Comprehensive Next Generation Sequencing Profiling in Combination with Transcriptomic-based Tumor Molecular Subtyping and Harmonized TMB Calculation using Paired Specimens from Late- Stage CRC Patients

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Danyi Wang[1], Lee McDaniel[2], Sean Boyle[2], Juergen Scheuenpflug[3], Zheng Feng [1#],

1. Clinical Genomics, Global Clinical Biomarkers & Companion Diagnostics , Translational Medicine | Biometrics & Precision Medicine , EMD Serono , A business of Merck KGaA, Darmstadt, Germany
2. Personalis, Inc. 1330 O' Brien Dr, Menlo Park, CA., 94025
3. Global Clinical Biomarkers and Companion Diagnostics, Translational Medicine, Global Development, Merck KGaA, Darmstadt, Germany

Corresponding author

BACKGROUND

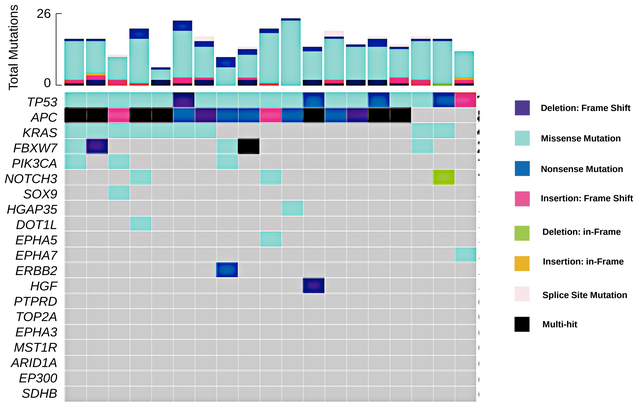
Colorectal Cancer (CRC) can be classified into transcriptomics subtypes such as, stromal or immunogenic. A previously demonstrated consensus molecular subtype (CMS) classification method was utilized on CRC samples [1,2]. We explored the potential clinical utility of combining a transcriptome derived subtypes approach with WES by demonstrating the association among subtypes and tumor characteristics such as whole exome-based microsatellite instability (MSI), tumor mutational burden (TMB) and karyotype.

METHODS

- Tumor tissue and paired blood samples were collected from 19 late-stage, treatment-naïve colorectal cancer (CRC) patients. gDNA and RNA were extracted and analyzed by the Personalis® ImmunoID NeXT Platform®[3].
- RNA-seq results were normalized with the R DESeq2 package, with CRC CMS classification performed by the R package CMScaller.
- Somatic variants and copy number variants and MSI were evaluated using paired tumor/normal (T/N) samples. Copy number (CN) was characterized both genome-wide as a ploidy estimate and focally as the number of amplified regions. TMB was computed in alignment with the Friends of Cancer Research phase I guidelines.

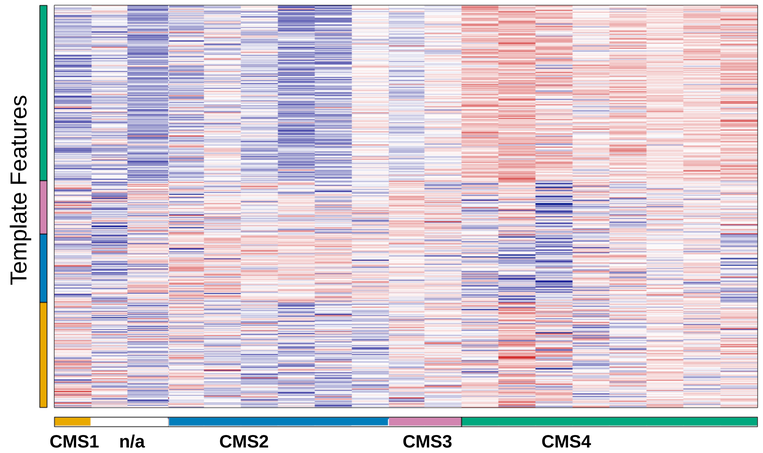
RESULTS

Commonly mutated cancer genes



The most commonly mutated genes included *TP53* (19/19), *APC* (16/19), and *KRAS* (9/19)

CMS Subtypes generated by CMScaller (RNA-seq)



CMS1: MSI, activated immune system

CMS2: Canonical

CMS3: Metabolic/epithelial

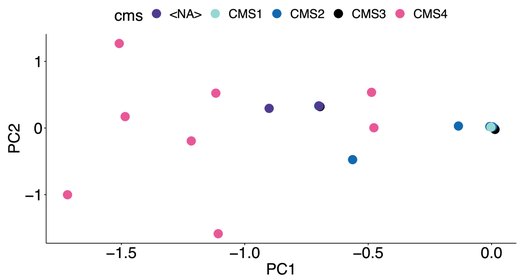
CMS4: Mesenchymal/stromal

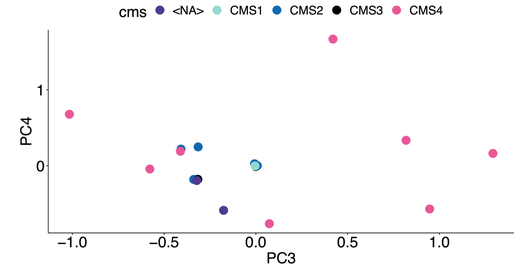
	CMS2	CMS3	CMS4
IVA	3	1	4
IVB	1	1	0
IVC	0	0	1

Above: RNA-seq molecular subtypes not associated with clinical subtype (Chi-squared, p=0.48)

Above: CMS2 vs CMS4 genes were identified with Boschloo's exact test and fitted to a PCA. Unidentified samples have mutation profiles most similar to CMS4(mesenchymal/stromal) subtype in DNA mutation space, whereas their RNA-seq profiles resemble CMS1 or CMS2.

Combine DNA-seq mutation profiles further stratify subtypes



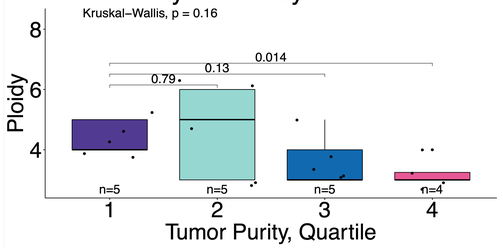


Gene	p-value
TSHZ3	0.44
RYR1	0.31
NUTM2A	0.31
NBEA	0.31
MTOR	0.31
KIAA1109	0.31
IGF2R	0.31
FGF23	0.31
DNM3	0.31
COL7A1	0.31
COL1A1	0.31
BRD4	0.31
AHNAK	0.31
SOX9	0.28
KRAS	0.11

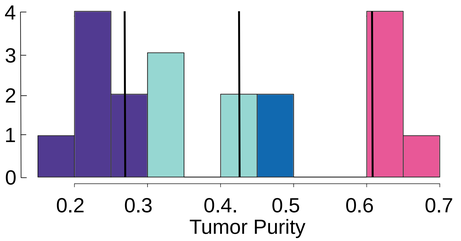
Above: CMS2 vs CMS4 genes were identified with Boschloo's exact test, taking the top 15 genes and embedding mutational profiles into a 0/1 encoded vector denoting the presence or absence of a mutation. These embeddings were dimensionally reduced via a principal component analysis(PCA)

Above: PCA analysis of the most differentially mutated genes between CMS2 and CMS4 CRC subtypes. By integrating the DNA-seq and RNA-seq capabilities of the ImmunoID NeXT platform, ambiguous RNA-seq based subtypes can be stratified according to SNV patterns.

CRC: Purity vs. Ploidy



Tumor Purity: Quartile Boundaries and Purity Distribution



- Tumor Ploidy vs Purity in CRC: Purity and ploidy were quantified with Sequenza, a component of the ImmunoID NeXT Platform. High purity was associated with lower ploidy (p=0.014 upper/lower quartile).
- ImmunoID NeXT somatic single nucleotide variant (SNV) detection contrasted tumor high throughput sequencing data (HTSD) against paired normal HTSD, allowing for direct detection of germline vs somatic variation consequent to the optional inclusion of paired normal samples.

CONCLUSIONS

- It has been demonstrated that the ImmunoID NeXT Platform's transcriptomics capabilities enable CMS classification in most samples (17/19, 89%), potential to, in the future, extend RNA-seq subtyping with DNA-seq data such as purity and ploidy.
- An association between tumor purity and WES using a broader exome-wide measurement was identified with transcriptomic based molecular subtyping.
- It has been corroborated that future comprehensive molecular classifiers can expand on transcriptomics-based classification by leveraging DNA-based measurements to further delineate subtypes and eventually lead to biomarker driven precision oncology focused patient selection .

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