Disclosure Statement

I have nothing to disclose

Antibody immunity to a panel of oncogenic proteins may predict presence of colon cancer and stage of disease.

Goodell V, Lu H, DeLong J, Disis M L.
Tumor Vaccine Group, Center for Translational Medicine in Women's Health, University of Washington; Seattle, WA.

Tumor-associated humoral immunity

- Patients with cancer can have tumor-associated antibody immunity, which is easily assessed by laboratory test.
- Antibody immunity to a single tumor associated antigen may be insufficient to be useful in predicting disease or outcome, but antibody immunity to a collection of antigens may have greater power to detect disease or predict outcome.
- Reports of antigen panel assays for detection of cancer have appeared in the literature, and support this hypothesis. For example…
- Erkanli et al. reported that responses to p53 alone did not discriminate between women with ovarian cancer and healthy controls, but a panel of 3 antigens resulted in an AUC of 86%.
  - Erkanli A, Taylor DD, Dean D, Eksir F, Egger D, Geyer J, Nelson BH, Stone B, Fritsche HA, Roden RBS. Application of Baysian modeling of autologous antibody responses against ovarian tumor-associated antigens. Cancer Res 66:1792-98.
- Zhang, et al. found that ~10-22% of patients with HCC had responses to a single TAA, but that the rate of positivity became progressively higher as 8 antigens were assessed to a final positive rate of~60%.
  - Zhang JY, Megliorino R, Peng XX, Tan EM, Chen Y, Chan EKL. Antibody detection using tumor-associated antigen mini-array in immunodiagnosing human hepatocellular carcinoma. J Hepatol 2007, 46:107-114.

Standards in biomarker development

- A multitude of studies on markers for cancer diagnosis/prognosis are reported, but very few reach a level of development which is clinically useful.

- The NCI Program for the Assessment of Clinical Cancer Tests (PACCT) and Reporting Recommendations for Tumor Marker Studies (REMARK) have issued guidelines for the study and report of potential tumor markers, and the Early Detection Research Network (EDRN) has proposed that biomarker studies follow a phased approach.

Phases of biomarker development

Preclinical Exploratory	**PHASE 1**	*Promising directions identified*
Clinical Assay and Validation	**PHASE 2**	*Clinical assay detects established disease*
Retrospective Longitudinal	**PHASE 3**	*Biomarker detects disease early before it becomes clinical and a "screen positive" rule is defined*
Prospective Screening	**PHASE 4**	*Extent and characteristics of disease detected by the test and the false referral rate are identified*
Cancer Control	**PHASE 5**	*Impact of screening on reducing the burden of disease on the population is quantified*

Pepe, M. S. et al. J. Natl. Cancer Inst. 2001 93:1054-1061.

JNCI

Phase 1: Identification of potential testing targets

- We hypothesized that assay of sera from patients with colorectal cancer and healthy controls could identify antigens which indicate presence of disease, and that antigens thus identified could be incorporated into a screening panel which distinguishes patients with colorectal cancer from healthy controls.

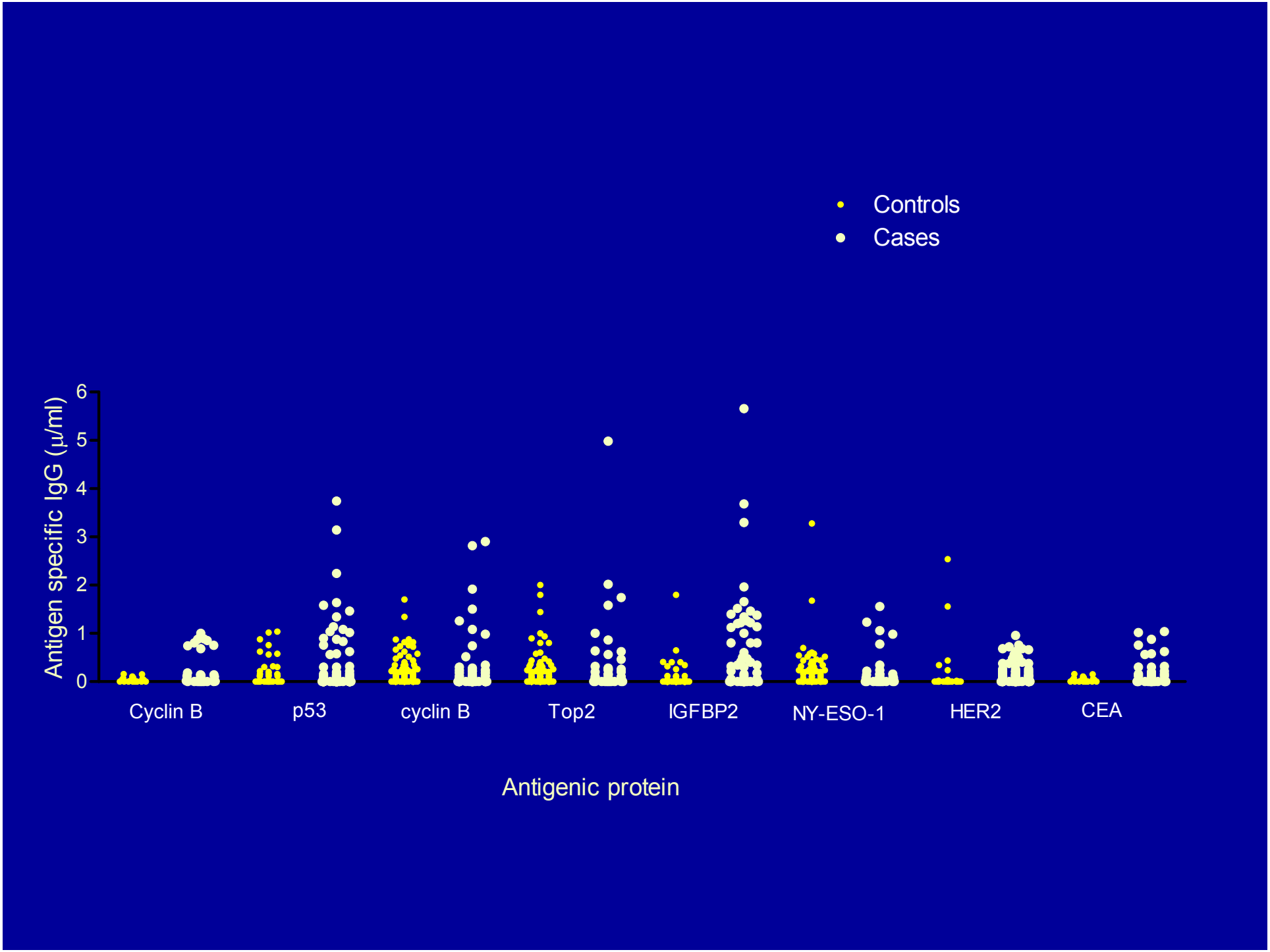
Phase 1: initial case/control sample set

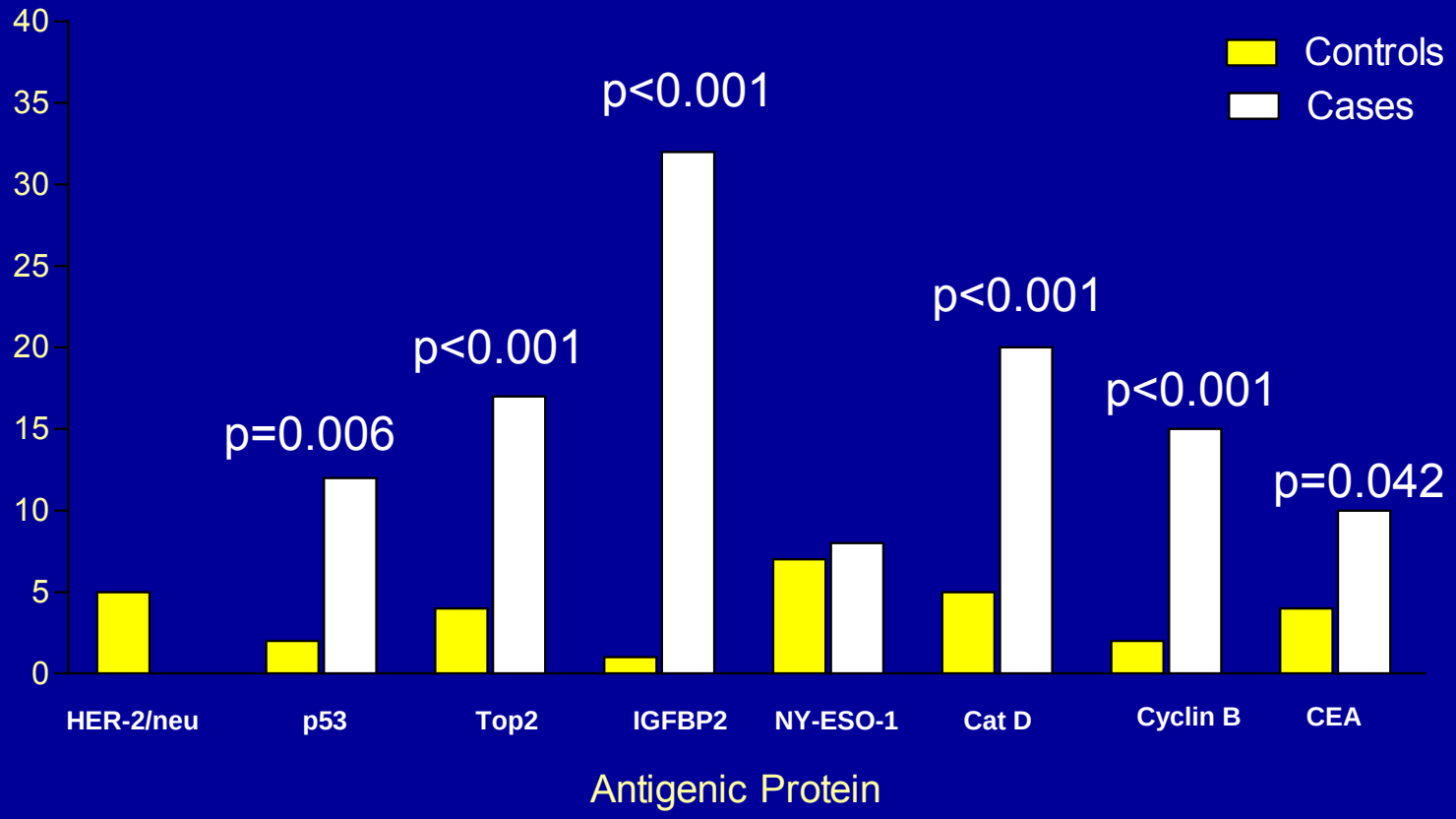
	Cases	Controls
Source	TVG serum repository	Puget Sound Blood Center
N	30	100
Disease state	Late stage (III/IV) colorectal cancer	Donors met standards for regional blood center
Sex	male & female	male & female
age	34-76	18-72
Total		130 samples

Phase 1 Assay

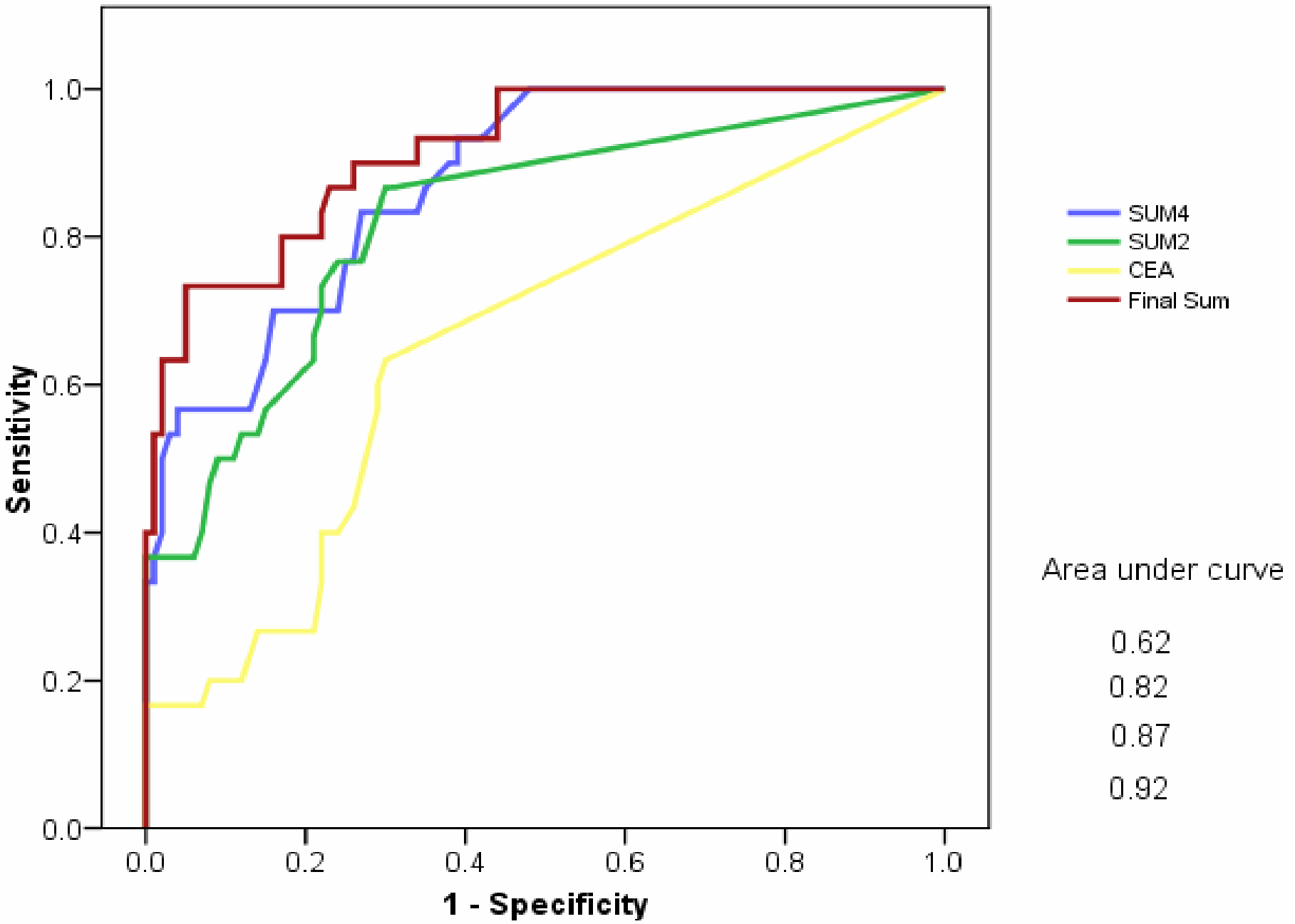
8 Antigens known to be antigenic in cancer patients and relevant to colon cancer:

Antigen	Expression in CRC	ELISA
p53	Overexpressed/mutated in ~50% of CRC	capture
HER-2/neu	Overexpressed in ~47% of CRC	capture
Cyclin B1	Overexpressed in ~57% of CRC	indirect
NY-ESO-1	Dysregulated expression in ~10% of CRC	indirect
CEA	Overexpressed/dysregulated in < 50% of CRC	indirect
Topoisomerase IIα	Expression in CRC associated with drug resistance and metastasis	indirect
IGFBP2	Expression associated with tumor growth in CRC	his-tag capture
Cathepsin D	Overexpressed in 42%-80% of CRC	indirect


Controls
Cases
Antigen specific IgG (µ/ml)
0
1
2
3
4
5
6
Cyclin B
p53
cyclin B
Top2
IGFBP2
NY-ESO-1
HER2
CEA
Antigenic protein



Based on results, we chose a panel of 6 antigens which stimulated significantly greater antibody responses in patients than in normal donors; p53, cathepsin D, CEA, cyclin B1, topoisomerase IIα and IGFBP-2.

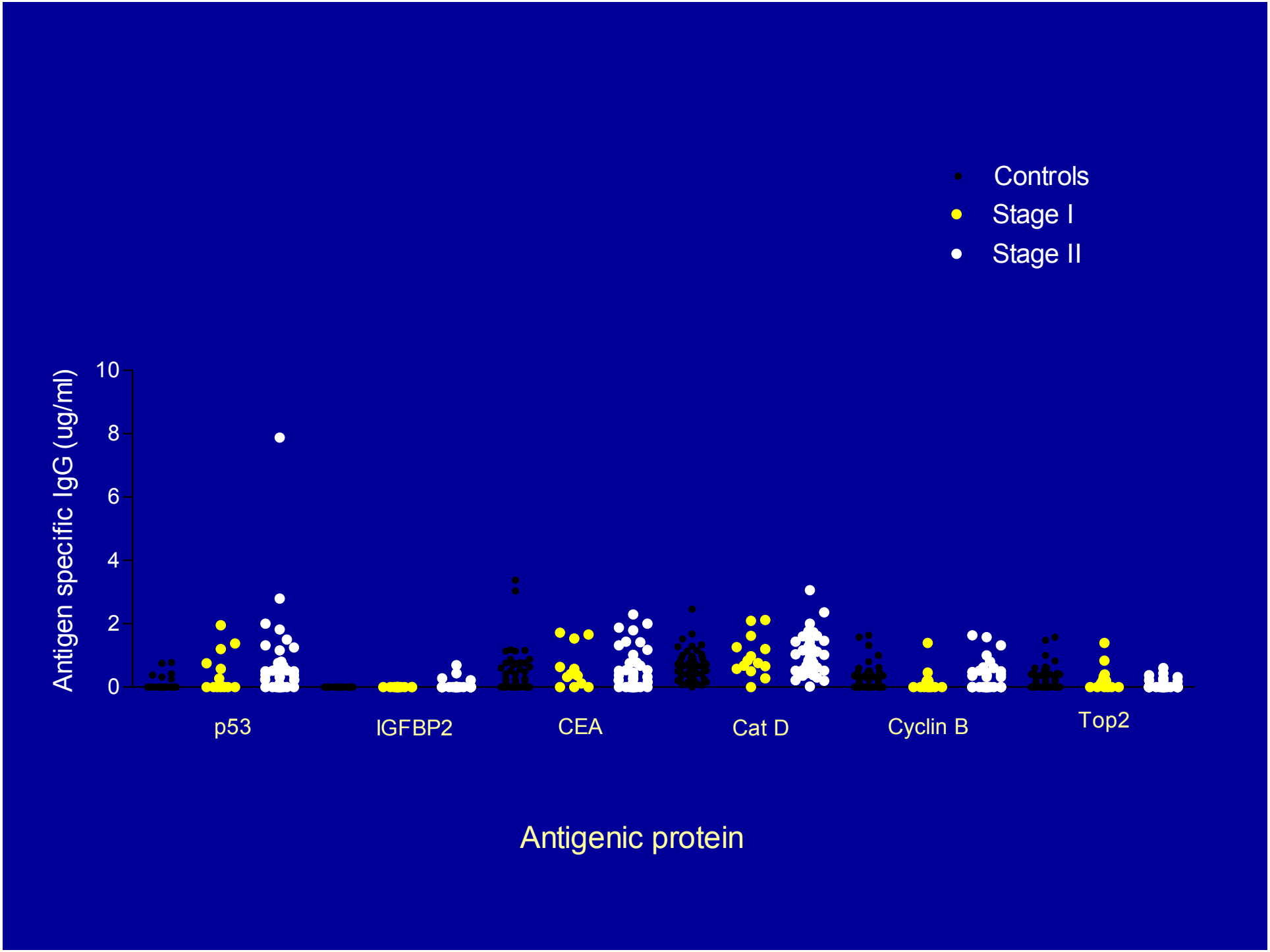

Sensitivity
1 - Specificity
0.0
0.2
0.4
0.6
0.8
1.0
SUM4
SUM2
CEA
Final Sum
Area under curve
0.62
0.82
0.87
0.92

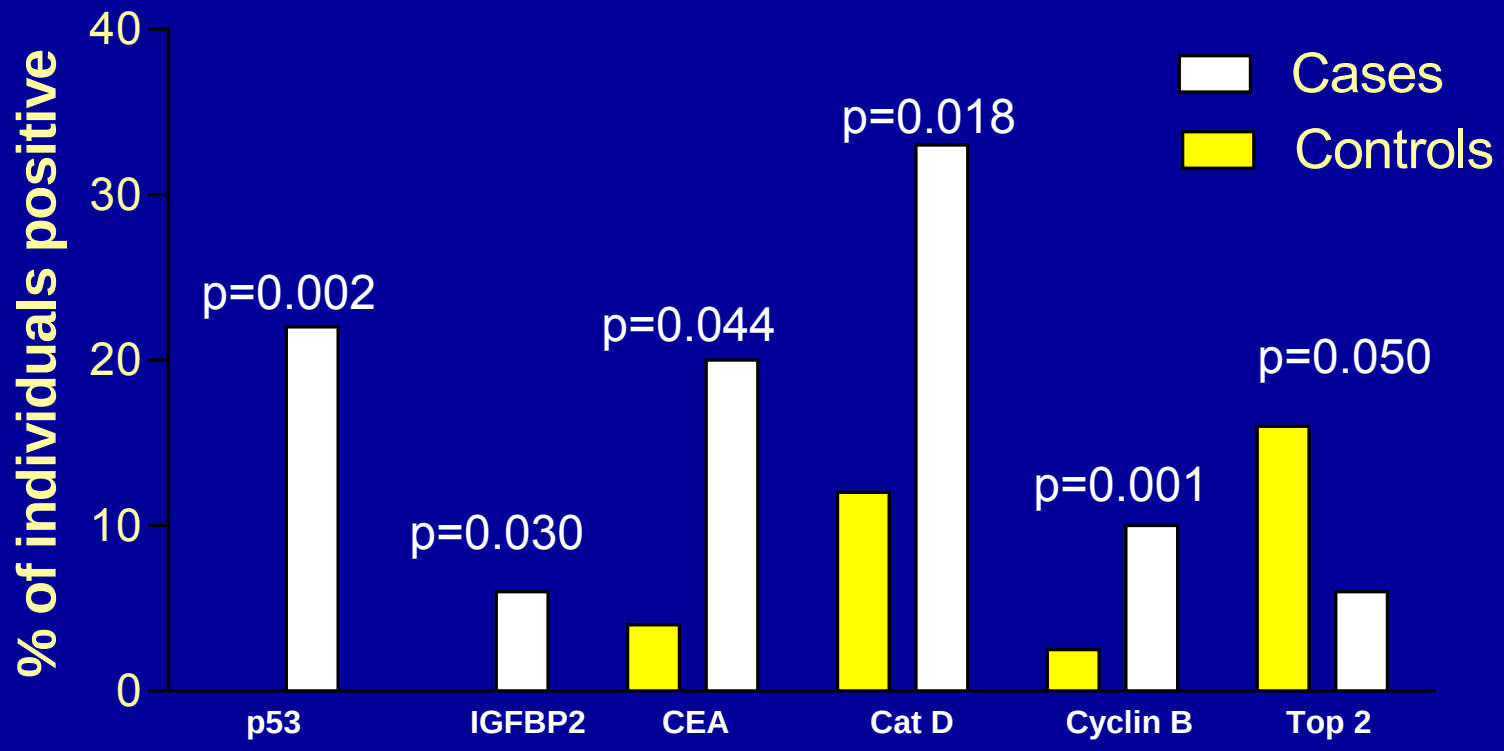
Phase 2: determine if test can detect existing disease

- We then obtained clinically characterized, commercially collected sera from patients with early stage colorectal cancer, and from age and sex matched healthy controls. Samples were coded, then assayed and analyzed in blinded fashion using validated ELISA.

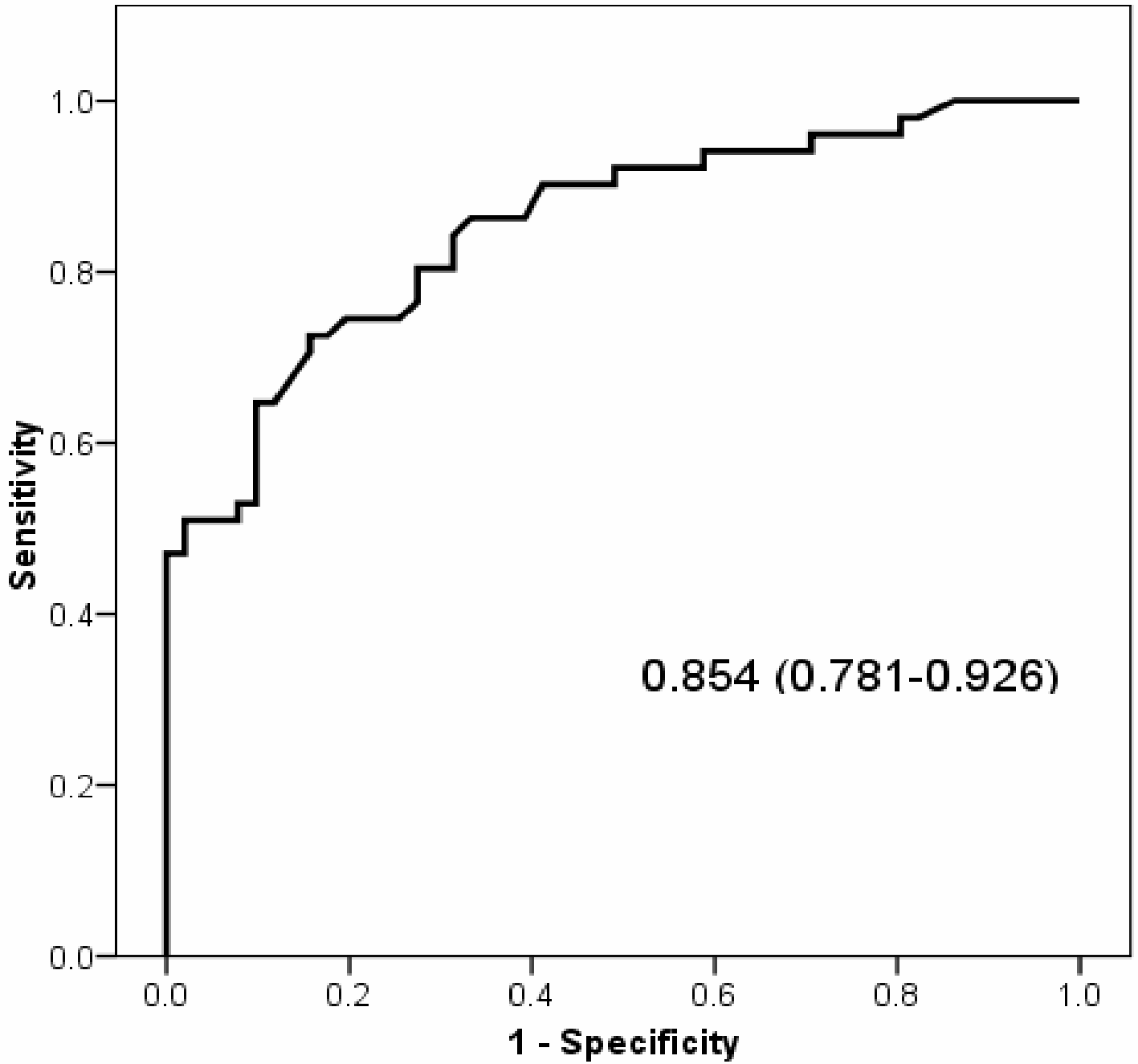
Phase 2: validation case/control sample set

	Cases	Controls
Source	Asterand	ProMeddx
N	51	51
Stage	Stage I: 13 Stage II: 38	Donors met standards for commercial blood donation
Sex	Male: 25 Female: 26	Male: 25 Female: 26
Mean age	67 (44-89)	67 (44-89)
Total		102 samples


Controls
Stage I
Stage II
Antigen specific IgG (ug/ml)
10
8
6
4
2
0
p53
IGFBP2
CEA
Cat D
Cyclin B
Top2
Antigenic protein

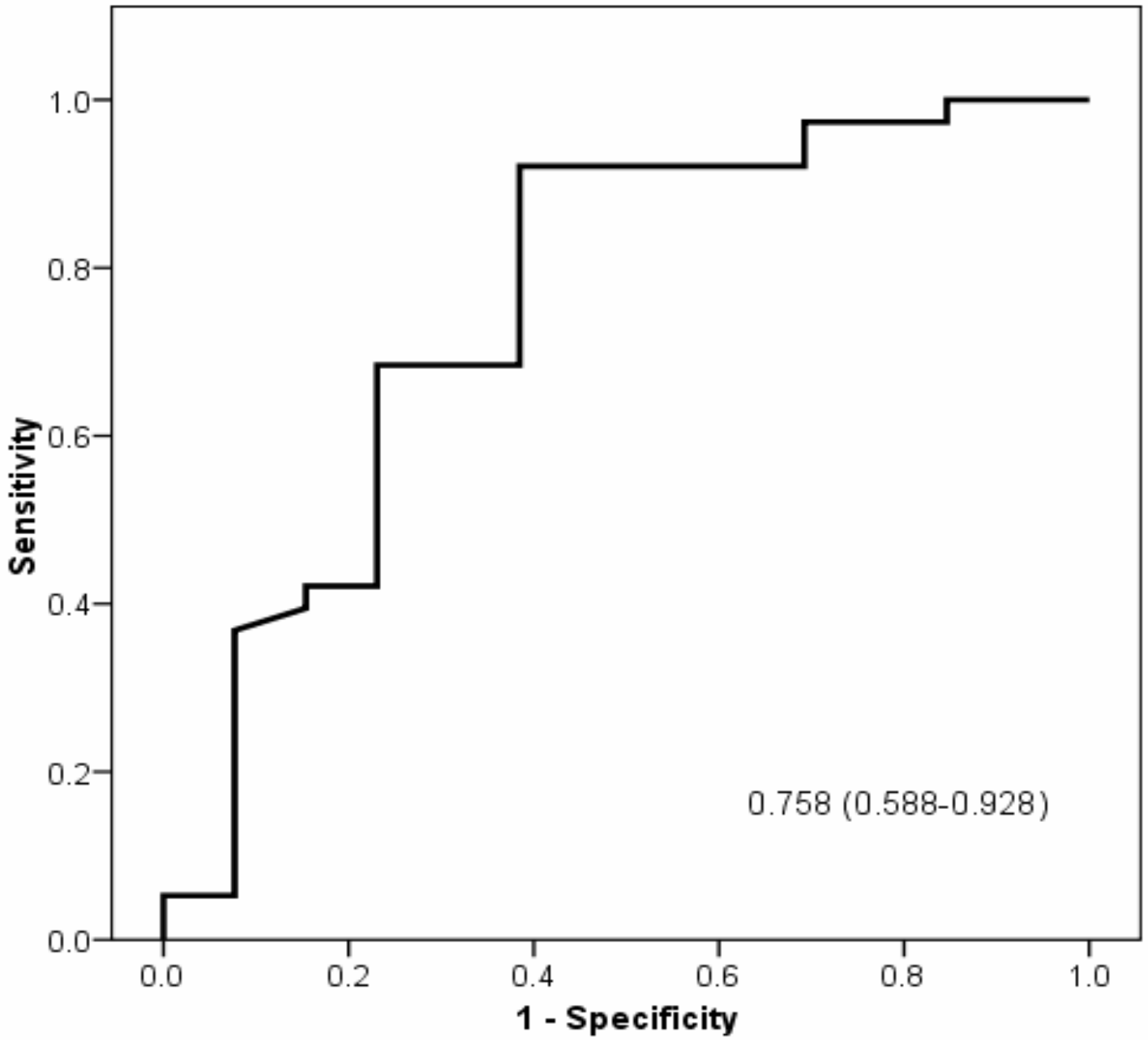


For each antigen, there was a significant difference between incidence in cases and incidence in controls. Results were combined and used to construct ROC curves.


1.0
0.8
0.6
0.4
0.2
0.0
Sensitivity
0.0
0.2
0.4
0.6
0.8
1.0
1 - Specificity
0.854 (0.781-0.926)

Diagnostic characteristics

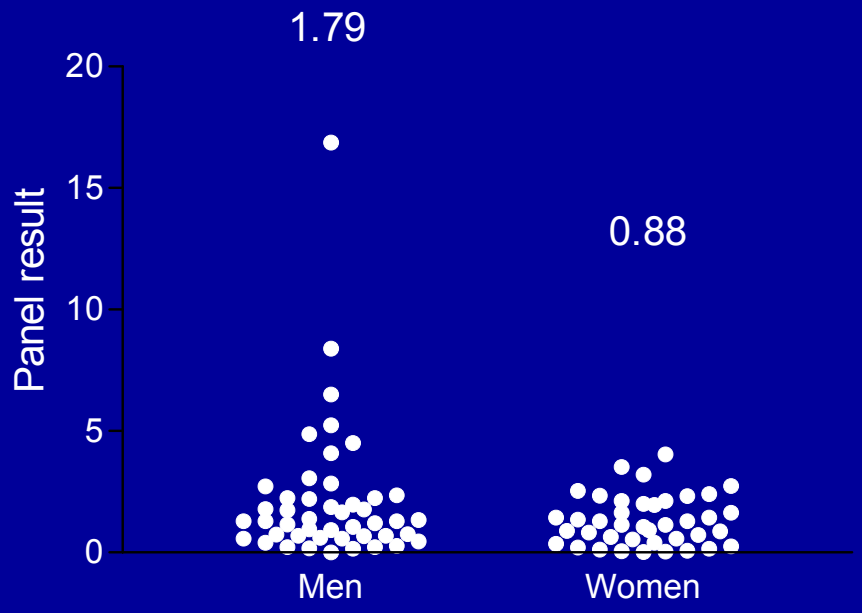
Cut-off:	0.1	0.35	1.0
Sensitivity	94%	92%	75%
Specificity	35%	51%	76%
# false -	3	4	13


Sensitivity
1.0
0.8
0.6
0.4
0.2
0.0
0.0
0.2
0.4
0.6
0.8
1.0
1 - Specificity
0.758 (0.588-0.928)

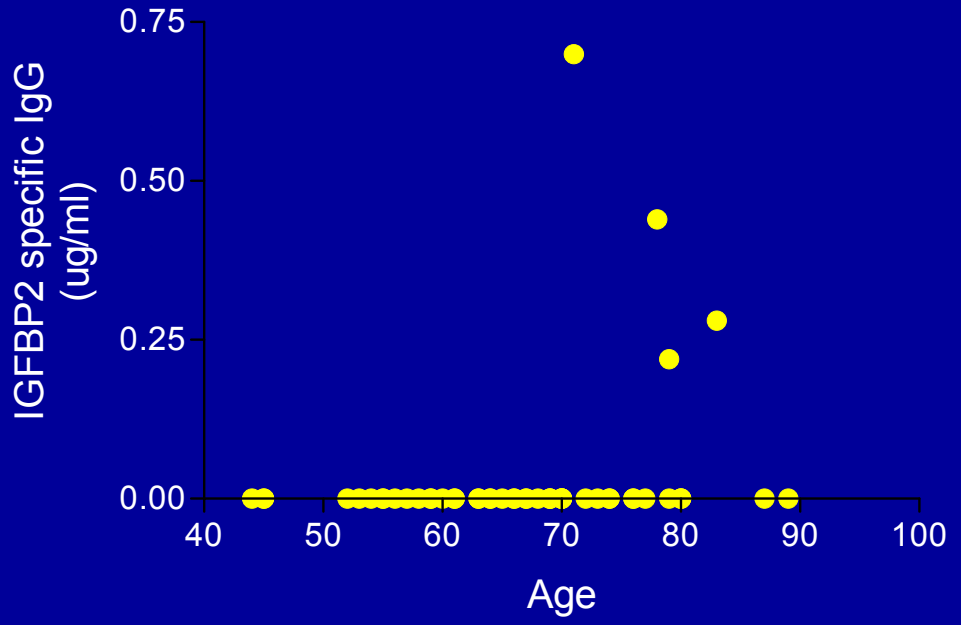
True stage

Panel prediction	Stage II	Stage 1
Stage II	35	5
Stage I	3	8

84% of patients accurately staged by panel results at a cut-off of 0.76.


1.79
0.88
Panel result
20
15
10
5
0
Men
Women

P= 0.042


IGFBP2 specific IgG (ug/ml)
0.75
0.50
0.25
0.00
40
50
60
70
80
90
100
Age

P= 0.018

Conclusions

Phase 1

- We identified a panel of antigens which evoke antibody responses in patients with colorectal cancer, and determined that combined ug/ml response may be able to distinguish between cases and controls.

Phase 2

- We validated the ability of the panel assay to distinguish between blinded case and control samples, and between Stage I and Stage II cases.

Future direction

Continue analysis of clinical data to determine association between clinical variables and immune responses

Phase 3

Determine the ability of the panel assay to detect pre-clinical disease in a retrospective longitudinal study

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www.tumorvaccinegroup.org