sensitivity can be meaningfully studied in various model systems. For instance, the biological response to ionizing radiation can be studied in cells irradiated in vitro or in biopsies from irradiated tissues. We have previously shown that the gene expression in fibroblasts irradiated in vitro is strongly associated with the risk of fibrosis after radiotherapy. Apart from being used for predictive purposes per se, a better understanding of the processes underlying the development of radiation-induced toxicity may facilitate a more focused search for genetic alterations affecting. For instance, SNPs shown to regulate the expression of genes involved in the response to irradiation could be tested for possible associations with risk of normal tissue complications. In addition, genes involved in the radiation-response could be subjected to targeted sequencing. This would decrease the 'multiple testing penalty' to be paid compared with an unconstrained genome-wide approach and hence reduce the sample size needed.

Figure 1: Size matters. Grey curves indicate the sample size needed to obtain 80% power for different genotype relative risks (GRRs) according to the risk allele frequency. Model assumptions: case:controlratio1:1, phenotype prevalence 25%, significance threshold 10−7 and multiplicative inheritance.

Figure 2: The genomic challenge. Normal tissue radiosensitivity is likely to be determined by the combined influence of large number different loci. These are to be selected from a very large pool of sequence variants of which the vast majority is not associated with the trait.

Both figures modified from Andreassen CN et al, Cancer Letters 2016.

SP-0482 GWAS in radiogenomics

G. Barnett[1]
[1]*Cambridge University Hospitals NHS Trust, Department of Oncology, Cambridge, United Kingdom*

Abstract text

Genome wide association studies (GWAS) have facilitated discoveries in population and complex-trait genetics, the biology of disease and translation towards new therapeutics. In the field of radiogenomics, several GWAS have reported associations between genetic variants and a variety of toxicity outcomes. Statistical power of radiogenomics studies depends on study size, prevalence of toxicity, minor allele frequency (MAF) and effect size of the variant and on the strength of the linkage disequilibrium (LD) between observed genotyped DNA variants and the unknown causal variants. To achieve adequate power, radiogenomics studies need to be large, requiring collaboration between international groups. To this aim, the Radiation Consortium was established and the STROGAR guidelines (Strengthening the Reporting of Genetic Association Studies in Radiogenomics) developed. The first meta-analysis of GWAS in radiogenomics identified three novel risk loci for late toxicity. With the establishment of the REQUITE project and the Oncoarray consortium sample size has increased further, whilst considering treatment- and patient-related factors in statistical analysis. Future directions are likely to involve the development of SNP risk profiles, whole genome sequencing and a systems biology approach to the analysis of Big Data. Combining different high-throughput unbiased 'omics' pathways may help identify pathways involving multiple genes important in the development of RT toxicity. Structural variation such as copy number variants and epigenetics may prove to be important. The ultimate aim of the Radiogenomics Consortium is to obtain a list of genuinely associated variants, produce SNP profiles with useful predictive value, recognize new biochemical pathways involved in RT toxicity and to personalize RT prescriptions.

SP-0483 The REQUITE project: integrating biomarkers and clinical predictors of radiotherapy side effects

C. Talbot[1], D. Azria[2], T. Burr[3], J. Chang-Claude[4], A. Dunning[5], C. Herskind[6], D. De Ruysscher[7], R. Elliott[8], S. Gutiérrez-Enríquez[9], P. Lambin[7], A. Müller[4], T. Rancati[10], B. Rosenstein[11], T. Rattay[1], P. Seibold[4], L. Veldeman[12], A. Vega[13], F. Wenz[6], R. Valdagni[10], A. Webb[1], C. West[8]
[1]*University of Leicester, Department of Genetics, Leicester, United Kingdom*
[2]*University of Montpellier, Institut du Cancer de Montpellier, Montpellier, France*
[3]*Source Bioscience, Research & Development, Nottingham, United Kingdom*
[4]*DKFZ German Cancer Research Center, Division of Cancer Epidemiology, Heidelberg, Germany*
[5]*University of Cambridge, Department of Oncology, Cambridge, United Kingdom*
[6]*Heidelberg University, Radiation Oncology, Mannheim, Germany*
[7]*Stichting Maastricht Radiation Oncology Maastro, Radiation Oncology, Maastricht, The Netherlands*
[8]*University of Manchester, The Christie NHS Foundation Trust, Manchester, United Kingdom*
[9]*Vall d'Hebron Institute of Oncology-VHIO, Oncogenetics, Barcelona, Spain*
[10]*Fondazione IRCCS Istituto Nazionale dei Tumori, Programma Prostata, Milan, Italy*
[11]*Icahn School of Medicine at Mount Sinai, Radiation Oncology, New York, USA*
[12]*Gent University, Radiotherapy, Gent, Belgium*
[13]*Fundación Pública Galega de Medicina Xenómica, Grupo de Medicina Xenómica, Santiago de Compostela, Spain*

Abstract text

The European Union funded REQUITE consortium aims to validate predictors of radiotherapy-related adverse reactions to develop clinically useful tools. Potential predictors include clinical and dose parameters, genetic markers, gene expression and the radiation-induced lymphocyte apoptosis (RILA).

REQUITE is a multi-centre, observational study (www.requite.eu). Enrolment was open for two and a half years through 10 hub centres (nine in Europe and one in the United States) each collecting through multiple hospitals. Follow-up is being collected for two years ending in September 2018. The primary endpoints are change in breast appearance at 24 months (breast), rectal bleeding at 24 months (prostate) and breathlessness at 12 months (lung). 4442 patients have been enrolled in REQUITE: 2071 breast, 562 lung and 1809 prostate cancer patients. In addition a further 383 lung cancer patients from another study have been integrated. All the patient data is held in a central database, including clinical, treatment, CTCAE scored toxicity, patient-reported outcomes, DVH & DICOM and biomarkers. All blood samples are held in the CIGMR Biobank at the University of Manchester.

All patients who complete the study are being SNP genotyped using Infinium OncoArrays, which tests for ~250,000 genome-wide SNPs and a similar number of cancer-specific SNPs, including some chosen from Radiogenomics studies. RILA was carried out in three of the European centres using a standardised protocol; it assesses the percentage radiation-induced apoptosis in lymphocytes, detected by flow cytometry, 48 hours after ex-vivo irradiation of whole blood. 1322 samples have been analysed using the apoptosis assay. The levels of apoptosis 48 hours after ex-vivo irradiation increase over baseline in a range from 2.4% to 62.4%, confirming large inter-patient variability. Factors that affect RILA have been identified, including cancer type and smoking status. Preliminary analysis has been carried out of acute toxicity data. A pilot RNA sequencing experiment has been carried out using 50 lung cancer cases to identify differentially expressed transcripts as potential

predictors.
18 sub-studies have been approved for use of the REQUITE data and/or samples to address a number of important questions e.g. the role of mitochondrial DNA, circadian rhythm effects, effect of integral dose on fatigue, modelling of the α/ß ratio for prostate toxicity, exploring patient attitudes to predictive testing. This large scale prospective observational study will be the largest to date to assess the use of predictive biomarkers for assessing radiotherapy related toxicity.

SP-0484 Machine Learning of radiogenomics SNP GWAS to predict complication risk and to identify key biological correlates

J. Deasy[1], S.K. Lee[1], J.H. Oh[1], S. Kerns[2], H. Orstrer[3], B. Rosenstein[4]
[1]*Memorial Sloan-Kettering Cancer Center, Medical Physics, New York- NY, USA*
[2]*University of Rochester Medical Center, Radiation Oncology, Rochester, USA*
[3]*Albert Einstein College of Medicine, Pathology and Pediatrics, New York, USA*
[4]*Icahn School of Medicine at Mount Sinai, Radiation Oncology and Genetics and Genomic Sciences, New York, USA*

Abstract text
We will review machine learning approaches to genome wide association studies with a focus on radiogenomics studies. The desire to develop machine learning approaches is motivated by the hypothesis that predictive models are best determined by building (what amounts to) "non-linear voting machines." Unlike standard statistical methods, the individual "voters" do not all need to be validated; instead, the wisdom of the crowd prevails. Genome wide association studies (GWAS) correlate a large number (typically ~ 1 million) of single nucleotide polymorphisms (SNPs) with an observed endpoint. When correlated with radiotherapy endpoints, the studies have been referred to as 'radiogenomics,' but many other endpoints have now been studied with GWAS. Typical GWAS analysis methods have focused on determining the statistical significance of the most highly correlated SNPs. These methods depend on having very large datasets and SNPs with large effect sizes in an attempt to overcome statistical noise inherent to extreme tails. Alternatively, some groups have applied machine learning approaches to GWAS analysis. We have developed a multistep machine learning method to build predictive models based on GWAS data and modest sized dataset (hundreds of patients.) The method relies on the crucial low-noise property of SNP measurements. The core machine learning step is based on the random forest methodology, which is well-suited to genomic biomarkers. The model itself discovers and emphasizes conditional relationships between SNPs through individual decision trees. These models can further be analyzed to understand key biological network sub-components that are critical to the observed endpoint. The overall impact of individual SNPs is ranked through permutation testing, and the resulting ranked list is analyzed using curated network databases to identify key biological interactions and processes. We will discuss the process and application to predicting toxicity following prostate radiotherapy, including erectile dysfunction, late rectal bleeding, and urinary dysfunction. We will also discuss limitations, alternative approaches, and potential applications.

Proffered Papers: RB 5: Head and neck radiobiology

OC-0485 Genetic variants associated with radiation-induced morbidity in a head-and neck cancer cohort

L.M.H. Schack[1], L. Dorling[2], L. Fachal[2], C. Luccarini[2], A.M. Dunning[2], J.G. Eriksen[3], C.N. Andreassen[3], J. Alsner[1], J. Overgaard[1], On behalf of DAHANCA[4]
[1]*Aarhus University Hospital, Experimental Clinical Oncology, Aarhus, Denmark*
[2]*University of Cambridge, Centre for Cancer Genetic Epidemiology- Department of Oncology, Cambridge, United Kingdom*
[3]*Aarhus University Hospital, Department of Oncology, Aarhus, Denmark*
[4]*The Danish Head and Neck Cancer Group, -, Denmark*

Purpose or Objective
Radiation-induced morbidity following cancer treatment affects the lives of cancer survivors. Radiotherapy (RT)-related factors account for a large part of the variance in morbidity seen in a cohort, but little is yet known about the individual inherent genetic susceptibility to the development of morbidity. We undertook a genome-wide association study in head and neck cancer patients from the DAHANCA biobank to identify single nucleotide polymorphisms (SNPs) associated with early and late radiation-induced morbidity.
Material and Methods
The cohort consisted of 1140 head- and neck cancer patients treated according to national DAHANCA guidelines with primary curative RT +/- concomitant treatment between 2000 and 2013. Toxicity scoring was done prospectively. Early endpoints were acute dysphagia and mucositis. Late endpoints (maximum grade between 600 days and 5 years after RT) included dysphagia, xerostomia, fibrosis and fibrosis + atrophy. Standardized Total Average Toxicity (STAT) scores were calculated for acute, late and global endpoints to analyze an overall association to the risk of developing radiation-induced morbidity.
We used the Infinium OncoArray-500K BeadChip (Illumina Inc. CA, USA) for SNP genotyping. Quality control adhered to OncoArray guidelines. Phasing and imputation of patient genotypes was carried out using SHAPEIT and IMPUTE2 software with the last version of 1000 Genomes Project as reference. Endpoints were analysed using a logistic or linear regression model in SNPTEST software. SNPs with p-values below $5{\cdot}10^{-8}$ were considered genome-wide significant.
The study was approved by the Data Protection Agency (j.no. 1-16-02-627-15) and the Scientifical Ethics Committee (j.no. 1-10-72-212-15) of Central Denmark Region.
Results
Two autosomal SNPs were significantly associated with acute endpoints (table I). The minor allele T in rs28419191 on chromosome 5 was associated with a decreased per-allele log-additive risk of developing mucositis with an OR=0.44 (95% CI 0.33-0.59), $p=4.39{\cdot}10^{-8}$. The minor allele T in rs448138 on chromosome 6 was associated with a decreased per-allele additive risk of overall acute morbidity (STATacute) with a coefficient=0.78 (95% CI 0.72-0.85), $p=4.36{\cdot}10^{-8}$. Manhattan plots, illustrating SNPs distributed by chromosome and the negative logarithm of the p-value, are shown in figure I. No significant associations were found between SNPs and late endpoints.