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IMMUNE RESPONSES AND LONG-TERM SURVIVAL WITH mRNA VACCINE TARGETING DIFFUSE MIDLINE GLIOMA

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Background Diffuse midline glioma (DMG) is a universal fatal glial brain cancer in children. We tested our novel multilamellar mRNA lipid particle aggregate vaccine (RNA-LPA, IND19304—Sayour),¹ a tumor-agnostic treatment platform that encapsulates tumor specific RNA and delivers the payload in a highly immunogenic fashion, as an approach to treating this currently incurable cancer.

Methods Using the K2 DMG model,² we implant H3K27M-expressing DMG cells into the 4th ventricle of P1-P3 neonatal C57BL/6 mice. RNA-LPA generated from predicated human H3K27M epitopes or total-tumor mRNA are administered intravenously beginning at day 35. We performed multiparameter 3D geospatial fluorescent microscopy to characterize mRNA transduction. Immunologic responses to treatment were evaluated by multiparameter flow cytometry, microscopy, and cytokine profiling.

Results Mice developed clinical neurological signs of disease by day 30–35. RNA-LPAs targeting human H3K27M epitopes were found to be immunogenic in wild-type mice. Intriguingly, nonspecific enhanced green fluorescent protein (eGFP)-RNA-LPAs resulted in statistically significant survival benefits compared to mice treated with empty LPs. However, tumor-specific RNA-LPAs (either H3K27M-specific or total tumor mRNA-derived) also enhanced survival and additionally resulted in a subset of mice with long-term survival. This survival benefit was observed despite the development of clinical hydrocephalus in mice treated with RNA-LPAs. 3D microscopy established that tumors demonstrated invasive disease and microvascular erosion in mice. We found that mRNA transduces fibroblastic reticular cells (FRCs) in the spleen and lymph nodes, prompting widespread immune activation. Treatment with RNA-LPA led to massive increases in production inflammatory cytokines (i.e. TNF- α) and chemokines (i.e. CCL2), which led to recruitment of the majority of circulating monocytes and lymphocytes to secondary lymphoid organs.

Conclusions RNA-LPAs extend survival in our highly aggressive DMG model, including curative outcomes in cohorts treated with either total tumor or H3K27M RNA-LPs. These data suggest that RNA-LPs are capable of stimulating host adaptive immune responses against established DIPG tumors. Signs of hydrocephalus in treated mice may indicate pseudo-progression due to immunologic response, yet mice were frequently able to survive this development. Future studies will further characterize the immunologic response in these mice and support expansion of our existing IND for a multi-institutional phase I clinical trial for children with DMG, who currently have no curative options.

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REFERENCES

1. Mendez-Gomez H, DeVries A, Castillo P, Stover B, Qdaisat S, Von Roemling C, Ogando-Rivas E, Weidert F, McGuinness J, Zhang D, Chung MC, Li D, Zhao C, Marconi C, Campaneria Y, Chardon-Robles J, Grippin A, Karachi A, Thomas N, Huang J, Milner R, Sahay B, Sawyer WG, Ligon JA, Silver N, Simon E, Cleaver B, Wynne K, Hodik M, Molinaro A, Guan J, Kellish P, Doty A, Lee J-H, Carrera-Justiz S, Rahman M, Gatica S, Mueller S, Prados M, Ghiaseddin A, Mitchell DA, Sayour EJ. mRNA aggregates harness danger response for potent cancer immunotherapy. *medRxiv*. 2023:2023.03.12.23287108. doi: 10.1101/2023.03.12.23287108.
2. Misuraca KL, Cordero FJ, Becher OJ. Pre-Clinical Models of Diffuse Intrinsic Pontine Glioma. *Front Oncol*. 2015;5:172. doi: 10.3389/fonc.2015.00172. PubMed PMID: 26258075; PMCID: PMC4513210.

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