EUROPEAN COMMISSION

RADIATION PROTECTION N° 171

EU Scientific Seminar 2011

"Individual radiosensitivity"

Proceedings of a scientific seminar held in Luxembourg on 22 November 2011

Working Party on Research Implications on Health and Safety Standards of the Article 31 Group of Experts

Directorate-General for Energy
Directorate D — Nuclear Safety and Fuel Cycle
Unit D4 — Radiation Protection
2012

Europe Direct is a service to help you find answers to your questions about the European Union

Freephone number (*):

00 800 6 7 8 9 10 11

(*) Certain mobile telephone operators do not allow access to 00 800 numbers or these calls may be billed.

More information on the European Union is available on the Internet (http://europa.eu).

Luxembourg: Publications Office of the European Union, 2012

ISBN 978-92-79-25858-9
doi: 10.2833/14386

Printed in Luxembourg

FOREWORD

Luxembourg, May 2012

Under the terms of the Treaty establishing the European Atomic Energy Community, the Community, amongst other things, establishes uniform safety standards to protect the health of workers and of the general public against the dangers arising from ionizing radiation. The standards are approved by the Council, on a proposal from the Commission, established taking into account the opinion of the Group of Experts referred to in Article 31 of the Treaty. The most recent version of such standards is contained in Council Directive 96/29/Euratom of 13 May 1996 laying down basic safety standards for the protection of the health of workers and the general public against the dangers arising from ionizing radiation.

The European Commission organises every year, in cooperation with the Group of Experts referred to in Article 31 of the Euratom Treaty, a Scientific Seminar on emerging issues in Radiation Protection – generally addressing new research findings with potential policy and/or regulatory implications. Leading scientists are invited to present the status of scientific knowledge in the selected topic. Based on the outcome of the Scientific Seminar, the Group of Experts referred to in Article 31 of the Euratom Treaty may recommend research, regulatory or legislative initiatives. The European Commission takes into account the conclusions of the Experts when setting up its radiation protection programme. The Experts' conclusions are valuable input to the process of reviewing and potentially revising European radiation protection legislation.

In 2011, the Scientific Seminar discussed *Individual radiosensitivity*. Internationally renowned scientists working in this field presented current knowledge on radiation sensitivity, genetic tools to address individual radiosensitivity and their limitations, genetic pathways for the prediction of radiation effects, potential of human genome sequencing, genetic signatures of radiation induced cancers, and ethical aspects of testing for individual radiosensitivity. The presentations were followed by a round table discussion, in which the speakers and invited additional experts discussed potential *policy implications and research needs*.

The Group of Experts discussed this information and drew conclusions that are relevant for consideration by the European Commission and other international bodies.

Augustin Janssens
Head of Radiation Protection Unit

CONTENTS

1 RADIATION SENSITIVITY – AN INTRODUCTION

Mike Atkinson

HelmholtzZentrum, München, Germany

1.1 Responses to ionizing radiation

The interaction of ionizing radiation with higher eukaryotes provokes a response that is both dose and time dependent. In the acute phase of the interaction localized damage to molecular components is produced by the deposition of energy. This occurs within nanoseconds and is followed by cellular damage response reactions aimed at restoring the lesions. Residual (unrepaired) damage can elicit an acute and prolonged local response at the tissue level, with cell death and local inflammation serving to repair radiation damage over hours and days. When normal repair and regeneration fails, the damage may compromise the integrity of the tissue, leading to a late chronic response characterized by wounding and local or systemic cytopenia. In severe situations this may result in infection, organ failure and even death. Even when the tissue level response appears to proceed without complications late pathological effects may develop that lead to serious long-term health effects. These may be the clonal growth of genetically transformed cells to form tumours, impairment of cerebro- and cardiovascular function, the development of lens opacities and cognitive impairment. Most commonly hypersensitivity is encountered in a small subset (around 1%) of cancer patients receiving radiation therapy, or in the even rarer group of individuals with a genetically determined failure of DNA repair (see below).

1.2 Individual variation in sensitivity to ionizing radiation

At each of these levels the extent to which an individual responds to a given dose of radiation is highly variable. Thus, although energy deposition itself is a finite physico-chemical event, the extent of the initial damage response, the subsequent cellular repair reactions, the tissue level responses and even the long-term health effects all differ between individuals. The responses are typical of the normal distribution seen for most biological parameters. Nevertheless, the extremes of the distribution curves are frequently interpreted as indicating an increased (or decreased) sensitivity to radiation (radiation hyper- and hypo-sensitivity) (Scott 2004). Classifications made in this way are somewhat arbitrary and subject to interpretation errors. No universally applicable rule is available that defines abnormal sensitivity, such as interpreting a value more than two standard deviations above of below the population mean as abnormal. Only for the acute and late clinical effects seen in radiation therapy patients are there clear guidelines for assessing the extent of an adverse reaction, leading to a quantifiable scoring of the severity of the sensitivity reaction.

1.3 Lack of a causal link between parameters quantifying the early damage response and the late effects of radiation

Individual variability has been demonstrated in the immediate damage response, where the level of DNA double strand breaks induced by radiation can vary considerably, with some individuals exhibiting greatly increased or decreased numbers of DNA damage repair foci (Andreassen et al 2002). Others may exhibit abnormal kinetics in the recovery from DNA damage, even retaining residual damage, or failing to remove damaged cells. This can be seen in the resolution of DNA repair foci or at later stages where extreme values in parameters such as G2 phase chromatid damage and micronuclei formation indicate altered sensitivity (Borgmann et al 2002, Huber et al 1989).

Whilst it would be logical to assume that an individual showing a greater than normal level of DNA damage would also show increased levels of residual damage this has not been demonstrated. Similarly, a correlation between unrepaired DNA damage and the later cellular parameters of radiation response are not reported. Most in vitro assays of hypersensitivity use lymphocytes as the model system. It remains to be seen if these cells have any relevance for non-lymphoid tissues, especially as many studies rely upon lymhoblastoid cells derived by infecting lymphocytes with the Epstein Barr virus. EBV subverts many cellular functions, including apoptosis and cell cycle progression, making infected cells a questionable model system.

1.4 An increased acute radiation sensitivity does not indicate elevated long-term susceptibility

Increased cancer risk (susceptibility), as a putative late consequence of radiation hypersensitivity is not associated with any parameter indicating sensitivity. Thus, suggestions that an increase in the extent of early effects may be indicative or even informative for subsequent susceptibility to late effects are not supported by available evidence. A good reason for this lack of a correlation is that parameters of cellular sensitivity are frequently assessed in peripheral leukocytes or skin fibroblasts. These are not target tissues involved in radiation carcinogenesis, so sensitivity to radiation in these tissues would not be expected to translate into susceptibility of those tissues (or even their stem cell components) where tumours are induced by radiation.

1.5 Susceptibility to radiation-induced cancer is multifactorial

A number of differences between individuals exert influence upon susceptibility to cancer. These include the chronological age of the individual at the time of exposure, where in particular developing or rapidly expanding tissues show disproportionate risks of cancer, as illustrated in both the A-Bomb survivors and Chernobyl victims. Gender too is an oft-overlooked predisposing factor determining long-term cancer susceptibility, with disproportionate rates of cancer evident between males and females. Although lifestyle factors such as diet, alcohol consumption and smoking affect cancer risk in general, little or no evidence is available to indicate if radiation exposure confers additional risk dependent upon lifestyle.

The role of lifestyle is well illustrated in epidemiological studies of migrant populations, in particular ethnic Japanese and Pacific islanders migrating to the USA. Generational studies

show that some cancer rates change from that of the country of origin to that of the new country. In particular, cancers whose aetiology is linked to dietary habits or infectious agents show the incidence of the new country within one generation (Ziegler et al 1993). Cancers of the breast and prostate show much slower changes in the rate, whilst some cancers such as those of the lung do not change from that seen in the original environment (Iwasaki 2004, Maskarinec & Noh 2004). Nevertheless, in no instance has it been demonstrated that such environmental factors have any influence over the susceptibility to radiation-induced cancers.

It will be interesting to observe the rates of radiation-associated cancers in the different ethnic populations from the same areas who were exposed to radioactivity from Chernobyl.

1.6 Genetic factors influence on susceptibility

Experience with the rare group of individuals suffering from inherited cancer syndromes reveals the genetic contribution to susceptibility to radiation-induced cancer. The presence of a germ-line mutation inactivating one allele of any of three tumour suppressor genes (Gorlin, Li-Fraumeni and Retnoblastoma syndromes) increases sporadic cancer incidence dramatically. Exposure of the affected individuals to ionizing radiation increases the cancer risk considerably (Mullenders et al 2009). Whilst other familial cancer syndromes may potentially confer an increased risk (e.g. CDKN2A) the effect of radiation is not confirmed due to the scarcity of cases.

Loss of DNA repair capacity is also linked to increased susceptibility to cancer (Berwick & Vineis 2000). These rare cases involve inheritance of two mutated alleles, most frequently lead to defective immune competence. The frequent appearance of leukemia in these individuals may be associated, in some cases, with radiation exposure, but the effect on risk of solid tumours is unclear due to the short life expectancy.

More problematic are the risks associated with heterozygous carriers of the DNA repair defects. The inheritance of only a single copy of the inactive repair gene may show a heightened risk of sporadic cancers (e.g. BRCA1 patients), but the effect of radiation exposure on the overall risk remains controversial.

The syndrome forms of cancer discussed above all represent the effects of single genes. All individuals inheriting the mutated copy show the susceptibility phenotype (high penetrance). An additional genetic contribution to susceptibility may come from the inheritance of common allelic variants present in any population. Genetic linkage studies have consistently indicated that the inheritance of these gene variants is associating with a very slight increase in the risk of developing sporadic cancers (as well as a number of other diseases such as diabetes, neurodegeneration and cardiovascular disease). Studies in cancer patients and in twin cohorts both indicate that the susceptibility to the DNA damage induced by radiation is influenced by multiple low penetrant genes (Scott 2004, Wu et al 2006). In a recent association study a possible link between allelic variants of DNA repair genes and acute cellular radiosensitivity concluded that mismatch repair genes showed the highest association (Mangoni et al 2011). However, no study has demonstrated such genetic influences on susceptibility extend to an influence on the risk of radiation-induced cancer in man. Nevertheless, the gastrointestinal cancers typically seen sporadically arising in mismatch repair deficiency syndromes are amongst those showing an elevated incidence in A-bomb survivors. A number of mouse studies have shown that variant genes may indeed make a significant contribution. Thus, the different susceptibility of inbred mouse strains to radiation-induced osteosarcoma and lymphoma (Santos 2010) has been shown to be influenced by the inheritance of allelic variants. In the case of osteosarcoma a set of at least

five genes is responsible for all of the observed difference in susceptibility between BALB/c and CBA strains (Rosemann et al 2006).

1.7 Relevance for radiation protection

The genetic arguments for including the majority of predisposed individuals within a scheme of radiation protection are to be separated from the ethical considerations. Thus, on a simple numerical basis, the number of individuals with an inherited tumour suppressor gene mutation predisposing to radiation-associated cancer is small. Estimates of the frequency within a population suggest only one case in tens of thousands. The more common mutations, leading to loss of DNA repair capacity, are associated with very poor prognosis, and a contribution of radiation to mortality may be insignificant.

More problematic are the individuals with a genetic mutation that may predispose to lifetime cancer risk (e.g. ATM, BRCA1). Here the risk of radiation exposure increasing cancer rate is uncertain, but a large number of individuals are potentially at risk. Even unclear is the situation for those individuals with no evident genetic deficiency, but who may inherit multiple variant genes each with a minor contribution. Animal studies show that these genes may make a significant contribution to risk.

Implementation of any protection measures directed at individual sensitivity requires the effective identification of any individuals at increased risk. As the causal genes are not yet identified there is considerable effort directed at using surrogate markers for sensitivity. Clearly these markers can identify individuals where the initial response to DNA damage is abnormal. However, at the time of this review there is no evidence to suggest that an increased sensitivity to damage responses translates into an increased sensitivity to cancer. The situation for non-cancer late effects is even less clear, as DNA damage mechanisms are not known to underlie the development of the disease. Moreover, no evidence for (or against) individual variability modifying the radiation response in non-cancer effects is known.

1.8 Future studies

Priority must be given to challenging the validity of the assumed link between early indicators of DNA damage responses and the risk of developing cancer or non-cancer late radiation effects.

Equally important is a continued effort to identify which low penetrant genes influence cancer susceptibility, but these studies should include efforts now to understand how these genes play a role in the mechanisms of carcinogenesis.

Some urgency should be given to establishing if the risks of developing non-cancer effects vary between individuals receiving comparable doses of radiation.

1.9 References

Andreassen CN, Alsner J, Overgaard J (2002) Does variability in normal tissue reactions after radiotherapy have a genetic basis—where and how to look for it? Radiother Oncol 64:131–140.

Berwick M, Vineis P.(2000) Markers of DNA repair and susceptibility to cancer in humans: an epidemiologic review. J Natl Cancer Inst 92: 874–97.

Borgmann K, Hoeller U, Nowack S et al (2008) Individual radiosensitivity measured with lymphocytes may predict the risk of acute reaction after radiotherapy. Int J Radiat Oncol Biol Phys 71:256–264.

Huber R, Braselmann H, Bauchinger M (1989) Screening for interindividual differences in radiosensitivity by means of the micronucleus assay in human lymphocytes. Radiat Environ Biophys 28:113–120.

Iwasaki M., Mameri CP., Hamada GS., Tsugane S. Cancer mortality among Japanese immigrants and their descendants in the state of Sao Paulo, Brazil, 1999-2001 (2004) Japn. J. Clin. Oncol 34:673-680.

Maskarinec G, Noh JJ. (2004) The effect of migration on cancer incidence among Japanese in Hawaii. Ethn. Diseases 14:431-9.

Mangoni M., Bisanzi S., Carozzi F., Sani C., Biti G., Livi L., Barletta E., Constantini AS., Gorini G. (2010) Association between genetic polymorphisms in the XRCC1, XRCC3, XPD, GSTM1, GSTT1, MSH2, MLH1, MSH3 and MGMT genes and radiisensitivity in breast cancer patients. Int J Radiat Oncol Biol Phys 81:52-8.

Mullenders L., Atkinson M.J., Paretzke H., Sabatier L., Bouffler S. (2009) Assessing cancer risks of low-dose radiation. Nature Rev Cancer. 9:596-604.

Rosemann M., Kuosaite V., Quintanilla-Martinez L., Richter T., Atkinson M.J. (2006) Multilocus inheritance determines predisposition to alpha-radiation induced bone tumorigenesis in mice Int J. Cancer 118:2132-2138.

Santos J., Gonzales-Sanchez L., Villa-Morales M., ors I., Lopez-Nieva P., Vaquero C., Ganzalez-Gugel E., Fernandez-Navorro P., Roncero AM., Guenet J-L., Montagutelli X., Fernandez-Piqueras J. (2010) The stromal gene encoding the CD274 antigen as a genetic modifier controlling survival of mice with gamma radiation-induced T-cell lymphoblastic lymphomas Oncogene 29:5265-73.

Scott D. (2004) Chromosomal radiosensitivity and low penetrance predisposition to cancer. Cytogenet Genome Res 104: 365–70.

Wu X., Spitz MR., Amos CI., Lin J., Shao L., Gu J., de Andrade M., Benowitz NL., Shields PG., Swan GE. (2006) Mutagen sensitivity has high heritability: Evidence from a twin study. Cancer Res 66:5993-97.

Ziegler RG, Hoover RN, Pike MC, Hildesheim A, Nomura AM, West DW, Wu-Williams AH, Kolonel LN, Horn-Ross PL, Rosenthal JF, Hyer MB. (1993) Migration patterns and breast cancer risk in Asian-American women. J Natl Cancer Inst 85:1819-1827.

2 GENETIC TOOLS TO ADDRESS INDIVIDUAL RADIOSENSITIVITY AND THEIR LIMITATIONS

Christian Nicolaj Andreassen, MD, PhD

Department of Oncology and Department of Experimental Clinical Oncology
Aarhus University Hospital, Denmark

2.1 Introduction

Radiotherapy may lead to severe acute or late side effects. Apart from causing pain and distress during treatment, acute normal tissue reactions can result in treatment interruptions or may develop into lasting consequential late damage. Late normal tissue reactions may cause chronic disability and compromise quality of life. Consequently, radiation induced normal tissue reactions constitutes a dose limiting factor in radiation therapy. Typically, radiotherapy regimens are designed to ensure that the risk of severe permanent effects does not exceed 5 to 10%. This basically means that a small fraction of radiosensitive patients limits the dose that can be given to the entire patient population, though the majority of patients could potentially tolerate a higher dose. Due to a relatively steep dose response relationship for tumor control, even a modest dose escalation would translate into a substantial increase in the chance of cure[1,2].

It has been estimated that more that 80% of the variability in normal tissue radiosensitivity can be attributed to patient-related factors rather than stochastic effects[3]. If this variability could be taken into account in the treatment planning phase, the therapeutic strategy could be individualized accordingly. Patients being relatively sensitive to the effects of ionizing radiation could (when possible) be offered a treatment strategy that does not include radiation therapy whereas the resistant patients could be dose escalated to some extent. This would lead to a substantial improvement in therapeutic index[2].

2.2 Genetic germline alterations as predictors of normal tissue radiosensitivity

In the late 1990'ies increasing interest was taken in the hypothesis that clinical normal tissue radiosensitivity is under genetic control and that normal tissue complication risk could be predicted from genetic analysis[4]. This concept received support from the observation that patients suffering from certain rare genetic syndromes such as ataxia telangiectasia, Blooms syndrome, Fanconi's anemia and Nijmegen breakage syndrome seem to experience devastating normal tissue reactions if treated with radiation therapy[2]. All these syndromes are related to mutations in genes involved in detection of DNA damage and initiation of DNA repair. Nevertheless, these syndromes characterized by Mendelian inheritance are extremely rare and probably of little relevance when addressing the average cancer patients. However, it was hypothesized that heterozygous carriers of pathogenic (truncating) mutations in ATM and BRCA 1 and 2 could constitute a radiosensitive subpopulation. This assumption was supported by the observation that cells from heterozygous carriers of ATM mutations exhibited cellular radiosensitivity that was intermediate compared to ataxia telangiectasia patients and normal controls. Even though heterozygous carriers of these mutations are

quite rare in general population (below 1 %), they may be more frequent among breast cancer patients due to their cancer predisposing effect. Nevertheless, a number of relatively small studies did not provide any indications that such genetic alterations are overrepresented among patients with excessive normal tissue reactions nor that carriers of truncating ATM or BRCA mutations exhibit a higher normal tissue complication risk than the average patient. Similarly, no obvious association was found between clinical normal tissue radiosensitivity and mutations in other DNA repair genes such as RAD50, RAD21, NBN or MRE11A. Nevertheless, the limited statistical power of these investigations should be taken into account in the interpretation of the results. A few studies have provided preliminary indications that patients with certain rare single base substitutions in the ATM gene may be more prone to adverse effects after radiation therapy[4].

2.3 SNPs and normal tissue radiosensitivity

Within the last decade, the efforts to unravel the genetics of normal tissue radiosensitivity have primarily focused on single nucleotide polymorphisms (SNPs)[5]. One reason for this is probably that this approach was in accordance with the quite influential 'common disease – common variant hypothesis'[6]according to which SNPs are assumed to constitute a substantial proportion of the genetic background underlying so called complex polygenic traits. Apart from this, the strategy certainly had an element of 'looking for the ring under the light post'. Rapid l assays for SNP genotyping had just been commercially available and comprehensive public SNP databases were developed at the turn of the millennium.

More than 50 studies have been carried out to establish associations between selected SNPs and risk of normal tissue complications after radiation therapy[2,5]. These studies have all been based on a so-called candidate gene approach[4] addressing genes involved in processes such as detection of DNA damage (i.e. ATM), DNA repair (i.e. XRCC1, XRCC3 and APEX), tissue remodelling (TGFB1 and TIMP) and scavenging of reactive oxygen species (i.e. SOD2 and GSTP1 and). More than 100 different genes have been investigated as part of this research. Remarkably, about 2/3 of these studies have reported significant associations between the assessed SNPs and various types of radiation induced normal tissue reactions[5]. Nevertheless, this does unfortunately not mean that a great deal of firm knowledge has been achieved. Generally, the findings have been hampered by conflicting results and lack of ability to replicate previous associations. Thus, none of the associations reported so far can by any reason be regarded as unambiguously proven. This lack of conclusive evidence can presumably be attributed to certain methodological problems related to most of the scientific work carried out until now. First of all, the studies have been very heterogeneous in terms of patient selection, tumor site, treatment regimens and the assessed normal tissue endpoints[7]. Consequently the studies are difficult to compare directly. Furthermore, most studies have been relatively small with sample sizes between 25 and 778 subjects. Seventeen studies included less than 100 subjects and only nine studies had more than 400 patients. The median sample size of the studies was 144[5]. This means that most studies have been severely underpowered to detect the impact of SNPs with only modest impact on normal tissue complication risk (as accounted for below, rather small effect sizes are probably what we should expect with SNPs). In that regard, it is thought provoking that a study with 150 participants and a 1:2 ratio between high risk and low risk genotypes has a power of less than 30% to detect a 1.5 fold increase in complication risk from 20% to 30% ($\alpha = 0.05$, two-tailed test). This lack of statistical power makes it particularly difficult to interpret negative studies. Furthermore, many of the studies investigated the impact of multiple SNPs and several different normal tissue reactions. In addition sub-group analyses were occasionally conducted. Despite this, measures were rarely taken to counteract a 'multiple testing problem'. This results in a high risk of false positives by chance. For

instance, in a study investigating the impact of 7 SNPs upon 2 different normal tissue endpoints (corresponding to 14 independent comparisons) the risk of getting at least 1 positive finding by chance is above 50% assuming a 5% significance level in each comparison. In this context, it should be kept in mind that the human genome contains a very high number of SNPs and other sequence alterations. This presumably means that the prior probability that a given genetic variant is 'truly associated' with the phenotype at interest may be very low. Given this assumption, the vast majority of positive associations reported are likely to be 'false positives' using a typical 1% or 5% significance level. To put it popular, too many of the studies conducted so far have been designed in such a way that the probability of detecting the presence of a 'true' association may be as low as 30% (in each comparison) whereas the risk of finding something that does not exist has been above 50% (in the entire study). This may very well explain why the results achieved so far have been rather inconsistent and conflicting[5].

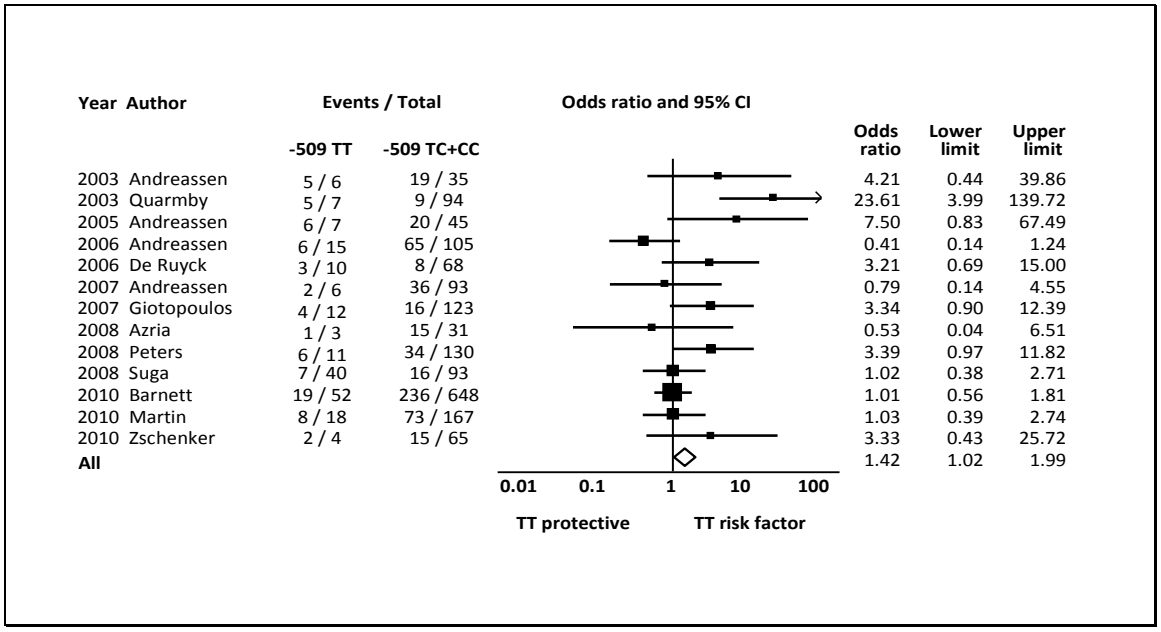
2.4 TGFB1 SNPs and risk of late toxicity – an illustrative case

TGFB1 is the gene of the versatile cytokine TGF-beta1. TGF-Beta 1 promotes the maturation of fibroblasts and stimulates the production of extracellular matrix proteins. TGF-beta1 is assumed to play a crucial role in the development of fibrosis. Within the promoter region and starting sequence of the TGFB1 gene a number of SNPs exist. Two of these SNPs designated the position -509 T/C and codon 10 Leu/Pro respectively have gathered particular interest in the field of normal tissue radiobiology. A few smaller studies have previously reported that these polymorphisms may affect the risk of various fibrosis related pathological conditions and influence the secretion rate of TGF-beta1. Consequently, these two SNPs represent obvious candidates for association studies addressing late normal tissue damage endpoints[4]. Due to relatively strong linkage disequilibrium, the position -509 C and codon 10 Pro (minority) alleles are often inherited together. Therefore, quite identical results are often achieved when the impact of these two SNPs are investigated[5].

Since 2003, a total of 15 studies have investigated the impact of one or both of these SNPs upon risk of various normal tissue reactions[5]. Most of these studies have been relatively small. Initially, relatively strong associations with risk of late toxicity were reported with regard to the position -509 C and/or codon 10 Pro alleles. A combined analysis of data from two studies addressing changes in the breast after post lumpectomy radiotherapy, demonstrated a three-fold increase of toxicity risk in patients with the TGFB1 position- 509 TT compared to those with the TC or CC genotype and an almost 15-fold increase when the TT genotype was compared to the CC genotype[8]. Though not all studies have been positive, the TGFB1 position -509 and codon 10 SNPs have for almost a decade been regarded as some of the most promising SNP markers for prediction of radiation induced late toxicity (particularly regarding endpoints that are assumed to have the development of fibrosis as a major underlying mechanism). Nevertheless, a more recent study with an accrual of almost 800 patients failed to demonstrate any association between the aforementioned SNPs and risk of various late reactions in the breast (including breast shrinkage and induration) after post lumpectomy radiation therapy[9]. With the possible exception of a relatively short length of follow-up (two years), the study was methodologically strong and by far the largest of its kind. Interestingly, the study was designed to provide 99% power to detect a three-fold increase in toxicity risk and was fairly well powered to detect a 1.5 fold increase. This finding seriously questions the assumption that the assessed TGFB1 SNPs has a major impact on risk of radiation induced fibrosis. A literature based meta-analysis of studies addressing the impact of the position -509 SNP upon risk of late radiation-induced toxicity has recently been published[5]. The analysis summarized the influence of this SNP in 1.888 patients assessed for various late normal tissue effects after radiation therapy for different cancers. The meta-

analysis demonstrated a modest enhancement of toxicity risk corresponding to an odds ratio of 1.42 with 95% confidence intervals not overlapping 1 (Figure 1). This could of cause be seen as an indication that the $TGFB_1$ position -509 SNP does in fact inflict a smaller increment in late toxicity risk. Nevertheless, the distribution of the observations (small studies reporting high odds ratios, larger studies clustering around the line of equality and a relative absence of small studies reporting an inverse association) is highly suggestive of publication bias. Thus, despite substantial research efforts for almost a decade, it is in fact still difficult to conclude whether or not an association exists between the $TGFB_1$ SNPs and risk of fibrosis related late toxicity[5].

Figure 1: Meta-analysis of studies addressing the impact of the TGFB1 position _509 C/T SNP upon risk of various late normal tissue reactions. The TT genotype was compared to the CT/CC genotype. The analysis indicates an enhanced risk of late toxicity in patients with the TT genotype (OR 1.42, 95% CI 1.02-1.99). Nevertheless, the distribution of the observations is highly suggestive of publication bias. Modified from reference 4 with permission.



2.5 Predictive models based multiple SNPs

A number of studies have investigated the combined impact of several SNPs and established predictive models based on multiple polymorphisms. Typically, the studies analyzed the association between normal tissue outcome and the total number of 'risk alleles' harboured at the assessed polymorphic sites. From a conceptual point of view, such approach is indeed appealing as it is perfectly in line with the assumption that clinical radiosensitivity should be considered a polygenic phenotype determined by the aggregate influence of several different loci[4]. Nevertheless, the models presented so far should certainly be interpreted cautiously. First of all, most SNPs included in these models have yielded rather conflicting results in studies investigating them individually[5]. Furthermore, there may be certain methodological problems related to the models. In most instances, the risk alleles (minority versus majority allele) were not defined based on any prior biological hypothesis or based on previous findings. Often, they were defined as risk alleles due to the observation that they were

(sometimes non-significantly) associated with a higher toxicity risk in the very study itself. Consequently, the models imply an inherent risk that coincidental statistical flukes are amplified into a statistically significant result when the apparent high-risk and-low risk alleles, respectively, are clustered together as part of the analysis. Thus, a significant finding related to the entire model cannot be used to indirectly verify the (occasionally non-significant) results obtained for the individual SNPs. This potential problem is underlined by the fact that different alleles have been appointed as 'risk alleles' in different models. For instance, four independent studies have established predictive models that had a number of SNPs in common. In two studies the majority allele in XRCC1 codon 399 (Arg) was appointed as the risk allele whereas in two others the minority allele (Gln) was appointed as the risk allele. Similar inconstancies between these models were also seen with regard to SNPs in ATM (codon 1853), SOD2 (codon 16), TGFB1 (codon 10), and XRCC3 (codon 241)[5]. This should indeed raise the suspicion that the apparent successfulness of these models might be a result of the aforementioned methodological problem rather than reflecting any true biological insights. Thus, a logical approach would presumably be to first investigate and validate associations for individual SNPs and then establish models based on multiple genetic markers.

A few studies have utilized what could be referred as a 'broad based candidate gene approach'. These studies assessed several hundreds of SNPs and established predictive models based on the findings[2,5]. In many of these investigations, the number of genotyped SNPs exceeded the number of participants which unavoidably leads to a 'multiple test problem'. Thus, independent validation of the results from such studies is of utmost importance.

2.6 Genome wide studies in normal tissue radiobiology

The genome wide association study (GWAS) provides a radical alternative to the candidate gene approach. The GWAS makes use of the fact that SNPs cluster into haplotypes due to linkage disequilibrium. This means that the majority of the estimated 11 million SNPs that exist in the genome can be indirectly assessed by means of a micro array which genotypes around 250,000 – 1 million well chosen 'tagging SNPs'. Thus, a GWAS provides the opportunity to conduct a hypothesis free search for SNP associations without any need for a prior understanding of the biology underlying the phenotype of interest. Obviously, the GWAS has an inherent (severe) 'multiple testing problem'. In order to keep the risk the risk of false positives at a reasonable level and still maintain statistical power, many GWASs have been based on very large patient cohorts occasionally approaching 50,000 subjects[6].

So far (December 2011), only one GWAS addressing normal tissue radiobiology has been published. This study investigated the risk of erectile dysfunction among 79 African Americans treated with radiation therapy for prostate cancer[10]. The study utilized a microarray that genotyped around 900,000 SNPs. This investigation identified a SNP in the gene of the follicle-stimulating hormone receptor (FSHR) that was significantly associated with erective dysfunction with a p value of 5×10^{-8} corresponding to a Bonferoni corrected p-value of 0,023. Furthermore, a predictive model was established based on the top-ranking four SNPs. These findings of cause need independent validation. A few other relatively small GWASs addressing the risk of normal tissue toxicity after radiation therapy are expected to be published in the near future.

2.7 Lessons learned in other scientific fields

Within the last decade, substantial efforts have been made to unravel the genetics of various phenotypes that are assumed to have a complex polygenic background. Until 2006 these efforts were entirely based on a candidate gene approach. Generally, the studies carried out have been relatively unfruitful and despite thousands of publications only a very limited number of irrefutable associations have been established[6]. One major reason for this is probably that many of the studies have suffered from the same shortcomings (primarily lack of statistical power and multiple testing problems) as those carried out in the field of normal tissue radiobiology. Furthermore, these studies have only investigated very small proportion of the genetic variation that exists throughout the human genome.

Since 2006, more than 950 GWASs addressing various biomedical phenotypes have been carried out (updated lists are available at www.genome.gov/gwastudies)[11]. For at least two reasons, these studies represent an important breakthrough in the attempts to unravel the genetics of complex traits. Firstly, GWASs have dramatically increased the number of convincing SNP associations reported. Thus, they have been productive in a context where the candidate gene approach has generally yielded very limited success[6]. Secondly, GWASs shed important new light on the allelic architecture that may underlie most complex traits. The experiences gained in various scientific fields can be summarized as follows:

2.7.1 The typical impact of SNPs is presumably relatively small

It is has been a common experience in most GWSSs that the majority of SNPs that have been convincingly related to various bio-medical traits only exhibited a modest impact on phenotype. Often, the effect size corresponded to an odds ratio around 1.2. Several of the GWASs were relatively well-powered to detect associations with odds ratios above 1.5 but such associations were rarely reported[6,11]. This probably implies that the typical impact of SNPs upon phenotype is rather small and emphasizes the need for well-powered studies[5].

2.7.2 The candidate gene approach may not be of much use

Another striking finding in most GWASs is that many of the identified SNPs with impact on phenotype were not located in any of the genes investigated as part of candidate gene studies or in genes involved in pathways assumed to be of major importance for the phenotype of interest. Often, the SNPs were located in non-coding sequences without any known function. This experience seriously questions the value of the candidate gene approach[5,6].

2.7.3 There is probably (much) more to complex trait genetics than SNPs

The genetic backgrounds of a number of different traits (i.e. cancer risk) have been quite extensively investigated using GWASs. Regardless of that, the identified SNPs affecting phenotype typically only accounted for a rather limited proportion of the expected heritable contribution to trait variance (often around only 5 – 25%). This can probably to some extent be attributed to methodological issues. Despite very large study cohorts, most GWASs provided limited statistical power to detect SNPs conferring odds ratios below 1.2. Thus, numerous ‘low impact’ SNPs’ may easily have been missed. Furthermore, many of the utilized SNP genotyping platforms offered limited coverage for SNPs with population frequencies below 5 – 10%. Nevertheless, the abovementioned observation first of all implies that other types of sequence variants than SNPs (e.g. copy number variants, translocations and inversions) probably have to constitute a substantial proportion of the genetic

background underlying many complex traits. It is likely that rare genetic alterations (that are not captured by the current generation of GWASs) are of major importance with regard to complex trait genetics[6,11]. This recognition is somewhat at odds with the aforementioned rather influential 'common disease – common variant hypothesis'. To conclude, the current evidence is consistent with the assumption that polygenic / complex traits are dependent on a spectrum ranging from common low penetrance alleles to rare alterations with a more dramatic impact on phenotype [7].

2.8 How to proceed?

The experiences gained from studies addressing the genetics of various complex traits should probably be taken into account in the future attempts to unravel the genetics if normal tissue complication risk after radiation therapy. As indicated above, a radical change in research strategy is probably needed if substantial progress should be made[5]. Statistical power and rigorous statistical testing are issues of immense importance. Under the assumption that each SNP only affect phenotype slightly (e.g. corresponding to an odds ratio around 1.2 – 1.5) future candidate gene studies should be based on thousands (rather than tens or hundreds) of subjects in order to provide precise risk estimates. Furthermore, careful correction for multiple testing and validation of previous results are needed. Given the limited success of the candidate gene approach, GWASs are certainly warranted in normal tissue radiobiology. As mentioned previously, the GWASs currently planned are of limited sample size (a few hundred subjects). Thus, these are only powered to detect very strong SNP associations (when the mandatory correction is made for multiple testing). Therefore, it seems quite likely that future GWASs will need to be expanded considerably with regard to sample size. In addition, novel catalogues of 'low-frequency genetic variants' (i.e. those with minor allele frequencies around 0.5 to 5%) and high-density microarrays will enable the exploration of sequence alterations in the 'sub-polymorphic' frequency range. However, such experiments also call for very large patient cohorts as the number of patients needed to obtain sufficient statistical power increases dramatically with decreasing minor allele frequencies[11]. Under the assumption that rare alterations (arbitrarily defined as those with an abundance below 0.5%) constitute a substantial proportion of the genetic variation underlying differences in normal tissue radiosensitivity, SNP based assays most likely need to be complimented by complete sequencing. Technologies that will enable cost efficient high throughput sequencing are currently emerging. Nevertheless, numerous obstacles (including immense statistical and financial challenges) still need to be overcome before association studies based on genome wide sequencing become feasible[6,11].

To summarize, it seems increasingly clear that it represents a massive undertaking to pursue a comprehensive understanding of the genetics assumed to underlie differences in normal tissue radiosensitivity[13]. However, it seems equally clear that advances in bio-informatics, genotyping technology and novel insights into population genetics provide unprecedented opportunities dissect the molecular and genetic basis of normal tissue radiosensitivity. Nevertheless, to fully exploit these new possibilities cooperation will be essential. One of the major challenges in that regard will be to establish sufficiently large cohorts of patients that are well categorized with regard to treatment characteristics and normal tissue outcome. This should encourage the formation of international networks and consortia such as the RAPPER, Radgenomics, Gene-pare, ESTRO GENEPI and the International Radiogenomic Consortium[2,5].

2.9 Should alternative strategies be considered?

As accounted for in the previous paragraphs, the genome has some inherent characteristics that makes it challenging to deal with (the number of variants to choose from is immense and many genetic determinants are likely to be either rare or of limited phenotypic impact). This may favour a return to research in predictive assays based on phenotype rather than genotype[12]. Analysis of gene expression profiles has proven a useful tool in various settings. A few recent studies have established predictive models for late normal tissue reactions derived from the transcriptional response to in vitro irradiation of fibroblasts or lymphocytes[13]. Typically, the genes included in these classifiers were involved in processes such as apoptosis, cell cycle arrest, reactive radical scavenging and tissue remodelling. These findings, of cause, need independent validation but may indicate that studies addressing the (radiation- induced) transcriptome may represent a rewarding alternative to studies addressing genetic germline sequence. Furthermore, this kind of experiments may (in addition to GWASs) provide new insights into molecular radiobiology. A possible spinoff from such insights could be the identification of pathways that could serve as targets for pharmacological intervention against radiation-induced normal tissue damage[14].

2.10 Conclusion

Over the last decades, various efforts have been made to establish a predictive test for normal tissue radiosensitivity based on genetic germline alterations. Despite this, a predictive assay applicable for clinical use has not been established. Nevertheless dramatic advances in molecular biology provide unique opportunities to pursue a more complete understanding of the biology and genetics underlying differences in normal tissue radiosensitivity. Hopefully, this will translate into improved treatment regimens for cancer patients.

2.11 References

1) Barnett GC, West CM, Dunning AM, Elliott RM, Coles CE, Pharoah PD, Burnet NG. Normal tissue reactions to radiotherapy: towards tailoring treatment dose by genotype. Nat Rev Cancer. 2009;9(2):134-42.

2) West CM & Barnett GC. Genetics and genomics of radiotherapy toxicity: towards prediction. Genome Med. 2011;3(8):52.

3) Safwat A, Bentzen SM, Turesson I, Hendry JH. Deterministic rather than stochastic factors explain most of the variation in the expression of skin telangiectasia after radiotherapy. Int J Radiat Oncol Biol Phys. 2002;52(1):198-204.

4) Andreassen CN. Can risk of radiotherapy-induced normal tissue complications be predicted from genetic profiles? Acta Oncol. 2005;44(8):801-15.

5) Andreassen CN. Searching for genetic determinants of normal tissue radiosensitivity--are we on the right track? Radiother Oncol. 2010;97(1):1-8.

6) Altshuler D, Daly MJ, Lander ES. Genetic mapping in human disease. Science. 2008;322(5903):881-8.

7) Andreassen CN, Alsner J. Genetic variants and normal tissue toxicity after radiotherapy: a systematic review. Radiother Oncol. 2009;92(3):299-309.

8) Giotopoulos G, Symonds RP, Foweraker K, Griffin M, Peat I, Osman A, Plumb M. The late radiotherapy normal tissue injury phenotypes of telangiectasia, fibrosis and atrophy in breast cancer patients have distinct genotype-dependent causes. Br J Cancer. 2007;96(6):1001-7.

9) Barnett GC, Coles CE, Burnet NG, Pharoah PD, Wilkinson J, West CM, Elliott RM, Baynes C, Dunning AM.No association between SNPs regulating TGF-β1 secretion and late radiotherapy toxicity to the breast: results from the RAPPER study. Radiother Oncol. 2010;97(1):9-14.

10) Kerns SL, Ostrer H, Stock R, Li W, Moore J, Pearlman A, Campbell C, Shao Y, Stone N, Kusnetz L, Rosenstein BS. Genome-wide association study to identify single nucleotide polymorphisms (SNPs) associated with the development of erectile dysfunction in African-American men after radiotherapy for prostate cancer. Int J Radiat Oncol Biol Phys. 2010;78(5):1292-300.

11) Juran BD, Lazaridis KN. Genomics in the post-GWAS era. Semin Liver Dis. 2011;31(2):215-22.

12) Ozsahin M, Crompton NE, Gourgou S, Kramar A, Li L, Shi Y, Sozzi WJ, Zouhair A, Mirimanoff RO, Azria D. CD4 and CD8 T-lymphocyte apoptosis can predict radiation-induced late toxicity: a prospective study in 399 patients. Clin Cancer Res. 2005;11(20):7426-33.

13) Mayer C, Popanda O, Greve B, Fritz E, Illig T, Eckardt-Schupp F, Gomolka M, Benner A, Schmezer P.A radiation-induced gene expression signature as a tool to predict acute radiotherapy-induced adverse side effects. Cancer Lett. 2011;302(1):20-8.

14) Bentzen SM. Preventing or reducing late side effects of radiation therapy: radiobiology meets molecular pathology. Nat Rev Cancer. 2006;6(9):702-13.

3 GENETIC PATHWAYS FOR THE PREDICTION OF THE EFFECTS OF IONISING RADIATION

Peter O'Neill

MRC/CR-UK Gray Institute for Radiation Oncology and Biology, University of Oxford, UK

3.1 Introduction

The fact that we are constantly exposed to low levels of natural background radiation makes the biological effects of low dose radiation a concern for the general population. The current model for estimating risk of low dose ionising radiation is currently based on linear extrapolation from experimental and epidemiological data obtained at high doses. Present estimates of the risks from radiation exposure are based largely on the average exposure to a population without consideration of **individual radiosensitivity**. Based on ICRP recommendations (ICRP Publication 79), radiation cancer risks relative to baseline are judged to be small at low doses for individuals with familial cancer disorders and insufficient to form the basis of special precautions. However risks to those with familial cancer disorders may become important at the high doses received during radiotherapy which is one of the most effective cancer treatments and is used alone or in combination with surgery and chemotherapy. Clinical evidence from diagnostic and therapeutic uses of ionising radiation clearly shows that individuals respond differently to ionising radiation. For instance, a fraction of patients react badly to radiotherapy. Adverse reactions to radiotherapy are seen in a percentage of patients although clinical observations indicate these reactions vary widely between individuals, in part reflecting the intrinsic sensitivity of normal tissue. These adverse reactions are commonly classified as acute (occurring during or within a few weeks of treatment) or late (occurring 6 months to many years later). As late effects can be permanent, they provide the basis for dose constraints to radiation toxicity. Ionising radiation can be seen as a double edged sword, induces cancer but also used to cure cancer. Consideration of individual radiosensitivity in contributing to induction of cancer for the individual and the radiation dose constrains required have to be taken into account for those cancer patients who may be more radiosensitive. Some biological endpoints have shown promise in the field of biomarkers for radiosensitivity coupled to radiation dose although the robustness of biomarker responses has often not been validated in appropriate studies. To date, all assays to develop biomarkers for individual radiosensitivity have generally fallen short of being reliable predictors of individual radiosensitivity. For instance one of the best known and most important genetic disorders with hypersensitivity to radiation is ataxia telangiectasia (AT). Evidence based on AT heterozygotes indicated that as yet genes other than ATM confer radiosensitivity and are involved in low penetrance predisposition to breast cancer in a high proportion of cases and may contribute to adverse reactions after therapy (Barber *et al*., (2000). The effectiveness of radiotherapeutic treatment of many tumours is limited by dose restrictions needed to minimise late effects of normal tissues in the irradiated area.

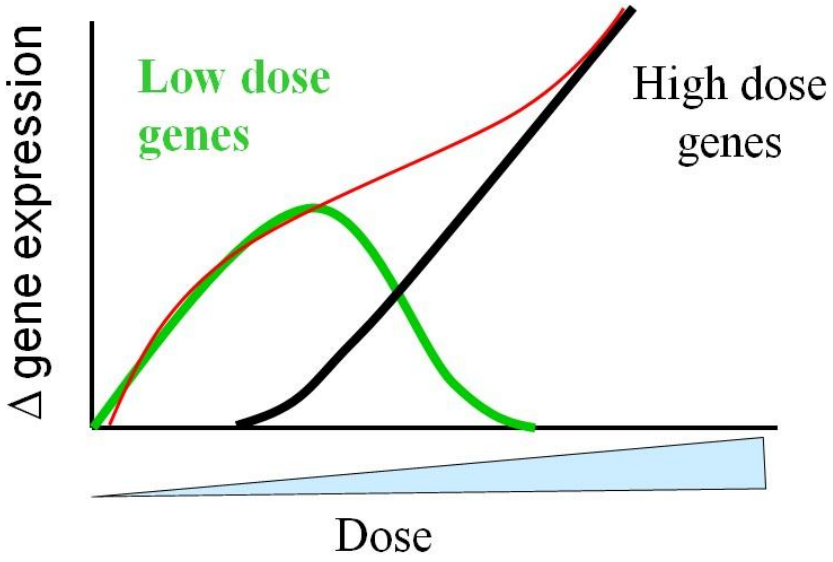
From both a radiation protection point of view and risk stratification of patients for radiotherapy, it is thus very important to identify radiation sensitive individuals and to understand the mechanisms involved to advance radiation protection and personalisation of radiotherapy treatment.

3.2 Genetic pathways for the prediction of the effects of ionising radiation: Low dose radiosensitivity and risk to normal tissue after Radiotherapy (GENEPI-lowRT, FP6 funded project)

3.2.1 Background at time of initiation of the project

One important late normal tissue complication after radiotherapy of breast cancer patients is skin fibrosis, an obliteration of normal tissue components with replacement by matrix and disordered collagen fibres. It occurs months after treatment and persists for many years. Although recent advances in radiation delivery have significantly reduced the occurrence and the severity of skin fibrosis in general, ~5% of the patients still suffer from marked late reactions, and the biological factors underlying this heterogeneity are currently not known. Early and late tissue responses to radiation have long been considered in terms of the “target cell concept” which assumes that the response of an organ/tissue to radiation is the direct consequence of the radiation-induced killing of specific target cells or functional units that are responsible for replication and regeneration of the tissue. For instance, many studies have investigated radiobiological endpoints such as colony forming ability, chromosome aberration formation and DNA damage induction and repair in cultured fibroblasts or lymphocytes to identify high dose responses which may correlate with late effects of normal tissue. Strategies linking normal tissue response to a variety of phenotypical responses, generally at high dose and dose rates, to predict individual risk of normal tissue response have not generally proven to be successful (Dikomey *et al*., 2000; Dickson *et al.*, 2002; Russell *et al.*, 1998). Based on the lack of identification of suitable biomarkers of adverse effects following radiotherapy, the EU funded a project entitled GENEPI-lowRT under FP6. The aim of this study was to test for associations between the risk of severe normal tissue toxicity following curative radiotherapy for early breast cancer and *in vitro* transcriptional and cellular responses induced in lymphocytes and dermal fibroblasts by low dose ionising radiation and to identify any links between radiosensitivity and genetic differences of individuals. The rationale as shown in Figure 1 was based on the hypothesis that any radiosensitivity classifier which may be linked to low dose responses resulting in modification of the levels of gene expression may be hidden underneath any high dose responses, such as those used in fractionated radiotherapy. This hypothesis was developed from the knowledge that the spectrum of genes and the levels of their expression induced by low dose of sparsely ionising radiation (20 mGy) is vastly different to the genes expressing modified levels for a high dose (4 Gy). At low doses mainly cell-cell signalling and signal transduction pathways are induced compared with apoptotic responses and cell proliferation genes at higher dose (Ding, L-H et al., 2005). These differences in gene expression on dose highlight the potential complication of high dose responses overshadowing an underlying low dose response. Additionally, non-targeted bystander responses and low dose hypersensitivity of cells have been identified at low dose.

Figure 1: Schematic to emphasis potential dose dependences of radiation induced differential gene expression at low (green line) and high doses (black line) respectively. The red line shows the overall dependence of differential dependence on dose for all genes.



Present indications with low dose gene profiling indicates variation in individual responses implying that expression of gene clusters and not individual genes may be better predictors and be more informative (Amundson, SA. *et al.*, 2004). Several studies (Amundson, SA. et al., 2003; Snyder, AR. et al., 2004; Snyder, AR. et al., 2005) have shown that transcriptional changes and protein modifications occur in response to very low doses of ionizing radiation. If sensitive and specific predictive test or biomarkers could identify which patients are more sensitive to radiotherapy, the treatment could then be tailored to deliver doses of ionising radiation at levels more appropriate to the patient's genetic make-up. The problem is that little is known about the biological factors underlying such normal tissue complications and attempts to link normal tissue responses in patients and various phenotypical cell and molecular responses to high doses in vitro have not generally been very successful.

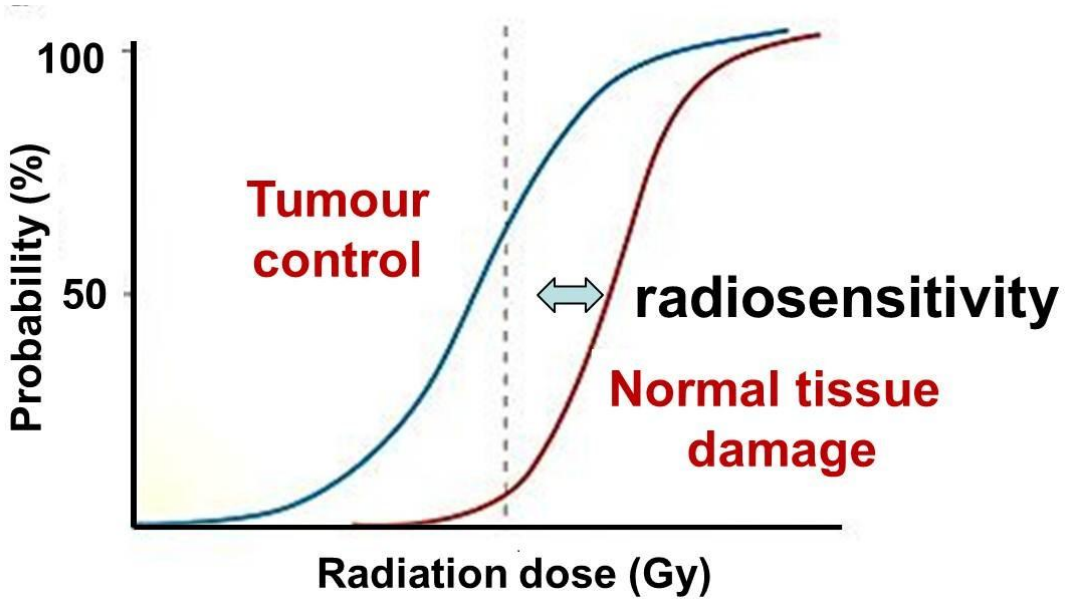
3.2.2 Preliminary findings from the Genepi-lowRT project

The data from the EU-funded Genepi-lowRT project is still being analysed by the bio-informaticians. It was verified that significant differences in gene expression were seen at high and low radiation doses with different gene sets being differentially expressed from the 108 clinical samples tested. From bioinformatic analysis of the gene profiles from lymphocytes and skin fibroblasts, several candidate biomarkers were identified. It was apparent though that a robust classifier(s) for radiosensitivity could not be established to identify those patients who showed adverse effects from the radiation (late effects). Functional analysis which focussed on the ability of the cells to repair DNA damage was also unable to distinguish between individual radiosensitivity, as is the case in many other studies based on functional analysis of DNA damage and repair. At this stage, a robust classifier for radiosensitivity to late effects of radiation could not be established due in part to unidentified confounding factors which may contribute to the radiosensitivity, in addition to any genetic contributions.

3.3 Potential biomarkers of radiosensitivity in radiotherapy patients

Quickly dividing tumour cells are generally more sensitive than the majority of body cells. Additionally tumour cells can be hypoxic and therefore less sensitive to radiation. However, the effectiveness of radiotherapeutic treatment of many tumours is limited by dose restrictions needed to minimise late effects of normal tissues in the irradiated area. To date many of the searches for a genetic biomarker of individual radiosensitivity have focussed on DNA repair pathways or functional analysis of pathways involved in DNA damage and repair and apoptosis. More recently, radiogenomics (Barnett *et al.* 2009) have been undertaken in the search for robust classifiers of individual radiosensitivity. Proteins containing single nucleotide polymorphisms have been proposed as predictors of individual radiosensitivity. As reported in Section 2 on differential gene expression, global gene expression responses to low and high dose radiation have now been undertaken in 3D tissue models, when the low dose effects tend to be associated with recovery and tissue repair whereas the high dose effects tend to be associated with loss of structural integrity and terminal differentiation (Mezentsev and Amundson, 2011).

Figure 2: The dose dependence for probability of either tumour control or normal tissue damage and the modulation of normal tissue response when radiosensitivity increases due to among others individual radiosensitivity.



Radical radiotherapy and surgery achieve similar cure rates in muscle-invasive bladder cancer. However the choice of which treatment is most beneficial cannot be predicted for individual patients. Studies aimed at identifying biomarkers following radiotherapy but at higher doses have identified MRE11, involved in DNA repair at the level of protein expression, shows a predictive factor associated with survival following bladder cancer radiotherapy but NOT a prognostic marker in bladder cancer (Choudhury, A., 2010). If validated, MRE11 as a predictive biomarker of sensitivity may allow patient selection for either radiotherapy or cystectomy.

Several studies have focussed on single polynucleotide polymorphisms (SNPs) with one , the RAPPER study, showing SNP in TGFβ as a predictor of late radiotherapy toxicity in

breast cancer, although the findings as in many studies was not subsequently validated (Barnett *et al.*, 2010). More recently, an association has been observed between clinical radiosensitivity in breast cancer patients and genetic variants in MSH2 and MSH3, proteins involved in mismatch repair mechanisms (Mangoni M *et al*., 2011). At this stage these mismatch repair genes remain interesting classifiers awaiting validation as biomarkers of acute radiosensitivity.

Overall, phenotypical changes have not proven to be a reliable approach to identify individual radiosensitvity. The use of Genome wide association studies have identified potential biomarkers, associations between pathways or have some predictive power but to date validation of potential classifiers has not been successful even at the higher radiation doses used in radiotherapy. Whether this bodes well for identification of biomarkers of individual radiosensitivity at low dose is predicted to be a major challenge for stochastic effects or even tissue reactions such as for cardiovascular effects, where the threshold dose tends to be much higher than the doses generally consider for low dose research into stochastic effects (ICRP 103).

3.4 Perspective

Many challenges are envisaged for future research in the quest to establish the extent to which individual sensitivity is dependent on genetic background in contrast to the role played by potentially modifiable lifestyle factors and/or inflammatory and immunological factors at low doses of sparsely ionising radiation. As severe syndromes are rare such as AT homozygotes which cause a high genetic predisposition to cancer, a major challenge remains in identifying heterozygote persons, with low penetrance genes, who may be slightly more radiosensitive than the majority of the population at low doses.

1) Potential approaches require the use of appropriate tissue banks or well-defined cohorts to define the role individual radiosensitivity to low and high dose radiation and latencies for different pathologies (cancer, non-cancer diseases). This may require setting up suitable (dosimetric and medical) cohorts that are well controlled. Accurate dosimetric data for any cohort is essential as is the heterogeneity of any radiation field to which they have been exposed. Appropriate infrastructures are required to facilitate high throughput screen enabling volumes of data to be collected and appropriately analysed.

2) Using well-defined cohorts may define the genetic background to individual radiosensitivity at low and high dose radiation and the latencies for different pathologies (cancer, non-cancer diseases). Based on past knowledge from gene expression, genetic polymorphisms or protein regulation, particular cellular pathways may be identified as biomarkers of individual radiosensitivity although the lifestyle and other confounding factors may dilute the ability to validate any classifier identified as robust indicators. Of particular concern within cohorts is the inclusion of groups potentially more sensitivity to ionising radiation such as infants, who may be up to 3 times (ICRP 103) more radiosensitive although neonates may be less radiosensitive than infants (Preston *et al.* 2008), and pregnant women.

3) Identification of the role of epigenetic effects in individual radiosensitivity or use of molecular epidemiology is becoming fashionable but it is important to use lessons learnt from previous searches for biomarkers. Whether these approaches will be sensitive or even have the statistical power at low doses <100 mGy remains an open question.

4) A potential spin-off from such research is also the development of bio-dosimeters for triage in 'radiation accidents'. Genomic and proteomic modulation induced by ionising radiation have identified cycline dependent kinase inhibitor (CDKN1A) apoptotic gene (BBC3) and DNA damage inducible protein 45 a gene (GADD45A) to name but a few as potential bio-dosimeters (Turtoi *et al.*, 2010; Badie *et al.* 2011).

From a radiation protection point of view and particularly for risk stratification of patients for radiotherapy and in diagnostic radiology, it would represent a significant step forward if radiation sensitive individuals could be identified through a biological classifier(s) and may lead to a better understand of the mechanisms involved in cancer induction and other health effects.

3.5 References

Amundson, SA, Bittner, M, Fornace Jr, AJ. Functional genomics as a window on radiation stress signaling. Oncogene 2003, 22: 5828-33.

Amundson, SA, Grace, MB, McLeland, CB, Epperley, MW, Yeager, A, Zhan, Q, Greenberger, JS, Fornace Jr, AJ. Human In vivo Radiation-Induced Biomarkers: Gene Expression Changes in Radiotherapy Patients. Cancer Research 2004, 64: 6368-71.

Barber, JBP, West, CML, Kiltie, AE, Roberts, SA, Scott, D, Detection of Individual Differences in Radiation-Induced Apoptosis of Peripheral Blood Lymphocytes in Normal Individuals, Ataxia Telangiectasia Homozygotes and Heterozygotes, and Breast Cancer Patients after Radiotherapy, Radiat. Res., 2000, 153: 570-578.

Barnett, GC, West CML, Dunning, AM, Elliott, RM, Coles, CE, Pharoah, PDP, Burnet, NG, Normal tissue reactions to radiotherapy: towards tailoring treatment dose by genotype. Nature reviews cancer 2009, 9: 134-142.

Barnett, GC, Coles, CE, Burnet, NG, Pharoah, PDP, Wilkinson, J, West, CML, Elliott, RM, Baynes, C, Dunning, AM, No association between SNPs regulating TGF-β1 secretion and late radiotherapy toxicity to the breast: Results from the RAPPER study. Radiother. Oncol., 2010, 97: 9-14.

Baumann M, Hoelscher T, Begg AC. Towards genetic prediction of radiation responses: ESTRO's GENEPI project. Radiother Oncol;2003, 69: 121-5.

Choudhury, A, Nelson, LD, Teo, MTW., Chilka, S, Bhattarai, S, Johnston, C.F, Elliott, F, Lowery, J, Taylor, CF, Churchman, M, Bentley, J, Knowles, MA, Harnden, P, Bristow, RG, Bishop, TD, Kiltie, AE, MRE11 Expression Is Predictive of Cause-Specific Survival following Radical Radiotherapy for Muscle-Invasive Bladder Cancer. Cancer Res; 2010, 70: 7017–26.

Dickson J, Magee B, Stewart A, West CM. Relationship between residual radiation-induced DNA double-strand breaks in cultured fibroblasts and late radiation reactions: a comparison of training and validation cohorts of breast cancer patients. Radiother Oncol 2002;62:321-6.

Dikomey E, Brammer I, Johansen J, Bentzen SM, Overgaard J., Relationship between DNA double-strand breaks, cell killing, and fibrosis studied in confluent skin fibroblasts derived from breast cancer patients. Int J Radiat Oncol Biol Phys 2000;46:481-90.

Ding, L-H, Shingyoji, M, Chen, F, Hwang, J-J, Burma, S, Lee, C, Cheng, J-F, Chen, DJ. Radiat. Res. 2005 164: 17-26.

ICRP Publication 79: Genetic Susceptibility to Cancer; Ann. ICRP, 1998, 28 (1-2).

ICRP Publication 103: The Recommendations of the International Commission on Radiological Protection, Ann. ICRP, 2007 37 (2-4).

Kabacik, S Mackay, A, Tamber, N, Manning, G, Finnon, P, Paillier, F, Ashworth, A, Bouffler, S, Badie, C, Proteomic and genomic modulations induced by γ-irradiation of human blood lymphocytes. Int. J. Radiat. Biol. 2011, 87: 115-129.

Mangoni, M, Bisanzi, S, Carozzi, F, Sani, C, Biti, G, Livi, L, Barletta, E, Costantini, AS, Gorini, G., Association between genetic polymorphisms in the XRCC1, XRCC3, XPD, GSTM1, GSTT1, MSH2, MLH1, MSH3, and MGMT genes and radiosensitivity in breast cancer patients. Int. J. Radiation Oncol. Biol. Phys., 2011, 81: 52-58.

Mezentsev, A, Amundson, SA, Global Gene Expression Responses to Low- or High-Dose Radiation in a Human Three-Dimensional Tissue Model . Radiation Res. 2011, 175: 677-688.

Preston, DL, Cullings, H, Suyama, A, Funamoto, S, Nishi, N, Soda, M, Mabuchi, K, Kodama, K, Kasagi, F, Shore RE, Solid Cancer Incidence in Atomic Bomb Survivors Exposed In Utero or as Young Chi ldren JNCI, 2008, 100: 428-436.

Russell NS, Grummels A, Hart AA, Smolders, IJH, Borger, J, Bartelink, H, Begg, AC, Low predictive value of intrinsic fibroblast radiosensitivity for fibrosis development following radiotherapy for breast cancer. Int J Radiat Biol 1998;73:661-70.

Snyder, AR, Morgan, WF. Gene expression profiling after irradiation: Clues to understanding acute and persistent responses? Cancer & Metastasis Rev. 2004, 23: 259-268.

Snyder, AR, Morgan, WF, Lack of consensus gene expression changes associated with radiation-induced chromosomal instability. DNA Repair 2005, 4: 958-70.

Turtoi, A., Sharan, R.N., Srivastava, A., Schneeweiss, F.H.A., Proteomic and genomic modulations induced by γ-irradiation of human blood lymphocytes Int. J. Radiat. Biol., 2010, 86: 888-904.

4 GENETIC PREDISPOSITION AND RADIATION SENSITIVITY: THE POTENTIAL OF GENOME SEQUENCING

Paul D.P. Pharoah

Reader in Cancer Epidemiology
University of Cambridge, United Kingdom

4.1 Introduction

Over the past decade there have been rapid developments in our understanding of the basis of inherited predisposition to many complex phenotypes ranging from height and body weight to different cancers. Despite this progress there is still little known about inherited predisposition to specific radiosensitivity phenotypes. However, it seems very likely that the progress seen with other phenotypes will soon also occur for radiation sensitivity.

In this paper I will discuss some of the key issues in genetic epidemiological study design and describe some of the barriers that have hindered progress in this field to date. I will then speculate on the likely outcomes of future research in this field.

4.2 The architecture of genetic predisposition to radiosensitivity phenotypes

In designing a study to investigate the association between any exposure and any outcome, the most efficient and appropriate design will be informed by the nature of the underlying association. Thus, genetic association studies that seek to identify association between germline genetic variation (exposure) and radiosensitivity (outcome) should be informed by the underlying genetic architecture of risk. The genetic architecture is an umbrella term to describe the range of risk alleles in terms of the allele frequency, the risks they confer and the genetic model for their effect (dominant, co-dominant or recessive). It should be noted that the phenotype of interest, radiosensitivity, is a complex construct encompassing a range of cellular and clinical phenotypes that are likely to be related, but which may differ in their underlying genetic architecture.

While there is good evidence for inter-individual variation in radiosensitivity phenotypes and good evidence that a substantial proportion of that variation is caused by germline genetic variation, there is very little data to provide any information about the likely underlying genetic architecture for any given radiosensitivity phenotype. Nevertheless, we can infer some broad generalisations from basic principles and from the genetic epidemiology of other complex human phenotypes. Firstly, highly penetrant alleles are likely to be rare or very rare. If this were not the case, the radiosensitive phenotype would be common in the population in which it is being studied. Such rare, highly-penetrant alleles would only account for a small proportion of the genetic component of the phenotypic variance. The remainder of the genetic component of phenotypic variance could be explained by a small number of common variants that confer modest risks to a very large number of very rare variants with small risks. Studies of other complex phenotypes have found few, if any, common variants that confer modest risks. For example, in breast cancer, common alleles that confer a relative risk of

disease of greater than 1.3 have not been found. Given that genetic association studies in breast cancer have virtually 100 percent power to detect such alleles, it seems reasonable to conclude that they do not exist. Common alleles conferring relative risks greater than 2 have not been identified for any complex disease phenotype. Genome-wide association studies for common, complex diseases have been very successful and have identified large numbers of common alleles conferring weak effects, with each allele explaining less than 2 percent of the genetic component of disease. In addition, rare and uncommon alleles conferring modest risks have been identified for many complex disease phenotypes.

It seems very likely that radiosensitivity phenotypes will have a similar genetic architecture to other complex phenotypes, with a small number of very rare or rare alleles with large effects and a wide range of rare to common variants with modest or small effects.

4.3 Searching for common alleles

Over the past decade there has been rapid progress in our understanding of the architecture of human genetic variation. There is a wide variety of different types of variation in the human genome, but the commonest form of variation is the single nucleotide polymorphisms (SNP). Projects such as the HapMap Project and the 1000 Genomes Project have provided a great deal of information about the extent and correlation structure of common variation across the genome in different populations. This combined with major developments in genotyping technology has made it possible to genotype tens of thousands of subjects for hundreds of thousands of single nucleotide polymorphisms that efficiently capture most of the common genetic variation across the genome.

4.3.1 What study design should be used?

The most appropriate study design depends on the radiosensitivity phenotype of interest. Some end-points are quantitative and can be measures on a continuous scale and other quantitative end-points may be measured on an ordinal scale. There are also end-points that simply represent the presence or absence of the phenotype of interest. The latter is perhaps the commonest and can be studied using a standard case-control design. In general, for a fixed sample size where sample size is fixed by constraints such as genotyping costs, it is most efficient to have an equal number of cases and controls. However, sample size is often limited by the availability of subjects with the phenotype of interest and power can be increased by increasing the ratio of controls to cases if additional controls are available.

4.3.2 What statistical test should be used?

The simple answer to this question is “the test that provides the greatest power to detect association”. However, the power of any given test for association depends on both the nature of the phenotype of interest (continuous, ordinal or dichotomous) and the underlying genetic model. Let us consider a bi-allelic SNP, which has three possible genotypes, common homozygote, heterozygote and rare homozygote. In a case-control study this will generate the standard 2x3 contingency table and simple tests can be used to test for association. A general Chi squared test for heterogeneity (2 degrees of freedom (d.f.)) can be used, but more powerful tests are available. Under a dominant genetic model the heterozygote and rare homozygote will confer the same risk and greatest power would be achieved by grouping these two genotype categories and carrying out a 1 d.f. Chi squared test on the resultant 2x2 contingency table. Similarly the common homozygote and

heterozygote genotypes could be combined in order to test for a recessive allele. Under a co-dominant genetic model the heterozygote will be at intermediate risk between common homozygote and rare homozygote. Here a Chi squared test for trend (1 d.f.) will have the greatest power to detect association. Note that the four tests – general, dominant, recessive and co-dominant – can detect association for all underlying genetic models, but at reduced power. Similar considerations apply to the analysis of ordinal phenotypes or continuous phenotypes where the test of association can be applied under the same four models.

As the genetic model is not usually known it is not possible to select in advance the test with the greatest power. Two possible approaches can then be used. The first is to apply all four tests and to choose the one with the smallest P-value. However, this P-value would need to be corrected for the fact that it was selected post hoc, usually using some sort of permutation procedure to allow for the fact that the tests are not independent. The alternative is to use the test that has the greatest power across a range of genetic models. The majority of common, disease susceptibility alleles detected to date seem to fit the co-dominant model best and so the chi squared test for trend, which has reasonable power across a range of genetic models, is commonly used as a single test for association.

The simple Chi-squared tests described above are generally used for univariate association tests. Where it is desirable to control for potentially confounding co-variates the equivalent tests can all be applied in a logistic regression framework. Note that there are unlikely to be many, if any, true confounders of a true genetic association.

4.3.3 Dealing with population stratification

Confounding can occur in the context of cryptic population stratification. If the phenotype frequency in cases and controls is different in different populations and the allele of interest is not associated with the phenotype, but differs in frequency between the populations, then spurious association will be observed.

Population stratification has not been found to be a major problem in carefully designed case-control studies restricted to populations of European origin. Furthermore, there are now well-established methods for dealing with the problem, such as principal components analysis.

However, perhaps the most important protection against false positives due to population stratification is through the replication of association signals in independent datasets. It is unlikely that population stratification causing a false positive for any given SNP in a study from one population will be the same in another study from a different population.

4.3.4 What should we consider "statistically significant"?

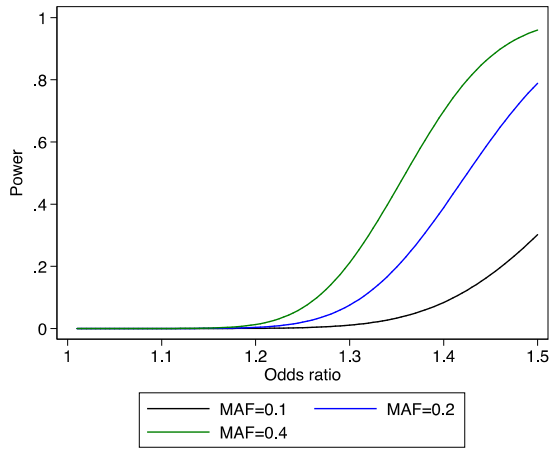
The early literature reporting genetic association studies was littered with reports of statistically significant associations that subsequently failed to be replicated by independent studies. Several possible reasons were put forward to explain this including population specific differences in i) risk allele frequencies, ii) the correlation structure between marker polymorphisms and causal variants, iii) differences in the frequency of interacting alleles and lefestyle/environmental factors and iv) limited power of small, replication studies to detect alleles with weak effects [1]. However, the major cause for failure of initial findings to replicate has been because most of the initial findings were false positives caused by using inappropriate threshold to declare statistical significance. The reason that the traditional $P<0.05$ threshold is inappropriate for genetic association studies, (and most other studies of observational epidemiology) is explained as follows.

The P-value, in itself, is not the probability of primary interest when interpreting data. The P-value is a conditional probability, namely the probability of observing data at least as extreme as those obtained if the null hypothesis is true. It is often incorrectly interpreted as the probability that the null hypothesis is true given the observed data. This latter probability can be considered to be the probability that an association that is declared as statistically significant is a false positive. The false positive probability depends on the prior probability of association (unknown), the power of the study to detect that association and the P-value. When the prior probability of association is small and the power to detect association is small, the false positive probability is high. The prior for a genetic association is unknown, but we know that there are around 10 million common variants in the human genome and there can be, at most, 100 variants that each explain 1 percent of the genetic variance, the prior probability of association for a random variant is 1:100,000 at best. While some would argue that this prior can be improved by judicious selection of variants in candidate genes, even if it were improved by an order of magnitude the prior would still be small (1:10,000). It is worth noting that candidate gene studies in the pre-GWAS era were notable for their lack of success and many risk allele identified by GWAS have been in regions without any obvious candidate genes based on known gene function. Furthermore, some risk alleles have been identified in so-called gene deserts containing no gene coding sequences. Even at an improbable prior of 1:1,000 a genetic association study with 80 percent power that declares a significant association at P=0.05 would have a 98 percent chance of being a false positive. As a consequence, well-powered studies with stringent criteria for declaring statistical significance are required in order to provide robust evidence of association. Various P-value thresholds have been suggested as appropriate for genetic association studies, but P<5x10-8 is widely accepted as denoting “genome-wide” significance.

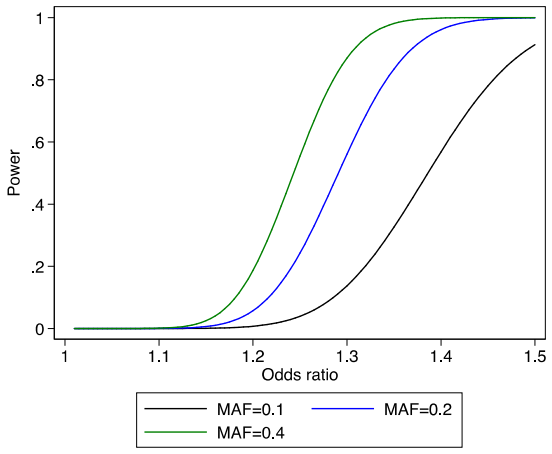
4.3.5 What sample size is required?

The power of a given study to detect a specific risk allele depends on the frequency of the risk allele in the population, the magnitude of the effect of the risk allele, the sample size, the ratio of cases to controls and the type I error rate (P-value threshold). An added level of complexity arises from genome-wide association studies where there is likely to be multiple risk alleles and the goal is to identify one or more risk alleles.

The figures show the power to detect a single risk allele by allele frequency and risk at a type I error rate of $5x10^{-8}$ for a phenotype with a frequency of 10 percent (case control ratio 1:9).

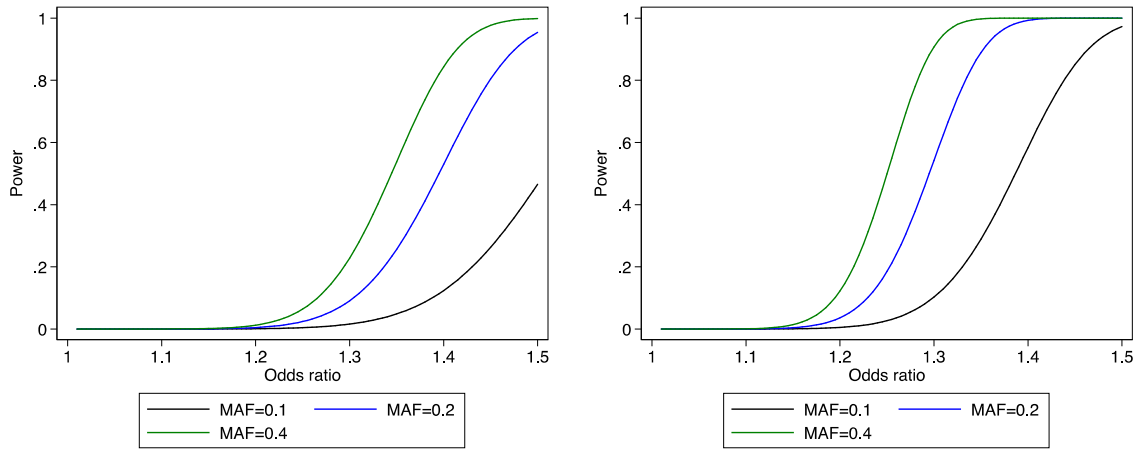


n=400/3600



n=800/7200

As can be seen, the power to detect specific, modest-effect alleles at highly stringent levels of significance is low. However, if we assume the presence of 10 risk alleles in a genome-wide study, the power to detect at least one is substantially greater.



n=400/3600 n=800/7200

4.3.6 Validation and replication

A fundamental requirement in evaluating genetic association is the need for replication of initial findings in independent data sets. What constitutes adequate replication is rarely defined and often misunderstood. There are two primary purposes of replication. First, where an initial association is of borderline statistical significance, replication can provide additional evidence of association such that when the data are combined the statistical evidence is strengthened. The strength of the statistical association as measured by the P-value in the replication study is less important than the requirement that the direction and magnitude of the effect in the replication study should be compatible with the initial association. Under these circumstances combining the data should result in a strengthening of the initial association. The second purpose of replication is to provide additional evidence that the initial association has not occurred as the result of bias or confounding. If a reported association is replicated in a second study with a different study design and carried out in a different population it reduces the likelihood that the initial association was simply due to some unidentified bias or confounding. For example, if an association is observed in a study based on a European population it is possible that that association occurred as the result of population stratification. However, if the association is replicated in a study from an Asian population the probability that population stratification accounts for the association is much less. Similarly, technical biases such as differences in DNA quality between cases and controls are unlikely to be replicated in an independent study.

There can be no definitive rules to define replication. In practice a pragmatic approach is needed. An association can be considered highly likely to be a true positive when the combined data from multiple studies carried out in different populations result in a highly significant association.

4.3.7 Future prospects

It is clear that the scope for modern science to identify common alleles associated with risk of specific radiation sensitivity phenotypes is critically dependent on the availability of large sample collections with carefully measured and relevant phenotypes. The most likely source of such studies comes from the filed of cancer radiotherapy, as large numbers of patients

treated with radiation are available for study. The Radiogenomics Consortium is an international collaboration that has recently been established to pool data and sample on large numbers of patients treated with radiotherapy in whom a variety of sensitivity endpoints have been carefully collected. It is clear that this international effort will yield some useful data, but it is very hard to predict the number of genetic loci for radiation sensitivity that will be detected or the likely effect sizes associated with such loci.

4.4 Searching for rare and uncommon risk alleles

Until recently the search for rare alleles for complex phenotypes was limited to phenotypes that had been identified in large multi-generation pedigrees, such as Mendelian disease phenotypes, as such families are amenable to gene finding by linkage. In general, linkage studies have not been possible for radiation sensitivity phenotypes due to lack of appropriate pedigree data. The exception to this has been some of the familial cancer syndromes characterised by radiation induced cancers. Advances in genotyping and sequencing technologies have made possible the search for uncommon and rare variants using alternative study designs. Rare variant genotyping chips that capture the majority of the uncommon SNPs in the genome are now being developed and the costs of high throughput sequencing (next-generation sequencing) bring the costs of targeted and even whole-genome sequencing to levels where is will be possible to sequence thousands of samples. However, there are many issues with data management and analysis and the probability of success of studies aimed at identifying uncommon or rare risk variants is not known.

As with common variants, one of the major problems in the search for uncommon or rare alleles for complex phenotypes is that the statistical power to detect single alleles, even with large sample sizes is modest. Recently published methods show that power to detect rare risk variation can be greatly enhanced by combining information across variants in a target region such as a gene, when multiple variants influence phenotype. The ‘‘cohort allelic sums test’’ (CAST) [2] and ‘‘combined multivariate and collapsing (CMC) method’’ [3] use this approach. CAST contrasts the number of individuals with one or more mutations between cases and controls. CMC, like CAST, pools all rare variants which are treated as a single count for analysis with common variants in a multivariate test. The CMC method permits a coherent test for common and rare variants (rare being defined arbitrarily, but usually at 1%). Madsen and Browning[4] introduced a non-parametric weighted sum test in which rare variants are grouped according to function (e.g. gene), and each individual is scored by a weighted sum of the mutation counts. The incorporation of weights improves the power of the test, and would be especially powerful when most of the rare variation is functionally relevant. While each of these rare variant tests differs in form, each seeks to assess the overall genetic burden due to rare variants, hence they are known as ‘‘burden tests’’. By design, they implicitly assume that all variation affecting phenotype acts in the same direction (increased risk). However, a gene harboring phenotypically relevant variation could include a handful of rare Mendelian mutations that cause the phenotype, some variants that moderately increase or decrease risk, along with numerous variants of no effect. A well-established and powerful test for the presence of a mixture of effect and neutral alleles is the C-alpha score-test [5 6], which has recently been adapted for the analysis of sequence-level, case-control data [7]. An alternative method proposed by Ionita-Laza and colleagues is based on assessing whether rare variants in a genetic region collectively occur at significantly higher frequencies in cases compared with controls (or vice versa) [8]. A main feature of the proposed methodology is that it is an overall test assessing a possibly large number of rare variants simultaneously, but the disease variants can be both protective and risk variants, with moderate decreases in statistical power when both types of variants are present. Simulations studies have shown that these approaches can be powerful under complex and general disease models, as well

as in larger genetic regions where the proportion of disease susceptibility variants may be small. Comparisons with previously published tests on simulated data show that the proposed approaches can have better power than the existing methods. It is likely that there will be further development of statistical methods for the analysis of sequence data over the next few years. For example, the admixture maximum likelihood (AML) test [9] was devised as a method for omnibus or "burden" testing of multiple common genetic variants within a gene or pathway [10]. We have now developed the method for the analysis of uncommon variants and have used the AML method in the analysis of rare sequence variants identified through resequencing of 13 genes involved in the metabolisms of cancer chemotherapy in 250 patients who had developed adverse, chemotherapy-related events after treatment (unpublished data). The AML method can also take account of variants that increase or decrease risk. Until the underlying architecture of uncommon and rare variants for complex disease susceptibility is elucidated we cannot know for certain what the most powerful statistical method will be for data analysis.

4.4.1 Future prospects

While it is difficult to predict the likely outcome of studies using the latest sequencing technologies, there is little doubt that such studies will be carried out in the next few years for a variety of radiation sensitivity phenotypes.

4.5 Conclusion

The application of new technology coupled to an increasing understanding of the range of common and rare variation in the human genome has led to rapid developments in our understanding of the inherited genetic basis of many complex disorders. Genetic association studies have been extremely successful in identifying common alleles associated with many physiological phenotypes and complex late-onset diseases in the past decade. More recently next-generation sequencing technologies have been applied to identify rarer alleles associated with the same phenotypes and these technologies are just beginning to bear fruit.

The study of the genetic epidemiology of radiation sensitivity phenotypes is relatively less well established. This is mainly due to the lack of suitable samples from individuals that have well annotated radiosensitivity phenotypes. However, initiatives such as the Radiogenomics Consortium, which is collecting data on radiation sensitivity after radiotherapy treatment for cancer in many thousands of subjects, will provide the raw material on which to apply well-established methods in genetic epidemiology. It seems highly likely that the next five to ten years will see rapid developments in our understanding of the genetic basis of interindividual variation in radiation sensitivity.

4.6 References

1. Pharoah PDP, Dunning AM, Ponder BAJ, Easton DF. Association studies for finding cancer-susceptibility genetic variants. *Nat.Rev.Cancer* 2004;4(11):850-60.
2. Morgenthaler S, Thilly WG. A strategy to discover genes that carry multi-allelic or mono-allelic risk for common diseases: a cohort allelic sums test (CAST). *Mutat Res* 2007;615(1-2):28-56.

3. Li B, Leal SM. Methods for detecting associations with rare variants for common diseases: application to analysis of sequence data. *Am.J.Hum.Genet.* 2008;83(3):311-21.

4. Madsen BE, Browning SR. A groupwise association test for rare mutations using a weighted sum statistic.*PLoS Genet* 2009;5(2):e1000384.

5. Neyman J, Scott E. On the use of c(alpha) optimal tests of composite hypotheses. *Bull.Int.Stat.Inst.*1966;41:477-97.

6. Zeleterman D, Chen C. Homogeneity tests against central-mixture alternatives. *J.Am.Stat.Assoc.* 1988;83(401):179-82.

7. Neale BM, Rivas MA, Voight BF, Altshuler D, Devlin B, Orho-Melander M, et al. Testing for an unusual distribution of rare variants. *PLoS Genet* 2011;7(3):e1001322.

8. Ionita-Laza I, Buxbaum JD, Laird NM, Lange C. A new testing strategy to identify rare variants with either risk or protective effect on disease. *PLoS Genet* 2011;7(2):e1001289.

9. Tyrer J, Pharoah PDP, Easton DF. The admixture maximum likelihood test: A novel experiment-wise test of association between disease and multiple SNPs. *Genet.Epidemiol.* 2006;30(7):636-43.

10. Pharoah PD, Tyrer J, Dunning AM, Easton DF, Ponder BA. Association between Common Variation in 120 Candidate Genes and Breast Cancer Risk. *PLoS Genet* 2007;3(3):e42.

4.7 Glossary of terms

Common	> 5%
Uncommon	1-5 percent
Rare	0.1 – 1 percent
Very rare	<0.1 percent

5 IDENTIFICATION OF CANDIDATE SUSCEPTIBILITY GENES IN HUMAN RADIATION-ASSOCIATED THYROID TUMORS

Sylvie Chevillard

Commissariat à l'Energie Atomique, DSV, iRCM
Fontenay-aux-Roses, France

The link between external radiation during childhood and thyroid cancer has been known since 1950 (Duffy and Fitzgerald, 1950), and up to recently this was the only demonstrated etiological risk factor for thyroid cancers (Ron et al, 1995). A higher incidence of thyroid cancer has been reported in epidemiological studies after either internal or external exposure (Ron et al., 1995; Cardis et al., 2005). A pooled analysis of seven studies established that the excess relative risk of thyroid cancer in subjects irradiated at a young age was very high - 7.7 per Gray (Gy) - the risk being significant for radiation doses as low as 0.1 Gy and increasing linearly with increasing doses (Ron et al., 1995). It has been estimated that 88% of thyroid carcinomas occurring in subjects exposed to radiation doses equal to 1Gy during childhood are radiation induced. The risk of developing a thyroid carcinoma is the highest 15-30 years after exposure, but is still present after more than 40 years (Ron et al., 1995). If exposure occurred at adulthood, the risk is much lower (Richardson, 2009).

In parallel, a worldwide increase in thyroid tumors, mainly papillary thyroid carcinomas (PTC), has been observed over the last 30 years (Enewold et al., 2009). This has led to debate concerning a potential link with changes in environmental exposure linked to nuclear tests, the nuclear industry and, in Western Europe, Chernobyl fallout. Some data suggest that this increase is at least partly related to the routine screening of thyroid nodules using neck ultrasound and fine needle biopsy which permit the detection of small papillary carcinomas that would otherwise have gone undetected (Leenhardt et al., 2004; Colonna et al., 2007). This high prevalence of such small cancers had already been reported in autopsy studies (Harach et al., 1985; Yamamoto et al., 1990). However, it is not possible to exclude that some of these thyroid tumors could have been radiation-induced.

Radiation-induced thyroid tumors have no specific histological characteristics (Rubino et al., 2002; Williams et al., 2008). They are either follicular thyroid adenomas (FTA) or PTC. These histological subtypes are also the most frequent sporadic thyroid tumors. For these reasons, it is of major interest to identify specific molecular signature of thyroid cancer developing after thyroid radiation exposure that would indicate, with a high probability, the etiology of any tumor at the individual level.

Molecular differences between sporadic and radiation-induced thyroid tumors were searched using microarray transcriptome analysis. A first study, including sporadic and post-Chernobyl PTC, did not show any specific radiation-induced gene expression signature (Detours et al., 2005). However, the authors were able to classify their series of tumors by using a signature that was previously found to discriminate between irradiated- and hydrogen peroxide treated-lymphocytes (Detours et al., 2007). Others studies found radiation-induced signatures in post-Chernobyl PTC (Port et al., 2007; Stein et al., 2010), but without blind validation of the signature. A recent study compared cell cycle protein expression in sporadic and post-radiotherapy PTC, but none of the tested markers could be associated with the etiology (Achille et al., 2009), while combinations of protein markers such as matrix metalloproteinases, cathepsins and neurotrophic tyrosine kinase receptor 1 allowed

discrimination of post-Chernobyl PTC as function of etiology by immunostaining (Boltze et al., 2009). Altogether, these studies must be considered as preliminary and others should be analyzed to establish a robust characterization of the gene expression differences between sporadic and radiation-induced PTC.

We first aimed at identifying ionizing radiation-related molecular specificities of human thyroid tumors developed in the radiation field of patients treated with radiotherapy. We compared the transcriptome profiles of sporadic and radiation-induced FTA and PTC (n = 28) obtained after hybrization on 25K oligonucleotide microarray and identified a signature of 322 genes that discriminated between radiation-induced tumors (FTA and PTC) and their sporadic counterparts. An independent testing set of 29 tumors was used to validate this signature by a blind classification in function of the etiology (Ory et al., 2011).

To identify molecular markers that could represent a radiation-induction signature of thyroid cancers, 57 tumor samples were collected at the Institut Gustave Roussy in collaboration with Dr Martin Schlumberger and at the Nice Human Biobank (Cancéropole PACA and CRB INSERM, CHU Nice) in collaboration with Dr Paul Hofman. The series comprised 12 radiation-induced PTCs (rPTCs), 15 radiation-induced follicular adenomas (rFTAs), 15 sporadic papillary thyroid carcinomas (sPTCs) and 15 sporadic follicular thyroid adenomas (sFTAs). Clinical data for sporadic and radiation-induced thyroid tumors are presented in Tables 1 and 2.

The thyroid tumors were split into 2 sets:

- the learning/training set, used to search for the molecular signature, comprised 7 rFTAs, 7 sFTAs, 7 rPTCs and 7 sPTCs.
- the testing set, used for blind classification, comprised the remaining 29 tumors (16 FTAs and 13 PTCs).

At the time of the blind classification, the tumor histology was known, but not any history of radiation exposure.

Sporadic tumors: To prevent any bias due to multiple comparison failure, patients with sporadic tumors of the learning/training set were selected to match, as far as possible, histology, TNM, sex, ethnicity and age at tumor diagnosis of patients with radiation-induced tumors. Specific enquiry on the absence of radiation exposure was indeed carried out.

To increase the likelihood that the leaning/testing tumors, used to search the signature, are radiation–induced and not sporadic, we paid special attention in the choice of patients and tumors included in this group. We strictly followed the Cahan criteria (Cahan et al., 1948): 1) after radiotherapy, the second neoplasm must arise in the irradiated field and be proved histologically, 2) a latent period of at least several years must have elapsed between radiation exposure and development of the second neoplasm and 3) the second tumor must be histologically different from the first tumor. Moreover, only patients treated before 15 year of age, which is considered as the period of high thyroid radiation sensitivity (Ron, 1995; Steliarova-Foucher et al., 2006), were included in the learning/training set of tumors. The estimated doses received by the thyroid gland are given in Table 1. These doses were retrospectively calculated using an approach and software developed by Diallo et al. (Diallo et al., 1996; Diallo et al., 2009).

Patients who received chemotherapy for the treatment of their primary tumors are indicated in Table 1.and 2. Importantly, for the validity of the data, it was shown that chemotherapy for the first cancer was not associated with thyroid cancer risk and it did not modify the effect of radiotherapy (Sigurdson et al., 2005). All patients were Caucasian except one who was African black.

To complete samples characterization, we searched for *RAS* and *BRAF* genetic alterations by PCR and sequencing and for *RET/PTC1 and RET/PTC3* rearrangements by PCR analysis (Table 3).

Most of methods used to analyze microarray data want to identify groups of genes that have coherent patterns of expression with large variance across groups of samples. Unfortunately, using these methods, we did not found any signature of tumor etiology, Gene shaving is a useful alternative method, which used Principal Component Analysis (PCA) to find the space direction, which capture the majority of variance in the whole data set and thus permits to find genes able to separate two groups of tumors, whatever the variance of individual gene expression. Unfortunately, gene shaving is not adapted to small series of samples, such as our series of tumors. For this reason, we have developed a method based on a similar strategy than that of gene shaving but adapted to limited number of samples. It permits to find the space direction which maximizes, if it exists, criteria discriminating two groups of tumors.

By using this method, we found a 322 gene expression signature permitting to distinguish sporadic and radiation-induced tumors, whatever the histology FTA or PTC. This set of genes included 137 over-expressed and 185 under-expressed in radiation-induced tumors compared with sporadic tumors.

In order to check that this molecular signature of tumor etiology was not specific for some DNA mutations, we searched for *RAS*, *RET/PTC* and *BRAF* genetic alterations in the learning/training set of tumors (Tables 1 and 2). Mutations at codon 61 of *N*-, *H*- or *K*-RAS were found in 1 rFTA and 2 sFTA. *RET/PTC* rearrangements were identified in 2 sPTC and *BRAF* mutations were detected in 4 sPTC and in 1 rPTC (with no overlap with *RET* rearrangements). Thus it is unlikely that the signature could be specific to any type of mutation.

For blind validation of the molecular signature, each testing tumor was projected into the classification space, allowing us to propose an etiology depending on the relative positioning of the testing tumors compared with the learning/training tumors (Figure 1).

This signature was robust, since it correctly predicted the etiology of 26 of the 29 tumors. The present signature of thyroid tumor etiology has a very good negative predictive value, as all testing tumors diagnosed as sporadic were indeed sporadic tumors, and a rather good positive predictive value, as 12 of the 14 radiation-induced testing tumors were well diagnosed (1 and 0.85, respectively).

To understand the molecular specificities of radiation-induced thyroid tumorigenesis, we searched for the biological function and relationship between the 322 genes of the discriminating signature. However, to be more exhaustive in the overview of the radiation-induced deregulated pathways, we also included the 651 pre-selected genes found to be deregulated during the training step. While not included in the final signature, these genes were able to classify tumors in several combinations of tumors of the learning/training set without misclassification.

Deregulated genes in radiation-induced tumors are mostly involved in molecular mechanisms such as cellular response to oxidative stress and irradiation, response to hypoxia, regulation of p53 function (Figure 2), immune response and signal transduction pathways including MAPK, EGFR, RAC/CDC42, hedgehog, TGF/BMP, calcium signaling and WNT canonical and noncanonical pathways (Figure 3). WNT/□catenin pathway has already been implicated in normal thyroid function and in thyroid carcinogenesis (Castellone et al., 2009; Garcia-Rostan et al., 2001).

5.1 Conclusion

For the first time we found a molecular signature of thyroid tumor etiology by comparing post-radiotherapy induced thyroid tumors with sporadic tumors. Moreover, this signature was successfully validated by a blind classification of in independent series of tumors.

Indeed, few studies on transcriptome analysis of post-Chernobyl thyroid tumors have been published. In our study, most of the patients were externally exposed to radiation to treat Hodgkin's disease or non-Hodgkin lymphoma. The estimated mean dose received by the thyroid gland varies between 0.1 and 43Gy, in repeated exposure at high dose rates (Table 1), In contrast, after the Chernobyl accident victims' thyroids were mainly contaminated chronically after ^{131}I ingestion. In the exposed population, the cumulative thyroid radiation doses ranged from less than 0.02Gy to more than 10Gy, but most people received doses less than 1Gy. A low iodine diet in the exposed population was reported to be an important parameter in the development of these tumors (Williams et al., 2008). The relevance of extrapolation of conclusions from data on tumors occurring after exposure to external radiation to post-Chernobyl tumors that occurred after internal ^{131}I contamination is unclear.

An overlap was found between post-radiotherapy deregulated genes, identified in our study, and already published post-Chernobyl deregulated genes. *EPB41L3*, a tumor suppressor included in the 322-gene signature, and *RERG*, *C13ORF33*, *GZMH*, *MST150*, *RARRES1*, *RIPK4* and *SFRP1*, found in the enlarged list of genes (see above), were reported in a set of genes deregulated in post-Chernobyl PTC (Port et al., 2007). Genes such as *ABI2*, *COL4A5*, *FAT3*, *IGFBP3*, *KRTAP3-2*, *SPOCK1* and several immunoglobulin chains were deregulated in post-radiotherapy tumors, while genes of the same family and/or function, such as *ABI3*, *COL4A6*, *FAT2*, *IGFBP1*, *KRTAP2-1*, *KRTAP2-4*, *SPOCK2* and other immunoglobulin chains, were also deregulated in the study of Port et al. (Port et al., 2007), suggesting common deregulated pathways in post-radiotherapy and post-Chernobyl tumors. Since we also observed genes in common with another previously published post-Chernobyl series of thyroid tumors (Detours et al., 2007), (genes from the signature: *C4A*, *CLU*; *DCI*, *DHCR24*, *EGFR*, *EGR3*, *GTF2H2*, *ICAM3*, *NRIP1*, *PLA2R1*, *RPS19* and from the enlarged set: *EFNA1*, *EIF2AK2*, *FAM38A*, *MED1*, *MGEF8*, *PPL*, *SCARA3*, *SMO* and *ZFHX4*), several of these genes could be potential markers of radiation-induced thyroid tumors, independently of the histology and the internal-external type of radiation exposure. It should be mentioned that *KLK10*, under-expressed in post-radiotherapy induced thyroid tumors, was also identified as a specific down-regulated gene in radiation-transformed human mammary epithelial cells (Liu et al., 1996).

Molecular signature discriminating sporadic from post-Chernobyl thyroid papillary carcinomas

As previously mentioned, to validate the efficiency of our method for identifying a transcriptomic signature, we analyzed a previously published series of 26 sporadic and post-Chernobyl PTCs (Accession number GSE3950). We chose this dataset because of our interest on finding radiation-induced specific signature in thyroid tumors and because the authors of the paper could not find a signature using several usual bioinformatic supervised and unsupervised classification methods (Detours et al., 2005; Detours et al., 2007). But importantly, by reading the manuscript, the existence of a signature could be suspected since these 26 samples were roughly classified, by the authors, by applying an empirical signature elaborated from previously published stress-specific signatures (Detours et al., 2007).

On this series of papillary thyroid tumors we applied our method of tumor classification based on a learning / training step followed by a testing step. For this analysis, we selected 14 tumors (7 sPTCs and 7 rPTCs) of the 26 samples for the learning training step and the

remaining 12 tumors (7 sPTCs and 5 rPTCs) were used for the blind validation. The 12 tumors of the learning / training step were chosen to match, as much as possible, age at tumor diagnosis when compared sporadic and radiation-induced groups (Table 3).

A signature of 106 genes discriminating sporadic versus Chernobyl thyroid tumors was found and this signature was robust enough to classify without error the all 12 remaining testing tumors in either the sporadic PTC or post-Chernobyl PTC subgroup (Figure 4).

Of course, we wanted to prove that the signature obtained, in the series of post-radiotherapy tumors, was not a signature of cancer predisposition but really a signature of tumor aetiology.

There are five common genes between the post-Chernobyl signature and the post-radiotherapy signature, these 5 genes classified both the post-radiotherapy and the post-Chernobyl papillary thyroid carcinomas as compared with the sporadic tumors (Figures 5). Moreover, part of each post-radiotherapy (37 probes) and post-Chernobyl signature (18 probes) cross-classified the respective series of thyroid tumors (Figure 6). Several molecular pathways deregulated in post-Chernobyl tumors matched with those found to be deregulated in post-radiotherapy tumors.

Overall, data suggest that thyroid tumors that developed following either external exposure or internal 131 iodide contamination shared common molecular features allowing their classification as radiation-induced tumors in comparison with sporadic counterparts, independently of doses and dose rates, and that a common radiation-induced signature may be identified in radiation-induced thyroid tumors. Analysis of the genes deregulated in radiation-induced thyroid tumors suggests that both response to stress and impact of genic susceptibilities may be associated with radiation-induced thyroid tumorigenesis.

5.2 References

Achille, M., H. Boukheris, et al. (2009). "Expression of cell cycle biomarkers and telomere length in papillary thyroid carcinoma: a comparative study between radiation-associated and spontaneous cancers." Am J Clin Oncol 32(1): 1-8.

Boltze, C., A. Riecke, et al. (2009). "Sporadic and radiation-associated papillary thyroid cancers can be distinguished using routine immunohistochemistry." Oncol Rep 22(3): 459-67.

Cahan, W. G., H. Q. Woodard, et al. (1948). "Sarcoma arising in irradiated bone; report of 11 cases." Cancer 1(1): 3-29.

Cardis, E. and M. Hatch (2011). "The Chernobyl accident--an epidemiological perspective." Clin Oncol (R Coll Radiol) 23(4): 251-60.

Castellone, M. D., V. De Falco, et al. (2009). "The beta-catenin axis integrates multiple signals downstream from RET/papillary thyroid carcinoma leading to cell proliferation." Cancer research 69(5): 1867-1876.

Colonna, M., A. V. Guizard, et al. (2007). "A time trend analysis of papillary and follicular cancers as a function of tumour size: a study of data from six cancer registries in France (1983-2000)." Eur J Cancer 43(5): 891-900.

Detours, V., L. Delys, et al. (2007). "Genome-wide gene expression profiling suggests distinct radiation susceptibilities in sporadic and post-Chernobyl papillary thyroid cancers." Br J Cancer 97(6): 818-25.

Detours, V., S. Wattel, et al. (2005). "Absence of a specific radiation signature in post-Chernobyl thyroid cancers." Br J Cancer 92(8): 1545-52.

Diallo, I., A. Lamon, et al. (1996). "Estimation of the radiation dose delivered to any point outside the target volume per patient treated with external beam radiotherapy." Radiotherapy and oncology : journal of the European Society for Therapeutic Radiology and Oncology 38(3): 269-271.

Diallo, I., N. Haddy, et al. (2009). "Frequency distribution of second solid cancer locations in relation to the irradiated volume among 115 patients treated for childhood cancer." International journal of radiation oncology, biology, physics 74(3): 876-883.

Duffy, B. J., Jr. and P. J. Fitzgerald (1950). "Thyroid cancer in childhood and adolescence; a report on 28 cases." Cancer 3(6): 1018-32.

Enewold, L., K. Zhu, et al. (2009). "Rising thyroid cancer incidence in the United States by demographic and tumor characteristics, 1980-2005." Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology 18(3): 784-791.

Garcia-Rostan, G., R. L. Camp, et al. (2001). "Beta-catenin dysregulation in thyroid neoplasms: down-regulation, aberrant nuclear expression, and CTNNB1 exon 3 mutations are markers for aggressive tumor phenotypes and poor prognosis." The American journal of pathology 158(3): 987-996.

Harach, H. R., K. O. Franssila, et al. (1985). "Occult papillary carcinoma of the thyroid. A "normal" finding in Finland. A systematic autopsy study." Cancer 56(3): 531-8.

Hess, J., G. Thomas, et al. (2011). "Gain of chromosome band 7q11 in papillary thyroid carcinomas.

Leenhardt, L., M. O. Bernier, et al. (2004). "Advances in diagnostic practices affect thyroid cancer incidence in France." Eur J Endocrinol 150(2): 133-9.

Liu, F. F. and R. P. Hill (1996). "Potential role of HSP70 as an indicator of response to radiation and hyperthermia treatments for recurrent breast cancer." International journal of hyperthermia : the official journal of European Society for Hyperthermic Oncology, North American Hyperthermia Group 12(2): 301-302.

Ory C, Ugolin N, Levalois C, Lacroix L, Caillou B, Bidart JM, Schlumberger M, Diallo I, de Vathaire F, Hofman P, Santini J, Malfoy B, Chevillard S 2011 Gene expression signature discriminates sporadic from post-radiotherapy-induced thyroid tumors. Endocr Relat Cancer 18:193-206.

Port, M., C. Boltze, et al. (2007). "A radiation-induced gene signature distinguishes post-chernobyl from sporadic papillary thyroid cancers." Radiat Res 168(6): 639-49.

Richardson, D. B. (2009). "Exposure to ionizing radiation in adulthood and thyroid cancer incidence." Epidemiology 20(2): 181-187.

Ron, E., J. H. Lubin, et al. (1995). "Thyroid cancer after exposure to external radiation: a pooled analysis of seven studies." Radiat Res 141(3): 259-77.

Ron, E., J. H. Lubin, et al. (1995). "Thyroid cancer after exposure to external radiation: a pooled analysis of seven studies." Radiat Res 141(3): 259-77.

Rubino, C., A. F. Cailleux, et al. (2002). "Thyroid cancer after radiation exposure." European journal of cancer 38(5): 645-647.

Sigurdson, A. J., C. M. Ronckers, et al. (2005). "Primary thyroid cancer after a first tumour in childhood (the Childhood Cancer Survivor Study): a nested case-control study." Lancet 365(9476): 2014-2023.

Stein, L., J. Rothschild, et al. (2010). "Copy number and gene expression alterations in radiation-induced papillary thyroid carcinoma from chernobyl pediatric patients." Thyroid 20(5): 475-87.

Steliarova-Foucher, E., P. Kaatsch, et al. (2006). "Quality, comparability and methods of analysis of data on childhood cancer in Europe (1978-1997): report from the Automated Childhood Cancer Information System project." European journal of cancer 42(13): 1915-1951.

Ugolin, N., C. Ory, et al. (2011). "Stategy to find molecular signatures in a small series of rare cancers: validation for radiation-induced breast and thyroid tumors " PloS One 6(8): e23581.

Williams, E. D., A. Abrosimov, et al. (2008). "Morphologic characteristics of Chernobyl-related childhood papillary thyroid carcinomas are independent of radiation exposure but vary with iodine intake." Thyroid 18(8): 847-52.

Yamamoto, Y., T. Maeda, et al. (1990). "Occult papillary carcinoma of the thyroid. A study of 408 autopsy cases." Cancer 65(5): 1173-9.

5.3 Tables and graphs

Table 1: Clinical data for radiation-induced follicular adenomas and papillary carcinomas (post radiotherapy series)

Patient	Histology	Sex	Primary tumor	Age at IR (yr)	Age at tumor diagnosis (yr)	Thyroid dosimetry (Gy)	Ethnicity	Tumor size (mm)	Chemotherapy	Detected by
Learning/training set										
RA1	FTA	F	Acne	13	46	20	C	20	-	Screening
RA2	FTA	F	Hodgkin's disease	3	36	42	C	22	-	Screening
RA3	FTA	F	Hodgkin's disease	3	36	42	C	13	-	Screening
RA4	FTA	M	Non-Hodgkin lymphoma	8	56	43	C	25	-	Incidental finding
RA5	FTA	M	Nasopharynx carcinoma	9	37	28	C	30	+	Screening
RA6	FTA	F	Non-Hodgkin lymphoma	5	25	43	C	12	-	Incidental finding
RA7	FTA	F	Hodgkin's disease	11	29	21	C	12	+	Screening
RP1	PTC	F	Hodgkin's disease	14	48	43	C	8	-	Incidental finding
RP2	PTC	F	Non-Hodgkin lymphoma	11	22	42	C	11	-	Incidental finding
RP3	PTC	M	Hodgkin's disease	12	30	15	c	15	+	Screening
RP4	PTC	F	Lymphoma	10	40	40	C	10	-	Incidental finding
RP5	PTCFV	M	Neuroblastoma	7	22	12	C	28	+	Screening
RP6	PTC	F	Hodgkin's disease	9	45	40	C	10	-	Incidental finding
RP7	PTC	F	Acute lymphoblastoid leukemia	6	20	12	C	9	+	Screening
				Mean = 8	Mean = 35			Mean = 16		
Testing set										
XA9	FTA	M	Hodgkin's disease	19	40	40	C	8	+	Screening
XA10	FTA	F	Hodgkin's disease	12	35	8	C	30	+	Screening
XA11	FTA	M	Hodgkin's disease	13	53	Unavailable	Unavailable	Unavailable	Unavailable	Screening
XA12	FTA	F	Hodgkin's disease	23	40	43	C	10	+	Unavailable
XA13	FTA	F	Hodgkin's disease	29	37	41	c	10	+	Screening
XA14	FTA	F	Hodgkin's disease	16	60	43	C	13	-	Screening
XA15	FTA	F	Non-Hodgkin lymphoma	19	43	41	C	45	-	Incidental finding
XA16	FTA	F	Uterus	28	60	48	C	30	-	Incidental finding
XP9	PTCFV	M	Hodgkin's disease	23	36	20	C	30	+	Incidental finding
XP10	PTC	F	Ovarian teratoma	13	30	0.1	AB	3	+	Screening
XP11	PTC	F	Lymphoma	24	59	44	C	12	-	Screening
XP12	PTC	F	Hodgkin's disease	11	61	40	C	100	-	Incidental finding
XP13	PTCFV	F	Graves disease	19	39	Unavailable	Unavailable	Unavailable	Unavailable	Incidental finding
				Mean = 19	Mean = 46			Mean = 24		

Table 2: Clinical data for sporadic tumors (post radiotherapy series)

Patient	Histology	Sex	Age at tumor diagnosis (yr)	Ethnicity	Tumor size (mm)	Chemotherapy	Detected by
Learning/training set							
SA1	FTA	F	59	C	26	-	Screening
SA2	FTA	M	63	C	30	-	Screening
SA3	FTA	M	48	C	20	-	Screening
SA4	FTA	F	22	C	40	-	Screening
SA5	FTA	M	44	C	33	-	Incidental finding
SA6	FTA	M	24	C	55	-	Screening
SA7	FTA	M	21	C	45	-	Incidental finding
SP1	PTCFV	F	54	C	50	-	Screening
SP2	PTC	F	27	C	10	-	Screening
SP3	PTC	F	25	C	20	-	Screening
SP4	PTCFV	F	44	C	32	-	Screening
SP5	PTC	F	39	C	18	-	Screening
SP6	PTC	F	34	C	13	-	Incidental finding
SP7	PTC	F	23	C	23	-	Incidental finding
			Mean = 37		Mean = 29		
Testing set							
XA1	FTA	M	58	C	35	-	Incidental finding
XA2	FTA	F	31	C	20	-	Screening
XA3	FTA	F	29	C	13	-	Screening
XA4	FTA	F	29	C	15	-	Screening
XA5	FTA	F	27	C	30	-	Screening
XA6	FTA	F	59	C	26	-	Screening
XA7	FTA	F	22	C	Unavailable	-	Screening
XA8	FTA	F	48	C	38	-	Screening
XP1	PTC	F	17	C	30	-	Screening
XP2	PTC	F	25	C	25	-	Screening
XP3	PTC	F	39	C	20	-	Screening
XP4	PTC	F	17	C	10	-	Screening
XP5	PTC	M	74	C	25	-	Screening
XP6	PTCFV	F	73	C	17	-	Screening
XP7	PTCFV	M	41	C	55	-	Screening
XP8	PTC	F	40	C	20	-	Screening
			Mean = 39		Mean = 25		

Table 3: Genic alterations in the thyroid tumors (post radiotherapy series)

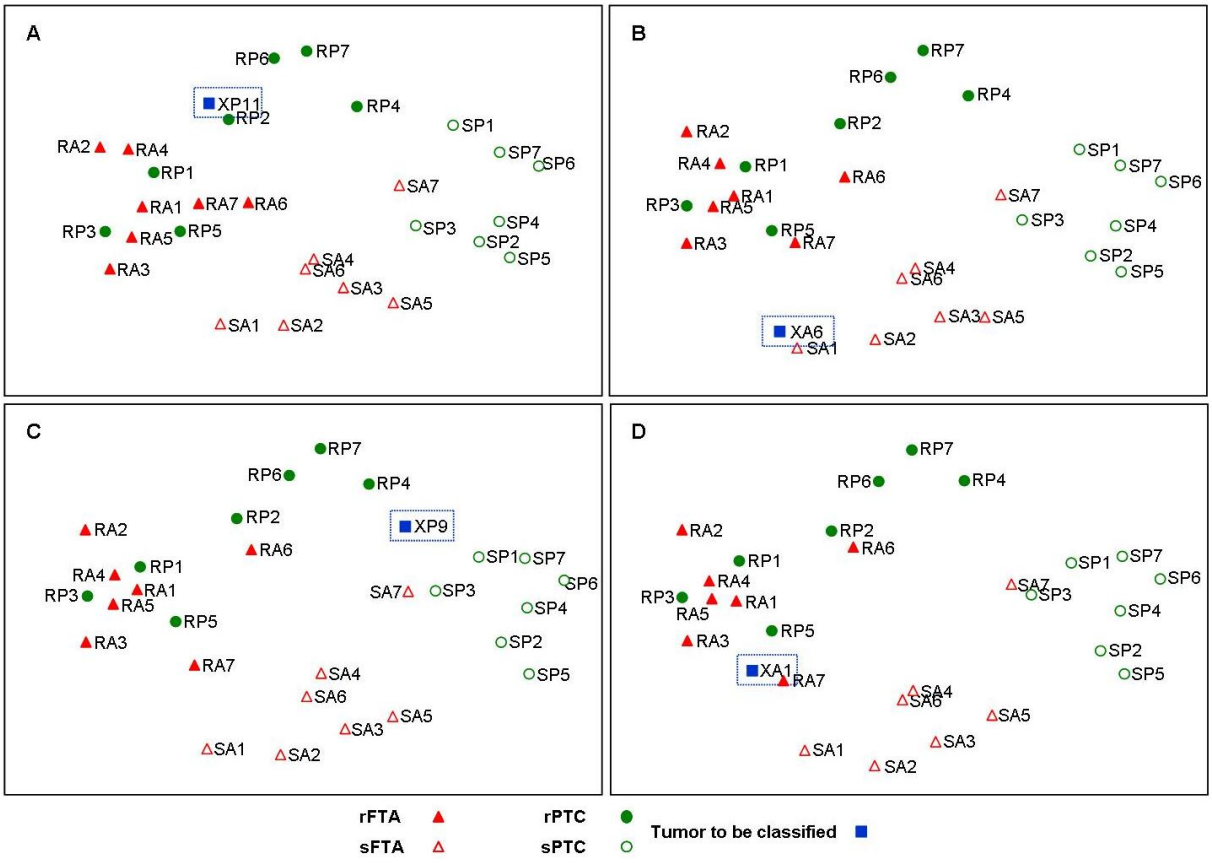
Patient	Genic alterations	Patient	Genic alterations
Learning/training set (radiation-induced tumors)		**Learning/training set (sporadic tumors)**	
RA1		**SA1**	
RA2	HRAS Q61R	**SA2**	
RA3		**SA3**	
RA4		**SA4**	
RA5		**SA5**	HRAS Q61R
RA6		**SA6**	
RA7		**SA7**	NRAS Q61R
RP1		**SP1**	BRAF V600E
RP2		**SP2**	BRAF V600E
RP3		**SP3**	
RP4		**SP4**	RET/PTC3
RP5		**SP5**	BRAF V600E
RP6	BRAF V600E	**SP6**	RET/PTC1
RP7		**SP7**	BRAF V600E
Testing set (radiation-induced tumors)		**Testing set (sporadic tumors)**	
XA9		**XA1**	
XA10		**XA2**	
XA11		**XA3**	
XA12		**XA4**	
XA13	KRAS Q61K	**XA5**	
XA14		**XA6**	
XA15		**XA7**	NRAS Q61K
XA16		**XA8**	
XP9	RET/PTC3	**XP1**	BRAF V600E
XP10	RET/PTC1	**XP2**	
XP11		**XP3**	
XP12	RET/PTC3	**XP4**	RET/PTC1
XP13	KRAS Q61R	**XP5**	BRAF V600E
		XP6	BRAF 3bp Del
		XP7	
		XP8	BRAF V600E

Table 4: Clinical data for sporadic and post-Chernobyl tumors (Chernobyl series)

Patient	Sex	Etiology	Age at IR (yr)	Age at tumor diagnosis (yr)	Genic alterations
Learning/training set					
RPTC7	F	**S**		29	BRAF V600E
RPTC14	M	**S**		32	
RPTC23	M	**S**		33	
RPTC8	M	**S**		36	RET/PTC
RPTC11	F	**S**		37	
RPTC6	M	**S**		37	
RPTC9	F	**S**		38	BRAF V600E
				Mean = 35 yr	
S418	M	**R**	10	27	BRAF V600E
S409	F	**R**	11	28	BRAF V600E
S415	M	**R**	12	28	BRAF V600E
S420	F	**R**	12	28	
S422	M	**R**	15	31	BRAF V600E
S414	F	**R**	16	33	RET/PTC
				Mean = 29 yr	
Testing set					
PTC26	F	**S**		47	BRAF V600E
PTC21	F	**S**		54	RET/PTC
PTC18	F	**S**		59	BRAF V600E
PTC22	F	**S**		60	
PTC25	F	**S**		60	
PTC19	M	**S**		68	RET/PTC
PTC20	F	**S**		68	BRAF V600E
				Mean = 59 yr	
S404	F	**R**	1	16	
S405	F	**R**	1	16	
V519	F	**R**	2	18	RET/PTC
S425	M	**R**	3	19	RET/PTC
S423	F	**R**	5	22	RET/PTC
V608	F	**R**	15	32	BRAF V600E
				Mean = 21 yr	

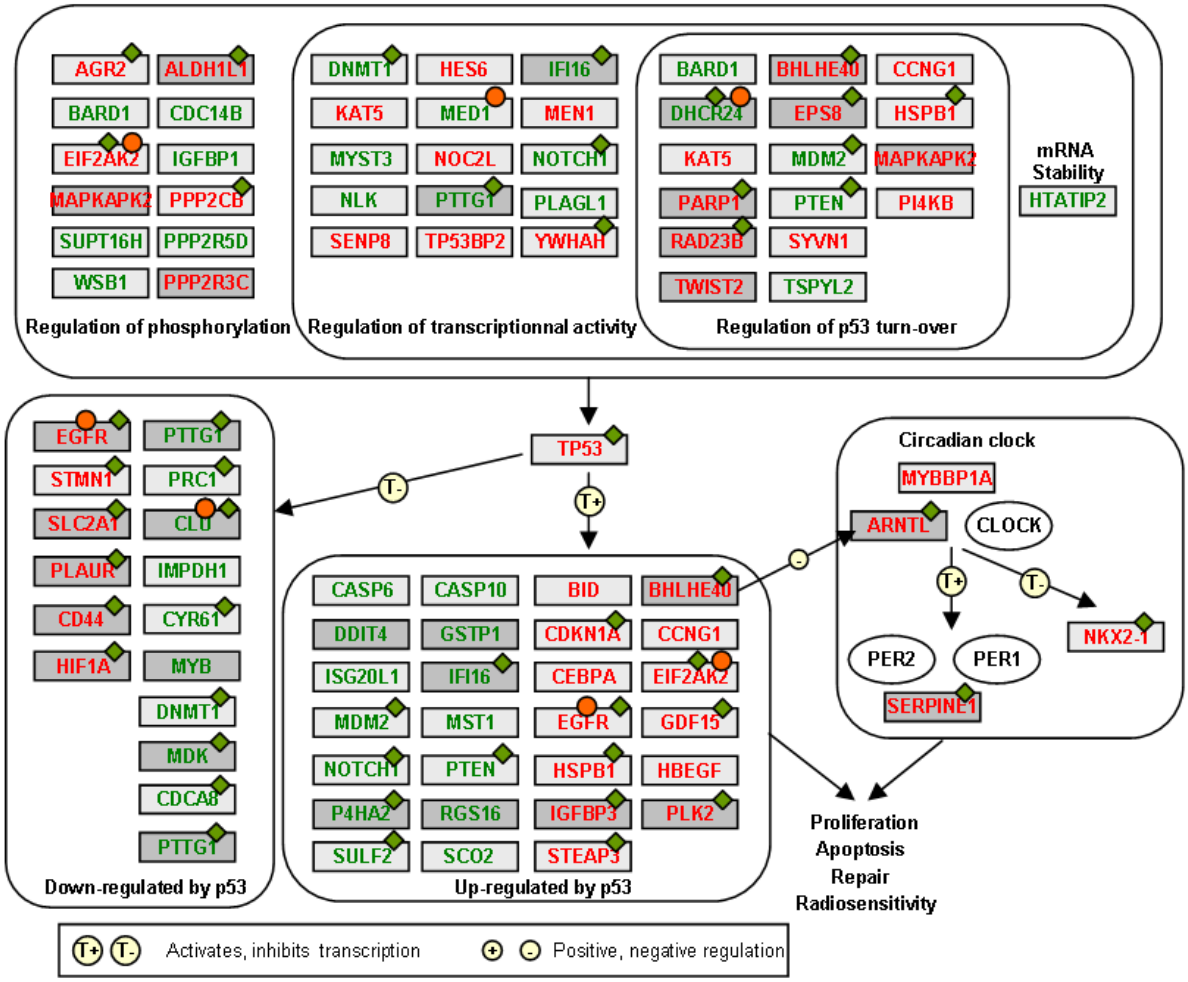
The data are reported as described in (Detours et al., 2007). PTCxx : sporadic PTC ; Sxx or Vxx : Chernobyl tumors

Figure 1: Blind validation of the post-radiotherapy-induced signature by PCA analysis of the testing tumors in the classification space defined by the tumors of the learning/training set (post-radiotherapy series)



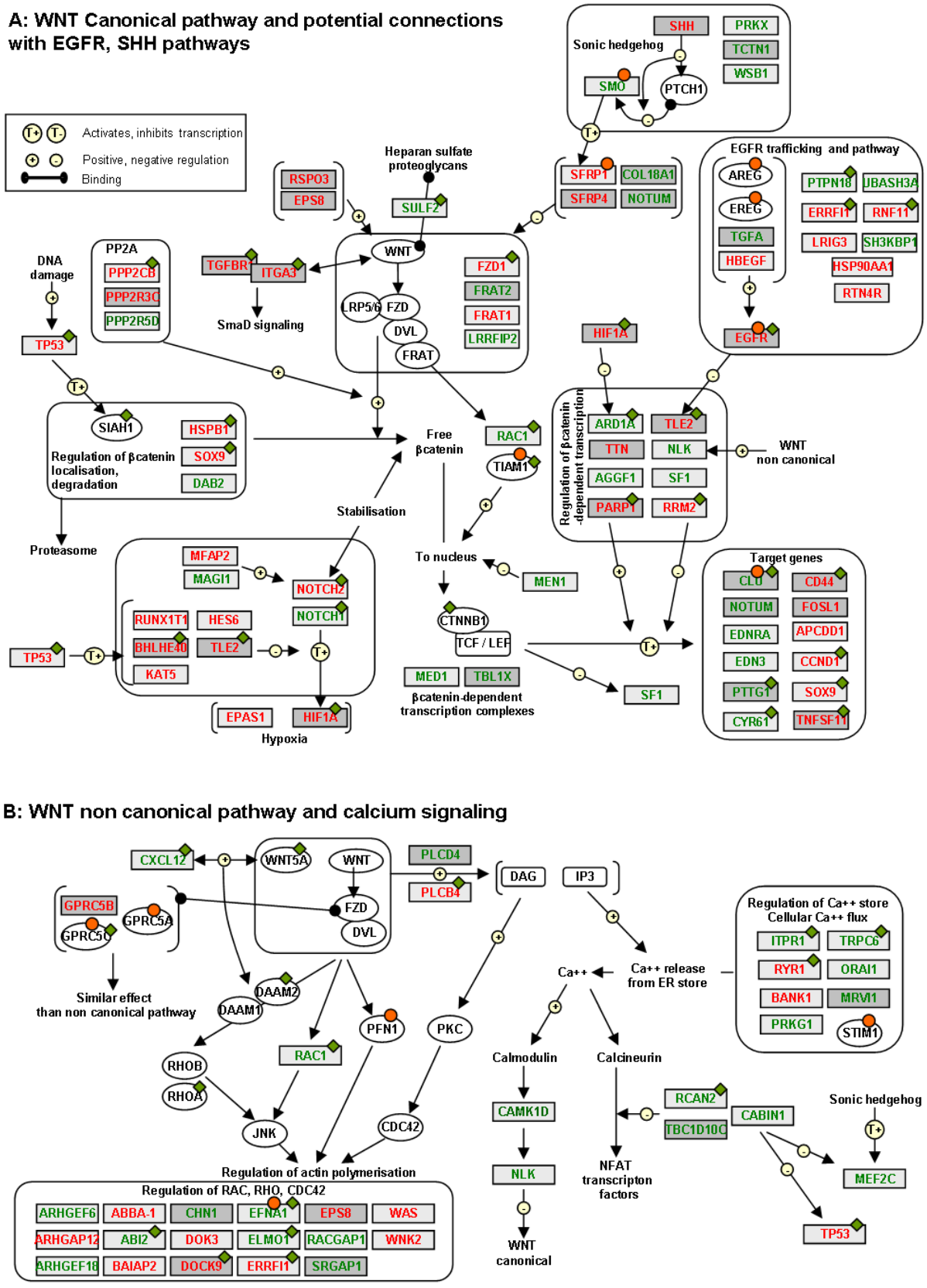
By the two-step PCA method, the tumors of the learning set, being FTA (red triangle) and PTC (green circle), either sporadic (empty symbols) or radiation-induced (full symbols), defined a validation space in which each tumor of the testing set is projected for identifying its etiology. The figure represents examples of the relative positioning of four testing tumors (blue square) in this validation space. A: a well-classified rPTC (XP11), B: a well-classified sFTA (XA6), C: the outlier rPTC tumors (XP9), positioned in the validation space between the rPTC and sPTC subgroups. D: a misclassified sFTA (XA1). Values of tumors used for hypothesis finding in A-D seem to differ slightly. This is an artefact due to data representation in two dimensions. The validation space is defined in 10 dimensions, according to the tumors of the learning/training set, and each tumor of the validation set is projected in this space to be classified. To visualize the results of tumor classification, the space is restrained to 3 dimensions and projected in two dimensions. During this reduction, the relative localization of the tumors could appear slightly modified.

Figure 2: Deregulated genes of the post-radiotherapy signature involved in the regulation of p53 turnover and/or function



Genes over-expressed (green) or under-expressed (red), either in the discriminating signature (322 genes) or deregulated with less recurrence in post-radiotherapy tumors are indicated by dark or light rectangles, respectively. Orange circles show genes found to be deregulated in post-Chernobyl tumors, while green diamonds indicate deregulated genes in thyroid sporadic tumors (data from published papers).

Figure 3: Deregulated genes of the post-radiotherapy signature involved in the WNT canonical and noncanonical pathways deregulated in radiation-induced thyroid tumors (post-radiotherapy series)



The figures represent a simplified overview of WNT canonical pathway with potential connections with EGFR, SHH or NOTCH pathways (A) and WNT noncanonical pathway (B). Genes over-expressed (green) or under-expressed (red), either in the discriminating signature (322 genes) or deregulated with less recurrence in post-radiotherapy tumors are indicated by dark or light rectangles, respectively. Orange circles show genes found to be deregulated in post-Chernobyl tumors, while green diamonds indicate deregulated genes in thyroid sporadic tumors (data from published papers).

Figure 4: Validation of the 106 genes Chernobyl signature: Blind classification of the 12 testing tumors of the Chernobyl series

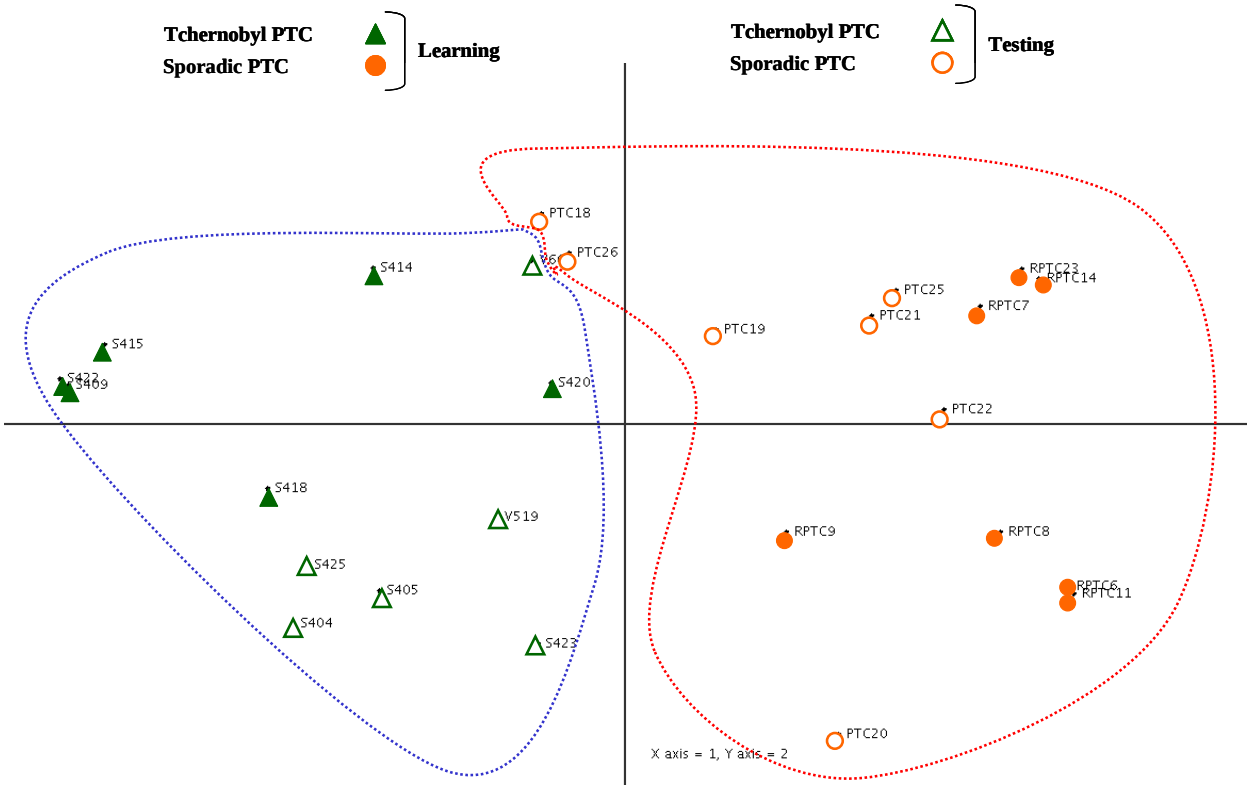


Figure 5: Clustering of the Chernobyl series of tumors using the 5 genes common to Chernobyl and post-radiotherapy signatures

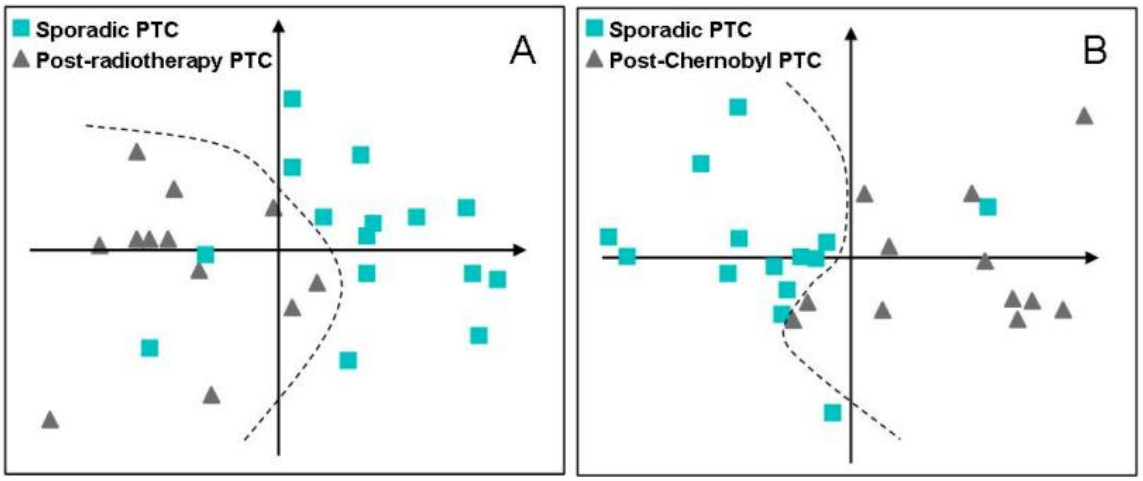
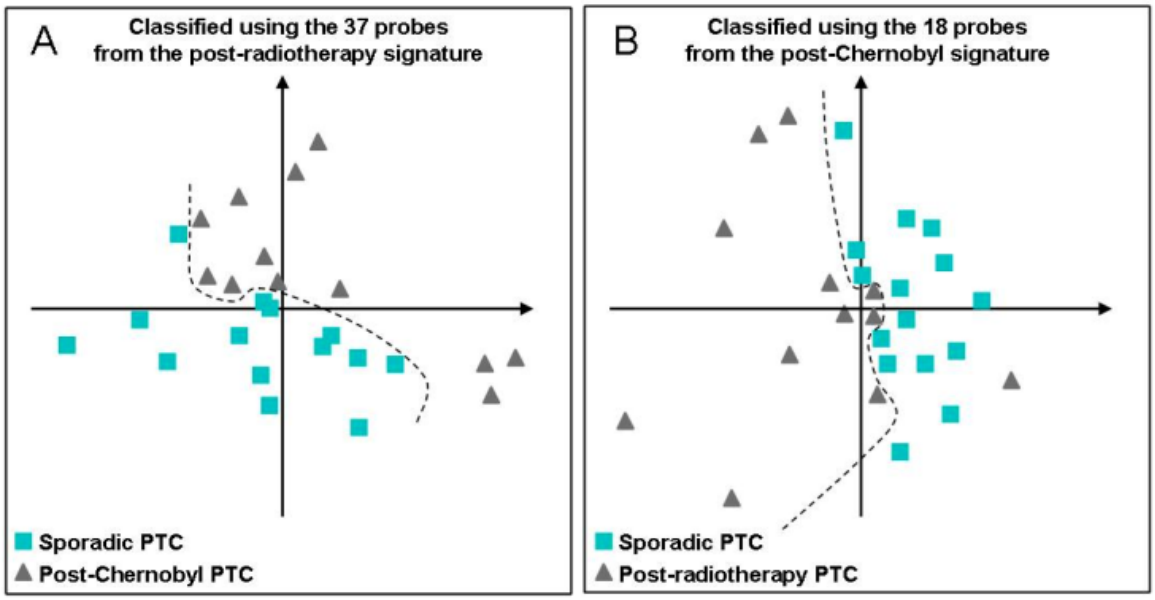


Figure 6: Clustering of the post-radiotherapy series of tumors using the gene signature of the Tchernobyl Tumors and of the posy-radiotherapy signatures, respectively



6 ETHICAL ASPECTS OF TESTING FOR INDIVIDUAL RADIOSENSITIVITY

Sven Ove Hansson

KTH Royal Institute of Technology, Stockholm, Sweden

6.1 Background on ethics and radiation protection

Some of the major problems in radiation protection are closely connected to issues that have a long, independent tradition in moral philosophy. This applies in particular to the relationship between protection of individuals and optimization on the collective level that is closely connected to the moral discussion on the relationship between individual rights and with the furthering of collective interests.

The moral discussion on this issue is dominated by two thought patterns, each of which has been distilled into a type of moral theory. The first of these thought patterns is often expressed with the metaphor of *weighing*. Whenever there are several actions that one can choose between, it would seem sensible to specify the advantages and disadvantages associated with each of the alternatives, and then choose the alternative that has the largest net advantage (sum of advantages minus sum of disadvantages). In moral philosophy, this way of thinking is associated with *utilitarianism*. According to utilitarian philosophers such as Jeremy Bentham (1748-1832) and John Stuart Mill (1806-1873), morality requires that we choose the actions that maximize utility. Classically, utility was defined as the total amount of happiness produced by an action, minus the total amount of unhappiness that it produces. In modern moral philosophy other definitions of utility have been used, such as the total amount of preference satisfaction. However, the thought pattern is the same. Utilitarians want us to choose between actions by weighing their positive and negative effects against each other. An essential feature of utilitarian weighing is that it makes no difference to what person a (negative or positive) effect pertains. An advantage for one person can be outweighed by a disadvantage for another person, just as easily and advantages and disadvantages for one and the same person can be weighed against each other. In this sense, persons are almost morally irrelevant for the utilitarian, except as carriers of utility and disutility.

The other approach consists in setting *limits*. In everyday life this is an equally important way of moral thinking as that of weighing. When we teach children ethical behaviour, we tell them that there are certain limits to what they may do. Moral philosophers have developed this mode of thinking into two closely related moral theories. One of them is *deontological ethics*, also called duty ethics, that has it focus on limits to what a person may do. The most famous deontologist was Immanuel Kant (1724-1804), who emphasized the strictness of moral limits. According to Kant, it is morally wrong to lie, and this applies even in cases when telling the truth can cause great harm. Other philosophers, most notably WD Ross (1877-1971), have developed less absolute variants of deontology, in which it is possible for a duty to be outweighed by other moral considerations. The other group of moral theories that are based on thinking in terms of limits are *rights-based ethics*, that focus on limits to what may be done (or not done) to a person.

Standard radiation protection makes use of a combination between on one hand weighing in a sense very close to that used in utilitarian ethics and on the other hand individual limit-setting in a sense very close to the principles of deontological or rights-based ethics. However, there is also an interesting difference. Philosophers typically identify themselves as

adherents to one specific moral theory, for instance as utilitarians or deontologists. On a conference in moral philosophy you will find utilitarians who argue that deontology is a misconceived form of moral philosophy and deontologists who say the same about utilitarianism. On a conference in radiation protection you will have a hard time finding optimizers who consider dose limits useless, or dose limit proponents who claim that optimization is irrelevant, or justification enthusiasts who regard optimization and dose limits as mistaken approaches. Instead you will find radiation protectors who try to combine these principles. This is no easy task, since the principles often conflict or at least seem to do so. The conflicts are particularly sharp between the principles of weighing and limit-setting, i.e. between optimization and dose limits. Since both weighing and limit-setting have strong support our moral intuitions, that are the ultimate source of any reasonable moral standpoint, attempts to combine them would seem to be a sensible approach. Moral philosophers have something to learn from radiation protectors in this respect. (On how it can be done, see Hansson 2007 and Wikman-Svahn et al 2006.).

In addition to the two approaches I have just described there is also a third approach that combines the idea of weighing with respects for individual rights. (Hansson 2004) Instead of utilitarian or collective weighing, in which all advantages and disadvantages, irrespective of whom they pertain to, are combined in one single calculation, we may combine the idea of weighing with that of treating each individual as a separate moral unit. Then advantages and disadvantages pertaining to each concerned person are weighed against each other in a separate act of weighing. A balance is thus struck separately for each individual. The most obvious way to make social decisions based on such individual weighing is to require a positive balance for each individual. This approach has a strong standing in economic theory. It is employed in fundamental equilibrium and welfare theory since the so-called new school in welfare economics was established the 1930's by Lionel Robbins when he showed how economic analysis can dispense with interpersonal comparability. (The central concept here is that of Pareto optimality. A state of the economy is Pareto optimal if and only if there is no other state that is better for one individual without being worse for at least one other individual.) Another field dominated by individual weighing is clinical medicine. When physicians talk about weighing risks against benefits they normally refer to the balance between risks and benefits for the individual patient. An interesting example of this is the ethical appraisal of clinical trials. It is an almost universally accepted principle in research ethics that a patient should not be included in a clinical trial unless there is genuine uncertainty on whether or not participation in the trial is better for her than the standard treatment that she would otherwise receive. That her participation is beneficial for others (such as future patients) cannot outweigh a negative net effect on her own health; in other words her participation has to be supported by an appraisal that is restricted to risks and benefits for herself.

Radiation protection can be found at the crossroads between these different ways of thinking; whereas clinical radiation protection (for instance in radiotherapy) has a strong focus on individual weighing, industrial radiation protection tends to use a combination of collective weighing with the (dose) limit approach.

6.2 Individual radiosensitivity

The first Annex of the 2007 Recommendations of the ICRP provides a detailed scientific background on what is currently known on individual differences in radiosensitivity: Radiogenic cancer risk at any given level of exposure is estimated to be 13 % higher for women in the ages 18-64 than for men in the same age interval. The detriment (the risk adjusted for differences in lethality etc.) is calculated to be 39 % higher for women than for

men. (ICRP 2007, p. 210) Furthermore, young children are identified as a "particularly sensitive subgroup" with respect to radiological exposure. (p. 193, cf. p. 131) The same applies to in utero exposure that results in a risk considered to be "similar to that following irradiation in early childhood, i.e., at most, about three times that of the population as a whole". (p 57, cf. pp. 169 and 195).

The Annex also reports that a number of known inherited syndromes give rise to increased radiosensitivity and cancer risk. Most of these syndromes involve mutations in DNA repair genes. In addition, there are indications that variations in DNA repair capacity in the general population can influence susceptibility to radiogenic cancer. (Filippi et al 2006. Chistiakov et al 2008.) The Commission notes that according to recent studies "most of the rare single gene, cancer prone disorders will show greater-than-normal sensitivity to the tumorigenic effects of radiation". (p. 56) It estimates that substantially less than 1 % of the general population have defects in DNA damage-sensing or repair genes that make them very radiosensitive. (p. 163, cf. p. 156).

6.3 Current regulatory approaches

The ICRP's current recommendations are consistently based on a population average of the expected detriment, rather than on data for subpopulations. According to the Annex referred to above, "[t]he Commission has made a policy decision that there should be a single set of w_T values [tissue weighting factors] that are averaged over both sexes and all ages". (p. 192) In particular, "sex-specific data are not recommended for the general purposes of radiological protection". (p. 187, cf. pp. 177, 192, and 211) Age-at-exposure data are averaged in the same way. (p. 177) High penetrance cancer genes are excluded from regulatory considerations since they are "too rare to cause significant distortion of population-based estimates of low-dose radiation cancer risk" (p. 56, cf. p. 195), which again confirms that the recommendations are based exclusively on the population average. (A similar conclusion was drawn in a previous ICRP document that treated sensitive groups more in detail; see ICRP 1999, pp. 136-138.).

Oversensitivity raises problem also in the regulation of chemicals. Occupational exposure limits for chemicals have largely focused on the protection of "healthy persons of working age". (Senatskommission 1996, p. 15.) In practice, occupational exposure limits differ in whether they protect persons who are particularly sensitive against specific substances, such as angina pectoris patients (carbon monoxide), alcoholics (carbon tetrachloride), smokers (asbestos), men (dibromochloropropane), women (lead), and pregnant women (embryotoxic and teratogenic substances). Carbon tetrachloride is a particularly interesting example, since different regulatory agencies have treated it differently. The substance is hepatotoxic, and much more so in persons with excessive alcohol consumption. The Australian occupational exposure limit for carbon tetrachloride (0.1 ppm) is intended to protect against the potentiating effect of alcohol consumption on the substance's hepatotoxicity. The US OSHA has set its exposure limit two orders of magnitude higher (10 ppm), since they do not intend to protect against these combination effects. (Schenk 2009).

6.4 The basic ethical issue

In order to tap our intuitions on the protection of sensitive groups, I will apply a standard method in philosophy. I will present a series of stylized and simplified, but reasonably realistic hypothetical examples. These examples will serve to elicit (intuitive or reasoned)

judgments. This method is based on the assumption that our intuitions or judgments should be consistent across the cases, so that if two cases are appraised differently in ethical terms, then an explicit and generalizable justification of the difference is required. If such a justification cannot be given, then our appraisals of the individual cases have to be reconsidered in order to achieve consistency. The examples are so chosen that they will bring us gradually from very obvious medical cases where our intuitions are quite clear-cut to the examples that are realistic and practically relevant in radiation protection.

Since exposure to lethal risks is a matter of life or death, medical ethics is a relevant framework for this investigation. For each of the scenarios to be discussed I will ask two questions. First, what information should a physician (or other expert) give an exposed person who wants to know the risks associated with the exposure? Secondly, should a risk management decision take the sensitive group into account? This is our first example:

> *Example 1:*
> An efficient drug against hypertension kills about 1 patient out of 100,000. This is because 1 in 100,000 has a metabolic abnormality that turns the drug into a killer.

Let us first consider the information question. What should a physician tell a person who is known to have the metabolic disorder in question? Should she say: “This drug is efficient against your high blood pressure. There is also a small risk, 1 in 100,000, of a lethal side-effect.” Of course she should not. For this patient, the drug is sure to be lethal, and this is what the patient should be told.

What should the physician tell a person who does not have the metabolic disorder? Should such a person be informed that there is a risk of 1 in 100,000 of a lethal side-effect? The answer is obviously no in this case as well. There is no reason to worry a patient with a risk that is not real. The patient should be told the truth, namely that as far as is known, the lethal effect is not relevant for her since she does not have the metabolic disorder that triggers it.

The question about risk management is equally easy to answer in this case. Patients with the metabolic disease should not be offered a prescription of this drug. The idea of offering such a person the drug, with the motivation that it is fairly safe for the average person, is too absurd to be taken seriously.

> *Example 2:*
> An efficient drug against hypertension kills about 1 patient out of 100,000. The risk is 1 in 50,000 for women and 0 for men.

Again, let us begin with the information question. Should physicians tell both women and men that they run a risk of 1 in 100,000 of being killed by the drug? Obviously not, since that information would be inaccurate in both cases. Men would be unnecessarily discommoded, and women would be given a too low estimate of the risk. The only acceptable approach is to provide both groups with the more specific information about risks that is available.

The question about risk management is also easily answered. A medical decision whether to offer this drug to female hypertension patients has to be based on the risk to women. The fact that the risk to men is smaller than to women does not contribute to the justification of offering the drug to women.

Both of these examples are staged in a medical setting. Let us therefore move out of healthcare. The following example has the same risk structure as the previous one, but it refers to an occupational instead of a medical exposure:

> *Example 3:*
> A chemical workplace exposure causes uterine cancer in 1 out of 50,000 exposed women. It causes no risks for men.

What should a physician tell a woman in this workplace who asks what the risks are to her personally? The obvious answer is that she should be told the truth, namely that she runs a risk of 1 in 50,000 of contracting uterine cancer. Telling her that the risk is 1 in 100,000 would be both untruthful and misleading. And what answer should a man receive who asks: "Doctor, I have heard that there is a cancer risk from the chemical we are working with. What kind of cancer is it, and how large is the risk?" Hopefully, he would not get the answer: "Well, it is uterine cancer, and the risk that you will contract it is 1 in 100,000."

Next, let us turn to the risk management question. The crucial issue here is whether, for the purposes of risk management, the risk should be treated as 1 in 100,000 or as 1 in 50,000. To make this clear, let us compare two hypothetical exposures. The two substances A and B are used in different workplaces. They both have the risk profile given in Example 3. In other words, each of these exposures causes uterine cancer in 1 out of 50,000 exposed women, but it causes no risks for men. In the workplace using substance A, all exposed employees are women. In the other workplace, equal numbers of female and male employees are exposed to substance B.

In the workplace using substance A it would be absurd to treat the risk as 1 in 100,000. The actual employees run a risk, individually and collectively, of 1 in 50,000. The fact that some other persons (in this case men) would have run no risk if they had been exposed cannot be allowed to determine the risk management decision. Risk managers are expected to deal with realities as they are. Treating a risk as smaller than it is because it *could have been* smaller if the workforce had been different looks more like wishful thinking than responsible risk management.

What about the workplace with substance B? Are the female workers there entitled to the same protection as their colleagues in the first workplace, or should they be treated as exposed to a smaller risk, 1 in 100,000? The latter option would mean that they receive lower priority for preventive measures merely because other workers are present who are unaffected by the risk. To see where this argument would lead us, suppose that the regulatory requirement is to keep the risk below 1 in 50,000. Then the manager at the workplace with substance A can "solve" the problem by providing half of the workforce with protective equipment that eliminates the risk. Although the remaining half are exposed to the same risk as before, according to this argument they have no reason to complain since the average risk level is now on the right side of the limit. This is an absurd conclusion. The protection to which a person is entitled should depend on the best estimate we can make of the risk to which she is exposed, not on the risk that some hypothetical person would have been exposed to under the same physical conditions. We can conclude that in Example 3, risk managers should acknowledge that the women are exposed to a risk level of 1 in 50,000, and act accordingly.

It could be argued that this example is rather special since uterine cancer only affects women. Let us therefore eliminate this feature from the example.

> *Example 4:*
> A chemical workplace exposure causes primary liver cancer in 1 out of 50,000 exposed women. It causes no risks in men.

The change from one disease to another does not affect the conclusion from the previous example that exposed persons should receive the most accurate risk information that is available. Here as well, women should be told that they are exposed to a risk of 1 in 50,000, and men should be told that this risk does not affect them. Neither group should be told that they run a risk of 1 in 100,000.

When it comes to risk management, the same argument (with the exposures A and B) can be used as in the former example. The presence of unaffected persons in the workplace with

substance B does not in any way make the risk exposure of those who are affected more acceptable. This is a conclusion that does not depend on what body organ is affected.

Example 4 is epistemically unrealistic. It is difficult to see how we could know with reasonable confidence that a substance that causes cancer in women does not at all cause cancer in men. We can modify the example to make it more realistic in this respect:

> *Example 5:*
> A chemical workplace exposure causes in 2 out of 50,000 exposed women and 1 out of 50,000 men.

There is no reason to repeat the argumentation from examples 3 and 4. The exposed individuals' right to receive as accurate information as possible applies here as well. The argument (with exposures A and B) used to show that risk management should be based on the best available estimates of the risks to which individuals are exposed is also straightforwardly transferable to this example.

Let us now turn to a radiological example:

> *Example 6:*
> A radiological workplace exposure causes cancer in 2 out of 50,000 exposed women and 1 out of 50,000 men.

Only one word differs between examples 5 and 6: the word "chemical" has been replaced by "radiological". It would be difficult to defend a claim that the two cases should therefore be treated differently. Admittedly, a viewpoint is sometimes encountered that can be called "radiation exceptionalism", namely that radiological risk factors should be treated very differently from other risk factors that have essentially the same effects. However, this is not a credible standpoint. Public health is concerned with the preservation and improvement of individuals' health. Therefore, the health effects of an exposure, not its biochemical mechanism, should be at focus in criteria setting. All the arguments that have been developed above for chemical exposures are equally applicable in the radiological case.

6.5 Ethical conclusions

Two major conclusions can be drawn from this series of examples for radiological protection:

1. *A person who is exposed to radiation has the right to receive the best possible information about the risk to herself. In particular, if she is a member of an identifiable group for which specific risk information is available, then she has a right to that group-specific information.*
2. *Regulations and recommendations in radiological protection have to be defensible in the perspective of each affected person. In order to justify that a certain person is exposed to a radiation dose, it is not sufficient to show that the hypothetical risk to which she* would have been *exposed if she had average radiosensitivity would have been acceptable. It is necessary to show that the risk to which she* is *exposed is acceptable.*

These conclusions refer to identifiable groups. A group is identifiable if it can be determined, for each person, whether or not she belongs to that group. Age groups and the sexes are identifiable in this sense, and so are some but currently not all groups with increased radiosensitivity due to genetic conditions. It follows from the second conclusion that the future identification of new radiosensitive groups may give us reason to reconsider some of the current recommendations and regulations on radiological exposure.

6.6 How can we protect sensitive groups?

There are two major ways in which we can protect the members of groups who are more sensitive than others to some exposure. *Differentiated protection* operates through measures targeted at the sensitive persons, specifically reducing or eliminating their exposure. It can take the form of excluding them from certain occupations or environments or providing them with special protective equipment. In *unified protection*, exposure limits and other regulations are kept the same for all individuals, but they are made sufficiently strict to protect the members of the sensitive group(s). Differentiated protection usually has economic advantages, but it can also have social disadvantages such as excluding parts of the population from certain employments. The following six considerations should have a role in the (often difficult) choice between these two methods:

1. The *difference in risk*: If the difference in risk is small between the sensitive group and the rest of the population, differentiated protection will not be meaningful. Hence, having an exposure limit of 5 (in some unit) for one of the sexes and 6 for the other would in most cases be impracticable due to lack of precision both in measurements and in abatement.
2. The *costs of abatement*: If it is inexpensive to reduce exposures, then there is little economic gain from choosing differentiated protection. This speaks in favour of unified protection.
3. *Identifiability*: If the identification of sensitive individuals is difficult or uncertain, then that speaks against differentiated protection. Unified protection has the important advantage of protecting sensitive individuals even if they are not identified. Probably the most important example when identifiability is problematic is exposure in utero. Birth defects due to disturbances of organogenesis occur during the 4th to 9th weeks, when the pregnancy is often unknown. (Weeks et al. 1991, pp. 489–501. Peters and Garbis-Berkvens 1996, pp. 935–936.) In spite of this, differentiated protection in the form of special provisions for pregnant women has often been resorted to in chemicals regulation. (Hansson 1998, pp. 45-47)
4. *Privacy*: In some cases the identification of sensitive groups is problematic from the viewpoint of privacy. The use of biochemical testing for such purposes in a workplace setting is often controversial. (Hansson 2005) This speaks in favour of unified instead of differentiated protection.
5. The *social exclusion* caused by differentiated protection: In some cases individuals who receive special protection will be disadvantaged for instance through loss of employment opportunities. If these effects are significant, then that is an argument in favour of unified protection.
6. *Previous discrimination*: If the persons who will be disadvantaged by differentiated protection are already subject to discrimination or otherwise underprivileged, then that is an argument against differentiated protection. As one example of this, in most countries it would be more problematic to weaken the position of black women on the labour market than that of white men.

6.7 Conclusion

ICRP's principle of basing radiation exposure standards exclusively on the population average is difficult to defend from an ethical point of view. Our ethical investigation points clearly in the opposite direction: Each radiation-exposed person has a right to the best

possible information about the risk to herself. If she is a member of some identifiable group that differs in radiosensitivity from the average, then she should receive the relevant group-specific information. If testing can provide information about whether or not she belongs to some radio-sensitive group, then she has a *prima facie* right to be tested if she so desires. Furthermore, risk exposures have to be defensible in the perspective of each such identifiable group for which a specific risk assessment can be made. Exposing a person to a high risk cannot be justified by pointing out that the risk to an average person would have been much lower.

6.8 References

Chistiakov, Dimitry A., Natalia V. Voronova, and Pavel A. Chistiakov (2008) "Genetic variations in DNA repair genes, radiosensitivity to cancer and susceptibility to acute tissue reactions in radiotherapy-treated cancer patients." *Acta Oncologica* 47(5):809-824.

Filippi, Andrea Riccardo, Pierfrancesco Franco, and Umberto Ricardi (2006) "Is clinical radiosensitivity a complex genetically controlled event?" *Tumori* 92(2):87-91.

Hansson, Sven Ove (1998) *Setting the Limit. Occupational Health Standards and the Limits of Science*. New York: Oxford University Press.

Hansson, Sven Ove (2004) "Weighing Risks and Benefits", *Topoi*, 23:145-152.

Hansson, Sven Ove (2005) "Privacy, Discrimination, and Inequality in the Workplace", pp 119-135 in Sven Ove Hansson and Elin Palm (eds.) *The Ethics of Workplace Privacy*. Brussels: Peter Lang.

Hansson, Sven Ove (2007) "Ethics and radiation protection", *Journal of Radiological Protection* 27:147-156.

ICRP (1999) *Genetic susceptibility to cancer*, J. Valentin (ed.), *Annals of the ICRP*, Publication 79. Oxford: Elsevier.

ICRP (2007) *The 2007 Recommendations of the International Commission on Radiological Protection*, J. Valentin (ed.), *Annals of the ICRP*, Publication 103. Oxford: Elsevier.

Peters, P. W. J. and J. M. Garbis-Berkvens (1996). "General Reproductive Toxicology." Pp. 929–946 in R. J. Niesink, J. de Vries, and M. A. Hollinger, *Toxicology. Principles and Applications*. Boca Raton: CRC Press.

Schenk, Linda (2009) "The use of primary data in risk assessment for occupational exposure limits", manuscript.

Senatskommission zur Prüfung gesundheitsschädlicher Arbeitsstoffe (1996). *List of MAK and BAT Values 1996*. Weinheim: Deutsche Forschungsgemeinschaft.

Weeks, J. L., B. S. Levy, and G. R. Wagner (1991) *Preventing Occupational Disease and Injury*. Washington DC: American Public Health Association.

Wikman-Svahn, Per, Martin Peterson and Sven Ove Hansson (2006) "Principles of protection: a formal approach for evaluating dose distributions", *Journal of Radiological Protection* 26:69-84.

7 SUMMARY

Prepared by Dr Anna Friedl, University of Munich, Germany on behalf of the Working Party on Research Implications on Health and Safety Standards of the Article 31 Group of Experts[1]

7.1 Introduction

This document provides the background, summarizes the presentations and the results of the round-table discussion, and tries to emphasize the potential implications of the Scientific Seminar on “Individual Radiosensitivity”, held in Luxembourg on 22 November 2011. It takes into account the discussions that took place during the seminar and during the subsequent meeting of the *Working Party on Research Implications on Health and Safety Standards*, although it is not intended to report in an exhaustive manner all the opinions that were expressed. The document has been submitted for comments to the lecturers, as far as their contributions were concerned.

7.2 The Article 31 Group of Experts and the rationale of the RIHSS seminars

The Article 31 Group of Experts is a group of independent scientific experts referred to in Article 31 of the Euratom Treaty, which assists the European Commission in the preparation of the EU Basic Safety Standards for the protection of the health of workers and the general public against the dangers arising from ionizing radiation. According to the Euratom Treaty and to their Code of Ethics, this group of experts has to give priority to the protection of health, to the safety and to the development of the best available operational radiation protection. For doing so, they have to follow carefully the scientific and technological developments and the new data coming from the world of research, particularly when these could affect the health of the exposed persons.

In this context, a Scientific Seminar is devoted every year to emerging issues in Radiation Protection – generally addressing new research findings with potential policy and/or regulatory implications. On the basis of input from the Directorate General Research of the European Commission and of information provided by individual members of the Article 31 Group of Experts, the Working Party RIHSS proposes relevant themes to the Article 31 Group that could be discussed during a subsequent seminar. After selection of the theme and approval of a draft programme by the Article 31 Group, the Working Party RIHSS deals with the preparation and the follow up of the seminar. Leading scientists are invited to present the status of scientific knowledge in the selected topic. Additional experts, identified by members of the Article 31 Group from their own country, take part in the seminars and act as peer reviewers. The Commission convenes the seminars on the day before a meeting of the Article 31 Group, in order that members of the Group can discuss the potential

[1] Besides A. Friedl (who was acting as rapporteur for the seminar), the following members of the Working Party on Research Implications on Health and Safety Standards of the Article 31 Group of Experts contributed to the preparation of this overview: L. Lebaron-Jacobs, R. Huiskamp, P. Olko, S. Risica, P. Smeesters (Chairperson of the WP), and R. Wakeford. They were assisted by the following official of the European Commission: S. Mundigl.

implications of the combined scientific results. Based on the outcome of the Scientific Seminar, the Group of Experts referred to in Article 31 of the Euratom Treaty may recommend research, regulatory or legislative initiatives. The European Commission takes into account the conclusions of the Experts when setting up its radiation protection programme. The Experts' conclusions are also valuable input to the process of reviewing and potentially revising European radiation protection legislation.

7.3 Key Highlights of Presentations at Scientific Seminar on Individual Radiosensitivity

Mike Atkinson – *Radiation Sensitivity – an Introduction*

Enhanced sensitivity towards ionizing radiation can be observed on many scales (molecules, cells, tissues, organs, individuals, populations) and using various endpoints (e.g., molecular damage, cell death, inflammation, cancer, cardiovascular disease), and hence there is no general definition of “radiosensitivity”. All implications depend on which type of enhanced sensitivity is being regarded. In addition, it is important to define what is meant by “enhanced”: e.g. a certain percentile in the distribution curve, or a certain deviance from the average response.

For radiation protection, two types of enhanced radiosensitivity are of special relevance:

a) acute response: 1-10% of radiotherapy patients (depending on clinics and irradiation regime used) experience an acute adverse tissue response.

b) chronic sensitivity: cancer development after irradiation (less than 0.01% of the population has a severe genetic predisposition, but an unknown proportion (maybe 10-50%) of the overall cancer risk may be due to low penetrance genetic sensitivity factors. In addition to genetic factors, it is known that susceptibility to radiation-induced cancer is affected by factors such as age and gender).

The prospective identification of individuals at enhanced risk, both regarding acute and chronic radiation effects, may rely either on genetic testing or on analysis of experimentally amenable surrogate markers. Genetic testing would require that susceptibility variants of all relevant genes and their influence on risk be known, which at present is clearly not the case (see e.g. contribution by Christian Nicolaj Andreassen). Also surrogate markers, such as e.g. measurement of induction and repair of DNA damage in tissue or blood samples, have so far not lead to a reliable identification of susceptible individuals in routine clinical situations.

It is important to note that there is no reason to assume a causal correlation between acute and chronic types of radiosensitivity. It must also be stressed that individual variation in sensitivity to another type of chronic radiation effect, namely the induction of cardiovascular diseases, has so far not been studied in detail.

Concerning radiation protection, it is at present not clear if and how individual differences in radiation sensitivity affect the dose-response curve. The relative impact may be higher in the low-dose region, since responses may be saturated in the high-dose region anyway.

Christian Nicolaj Andreassen – *Genetic tools to address individual radiosensitivity and their limitations*

In the past, many studies have been conducted searching for genetic variants that affect normal tissue response in radiotherapy. In a meta-analysis of 47 studies investigating SNPs (single nucleotide polymorphisms) in a variety of candidate genes, about two third reported

significant associations between a certain SNP and enhanced normal tissue toxicity. However, studies were mainly conducted with limited numbers of patients (median 144), and data were conflicting and/or not reproducible. Often, studies did not correct for multiple testing. In a somewhat pointed way, Dr Andreassen states that “We have far too often conducted studies in which the probability of detecting the presence of a ‘true’ association may have been less than 30% (in each comparison) while the risk of finding something that does not exist has been above 50% (in the entire study)”. He concluded that at present there are no unambiguously proven associations.

The situation is complicated by the fact that in an individual many SNPs (both with positive and negative effects on risk) interact. Individual SNPs that are present in a significant proportion of the population in general are expected to contribute very small increased risks (odds ratios in the range of 1.1-1.5), whereas variants conferring strongly enhanced risks are very rare. While the experiences gathered so far suggest that candidate gene approaches may not be useful, the usefulness of genome-wide association studies for identification of radiosensitivity alleles remains to be tested. So far, only one such study (on erectile dysfunction after radiotherapy of prostate cancer) has been published.

Thus, for statistically meaningful investigations, studies must be performed on many thousands of patients. The number of clinically identified radiotherapy overreactors is, however, quite low. Therefore, international co-operations will be necessary. Some international cooperation studies have already been implemented (e.g. ESTRO GENEPI, RAPPER GENEPAR, RadGenomics, International Radiogenomic Consortium). Alternatively, instead of genetic profiling, gene expression analysis in patient cells after irradiation may turn out to have predictive value for radiation side effects.

In the discussion following the presentations, it was stressed that there are different types of clinical radiosensitivity, affecting different organs and tissue types, which often are not causally linked (e.g. teleangiectasia and fibrosis). Thus, the genetic basis of each type may differ. It was also stressed that, while genetic testing may appear expensive in time and money, simpler potentially predictive methods, such as measuring DNA damage or repair outcomes in patients’ cells, so far have not successfully been applied in a predictive manner with “normal” patients in routine clinical situations.

Peter O’Neill – *Genetic Pathways for the Prediction of the Effects of Radiation*

This talk was given by Mike Atkinson using Peter O’Neill’s power-point presentation, since Dr O’Neill could not attend the seminar due to bad weather conditions.

The rationale of the Euratom-funded research project “GENEPI-lowRT” is the assumption that the influence of genetic variants on radiosensitivity may become especially relevant at higher doses, such as for example encountered during radiotherapy. The project combines establishment of T-cell and dermal fibroblast cultures from breast cancer patients, genotyping and gene expression analyses, as well as functional testing of DSB repair, senescence / differentiation, apoptosis and radiosensitivity. Functional testing has not demonstrated predictive power for the identification of radiosensitive (with regard to development of skin fibrosis) patients. The results of the gene expression studies are still preliminary, but hint at a differential response at low (0.1 Gy) and high (2 Gy) dose. At this stage it was not possible to identify a robust classifier for radiosensitivity. It is suspected that some unidentified confounding factors may contribute to the radiosensitivity phenotype. The contribution of other factors such as life-style and immunological or inflammatory processes etc. on radiosensitivity must therefore be elucidated in the future. In addition, it is important that cohorts are well-defined and that accurate dosimetric data are available.

Paul Pharoah – *Genetic Predisposition and Radiation Sensitivity: the Potential of Genome Sequencing*

Due to bad weather conditions, Dr Pharoah was unable to attend the seminar. Therefore, this talk was not given. Dr Pharoah did, however, provide his power-point slides and a manuscript. He emphasizes that the genetic architecture of radiosensitivity phenotypes is expected not to differ from that of other complex human phenotypes. Thus, the experience from studies on common genetic variants affecting other phenotypes should be used with regard to determining study design, choice of statistical testing, dealing with potential confounders, and strategies for validation and replication of results. Dr Pharoah also presents strategies for the search of rare and uncommon risk alleles.

Sylvie Chevillard – *Identification of Candidate Susceptibility Genes in Human Radiation-Associated Thyroid Tumors*

Any testing for association between genetic markers and development of radiation-induced disease phenotypes requires that the disease phenotype in question can with high probability be attributed to the radiation exposure. While identification of adverse side effects during or after radiation therapy is quite straight-forward, a radiation etiology of tumours is more difficult to determine, given the high frequency of spontaneous tumours and the long lag phase between radiation exposure and tumour development. Therefore, in recent years many researchers have tried to define molecular signatures allowing differentiation of radiation-induced tumours from spontaneous tumours. Dr Chevillard reports on results from transcriptome analysis of radiation-induced cancers (post-radiotherapy thyroid cancers, post-Chernobyl thyroid cancers, post-radiotherapy sarcomas and post-radiotherapy breast tumours). A molecular signature, based on 322 deregulated genes, was found in a training set of post-radiotherapy thyroid tumours and could be validated in an independent set of post-radiotherapy thyroid tumours. A signature based on 106 deregulated genes was identified and validated in a series of post-Chernobyl thyroid tumours. Both signatures show an overlap of 5 deregulated genes and the authors claim that thyroid tumours developing both after external and after internal (^{131}I) exposure may be classified as radiation-induced, based on the signature overlaps.

Sven Ove Hansson – *Ethical Aspects of Testing for Individual Radiosensitivity*

Currently, dose limit recommendations are consistently based on a population average of the expected detriment, rather than on data for subpopulations differing e.g. with regard to age, gender, or genetic make-up. Based on a series of hypothetical case examples, Dr Hansson comes to the conclusion that “a person who is exposed to radiation has the right to receive the best possible information about the risk to herself. In particular, if she is a member of an identifiable group for which specific risk information is available, then she has a right to that group-specific information”. He further concludes that members of groups who are more sensitive than others should be protected either by differentiated protection procedures, or by setting exposure limits and other regulations for all groups so that the most sensitive group is protected adequately (unified protection). Whether differentiated or unified procedures and regulations should be applied depends on a variety of factors, such as the extent of difference in risk, the costs of abatement, the identifiability of the most sensitive subgroups, the protection of privacy and avoidance of social exclusion, as well as avoidance of further discrimination if the persons who will be disadvantaged by differentiated protection are already subject to discrimination.

7.4 Summary of the Roundtable discussion

Christian Nicolaj Andreassen, Mike Atkinson, Michel Bourgignon, Sylvie Chevillard, Sven Ove Hansson, Ausra Kesminiene, Patrick Smeesters (Moderator)

Before the actual round table discussion, Dr Kesminiene gave a short overview over the EPI-CT study and Dr Bourguignon made a presentation on testing of individual radiosensitivity by immunofluorescence-based detection of radiation-induced foci.

The first question addressed by the discussion focused on the feasibility of predictive testing of radiotherapy patients by surrogate functional tests (such as DNA damage induction and repair). Most panellists expressed a sceptic view although such predictive testing seems possible in a research laboratory. Indeed, while many studies initially looked quite promising, so far none has proved convincing in large-scale validation assays.

Concerning radiation risks after low-dose exposure, one example where knowledge on genetic factors is already used is mammography screening schemes of women from breast cancer families. While the actual risk is not clear, the precautionary principle asks for specific tailoring of screening measures, e.g. by using MRI instead of mammography, where applicable.

Also the implications of predictive testing were discussed. Current ICRP values were regarded as reflecting already the protective needs of the most sensitive groups in the population. In addition the opinion was expressed that specific rules for specific subgroups in the population may be very difficult to implement and also to control. On the other hand, predictive testing may be an issue for emergency workers, who may be exposed to relatively higher doses. It should be noted, however, that genotypic testing in many European countries is not allowed.

8 CONCLUSIONS

Working Party "Research Implications on Health and Safety Standards" of the Article 31 Group of Experts[2]

Consideration of individual radiosensitivity in radiation protection has in recent years gained increasing interest. The seminar showed that it is very important to clearly define the term „enhanced radiosensitivity" in the discussions. An enhanced sensitivity towards development of side effects during radiotherapy will have other implications than an enhanced sensitivity towards development of cancer after radiation exposure at doses relevant in diagnosis, at workplaces, or in everyday life. Also the molecular pathways involved will be different, and therefore it is very likely that the sensitivity profiles for different endpoints will be different.

Also a clear definition is necessary of what is considered to be "enhanced". Like many complex traits, variation in normal tissue reactions appears to approximately follow a Gaussian distribution. In addition to the genetic profile, many factors, such as treatment modalities, concomitant chemotherapy or surgery, age, co-morbidities (diabetes, hypertension, vascular and connective tissue disease), smoking habits, diet, and chance determine whether a patient experiences adverse radiation effects. Assuming a Gaussian distribution of sensitivity towards development of side effects in therapy, one could define the tails of the distribution as being hypersensitive or, on the other side, hyper-resistant. There may, however, also be true outliers, for example patients that suffer from certain severe syndromes (e.g. Ataxia teleangiectasia, Nijmegen breakage Syndrome, Bloom's Syndrome) that confer a high degree of radiation sensitivity. In practice, however, this group of patients is easily identified and specific treatment regimens are generally followed in treating these patients.

Considering radiotherapy, a basic and well-proven concept is that the dose-effect curve for tumour control and the dose-effect curve for the induction of side effects (especially irreversible late effects) define the therapeutic window. From the steepness of these curves it is evident that even small alterations in dose may have a large impact on tumour control. To increase the probability of tumour control, the dose to the treatment volume should be as large as possible. Since the probability of side effects is increasing with increasing dose, a certain trade-off is necessary. Conventionally, the trade-off chosen is that the doses applied are so high that only a certain small proportion (about 5%) of the patients develops side effects. Thus, in this case the definition of hypersensitive person depends largely on the doses chosen for therapy and a statement that about 5% of the population have to be considered as radiation sensitive (or hypersensitive) may at least in part suffer from circular reasoning. It is, however, true that treatment doses could be increased for the other say 95%, if the most sensitive 5% could with high accuracy be identified before radiotherapy begins.

For the last 20 years many groups have tried to establish predictive functional assays to identify patients likely to develop unwanted side effects in the course or after radiation therapy. In the discussions at the scientific seminar it became clear that in several hospitals programs for such a predictive testing are in operation. However, there is so far no generally accepted procedure and it has in the past often be observed that protocols which seem to work well in a certain setting with a limited number of patients did not come up to the expectations when used in larger settings. These functional assays are developed on the

[2] The following members of the Working Party on Research Implications on Health and Safety Standards of the Article 31 Group of Experts contributed to the preparation of these conclusions: A. Friedl, L. Lebaron-Jacobs, R. Huiskamp, P. Olko, S. Risica, P. Smeesters (Chairperson of the WP), and R. Wakeford. They were assisted by the following official of the European Commission: S. Mundigl.

basis of pre-conceived opinions on the relevance of certain end-points for the development of the side effects. For example, after ex-vivo irradiation of patient cells (e.g. blood cells), the capabilities to repair radiation-induced DNA damage such as double-strand breaks, the frequency of formation of misrepair products such as chromosome aberrations or micronuclei, or the induction of cell killing are investigated. While these endpoints may well be relevant for side effects caused by excess cell killing, other side effects, such as fibrosis, depend on other pathways and may thus not be covered.

Theoretically, genetic testing is less cost- and time expensive than functional testing, once the genetic variants associated with enhanced sensitivity are known. In order to identify genetic variants that predict enhanced susceptibility for adverse side effects in radiotherapy, so far mainly candidate gene-driven association studies have been performed. Although many studies described statistically significant associations in patient groups of limited size, a general lack of reproducibility has so far precluded the use of any of the markers thus identified for predictive testing in a routine clinical setting. Modern high-throughput methods such as analysis of genetic variations in the population or radiation-induced alterations in gene expression patterns have the potential advantage that analysis is not limited to pre-conceived pathways. Since many (up to several 100 000) markers are tested at the same time, very large cohorts, stringent statistical evaluation, and data validation in independent cohorts are necessary if useful information is to be obtained. Large-scale international co-operations have been set up to fulfil these criteria.

It is important to stress that even if it will become possible to identify patients likely to develop side effects in radiotherapy beforehand, the consequences are not yet entirely clear. Up to now it is not yet known whether and to what degree the dose-effect curve for tumour control is shifted to smaller doses if the dose-effect curve for development of side effects is shifted towards smaller doses. If the dose to the treatment volume simply is reduced in patients likely to develop side effects, there is a danger of under-treatment resulting in reduced tumour control.

The identification of persons carrying genetic variants that make them more susceptible for radiation-induced tumours is even more difficult than the identification of persons susceptible to side effects of radiotherapy. This is, because up to now the causal pathway from radiation damage to tumour development is not well understood, even in the case of “average” susceptibility. Because of the long lag phase, a causal link between radiation exposure and tumour development is difficult to make, and only few cohorts of patients with tumours likely due to previous radiation exposure are available. While some preliminary data suggest that certain gene expression patterns may be used to differentiate radiation-induced from spontaneous tumours, validation of these data has not yet been obtained.

Still, even if the identification of persons carrying a genetically determined enhanced risk for development of tumours (or other late effects such as cardiovascular disease) is not yet possible, the legal and societal implications should already now be considered. So far, according to ICRP Publication 103, calculation of effective doses for the purpose of radiation protection does not account for possible age- or sex-specific differences, and tissue weighting factors are averaged over sex and age. ICRP justifies this, among other reasons, by the large degree of uncertainty associated with determination of tissue weighting factors and, in the case of sex-specific differences, by the inherent danger of discrimination at workplaces. It should be stressed that indeed the question of an enhanced risk for women to develop tumours after radiation exposure, although often purported, has not been unequivocally clarified. In many cases, estimated relative risks for women are higher than for men, but due to lower spontaneous tumour incidence and mortality at many tumour sites, absolute risks may be comparable or even lower. In addition, data from different cohorts do not necessarily agree. Concerning age effects, an enhanced sensitivity in utero and for children and adolescents is shown by many studies. Less well investigated is the question whether the elderly also are characterized by enhanced sensitivity. It is therefore very

important that all future epidemiological studies on radiation-induced cancer (and also non-cancer diseases) carefully investigate risk coefficients after sex- and age-specific stratification.