A novel and noninvasive FIT, DNA, mRNA and machine learning / AI-based early Colorectal Cancer and Advanced Adenoma detection test

First interim data review of the international COLOFUTURE study

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INTRODUCTION

Colorectal cancer (CRC) ranks as the **second leading cause** of global cancer mortality, with incidence particularly rising among younger demographics. Early detection of polyps and lesions that can progress to CRC at the **advanced adenoma (AA) stage** or earlier is crucial as it prevents them from progressing further and leads to better treatment outcomes. As colonoscopy participation rates remain poor, reliable noninvasive screening methods like Fecal Immunochemical Test (FIT) or novel alternatives are needed. Here, we describe a **new approach** applying state-of-the-art PCR based **DNA and mRNA-based screening** in combination with FIT and a ML/AI driven algorithm, leading to a highly sensitive and specific clinical test for CRC and AA.

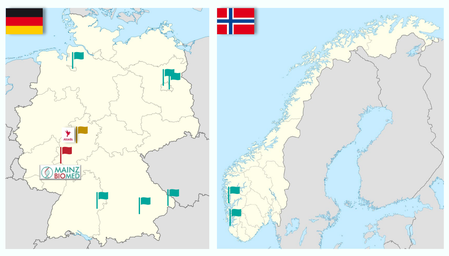
OBJECTIVES

- Interim analysis of the clinical performance of a novel noninvasive FIT, DNA and mRNA-based CRC screening test using a proprietary reagent stabilization formulation, small stool sample volumes, state-of-the art machine learning (ML) and an artificial intelligence (AI) driven algorithm.
- Demonstrate high-confidence clinical classification based on minimal sample input.
- Discrimination of CRC and its precursor lesions (including AAs) from normal control samples with high sensitivity and specificity.

METHODS

COLOFUTURE Trial Overview

- Prospective, multi-center study across Germany and Norway.
- Study participants were 40-85 years old, referred for colonoscopy (screening or diagnostic), or had a diagnosis of colorectal adenocarcinoma with no previous treatment.



Laboratory Process Overview

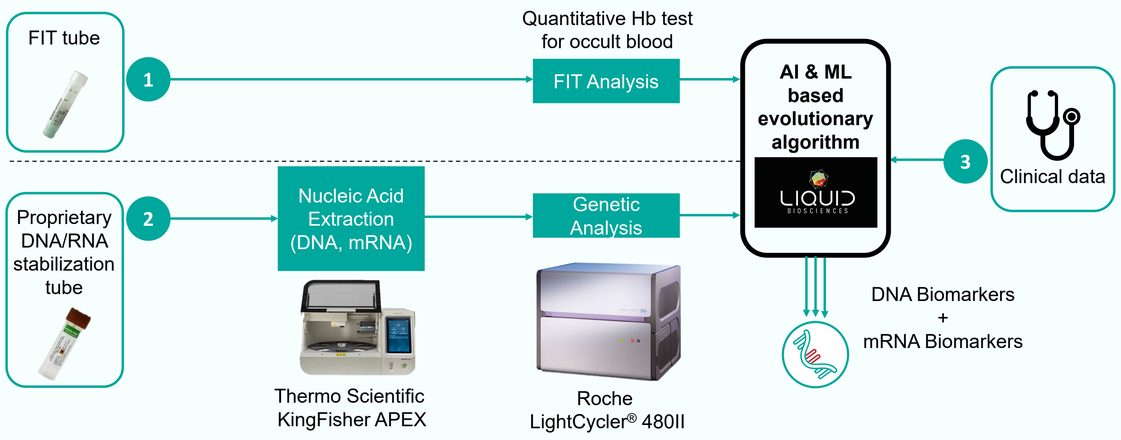


Figure 1: COLOFUTURE laboratory process overview.

- Occult blood was determined by hemoglobin quantification.
- The proprietary DNA/mRNA stool collection tube and sample underwent an automated silica-bead based extraction method followed by (RT)-qPCR for novel mRNA and DNA biomarkers.
- The mRNA biomarkers were analyzed by absolute and relative quantification approaches. For relative quantification, normalization by a housekeeping gene (GAPDH) was used.
- The artificial intelligence/ machine learning algorithm was developed in partnership with Liquid Biosciences of Aliso Viejo, California.

COLOFUTURE Patient Age and Diagnosis

	Mean age	Control	Non-AA	AA	CRC	All groups
Male	62.2	56	6	37	23	122
Female	62.4	61	4	18	15	98
All genders	62.3	117	10	55	38	220

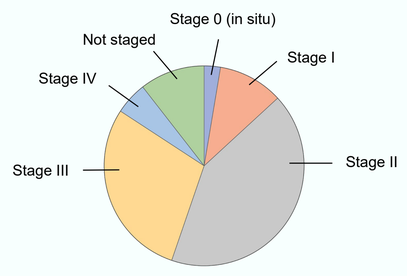
RESULTS

Genetic Biomarkers Analyzed

Biomarker	Name	Function	Target
BRAF, somatic	Serine/ threonine kinase	MAP kinase/ ERK signaling pathway	DNA
KRAS, somatic	Kirsten ras oncogene homolog	GTPase, regulation of cell proliferation	DNA
B2M	Beta-2-microglobulin	Antigen presentation in immune system	mRNA
CEACAM5	Carcinoembryonic antigen-related cell adhesion molecule 5	Cell adhesion, Intracellular signaling	mRNA
ITGA6	Integrin alpha-6 heavy chain	Cell surface adhesion and signaling	mRNA
MACC1	Metastasis-associated in colon cancer protein 1	Transcription activator	mRNA
PTGS2	Prostaglandin G/H synthase 2	Production of inflammatory prostaglandins	mRNA
S100A4	S100 calcium binding protein A4	Regulation of cellular processes such as cell cycle progression and differentiation	mRNA
GAPDH	glyceraldehyde-3-phosphate dehydrogenase	Moonlighting protein based on its ability to perform mechanistically distinct functions	mRNA

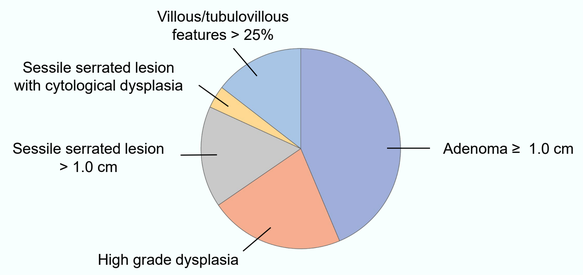
Distribution of Pathological Results for CRC and AA

Colorectal Cancer



Total: 38

Advanced Adenomas



Total: 55

Clinical Performance

Performance of advanced nucleic acid screening for AA and CRC combined with classical FIT. Performance is displayed as sensitivity and specificity for CRC, AA and a combined group of diseased subjects with two-sided 95% Clopper-Pearson confidence intervals (CI).

	DNA+mRNA+FIT		FIT only*	
Category	**Sensitivity (%)**	**Specificity (%)**	**Sensitivity (%)**	**Specificity (%)**
CRC vs. Normal + non-AA (95% confidence interval)	**94.4** (81.3-99.3)	**97.5** (92.9-99.5)	**73.7** (56.9-86.6)	**93.7** (88.0-97.2)
AA vs. Normal + non-AA (95% confidence interval)	**80.0** (66.3-90.0)	**95.2** (89.8-98.2)	**29.1** (17.6-42.9)	**93.7** (88.0-97.2)
CRC + AA vs. Normal + non-AA (95% confidence interval)	**87.1** (78.5-93.2)	**92.9** (87.0-96.7)	**47.3** (36.9-57.9)	**93.7** (88.0-97.2)

**calculated on all available samples*

CRC Stage Sensitivities

Stage	Pathology staging*	Detected	Sensitivity (%)
Stage 0	1/34	0	**0**
Stage 1	4/34	4	**100**
Stage 2	16/34	15	**94**
Stage 3	11/34	11	**100**
Stage 4	2/34	2	**100**

** 4 CRC samples were not staged at the time of the interim analysis*

Sensitivity for high grade dysplasia: 75% (9/12)

CONCLUSION

Applying this novel approach, **sensitivity** for detection of **CRC** was found to be **94.4%** while **precursor lesions including AAs** were detected with **80% sensitivity**. Specificity (negative colonoscopies plus non-AAs) was calculated to be 97.5% and 95.2% for CRC and AA, respectively. This innovative setup represents the inaugural instance of a multimodal analysis involving DNA, mRNA expression, FIT results and an AI/ML developed algorithm. Results indicate a substantial and meaningful enhancement in the effectiveness of non-invasive CRC screening, particularly for the detection of AA, where increased sensitivity is urgently needed to significantly reduce CRC incidence and mortality.

References:

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