Poster# MP-103

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

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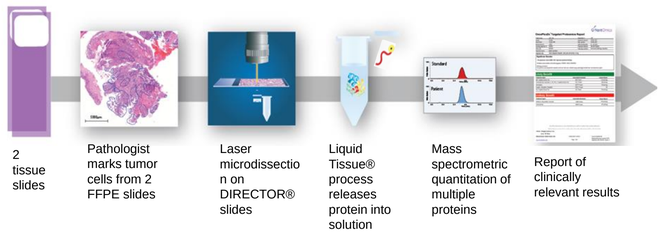
Background

- 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 O^6-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

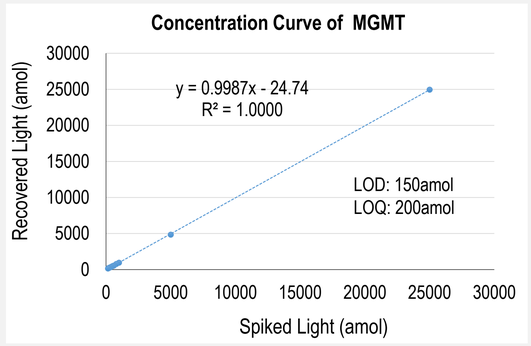
- A selected reaction monitoring (SRM) mass spectrometry assay was developed to quantify MGMT protein in formalin-fixed, paraffin-embedded (FFPE) tissue.
- Archived tissue sections were obtained from patients with CRC (n=41) who had received TMZ in a clinical trial. A pathologist marked the tumor areas, which were laser microdissected and solubilized to tryptic peptides using the Liquid Tissue® process[1,2].
- In each liquefied 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.

Figure 1. Selected reaction monitoring (SRM) in FFPE tumor tissue[1,2]



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.



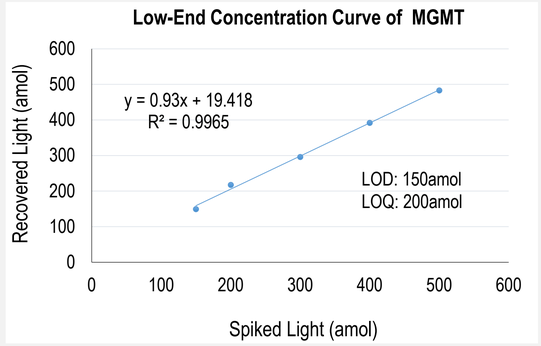


Figure 3. Precision of the MGMT SRM assay was assessed in 10 spiked FFPE tissue samples analyzed on 2 different MS systems.

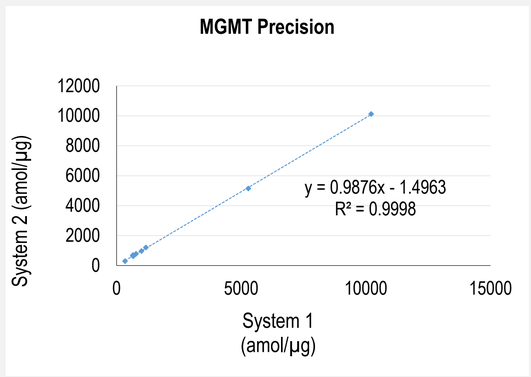


Figure 4. Progression-free survival (PFS). The cancer progressed in all patients treated with TMZ. However, the patients with MGMT levels <200 amol/ug had longer median PFS (mPFS) than those with higher MGMT levels (3.7 vs 1.8 months).

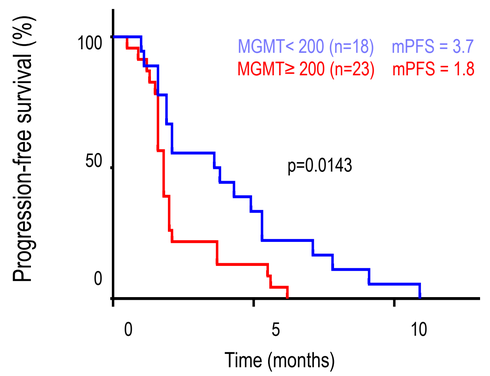
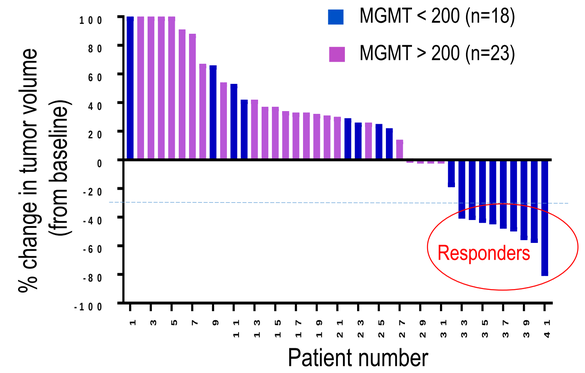


Figure 5. Responders to TMZ were retrospectively identified by MGMT protein quantitation. Percent change in tumor volume from baseline by patients with MGMT< 200 amol/ug (n = 18; dark blue) and MGMT ≥ 200 amol/ug (n = 23; purple). Response was defined by RECIST 1.1.



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

1. Hembrough et al (2013) Application of selected reaction monitoring for multiplex quantification of clinically validated biomarkers in formalin-fixed, paraffin-embedded tumor tissue.
2. Hembrough et al (2016) Quantification of Anaplastic Lymphoma Kinase Protein Expression in Non–Small Cell Lung Cancer Tissues from Patients Treated with Crizotinib.