DETECTION OF COLORECTAL CANCER AND ADENOMAS BY EPIGENETIC PROFILES OF CIRCULATING NUCLEOSOMES: A PILOT STUDY WITH 58 SUBJECTS

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BACKGROUND
Immunohistochemistry studies show genome-wide epigenetic changes in the chromatin of cancer tissue and have identified histi-oncoproteins - histone modifications and other epigenetic changes linked to cancer. In cancer patients, ctDNA circulates as nucleosome fragments of tumor chromatin consisting of short, less than 200 base pair, DNA sequences wrapped around four pairs of histone proteins. Nucleosome bound DNA fragments contain mutations found in cancer tissue suggesting a tumor chromatin origin for at least some circulating nucleosomes. Profiling of global levels of epigenetic alterations in circulating nucleosomes can provide disease-specific diagnostic information.

MATERIAL and METHODS
VolitionRx has developed serum ELISA assays that measure circulating nucleosomes containing specific epigenetic signals and used these to investigate global epigenetic profiles in colorectal cancer (CRC) and adenomas patients. The assays employ one antibody targeted to bind a common nucleosome epitope and a second antibody targeted to bind to the epigenetic structure of interest.

Twelve circulating nucleosome structures were measured in serum samples collected from 58 symptomatic subjects referred for a colonoscopy at the Academic Hospital, CHU UCL Namur (Belgium), using specific ELISA assays (NuQ®, Belgian Vivotom SA) and analysed using univariate and multivariate approaches. Linear models, based on a weighted sum of one to five variables were developed using Fisher's linear regression (LDA) optimised for the best Area Under the Curve (AUC).

RESULTS
VolitionRx has developed five patient-protected families of NuQ® standard ELISA assays, each of which captures intact nucleosomes and labels (identifies) a specific structural feature:

- NuQ®-X specific DNA modifications
- NuQ®-Y: histone variants
- NuQ®-M: histone modifications
- NuQ®-A: nucleosome-protein adducts
- NuQ®-T: total nucleosomes

Using a novel ELISA platform - NuQ®-combin®, we evaluated 12 specific epigenetic features of circulating nucleosomes as potential blood-based biomarkers for colorectal cancer.

CONCLUSIONS
Serum profiles of epigenetically altered circulating nucleosomes measured by ELISA can be used to detect CRC early stage and precocious bowel lesions in a simple blood test. Epigenetic nucleosome assays have the potential for improved patient compliance and accuracy in the early detection of CRC. Further studies in larger patient cohorts will be required to validate the usefulness of these NuQ® biomarkers in CRC early diagnosis.

Conflict of interest
MH, EJ, KS are employees of Belgian Vivotom SA.
JM is a consultant of Belgian Vivotom SA.
MH, EJ, KS, JM have a financial interest in Belgian Vivotom SA.

SUMMARY
VolitionRx has developed five patient-protected families of NuQ® standard ELISA assays, each of which captures intact nucleosomes and labels (identifies) a specific structural feature:

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Providing disease-specific diagnostic information.

The study comprised 58 individuals above 50 years old at high risk, or displaying symptoms of colorectal cancer (CRC). Patients were classified into three groups based on their colonoscopy results: CRC patients, patients with colorectal polyps, healthy controls with normal epithelium.

Comparison of criteria for the detection of CRC using different ELISA assays.

We evaluated the cumulative performance of our NuoX biomarker panel in combination with CEA, and adjusted for age using a multivariate analysis.

At 90% of specificity:
The tumor marker CEA gave a sensitivity of 32%.
Combination of 4 circulome nucleosome increased the sensitivity to 74%.
Age-adjusted circulome nucleosome panel increased the sensitivity to 91%.

We developed a new combination of circulome nucleosome assays for early-stage CRC detection.

DETECTION OF OF UP TO 91% OF CRC CASES

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DETECTION OF EARLY STAGE CANCER

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DETECTION OF 62% OF ADENOMA CASES

DETECTION OF 62% OF ADENOMA CASES

The single biomarker CEA showed a relatively good sensitivity in stage IV but performed poorly in the earliest stages. Conversely, the 4 circulome nucleosomes showed markedly increased sensitivity across all stages of CRC.

A second algorithm, optimised for the discrimination between the polyp group and the healthy controls was developed. A combination of 4 circulome nucleosome biomarkers significantly improved the discrimination of polyp vs healthy (p<0.001).

The score was significantly higher (p<0.001) in serum of patients with colorectal cancer compared to healthy controls and to the polyp group.

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