therapy drugs in order to avoid excessive damage to organs and avoid the onset of late effects. Cannabidiol (CBD) has been shown to have cytotoxic properties on paediatric brain tumour cell lines. Although CBD is far less toxic and damaging than the classical chemotherapy options which are currently available to children suffering with brain tumours, there are some possible side effects. Given that the half-life of the drug is 24 hours, it was important to establish the nature of the effect of cumulative dosing on top of the remaining drug in the system. The pHGG cell line, SF188 was cultured in different concentrations of CBD with either 1, 2 or 3 doses being given on consecutive days. 24 hours after the last dose the cells were analysed using the resazurin assay. It was observed that the amount of drug required for an EC_{50} to be obtained decreased; 17.6μM (1 dose), 8μM (2 doses), 5μM (3 doses) and that cell survival was reduced to nearly 0% in those cells which received multiple does of CBD at 17.6μM. In order to mimic the intermittent dosing regime, the cells were returned to the incubator for 4 days before the resazurin assay was repeated. The decrease in viability was maintained over the extended culture period meaning that the ability of even the apparent "healthy" cells to proliferate had been permanently affected.

THER-04. IS THERE A ROLE FOR CANNABIDIOL IN THE TREATMENT OF CHILDHOOD BRAIN TUMOURS?

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Brain tumours are the leading cause of cancer related death in children with limited treatment options and high recurrence rates. Recent evidence suggests there may be anti-tumoral properties of cannabinoids, and of cannabidiol (CBD) in particular. We evaluated the effect of CBD on paediatric brain tumour cell lines in 2D and 3D spheroids; pHGG (SF188), ependymoma (BxD-1425EPN) and human astrocytes. At the CBD EC_{50} concentration, astrocytic cell death was insignificant. 3D spheroids decreased in size by approximately 20% when cultured in CBD compared to cells only after 5-day exposure. Cell death increased with time after a single dose of CBD. Western Blot showed an increase in Lc3b expression (autophagy) after 24 hours incubation (early cell death) with CBD in both BxD-1425EPN and SF188 with PARP expression (apoptosis) increased after 5 days incubation (late cell death). Cell cycle analysis showed a decrease of cells in G_1 and no change in G_2 indicating cell cycle arrest. In hypoxia, SF188 and BxD-1425EPN cells showed decreased cell death after 24 hours and 5 days when compared to normoxia and an EC_{50} within acceptable limits could not be achieved. SF188 cells pre-treated with receptor antagonists indicate that CBD was not acting through CB_1, CB_2, GPR18, PPARα or PPARγ receptors but may act as a partial agonist of the TRPV1 and 5-HT_{1A} receptors and a full agonist of the GPR55 receptor (resazurin assay). This provides evidence that CBD is effective at killing paediatric brain tumour cells and does not have a significant effect on normal astrocytes.

THER-05. GENETICALLY STABLE POLIOVIRUS VECTOR CARRYING H3.3K27M ANTIGEN FOR TREATMENT OF DIFFUSE MIDLINE GLIOMA BY INTRAMUSCULAR INJECTION

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BACKGROUND: H3 K27M-mutant diffuse midline glioma (DMG) is invariably lethal. Viruses naturally engage innate immunity, induce antigen presentation, and mediate CD8 T cell priming against foreign antigens. Polioviruses, in particular, are uniquely tropic for dendritic cells (DC) and potently activate DC, inducing Th1-dominant cytokine profiles, CD8 T cell immunity, and enhanced epitope presentation. Thus, poliovirus is ideally suited for vectored delivery of signature tumor neoantigens, e.g. the H3 K27M feature of DMG. However, poliovirus vector design is inherently limited by genetic instability and the underlying neuropathogenicity of poliovirus. METHODS: We created a genetically stable, polio:rhinovirus chimera vector devoid of neuropathogenicity and modified for stable expression of the HLA-A2 restricted H3.3 K27M antigen (RIPO (H3.3)). RESULTS: RIPO(H3.3) infects, activates, and induces H3.3K27M antigen presentation in DCs in vitro. Given intramuscularly in vivo, RIPO(H3.3) recruits and activates DCs with Th1-dominant cytokine profiles, efficiently primes H3.3K27M-specific CD8 T cells, induces antigen-specific CD8 T cell migration to the tumor site, delays tumor growth, and enhances survival in murine tumor models. CONCLUSION: This novel approach leverages the unique ability of polioviruses to activate DCs while simultaneously introducing the H3.3 K27M antigen. In this way, DCs are activated optimally in situ, while being simultaneously infected to express/present tumor antigen. RIPO(H3.3), given by intramuscular injection, will be evaluated in a clinical trial for children with H3 K27M-mutant diffuse midline glioma.

THER-06. THERAPEUTIC EFFICACY OF RRV-MEDIATED PRODRUG ACTIVATOR GENE THERAPY IN CLINICAL TRIALS OF RECURRENT HIGH-GRADE GLIOMA AND IN MURINE ORTHOTOPIC MODELS OF INTRACEREBRAL GLIOMA AND INTRACEREBELLAR MEDULLOBLASTOMA

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Toca 511, a clinical-stage tumor-selective retroviral replicating vector (RRV), encodes optimized yeast cytosine deaminase (CD), which converts the prodrug 5-fluorocytosine (5-FC) to the active drug 5-fluorouracil (5-FU) within infected cancer cells. In preclinical models of intracerebral glioblastoma, 5-FU generated locally by Toca 511 (RRV-CD) prodrug activator gene therapy has also been shown to kill immunosuppressive myeloid cells in the tumor microenvironment, leading to anti-cancer immune activation and long-term survival. Early-phase clinical trials of Toca 511 in recurrent high-grade glioma showed highly promising evidence of therapeutic benefit, leading to a Phase III trial completed in late 2019 (n=400 patients, randomized 1:1 vs. standard chemotherapy), which appeared to show negative results overall. However, additional analysis showed possible efficacy in prespecified subgroups, and further clinical investigation is being pursued. In preclinical studies, we have also evaluated RRV for use in medulloblastoma, the most common malignant tumor of the pediatric nervous system. Both established and primary human medulloblastoma cell lines supported efficient RRV replication in vitro, with spread to >90% of cells by day 10 post-inoculation, and RRV-CD-transduced medulloblastoma cells showed significant dose-dependent reduction of viability upon exposure to 5-FC, compared to controls. In an intracerebellar HDMB03 medulloblastoma model, RRV-CD-treated mice exhibited long-term survival while on sequential cycles of 5-FC prodrug, until prodrug treatment was stopped, after which 25% long-term survival was observed (median survival 110 days) as compared to controls (median survival 28 days, 100% lethality) (p=0.00007). These results support further evaluation of RRV-mediated prodrug activator gene therapy for pediatric brain tumors.

THER-07. INHIBITION OF THE RAS SIGNALING ENHANCES VIRAL ONCOLYSIS IN MALIGNANT GLIOMAS

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Pediatric malignant glioma indicates rapid proliferation, widely infiltrative properties and resistance to various therapies, and carries a very poor prognosis. There are methods of using virus within novel therapies under development against malignant neoplasms, which have been studied for many years already. We examined the treatment with sunitinib or GW5074 to our experimental model of vaccinia virus therapy for malignant glioma, and then evaluated changes in the tumoricidal activity, the viral infectivity, and the impact on the Ras signaling pathway. Glioma cells (U251MG, LN229, LN18, rat C6) infected with vaccinia virus was fatal, in its course of death, apoptosis and autophagy were induced. The activity of Ras signaling in vaccinia-infected cells heightened in the early stage and declined in the late stage Inhibition of the Ras signaling pathway at the early stage of viral infection prevented vaccinia virus replication, while viral oncolysis was not inhibited when the pathway was blocked after sufficient viral spread. Glioma cells infected with vaccinia virus are led to cell death. Vaccinia virus regulates Ras or other survival signaling pathways in the infected cells. It enhances the signaling in the early stage (viral replicative period), however