Identifying Responders to Temozolomide Among Patients with Metastatic Colorectal Cancer Using Proteomic Quantitation of MGMT

**Background**
- Temozolomide (TMZ) is a standard treatment for glioblastoma and melanoma and it has shown limited but encouraging activity in patients with metastatic colorectal cancer (mCRC).
- The DNA repair protein O6-methylguanine-DNA methyltransferase (MGMT) is a resistance marker for TMZ. MGMT promoter methylation is associated with loss of MGMT protein expression and response to TMZ.
- The MGMT expression status of tumors can be assessed by a digital polymerase chain reaction (PCR) method known as methyl-BeAMing (MB); a cutoff of >60% MGMT methylation predicts benefit from TMZ.
- Mass spectrometry (MS) proteomic analysis objectively quantifies MGMT protein and other actionable protein biomarkers in formalin-fixed, paraffin-embedded (FFPE) tissue sections.

**Hypothesis**
- Tumor expression of MGMT protein <200 amol/ug is predictive of response status.
- TMZ-treated mCRC patients with low MGMT protein expression (<200 amol/ug) had longer progression-free survival (PFS) than patients with higher MGMT protein levels.
- The agreement of MGMT methylation status by proteomics with MB (red bars; n = 35) with positive status defined as >60% (red line).

**Methods**
- Archived FFPE tissue sections were obtained from 41 patients without metastases who had received TMZ in phase II trials. A pathologist marked the tumor areas, which were microdissected and solubilized. In each tumor sample, multiple protein biomarkers including MGMT were quantified with MB, and MGMT methylation status was assessed by MB.

**Results**
- Figure 2. A. Among TMZ-treated patients (n = 41), those with MGMT protein levels <200 amol/ug (n = 18) had higher median PFS (mPFS) than patients with higher MGMT protein levels. (A) All patients eventually progress on TMZ. (B) Progression is redefined by the RECIST criteria to reflect clinical response: patients with partial response or stable disease for >6 months were defined as responders (n=18), non-responders (n=23). Results for overall survival were consistent and nearly statistically significant (8.7 vs 7.4 months, HR=0.6, p=0.077).

**Responses to TMZ are identified by MGMT protein quantitation (by MB) and MGMT promoter methylation (by MB)**

**Figure 4. **
A. Percent change in tumor volume (from baseline) by patients with MGMT protein ≥ 200 amol/ug (n = 18; dark blue) and MGMT protein ≤ 200 amol/ug (n = 23; light blue). MGMT methylation status by MB (red bars; n = 35) with positive status defined as >60% (red line).
B. Response rates according to RECIST 1.1.

**Conclusions**
- Among TMZ-treated patients with MCRc, whose tumors expressed low or undetectable levels of MGMT protein had a longer mPFS than their counterparts with higher MGMT protein levels.
- A correlation of 80% was observed between MGMT protein expression and methyl-BeAMing by MB.
- Quantitative proteomics objectively measured MGMT protein in FFPE tumor samples and retrospectively identified 9 of 9 responders to TMZ.
- Digital PCR methylation assay (methyl-BeAMing) retrospectively identified 7 of 8 responders to TMZ.
- Quantitative proteomic analysis of MGMT could potentially be used to predict responders to TMZ therapy.

**Future Plans**
- Assess post progression tissue, to understand whether MGMT expression was altered during the course of therapy.
- Validate the study results in other indications including glioblastoma and melanoma.