Signal Genetics Announces Publication of Meta-Analysis of its Colon Cancer Test-Previstage™ GCC Confirming Prognostic Capabilities in Patients with Colon Cancer.

New York, NY, January 23, 2012 – Signal Genetics, a privately held personalized medicine company focused on cancer, announced today the presentation of a paper entitled "Guanylyl Cyclase C (GCC) Lymph Nodes (LN) Classification as a Prognostic Marker in Patients with Stage II Colon Cancer: A Pooled Analysis" at the 2012 American Society of Clinical Oncology Gastrointestinal Cancer Symposium held in San Francisco, CA January 21, 2012. The data from the paper demonstrate the value of implementing Previstage testing in patients with colorectal cancer to identify those patients at risk of relapse.

The paper is the culmination of the work conducted by a team of researchers and collaborators from several centers, including: Rhode Island Hospital, Brown University, University of Massachusetts Medical School, University of North Carolina, Lahey Clinic, Brigham and Women's Hospital, British Columbia Cancer Agency and DiagnoCure Inc. These researchers conducted a pooled individual data analysis on 310 patients to confirm whether molecular detection of GCC in Lymph Nodes indicates high risk of disease recurrence and poor survival in untreated stage II colon cancer.

GCC is a colon-specific biomarker normally found in gastrointestinal epithelium whose expression is preserved in primary and metastatic colorectal cancer cells. Studies to date have suggested that the presence of GCC gene expression in lymph nodes increased the likelihood of disease recurrence in stage II colon cancer patients, independent of traditional high risk features. The results of this study suggest that detection of GCC mRNA in lymph nodes is associated with risk of disease recurrence in stage II colon cancer patients not treated with adjuvant chemotherapy. These findings are consistent with several other studies conducted over the past 10 years.

Based on GCC levels, the estimated 5 year recurrence risks were 11% and 32% for the low and high risk groups respectively, clearly showing that GCC is a strong prognostic marker that effectively stratifies patients between those that are essentially cured from those at risk of disease recurrence. Higher detection levels of GCC in lymph nodes is also significantly associated with increased risk of all-cause mortality, disease-specific survival, and disease-free survival.

According to Joe Hernandez, President and CEO of Signal Genetics, "This paper represents further validation of GCC as a strong prognostic test that provides physicians and their patients an important insight that helps them make critical treatment decisions."

About Signal Genetics
Signal Genetics, the parent company of Myeloma Health, Respira Health, and CC Health, is a privately held personalized medicine genetic testing company focused on bringing novel insights to physicians and their patients with various types of cancer. The goal of Signal Genetics is to provide information regarding disease status, stage, odds of relapse, predicting response to therapy, and prognosis through an array of proprietary tools to help guide physicians to the optimal treatment for each individual patient. Additional information is available at www.signalgenetics.com.

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Guanylyl Cyclase C (GCC) Lymph Nodes (LN) Classification as a Prognostic Marker in Patients with Stage II Colon Cancer: A Pooled Analysis

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Abstract #441

Background

- Heterogeneity in risk of recurrence exists in stage II CC patients
- Molecular markers and gene signatures have been recently evaluated to identify patients with a higher risk of recurrence.
- GCC is a colon-specific biomarker normally found in gastrointestinal epithelium whose expression is preserved in primary and metastatic colorectal cancer cells[1]
- Preliminary studies have suggested that the presence of GCC gene expression in LN increased the likelihood of disease recurrence in stage II CC patients, independently of traditional high risk features[2-3]
- A GCC LN risk classification was established based on the analysis on pre-planned training set in a multi-center cohort study[4]

Objective

We conducted a pooled individual data analysis to confirm whether molecular detection of GCC in LNs indicates high risk of disease recurrence and poor survival in untreated stage II CC.

Methods

- **GCC expression**
  - GCC mRNA was quantified by RT-qPCR using formalin fixed LN tissues from untreated stage II CC patients blinded to clinical outcome.
  - Individual LN GCC status was determined by relative quantification of GCC (ΔCt = Ct GUSB – Ct GCC) with a validated cut-off (-5.9).
- **Populations**
  - **Sargent 2011[4]: N = 241,**
    - 5-year recurrence rate = 15.8%
    - Median age = 74 yrs, range = 34 – 96 yrs
    - Male = 42%
    - Surgery in 2004 – 2006: 47%
    - Grade: G1 = 8%; G2 = 74%; G3 = 18%
    - T stage: T3 = 95%; T4 = 5%
  - **Haince2010[5]: N = 69,**
    - 5-year recurrence rate = 24.9%
    - Median age = 68 yrs, range = 23 – 94 yrs
    - Male = 41%
    - Surgery in 2004 – 2006: 6%
    - Grade: G1 = 24%; G2 = 58%; G3 = 18%
    - T stage: T3 = 99%; T4 = 1%

Table 1 Baseline Characteristics (n=310)

	LNR 0 -0.1 (N=207)	LNR 0.1+ (N=103)	Total (N=310)	p value
Age at Surgery *, years**				0.7447
Median	73.0	71.0	72.5	
Range	(23.0-94.0)	(34.0-96.0)	(23.0-96.0)	
Race, n (%) **				0.0067
African American	11 (7.3%)	14 (17.9%)	25 (10.9%)	
Asian	0 (0.0%)	1 (1.3%)	1 (0.4%)	
Caucasian	140 (92.7%)	62 (79.5%)	202 (88.2%)	
Hispanic	0 (0.0%)	1 (1.3%)	1 (0.4%)	
Gender, n (%) *				0.4928
Female	123 (59.4%)	57 (55.3%)	180 (58.1%)	
Male	84 (40.6%)	46 (44.7%)	130 (41.9%)	
Surgery Year Group, n (%) *				0.1502
1993-2003	134 (64.7%)	58 (56.3%)	192 (61.9%)	
2004-2006	73 (35.3%)	45 (43.7%)	118 (38.1%)	
Tumor Grade, n (%) *				0.9776
G1	23 (11.3%)	11 (10.7%)	34 (11.1%)	
G2	144 (70.9%)	73 (70.9%)	217 (70.9%)	
G3	36 (17.7%)	19 (18.4%)	55 (18.0%)	
T stage, n (%) **				0.7654
T3	199 (96.1%)	98 (95.1%)	297 (95.8%)	
T4	8 (3.9%)	5 (4.9%)	13 (4.2%)	
LVI, n (%) *				0.3269
No	166 (87.4%)	84 (83.2%)	250 (85.9%)	
Yes	24 (12.6%)	17 (16.8%)	41 (14.1%)	
LN Assessed, n (%) *				0.4239
<12	42 (20.3%)	17 (16.5%)	59 (19.0%)	
>= 12	165 (79.7%)	86 (83.5%)	251 (81.0%)	

Abbreviations: LVI, Lymphovascular Invasion; LN, Lymph Node; LNR, Lymph Node Ratio
*Chi-Squared test; ** Fisher's exact test; ***Wilcoxon rank-sum test

Statistical Analyses

- **Clinical outcomes:**
  - **Time to Recurrence (TTR)** -- time from surgery to first event of recurrence (local or distant), or death related to first primary
  - **Overall Survival (OS)** – time from surgery to death due to any cause
  - **Disease-specific survival (DSS)** – time from surgery to death due to disease
  - **Disease-free survival (DFS)** – time from surgery to first event of recurrence or death due to any cause
- **LN ratio (LNR)**: number of positive nodes by GCC divided by number of informative nodes
  - **High risk group:** LNR ≥ 0.1
  - **Low risk group:** LNR < 0.1
- **Stratified log-rank testused to examine the relationship between GCC LN risk classification and primary and other clinical outcomes.**
- **Stratified Cox models (univariate and multivariate) used to estimated unadjusted and adjusted Hazard Ratios (HRs), comparing the risk of recurrence and/or death between risk groups defined by GCC LNR values**

Figure 1A: TTR

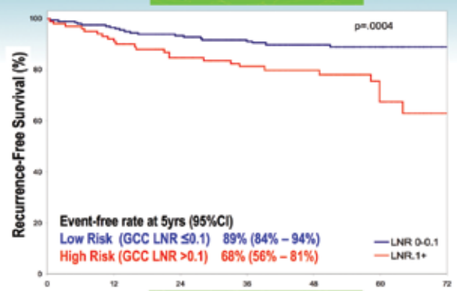

Figure 1B: OS

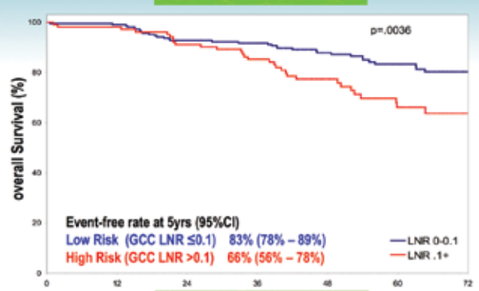

Figure 1C: DSS

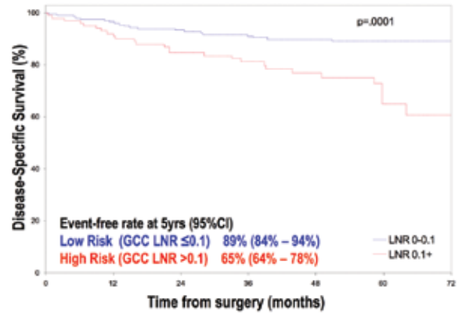

Figure 1D: DFS

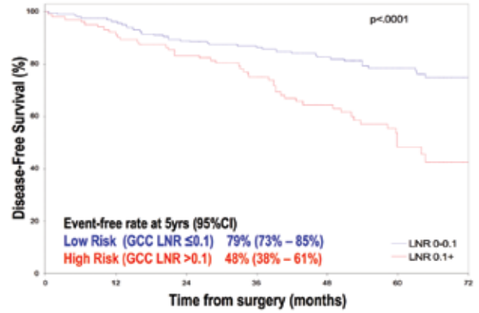

Table 2 Unadjusted and Adjusted HRs comparing outcomes between high and low risk groups

Table 2A: Overall stage II pts (N = 310)

Study Endpoint	LNR Risk Group	Univariate Analysis HR (95% CI)	P	Multivariate Analysis* HR (95% CI)	P
TTR	0.1 +	2.988 (1.65, 5.42)	0.0003	2.635 (1.45, 4.78)	0.0014
	0 – 0.1	ref		ref	
OS	0.1 +	1.831 (1.17, 2.87)	0.0083	1.724 (1.06, 2.80)	0.0279
	0 – 0.1	ref		ref	
DSS	0.1 +	3.074 (1.73, 5.47)	0.0001	2.698 (1.51, 4.81)	0.0008
	0 – 0.1			ref	
DFS	0.1 +	2.234 (1.51, 3.31)	<.0001	2.160 (1.42, 3.28)	0.0003
	0 – 0.1	ref		ref	

*Adjusting for age, T stage, grade, number of LNs examined, and presence of lymphovascular invasion

Table 2B: Low risk stage II pts (N = 241, T3, ≥ 12 LNs examined, negative surgica l margins)

Study Endpoint	LNR Risk Group	Univariate Analysis HR (95% CI)	P	Multivariate Analysis* HR (95% CI)	P
TTR	0.1 +	4.344 (2.07, 9.13)	0.0001	3.859 (1.82, 8.16)	0.0004
	0 – 0.1				
OS	0.1 +	2.095 (1.21, 3.62)	0.0081	2.668 (1.42, 5.00)	0.0022
	0 – 0.1				
DSS	0.1 +	4.145 (2.03, 8.48)	<.0001	3.699 (1.79, 7.62)	0.0004
	0 – 0.1				
DFS	0.1 +	2.548 (1.61, 4.04)	<.0001	2.916 (1.76, 4.84)	<.0001
	0 – 0.1	ref		ref	

*Adjusting for age, grade, number of LNs examined, and presence of ly mphovascular invasion

Results

- In this pooled analysis, the prognostic value of the ratio of the number of GCC+ LNs over the total number of informative LNs was confirmed.
- High LNR significantly predicted higher recurrence risk. The estimated 5-yr recurrence risks were 11% and 32% for the low and high risk group. ***Figure 1A (HR = 2.98, 95% CI 1.65 – 5.42, p=0.0003)***
- Higher GCC LNR (≥ 0.1) is also significantly associated with increased risk of all cause mortality (HR = 1.83), disease-specific survival (HR=3.07), and disease-free survival (HR = 2.23).
- In a subset of pts with traditionally favorable prognostic factors (negative margins, T3 tumor, and ≥12 LNs examined), the GCC LNR had a HR for recurrence of 4.34 (95% CI 2.07-9.13, p=0.0001), translating into 5-yr recurrence rates of 8% among low risk patients and 34% for the high risk group.
- In a multivariate analysis adjusted for age, T stage, grade, number of LNs assessed, and LVI, the GCC LNR significantly predicted higher recurrence risk (HR, 2.64; 95% CI: 1.45-4.78, p=0.0014). ***Table 2A***

Conclusions

- Our results suggest that detection of GCC mRNA in LNs is associated with risk of disease recurrence in stage II CC patients not treated with adjuvant chemotherapy.
- The GCC LNR appears to have the greatest clinical utility for T3 patients with ≥ 12 LNs assessed, which constitute the majority of stage II CC patients.

References

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