Detection of colorectal cancer and adenomas by epigenetic profiles of circulating nucleosomes: a pilot study with 58 subjects

L. D’Hondt†, M. Herzog†, J.F Rahier†, L. Faugeras†, A. Druez†, E. Josseaux†, K. Scoubeau‡, F. George‡, T. De Ronde‡, J. Micalef‡

1 CHU UCL Namur, Department of Oncology, Yvoir, Belgium. Correspondence: lionel.dhondt@uclouvain.be
2 Belgian Volition SPRL, 22 Rue Phocas Lejeune, Parc Scientifique Crealys, 5032 Isnes, Belgium. Correspondence: m.herzog@volitionrx.com
3 CHU UCL Namur, Department of Gastroenterology, Yvoir, Belgium.
4 CHU UCL Namur, Biobank-USS, Yvoir, Belgium

Background
Immunohistochemistry studies show genome-wide epigenetic changes in the chromatin of cancer tissue and have identified histo- oncoproteins – histone modifications and other epigenetic changes linked to cancer.

In cancer patients, cDNA 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 use these to investigate global epigenetic profiles in colorectal cancer (CRC) and adenomas. The assays employ one antibody targeted to bind to a common nucleosome epitope and a second antibody targeted to bind to the epigenetic structure of interest.

Twelve circulating c-nucleosomes 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 Volition SA) and analysed using univariate and multivariate approaches. Linear models, based on a weight sum of one to five variables were developed using Fisher’s linear regression (LDA) optimised for the best Area Under the Curve (AUC).

Conclusions
Serum profiles of epigenetically altered circulating nucleosomes measured by ELISA can be used to detect CRC including early stage and precancerous 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 are warranted to validate the usefulness of these NuQ® biomarkers in CRC early diagnosis.

Detection of early stage cancer
Table: Percentage of sensitivity at 90% specificity at the different CRC cancer stages for the established tumor marker Carcinoembryonic antigen (CEA) and the c-nucleosome biomarkers.

<table>
<thead>
<tr>
<th>% sensitivity at 90% specificity</th>
<th>CRC</th>
<th>CEA</th>
<th>Combination of 4 NuQ® assays</th>
<th>Combination of 4 NuQ® assays age-adjusted</th>
</tr>
</thead>
<tbody>
<tr>
<td>All stages</td>
<td>55</td>
<td>75</td>
<td>85</td>
<td>95</td>
</tr>
<tr>
<td>Stage I</td>
<td>10</td>
<td>75</td>
<td>90</td>
<td></td>
</tr>
<tr>
<td>Stage II</td>
<td>14</td>
<td>84</td>
<td>86</td>
<td></td>
</tr>
<tr>
<td>Stage III</td>
<td>17</td>
<td>71</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Stage IV</td>
<td>75</td>
<td>50</td>
<td>50</td>
<td></td>
</tr>
</tbody>
</table>

Detection of 62% of adenoma cases

The single biomarker CDA showed a relatively good sensitivity in stage IV but performed poorly in the earliest stages, whereas the combination of NuQ® biomarkers significantly improved the sensitivity at all stages of CRC.

Detection of up to 91% of CRC cases

We evaluated the cumulative performance of c-nucleosome biomarkers alone, in combination with CDA and adjusted for age using a multivariate analysis. At 90% of specificity, the tumor marker CEA gave a sensitivity of 35%. Combination of c-nucleosomes increased the sensitivity to 74% in CRC and to 91% in c-nucleosome biomarker panel increased the sensitivity to 91%.

Demographic of the study group

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.

\* \* \*