not able to form new tumors and have high expression of CD127 on their T cells, a marker for immunological memory. This new treatment will be further investigated in a Phase I study for patients with an oligometastatic solid tumor (NCT02086721).

**OC-0235**

Enhancing stereotactic radiation schedules using the vascular disrupting agent OXi4503

M.R. Horsman1, T.R. Wittenborn1

**Aarhus University Hospital, Department of Experimental Clinical Oncology, Aarhus C, Denmark**

Purpose or Objective: The novel combretastatin analogue, OXi4503, is a vascular disrupting agent (VDA) that has recently been shown to significantly enhance a stereotactic radiation treatment. This was achieved using an OXi4503 dose of 10 mg/kg combined with a stereotactic treatment of 3 x 15 Gy. The current study was undertaken to determine the OXi4503 dose dependency when using different stereotactic radiation dose schedules.

**Material and Methods:** A C3H mammary carcinoma grown in the right rear foot of female CDF1 mice was used in all experiments. Tumours were periodically monitored and tumours were excised and single cell suspensions were generated and plated for clonogenic survival. Tumor-free intervals were estimated. A Chi-squared test (p<0.05) was used to determine significant differences between the TCD50 values.

**Results:** The clamped top-up TCD50 values (with 95% confidence intervals) obtained following irradiation with 3 treatments of 10, 15 or 20 Gy were found to be 42 Gy (38-47), 30 Gy (23-39), and 0.8 Gy (0.3-2.3), respectively. A plot of the TCD50 values against the stereotactic doses gave rise to a linear response (slope = -4.1; correlation coefficient = 0.97). OXi4503 significantly decreased the clamped radiation top-up TCD50 values and this effect appeared to be independent of both the ambient radiation dose applied with each of the 3 fractions and the VDA dose; the curve showing the TCD50 values against stereotactic radiation dose was similar to that for radiation alone (slope = -4.3; correlation coefficient = 0.94), but the radiation + OXi4503 curve was observed to be a simple additive effect independent of both the radiation dose applied with each fraction and the VDA dose used.

**Conclusion:** OXi4503 is an effective agent for enhancing a stereotactic radiation treatment. But, the enhanced response appeared to be a simple additive effect independent of both the radiation dose applied with each fraction and the VDA dose used.

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**OC-0236**

DTP-006: a novel, orally bioavailable hypoxia-activated prodrug

R. Niemans1, A. Varominia1, J. Thyes1, A. Ashoorzadeh2, R. Anderson2, M. Bull1, C. Guise1, H.L. Hus1, M. Abbattista1, A. Howdary1, A.V. Patterson1, J.B. Small1, D.F. Ackerley1, L. Dubois1, P. Vanhoof6, S. Short6, F. Verhaegen1, M. Vooijs1

1Maastricht University- GROW - School for Oncology and Developmental Biology, Maastricht Radiation Oncology MAASTRO Lab, Maastricht, The Netherlands

2University of Auckland, Auckland Cancer Society Research Centre, Auckland, New Zealand

3Victoria University of Wellington, School of Biological Sciences, Wellington, New Zealand

**Purpose or Objective:** Hypoxia is a common feature of solid tumors. Conventional treatments such as chemo- and radiotherapy (RT) are less effective against hypoxic tumor cells. Hypoxia-activated prodrugs (HAPs) are specifically activated in hypoxia to target hypoxic cells as well as adjacent oxygenated tumor cells via their bystander effect. DTP-006 is a newly synthesized nitroaromatic HAP with highly favorable properties: 1) activation under hypoxia, 2) high bystander effect, 3) excellent aqueous solubility, 4) murine oral bioavailability and 5) no off-mechanism activation by human hepatic reductases NQO1 and AKR1C3. Here we show the effects of DTP-006 on tumor cell viability, spheroid growth and radiation resistant tumor cells in vivo, and assess its pharmacokinetics and oral bioavailability in mice.

**Material and Methods:** The one-electron reduction potential (E1) of DTP-006 was determined by pulse and steady state radiolysis. IC50 viability ratios were assessed in 2D cell culture exposed to normoxic or anoxic (≤0.2% O2) conditions. H460 multicellular layers (MCLs) under aerobic (5% CO2, 95% O2) or anoxic (5% CO2, 95% H2) conditions were incubated with DTP-006 for 5 h after which cells were plated for clonogenic survival. H460 spheroids were incubated with DTP-006 upon confirmation of a hypoxic core. NIH-III mice bearing H460 tumors received a single i.p. dose of DTP-006 (781 mg/kg) after irradiation (10 Gy) of tumors. 18 h later tumors were excised and single cell suspensions were generated and plated for clonogenic survival. Tumor-free female NIH-III mice received a single i.v. or oral dose of DTP-006 (383 mg/kg). Terminal blood samples collected at time points via cardiac puncture were analyzed by LC/MS/MS. Plasma half-life (T1/2) and absolute oral bioavailability (Fabs) were calculated.

**Results:** DTP-006 has an E1 value of -351 mV, indicating strong oxygen inhibition of nitro radical formation. IC50 were lower in anoxia than normoxia by factors of 293 (MDA-MB-468), 55 (C33A), and 20 (HCT116). In a H460 MCL clonogenic assay, 100 µM DTP-006 caused 99% cell kill under anoxia but exhibited no aerobic cell kill. It caused a concentration-dependent growth delay in spheroids, where 250 µM completely halted growth. A single dose of DTP-006 caused a significant loss of clonogenicity when combined with RT in an in vivo excision assay (log cell kill 2.35 relative to control). T1/2 after oral administration was 0.82 h and bioavailability was 47%.

**Conclusion:** DTP-006 kills tumor cells only in severe hypoxic conditions in vitro, reduces growth of tumor cell spheroids, and stabilizes radiation resistant tumor cells in vivo. It has clinically relevant bioavailability after oral administration. As such, DTP-006 is a promising new HAP with potentially favorable properties for clinical use. Further studies to determine the antitumor effects of DTP-006 as a monotherapy and in combination with RT in several preclinical tumor models are ongoing.

**OC-0237**

Adding Notch inhibition increases efficacy of standard of care treatment in glioblastoma

S. Yahyanejad1, H. King2, V. Iglesias1, P. Granton3, L. Barbeau1, S. Van Hoof1, A. Groot1, R. Habets1, J. Prickaerts1, A. Chalmers1, J. Theys1, S. Short1, F. Verhaegen1, M. Vooijs1

1University of Maastricht GROW Research Institute, Department of Radiation Oncology, Maastricht, The Netherlands

2Leeds Institute of Cancer and Pathology, Department of Radiation Biology and Therapy, Leeds, United Kingdom

3London Health Sciences Center, Department of Oncology, London- Ontario, Canada

**Purpose or Objective:** The novel combretastatin analogue, OXi4503, is a vascular disrupting agent (VDA) that has recently been shown to significantly enhance a stereotactic radiation treatment. This was achieved using an OXi4503 dose of 10 mg/kg combined with a stereotactic treatment of 3 x 15 Gy. The current study was undertaken to determine the OXi4503 dose dependency when using different stereotactic radiation dose schedules.

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**Conclusion:** OXi4503 is an effective agent for enhancing a stereotactic radiation treatment. But, the enhanced response appeared to be a simple additive effect independent of both the radiation dose applied with each fraction and the VDA dose used.

Supported by grants from the Danish Cancer Society and the Danish Council for Independent Research: Medical Sciences.
Akt1 facilitates DNA double-strand breaks repair through a prolonged survival. Strikingly, the longest tumour growth or RT irradiation resulted in a significant growth delay and (GSI+RT+TMZ), with 1 out of 4 mice showing tumour cure. The observed therapeutic model that adding a clinically approved Notch inhibitor to the guided micro-CT and radiotherapy platform treatments with Notch inhibitors markers SOX2 and CD133 was blocked by single or combined glioma spheroid growth. The expression of glioma stem cell markers. Luciferase-expressing U87 cells were placed at the center of the tumour.

Material and Methods: Treatment efficacy in vitro was tested in 2D cultures using proliferation and clonogenic survival assays. 3D sphere assays were used as a model for pharmacological treatment response with quantification of sphere number and delay in the different treatments arms. Flow cytometry was used to detect cells expressing stem cell markers. Luciferase-expressing U87 cells were intracranially injected into the brain of CD-1 mice. Tumor volume was quantified using contrast-enhanced microCT and bioluminescence imaging. Animals received TMZ (ip), RO4929097 in tumor control in combination with standard care of treatment (TMZ+RT) in an orthotopic glioma tumour model.

Results: GSI in combination with RT and TMZ attenuated tumour cell proliferation, clonogenic survival as well as glioma sphere growth. The expression of glioma stem cell markers SOX2 and CD133 was blocked by single or combined treatments with Notch inhibitors in vitro. Using our image guided micro CT and radiotherapy platform in vivo, a significant growth delay was observed in GSI-, RT- and TMZ-only treated groups compared to the control group. Standard of care treatment (RT + TMZ) or addition of GSI to either TMZ or RT irradiation resulted in a significant growth delay and prolonged survival. Strikingly, the longest tumour growth delay together with an increase in median survival was observed in mice treated with the triple combination (GSI+RT+TMZ), with 1 out of 4 mice showing tumour cure.

Conclusion: We show in an orthotopic glioblastoma mouse model that adding a clinically approved Notch inhibitor to the TMZ/RT standard of care results in a significant growth delay and increased overall survival. The observed therapeutic benefit is promising for clinical translation in order to increase survival in patients bearing glioblastoma with active Notch signaling.

OC-0238 Akt1 facilitates DNA double-strand breaks repair through a direct physical interaction with DNA-PKcs

M. Toulany1, J. Maier2, U. Rothbauer2, H.P. Rodemann1
1Division of Radiobiology & Molecular Environmental Research, Department of Radiation Oncology- University of Tuebingen, Tuebingen, Germany
2Natural and Medical Sciences Institute at the University of Tuebingen, Reutlingen, Germany

Purpose or Objective: It is well known that PI3K/Akt pathway is hyperactivated in K-RAS mutated tumor cells and is involved in radioresistance. Exposure to ionizing radiation induces activation of DNA-dependent protein kinase catalytic subunit (DNA-PKcs) as an essential enzyme for repair of DNA double-strand breaks (DSBs) through non-homologous end joining. Radiation-induced DNA-PKcs activity is partially dependent on serine/threonine kinase Akt1. In this study, role of DNA-PKcs in Akt1-mediated DSBR activity and post-irradiation cell survival was investigated. Likewise, a direct physical interaction of Akt1 with DNA-PKcs was studied.

Material and Methods: Non-small cell lung cancer cell line A549 and colorectal cancer cell line HCT116 with point mutations in K-RAS gene were utilized. Complex formation of Akt1 with DNA-PKcs and role of Akt1 in DSBs repair were tested by immunoprecipitation and γH2AX foci assays, respectively. Localization of Akt1 to DSB site was tested by immunofluorescence staining and confocal microscopy of P-Akt (S473) and γH2AX following microbeam laser irradiation and after exposure to ionizing radiation. To determine the potential interacting domain of Akt1 with DNA-PKcs; GST, GST-Akt1 full-length, GST-Akt1-N-terminal fragment (1-150 a.a.), and GST-Akt1-C-terminal (151-480 a.a.) proteins were incubated with purified DNA-PKcs and pull-down assay was performed. In order to identify the domain of DNA-PKcs that interacts with Akt1, constructs expressing four distinct fragments of DNA-PKcs (1-426, 427-1400, 2401-3850, 3700-4128 a.a.) tagged with EGFP and full length Akt1 tagged with mCherry were produced. Akt1/DNA-PKcs was studied in A549 cells, transiently transfected with the appropriate constructs.

Results: Akt1 formed a complex formation with DNA-PKcs in the nuclear fraction immediately after irradiation. Nuclear Akt1 was co-localized with γH2AX foci and found to be essential for the efficient repair of ionizing radiation-induced DSBs and post-irradiation cell survival, in a DNA-PKcs dependent manner. A direct physical interaction of DNA-PKcs to the C-terminal domain of Akt1 could be demonstrated. Additionally, Akt1 was found to make physical interaction not only with the C-terminal domain of DNA-PKcs (3700-4188 a.a.) but also with the N-terminal domain (1-426 a.a.).

Conclusion: Akt1, through a direct physical interaction with DNA-PKcs, regulates repair of ionizing radiation-induced DSBs. Thus, due to overexpression of Akt1 in tumor cells and constitutive Akt activity in K-RAS mutated tumor cells, Akt1 can be proposed as a tumor specific target for radiosensitization.

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Proffered Papers: Clinical 5: Upper and lower GI

OC-0239 Survival of clinical stage I-III rectal cancer patients: a population-based comparison

I. Joye1, G. Siersmens2, E. Van Eycken2, A. Debuquoy2, T. Vandendael2, F. Penningts2, K. Haustermans2
1KU Leuven/University Hospitals Leuven, Department of Radiation Oncology, Leuven, Belgium
2Belgian Cancer Registry, Statistics, Brussels, Belgium

Purpose or Objective: Total mesorectal excision is the cornerstone of rectal cancer treatment and preoperative (chemo)radiotherapy and adjuvant chemotherapy are often administered. This population-based study compares the survival in clinical stage I-III rectal cancer patients who received either preoperative radiotherapy, preoperative chemoradiotherapy or no preoperative therapy. The effect of type of radical resection and adjuvant chemotherapy on survival was also investigated.

Material and Methods: Patients diagnosed between January 2006 and December 2011 with clinical stage I-III rectal adenocarcinoma were retrieved from the national Cancer Registry database. Only first primary invasive rectal tumors were included and only patients who underwent a radical resection were retained. The observed survival was