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 formalin-fixed, paraffin-embedded (FFPE) tissue sections.

Hypotheses

- An MGMT protein cutoff of 200 amol/ug (predefined based on the assay’s limit of detection) is predictive of benefit in mCRC patients treated with TMZ.
- MGMT protein quantity may correlate with MGMT methylation status.

Methods

- Mass spectrometry (MS) proteomic analysis objectively quantifies multiple protein biomarkers including MGMT.
- Among TMZ-treated mCRC patients with low MGMT protein expression (>200 amol/ug) had longer progression-free survival (PFS).

Results

- 10 of 18 patients with low MGMT protein levels (defined as responders (n=18) MGMT protein levels <200 amol/ug had longer mPFS than patients with higher MGMT protein levels. (A) All patients eventually progressed on TMZ (B) Progression was 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 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 sputumized. 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.

Figure 2. (A, B) Among TMZ-treated patients (n = 41), those with MGMT protein levels <200 amol/ug had longer median PFS (mPFS) than patients with higher MGMT protein levels. (A) All patients eventually progress on TMZ (B) Progression was 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).

Figure 3. In 35 tumors analyzed by MB, the agreement rate between the MGMT protein assay and MB was 80%; p=0.001 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.

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.