Radioresistance of glioblastoma stem-like cells is associated with replication stress.

**Material and Methods:** Patient-derived GBM-SCs were cultured under stem cell culture conditions. Western blot was used for protein expression analyses. Sphere formation served as a surrogate assay for self-renewal and cell death was assessed flow cytometrically. Lentiviral RNA-knockdowns or overexpression of p53 and FoxO proteins were employed for molecular studies. ChIP assay was used to assess binding of FoxO transcription factors to the regulatory region of the sox2 gene.

**Results:** p53-proficient GBM-SCs lost stem cell markers and self-renewal ability and underwent differentiation a few days after the combination treatment with γ-IR and a PI3K/mTOR inhibitor (PI-103 or NVP-BEZ235). Expression of FoxO proteins was also lost. In contrast, stem cell markers and FoxO proteins were not lost anymore upon p53 shRNA knockdown or in p53-deficient GBM-SCs. FoxO1/3 knockdown also caused reduced sphere formation and cell survival after the combination treatment in p53-proficient but not in p53-deficient GBM-SCs. Furthermore, FoxO1 and FoxO3 were found to bind to the sox2 regulatory region in GBM-SCs, and combined FoxO1/3 deletion abolished Sox2 expression which was confirmed with a novel synthetic FoxO1 inhibitor. Finally, FoxO overexpression prevented GBM-SC differentiation upon combination treatment with γ-IR and dual PI3K/mTOR inhibitors.

**Conclusion:** Our results suggest that FoxO proteins are crucial for functional stemness and survival in p53-proficient GBM-SCs and that non-functional p53 can maintain these functions instead.

**OC-0133**

Radioresistance of glioblastoma stem-like cells is associated with replication stress.

**Purpose or Objective:** Tumour recurrence in glioblastoma (GBM) patients is inevitable despite multi-modality treatment with surgery, radiotherapy and chemotherapy. Tumour recurrence is thought to be driven by a small population of glioblastoma stem-like cells (GSCs) that are resistant to conventional therapies. DNA damage response (DDR) signalling has been shown to be up-regulated in GSCs and implicated in radioresistance and treatment failure. However the cause of enhanced DDR signalling in GSCs and its contribution to radiation resistance and tumour recurrence is not well understood. The objectives of this study were to investigate the underlying cause of DDR upregulation and treatment resistance in GSCs and to identify novel therapeutic targets.

**Material and Methods:** A panel of primary GBM cell lines cultured under conditions to enrich for or deplete the tumour stem cell population (GSC vs bulk respectively) were utilised to investigate enhanced GSC DDR under basal conditions and were radioresistant compared to paired bulk populations. Augmented DDR in GSCs has been linked to increased reactive oxygen species levels by other authors, however we were unable to demonstrate this in our GSC cultures. Instead, we show that RPA is significantly higher in replicating GSCs and confirm by DNA fibre assays that GSCs and CD133+ cells have increased numbers of stalled replication forks, fewer new origins and slower DNA replication compared to bulk or CD133- populations, suggesting that replication stress may be important to constitutive DDR activation seen in GSCs. Importantly, inhibition of ATR or CHK1 was cytotoxic to GSCs and when combined with PARP inhibition caused DNA double strand breaks and reduced neurosphere formation.

**Conclusion:** This study demonstrates that replication stress is a hallmark of GSCs. We implicate replication stress in GSCs as the driver of enhanced DDR and radioresistance in GSCs and therefore a cause of tumour recurrence in GBM. This suggests that replication stress is a GSC specific therapeutic target, and we are able to demonstrate the effectiveness of inhibitors of replication stress response in targeting this treatment resistant tumour subpopulation.

**OC-0134**

Irradiation-induced plasticity of the cancer stem cell population in prostate cancer

**Purpose or Objective:** Although prostate cancer is the most common malignancy in men, the cellular and molecular mechanisms underlying tumor progression and therapy resistance remain poorly understood. Within this study we discovered cancer stem cell (CSC)-related properties, CSC plasticity and tumor heterogeneity as a source for radiotherapy resistance. Therefore, analysis of CSC-based biomarkers might be an important predictive tool for individualized radiotherapy and treatment.

**Material and Methods:** Global gene expression and membrane proteomic profiling of radioresistant sublines from established prostate cancer cell lines identified novel biomarker for prostate cancer radioresistance, which were validated in NMRI nu/nu mice in vivo, with immunohistochemical analysis of tumor sections and in short-term ex vivo cultures of primary prostate cancer tissue.

**Results:** Within this study we found that the aldehyde dehydrogenase (ALDH) activity is a predictive marker of a radioresistant prostate cancer progenitor population with enhanced DNA repair capacity and activation of epithelial-mesenchymal transition (EMT). The activation of the WNT/B-catenin signaling pathway was identified as a key molecular mechanism, which link CSC-related properties to radioresistance. We found that the B-catenin/TCF transcriptional complex is directly activating the ALDH1A1 gene transcription, and molecular targeting of the WNT pathway with XAV939 leads to radiosensitization. Moreover, our study revealed that irradiation causes long-term upregulation of stem cell markers and induces tumor cell reprogramming. This phenotypic plasticity is associated with genetic and epigenetic changes induced by irradiation, such as the histone H3 methylation within the promoter sequence of the ALDH1A1 gene. The inhibition of histone methylation by DZNep triggered radiosensitization by apoptosis induction in vitro and in vivo.

**Conclusion:** Our findings suggest that ALDH-positive CSCs contribute to tumor radioresistance, but these radioresistant...
properties are dynamic in nature. Therapeutic agents inhibiting tumor cell reprogramming may have the potential to increase the effectiveness of radiotherapy. Moreover, monitoring of CSC-related biomarker before and during the course of radiotherapy may be able to predict therapy response and clinical outcome.

Conclusion: General patient characteristics together with comorbidity data, especially the history of a previous malignancy, can predict early death, however, prediction accuracy is insufficient to select patients to wait and see instead of offering SBRT as a curative treatment.

OC-0136
Primary Study Endpoint Analysis of NRG Oncology/RTOG 0813 Trial of SBRT for centrally located NSCLC

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Purpose or Objective: This study analyzed whether short-term death of patients with peripheral stage I NSCLC can be predicted reliably to select a sub-group of patients, which will not have a benefit from SBRT and which can be referred to wait and see.

Material and Methods: 802 patients with early stage NSCLC treated with SBRT in 5 institutes for whom information on overall survival within the first six months after treatment was available were included in this analysis. The probability of dying within six months after treatment was modeled by multivariate logistic regression; this interval was chosen because death of early stage NSCLC is a rare event within six months after diagnosis. Model fitting was performed using the LASSO method which simultaneously serves to select the features most closely related to the outcome. The performance of the model that would be achieved on an independent dataset was estimated using double 10-fold cross validation (CV). Because with CV the estimation of test performance depends somewhat on the splitting of the data sets, double 10-fold CV was repeated 100 times, resulting in 1000 models from which the variance in the performance measure could be obtained. The variables age, gender, ECOG status, operability, FEV1 and Charlson comorbidity index (CCI) where considered for model building.

Results: Using different variable combinations for model building resulted in different sample sizes and model performances (Table 1). Common among all models was the identification of the CCI as the most frequently selected and thus most important variable predicting six-months death, with increasing values predicting higher probability of death. Gender was consistently the second-most frequently selected variable. Regressing on the individual components of the CCI with the LASSO method showed that presence of a second solid tumor was the most important predictor, followed by various forms of heart disease (Figure 1). Replacing the CCI by these individual components in model building confirmed the strong relation between the presence of a second tumor and early death, but led to a worse model performance than with the full CCI (Table 1). Overall the accuracy of all models predicting six-months death was poor with maximum AUC=0.62.

Table 1: Results of the model fitting procedure applied to three sub-samples of the data. MI: Myocardial infarct; PVD: Peripheral vascular disease; CVD: Cardiovascular disease.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Age, Gender, KLOP, Operability, FEV1, CCI</th>
<th>Age, Gender, CCI</th>
<th>Age, Gender, MI, PVD, CVD, second tumor</th>
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<tbody>
<tr>
<td>Sample size</td>
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<tr>
<td>Number of events</td>
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<tr>
<td>Specificity [%]</td>
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<td>0.72</td>
<td>0.72</td>
</tr>
</tbody>
</table>

Figure 1: Results of variable selection by the LASSO method when using the individual components of the CCI for predicting death within six months after treatment. 10-fold CV was repeated 1000 times, resulting in a total of 1000 individual models. Shown is the frequency with which a variable was selected into a model.