Material and Methods: Chemically synthesized, RGD-/PEG-functionalized gold nanoparticles (RGD:AuNP; 2-3 nm) were characterized using STEM, TEM, and LIBS imaging. Following clonogenic assay, radiation damage was induced in Panc1 xenographs with 10 Gy and 220 kVp (Xtralh, Inc). γ-H2AX, 3D- (confocal) vessel imaging and IHC were performed.

Results: Tumor vessel-targeted gold nanoparticles were subjected to conformation image-guided irradiation in Panc1 tumor xenograft to induce tumor vascular disruption. By specifically targeting the early angiogenic tumor endothelium, RGD:AuNP circumvent the dense stromal diffusion pathways that often limits the penetration and permeation of anti-cancer drugs/ nanoparticles to the cancer cells - a limitation of current radiosensitization approaches. In vitro testing in HUVEC displayed -fold difference (**P<0.0001) in radiation damage in the +RGD:AuNP/+IR compared to the controls. More to it, the sub-millimeter accuracy of image-guided radiation therapy facilitated improved therapeutic efficacy (95%-100% tumor dose distribution) and less off-target toxicities. Quantification of the DNA-strand breaks (by γH2AX) showed 3-fold increase (P<0.001) in the radiation specific DNA damage in the nanoparticle group (+RGD:AuNP/+IR: 97%) compared to the ‘radiation’ group (+RGD:AuNP/+IR:19%) and almost ≈10-fold difference (P<0.001) compared to ‘nanoparticle-radiation’ cohort (+RGD:AuNP/+IR: 57%).

Conclusion: This dual-targeting strategy holds great translational potential in radiation oncology. The resulting vascular disruption substantially improved the therapeutic outcome and subsidized the radiation/ nanoparticle toxicity, extending its utility to intransigent/ non-resectable tumors that barely respond to standard therapies. This abstract extends its utility to intransigent/ non-resectable tumors.

PO-0984 Combined inhibition of Chk1 and Wee1 kinases for cancer treatment
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Purpose or Objective: Inhibition of checkpoint kinases Wee1 or Chk1 causes G2-checkpoint abrogation and mitotic catastrophe, particularly in p53 defective tumors. Based on this, Wee1 and Chk1 inhibitors are currently in clinical trials, combined with radiation or chemo-therapy. However, our previous work has shown that inhibition of Wee1 or Chk1 also causes DNA breakage in S-phase, largely due to high Cyclin-Dependent-Kinase (CDK)-activity followed by unscheduled replication initiation. Furthermore, recent work by others has shown synergistic anti-cancer effects after combined Wee1 and Chk1 inhibition. The aim of this study was to investigate whether S-phase DNA damage may contribute to the synergistic effects after combined Chk1/Wee1 inhibition.

Results: We observed a strong synergy in induction of S-phase damage after combined Wee1 and Chk1 inhibition. Also, clonogenic survival was strongly decreased after the combined treatment. Surprisingly, this synergy could not be explained by increased CDK-activity, as S-phase CDK-activity did not correlate with induction of DNA damage after Wee1 and Chk1 inhibition. Wee1 inhibition caused a bigger increase in CDK-activity than Chk1 inhibition. However, Chk1 inhibition caused more S-phase damage and loading of the replication factor CDC45. The combination of Wee1 and Chk1 inhibitors further increased the CDC45 loading, and the extent of CDC45 loading correlated with DNA damage induction.

Conclusion: We have shown for the first time that combined Wee1 and Chk1 inhibition causes synergistic S-phase DNA damage, due to distinct effects of Wee1 and Chk1 kinases in regulation of CDK activity and CDC45 loading, respectively. This synergy can explain the synergistic anti-cancer effects obtained by simultaneous Chk1/Wee1 inhibition. We propose that combined Chk1/Wee1 inhibition may be useful together with radiation therapy to eliminate radiosensitive S-phase cells.

PO-0985 Anti-GRP 78 antibodies bind specifically to cancers enhance efficacy of radiotherapy in cancer
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Purpose or Objective: Glioblastoma demonstrates progression of disease within the high dose region of radiotherapy, in nearly all cases. The physiologic response within glioblastoma to radiation is in part dependent upon a pro-survival signaling. GRP78 was first described to regulate cellular stresses, including hypoxia, hyperosmolarity, and ER stress response. GRP78 is an important regulator of cell stress, and binds to several pro-survival proteins. Antagonists to GRP78 include Kringle-3 and PAR4 which induce apoptosis in tumor vasculature endothelium and cancer cells. The molecular events that result from the ER stress response can enhance cell viability. GRP78 is overexpressed in poor prognosis cancers and is a molecular therapeutic target in poorly differentiated cancers.

Material and Methods: Methods: We studied radiation induction of GRP78 by western immunoblot and flow cytometry. We used siRNA to knock down GRP78 in human GBM and NSCLC cell lines. In order to study the potential relationship between radiation dose and induction of ATF6 activity, we treated D54 cells with 3 Gy and 6 Gy and analyzed GRP78 protein expression 48h after irradiation. We utilized Anti-GRP78 antibodies administered IV to mouse models of human cancer xenografts. We measured tumor growth delay using subcutaneous implants of human cancer xenografts.

Results: We found that radiation induces the expression of GRP78 in glioblastoma. Antibodies to GRP78 enhanced radiation-induced cytotoxicity in glioblastoma but not normal cells. We found that radiation induced GRP78 expression is regulated through the ER stress response, and that ATF6 is responsible for the transcriptional induction of GRP78. Knockdown of ATF6 abrogates GRP78 induction and enhanced cytotoxicity from radiation. Moreover, interruption of GRP78 signaling enhances therapeutic effects of radiation. GRP78 antibodies enhanced cytotoxicity from radiation in human glioblastoma and NSCLC cell lines. We found that the levels of GRP78 protein were elevated at the 48 and 72h time points. Knockdown of ATF6 was sufficient to abrogate GRP78 induction. We observed dose dependent increases in GRP78 levels, which were reproducible when the experiment was repeated with LN827 cells. Similar changes were observed in GRP78 mRNA levels 48h after IR, where a 75% and 100% increase was observed in D54. Anti-GRP78 antibodies bind specifically to irradiated cancers enhanced the efficacy of radiation.
radiotherapy in human cancer models in vitro and in mouse xenografts.

Conclusion: Conclusion: GRP78 is a molecular target for the development of novel radiation sensitizing agents. Anti-GRP78 antibodies enhance the efficacy of radiotherapy when administered IV to mouse models of human cancer.

Poster: Radiobiology track: Tumour biology and microenvironment

PO-0986
MiR-143 inhibits tumour progression by targeting STAT3 in esophageal squamous cell carcinoma
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Purpose or Objective: The objective of this study was to investigate the biological role of miR-143 in esophageal squamous cell carcinoma (ESCC) progression and its underlying mechanism.

Material and Methods: Surgical tumor tissue samples were obtained from 40 patients. MiR-143 and STAT3 protein expression levels in these clinical samples and three ESCC cell lines were determined by quantitative RT-PCR and western blot. The relationship between expression level of miR-143 and clinical parameters were explored by one-way ANOVA. The specific targeting site of miR-143 in the 3’-UTR of STAT3 was identified using dual-luciferase reporter assays. Then, the effects of ectopic miR-143 or STAT3 expression on proliferation, cell cycle distribution, migration and invasion were determined in colony-forming assay, flow cytometry and transwell assay. The effect of miR-432 on tumor progression in vivo was determined by performing tumor formation assay in nude mice. The role of miR-143 in regulating cell cycle signaling, epithelial-mesenchymal transition and MMP up-regulation through repressing STAT3 was explored by analyzing the expression level of the downstream proteins.

Results: MiR-143 expression was downregulated in 90% of the ESCC clinical samples and its expression level was associated with the lymph node metastasis(LNM), invasion and TNM stage in ESCC patients. Functional experiments showed that over-expression of miR-143 induced an inhibition of cell proliferation, promotion of apoptosis and suppression of cell migration and invasion in vitro by targeting IGSF3.

Conclusion: In conclusion, our results established a functional link between miR-432 and IGSF3 expression in esophageal cancer, demonstrating that IGSF3 was directly repressed by miR-432, which subsequently effects the tumor biology process. Collectively, this finding not only helped us understand the molecular mechanism of esophageal carcinogenesis, but also gave us a strong rationale to further investigate miR-432 as a potential biomarker and therapeutic target for esophageal cancer.

PO-0988
Combined treatment strategies for microtubule interfering agent-resistant tumors
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Purpose or Objective: Tumor cells are the major targets for classic anticancer treatment modalities. At the same time other cell types within the tumor microenvironment are also targeted and co-determine the treatment response. Resistances to specific treatment modalities are therefore not only linked to the mutated genetic background of the tumor cells but also to the interaction of tumor cells with the tumor microenvironment. Thus targeting of important elements of the microenvironment is a promising strategy to overcome treatment resistances in solid tumors. Here we mechanistically investigate in different clinically relevant microtubule-stabilizing agent (MSA)-refractory tumor models the potency of combined treatment modalities of MSAs, inhibitors of angiogenesis and ionizing radiation to overcome MSA-resistance.

Material and Methods: Rationally designed single and combined treatment regimens of ionizing radiation, microtubule-stabilizing (taxane, epothilone) and destabilizing agents and anti-angiogenics compounds were investigated in genetically defined MSA-sensitive and MSA-resistant lung and colon adenocarcinoma cell lines in vitro and in the corresponding tumor xenografts in vivo.

Results: While MSAs potently inhibited A549 wt and endothelial cell proliferation, no anti-proliferative effect was observed in the corresponding mutated MSA-resistant tumor cells. Importantly, MSAs did not block anymore pro-survival auto- and paracrine signaling from resistant tumor cells by downregulation of HIF-1alpha transcriptional activity and subsequent secretions of HIF-1alpha-mediated growth factors and cytokines like VEGF. Thereby continuous pro-survival...