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**Purpose or Objective:** Selecting good responders after chemoradiation (CRT) for locally advanced rectal cancer (LARC) could lead to the omission of total mesorectal excision (TME) in patients with pathologic complete response (pCR). In the current study, we assessed the value of several blood biomarkers associated with the fibrotic response to CRT (IGF-1, IGFBP-2, HGF & GDF-15) as markers for general fibro-inflammatory response and as tumor response predictors in a group of 80 patients.

**Material and Methods:** ELISA analysis of IGF-1, IGFBP-2, HGF and GDF-15 was conducted on prospectively collected serum samples of 80 LARC patients on 3 time points (before, during, after CRT). The fibro-inflammatory response was scored on H&E sections of the resection specimen. Changes in concentration were analysed using a Kruskal-Wallis test. Correlation of concentration at each time point and the difference between these time points (Δ) with fibro-inflammatory response and tumor response (pCR and ypT0-1) were assessed using a Mann-Whitney-U test.

**Results:** Higher Growth Differentiation Factor 15 (GDF-15) concentration before CRT correlated with the presence of a fibro-inflammatory response ($p = 0.04$), but was not observed for the other proteins nor for GDF-15 at other time points.
General increase in GDF-15 concentration during treatment (median 0.81 ng/ml before, 2.16 ng/ml during, 2.37 ng/ml after CRT; $p < 0.0001$) was measured (Figure 1). Although no significant general concentration changes occurred for IGF-1, IGFBP-2 or HGF, we did find a correlation between the variation in expression of IGFBP-2 during treatment (ΔIGFBP-2 TP3-TP2) with tumor response (pCR $p = 0.02$; ypT0-1 $p = 0.02$). Other proteins did not correlate with tumor response.

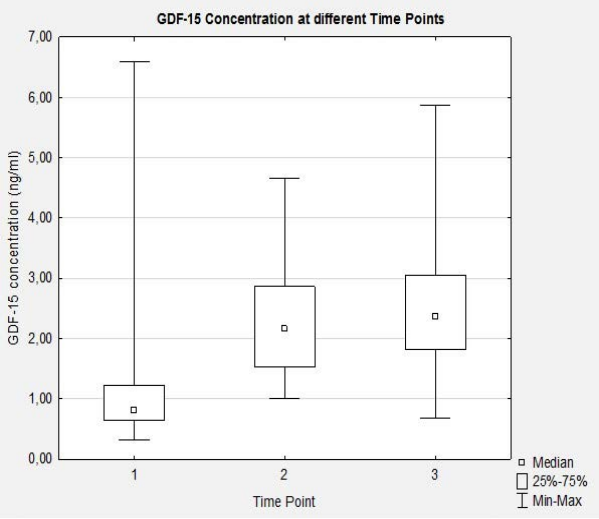


**Figure 1.** GDF-15 Concentration at different time points (TP) during treatment. (TP1: before CRT; TP2: during CRT; TP3: after CRT)

**Conclusion:** GDF-15 serum concentration increases during CRT for LARC and a higher concentration measured before start of treatment is correlated with the presence of a fibro-inflammatory response. These results suggest that GDF-15 could be used as an early predictor of fibro-inflammatory response and thereby indirectly as predictor for disease-free survival. This will be evaluated when follow-up data are available for this patient cohort.
The correlation of variation in expression of IGFBP-2 with tumor response (pCR and ypT0-1) opens a novel possibility for selecting good responders to CRT. We aim to combine these findings with imaging analyses (DW-MRI, PET) at different time points during treatment to develop a predictive model for selecting LARC patients in whom surgery could be omitted.

**EP-2056**
**Preclinical investigation of hypoxia induced genes in different prostate cancer cell lines.**
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**Purpose or Objective:** Hypoxia is a common feature in prostate cancer and is known to reduce the response to radiotherapy. Hypoxic modifiers can to a large extent overcome these obstacles, and a proper classification of tumors into hypoxic and non-hypoxic fractions is necessary. Previously our department has developed a gene profile consisting of 15 genes, which demonstrated prognostic and predictive impact for hypoxic modification in head and neck squamous cell carcinomas (HNSCC). In the current study we investigated the 15 gene profile in different prostate cancer cell lines.

**Material and Methods:** For the in vitro experiments the prostate cancer cell lines investigated were PC-3, DU-145, and LNCaP. Cell lines were cultured under normoxic (21% O2) or hypoxic conditions (0% O2) for 24 hours, totRNA was extracted and gene expression levels measured by qPCR. Individual reference genes were selected (PSMC4, TBP, NDFIP1) and applied in the normalization of the relative expression levels, together with the reference genes previously used in the HNSCC study. For in vivo experiments, the PC3 cell line was inoculated on the flank of female NMRI nu/nu mice, whereas the LNCaP and DU-145 cell lines were inoculated on the flank of severely immunocompromised CIEA/NOG mice. Two hypoxia-sensitive tracers (18F-FAZA and Pimonidazole) were administered in order to determine hypoxic and non-hypoxic regions on excised tumor sections. These regions were isolated by laser-assisted microdissection, after which totRNA was extracted and gene expression levels measured by qPCR.

**Results:** In the in vitro experiments, all prostate cancer cell lines had 14 of the 15 genes induced by hypoxia. The only discrepancy was ALDOA, which was not upregulated in the hypoxic cells. In vivo experiments are still ongoing but preliminary results from PC3 xenografts have been produced. These show a hypoxia induced upregulation in 10 out of the 15 genes, of which 4 were significantly upregulated (ADM, ANKRD37, FAM162A, and LOX).

**Conclusion:** In this study we investigated the 15 gene hypoxic profile in three different prostate cancer cell lines. A hypoxia dependent induction of genes was observed in both in vitro and in vivo experiments. From the performed experiments, and looking only at oxygen dependency, it appears that the gene profile could be suitable for prostate cancers as well as HNSCC.

**EP-2057**
**Radiotoxicity prediction by gene expression profiling when simulating therapy in matched fibroblasts**
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**Purpose or Objective:** Acute radiotoxicity might put a vital threat to the patient and may require interruption or

preterm stop of the therapy thus jeopardizing the intended treatment outcome. Despite numerous research attempts there is still no robust feature established in clinical routine to predict radiotherapy-induced toxicity prior to therapy start.

**Material and Methods:** The study cohort comprised 40 patients who underwent neoadjuvant radiochemotherapy (N-RCT) for rectal cancer (28x1.8 Gy, 5 times weekly, concomitant with two cycles 5-FU-based chemotherapy). From each of those patients dermal fibroblasts were cultured from skin specimen gained outside of the radiotherapy planning target volume at occasion of surgery conducted about six weeks upon N-RCT completion. Acute radiotoxicity was thoroughly monitored throughout the N-RCT series and documented according to CTC classification. Maximal acute toxicity (MAT) was defined by the highest CTC grade of the four items "cystitis", "proctitis", "enteritis", and "dermatitis". MAT was grouped into grades 0/1 (n=16), 2 (n=16), and 3/4 (n=8). N-RCT was simulated in the cultured fibroblasts for five consecutive days (1.8 Gy each at d1-d5 with addiation of 5-FU at a concentration reflecting clinical steady-state levels) followed by a 7-day wash-out period. Gene expression of nine candidate genes (*CAT*, *CDKN1A*, *CTGF*, *SMAD2/3/4/7*, *TGFB1*, *TGFBR1*) supposed to mediate early radiation-induced toxicity was ascertained by quantitative real time PCR. Samples for these RNA analyses were harvested at d2 and d5 (each 4 hours upon application of the radiation fraction) as well as at day 12 upon the wash-out period. *GAPDH* and *HPRT1* transcript levels served as reference.

**Results:** MAT was related to radiation-induced expression changes of four of the considered genes in fibroblasts. The strongest impact was obtained for *SMAD7* and *CAT* at d5. The higher the MAT score, the lower the induction of *SMAD7* and *CAT* by radiation was (p=0.001 and 0.003). However, upon the wash-out period at d12 no statistical differences in dependence on the MAT score were seen anymore for these two genes. In contrast, a high MAT score was linked to low radiation-induced induction of *CTGF* (p=0.005) and to a faster decrease of the massively induced *CDKN1A* (p=0.03) at d12. At d2, a trend (p=0.06) for *CAT* in relation to MAT in the same direction as at d5 was noticed with no correlation of any of the other genes at this early time point.

**Conclusion:** Radiation-induced expression changes in patient-matched fibroblasts may serve as biomarkers to predict clinical radiotoxicity. Induction of *SMAD7* and *CAT* may mitigate TGFbeta signalling and reactive oxygen species load thus saving normal tissue during radiotherapy. A protective role might also be attributed to sustained elevation of *CDKN1A*. The link between post-radiation induction of *CTGF* in fibroblasts and low MAT remains to be clarified. Understanding the mechanistic basis of these findings might pave the way for better protection of irradiated normal tissue.

## EP-2058

### A novel multi-SNP model predictive of erectile dysfunction following radiotherapy in prostate cancer

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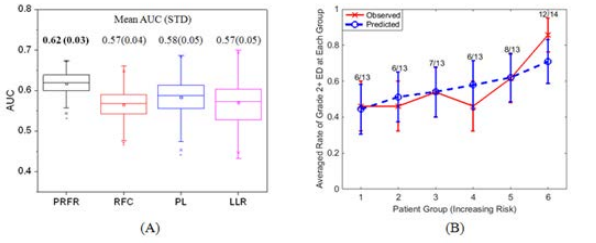
**Purpose or Objective:** Erectile dysfunction (ED) is one of the most common complications encountered after radiotherapy in prostate cancer patients. The goal of this study was to investigate whether single nucleotide polymorphisms (SNPs) are associated with late ED in men treated with radiotherapy for prostate cancer. To this end, we designed a novel machine learning-based multi-SNP model using a genome-wide association study (GWAS) dataset.

**Material and Methods:** We analyzed 236 evaluable patients with at least one year of follow-up for the development of ED. The severity of ED was assessed using either the patient-administered Sexual Health Inventory for Men (SHIM) or the clinician-assigned Mount Sinai Erectile Function (MSEF) scoring schema. There were 133 patients with Grade 2 or more ED. For our analysis, the cohort was split into two groups (cases/controls: MSEF 0,1 / 2,3; cases/controls: SHIM ≤7 / ≥16). Genome-wide SNP data were available from Affymetrix Genome-Wide Human SNP Array 6.0. After a quality test including SNP missing rate>5%, minor allele frequency (MAF)<5%, and Hardy-Weinberg equilibrium (p<10E-5), 613,496 SNPs remained.
For the validation purpose of our proposed model, the dataset was split into a training dataset (2/3 of samples) and a validation dataset (1/3 of samples). Our model building process was performed using the bootstrapped data from the training dataset. Our idea is to convert the binary outcomes into preconditioned continuous outcomes based on normal tissue complication probability (NTCP) using principal component analysis (PCA) and logistic regression. The preconditioned outcomes were used in the model building process using random forest regression. Then, the model was tested using the validation dataset. The final predicted outcomes were compared with the original binary outcomes to estimate the predictive performance. We iterated this process 100 times and the performance was averaged. We compared the performance of our proposed method (preconditioning random forest regression: PRFR) with other methods including preconditioning lasso (PL), lasso logistic regression (LLR), and random forest classification (RFC).

**Results:** Univariate analysis was performed using the training dataset. With a threshold of p=0.001, 367 SNPs remained. These SNPs were fed into our model. As shown in Figure (A), the averaged performance with the validation dataset was AUC=0.62, which is better than other methods: RFC (0.57), PL (0.58), and LLR (0.57). The 79 patients in the validation dataset were binned into 6 groups according to the predicted risk of ED. Figure (B) shows the comparison of the model-predicted incidence of ED with observed incidence.

**Conclusion:** Our machine learning-based multi-SNP model showed the potential to better predict the radiation-induced late ED. However, we need to validate our model using other datasets.

Figure. Comparison of our machine learning-based multi-SNP model with other methods (A) and comparison of the model-predicted incidence of ED with observed incidence with events/patients on the top of the standard error bars in 6 bins (B).




## EP-2059

### Changes in hypoxia in serial F-MISO/PET-CT during chemoradiation in HNSCC

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**Purpose or Objective:** Tumor hypoxia, a common feature of locally advanced head and neck cancer (HNSCC), is associated with higher malignancy and increased radioresistance. The decrease of tumor hypoxia during