#EACR21v-0513 Accurate Detection of Colorectal Advanced Adenomas Through Analysis of Cell-free circulating tumor DNA (ctDNA) Methylation Patterns

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BACKGROUND

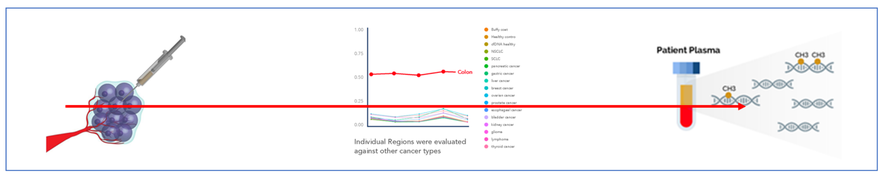
- 80% of sporadic colorecatal cancers arise from **pre-malignant advanced adenomas (AA)** [1-2]
- **Detection** and subsequent removal of colorectal adenomas **could save lives**[3]
- Measuring the **methylation status of ctDNA** in plasma could enable identification of AA
- We report here a **plasma targeted methylation panel** performance for detection of patients with varied subtypes of **AA with good accuracy**

METHODS

This was a **prospective, international multicenter observational cohort study**. Plasma samples were collected prior to a scheduled colonoscopy as part of standard colorectal cancer screening.

Characteristics	Controls (n=136)	Advanced adenoma (n=80)
Age (years, mean (IQR))	63 (46-78)	63 (50-79)
Gender (n (%))		
Female	69 (51%)	41 (51%)
Male	67 (49%)	39 (49%)
Body mass index (kg/m2, mean (IQR))	28 (19.5-42)	28 (19-48)
Histology		
Tubular		29
Tubulovillous		29
Serrated		15
Carcinoma in situ		7
Dysplasia grade		
Tis		7
High grade dysplasia		25
>= 1cm low grade		40
<1 cm (serrated with dysplasia or tubulovillous low grade)		8

Table 1 *Sample set demographics*



Paired tissue analysis for putative biomarker regions → **Filtering against buffy coat, healthy cfDNA, other cancer and tissue** → **Transfer to targeted capture analysis from plasma**

Figure 1 *Biomarker selection process*

Differentially methylated regions (DMRs) were initially selected by analyzing **colon cancer and adenoma and control tissue samples** with whole genome bisulfite sequencing. Regions were further **filtered,** and a **targeted hybrid capture methylation sequencing assay** was designed to capture these DMRs in plasma ctDNA.

Individual sequencing reads were **evaluated for tumor-specific methylation signal** and scores calculated for each DMR in a sample. A panel of methylation scores originating from **220 DMRs** was used to train and evaluate a support-vector machine (**SVM**) model in 50-fold cross-validation setting.

AMBER score*

****Abnormally methylated biomarker early recognition algorithm used for read-based signal scoring per region per sample***

Model training

Monte Carlo cross-validation with SVM model building based on 220 DMRs

RESULTS

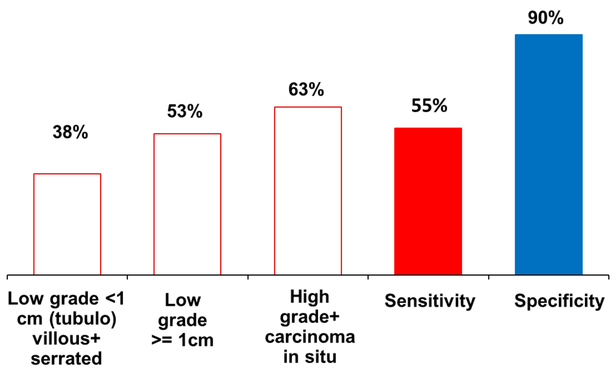


Figure 2 *Prediction accuracy values of 220-marker SVM algorithm severity grade of sub-groups of AA indicating improved sensitivity with advanced stage*

Further analysis of histological subtypes showed a sensitivity of **52% (15/29)** for **tubulovillous**, **57% (4/7)** for **Tis, 59% (17/29)** for **tubular** and **60% (9/15)** for **serrated** histology

The SVM model based on read-wise score of **220 DMRs** targeted in ctDNA had **55% (44/80) overall sensitivity** of detecting AA patients at **specificity of 90% (123/136).** Sensitivity increased with the severity of the AA finding from **38% (3/8) for <1 cm group** (<1 cm serrated adenomas with dysplasia or <1 cm (tubulo)villous low grade dysplasia adenoma), **53% (21/40) for low grade >=1cm adenomas**, **63% (20/32)** for combined group of patients with **high grade dysplasia or carcinoma in situ** findings.

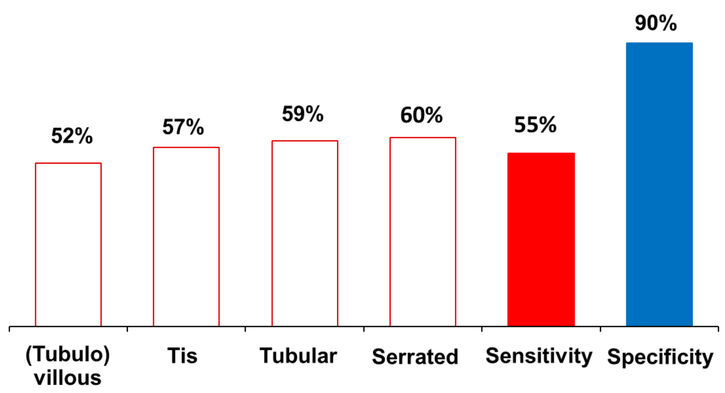


Figure 3 *Prediction accuracy values of 220-marker SVM algorithm on for histological groups of AA, indicating good sensitivity regardless of the histological type*

DAVID KEGG pathways
Term
hsa05200:Pathways in cancer
hsa04510:Focal adhesion
hsa04810:Regulation of actin cytoskeleton
hsa04015:Rap1 signaling pathway
hsa05214:Glioma
hsa04010:MAPK signaling pathway
hsa05218:Melanoma
hsa04350:TGF-beta signaling pathway

Individual marker regions are connected to **cancer pathways** and **signalling pathways** linking methylation regions to biological processes in cancer

CONCLUSIONS

- ctDNA methylation sequencing data analysis using read-wise scoring approach combined with a machine-learning algorithm is highly diagnostic for advanced colonic adenomas (**55% sensitivity at 90% specificity**).
- This method could serve as the basis for a **highly accurate and minimally invasive blood-based screening test** with significant implications for early detection and cancer prevention.
- **Prospective clinical trials are underway** to validate the performance of this novel biomarker panel for advanced adenoma detection in representative screening cohorts.

REFERENCES

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DISCLOSURE

Universal Diagnostics S.L. sponsored this study. PCN, MB, KK are employees of Universal Diagnostics S.L.

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