RT response, RT treated tumors have increase in cell cycle regulatory genes such as CdK1a, across all clusters. In non-resident myeloid cells, compared to untreated tumor, RT is associated with a robust upregulation of interferon responses genes in brain tumor macrophages (log12 FC 1.64; log12 FC 2.02; Cxcl10 FC 2.29) and dendritic cells (log12 FC 1.67; fit1 FC 1.72; fit1 FC 2.06; Cxcl10 FC 1.50). We also find differential expression of immune checkpoints in RT-treated versus untreated tumor with decreased expression of Lag3, Tim3 (H2-A2), and CD47 and increased expressions of C4d7, Sipa and Gitr (Trnfsf18) post RT. In summary, RT stimulates a pro-inflammatory response and alters immune checkpoints in DMG, highlighting the potential for combining RT and immunotherapy in these tumors.

DIPG-46. RADIATION INDUCED SENESCENCE IN DIFFUSE INTRINSIC PONTINE GLIOMA CELLS REVEALS SELECTIVE VULNERABILITY TO BCL-XL INHIBITION.
Ashley Vardon1, Romain Guibo1, Diana Carvalho2, Jessica Boul2, Rebecca Carter1, Yura Grabovsk3, Alan Mackay4, Guangrong Zheng5, Daoshong Zhou1, Crispin Wiley1, Mark Lythgoe1, Chris Jones6, Darren Hargrave7, Juan-Pedro Kambhampati8,13.

1University College London, London, United Kingdom. 2University of Florida, Florida, USA.

Diffuse intrinsic pontine glioma remains a devastating condition with a dismal five year survival rate less than 5%. New approaches for treating this aggressive disease are critical to driving progress. Radiotherapy remains the cornerstone of treatment, with no chemotherapeutic agent found to improve survival. However, radiotherapy is often delivered as a palliative treatment, and disease often recurs 3-6 months after Radiation causes DNA damage and oxidative stress yielding a senescent state of replicative arrest in susceptible cells. However, increasing evidence demonstrates melanoma has also escape senescence following radiation. Targeted ablation of non-replicating senescent tumour cells following radiation could negate tumour recurrence. It remains unknown whether DIPG undergoes senescence following radiation, and furthermore, whether senectics can be used as a senescent DIPG. We employed radiation to induce a senescent state in primary human DIPG cell lines. Senescence was confirmed using SA-beta-gal staining, lack of EdU incorporation and qRT-PCR to characterise the SASP in three primary human DIPG cell lines. RNA-seq analysis of cells following radiation and SASP signatures. Likewise, expression of senescence markers has been detected in human tumours. Visible cells that survive radiation were then utilised to screen candidate senolytic drugs, only Bcl-XL inhibitors demonstrated reproducible senolytic activity in radiation treated DIPG cells. In addition, Bcl-Xl degradation using PROTACs (proteinolytic targeting chimeras) resulted in a significant increase in senolyis of susceptible tumour cells. Conversely, Bcl-2 inhibitors failed to show any consistent senolytic activity. We are currently performing preclinical studies in the mouse to test the efficiency of senolics against DIPG. These results demonstrate future possibilities of targeting radiation induced senescence in DIPG, using novel senolytic therapies and highlight Bcl-Xl dependency as a potential vulnerability of surviving DIPG cells following exposure to radiation.

DIPG-47. T5050CTDNA SEQUENCING REVEALS ONCOGENIC MUTATIONS AND COPY NUMBER VARIATIONS IN THE LIQUID BIOME OF CHILDREN WITH DIFFUSE MIDLINE GLIOMA.
Erin R. Bonner1,2, Robinson Harrington3, Augustine Eze3, Mariam Bornhorst4,5.

1Institute of cancer research, London, United Kingdom. 2Department of Neurology, Neurosurgery and Pediatrics, University of California San Francisco, San Francisco, CA, USA.

INTRODUCTION: Diffuse midline glioma (DMG) is a fatal childhood CNS tumor. Magnetic resonance imaging (MRI) is the gold standard for DMG diagnosis and monitoring of response to therapy. Leveraging novel MRI analytical approaches, including volumetric and machine learning based analyses, may aid in the prediction of patient overall survival (OS) and help to identify high-risk cases. METHODS: T1- and T2-weighted MR images were retrospectively collected from children and young adults diagnosed with DMG (n=43). MRI feature analysis was performed using 3D computer vision (2D and 3D). T2 volume (T2), T1 contrast-enhancing tumor volume, T1 relative to whole brain volume, tumor average T2 volume (T1/T2), tumor relative to whole brain volume, tumor average intensity, and tumor heterogeneity (i.e., intensity skewness and kurtosis), were evaluated at upfront diagnosis. MRI features were analyzed to identify significant features that correlate with OS or progression-free survival (PFS). RESULTS: On univariate analysis, T1/T2 ratio was identified as OS independent predictor of OS. On multivariate analysis, T1/T2 ratio (p=0.0011; hazard ratio 2.51; 95% CI 1.08-5.79) predicted significantly worse OS. However, feature selection identified T2 mean intensity (p=0.001), T1/T2 ratio (p=0.05), and T1 volume relative to whole brain volume (p=0.03) as significant predictors of OS outcome (short versus long). Combining T2 mean intensity, T2 image skew, T1 segment kurtosis and patient gender resulted in OS outcome prediction accuracy of 83.3% (sensitivity=85%, specificity=81.8%, n=42 cases). CONCLUSION: We have identified MRI volume and imaging features that significantly predict OS outcome in children diagnosed with DMG. Our findings provide a framework for incorporating MRI volumetric and machine learning analyses into the clinical setting, allowing for the customization of treatment based on tumor risk characteristics.

DIPG-48. MRI VOLUMETRIC AND MACHINE LEARNING BASED ANALYSES PREDICT SURVIVAL OUTCOME IN PEDIATRIC DIFFUSE MIDLINE GLIOMA.
Erin R. Bonner1,2, Xinyang Liu2, Carlos Tor-Diez2.

1Department of Neurology, Neurosurgery and Pediatrics, University of California San Francisco, San Francisco, CA, USA.

INTRODUCTION: Diffuse midline glioma (DMG) is a fatal childhood CNS tumor. Magnetic resonance imaging (MRI) is the gold standard for DMG diagnosis and monitoring of response to therapy. Leveraging novel MRI analytical approaches, including volumetric and machine learning based analyses, may aid in the prediction of patient overall survival (OS) and help to identify high-risk cases. METHODS: T1- and T2-weighted MR images were retrospectively collected from children and young adults diagnosed with DMG (n=43). MRI feature analysis was performed using 3D computer vision (2D and 3D). T2 volume (T2), T1 contrast-enhancing tumor volume, T1 relative to whole brain volume, tumor average intensity, and tumor heterogeneity (i.e., intensity skewness and kurtosis), were evaluated at upfront diagnosis. MRI features were analyzed to identify significant features that correlate with OS or progression-free survival (PFS). RESULTS: On univariate analysis, T1/T2 ratio was identified as OS independent predictor of OS. On multivariate analysis, T1/T2 ratio (p=0.0011; hazard ratio 2.51; 95% CI 1.08-5.79) predicted significantly worse OS. However, feature selection identified T2 mean intensity (p=0.001), T1/T2 ratio (p=0.05), and T1 volume relative to whole brain volume (p=0.03) as significant predictors of OS outcome (short versus long). Combining T2 mean intensity, T2 image skew, T1 segment kurtosis and patient gender resulted in OS outcome prediction accuracy of 83.3% (sensitivity=85%, specificity=81.8%, n=42 cases). CONCLUSION: We have identified MRI volume and imaging features that significantly predict OS outcome in children diagnosed with DMG. Our findings provide a framework for incorporating MRI volumetric and machine learning analyses into the clinical setting, allowing for the customization of treatment based on tumor risk characteristics.
INTRODUCTION: DMG-ACT (DMG- multi-arm Adaptive and Combinatorial Trial) will implement an innovative clinical trial design of combinatorial arms for patients with DMG at all disease stages, that is adapted to both clinical and correlative data generated in collaborating institutions. The goal of the team is to rapidly identify and validate i) promising drugs and drug combinations for clinical use, and ii) predictive biomarkers of promising drugs. METHODS: In vitro (n=30) and in vivo (n=8) models of DMG across fourteen institutions were used to assess single and combination treatment of over 80 drugs and drug combinations. Predictive biomarkers of response for top candidate drugs were identified using extensive molecular assays including proteomics, CRISPR, RNA-seq, Envoy ESEA, and IHC. Results were validated in Marizomib was highly toxic in murine PDX and zebrafish larvae assays. Murine pharmacokinetic analysis showed peak brain levels of ONC201 and ONC206 above pre-clinical IC50 concentrations. Molecular testing in a series of existing drug screen across 758 cancer cell lines validated the onchondrial stress and additional proteins, as the main targets induced by ONC201/6. CONCLUSION: Thorough preclinical testing in a multi-site laboratory setting identified promising therapeutics for DMGs, resulting in i) promising drugs and drug combinations for clinical use, and ii) preclinical IC50 concentrations. Molecular testing and a series of existing drug screen across 758 cancer cell lines validated the mitochondrial protease ClpP, leading to increased mitochondrial stress. Increased expression of activating transcription factor 4 (ATF4) indicates increased expression of activating transcription factor 4 (ATF4) and activates the mitochondrial protease ClpP, leading to increased mitochondrial stress. Increased expression of activating transcription factor 4 (ATF4) and activates the mitochondrial protease ClpP, leading to increased mitochon...