Interrogating Clonal Repopulation Dynamics in Glioblastoma

The aim of this project will be to investigate clonal repopulation dynamics to determine if clonal diversity is a predictor of early disease progression through the emergence of resistant clones driven by specific genes.

The project will test the hypothesis that inherent genetic diversity results in variegated clonal architecture through a process of clonal selection that is modulated by environmental selection pressures brought about by clinical therapy, leading to the emergence of treatment resistant clones that drive disease progression.

Background

Little is known about the impact of intra-tumour heterogeneity on tumour evolution, re-population dynamics and the consequences of such heterogeneity on the emergence of treatment-resistant disease. This is an important question in brain tumour research because it is the emergence of treatment-resistant disease that kills patients with glial cancers and it is variability in this biological process that is responsible for clinical phenotypic heterogeneity in tumour responses and clinical outcomes[1].

Therapeutic intervention in cancer imposes novel environmental selection pressures allowing the evolution of variegated clonal populations united in their ability to resist the intervention[2, 3]. Consistent with this in glioma clinical phenotypic diversity persists between different patients with the same histopathological grade. Some GB patients progress during (or immediately upon cessation of) treatment (unstable disease) while others enter a variable period of disease stability before further progression[4]. It is not possible prospectively to distinguish this cohort of GB patients although promotor methylation of the DNA repair enzyme MGMT is predictive of response to Temozolomide (TMZ)[5] allowing some limited patient stratification.

Recent technological advances have allowed interrogation of cancer genomes at high resolution [6]. This has revealed that tumours of the same histological subtype share only a minority of genetic aberrations and this might explain differences in response to therapies among patients with the same tumour. The basis of clinical phenotypic diversity is the genetic heterogeneity between patients that is further exacerbated by intra-tumour genetic heterogeneity, clonal diversity and epigenetic instability at both spatial and temporal levels [3, 7-11].

Figure 1: We have developed a real-time multiple sampling scheme to interrogate high-grade glioma during surgery. FGMS (Fluorescence-Guided Multiple Sampling) is based on fluorescence-guided resection technology to obtain spatially distinct tumour biopsies from individual tumours in real time[12].

Objective 1: to test the hypothesis that clonal diversity at initial presentation is a predictor of poor outcome

Cellular and clonal diversity are fundamental to neoplastic progression and establish linkage between molecular mechanisms that maintain genome integrity and evolution of neoplastic cell lineages. Genetic
instability produces cellular diversity on which natural selection can act to generate expanded clones able to drive tumour evolution. Consistent with this hypothesis clonal diversity predicts tumour progression in oesophageal adenocarcinoma[13] and head & neck cancer[14]. We, and others, have identified high levels of diversity and spatial heterogeneity in GBM clones[12, 15, 16] but the impact on disease progression is unknown.

To investigate this we will use our multiple, spatially segregated samples from primary GB patients with distinct clinical phenotypes (unstable vs stable disease). We will use exome sequencing to identify CNAs, which will be used to quantify and measure diversity. We will identify mutations and use these data to describe genetic divergence with respect to key mutations including EGFR, PDGFRα, TP53, CDKN2A/B, PTEN and PI3K [17].

**Key deliverable:**
- We will determine if clonal diversity is a predictor of early disease progression in glioblastoma

**Objective 2: to test the hypothesis that glioblastoma treatment introduces environmental selection pressures favouring the emergence of resistant clones that drive recurrent disease**

Treatment with the alkylating agent TMZ in GB patients with MGMT promotor methylation is associated with inactivating mutations of mismatch repair genes[17-19]. Deficiency in mismatch repair in association with MGMT methylation results in a profound shift in the frequency and pattern of somatic point mutations in treated glioblastoma patients[17]. Therefore TMZ treatment in MGMT methylated GB patients could lead to accelerated mutagenesis and enhanced clonal diversity with implications for the evolution of treatment resistance. We propose to investigate this hypothesis using exome sequencing on paired samples from primary & recurrent GB in treated patients stratified by MGMT promotor methylation. We will use copy number aberration and mutation data to describe and quantify clonal diversity and correlate these data with disease progression & overall survival.

**Key deliverable:**
- We will determine the clonal architecture of recurrent GB and establish if recurrence is driven by specific genes

**Educational Objectives**

The student will work in a collaborative environment that is unique in the UK on a cancer that represents one of the most challenging areas in oncology. The student will be based within the University Department of Clinical Neurosciences in the Watts group, which is part of the Cambridge Cancer Centre. The student will liaise closely with the Tavaré group based in the CR-UK Cambridge Cancer Institute. The student will gain experience in a wide range of practical techniques including molecular biology, microscopy, cancer cell derivation and culture, cancer stem cell biology, immunohistochemistry & immunocytochemistry. The student will gain insight into the application of bioinformatics and computational analyses applied to cancer. The student will gain direct exposure to patients with brain cancer and gain insight into the human and clinical aspects of cancer. The student will be expected to present their work at national and international meetings. They will learn generic skills in communication & presentation of information/data to specialized and lay audiences.
References


