

**2016-2017 LETTER INTENT**

**Using CRISPR Induced Deletions on Chromosome 1p and 19q of human tissue models to Determine the Effectiveness of Temozolomide Chemotherapy Treatment on Grade II Oligodendroglioma**

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## **Background**

Oligodendrocytes are the cells that myelinate the axons of neurons in the central nervous system. It is important for them to proliferate and migrate in a timely manner; therefore, they are considered vulnerable cells (1). Oligodendrogliomas are tumors that begin from mutated oligodendrocytes. These are one of the types of cells that make up the glial tissue of the brain. Oligodendrogliomas are soft, grey/pink tumors that are often calcified and contain small areas of blood and/or cysts (2,4). They can be either low- grade (Grade II) or high- grade (Grade III, or anaplastic). According to Brain Tumor Foundation of Canada, about 2.5% of primary brain tumors are oligodendrogliomas; which is about 5-6% when compared to all gliomas. This type of glioma is also more common in men than women and tends to occur within the age group of 20-40; however, it can occur in children as well (3). The most common symptoms of oligodendrogliomas are seizures, headaches, personality changes, weakness and/or paralysis (2-4). Other symptoms may also present themselves based on the grade and location of the tumor. An MRI is necessary to determine the location of the tumor as it may affect brain function in vital areas. Standard treatment for these types of tumors is surgical removal of as much of the tumor as possible. In low grade gliomas, this is followed by regular MRI scans. If any residual tumor remains after surgery, chemotherapy or radiation treatment is recommended. High grade gliomas are treated with both radiation therapy and chemotherapy following surgery (2-4). There is no standard prognosis time for this type of tumor as it is heavily dependent on the complexity, size, location, and grade of the tumor. The patient's age and whether their tumor has 1p or 19q deletions play as factors as well, in prognosis time (2).

Oligodendrogliomas are strongly associated with 1p and/or 19q chromosomal deletion on chromosomes 1 and 19. In recent studies, it has been found that the co-deletion is mediated by unbalanced translocations of the chromosomes (5). Individuals with combined losses have also shown better chemotherapeutic response and overall patient survival (6). Some patients do not have any deletions of 1p or 19q at all. A study was performed in 2003 to determine the ratio of patients with a deletion in either 1p or 19q, co-deletion or no deletions. It was found that out of 31 patients: 1 patient had only a 1p deletion, 4 patients had only a 19q deletion, 13 patients were detected with co-deletions, and 13 patients had no deletions at all. Patients not accounted for had deletions in other areas (6).

Individuals born with a 1p chromosome deletion tend to have developmental delay, intellectual disability, behavioral problems, and distinctive facial features (7). Therefore, it is most likely necessary for brain development. The incidence of oligodendrogliomas has significantly increased over the past few years (3) and, therefore, sparks an area of interest for research.

## **Research Problem**

Natural co-deletions of 1p/19q are proven to increase the efficacy of chemotherapy in patients with grade (II) oligodendrogliomas increasing life expectancy. Therefore can artificial co-deletion of 1p/19q have the same efficacy with patients that do not possess these deletions naturally and improve the performance of Temozolomide in oligodendroglioma patients?

## **Hypothesis**

The induced deletion of the 1p/ 19q in patients with grade (II) oligodendroglioma is related to the prognosis of the tumor and overall efficacy of chemotherapy treatments similar to patients with natural deletions in the 1p/19q gene (8). This signifies that people with the aforementioned gene deletion will respond better to the overall chemotherapy treatment and have increased survival rates 5 years after treatment.

The induced deletion of the 1p/ 19q in patients with grade (II) oligodendroglioma is not related to the prognosis of the tumor and overall efficacy of chemotherapy treatments similar to patients with natural deletions in the 1p/19q gene. This signifies that people with the aforementioned gene deletion will not respond better to the overall chemotherapy treatment and have increased survival rates 5 years after treatment.

## **Specific Aims**

The 1p/19q chromosomal arm segments will be deleted in human tissue models with gliomas using CRISPR technology to determine the effect of the deletion on human oligodendrogliomas (8). The human tissue models derived from brain cells affected by oligodendroglioma will be used to provide a working model that will give insight into the effects of the specific deletion of 1p/19q in patients with low-grade oligodendroglioma. The CRISPR technology and associated Cas9 system will be used to conduct the gene deletion in the isolated brain tissue (9).

The deletion of 1p/19q in patients with oligodendroglioma will improve the prognosis of the tumor and increase their longevity (8). The study seeks to explore this relation between deletion and the longevity of affected patients. The study of the development of the tumour in human tissue will provide insight into the progression of the tumour in patients with this deletion (10). The tumour progression in human tissue is expected to be slowed compared to that in tissue without this deletion (17).

The 1p/19q deletions will allow oligodendroglioma tumors to be more susceptible to chemotherapy treatment (8). The deletion of 1p/19q in subjects indicates a better response to the treatment. The response rate of the tumour is expected to be higher in the malignant human tissue with the 1p/19q deletion than in the malignant human tissue without the deletion (11). The high chemosensitivity of the tissue with 1p/19q deletions will result in much faster tumour size reduction when treated. The treatment of tissue with 1p/19q deletions using chemotherapy will provide insight into the response of subjects with 1p/19q deletions and the possible effects of using bioengineering to delete the 1p/19q genes.

## **Experimental Procedures**

In order for the experiment to take place, a tissue sample with patients diagnosed with (grade II) oligodendroglioma must be obtained by surgical resection, with the patient's consent (12). Tissue samples must be kept in the right environment to sustain their normal activity (15). Typically the time it takes for the tissue sample to render useless is one week under peak conditions; therefore this experiment has a time constraint (16). Once the tissue samples are obtained, the presence of 1p/19q on certain chromosomes can be identified using metaphase fluorescent in situ hybridization (FISH) (13). The presence or absence of the 1p/19q genetic material can be found using microsatellite markers (13). Patients' tissue samples will then be grouped based on the status of their 1p/19q genetic material since the presence of 1p/19q is not the same for all patients (14). The tissue samples will be grouped according to the presence of a single deletion of either 1p or 19q, co-deletion, and no deletion. The tissue samples with naturally occurring co-deletions within the chromosome will be the positive control group. Half of the "no deletions" group (ND1) will be associated with the negative control. The experimental groups will be the groups with a "single" deletion and the other half of the "no deletion" (ND2) in the 1p/19q genetic material. However, these groups will remain separate at all times.

CRISPR/Cas9 methods will then be used to delete either gene that is present in the "single gene" deletion group or both genes in the ND2 group. The CRISPR/Cas9 system will perform RNA-guided site-specific DNA cleavage to create the 1p/19q deletion (17). Once 1p/19q is deleted, it must be quickly treated with chemotherapy. For this experiment Temozolomide (TMZ) will be used rather than PCV (procarbazine, CCNU, and vincristine) as it is less toxic in comparison. The positive control group will also be treated with TMZ. The data that will be collected will be based on the size of the tumor tissue before and after the treatment for both experimental groups and the positive control group. Data will also be collected on the sizes of the tumors in the negative control group. The samples will then be analyzed by comparing the sizes of the tumors between the experimental, positive and negative groups.

## **Therapeutic Relevance**

Grade II oligodendrogliomas are a great cause for concern, as they are difficult to completely remove through therapy or surgery (18). Studies have shown that patients diagnosed with this type of tumor tend to naturally translocate 1p/19q genes and have a greater response to treatment with a higher rate of patient survival (19). Therefore, through the use of CRISPR these two chromosomal segments will be deleted with the expectation of the tumor cells becoming more susceptible to chemotherapy. If successful, this method will be applied to mice with the hopes of eventual patient trials. It is anticipated that this will result in an improvement in chemotherapy efficiency as well as an increase in patient survival rate.

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