sex and treatment modality (irradiated/unirradiated), but do not make statements about possible sex-specific differences among irradiated patients. For example, Tward et al. studied the risk of secondary malignancies among approximately 78 000 patients treated for non-Hodgkin's lymphoma (NHL) between 1973 and 2001 [Twa 06]. According to their survey, male and female NHL survivors have similarly elevated risks of developing secondary malignancies (O/E = 1.15 for males and O/E = 1.12 for females). Radiation therapy, in this study, had no significant influence on risk (O/E = 1.18 [95% CI: 1.12; 1.23] for irradiated survivors vs. O/E = 1.13 [95% CI: 1.10; 1.17] for unirradiated survivors). In a smaller study of 563 NHL survivors [Sac 08], by contrast, a significantly increased risk for male patients (SIR=2.72 [1.76; 4.06]) compared with female patients (SIR=1.10 [0.50; 2.10]) was observed, but no significant influence of radiation therapy was noted (SIR=2.48 [0.91; 5.40] with radiation therapy vs. SIR=1.88 [1.25; 2.71] without radiation therapy).

Brown et al. [Bro 08] studied the incidence of second primary malignancies in more than 30 000 survivors of thyroid cancer (diagnosis period 1973-2002). The relative risk of second malignancies was higher among persons who had received treatment with radioisotopes than among patients who had not undergone such treatment (RR=1.10 [1.05; 1.27]; $p < 0.05$). In general, sex had no influence on the risk of developing second primary malignancies (O/E=1.09 for males and females), but the combination of the two parameters was not investigated.

References

- [Ale 02] Alexiou, C.; Onyeaka, C. V.; Beggs, D.; Akar, R.; Beggs, L.; Salama, F. D.; Duffy, J. P.; Morgan, W. E.: Do women live longer following lung resection for carcinoma? *Eur J Cardiothorac Surg* 21:319-325, 2002
- [Bat 05] Batevik, R.; Grong, K.; Segadal, L.; Stangeland, L.: The female gender has a positive effect on survival independent of background life expectancy following surgical resection of primary non-small cell lung cancer: a study of absolute and relative survival over 15 years. *Lung Cancer* 47:173-181, 2005
- [Ben 07] Bentzen, S. M.; Trotti, A.: Evaluation of early and late toxicities in chemoradiation trials. *J Clin Oncol* 25:4096-4103, 2007
- [Bha 02a] Bhatia, S.; Paulino, A. C.; Buatti, J. M.; Mayr, N. A.; Wen, B. C.: Curative radiotherapy for primary orbital lymphoma. *Int J Radiat Oncol Biol Phys* 54:818-823, 2002

- [Bha 02b] Bhatia, S.; Sklar, C.: Second cancers in survivors of childhood cancer. *Nat Rev Cancer* 2(2):124-32, Feb 2002
- [Bir 06] Birim, O.; Kappetein, A. P.; van Klaveren, R. J.; Bogers, A. J.: Prognostic factors in non-small cell lung cancer surgery. *Eur J Surg Oncol* 32:12-23, 2006
- [Blu 08] Bluhm, E. C.; Ronckers, C.; Hayashi, R. J.; Neglia, J. P.; Mertens, A. C.; Stovall, M.; Meadows, A. T.; Mitby, P. A.; Whitton, J. A.; Hammond, S.; Barker, J. D.; Donaldson, S. S.; Robison, L. L.; Inskip, P. D.: Cause-specific mortality and second cancer incidence after non-Hodgkin lymphoma: a report from the Childhood Cancer Survivor Study. *Blood* 111(8):4014-4021, 2008
- [Bor 09] Borgmann, K.; Dikomey, E.; Petersen, C.; Feyer, P.; Hoeller, U.: Sex-specific aspects of tumor therapy. *Radiat Environ Biophys* 48:115-124, 2009
- [Bos 06] Bosset, J. F.; Collette, L.; Calais, G.; Mineur, L.; Maingon, P.; Radosevic-Jelic, L.; Daban, A.; Bardet, E.; Beny, A.; Ollier, J. C.: Chemotherapy with preoperative radiotherapy in rectal cancer. *N Engl J Med* 355:1114-1123, 2006
- [Bou 08] Boukheris, H.; Ron, E.; Dores, G. M.; Stovall, M.; Smith, S. A.; Curtis, R. E.: Risk of radiation-related salivary gland carcinomas among survivors of Hodgkin lymphoma: a population-based analysis. *Cancer* 113(11):3153-9, 2008
- [Bro 08] Brown, A. P.; Chen, J.; Hitchcock, Y. J.; Szabo, A.; Shrieve, D. C.; Tward, J. D.: The risk of second primary malignancies up to three decades after the treatment of differentiated thyroid cancer. *J Clin Endocrinol Metab* 93(2):504-515, 2008
- [Buj 06] Bujko, K.; Nowacki, M. P.; Nasierowska-Guttmejer, A.; Michalski, W.; Bebenek, M.; Kryj, M.: Long-term results of a randomized trial comparing preoperative shortcourse radiotherapy with preoperative conventionally fractionated chemoradiation for rectal cancer. *Br J Surg* 93:1215-1223, 2006
- [Cal 07] Caldarella, A.; Crocetti, E.; Comin, C. E.; Janni, A.; Pegna, A. L.; Paci, E.: Gender differences in non-small cell lung cancer: A population-based study. *Eur J Surg Oncol* 33:763-768, 2007
- [Chan 05] Chansky, K.; Benedetti, J.; Macdonald, J. S.: Differences in toxicity between men and women treated with 5-fluorouracil therapy for colorectal carcinoma. *Cancer* 103:1165-1171, 2005
- [Chat 04] Chatkin, J. M.; Abreu, C. M.; Fritscher, C. C.; Wagner, M. B.; Pinto, J. A.: Is there a gender difference in non-small cell lung cancer survival? *Gend Med* 1:41-47, 2004
- [Con 08] Constine, L. S.; Tarbell, N.; Hudson, M. M.; Schwartz, C.; Fisher, S. G.; Muhs, A. G.; Basu, S. K.; Kun, L. E.; Ng, A.; Mauch, P.; Sandhu, A.; Culakova, E.; Lyman, G.; Mendenhall, N.: Subsequent malignancies in children treated for Hodgkin's disease: associations with gender and radiation dose. *Int J Radiat Oncol Biol Phys* 72(1):24-33, 2008

- [Dah 90] Dahl, O.; Horn, A.; Morild, I.; Halvorsen, J. F.; Odland, G.; Reinertsen, S.; Reisaeter, A.; Kavli, H.; Thunold, J.: Low-dose preoperative radiation postpones recurrences in operable rectal cancer. Results of a randomized multicenter trial in western Norway. *Cancer* 66:2286-2294, 1990
- [Dor 02] Dores, G. M.; Metayer, C.; Curtis, R. E.; Lynch, C. F.; Clarke, E. A.; Glimelius, B.; Storm, H.; Pukkala, E.; van Leeuwen, F. E.; Holowaty, E. J.; Andersson, M.; Wiklund, T.; Joensuu, T.; van't Veer, M. B.; Stovall, M.; Gospodarowicz, M.; Travis, L. B.: Second malignant neoplasms among long-term survivors of Hodgkin's disease: a population-based evaluation over 25 years. *J Clin Oncol* 20(16):3484-94, 2002
- [Fer 03] Fernandes, O. J.; Almgren, S. O.; Thaning, L.; Filbey, D.; Helsing, M.; Karlsson, M.; Magnusson, A.; Souza, D.: Prognostic factors for the survival of surgically treated patients for non-small cell lung cancer. *Acta Oncol* 42:338-341, 2003
- [Fie 07] Fietkau, R.; Rodel, C.; Hohenberger, W.; Raab, R.; Hess, C.; Liersch, T.; Becker, H.; Wittekind, C.; Hutter, M.; Hager, E.; Karstens, J.; Ewald, H.; Christen, N.; Jagoditsch, M.; Martus, P.; Sauer, R.: Rectal cancer delivery of radiotherapy in adequate time and with adequate dose is influenced by treatment center, treatment schedule, and gender and is prognostic parameter for local control: results of study. CAO/ARO/AIO-94. *Int J Radiat Oncol Biol Phys* 67:1008-1019, 2007
- [Foe 07] Foegle, J.; Hedelin, G.; Lebitasy, M. P.; Purohit, A.; Velten, M.; Quoix, E.: Specific features of non-small cell lung cancer in women: a retrospective study of 1738 cases diagnosed in Bas-Rhin between 1982 and 1997. *J Thorac Oncol* 2:466-474, 2007
- [Fol 05] Folkesson, J.; Birgisson, H.; Pahlman, L.; Cedermark, B.; Glimelius, B.; Gunnarsson, U.: Swedish Rectal Cancer Trial: long lasting benefits from radiotherapy on survival and local recurrence rate. *J Clin Oncol* 23:5644-5650, 2005
- [Gan 04] Gandhi, M.; Aweeka, F.; Greenblatt, R. M.; Blaschke, T. F.: Sex differences in pharmacokinetics and pharmacodynamics. *Annu Rev Pharmacol Toxicol* 44:499-523, 2004
- [Ger 06] Gerard, J. P.; Conroy, T.; Bonnetain, F.; Bouche, O.; Chapet, O.; Closon-Dejardin, M. T.; Untch, M.; Leduc, B.; Francois, E.; Maurel, J.; Seitz, J. F.; Buecher, B.; Mackiewicz, R.; Ducreux, M.; Bedenne, L.: Preoperative radiotherapy with or without concurrent fluorouracil and leucovorin in T3-4 rectal cancers: results of FFCD 9203. *J Clin Oncol* 24:4620-4625, 2006
- [Ins 07] Inskip, P. D.; Curtis, R. E.: New malignancies following childhood cancer in the United States, 1973-2002. *Int J Cancer* 121(10):2233-2240, 2007
- [Jag 09] Jagsi, R.; Motomura, A. R.; Amarnath, S.; Jankovic, A.; Sheets, N.; Ubel, P. A.: Under-representation of women in high-impact published clinical cancer research. *Cancer* 115(14):3293-301, 2009

- [Kap 01] Kapiteijn, E.; Marijnen, C. A.; Nagtegaal, I. D.; Putter, H.; Steup, W. H.; Wiggers, T.; Rutten, H. J.; Pahlman, L.; Glimelius, B.; van Krieken, J. H.; Leer, J. W.; van de Velde, C. J.: Preoperative radiotherapy combined with total mesorectal excision for resectable rectal cancer. *N Engl J Med* 345:638-646, 2001
- [Mar 06] Marosi, C.: Gender aspects of treatment and drug related toxicity in medical oncology. *Wien Med Wochenschr* 156:534-540, 2006
- [Mel 07] Mell, L. K.; Schomas, D. A.; Salama, J. K.; Devisetty, K.; Aydogan, B.; Miller, R. C.; Jani, A. B.; Kindler, H. L.; Mundt, A. J.; Roeske, J. C.; Chmura, S. J.: Association Between Bone Marrow Dosimetric Parameters and Acute Hematologic Toxicity in Anal Cancer Patients Treated with Concurrent Chemotherapy and Intensity-Modulated Radiotherapy. *Int J Radiat Oncol Biol Phys* 70(5):1431-1437, 2007
- [Moo 04] Moore, R.; Doherty, D.; Chamberlain, R. and Khuri, F.: Sex differences in survival in non-small cell lung cancer patients 1974-1998. *Acta Oncol* 43:57-64, 2004
- [MRC 96] Medical Research Council Rectal Cancer Working Party: Randomised trial of surgery alone versus radiotherapy followed by surgery for potentially operable locally advanced rectal cancer. *Lancet* 348:1605-1610, 1996
- [Oka 03] Okada, M.; Nishio, W.; Sakamoto, T.; Harada, H.; Uchino, K.; Tsubota, N.: Longterm survival and prognostic factors of five-year survivors with complete resection of non-small cell lung carcinoma. *J Thorac Cardiovasc Surg* 126:558-562, 2003
- [Pae 04] Paez, J. G.; Janne, P. A.; Lee, J. C.; Tracy, S.; Greulich, H.; Gabriel, S.; Herman, P.; Kaye, F. J.; Lindeman, N.; Boggon, T. J.; Naoki, K.; Sasaki, H.; Fujii, Y.; Eck, M. J.; Sellers, W. R.; Johnson, B. E.; Meyerson, M.: EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. *Science* 304:1497-1500, 2004
- [Pal 08] Palazzi, M.; Tomatis, S.; Orlandi, E.; Guzzo, M.; Sangalli, C.; Potepan, P.; Fantini, S.; Bergamini, C.; Gavazzi, C.; Licitra, L.; Scaramellini, G.; Cantu, G.; Olmi, P.: Effects of Treatment Intensification on Acute Local Toxicity During Radiotherapy for Head and Neck Cancer: Prospective Observational Study Validating CTCAE, Version 3.0, Scoring System. *Int J Radiat Oncol Biol Phys* 70:330-337, 2008
- [Pao 04] Pao, W.; Miller, V.; Zakowski, M.; Doherty, J.; Politi, K.; Sarkaria, I.; Singh, B.; Heelan, R.; Rusch, V.; Fulton, L.; Mardis, E.; Kupfer, D.; Wilson, R.; Kris, M.; Varmus, H.: EGF receptor gene mutations are common in lung cancers from "never smokers" and are associated with sensitivity of tumors to gefitinib and erlotinib. *Proc Natl Acad Sci USA* 101:13306-13311, 2004
- [Pie 06] Pieters, R. S.; Niemierko, A.; Fullerton, B. C.; Munzenrider, J. E.: Cauda equina tolerance to high-dose fractionated irradiation. *Int J Radiat Oncol Biol Phys* 64:251-257, 2006
- [Pre 07] Preston, D. L.; Ron, E.; Tokuoka, S. et al. : Solid cancer incidence in atomic bomb survivors: 1958-1998. *Radiat Res* 168:1-64, 2007

- [Rad 02] Radzikowska, E.; Glaz, P.; Roszkowski, K.: Lung cancer in women: age, smoking, histology, performance status, stage, initial treatment and survival. Population-based study of 20 561 cases. *Ann Oncol* 13:1087-1093, 2002
- [Rob 09] Robison, L. L.; Armstrong, G. T.; Boice, J. D.; Chow, E. J.; Davies, S. M.; Donaldson, S. S.; Green, D. M.; Hammond, S.; Meadows, A. T.; Mertens, A. C.; Mulvihill, J. J.; Nathan, P. C.; Neglia, J. P.; Packer, R. J.; Rajaraman, P.; Sklar, C. A.; Stovall, M.; Strong, L. C.; Yasui, Y.; Zeltzer, L. K.: The Childhood Cancer Survivor Study: a National Cancer Institute-supported resource for outcome and intervention research. *J Clin Oncol* 27(14):2308-18, 2009
- [Rou 02] Rousseau, A.; Marquet, P.: Application of pharmacokinetic modelling to the routine therapeutic drug monitoring of anticancer drugs. *Fundam Clin Pharmacol* 16:253-262, 2002
- [Sac 08] Sacchi, S.; Marcheselli, L.; Bari, A.; Marcheselli, R.; Pozzi, S.; Luminari, S.; Lombardo, M.; Buda, G.; Lazzaro, A.; Gobbi, P. G.; Stelitano, C.; Morabito, F.; Quarta, G.; Brugiattelli, M.: Secondary malignancies after treatment for indolent non-Hodgkin's lymphoma: a 16-year follow-up study. *Haematologica* 93(3):398-404, 2008
- [San 98] Santoni, R.; Liebsch, N.; Finkelstein, D. M.; Hug, E.; Hanssens, P.; Goitein, M.; Smith, A. R.; O'Farrell, D.; Efid, J. T.; Fullerton, B.; Munzenrider, J. E.: Temporal lobe (TL) damage following surgery and high-dose photon and proton irradiation in 96 patients affected by chordomas and chondrosarcomas of the base of the skull. *Int J Radiat Oncol Biol Phys* 41:59-68, 1998
- [Sau 04] Sauer, R.; Becker, H.; Hohenberger, W.; Rodel, C.; Wittekind, C.; Fietkau, R.; Martus, P.; Tschmelitsch, J.; Hager, E.; Hess, C. F.; Karstens, J. H.; Liersch, T.; Schmidberger, H.; Raab, R.: Preoperative versus postoperative chemoradiotherapy for rectal cancer. *N Engl J Med* 351:1731-1740, 2004
- [Sch 06] Scharf, O.; Colevas, A. D.: Adverse event reporting in publications compared with sponsor database for cancer clinical trials. *J Clin Oncol* 24:3933-3938, 2006
- [Schn 98] Schneider, A. B.; Lubin, J.; Ron, E.; Abrahams, C.; Stovall, M.; Goel, A.; Shore-Freedman, E.; Gierlowski, T. C.: Salivary gland tumors after childhood radiation treatment for benign conditions of the head and neck: dose-response relationships. *Radiat Res* 149:625-630, 1998
- [Seb 09] Sebag-Montefiore, D.; Stephens, R. J.; Steele, R.; Monson, J.; Grieve, R.; Khanna, S.; Quirke, P.; Couture, J.; de Metz, C.; Myint, A. S.; Bessell, E.; Griffiths, G.; Thompson, L. C.; Parmar, M.: Preoperative radiotherapy versus selective postoperative chemoradiotherapy in patients with rectal cancer (MRC CR07 and NCIC-CTG C016): a multicentre, randomised trial. *Lancet* 373:811-820, 2009
- [Sin 05] Singh, S.; Parulekar, W.; Murray, N.; Feld, R.; Evans, W. K.; Tu, D.; Shepherd, F. A.: Influence of sex on toxicity and treatment outcome in small-cell lung cancer. *J Clin Oncol* 23:850-856, 2005

- [SRCT 97] Swedish Rectal Cancer Trial: Improved survival with preoperative radiotherapy in resectable rectal cancer. *N Engl J Med* 336:980-987, 1997
- [Tep 02] Tepper, J. E.; O'Connell, M.; Niedzwiecki, D.; Hollis, D. R.; Benson, A. B. 3rd; Cummings, B.; Gunderson, L. L.; Macdonald, J. S.; Martenson, J. A.; Mayer, R. J.: Adjuvant therapy in rectal cancer: analysis of stage, sex, and local control – final report of intergroup 0114. *J Clin Oncol* 20:1744-1750, 2002
- [Til 09] Tillack, A.: Zum Risiko eines Zweitmalignoms bei Krebskranken anhand der Daten des klinischen Krebsregisters des Tumorzentrums Land Brandenburg. Dissertation, Medizinische Fakultät, Charité–Universitätsmedizin Berlin, 19.08.2009
- [Tra 06] Travis, L. B.: The epidemiology of second primary cancers. *Cancer Epidemiol, Biomarkers Prev* 15(11):2020-2026, 2006
- [Tve 97] Tveit, K. M.; Guldvog, I.; Hagen, S.; Trondsen, E.; Harbitz, T.; Nygaard, K.; Nilsen, J. B.; Wist, E.; Hannisdal, E.: Randomized controlled trial of postoperative radiotherapy and short-term time-scheduled 5-fluorouracil against surgery alone in the treatment of Dukes B and C rectal cancer. Norwegian Adjuvant Rectal Cancer Project Group. *Br J Surg* 84:1130-1135, 1997
- [Twa 06] Tward, J. D.; Wendland, M. M.; Shrieve, D. C.; Szabo, A.; Gaffney, D. K.: The risk of secondary malignancies over 30 years after the treatment of non-Hodgkin lymphoma. *Cancer* 107(1):108-115, 2006
- [Vis 04] Visbal, A. L.; Williams, B. A.; Nichols, F. C. 3rd; Marks, R. S.; Jett, J. R.; Aubry, M. C.; Edell, E. S.; Wampfler, J. A.; Molina, J. R.; Yang, P.: Gender differences in non-small-cell lung cancer survival: an analysis of 4,618 patients diagnosed between 1997 and 2002. *Ann Thorac Surg* 78:209-215; discussion 215, 2004

ANNEX 3: Summary of Biological Findings

1 Genetic and Molecular Basis of Sex Determination

1.1 Sex chromosomes in humans

In human beings, as indeed in most other mammals, sex is determined and inherited by sex chromosomes. Males have one Y chromosome and one X chromosome, while females have two X chromosomes. The two sex chromosomes differ in size, structure, and the number and type of genes. In humans, the X chromosome is approx. 160 Mb in length and contains more than 1 000 genes, whereas the Y chromosome is approx. 65 Mb in length and contains only around 100 genes. These genes are mainly found in a euchromatic¹ region (approx. 25 Mb), containing the short arm and parts of the long arm of the chromosome; the long arm also contains a very large heterochromatic gene-free sequence block. At the chromosome ends (near the telomeres), there are the pseudoautosomal regions (PAR1 and PAR2) which are homologous sequences of nucleotides on the X and Y chromosomes and allow recombination between both chromosomes. In the remaining areas of the X and Y chromosome, there are mosaic-like sequences which originate in the shared “autosomal ancestor”² and are therefore similar to each other, and those which are X- or Y-specific [Ska 03, Ros 05].

1.2 Y chromosome specific genes

The Y-specific genes are expressed preferentially or exclusively in testicular tissue and are significant for spermatogenesis and for male sexual differentiation. The *SRY* gene (*sex-determining region Y*) is most important for determining male sex. For male fertility (spermatogenesis), other Y chromosome genes are required, which are generally present in multiple copies and arranged as palindromes in blocks of 10–100 kb; for example, there are 35 copies of *TSPY* (testis-specific protein Y).

1.3 Sex differentiation

During embryogenesis in mammals, “bipotential” gonads and reproductive organs are initially formed, whose development generally follows the female pathway if no active SRY protein – which would drive development along the male pathway – is present. The tissue of the gonads consists of three types of cells: the precursors of the sex cells, the nurse and supporting cells with metabolic functions, and the hormone-producing cells. After SRY-dependent differentiation into male and female gonads, the Sertoli and Leydig cells form in the male gonads, whose hormones (Anti-Müllerian hormone / testosterone) serve to suppress phenotypically female structures or, in the case of testosterone, support the development of phenotypically male sex organs. Without SRY protein, the gonads develop into ovaries and, in the absence of testosterone and Anti-Müllerian hormone, the primordial reproductive organs develop into female sex organs.

1.4 X chromosome inactivation

Male mammals have one X chromosome; females, on the other hand, have two with all the

¹ Chromatin is a combination of DNA, which wraps around histones, and other proteins. It is present in two forms: *euchromatin*, which does not condense significantly, and which allows the transcription of the genes it contains, and strongly condensed *heterochromatin*, which is genetically inactive. The condensation of the chromatin is controlled mainly through epigenetic processes.

² The precursor of the sex chromosomes was a normal chromosome pair (called an autosomal pair). After one partner had developed a sex-determining sequence and meiotic recombination between the partners had been prevented because of inversions, the two chromosomes developed separately.

genes located on them. During early embryonic development, however, one X chromosome in the cells of the female embryo is genetically “inactivated”. The choice of which X chromosome will be inactivated is random and can apply to the paternally derived or the maternally derived X chromosome. The inactivation is then transmitted through all successive cell divisions. The effect of the inactivation is the formation of the heterochromatic Barr body which, due to strong condensation, is visible under the microscope. The overwhelming majority of genes (80%) on the inactivated X chromosomes are transcriptionally silenced and all the epigenetic signs of transcriptionally inactive chromatin can be found: DNA methylation on the CpG islands (regulatory elements in the promoter region of coding genes), reduced or absent histone acetylation, methylation patterns that are typical of the histone H3 and a variant of histone H2A, macroH2A1. One effect of heterochromatisation is the late replication of the inactivated X chromosome in the cell cycle, whereas the active X chromosome replicates early in the S phase.

1.5 Epigenetic regulation of X chromosome inactivation

An essential role in X chromosome inactivation is played by the *X inactivation centre* (XIC), found in the region of the centromere. If this region is deleted, the inactivation of the X chromosome does not take place. Located in the XIC region is the *XIST* gene which is expressed exclusively from the inactive X chromosome and codes for the *X inactive specific transcript*. The *XIST* transcript does not encode a protein; with its length – approx. 17 kb – it belongs to the category of macro non-coding (nc) RNAs. In early embryogenesis, the *XIST* transcript is expressed by both X chromosomes, but later, it is only expressed by the inactivated X chromosome. For the biological function of *XIST* ncRNA, the following mechanism is postulated: *XIST* ncRNA binds to the X chromosome and starts a series of events leading to the chromosome's inactivation. A kind of structure forms to which enzymes bind, such as those which methylate histone H3 at lysine 9 and 27 (histone methyltransferases) and deacetylate histone H4 (histone deacetylases). These mechanisms are part of epigenetic regulation.

The inactivation of one of the two X chromosomes is an essential process, at least in mice. Mouse mutants in which inactivation does not take place display strongly disrupted embryogenesis, which ultimately leads to the death of the embryo. X inactivation is subject to complex genetic control which also affects the expression and stability of *XIST* ncRNA. Disruptions can result in the inactivation of the paternal or maternal chromosome not taking place at random.

1.6 Genomic Imprinting

Another case of epigenetic regulation is genomic imprinting, in which paternal and maternal genes (located on the paternal/maternal chromosomes) are expressed differently even though they have the same nucleotide sequence. Fertilised ovules of mice which, after manipulation, only possess maternal or paternal genomes die shortly before or after implantation in the uterus wall.

In the human genome, there are around 90 genes which are arranged in clusters of two or more genes, in which this parent-of-origin-specific inactivation of gene expression takes place. In imprinting too, epigenetic mechanisms play a role. Firstly, it involves ncRNAs of different sizes, which operate either through structure-forming functions in the chromatin (like the *XIST* transcript) or which are coupled to the transcription. Secondly, specific DNA regions (insulators) are involved, whose methylation status varies according to parental source [Pau 07].

Genes which are subject to imprinting are functionally haploid, i. e. only one of the two

copies is active. This means that they lack the protection normally afforded by the presence of two copies, whereby damage and inactivation of one of the two copies can be compensated, at least in part, by the second, still functioning copy. Males and females do not differ in terms of the genes that are subject to imprinting, or their regulation. In the event of DNA damage in the germline, however, due to imprinting the consequences for progeny may vary according to whether the paternal or maternal germline is affected.

(Details of the topic covered in this Section 1 can be found in the textbook by R. Knippers [Kni 06]).

2 The Issue and Possible Experimental Approaches

Epidemiological studies show a significantly lower spontaneous cancer incidence for females than for males [Rie 08]. Epidemiological studies also indicate that there may be sex-specific differences in radiation sensitivity (see Annex 1). For biological studies to underpin the epidemiological findings, suitable experimental models must be used which, assuming sufficient scope of the experiment, can provide clear evidence of the postulated sex-specific radiation sensitivity. Apart from possible genetic and epigenetic control, other factors, especially hormones, may be influential. Animal models in which cancer incidence and, if appropriate, other endpoints can be analysed as indicators of radiation sensitivity therefore appear to be a suitable method of identifying these various factors. Indeed, mouse models have been successfully established, allowing the various analyses to be carried out under standardised conditions (see Section 3).

In order to obtain information about the mechanisms underlying the postulated sex-specific radiation sensitivity, cell models have also been used alongside the animal models. Human peripheral blood cells from healthy subjects were also irradiated and analysed, as were human cell lines or cells from selected organs of the mouse models, which were developed to provide evidence of sex-specific radiation sensitivity and treated in accordance with appropriate radiation protocols. Cellular responses to irradiation, e.g. DNA repair, cell death and unlimited proliferation as well as gene expression were investigated [Bor 09].

In some cases, whole genome screening processes were used, such as microarrays to analyse gene expression (quantification of transcript levels) and various techniques to analyse epigenetic regulatory mechanisms, such as DNA and histone methylation patterns and expression of microRNAs (miRNA). The miRNAs are endogenous single-stranded RNA molecules which, unlike the macro ncRNAs mentioned above, are very short (19-21 nucleotides in length). MiRNAs are now thought to control the activity of approximately 30% of the genes in the mammalian genome. Through their sequence homology, miRNAs appear to bind to messenger RNAs (mRNA) and prevent their translation. In this way, the expression of 10-100 genes can be regulated simultaneously. It is thought that in the human genome, thousands of miRNAs are expressed, but to date, only around 500 have been described (for an overview, see articles [Mac 07, Tan 08, Gol 07]).

Epigenetic regulatory factors are an extremely interesting topic in the context of sex-specific radiation sensitivity, in terms of the following aspects. The question which arises is whether in humans, X inactivation, which is controlled by around 90 genes, or imprinting processes can be affected by radiation or genotoxic agents. In fact, there is very good evidence that low and in some cases chronic doses of radiation influence gene expression via epigenetic mechanisms [Kov 08b, Kov 08c]. These potential interactions have yet to be studied in detail.

3 Animal Models

Animal models confirm and expand the evidence obtained from the epidemiological studies. For example, Swiss female mice exposed to an acute dose (3 Gy) of whole body irradiation developed a significantly higher incidence of thymic lymphoma (83%) after three to four weeks of exposure than male mice which received the same treatment (72%); the growth of tumour was also more aggressive in the female mice. The difference was far more pronounced in mice aged 12-13 weeks: 74% of the female mice developed thymic lymphoma but only 14% of the male animals did so [Dan 07], which could be due to the influence of hormonal status. The osteosarcoma incidence after treatment with ^{227}Th (4 Gy) was between 25-60% in female mice, whereas in males it was 10-30% [Mue 78]. By contrast, after chronic exposure to equal doses of UVB, male mice developed squamous cell carcinoma (SCC) earlier and had more tumours than female mice; tumours in male mice tended to be larger, and the total tumour burden was greater than in females, with a poorer prognosis for the male mice compared with the females [Tho 07]. In relation to sensitivity to chemical carcinogens, too, in a meta-analysis of 1 394 experiments, sex-specific differences were found in the incidence of organ-specific cancers. For example, 25 different chemicals induced kidney tumours in male rats, but only 9 substances did so in females [Huf 91].

Very many of the studies mentioned below, which include cellular and molecular analyses of sex-specific effects of radiation, were carried out under standardised conditions by the research group headed by O. Kovalchuk. C57/BL6 mice were used; depending on the experiment, the animals were 45-60 days old at the time of irradiation, had identical body weight and were irradiated according to standardised protocols. “Chronic” whole body irradiation was simulated using 10 applications of 5cGy/day X-ray irradiation, with an acute dose of 50 cGy being administered once on the 10th day. Control animals underwent corresponding simulated irradiation [Bes 05, Cas 06, Iln 08, Iln 09, Tam 08b].

The same group also developed a radiation protocol in order to investigate the bystander¹ effect *in vivo*. C57/BL6 mice were subjected to head exposure (1 Gy) when the rest of the body was protected by a shield (controls received 1 Gy of whole body irradiation) and the effects on the spleen were analysed [Kot 08a, Kot 08b, Kot 08c]. Similar experiments have also been carried out on rats (Long Evans, 3 months old) [Kot 07, Tam 08a].

4 Cellular Responses to Radiation

Cellular responses to radiation are highly complex and involve a large number of molecular processes, such as (1) changes in gene expression, (2) signal transduction chains, which can be initiated both by DNA damage and by conformational change in the receptors found in the membrane, (3) repair processes, by means of which a great deal of DNA damage can be removed or functionally tolerated, (4) cell proliferation, and (5) responses such as cell cycle arrest and programmed cell death (apoptosis) (for an overview, see [SSK 08]). In principle, sex-specific differences in these processes and other physiological mechanisms such as hormonal factors and viral infections, and in some cases differences in lifestyle could cause a sex-specific reaction to radiation in the whole organism. However, as a very large number of

¹ The authors themselves call the observed effects in organs which were not irradiated “bystander effects”. Similar effects were previously known as abscopal effects, whereas the term “bystander effect” was mainly used to describe effects in non-irradiated cells in cell cultures or in three-dimensional cell-culture systems.

genes are involved in these various mechanisms, the use of whole genome screening processes would appear to be sensible, but are not often carried out as yet, probably due to the high costs involved.

4.1 Quantification of gene expression (microarray studies)

Gene expression as a marker for interindividual variation has been investigated in numerous studies using peripheral blood and cell cultures as well as animal models with microarrays, and these procedures are increasingly acquiring clinical relevance for therapy design [Whi 03, Mea 08]. Possible sex-specific differences are therefore only one of many parameters in the analyses. Whitney et al. [Whi 03] surveyed variation in gene expression patterns in peripheral blood from 75 healthy volunteers by using microarrays and were able to identify sex-specific patterns of gene expression. While many encode on the X or Y chromosome, a clear sex-specific bias was also observed for some autosomal genes, e. g. for genes which play a role in interferon response and possibly in autoimmune diseases. Meadows et al. [Mea 08] describe specific gene expression profiles which enable irradiated patients (1.5 or 2 Gy whole body irradiation for transplant conditioning) to be distinguished from unirradiated persons. In studies with mice, these authors also found evidence that gene expression profiles specific to radiation exposure differ in many genes in males and females [Mea 08].

There are other studies in which gene expression profiles in mice in response to radiation have been analysed; they include comparisons of acute and chronic exposure to low-dose radiation (LDR) [Bes 05, Kov 04]. Using the C57/BL6 mouse model presented in Section 3 above, the research group of Kovalchuk and co-workers has investigated radiation-induced gene expression after acute (50 cGy whole body dose, administered once) and chronic (5 cGy whole body dose/day, administered over 10 days) X-ray irradiation of male and female mice. Muscle, spleen and liver tissue was analysed using murine Affimetrix microassays and the findings from selected candidate genes were checked using RT-PCR and Western blot. There was found to be a remarkably large number of statistically proven sex-specific differences in radiation-induced changes in gene expression, particularly in genes which code factors for signal chains (growth factors and growth-factor receptors, cytoplasmic serine/threonine protein kinases, G proteins etc.) and in genes for cell-cell interactions. Significant changes in gene expression were found more often in male tissue, after both acute and chronic irradiation (examples *Smarcf*, *Cox6c*), whereas in female mice, little or no changes were found in most cases. On the other hand, the gene for the transcription initiation factor *Eif1* was strongly induced in female mice (8-fold after acute and 10-fold after chronic irradiation), whereas in male mice, it was clearly down-regulated [Kov 04]. Increased expression of the *Eif1* gene in female mice has been found even without irradiation, however [Whi 03]. In another study [Cas 06], with a study design similar to that described by Kovalchuk et al. 2004 [Kov 04], the in male mice clearly increased, but in females significantly decreased expression of the RBBP9 protein (retinoblastoma binding protein 9) in spleen tissue was analysed as to their impact on the phosphorylation status of the retinoblastoma protein as well as on apoptosis and cell proliferation. Differences between male and female animals and also between acute and chronic radiation were observed. As with the other whole genome analyses of gene expression, the possible relevance of these findings to the issue of sex-specific radiation sensitivity in humans remains uncertain, however, as it is still unclear how the identified changes in expression influence tumour formation.

4.2 Epigenetic regulation of gene expression

Epigenetic regulation of gene expression takes place (1) through DNA methylation of CpG islands, i. e. local clustering of sequences of cytidines (C) and guanines (G) in the promoter region of genes, (2) through histone modification (3), and through miRNA-related inhibition

of transcript translation.

Hypomethylation of CpG islands leads to activation, and hypermethylation leads to the silencing of expression of the relevant genes. Radiation (and many other agents) alters methylation patterns and, in consequence, causes changes in gene expression. In a study whose design was similar to that described above, after chronic exposure to radiation, female mice were found to have more strongly reduced whole genome methylation than male mice, and this was also found to be tissue-dependent [Kov 04, Pog 04, Sil 04]. A correlation to sex-specific tumour incidence, which has been shown in epidemiological studies (see Chapter 2 and Annex 1) is not apparent, however. Thus whole genome methylation in the liver of female mice is significantly higher than in male mice [Pog 04]; however, according to Table A4 and Table A11, there is no difference in the ERR for radiation-induced liver cancer in males and females. It should also be noted here that the relevance of molecular parameters such as expression profiles of genes or whole genome methylation patterns has not been clarified in terms of our understanding of radiation sensitivity. Problems include the different model systems used by various groups, and, where relevant, the differences between humans and mice in their cellular and organism responses to radiation. Above all, however, we do not yet have enough information available about the significance of individual genes in the formation of spontaneous and radiation-induced cancers.

MicroRNAs (miRNAs) – short, single-stranded non-coded RNAs – are important regulators of gene expression. They also play a role in embryogenesis and germ cell maturation, and there is increasingly good evidence that cellular miRNAs are influenced qualitatively and quantitatively by genotoxic stress from chemicals and radiation [Kot 08a, Kot 08c, Kov 08a]. However, their role in spontaneous and radiation-induced tumour genesis has also not been sufficiently analysed and is therefore not yet understood.

4.3 Signal transduction

In whole-genome gene expression analyses, it is apparent that sex-specifically expressed proteins can often be assigned to the signal transduction network [Bes 08, Kov 04]. In this network, the receptor for the epidermal growth factor (EGFR) located in the membrane has a very important key function. The EGFR is responsible for responses to various growth factors and to exogenous stress factors such as radiation, and for the subsequent regulation of cell growth, differentiation and cell death. In combined radio- and chemotherapy, overexpression of the EGFR, which is often found in tumours, leads to the development of resistance which can be controlled by EGFR inhibitors [Rod 07]. There are known to be mutations in the *EGFR* gene which occur more frequently in females than in males (18.5% vs. 8.6%; $p=0.0004$) and lead to better treatment outcomes in the persons concerned [Bor 09, Pao 04, Bel 05]. The EGFR receptor is therefore a very good example of how molecular findings can help to improve our understanding of sex-specific aspects of radiation sensitivity.

4.4 DNA repair

It is generally accepted that radiation-induced DNA damage, its extent and the efficiency and quality of its repair is a key causal factor in determining whether cell death or cell survival occurs. It is therefore reasonable to assume that sex-specific radiation sensitivity is a causal factor in the different extents of DNA damage and/or differences in the expression of repair genes, and consequently in repair processes of varying effectiveness. The data situation is highly contradictory, however, and indeed unsatisfactory given the numerous studies undertaken. In contrast to the clear sex-specific differences in expression of genes involved in signal transduction, gene expression analyses of DNA repair genes show virtually no differences in radiation-induced expression in muscle tissue of male and female mice [Kov

08c].

For the quantity of DNA damage (chromosomal breakage, micronuclei, DNA breaks measured in the comet assay, and double-strand breaks (DSBs) measured with γ H2AX foci assay etc.), which was analysed after irradiation of mice and irradiation of human cells in culture using a variety of methods, no trend towards sex-specific differentiation could be observed. The reasons may lie in the strong heterogeneity of the cohorts, with no account being taken of possible influential factors (confounders) such as age, smoking, alcohol consumption etc. [Jos 04, Kot 06a, Kot 06b, Maf 02, Maf 04, Mar 03, May 91, Mul 01, Pog 04, Sor 00, Thi 00, Wan 00]. Studies of radiation sensitivity of human diploid fibroblasts under standardised conditions also displayed no sex differences, however [Cox 80, Lit 88].

By contrast, UVB-treated male mice showed significantly more oxidative DNA damage than females and lower antioxidant levels [Tho 07]. An increase of radiation-induced chromosome breakage was observed in lymphocytes of pregnant mice [Ric 91]. Similar findings have been obtained for pregnant women, with analyses showing a very strong correlation between the amount of pregnancy hormones, progesterone in particular, and the number of breaks. The breakage rate returned to that of non-pregnant women after the birth [Ric 97]. Females on therapeutic oestrogen had significantly elevated plasma cortisol and suppression of DNA repair capacity [O'Bri 93]. These findings indicate that sex hormones have an important role to play in terms of our understanding of sex-specific differences in radiation sensitivity (see also Chapters 5 and 6). This confirms the assumption, already mentioned, that animal experiments are needed to investigate the issue of sex-specific radiation sensitivity. It is understandable that the experiments mentioned above using cell models have not found any sex-specific differences in the development/repair of DNA damage.

4.5 Cell proliferation and apoptosis

In two studies, human lymphocytes [Schm 03] and murine spleen cells [Cas 06] were analysed to investigate sex-specific differences in apoptosis and cell proliferation after irradiation with X-rays. Both studies describe greater sensitivity for apoptosis in male subjects, although in mice, this did not affect proliferation [Cas 06]. UVB irradiation caused a greater inflammatory response in female mice than in males; however, as the rate of skin cancer (non-melanoma) was significantly higher in male animals than in females, inflammatory response obviously does not play a part in sex-specificity ([Tho 07]. This too is an example of how the findings for different endpoints, in this case inflammatory response vs. formation of skin tumours, are contradictory.

Summing up, using modern screening processes, a large number of genes have been identified which show sex-specific differences in expression in response to radiation. These findings enable targeted experiments to be conducted in order to improve our understanding of potentially sex-specific *cellular* responses to radiation. Based on the findings relating to cellular functions such as cell proliferation and survival, DNA repair etc., the statements that can be made about sex-specificity in radioreaction are far more equivocal than those resulting from gene expression analyses, and are in some cases highly contradictory.

5 Bystander Effect and Sex-specific Radiation Sensitivity

The “bystander effect” is defined as the induction of radiation effects in cells (known as bystander cells) that are not irradiated but are in close proximity to cells that are. In these unirradiated cells, destabilisation of the genome and other phenotypic reactions may occur, similar to those found in directly irradiated cells. These bystander effects have been demonstrated in cell cultures, 3D cell models and, recently, *in vivo*. Kovalchuk and her co-

workers have developed mouse and rat models in which, after local irradiation of the head with X-rays, the bystander effects can be demonstrated in the gonads, which were shielded from the radiation, in progeny, and particularly in the spleen [Kot 06a, Kot 07, Kot 08a, Kot 08c, Iln 08]. It is apparent that the spleen plays a key role in the bystander effect *in vivo* [Kot 08]. X-ray irradiation caused clear epigenetic deregulation in the bystander tissue of the spleen and led to a significant reduction in global DNA methylation, down-regulation of key proteins which regulate histone methylation patterns and chromatin density as well as to significantly increased expression of microRNAs, which play an important role in the silencing of genes/chromatin. All these reactions lead to active chromatin and hence to activation of genes such as LINE-1 retroposons, whose activation through transposition leads to genome destabilisation, and of other genes whose activation can contribute to radiation-induced carcinogenesis.

By means of selective irradiation of the head of their C57/BL6 mouse model, Koturbash et al. provide evidence that the bystander effect differs significantly in the spleen of male mice compared with females [Kot 08a]. They analysed DNA breaks, global DNA methylation and cellular parameters such as apoptosis and cell proliferation found in distant spleen tissue that had been completely shielded during irradiation, and were able to provide evidence of clear sex-specific differences. More DNA breaks and a reduction of DNA methylation were found in male mice, whereas in female mice, apoptosis rates were higher than in male animals. These differences were far less pronounced when animals whose gonads had been removed were treated. These findings clearly point to a role of sex hormones in the bystander effect, or to the influence of the bystander effect on sex-specific differences in radiation sensitivity.

This raises the question of the nature of the bystander signal *in vivo* and the molecular mechanisms which lead to a sex-specific reaction. One possible explanation is that part of the blood cells which receive a certain dose of radiation during cranial irradiation then undergo apoptosis or necrosis and produce various soluble factors such as cytokine and small RNAs which could act as bystander signals [Kot 07]. However, these findings are not consistent with those of Koturbash et al., who describe a higher apoptosis rate in the spleen affected by the bystander effect in female compared with male mice, but a lower bystander effect for the induction of DNA breaks and the reduction of global DNA methylation for female compared with male mice [Kot 08a, Kot 08b, Kot 08c].

The data on localised cranial irradiation [Kot 08a, Kot 08b, Kot 08c] and earlier publications by the Kovalchuk research group which analysed animals after whole body irradiation [Kov 04, Pog 04, Rai 04] have provided conclusive evidence that male mice react with greater sensitivity to radiation and show a stronger bystander effect in spleen for various factors such as DNA breaks and various epigenetic parameters. These data prompt the authors to hypothesise that male mice, due to the indirect radiation effect mediated by the bystander effect, should have a higher tumour incidence after irradiation than females [Kot 08a]. However, this would conflict with the findings of epidemiological studies in humans.

6 Hormonal Factors

The findings of Koturbash et al. [Kot 08a] presented in Chapter 5 showed, using selective cranial irradiation of C57/BL6 mice, that the bystander effect differs significantly in the spleen of male mice compared with females. These differences are greatly reduced if animals whose gonads have been removed after birth are treated, clearly indicating that sex hormones play a role in the bystander effect, or that the bystander effect influences sex-specific differences in radiation sensitivity. At present, however, no statements can be made about possible molecular mechanisms.

This raises the question of further studies on animal models and in humans which may provide indications of hormone influences on sex-specific radiation sensitivity. It is well-known that circulating hormones, especially sex steroids and peptide hormones secreted from the pituitary gland, influence various cellular activities. For that reason, hormones can certainly be regarded as possible causes of sex-specific differences in cellular responses to ionising radiation. However, no *systematic* studies are available about the influence of the endocrine system on radioreaction. The existing information is limited and relates only to breast cancer and the role of oestrogens.

The carcinogenic effect of oestrogens has been demonstrated in epidemiological studies which have provided substantial evidence that breast cancer risk is associated with prolonged exposure to female hormones: early onset of menarche, late menopause, hormone replacement therapy and postmenopausal obesity are associated with greater breast cancer incidence [Rus 98]. By contrast, breast glands atrophy in the absence of oestrogens and thus become resistant to carcinogenic agents [Rus 98].

Treatment with natural or synthetic oestrogens greatly increased the induction of breast cancer from ionising radiation in rats [Ina 00]. At present, however, there is insufficient evidence of similar responses in humans. One epidemiological study has indeed established a link between inheritance of a polymorphic variant of the *CYP1B1* gene, which plays a role in oestrogen metabolism, and increased incidence of breast cancer among US radiologic technologists who were exposed to low doses of radiation [Sig 09].

It is still unclear how oestrogens exert a molecular function in increasing cancer risk. One possible explanation is that by stimulating proliferation, the effect of oestrogen on epithelial cells in the mammary glands could increase the production of reactive oxygen and therefore cause oxidative damage. However, it has also been shown that oestrogens have direct genotoxic effects [Miz 04, Schn 09]. Paradoxically, it has also been shown that oestrogens stimulate productive mechanisms for DNA, such as phosphorylation of the DNA repair protein BRCA2 [Mal 09], thereby reducing radiation-induced mutagenesis [Puc 99] and p53-dependent apoptosis [Dun 08]. These findings may explain the observed protective effect of oestrogens in certain types of cancer, such as basal cell carcinoma and squamous cell carcinomas of the skin [Man 09].

References

- [Bel 05] Bell, D. W. et al.: Epidermal growth factor receptor mutations and gene amplification in non-small-cell lung cancer: molecular analysis of the IDEAL/INTACT gefitinib trials. *J Clin Oncol* 23(31):8081-92, 2005
- [Bes 05] Besplug, J. et al.: Sex and tissue-specific differences in low-dose radiation-induced oncogenic signaling. *Int J Radiat Biol* 81(2):157-168, 2005
- [Bor 09] Borgmann, K. et al.: Sex-specific aspects of tumor therapy. *Radiat Environ Biophys* 48(2):115-124, 2009
- [Cas 06] Cassie, S. et al.: Novel retinoblastoma binding protein RBBP9 modulates sex-specific radiation responses in vivo. *Carcinogenesis* 27(3):465-474, 2006
- [Cox 80] Cox, R. and Masson, W. K.: Radiosensitivity in cultured human fibroblasts. *Int J Radiat Biol Relat Stud Phys Chem Med* 38(5):575-576, 1980
- [Dan 07] Dange, P. et al.: Radiation-induced incidence of thymic lymphoma in mice and its prevention by antioxidants. *J Environ Pathol Toxicol Oncol* 26(4):273-279, 2007

- [Dun 08] Dunphy, K. A. et al.: Estrogen and progesterone induce persistent increases in p53-dependent apoptosis and suppress mammary tumors in BALB/c-Trp53^{+/+} mice. *Breast Cancer Res* 10(3):R43, 2008
- [Gol 07] Goldberg, A. D.; Allis, C. D.; Bernstein, E.: Epigenetics: a landscape takes shape. *Cell* 128(4):635-638, 2007
- [Huf 91] Huff, J. et al.: Chemicals associated with site-specific neoplasia in 1394 long-term carcinogenesis experiments in laboratory rodents. *Environ Health Perspect* 93:247-270, 1991
- [Ina 00] Inano, H. et al.: Radiation-induced mammary tumors in virgin and parous rats administered contraceptive steroids, 17alpha-ethinylestradiol and norethisterone. *Carcinogenesis* 21(5):1043-1050, 2000
- [Iln 08] Ilnytsky, Y. et al.: Altered microRNA expression patterns in irradiated hematopoietic tissues suggest a sex-specific protective mechanism. *Biochem Biophys Res Commun* 377(1):41-45, 2008
- [Iln 09] Ilnytsky, Y.; Koturbash, I.; Kovalchuk, O.: Radiation-induced bystander effects in vivo are epigenetically regulated in a tissue-specific manner. *Environ Mol Mutagen* 50(2):105-113, 2009
- [Jos 04] Joseph, L. J.; Patwardhan, U. N.; Samuel, A. Met al.: Frequency of micronuclei in peripheral blood lymphocytes from subjects occupationally exposed to low levels of ionizing radiation. *Mutat Res* 564(1):83-88, 2004
- [Kni 06] Knippers, R.: *Molekulare Genetik*. Vol. 9. Stuttgart: Thieme Verlag, 2006
- [Kot 06a] Koturbash, I. et al.: Irradiation induces DNA damage and modulates epigenetic effectors in distant bystander tissue in vivo. *Oncogene* 25(31):4267-4275, 2006
- [Kot 06b] Koturbash, I. et al.: Epigenetic dysregulation underlies radiation-induced transgenerational genome instability in vivo. *Int J Radiat Oncol Biol Phys* 66(2):327-330, 2006
- [Kot 07] Koturbash, I. et al.: Role of epigenetic effectors in maintenance of the long-term persistent bystander effect in spleen in vivo. *Carcinogenesis* 28(8):1831-1838, 2007
- [Kot 08a] Koturbash, I. et al.: Radiation-induced bystander effects in vivo are sex specific. *Mutat Res* 642(1-2):28-36, 2008
- [Kot 08b] Koturbash, I. et al.: In vivo bystander effect: cranial X-irradiation leads to elevated DNA damage, altered cellular proliferation and apoptosis, and increased p53 levels in shielded spleen. *Int J Radiat Oncol Biol Phys* 70(2):554-562, 2008
- [Kot 08c] Koturbash, I. et al.: Sex-specific microRNAome deregulation in the shielded bystander spleen of cranially exposed mice. *Cell Cycle* 7(11):1658-1667, 2008
- [Kov 04] Kovalchuk, O. et al.: Dissimilar genome response to acute and chronic low-dose radiation in male and female mice. *Mutat Res* 550(1-2): 59-72, 2004
- [Kov 08a] Kovalchuk, O. et al.: Involvement of microRNA-451 in resistance of the MCF-7 breast cancer cells to chemotherapeutic drug doxorubicin. *Mol Cancer Ther* 7(7):2152-2159, 2008
- [Kov 08b] Kovalchuk, O.: Epigenetic research sheds new light on the nature of interactions between organisms and their environment. *Environ Mol Mutagen* 49(1):1-3, 2008

- [Kov 08c] Kovalchuk, O. and Baulch, J. E.: Epigenetic changes and nontargeted radiation effects – is there a link? *Environ Mol Mutagen* 49(1):16-25, 2008
- [Lit 88] Little, J. B. et al.: Survival of human diploid skin fibroblasts from normal individuals after X-irradiation. *Int J Radiat Biol* 54(6):899-910, 1988
- [Mac 07] Mack, G. S.: MicroRNA gets down to business. *Nat Biotechnol* 25(6):631-638, 2007
- [Maf 02] Maffei, F. et al.: Micronuclei frequencies in hospital workers occupationally exposed to low levels of ionizing radiation: influence of smoking status and other factors. *Mutagenesis* 17(5):405-409, 2002
- [Maf 04] Maffei, F. et al.: Spectrum of chromosomal aberrations in peripheral lymphocytes of hospital workers occupationally exposed to low doses of ionizing radiation. *Mutat Res* 547(1-2):91-99, 2004
- [Mal 09] Malone, J. L. et al.: Oestrogen-mediated phosphorylation and stabilization of BRCA2 protein in breast. *J Pathol* 217(3):380-388, 2009
- [Man 09] Mancuso, M.; Gallo, D.; Leonardi, S. et al.: Modulation of basal and squamous cell carcinoma by endogenous estrogen in mouse models of skin cancer. *Carcinogenesis* 30(4):721, 2009
- [Mar 03] Marcon, F. et al.: Assessment of individual sensitivity to ionizing radiation and DNA repair efficiency in a healthy population. *Mutat Res* 541(1-2): 1-8, 2003
- [May 91] Mayer, P. J. et al.: Gender differences in age-related decline in DNA double-strand break damage and repair in lymphocytes. *Ann Hum Biol* 18(5):405-415, 1991
- [Mea 08] Meadows, S. K. et al.: Gene expression signatures of radiation response are specific, durable and accurate in mice and humans. *PLoS ONE* 3(4):e1912, 2008
- [Miz 04] Mizutani, A. et al.: Extensive chromosomal breaks are induced by tamoxifen and estrogen in DNA repair-deficient cells. *Cancer Res* 64(9):3144-3147, 2004
- [Mue 78] Mueller, W. A.: Late effects after incorporation of the short-lived alpha-emitters ^{224}Ra and ^{227}Th in mice. *Health Phys* 35(1):33-55, 1978
- [Mul 01] Muller, W. U. et al.: Radiation sensitivity of lymphocytes from healthy individuals and cancer patients as measured by the comet assay. *Radiat Environ Biophys* 40(1):83-89, 2001
- [O'Bri 93] O'Brien, S. N.; Larcom, L. L.; Baxley, E. G et al.: Correlates of plasma cortisol and DNA repair in human peripheral lymphocytes: suppression of repair in women taking estrogen. *Horm Res* 39(5-6):241-246, 1993
- [Pao 04] Pao, W. et al.: EGF receptor gene mutations are common in lung cancers from "never smokers" and are associated with sensitivity of tumors to gefitinib and erlotinib. *Proc Natl Acad Sci USA* 101(36): 13306-13311, 2004
- [Pau 07] Pauler, F. M.; Koerner, M. V.; Barlow, D. P.: Silencing by imprinted noncoding RNAs: is transcription the answer? *Trends Genet* 23(6):284-292, 2007
- [Pog 04] Pogribny, I. et al.: Dose-dependence, sex- and tissue-specificity, and persistence of radiation-induced genomic DNA methylation changes. *Biochem Biophys Res Commun* 320(4):1253-1261, 2004

- [Puc 99] Puck, T. T.; Johnson, R.; Webb, P.: Mutation inhibition by beta-estradiol after low doses of gamma-irradiation of mammalian cells. *Somat Cell Mol Genet* 25(2):59-65, 1999
- [Rai 04] Raiche, J. et al.: Sex- and tissue-specific expression of maintenance and de novo DNA methyltransferases upon low dose X-irradiation in mice. *Biochem Biophys Res Commun* 325(1):39-47, 2004
- [Ric 91] Ricoul, M. and Dutrillaux, B.: Variations of chromosome radiation sensitivity in fetal and adult mice during gestation. *Mutat Res* 250(1-2):331-335, 1991
- [Ric 97] Ricoul, M.; Sabatier, L.; Dutrillaux, B. et al.: Increased chromosome radiosensitivity during pregnancy. *Mutat Res* 374(1): 73-78, 1997
- [Rie 08] Ries, L. A. G. et al.: SEER Cancer Statistics Review, 1975-2005. National Cancer Institute: Bethesda, MD, 2008
- [Rod 07] Rodemann, H. P.; Dittmann, K.; Toulany, M.: Radiation-induced EGFR-signaling and control of DNA-damage repair. *Int J Radiat Biol* 83(11-12):781-791, 2007
- [Ros 05] Ross, M. T. et al.: The DNA sequence of the human X chromosome. *Nature* 434(7031):325-337, 2005
- [Rus 98] Russo, I. H. and Russo, J.: Role of hormones in mammary cancer initiation and progression. *J Mammary Gland Biol Neoplasia* 3(1):49-61, 1998
- [Schm 03] Schmitz, A. et al.: Intrinsic susceptibility to radiation-induced apoptosis of human lymphocyte subpopulations. *Int J Radiat Oncol Biol Phys* 57(3):769-778, 2003
- [Schn 09] Schnyder, S. et al.: Estrogen treatment induces MLL aberrations in human lymphoblastoid cells. *Leuk Res*, 2009.
- [Sig 09] Sigurdson, A. J. et al.: Polymorphisms in estrogen biosynthesis and metabolism-related genes, ionizing radiation exposure, and risk of breast cancer among US radiologic technologists. *Breast Cancer Res Treat*, 2009
- [Sil 04] Silasi, G. et al.: Selective brain responses to acute and chronic low-dose X-ray irradiation in males and females. *Biochem Biophys Res Commun* 325(4):1223-1235, 2004
- [Ska 03] Skaletsky, H. et al.: The male-specific region of the human Y chromosome is a mosaic of discrete sequence classes. *Nature* 423(6942):825-837, 2003
- [Sor 00] Sorensen, K. J. et al.: The in vivo dose rate effect of chronic gamma radiation in mice: translocation and micronucleus analyses. *Mutat Res* 457(1-2):125-136, 2000
- [SSK 08] Strahlenschutzkommission (SSK): Einfluss der natürlichen Strahlenexposition auf die Krebsentstehung in Deutschland. Veröffentlichungen der Strahlenschutzkommission, Band 62. Stellungnahme der Strahlenschutzkommission mit wissenschaftlicher Begründung, H. Hoffmann GmbH - Fachverlag, Berlin, 2008
- [Tam 08a] Tamminga, J. et al.: Paternal cranial irradiation induces distant bystander DNA damage in the germline and leads to epigenetic alterations in the offspring. *Cell Cycle* 7(9):1238-1245, 2008

- [Tam 08b] Tamminga, J. et al.: DNA damage-induced upregulation of miR-709 in the germline downregulates BORIS to counteract aberrant DNA hypomethylation. *Cell Cycle* 7(23): 3731-3736, 2008
- [Tan 08] Tang, G.: MicroRNAs: an exciting and open field calls for extensive study from initial and established investigators. Preface. *Biochim Biophys Acta* 1779(11):653-654, 2008
- [Thi 00] Thierens, H. et al.: Cytogenetic monitoring of hospital workers occupationally exposed to ionizing radiation using the micronucleus centromere assay. *Mutagenesis* 15(3):245-249, 2000
- [Tho 07] Thomas-Ahner, J. M. et al.: Gender differences in UVB-induced skin carcinogenesis, inflammation, and DNA damage. *Cancer Res* 67(7):3468-3474, 2007
- [Wan 00] Wang, L. E. et al.: Gender difference in smoking effect on chromosome sensitivity to gamma radiation in a healthy population. *Radiat Res* 154(1):20-27, 2000
- [Whi 03] Whitney, A. R. et al.: Individuality and variation in gene expression patterns in human blood. *Proc Natl Acad Sci USA* 100(4):1896-1901, 2003