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 xenografts with 10 Gy and 220 kVp (Xtrahl, Inc). γ-H2AX, 3D-(confocal) vessel imaging and IHC were performed.

Results: Tumor vessel-targeted gold nanoparticles were subjected to conformational 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 a 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 radiotherapy facilitated improved therapeutic efficacy (95%-100% tumor dose distribution) and less off-target toxicities. Quantification of the DNA-strand breaks (by γH2AX) showed a 3-fold increase (P=0.001) in the radiation specific DNA damage in the nanoparticle-coated samples (+RGD:AuNP-/-IR: 6%) compared to the ‘radiation’ group (+RGD:AuNP+/IR:19%) and almost 10-fold difference (P=0.001) compared to (+RGD:AuNP-/IR: 6% and -RGD:AuNP+/IR: 6%).

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 presents the first in-depth experimental investigation of tumor vascular disruption with nanoparticles, a novel strategy in radiation therapy.

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 and 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.

Material and Methods: Osteosarcoma U2OS and lung cancer A549, H460 and H1975 cells were exposed to the Wee1 inhibitor MK1775 and/or the Chk1 inhibitors AZD7762, LY2606368, MK8776 and UCN01. The DNA damage marker γH2AX was analyzed in S-phase cells by flow cytometry. DNA damage signaling and inhibitory phosphorylation of CDK1 and CDK2 were examined by immunoblotting, and cell survival by clonogenic survival assays. CDK activity was measured in S-phase cells by a novel flow cytometry barcoding method. In this method, CDK-dependent phosphorylations (antibodies to phospho-BRCA2 S3291, phospho-BMyb T487 and phospho-Mmp2) versus DNA content ( Hoechst staining) were examined in individual cells. Barcoding with Pacific Blue was included to reduce sample-to-sample variations. Looting of the replication initiation factor CDC45 was measured by a similar flow cytometry method and by immunoblotting after removal of unbound proteins by extraction with salt and detergent.

Results: We observed a strong synergy in induction of S-phase damage after combined Wee1 and Chk1 inhibition. Also, clonogenic survival was strongly increased 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-GRP78 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 hypoglycemia, hypoxia and the 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 PARK4 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: 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 LN227 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...