

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

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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 benefit in mCRC patients treated with TMZ.
- MGMT protein quantity may correlate with MGMT methylation status.

Methods

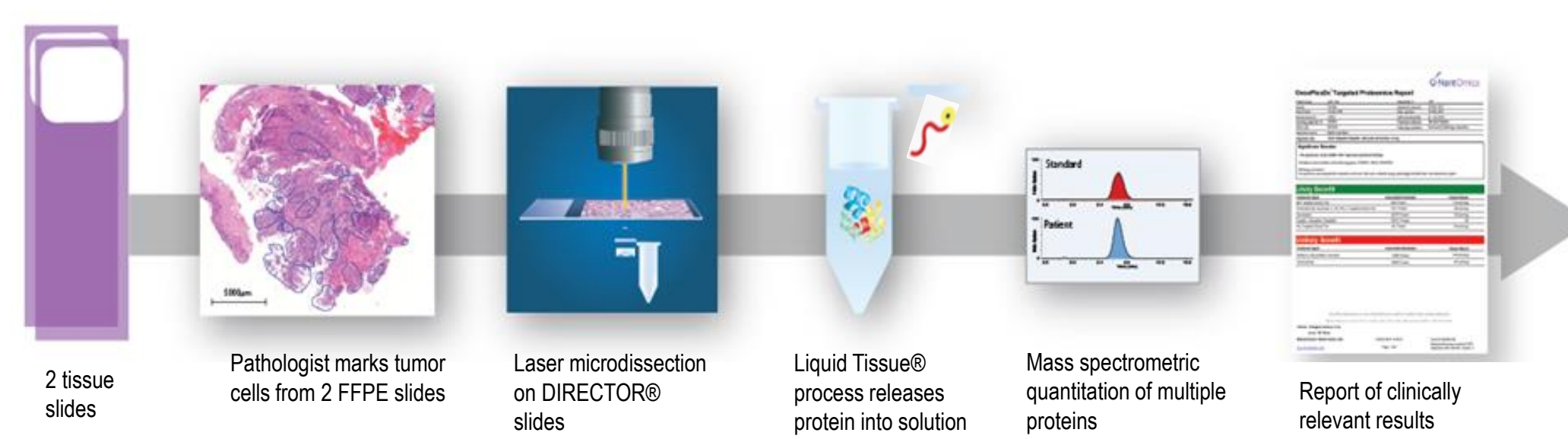


Figure 1. Archived FFPE tissue sections were obtained from 41 patients who had received TMZ in phase II trials. A pathologist marked the tumor areas, which were microdissected and solubilized. In each tumor sample, multiple protein biomarkers including MGMT were quantified with a MS-based assay. MGMT methylation status was assessed by MB. Mantel-Cox log-rank and Fisher's exact tests were used for survival comparisons.

Results

TMZ-treated mCRC patients with low MGMT protein expression (<200 amol/ug) had longer PFS

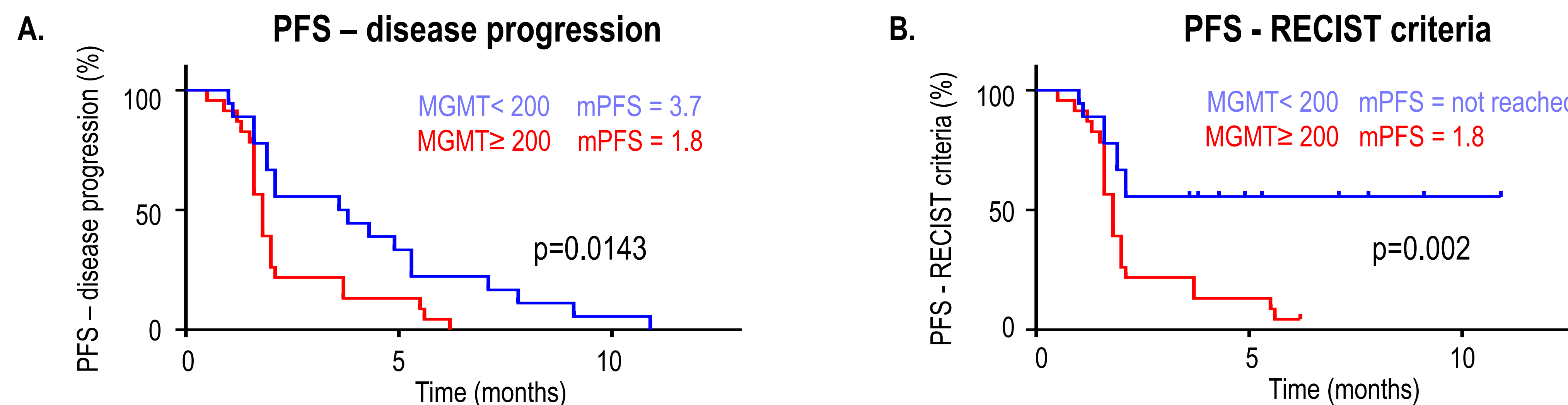


Figure 2. (A, B) Among TMZ-treated patients (n = 41), those with MGMT protein levels <200 amol/ug (n = 18) had longer median PFS (mPFS) than patients with higher MGMT protein levels. (A) All patients eventually progress on TMZ. (B) Progression is redefined by the RECIST criteria to reflect clinical response: 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).

Responders to TMZ are identified by MGMT protein quantitation (by MS) and MGMT promoter methylation (by MB)

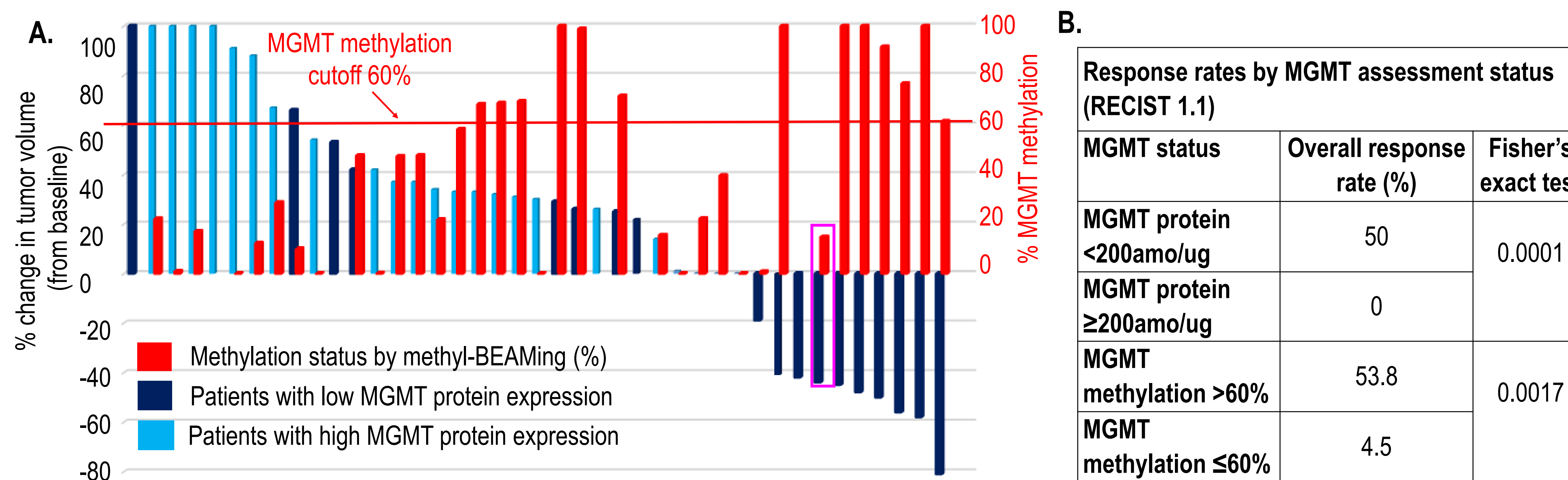


Figure 4. A. Percent change in tumor volume (from baseline) by patients with MGMT protein < 200 amol/ug (n = 18; dark blue) and MGMT ≥ 200 amol/ug (n = 23; light blue). MGMT methylation status by MB (red bars; n = 35) with positive status defined as > 60% (red line). **B.** Response rates according to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

80% agreement between MGMT protein quantity (by MS) and MGMT methylation (by MB)

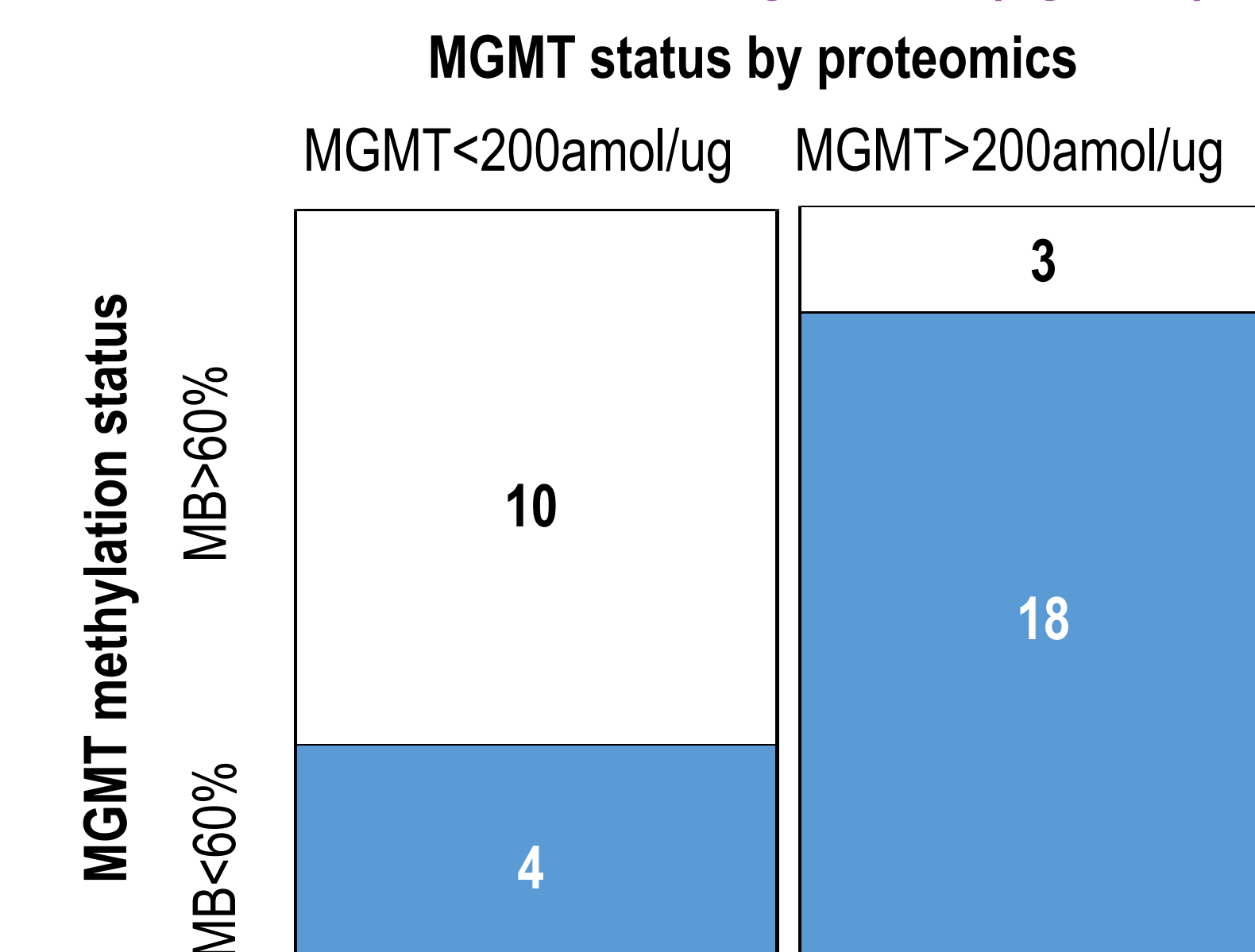


Figure 3. In 35 tumors analyzed by MB, the agreement rate between the MGMT proteomic assay and MB was 80%; p=0.0011 Fisher's test.

Conclusions

- Among TMZ-treated patients with mCRC, those 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 as quantified by mass spectrometry and MGMT methylation status 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 select mCRC patients for 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.