(ITVT) approach was applied. To evaluate the clinical benefit of DTIT, the tumor motion amplitude on 4DCT was compared to the mean maximal peak-to-peak amplitude on fluoroscopy sequences acquired during DTIT and the difference in PTV volume (DTIT versus ITVT) was calculated. Treatment-related toxicity was scored according to the Common Terminology Criteria for Adverse Events v.4.0.

**Results:** A total of 38 lesions were treated in 35 patients. The delivered dose schedules were as follows: 48Gy/4 fractions (n=32), 51Gy/3 fractions (n=4), 60Gy/8 fractions (n=2). Mean superior-inferior (SI) motion exceeded 8 mm in 14 out of 38 lesions. DTIT was used for 7 lesions. Reasons for omitting DTIT were: pulmonary function or lesion location not allowing visiocll insertion and history of prior pneumothorax. Mean treatment time for a DTIT session was 28.6 minutes (20-34.8 minutes). Mean SI motion on 4DCT in DTIT lesions was 11.8 mm (8.6-16.9 mm). The mean maximal peak-to-peak amplitude observed during fluoroscopy was 20.4 mm (8.2-50.5 mm) demonstrating a significant variability in respiration induced tumor motion. DTIT achieved a median reduction of 58% in PTV volume. With a median follow-up of 7 months (3-19 months), 1 local failure was observed in a centrally located lesion treated with an ITVT approach. Only 1 patient experienced a grade 2 radiation pneumonitis and 2 patients presented with a COPD exacerbation in the weeks following radiation. No toxicity was observed in the patients treated with DTIT.

**Conclusions:** DTIT with the Vero4DRT system using a single fiducial marker proved to be clinically feasible and safe. DTIT can be performed in an acceptable time frame, is able to account for respiratory variability and results in a substantial reduction in PTV volume.


**Proffered Papers: Radiobiology 1: Prediction of response using genetics**

**OC-0081**

Prediction of normal tissue radiosensitivity from random numbers?? Be cautious out there!

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**Purpose/Objective:** Background: During the last decade, several studies have established predictive models for normal tissue radiosensitivity based on multiple SNPs (1-8). Typically, these studies assessed a limited number of SNPs. For some of these SNPs, a ‘risk allele‘ was defined and the numbers of risk alleles and normal tissue complication risk. Even though many of these models have yielded highly significant results, the models have often been inconsistent with each other (table 1). This probably relates to the way these models were constructed. The process had three steps: 1) For each of the included SNPs, a risk allele (minority vs. majority allele) was defined based on the observation that it was (often non-significantly) associated with the outcome parameter of the study (radiosensitivity). 2) A model was established based on these risk alleles. 3) A statistical test was carried out to determine if the number of risk alleles was significantly associated with radiosensitivity (the same parameter as used for the selection of the risk alleles). By doing so, a circularity is introduced into the analysis that makes it likely that random fluctuations (for the individual SNPs) are amplified into significant associations (for the entire model).

**Materials and Methods:** In order to further explore this potential problem, we reanalyzed the dataset originally used to establish the multiple SNP model published by Andreassen et al. in 2003 (1). Instead of the actual SNP genotypes we randomly assigned ‘genotypes‘ to the patients for 7 fictitious SNPs that had the same relative distribution as the SNPs in the original dataset. Subsequently, we selected risk alleles for these ‘SNPs‘ and established a multiple SNP model exactly as in the original study. This procedure was repeated 10 times...

**Results:** In 8 out of 10 times a significant result was found for the model. This clearly demonstrates that the process of actively fitting the model to the dataset is indeed per se capable of producing nominally significant results for the entire model.

**Conclusions:** Great caution should be taken when a predictive model is established and tested within the same patient cohort. A significant finding for a multiple SNP model established in this way cannot be used to indirectly validate the underlying SNPs. Thus, we have to establish robust associations for the individual SNPs that can be entered into a predictive multiple SNP model that should finally be validated in an independent dataset.

**Table 1: Alleles assigned as ‘risk alleles’ in four different multiple SNP models**

<table>
<thead>
<tr>
<th>Author, year</th>
<th>N</th>
<th>XRC1 codon 309 Arg/Gln</th>
<th>XRC2 codon 244 Thr/Met</th>
<th>TOP1 codon 10 Leu/Pro</th>
<th>ATM codon 1812 Asn/Asp</th>
</tr>
</thead>
<tbody>
<tr>
<td>Andreassen, 2003</td>
<td>41</td>
<td>Arg</td>
<td>Thr</td>
<td>Leu</td>
<td>Asp</td>
</tr>
<tr>
<td>Ause, 2006</td>
<td>57</td>
<td>Gln</td>
<td>-</td>
<td>-</td>
<td>Res</td>
</tr>
<tr>
<td>Ackermann, 2010</td>
<td>09</td>
<td>Arg</td>
<td>Met</td>
<td>Leu</td>
<td>-</td>
</tr>
<tr>
<td>Zacherl, 2012</td>
<td>09</td>
<td>Gln</td>
<td>-</td>
<td>-</td>
<td>Res</td>
</tr>
</tbody>
</table>

*N* SNP not included in the model. Note: other SNPs were included in some of the models.


**OC-0082**

A machine learning method demonstrates that a large number of SNPs contribute to clinical radiosensitivity

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**Purpose/Objective:** Rectal bleeding is one of the common radiation-induced complications following radiotherapy in prostate cancer patients, which can greatly impair the quality of life for cancer survivors. The purpose of this study was to investigate whether single nucleotide polymorphisms (SNPs) are associated with susceptibility to late rectal bleeding in men treated with radiotherapy for prostate cancer using a genome-wide association study (GWAS) dataset.
Materials and Methods: There were 365 evaluable patients with at least one year of follow-up for the development of rectal bleeding. Rectal bleeding was assessed using the Radiation Therapy Oncology Group (RTOG) late radiation morbidity scoring schema. There were 74 patients with Grade 2 or more late rectal bleeding. For all patients, DNA was genotyped using Affymetrix Genome-Wide Human SNP Array 6.0. A quality control test was performed with SNP missing rate > 5%, minor allele frequency (MAF) < 5%, and Hardy-Weinberg equilibrium (p < 10E-5). As a result, 613,496 SNPs remained. For the analysis, we split the patients into two groups: a group of patients with Grade 0 or 1 late rectal bleeding and the other group of patients with Grade 2 or more.

To predict late rectal bleeding toxicity, we designed a machine learning-based multi-SNP model. Our model mainly consists of two steps: in the first step, principal component analysis (PCA) is used to filter out irrelevant SNPs and in the second step L1-penalized regression (LASSO) is used to remove redundant SNPs and to build a sparse regression model. In particular, normal tissue complication probability (NTCP), which was calculated using logistic regression with a few principal components derived from the first step, was used as a response variable in the second step. For unbiased assessment of the predicted model, the dataset was split into two groups: training dataset (2/3 of samples) and validation dataset (1/3 of samples). In the model design, only the training dataset was used with 10-fold cross validation. After 50 iterations of the proposed method, SNPs were ranked based on the frequency that each SNP was used in the LASSO models. In a manner of forward feature selection, the validation dataset was tested with the LASSO models that were constructed using the training dataset. The area under the receiver operating characteristic (ROC) curve (AUC) was used as a performance metric.

Results: Using the training data, univariate analysis was performed using Chi-square test. With a threshold of p<0.001, 749 SNPs remained. These SNPs were fed into our model. The cross-validation with the training dataset resulted in AUC=0.85 (standard deviation: 0.02) and the best final model with the validation dataset resulted in AUC=0.68 (p=0.004) with 360 SNPs as shown in Fig. 1.

![Fig 1. Performance assessment of final models using a validation dataset in a manner of forward feature selection.](image)

Conclusions: A novel machine learning method demonstrated that a large number of SNPs contribute to clinical radiosensitivity for the radiation-induced late rectal bleeding. However, evaluation on other datasets is necessary to validate our model.

Purpose/Objective: Single-nucleotide polymorphisms (SNPs) in the ataxia telangiectasia mutated (ATM) gene have been associated with clinical radiation pneumonitis, but those findings have not been correlated with the pulmonary function impairment. Therefore, we investigated the association between SNPs in the ATM gene and the risk of difusing capacity of the lung for carbon monoxide (DLCO) change in patients with non-small-cell lung cancer (NSCLC) treated with radiation therapy (RT).

Materials and Methods: From November 1998 through June 2009, 448 consecutive patients with inoperable or unrespectable primary NSCLC underwent definitive (≥60 Gy) radio(chemo)therapy; exclusion criteria were patients with a history of thoracic surgery, RT, or lung cancer or those who did not have undergone pulmonary function test before and after RT within one year. Ultimately, 100 patients met the selection criteria for this study. We genotyped two SNPs of the ATM gene (rs189037 and rs228590), and assessed correlation with DLCO impairment using logistic regression analysis.

Results: The dataset consisted of 58 men and 42 women, with a median age of 64 years (range, 38-83 years). Of all these patients, 86 were whites and 82% had stage III/IV diseases according to the 6th edition of the AJCC stage grouping criteria. The median mean lung dose was 17.5 Gy (range, 4.7-29.5 Gy). Median DLCO change within one year after RT was 0.81 (range 0.22-1.79). Early DLCO change (3-6 months after RT) had the same range but the median value was 0.77. The genotype distribution of all studied SNPs was: rs189037, 29% AA, 49% AG, 22% GG; and rs228590, 33% CC, 49% CT, 19% TT. Univariate and multivariate analyses showed that the AA genotype of ATM rs189037 was associated with significantly higher DLCO impairment after definitive radiation than the GG/AG genotypes (univariate beta regression coefficient -0.12; 95% confidence interval [CI], -0.24–0.008; P = 0.037; multivariate beta regression coefficient -0.10; 95% CI, -0.20–0.005; P = 0.04). However, similar results were not observed for rs228590 (univariate beta regression coefficient -0.10; 95% CI, -0.25–0.12; P = 0.096).

Conclusions: The AA genotype of ATM rs189037 was associated with higher risk of lung injury, compared with the GG/AG genotypes in patients with NSCLC treated with radio(chemo)therapy. This response marker may be used for guiding therapy intensity in an individual patient, which would further the goal of individualized therapy.

OC-0084
SNP analysis on late radiation induced toxicity analyzed by anal physiological methods in prostate cancer patients

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Purpose/Objective: Normal tissue toxicity sets the dose limit for radiotherapy (RT) in cancer treatment and hence has significance for its curative potential. Individual differences in the severity of radiation induced toxicity suggests that there is a possibility of dose escalation to the more radiosensitive group. In theory prediction of individual radiosensitivity could augment cure rates of RT. From a previous study we have access to a cohort of 42 prostate cancer patients treated with External Beam RT (EBRT) and clinical data for radiation induced toxicity measured by anal