



# Update on TRIDENT, a three-gene biomarker assay for colorectal neoplasia

EDRN Steering Committee-GI Collaborative  
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*better detection is essential to improving survival*





## COLVERA™

(in market)

### Monitoring

#### Value Proposition:

Timely identification saves lives <sup>(1,2)</sup>



## COLVERA™

#### Product:

- 2-gene ctDNA blood test for CRC recurrence monitoring
- 68% sensitivity and 93% specificity <sup>(3)</sup>
- Sophisticated PCR methods deliver performance at low cost to deliver superior health economics

#### Goal:

- Detect recurrence when case amenable to curative intent <sup>(4)</sup>
  - 80% of recurrences occur within 2 years of primary treatment <sup>(4)</sup>
  - Detects > 2x the cancers as the current standard of care <sup>(5)</sup>

## TRIDENT PROGRAM

#### Product:

- 3-gene ctDNA blood test with IC2 technology for CRC screening
- 74% sensitivity and 91% specificity <sup>(7)</sup>
- Sophisticated PCR methods deliver performance at low cost to deliver superior health economics even used on an annual basis

#### Goal:

- A blood test offers more compliant annual option <sup>(8)</sup>
- Address unscreened population
  - Blood test can be part of annual testing panel



## TRIDENT PROGRAM

(in development)

### Screening

#### Value Proposition:

Improved detection saves lives <sup>(6)</sup>





- TRIDENT identifies **circulating fragments of tumor DNA (ctDNA)** in blood
- Uses a sensitive PCR-based method to detect three genes (*BCAT1/IKZF1/IRF4*) **methylated** (or silenced) in colorectal cancer
- ***Does not*** depend on the presence of specific gene mutations commonly found in subsets of CRC cases (i.e. *KRAS, BRAF*)

# TRIDENT Biomarkers

## IKZF1: IKAROS family zinc finger 1

- Involved in cell differentiation control through Notch signaling pathway
- Hypermethylation down regulates DNA repair (MSH2), up regulates cell-cycle progression genes, inhibits apoptosis and stem cell renewal
- Directly regulates c-myc and IRF4

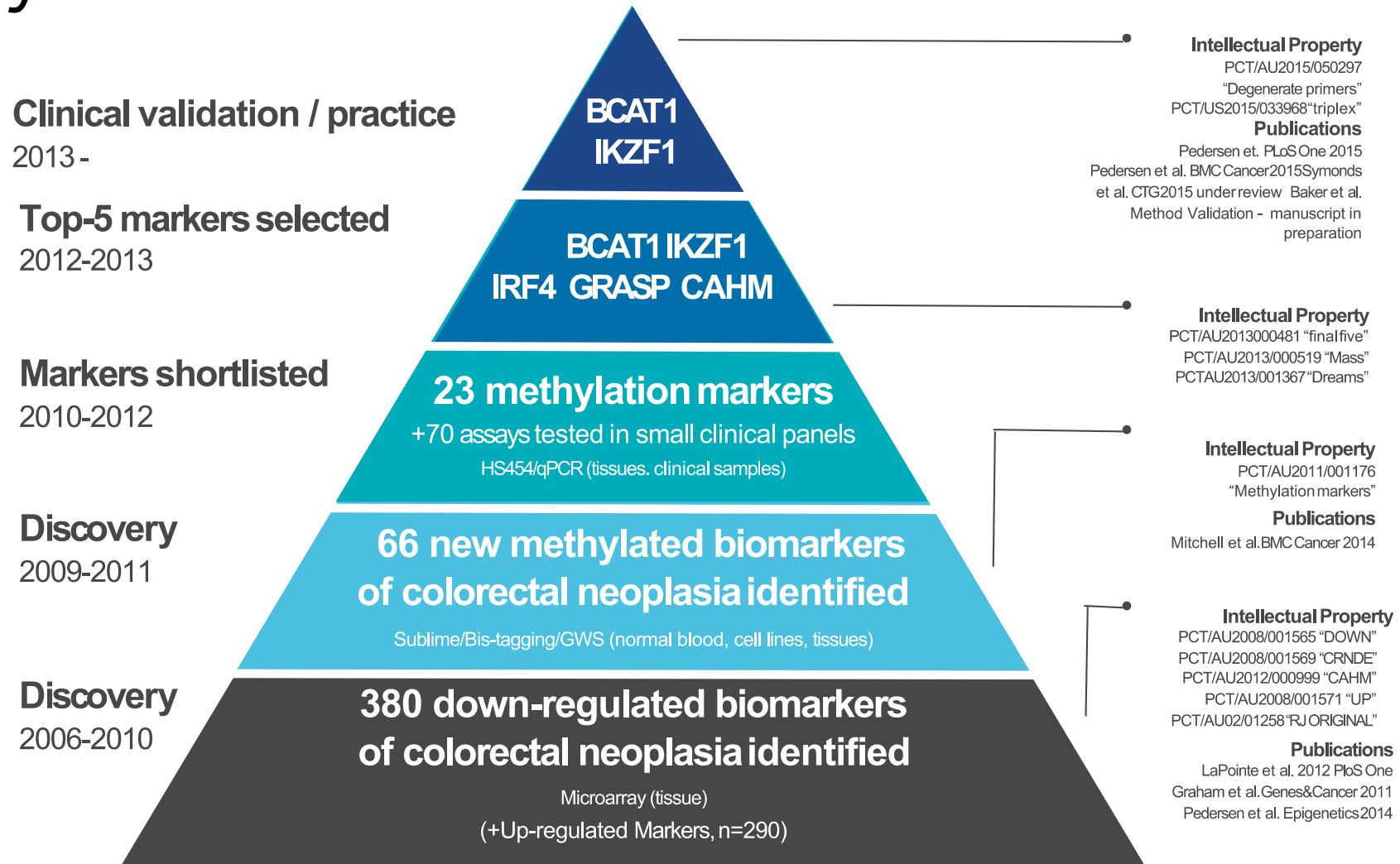
## IRF4: Interferon Regulatory Factor 4

- Regulation of immune response, apoptosis, cell-cycle control

## BCAT1: Branched chain amino acid transaminase 1

- Regulates catabolism of essential branched-chain amino acids (VAL, LEU, ILE) for energy purposes
- Regulated by c-myc
- Implicated in methylation status through control of alpha keto-glutarate levels and

# Discovery...





# Validation.

<https://www.clinicalgenomics.com/technology.html>

## 2019

Symonds et al. [A randomised controlled trial testing provision of fecal and blood test options on participation for colorectal cancer screening](#). Cancer Prevention Research, 2 July, 2019.

## 2018

Saluja et al. [The Use of Circulating Tumor DNA for Prognosis of Gastrointestinal Cancers](#). Frontiers in Oncology, 24 July 2018.

Murray et al. [Relationship between post-surgery detection of methylated circulating tumor DNA with risk of residual disease and recurrence-free survival](#). Journal of Cancer Research and Clinical Oncology, 10 July 2018.

Symonds et al. [Circulating tumour DNA for monitoring colorectal cancer—a prospective cohort study to assess relationship to tissue methylation, cancer characteristics and surgical resection](#). Clinical Epigenetics, 16 May 2018.

Jedi et al. [Methylation and Gene Expression of BCAT1 and IKZF1 in Colorectal Cancer Tissues](#). Clinical Medicine Insights: Oncology, 10 May 2018.

## 2017

Shapiro et al. [A Comparison of Fecal Immunochemical and High-Sensitivity Guaiac Tests for Colorectal Cancer Screening](#). Am J Gastroenterol advance online publication, 10 October 2017; doi: 10.1038/ajg.2017.285

Murray et al. [validation-of-a-circulating-tumor-derived-dna-blood-test-for-detection-of-methylated-bcat1-and-ikzf1-dna.pdf](#)Validation of a Circulating Tumor-Derived DNA Blood Test for Detection of Methylated BCAT1 and IKZF1 DNA. JALM 2017, doi: 10.1373/jalm.2017.023135

## 2016

Mitchell et al. [Evaluation of Methylation Biomarkers for Detection of Circulating Tumor DNA and Application to Colorectal Cancer](#). Genes 2016, 7(12), 125; doi:10.3390/genes7120125

Young et al. [A Cross-sectional Study Comparing a Blood Test for Methylated BCAT1 and IKZF1 Tumor-derived DNA with CEA for Detection of Recurrent Colorectal Cancer](#). Cancer Medicine. doi:10.1002/cam4.868

Symonds et al. [A Blood Test for Methylated BCAT1 and IKZF1 vs. a Fecal Immunochemical Test for Detection of Colorectal Neoplasia](#). Clin Trans Gastro. 2016; 7, e137.

## 2015

Pedersen et al. [a-two-gene-blood-test-for-methylated-dna-sensitive-for-colorectal-cancer.pdf](#)A Two-Gene Blood Test for Methylated DNA Sensitive for Colorectal Cancer. PLoS One 2015;10(4):e0125041.

Symonds et al. [Improving Participation in Colorectal Cancer Screening: a Randomised Controlled Trial of Sequential Offers of Fecal then Blood Based Non-Invasive Tests](#). Asian Pac J Cancer Prev, 16 (18), 8455-8460.

Pedersen et al. [Evaluation of an Assay for Methylated BCAT1 and IKZF1 in Plasma for Detection of Colorectal Neoplasia](#). BMC CANCER 15(1):654, October 2015.

## 2014

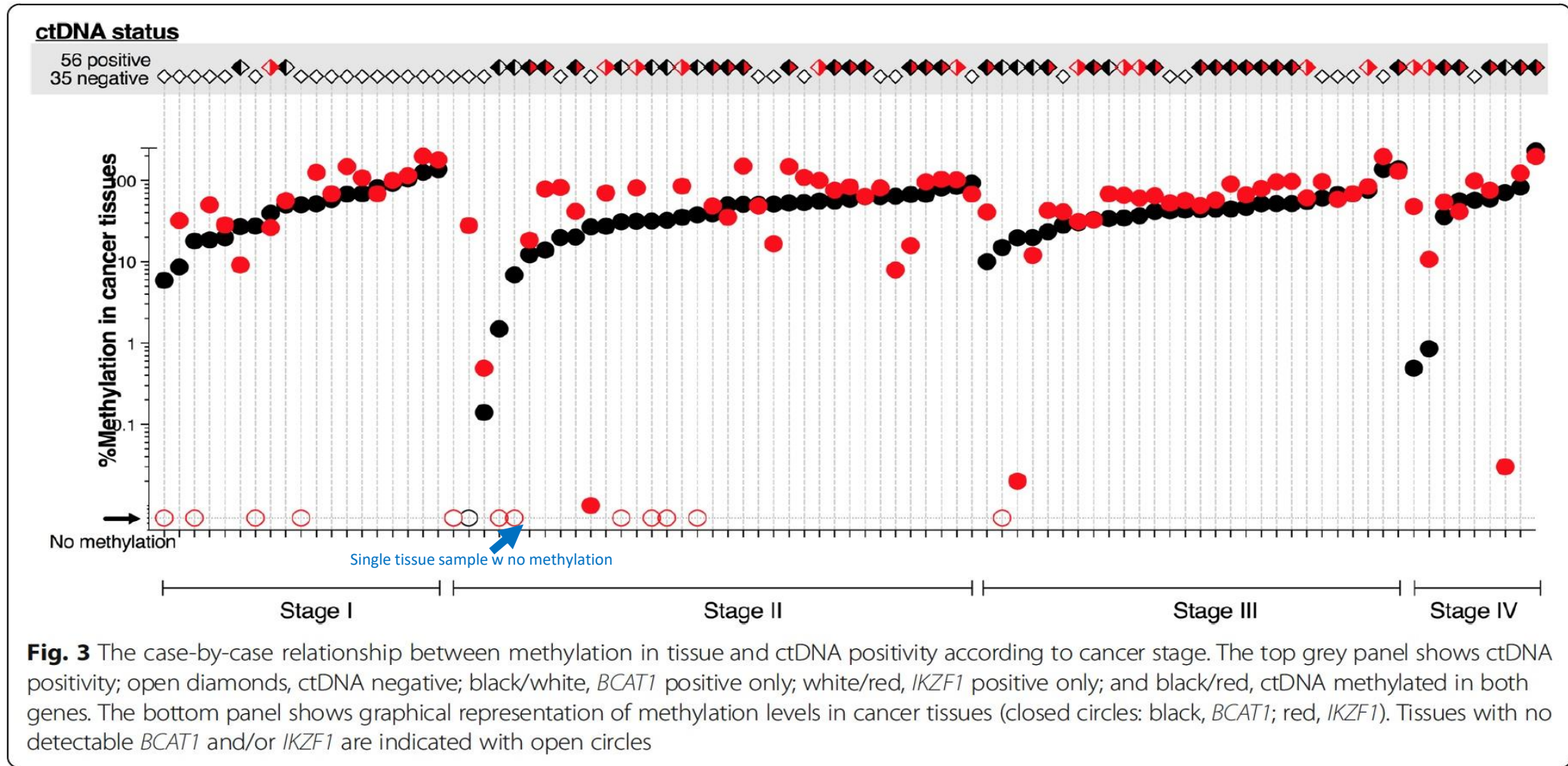
Mitchell et al. [A Panel of Genes Methylated with High Frequency in Colorectal Cancer](#). BMC Cancer. 2014;14:54.

Pedersen et al. [CAHM, a Long Non-Coding RNA Gene Hypermethylated in Colorectal Neoplasia](#). Epigenetics. 2014 Aug;9(8):1071-82.

# BCAT1/IKZF1 methylation

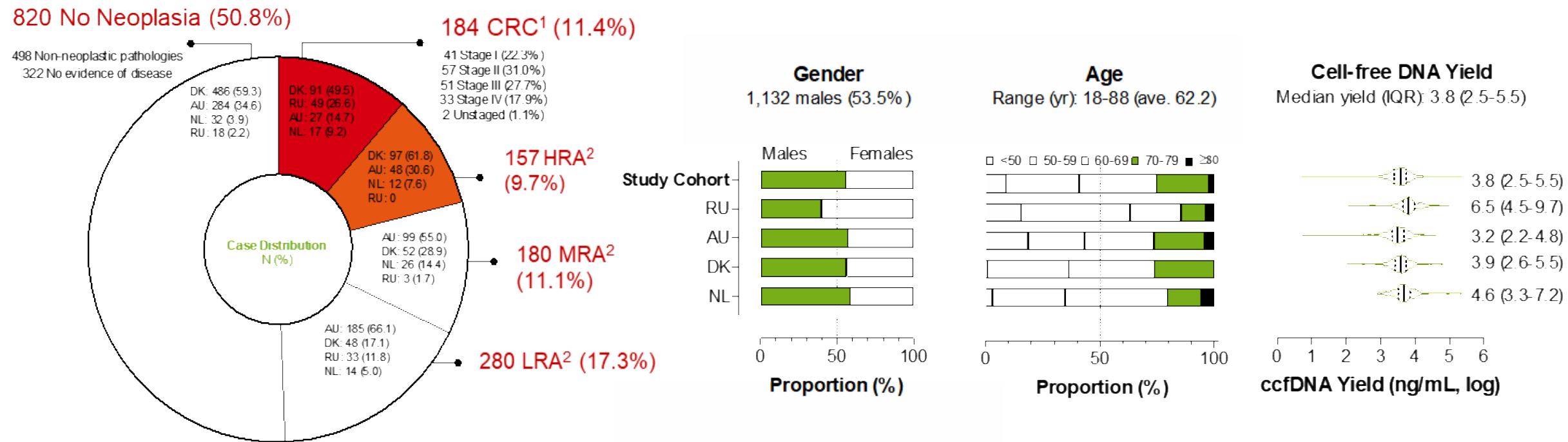
Symonds et al, *Clinical Epigenetics*. 2018

Of 91 tumor tissue samples, 90 methylated in one or both target genes





# TRIDENT: Initial validation cohort (2019)



<sup>1</sup> CRC – invasive adenocarcinoma

<sup>2</sup> Adenomas were classified as high- (HRA), medium- (MRA) or low-risk (LRA). [Vieth M et al. Endoscopy 2012;44 (Suppl 3):131].

# TRIDENT: Initial validation cohort (2019)

Diagnosis	N	Biomarker Positivity Rate (% 95% Conf Interval)
<b>CRC</b>	<b>184</b>	<b>73.9% (68-80)</b>
Stage I	41	39.0% (23-55)
Stage II	57	87.7% (79-97)
Stage III	51	78.4% (67-90)
Stage IV	33	84.8% (72-98)
Stage Uncertain	2	100% (16-100)
<b>Adenoma</b>	<b>616</b>	<b>12.8% (10-15)</b>
Low-risk Adenoma	279	9.3% (6-13)
High-risk Adenoma	337	15.7% (12-20)
<b>No Neoplasia</b>	<b>820</b>	<b>9.9% (8-12)</b>
Non neoplastic pathology	498	11.6% (8.8-15)
Normal	322	7.1% (4-10)

## Clinical cohort:

- 1,620 plasma specimens from enriched, screening age population – all colonoscopically confirmed.

## TRIDENT assay:

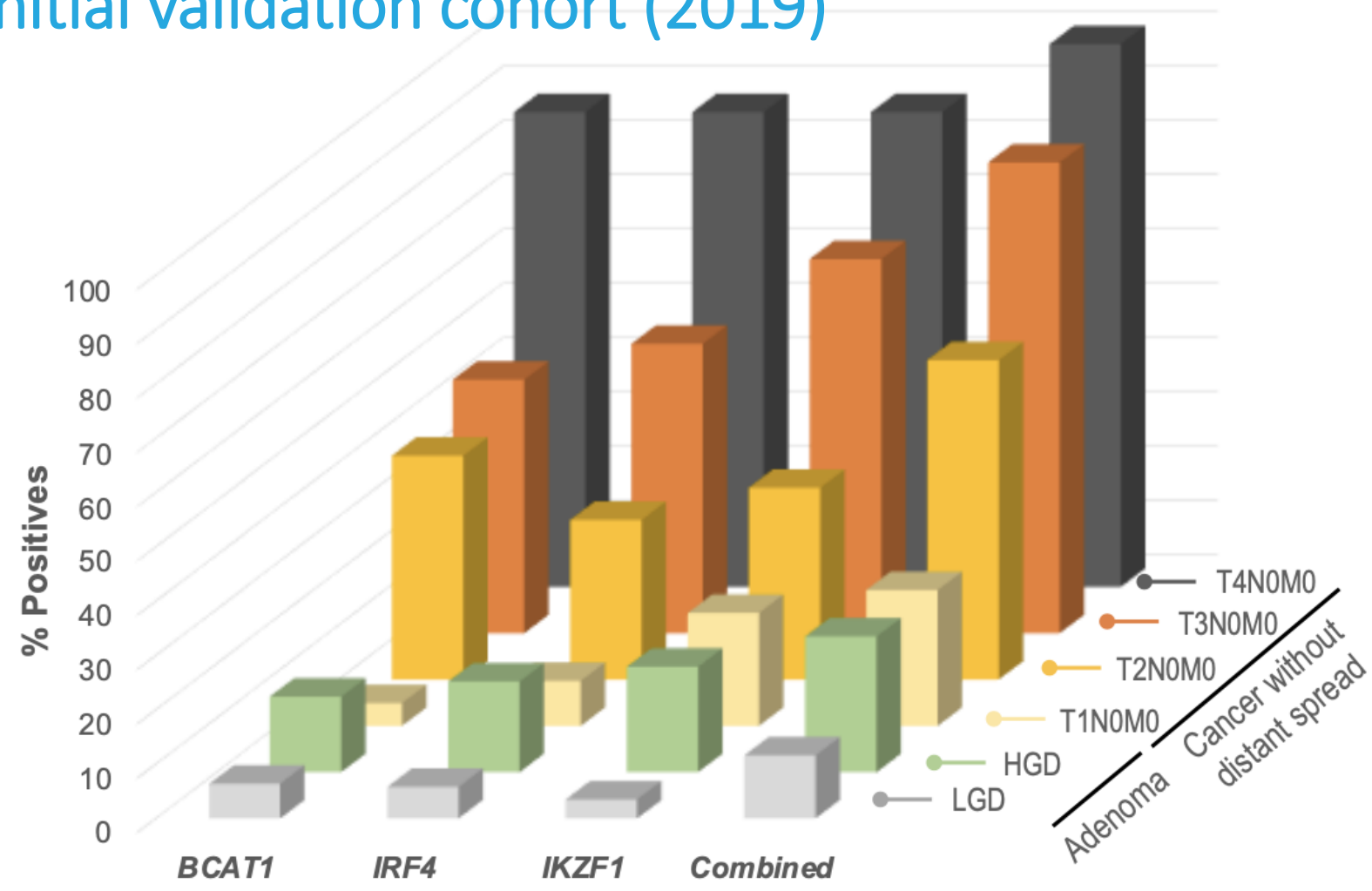
- *BCAT1*, *IKZF1* and *IRF4* genes.
- ~30 CpG targets

**Sensitivity for CRC was 73.9% (67.1 – 79.7).**

**Specificity for neoplasia was 90.1% (88.0-92.0).**

SK Pedersen, et al. DDW 2020, May 2-5, 2020. Manuscript under review.

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# Next steps

Building from Colvera® foundation (>4,000 subjects assayed, published)

TRIDENT: Design complete

- Initial validation cohort (1,600+ patients) submitted for publication

Positive discussions with FDA and CMS

Collaboration with GLNE team @ EDRN

- Applying GLNE007 + GLNE010 specimens to FDA pivotal trial
- Challenge: ensuring specimens from screen-relevant subjects are used

Additional validation partnerships under way: Open door to collaborators

# THANK YOU





# 5 minute Q&A

Chair/Co-Chair/NCI

feed Zoom Chat questions to presenter  
and Track Time

NCI and Production Team

answer Chat questions not related to presentations  
and use Slack