SECTION: II

A. Executive Summary

Diffuse Intrinsic Pontine Glioma (**DIPG**) is a rare high-grade glial tumor that occurs in young children and is nearly uniformly fatal. Several factors have contributed to the high fatality in children with DIPG. It occurs in, a region, making surgery potential dangerous. Complete surgical resection of tumor is impossible because of the highly invasive nature and ill-defined boundaries of the tumor. Radiation has been used in DIPG treatment, but it is not curative and merely extends life by a very short time. Delivery of chemotherapeutic agents to the tumor has been hindered by the existence of the blood brain barrier. Doses of drugs that result in significant systemic toxicity have to be administered to obtain miniscule reduction in tumor growth. Thus, there is a dire need for novel therapeutic approaches to improve survival in children with DIPG.

In the current application, we propose to evaluate the feasibility of using **ex-vivo expanded natural killer (NK) cells to target DIPG tumor cells in pre-clinical studies.** NK cells are a subset of lymphocytes characterized by CD56 positivity and CD3 negativity (CD56+, CD3-). Their use for cancer therapy is particularly appealing because these immune cells unlike T- and B-cells can lyse tumors without prior immune sensitization. NK cell-mediated cytotoxicity is highly dependent on their ability to distinguish "self" (normal cells) from non-self (tumor cells). Our vitro data show that (a) DIPG cells are sensitive to NK-mediated cytolysis and (b) histone deacetylase inhibitors (HDACIs) upregulate genes such as NKP30 and NKG2D, which are critical for NK-tumor recognition of tumor cells. Here, we will build upon these findings to assess if loco-regional administration of NK cells [using a guide screw or convection enhanced delivery (CED)] in mouse orthotopic models of DIPG will promote tumor shrinkage. We will also ask if HDACI-mediated increase in tumor cell recognition by NK cells will augment DIPG cell cytolysis.

The **specific aims** of our study are:

Aim I. To study the cytolytic activity of NK cells delivered loco-regionally in mouse orthotopic models of DIPG. We will first evaluate the cytolytic activity of ex vivo expanded NK cells from five other anonymous donors using fluorescence-based cytolysis assays. NK-sensitive, firefly luciferase marked DIPG cells will be implanted in the brain stem of mice and treated with graded doses of NK cells labeled with a fluorescent lipophilic dye (DIR) and delivered by a guide screw or convection enhanced delivery (CED). Tumor response will be measured by bioluminescence imaging. NK persistence will be assessed by fluorescence imaging.

Aim II. NK cells will be combined with an HDACI, MS-275, which we previously showed to upregulate tumor recognition molecules on NK cells to evaluate its effect on NK cell cytolytic activity in vitro. In vivo, we will combine the two agents at doses where each alone promotes 20% killing. The involvement of tumor recognition molecules in augmentation of NK function will be shown by using specific blocking antibodies.

Feasibility and Innovation: Feasibility is shown by: (a) the proven expertise of our multi-institutional and multi-disciplinary team in basic and translational neuro-oncology (b) availability of platform technologies that we have developed, using artificial antigen presenting cells (aAPC) to expand clinical-grade NK cells (b) our unique brainstem DIPG mouse model and cell lines generated from autopsy material and finally (c) our expertise in delivering chemotherapy to the brain stem using guide screw and CED.

Clinical Relevance and Significance: The proposed project is based on our unique access to and ability to use clinically-adaptable agents: (i) aAPC, (ii) NK cells and (iii) narrow-spectrum HDACi, Our group has ongoing clinical trials infusing NK cells to treat children with non-neural tumors. Based on our recent pre-clinical research on medulloblastoma (MB) and atypical teratoid rhabdoid tumor (ATRT) (section III-C and data not shown), we are in the process of seeking regulatory approval to open the first in pediatrics immunotherapy trial for children with fourth ventricular tumors through loco-regional administration of ex vivo expanded NK cells. The successful completion of this study will (a) provide pre-clinical evidence for the therapeutic application of NK cells in children with DIPG and (b) provide a rationale for novel synergistic combinations of NK cells and other therapeutic agents. The proposed work has the potential to create a paradigm shift in DIPG treatment.