Natural Killer Cell Transfusion for Glioblastoma Tumor Volume Analysis via MR/PET Imaging Coregistration with Histology

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Abstract

Hypothesis: Immunotherapies hold great promise for the treatment of highly resistant cancers, such as glioblastoma (GBM). We hypothesized that high powered imaging modalities can be effectively combined to quantitatively assess the therapeutic efficacy of human derived natural killer (hNK) cells in an orthoptic xenografted mouse model of GBM.
Methods: Cells derived from recurrent human GBM were implanted intracranially into 10 mice. Mice in the treatment (n=5) and control (n=4) groups were given IV hNK cells and physiological saline, respectively. MRI and PET scans were performed 4 and 6 weeks after implantation. Ex vivo validation with histology was performed at week 6. Software analysis was conducted via Qimage (courtesy of Dr. Hutchins) and Indica Labs - HALO.
Results: Mean growth rates are as follows: T1 volume (μL) – 4.1 (control) vs 2.3 (hNK treated) ($p < 0.01$). T2 volume (μL) – 6.0 (control) vs 2.7 (hNK treated). PET volume (μL) – 3.1 (control) vs 2.1 (hNK treated), SUV – 5.4 (control) vs 3.0 (hNK treated).
Conclusion: Tumor volume and SUV were reduced in hNK treated mice compared to control, with a correlated lower histology TBR, suggesting MR/PET imaging is effective for in vivo assessment of therapeutic efficacy in the mouse model. Phase II of this model will include genetically engineered NK cells with greater tumor localization ability and killing capacity. Clinical trials of NK cell immunotherapy with MR/PET imaging may one day offer remission to patients suffering from an, as yet, incurable cancer.

Introduction

Glioblastoma Multiforme (GBM) is a deadly astrocytoma brain cancer with a 5-year survival rate of under 10%, comprising 17% of all brain tumors diagnosed. Even with advancements in our understanding of its molecular composition, GBM remains largely resistant to traditional treatment options, in part, because it can mutate to create an immunosuppressive extra-cellular microenvironment. Preclinical models of disease are of particular importance for establishing novel clinical treatments. Immunotherapy is a novel class of systemic cancer therapies that holds great promise in the treatment of highly resistant brain tumors such as GBM. Immunotherapy is based on activating the body's immune system to target cancer. Among immune cells, natural killer (NK) cells, in particular, play a critical role in the early host defense against cancer, which can be harnessed to become more effective cancer killing agents. In vivo neuroimaging has become an essential tool to assess such novel cancer therapies in real time, but its adaptation in the mouse model is uncommon. Combination imaging techniques provide both the high anatomical detail of MRI with the powerful quantitative detection sensitivity of PET. Additionally, PET radiotracers, such as 18F-FET, Cu-PTSM, and Cu-ETS, have been developed to assess a wide range of tumor characteristics using standard uptake value (SUV), a quantitation of initial injected dose, animal weight and signal intensity.

Methods

TT was validated via ProSense 680 Fluorescence (PF) cathepsin detection. Approximately 5 x 106 hNK cells (n=5) or saline control (n=4) were weekly injected IV into an intracranial mouse model of GBM (GB10) tumors. Therapy mice received supplemental IL-2 IP three times weekly for NK cell stimulation. MRI and PET scans were performed four and six weeks after implantation. At time of death brains were surgically removed, stored in sucrose, and histological slices were prepared at the pathology lab. Multimodal imaging of MR T1CE, T2, and 18F-FET PET was co-registered and analyzed to track tumor growth. Analysis of tumor volume and SUV was conducted using Qimage and TBR using Indica Labs – HALO software. MR/PET volumes of interest (VOI)s were generated using histogram parameters with our algorithm for rejecting background brain intensity. Histology regions of interest were hand drawn within HALO software, cell type was analyzed and TBR area was computed via proprietary algorithm. CD56 histological staining was conducted to detect NK cell localization, quantity and characteristics of immune response. Cu-ETS and Cu-PTSM PET radiotracers were administered IV, in a subset of mice, to assess metabolic activity and blood brain barrier (BBB) intactness.

Conclusion

Multimodality MR/PET imaging analysis demonstrated significantly lower T1 contrast-enhanced MRI tumor volume in the hNK treated group compared with the saline (control) treated group. hNK treatment showed a trend of reduction in tumor growth rate, SUV and variability following analysis of MRI T2 sequence and PET 18F-FET images with histological correlation of lower TBR. Histological staining showed CD56dim NK cells localized in the tumor, preferentially, and at higher concentrations in the treatment group. PET analysis suggests that brain perfusion and BBB intactness can be effectively assessed via Cu-PTSM and Cu-ETS, respectively.

Discussion

Despite positive trends, only T1CE volume data expressed significance, likely due to the inherent variability in each analysis modality and low sample size. T2 is weighted for fluid, meaning it may pick up edema surrounding tumor upon analysis, accounting for increased variability. A T2 FLAIR sequence may be considered to suppress fluid signal from CSF. PET can also be susceptible to variability due to its lower spatial resolution. This can make it difficult to accurately assess exact perimeter of tumors. Co-registration using MRI in combination with PET imaging is critical to overcoming the inherent limitations within each modality. The CD56 staining in hNK treated mice suggests that the NK cells injected were not of the CD56bright variety, theorized as a precursor to mature CD56dim/CD16+ NK cells. Moving forward, CD16 staining would be helpful in further identifying the cell types within these tumors. Decreased tumor variability in the treatment group could be explained by the hypothesis that GBM growth was made more uniform by NK cell presence. Perhaps through NK cell interruption of the extracellular matrix surrounding the tumor, GBM was unable to provide itself nutrients for proliferation. The lower sample size in this study will be supplemented with future trials to establish multimodal statistical significance in overall growth rate reduction. This and future studies would also benefit from longer NK treatment times.

Future Impact

Phase II of this animal model development and cellular therapy pipeline creation coupled with multi-modality imaging was recently funded through the Walther Oncology Embedding Program to build on the current work. Phase II will include genetically engineered NK cells with greater tumor localization ability and tumor killing capacity by Dr. Sandro Matosevic and his team at Purdue University, Lafayette. If approved for clinical trials, NK cell immunotherapy with multimodal MR/PET imaging could one day offer remission to patients suffering from an, as yet, incurable cancer.

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Acknowledgements

I would like to thank faculty advisors, Dr. Michael Veronesi and Dr. Gary Hutchins, of the Department of Radiology as well as lab teammates, Dr. Yeonhee Yun and Shelby for their expertise on the project. This project was funded, in part, with the support of Indiana Clinical and Translational Sciences Institute through grant number UL1TR002529 from the National Institutes of Health as well as the Walther Oncology Embedding Grant Program and the PI's on the grant (Dr. Matosevic, Dr. Renbarger, Dr. Veronesi and Dr. Pollok). The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.