EXECUTIVE SUMMARY

TARGETING TOP3A-AMPLIFIED DIPG CELLS BY SIRTUIN INHIBITION

We identified TOP3A as a novel target in H3.3K27M-mutant DIPG, with treatment with sirtuin inhibitors as an innovative therapeutic option in this subgroup of tumours. We will explore the underlying mechanisms of this using gene editing, and will screen a range of possible drugs in patient-derived DIPG models in vitroand in vivoin order to provide biological rationale for translating these observations to the clinic.

Scientic Merit

TOP3Aencodes DNA topoisomerase III alpha, which has been shown to play an important role in homologous recombination and the maintenance of the alternative lengthening of telomeres (ALT) phenotype. In DIPG, TOP3Aamplication is found to be mutually exclusive with ATRXmutation, suggesting a novel, potentially targetable mechanism by H3.3K27M tumours preserve an ALT phenotype. TOP3A forms a complex with BLM, which in common with another RecQ helicase WRN localizes with ALTassociated PML bodies and associates with telomeric proteins TRF1 and TRF2 in ALT cells. Knockdownof TOP3Areduces TRF2 levels, loss of G-strand overhangs and a reduction of ALT cell viability. In a drug screen of patient-derived DIPG cells, the SIRT1 inhibitor sirtinol was found to be an outlier hit in the single TOP3A-amplied cell line HSJD-DIPG-013. Sirtuins belongs to a family of NAD -dependent lysine deacetylases, with SIRT1 shown to regulate several key cellular processes, among them chromatin remodeling, transcriptional silencing, and genomic stability. SIRT1 deacetylates both WRN and BLM and may have acetylation-independent functions on subcellular localisation and appearance in nuclear PML bodies. The current proposal aims to explore the function of TOP3A in DIPG cells, and will provide insight into novel DNA repair and telomere maintenance mechanisms in these tumours. Moreover, we will explore the role of sirtuin inhibitors as a unique therapeutic vulnerability in this subset of DIPGs, which will be tested in patient-derived models in vitroand in vivo.

Feasibility

All gene editing, cell biology, and preclinical techniques are well established within the Jones lab and the ICR's Biological Services Unit and Centre for Cancer Imaging. The proposal supports an experienced scientist with the relevant expertise required for the success of this project. We are building on previously funded infrastructure in respect of drug (INSTINCT) and CRISPR (Brain Tumour Charity / Billie Buttery Fund) screening, providing added value to the current proposal. This initial grant is aimed at generating an achievable amount of data which would then be much more widely validated across multiple labs in a future application.

Expertise

The Jones lab is an international leader in the genomic characterisation of pGBM / DIPG samples, and has published extensively on the molecular proling of these tumours as well as detailed functional assessment of their dening mutations – our group discovered the presence of TOP3Aamplication in DIPG. We form part of the INSTINCT network with Great Ormond Street Hospital and Newcastle University, and the CRUK Children's Brain Tumour Centre of Excellence (with the University of Cambridge), particularly focused on drug development for high risk paediatric brain tumours. Chris Jones is biology lead on the HERBY and BIOMEDE clinical trials, and former Chair of the Biology Subcommittee of the SIOPE HGG / DIPG Working Group, allowing rapid dissemination of results and clinical translation.