Quantitative proteomic analysis of MGMT may predict response of colorectal cancer patients to treatment with temozolomide

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**Background**

- About half of patients with metastatic colorectal cancer (mCRC) do not respond to standard fluorouracil-based chemotherapy.
- Temozolomide (TMZ) is standard chemotherapy treatment for glioblastoma and melanoma and it has shown modest but encouraging efficacy in CRC.
- Tumor expression of O6-methylguanine-DNA methyltransferase (MGMT) is a marker of resistance to TMZ in multiple cancer types; MGMT promoter methylation is associated with loss of MGMT expression and response to TMZ.
- We hypothesized that tumor expression of MGMT <200 amol/ug is predictive of response in mCRC patients treated with TMZ.

**Methods**

- A selected reaction monitoring (SRM) mass spectrometry assay was developed to quantify MGMT protein in formalin-fixed, paraffin-embedded (FFPE) tissue.
- Archived tissue sections were obtained from patients with CRC (n=41) who had received TMZ in a clinical trial. A pathologist marked the tumor areas, which were laser microdissected and solubilized to tryptic peptides using the Liquid Tissue® process1,2.
- In each liquified tumor sample, multiple proteins including MGMT were quantified. The total protein concentration of each sample was measured using a Micro BCA Protein Assay Kit. A mixture of stable isotope-labeled heavy peptides were added prior to analysis and used as internal standard.
- Relationships between MGMT expression and the patients' clinical response to TMZ were retrospectively assessed.

**Results**

**Figure 2. Performance of SRM assay for MGMT.** Eleven points were spiked in complex background with a constant amount (5fmol) of heavy synthetic peptide and varying amounts (from 0 to 25000 amol) of light synthetic peptide to build the concentration curve.

**Figure 3. Precision of the MGMT SRM assay was assessed in 10 spiked FFPE tissue samples analyzed on 2 different MS systems.**

**Figure 4. Progression-free survival (PFS).** The cancer progressed in all patients treated with TMZ. However, the patients with MGMT levels <200 amol/ug had longer median PFS (p=0.0143).

**Figure 5. Responders to TMZ were retrospectively identified by MGMT protein quantitation.** Percent change in tumor volume from baseline by patients with MGMT<200 amol/ug (n = 18; dark blue) and MGMT ≥ 200 amol/ug (n = 23; purple). Response was defined by RECIST 1.1.

**Conclusions**

- We developed a quantitative SRM assay for MGMT protein in FFPE tumor tissue with high linearity and precision across the entire dynamic range.
- TMZ-treated CRC patients with low MGMT protein expression (<200 amol/ug) had longer median progression free survival (p=0.0143).
- MGMT protein quantified by SRM retrospectively identified 9 of 9 responders to TMZ treatment.
- SRM analysis of MGMT could potentially be used to select patients who will be likely to respond to TMZ therapy.

**References**