Molecular Profiling of Appendix-Derived Pseudomyxoma Peritonei (PMP)

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Abstract

Abstract # 571

Background: PMP is a rare malignancy originating from the appendix, characterized by disseminated mucinous tumor implants on peritoneal surfaces. We examined the role of multiplatform molecular profiling to study biomarker-guided treatment strategies for this rare malignancy.

Methods: 54 patients with appendix-derived PMP were included in the study. Tests included one or more of the following: gene sequencing (Sanger or next generation sequencing [NGS]), protein expression (immunohistochemistry [IHC]) and gene amplification (C/FISH).

Results: Targeted sequencing of 47 genes detected variants in KRAS (79%), GNAS (73%) and SMAD4 (18%). Mutations were found at low frequencies (n=1-2) in APC, ATM, BRAF, PIK3CA, MLH1 and TP53. GNAS and KRAS co-occurrence was found in 78%. Protein overexpression was found in EGFR (83%), cMET (59%), cKIT and PDGFRA (58%), respectively. Immune checkpoint expression was found in 36% (PD1) and 9% (PDL1). Surrogate markers of cell proliferation were found at low rates (TLE3 27%, TOP2A 22%), consistent with the slow-growing biology of PMP. PTEN was intact (wild type [100%] and positive IHC [80%]). Patients exhibited stable microsatellite status and mismatch repair proficiency (93%). Importantly, multidrug resistance protein expression was elevated (100% BCRP, 94% MRP1, 88% PGP). Markers for gemcitabine (RRM1), fluorouracil (TS), oxaliplatin (ERCC1) and irinotecan (TOPO1) chemosensitivities were detected at favorable rates: 93%, 87%, 77% and 65%, respectively.

Conclusion: Molecular profiling by multiple platforms identified potential therapies for the non-targetable KRAS-mutated population. The role of cMET-targeted therapeutics and immune checkpoint inhibitors merits further investigation. Biomarker-guided selection of cytotoxic chemotherapies may facilitate responses to systemic treatment.

Background

Pseudomyxoma peritonei (PMP) is a clinical syndrome that is characterized by mucinous ascites that results from rupture of a mucin-producing neoplasm, typically from appendiceal origin.

Current treatment largely entails cytoreductive surgery (CRS) with mitomycin-based heated intraperitoneal chemotherapy (HIPEC) despite pathological classification/underlying tumor biology (Diffuse Peritoneal Adenomucinosis [DPAM], Peritoneal Mucinous Carcinomatosis [PMCA] or intermediate variant).

Molecular characterization and application of molecular profiling to guide systemic treatment is largely unknown for this disease.

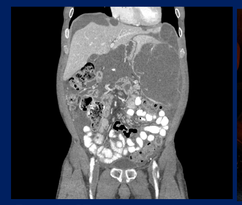


Figure 1. CT scan coronal imaging of PMP mucinous ascites with resultant mass effect on intra-abdominal viscera.

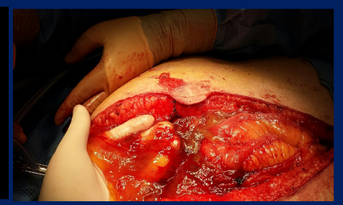


Figure 2. Intra-operative picture of PMP mucinous ascites.

Methods

In an academic medical center and commercial biomarker data repository (Drexel University College of Medicine, Philadelphia, PA and Caris Life Sciences, Phoenix, AZ), 54 appendix-derived PMP were identified (2010-2015) and included in this retrospective analysis.

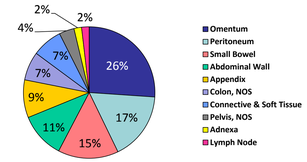
Specific testing was performed and included a multiplatform approach:

- Protein expression (IHC)
- Gene amplification (CISH/FISH)
- Sequencing (Sanger, NGS [truSeq=47 gene panel/MiSeq=600 gene panel])

Four patients with clinically aggressive underlying tumor biology were selected for further sequencing (MiSeq=600 gene panel).

Results

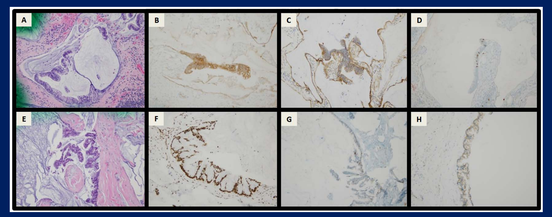
Figure 3. Specimen Sites Utilized for Profiling



Primary Tumor Sites: 89% Appendix, 4% Colon, 4% Peritoneum, 2% GI Tract, NOS, 2% Unknown

Table 1. Clinicopathological Parameters

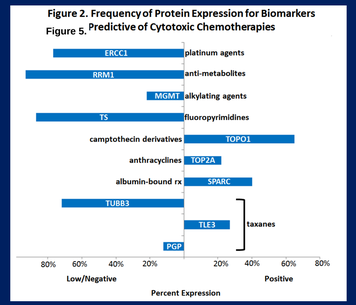
n (%)	Peritoneal Disease Grade			
	All	DPAM	PMCA	Unclassified
Total Cases	54	29 (54)	9 (17)	16 (29)
Gender (m)	23 (43)	13 (45)	6 (67)	4 (25)
Gender (f)	31 (57)	16 (55)	3 (33)	12 (75)
Age (Median)	55	58	53	60
Age (Range)	27-77	27-77	38-62	34-67



Figures 4A-4H. Representative images of IHC staining, all 20X magnification. (A) H&E of 50 y.o. KRAS wildtype male patient with high-grade PMP, peritoneal fluid with malignant cells, (B) cMET+ (2+90%), (C) EGFR+ (1+ 90%), (D) TOP2A - (2+5%), (E) H&E of 42 y.o. male KRAS G12D male patient with low-grade PMP, umbilicus, (F) TOPO1+ (2+90%), (G) TS+ (1+10%), (H) PGP (1+85%).

Results, contd.

Figure 2. Frequency of Protein Expression for Biomarkers Predictive of Cytotoxic Chemotherapies
Figure 5.



Figures 5-6. Protein expression rates of biomarkers that are predictive of cytotoxic (2) and targeted (3) therapies. N per biomarker varies depending on time of testing : ERCC1 (30), RRM1 (44), MGMT (46), TS (45), TOPO1 (43), TOP2A (45), SPARC (47), TUBB3 (25), TLE3 (30), PGP (49), PDL1 (11), PD1 (11), COX-2 (11), PTEN (44), EGFR (23), HER2 (52), cMET (27), PDGFRA (19), cKIT (24). MSH2, MSH6, MLH1 and PMS2 were also performed on 14 patients, of which 1 patient exhibited MMR deficiency.

Figure 6. Frequency of Protein Expression for Biomarkers Predictive of Targeted Therapies

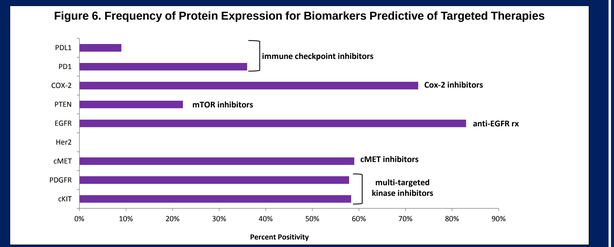


Figure 7. Frequency of mutations detected by sequencing

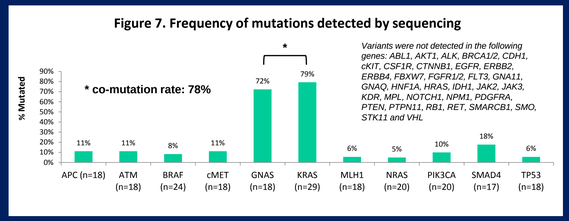


Figure 7. Frequency of mutations detected by sequencing. N per biomarker varies: with the exception of BRAF, KRAS, NRAS and PIK3CA, 18 PMP patients were assayed with NGS, where the former genes also include sanger data. Variants detected are as follows: KRAS (G12D-57%; G12V 43%), GNAS (R201C 46%; R201H 54%), SMAD4 (G386D, R496H, S474X), ATM (R2443Q, A1309T), BRAF (V600E, D594G), PIK3CA (E545K, E545G), cMET (A319T, T1010I), APC (L1129S, T1556fs), TP53 (L194R), NRAS (Q61R), MLH1 (S406N).

Results, contd.

Table 2. Clinical Application and NGS

Patient	CMI - IHC	CMI - NGS	Treatments	Time on Rx	Fusions	CNV	Clinical Genes (600 Assays)	Unclassified Genes
Case #1	TS Negative (0+ 100%)	no variants detected	capecitabine	5 months	none detected	none detected	NOTCH1 (R912W; VUS; 52%)	IGF1R (R511Q), VEGFB (T166N; UV; 43%), PICALM (I34M; UV; 48%), **SBDS (K62X, UV, 12%)**, MDS2 (R95dup; UV; 39%), c11orf30 (P629T, UV, 46%), 8RD3 (A172V, UV, 48%), BCR (D1106N, UV, 13%)
Case #2	TS Negative (0+ 100%)	no variants detected	5-fluorouracil, leucovorin, bevacizumab	18 months	none detected	H3F3A - amplified	PTCH1 (T1052M, VUS, 62%), KRAS (G12D, P, 54%), GNAS (R201C, P, 28%), ERBB3 (A133T, VUS, 58%), ATM (L121X, P, 90%)	EPHA5 (G410D, UV, 15%), FLT4 (E951del, UV, 16%), IL6ST (A200T, UV, 39%), KDM5C (P1325S, UV, 54%), MN1 (Q533dup, UV, 29%), RUNX1 (L56S, UV, 77%)
Case #3	MSI-High	BRAF V600E, PIK3CA E545G, APC T1556fs, cMET T1010I	panitumumab bevacizumab	3 months 5 months	none detected	none detected	NOTCH1 (R912W; VUS; 49%), **KRAS** (G12D; P, 35%), **GNAS** (R201H; P, 29%), cMET (T1010I, PB, 51%), DDR2 (S173C, VUS, 52%), CTNNB1 (N287S, PB, 49%), BRCA2 (H2021R, VUS, 52%)	TRIP11 (N701S, UV, 51%), TET2 (K1090N, UV, 53%), TET1 (V128F, UV, 51%), TERT (H412Y, UV, 51%), SMARCA4 (Y372H, UV, 50%), SDHB (T60A, UV, 51%), RUNX1 (L56S, UV, 50%), PCSK7 (c.1691+1G>A, UV, 24%), MN1 (Q533dup, UV, 20%), KIAA1549 (S1855L, UV, 31%), GRIN2A (R1011Q, UV 46%; Y1292C, UV, 50%),
Case #4	n/a	no variants detected	palliative HIPEC	n/a	ADCK4-NUMBL	none detected	PTCH1 (G38V, VUS, 45%), PDGFRB (E112K, VUS, 52%), NOTCH1 (A1343V, PB, 41%), KRAS (G12D, P, 30%, GNAS (R201C, P, 31%), CSF1R (T393M, VUS, 48%), ATM (E2096X, P, 15%), SMAD4 (P215R, UV, 49%; Y131C, UV, 32%)	NOTCH2 (R1640fs, UV, 15%), MDS2 (R95dup, UV, 35%), TET2 (V1718L, UV, 47%), TET1 (V128F, UV, 48%), SFPQ (G24dup, UV, 42%), MED12 (H2116_Q2119dup, UV, 59%), KAT6B (E1366_E1368del, UV, 33%), HIP1 (S319L, UV, 50%), BCR (T845M, UV, 52%)

Summary

- Low levels of TOP2A positivity (22%) are supportive of a less proliferative phenotype, which mirrors the slow-growing biology of PMP tumors.
- Expression of oncogene targets suggest a potential role for cMET and EGFR targeted therapies, based on high rates of protein overexpression (59%, 83%, respectively).
- KRAS mutations are the predominant mutational aberration, detected in 79% of PMP, with co-occurrence of GNAS occurring in 78% of KRAS+ PMP.

Conclusions

- Predictive biomarker expression rates suggest potential sensitivity to several cytotoxic chemotherapies including platinum agents, fluoropyrimidines and taxanes.
- IHC, gene amplification and NGS may improve identification of genes with targeted therapies.

References

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