

Abstract

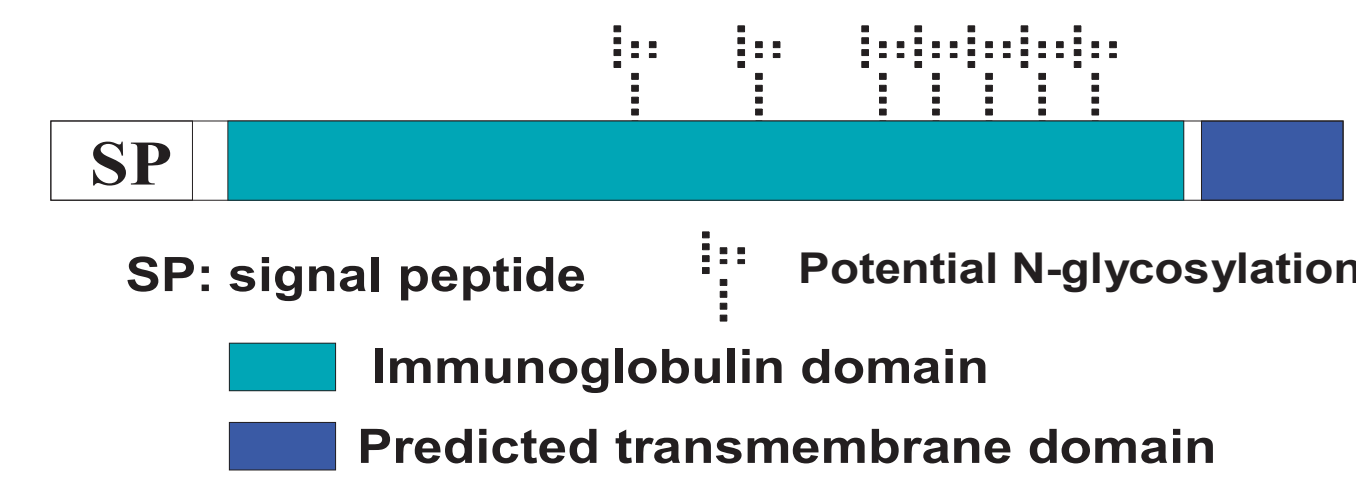
B7-H4 (DD-O110) is a novel membrane protein that functions as a negative regulator of T-cell response. We have developed a sensitive ELISA for B7-H4 and have previously shown that the B7-H4 levels in serum are elevated in ovarian cancer patients compared to normal controls and patients with benign gynecological diseases. To further characterize the over-expression of B7-H4 in ovarian cancer, we evaluated the expression of B7-H4 with CA125 proteins in tissue lysates from ovarian cancers of various histological types and stages.

Ovarian tissue lysates from 256 patients with ovarian carcinoma were assessed for the levels of B7-H4 and CA125. Patients were diagnosed with either early stage cancer (n=87) or late stage cancer (n=169). For comparison, ovarian tissue from patients with benign diseases (n=43) and from normal control patients (n=32) were tested. The concentrations of B7-H4 and CA125 in ovarian tissue extracts were correlated with clinicopathological variables documented at the time of surgical excision and with patient outcome. The 95th percentile of B7-H4 concentration in the control group was selected as a cut-point for a positive signal. Using this cut-off value, B7-H4 was over-expressed in 48% of stage I cancer, 57% of stage II cancer, and 67% of late stage cancer. More importantly, B7-H4 was elevated in 46 of 87 patients with early stage cancer, while CA125 was elevated in 26 patients. In total, 58 patients (67%) with early stage disease were positive for either B7-H4 or CA125. Correlation of the marker expression levels to outcome showed that B7-H4 and CA125 levels increased with poor outcome. However, the effect was not significant when stage of the disease was included in multivariate analysis.

In summary, B7-H4 level is correlated with stage where more than 50% of early stage and 67% of late stage cancers over-expressed B7-H4 in the primary tumor. The data support the biological rationale for elevation of B7-H4 in serum of women with early stage disease, and suggest B7-H4 as a promising new diagnostic marker and therapeutic target, which may complement CA125 in the detection of early stage ovarian cancer.

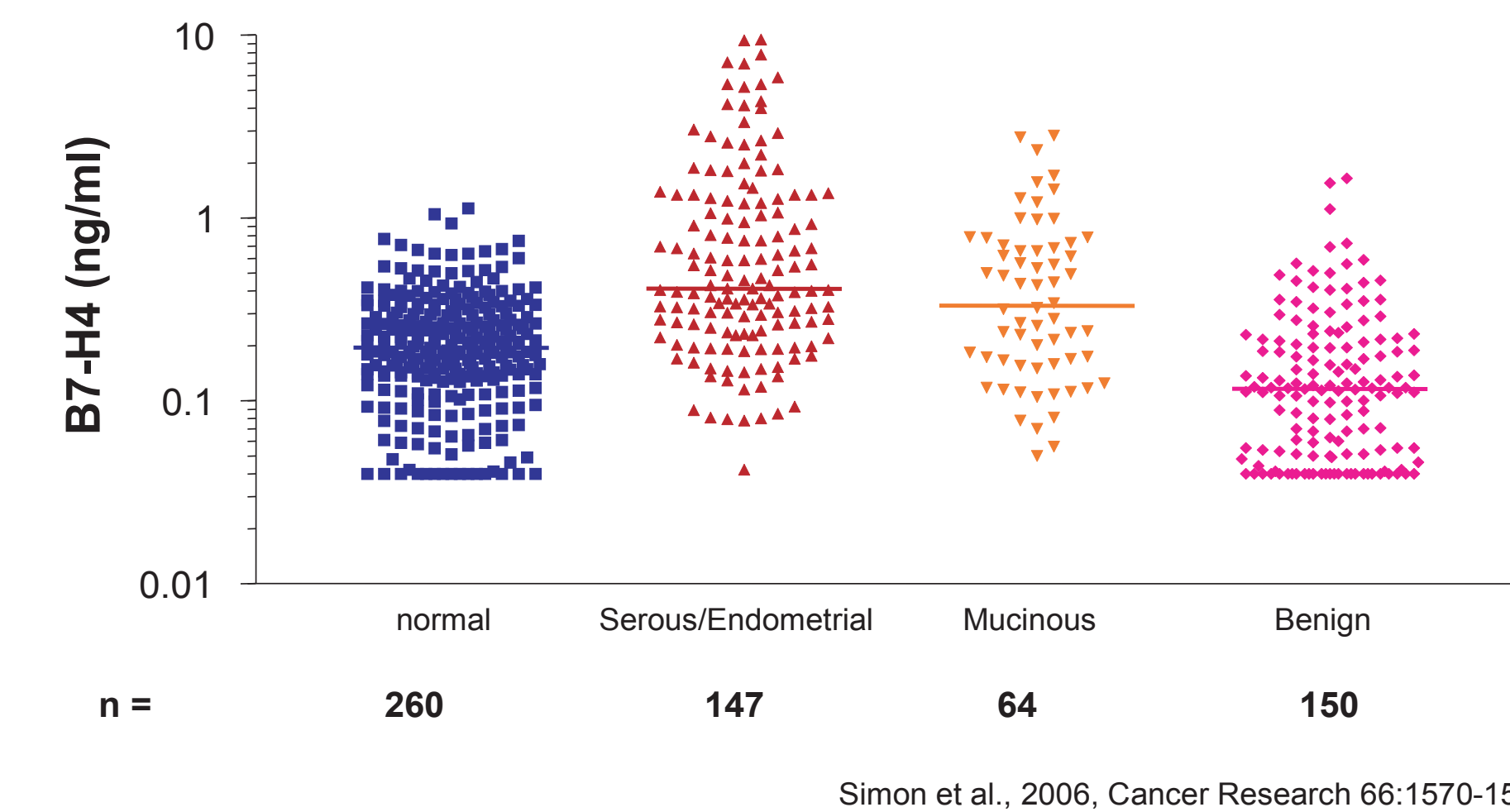
B7-H4 detection in serum and tissue

B7-H4 (DD-O110) Background

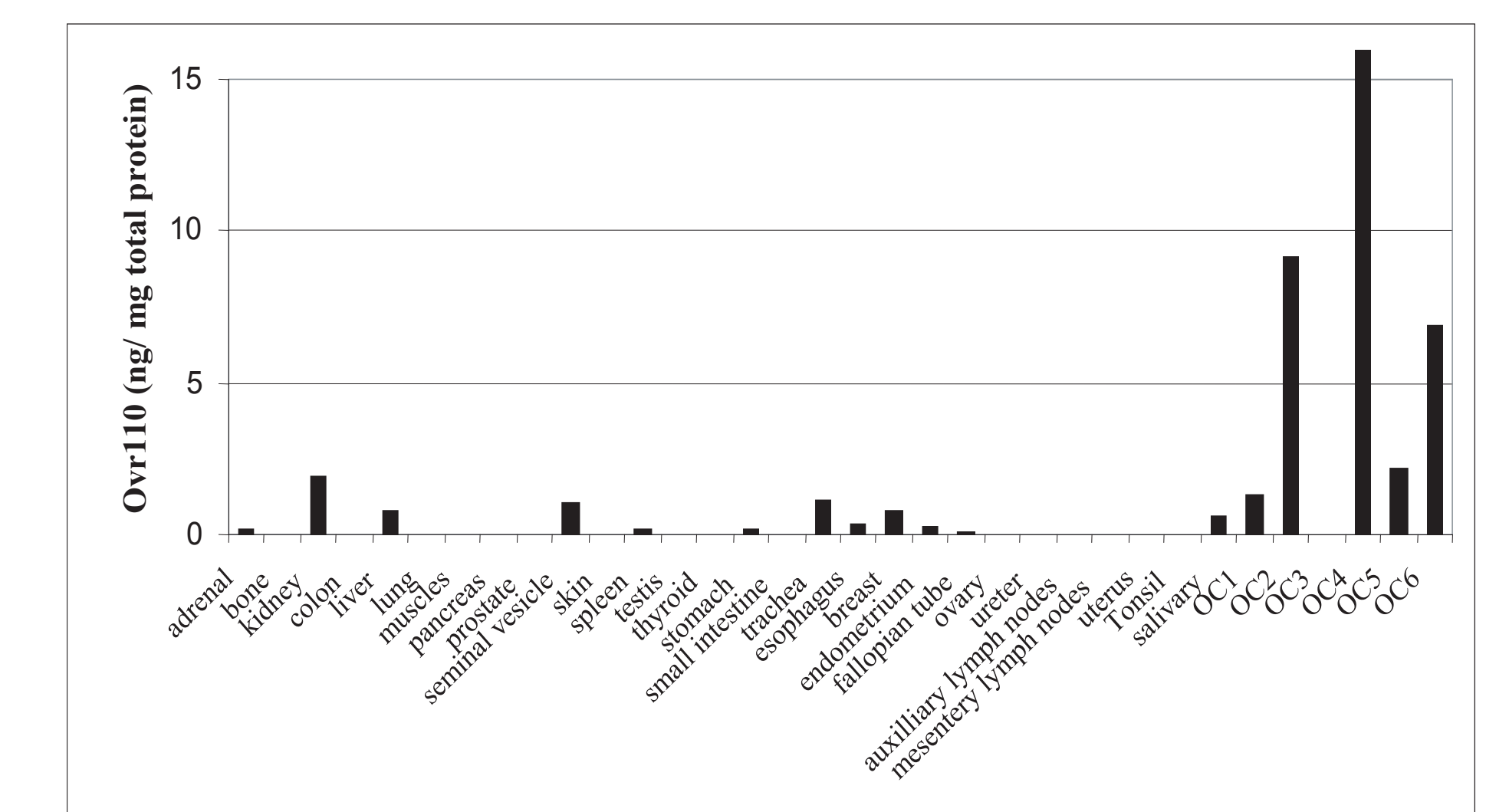


- B7-H4 (DD-O110) is a transmembrane protein with an IgG domain, 282 aa in length, and is also known in the literature as B7x.
- B7-H4 is a new member of the B7 protein family and has been shown to negatively regulate immune response. The function of B7-H4 in ovarian cancer is not yet known, but the over-expression of B7-H4 in cancer cells may play a role in evading immune system surveillance.
- Cell surface expression has been demonstrated by FACS, IHC and biotinylation experiments in breast and ovarian cancer tissue and cell lines.

B7-H4 elevation in ovarian cancer serum samples

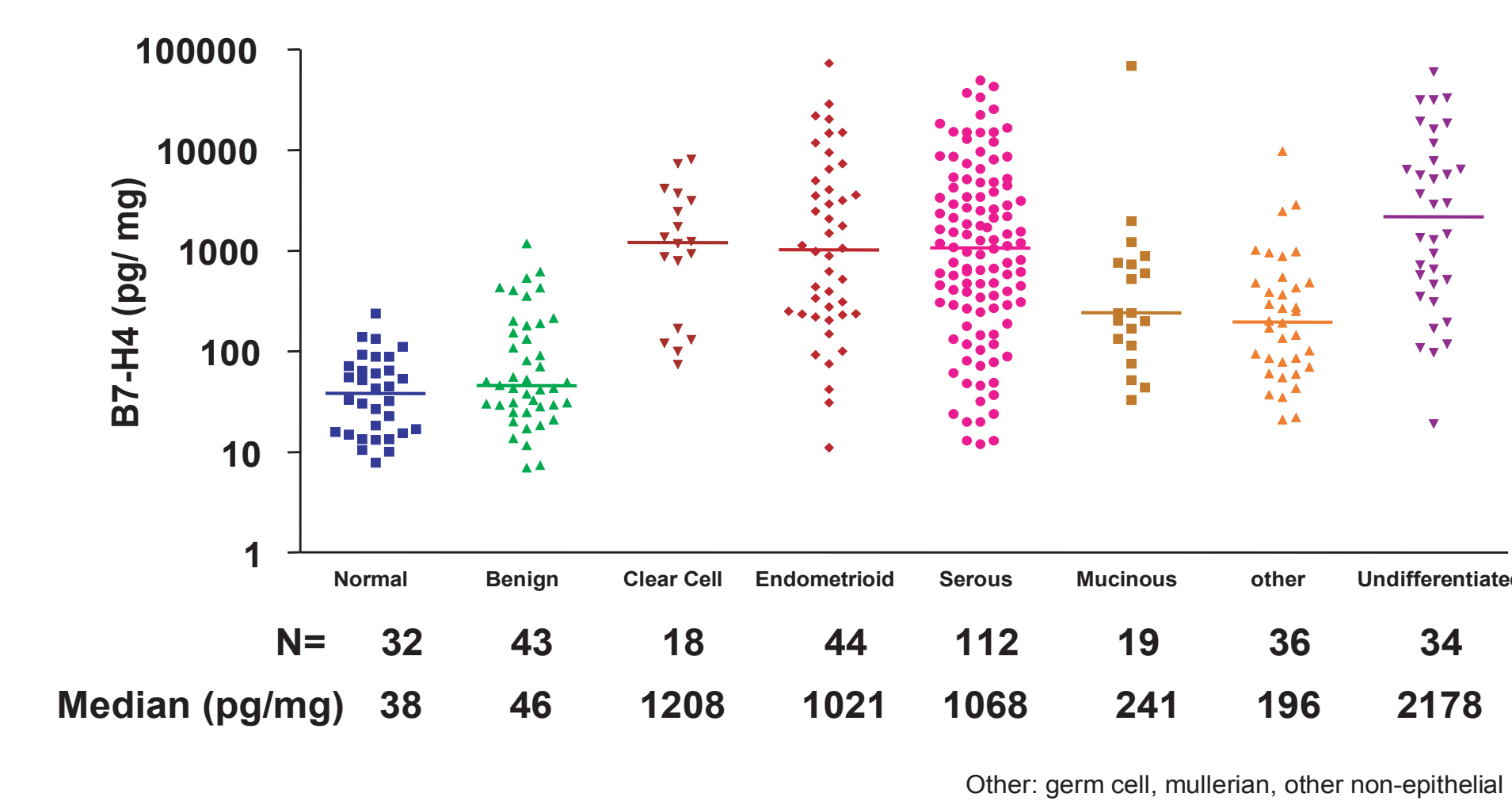


B7-H4 protein over-expression in ovarian cancer and normal tissues

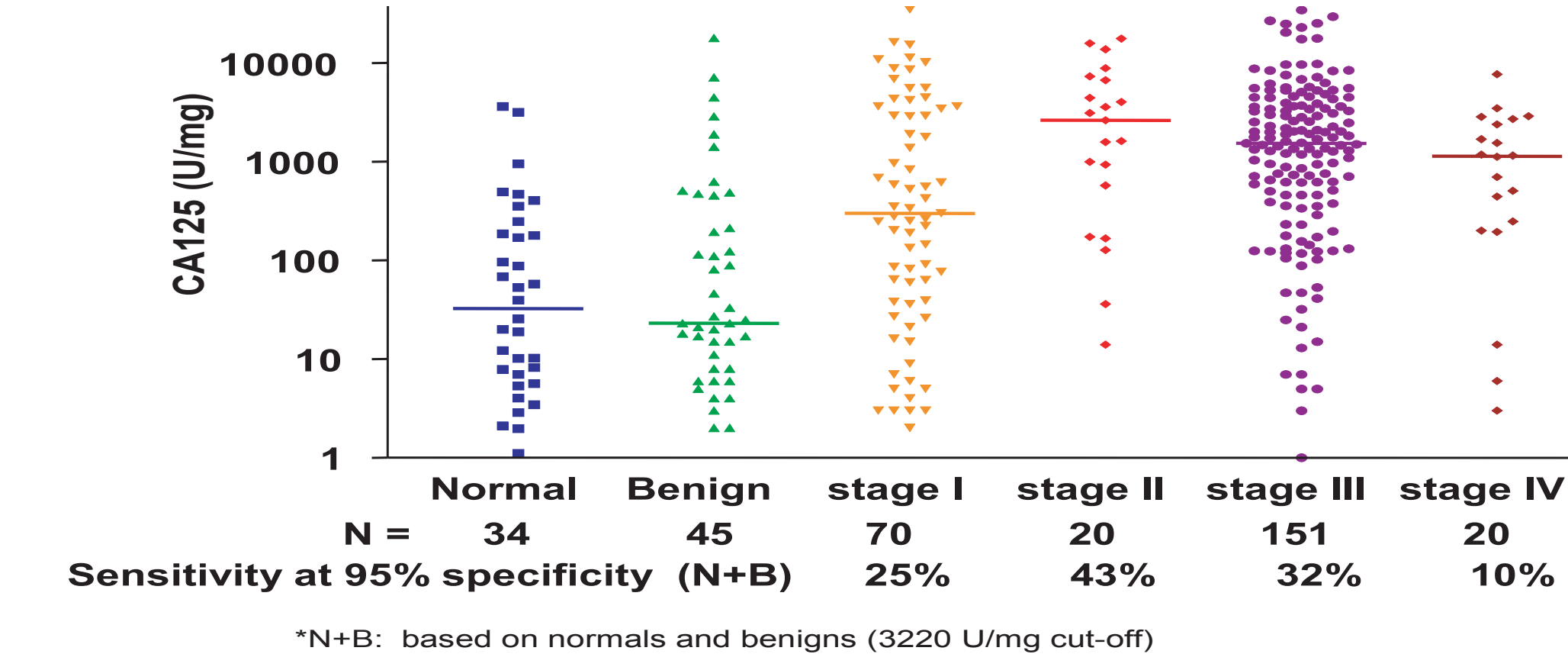
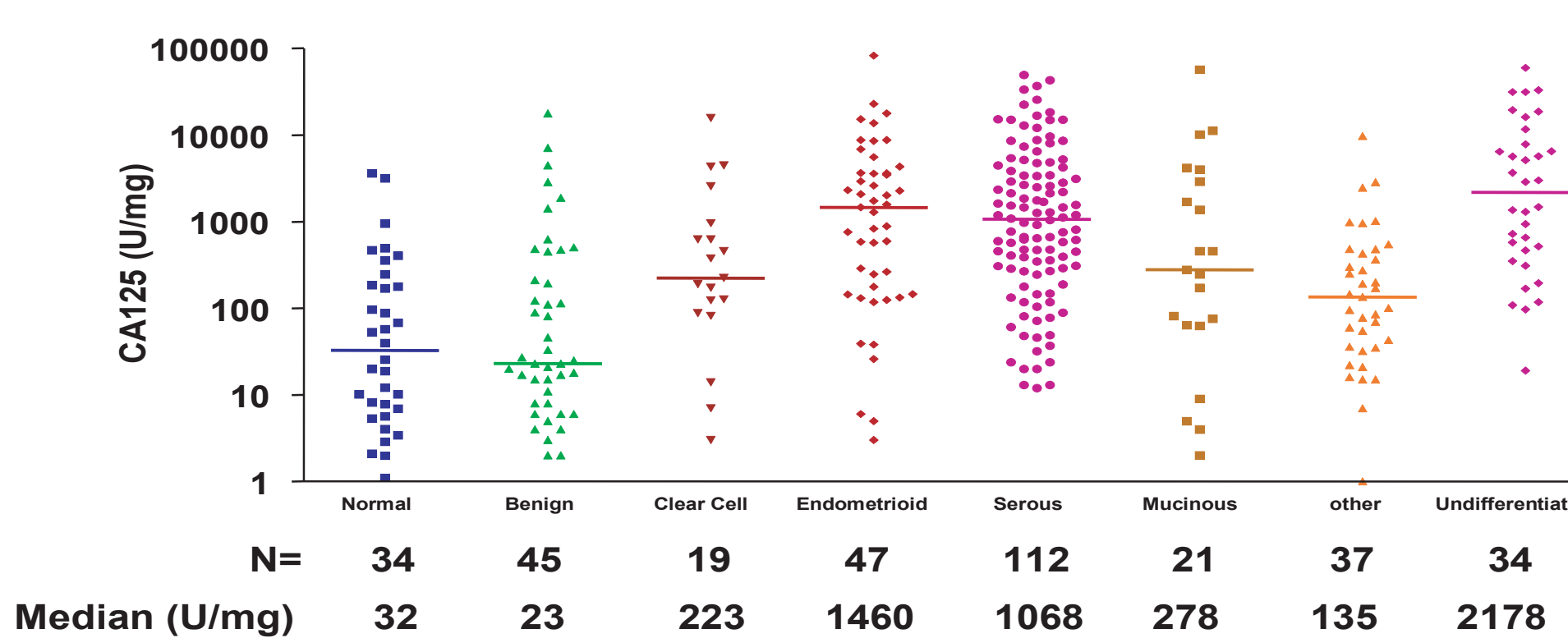
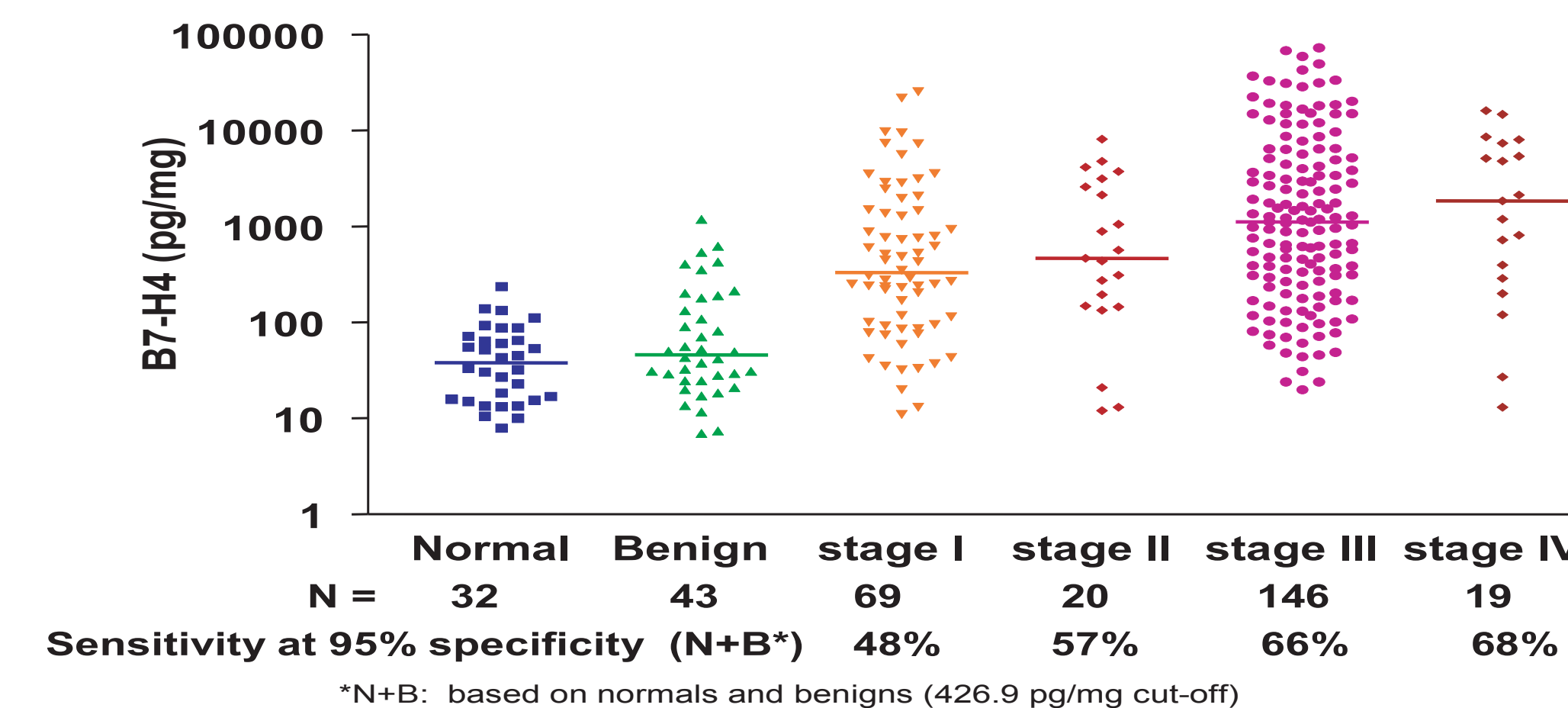


B7-H4 and CA125 detection in ovarian tissue lysate

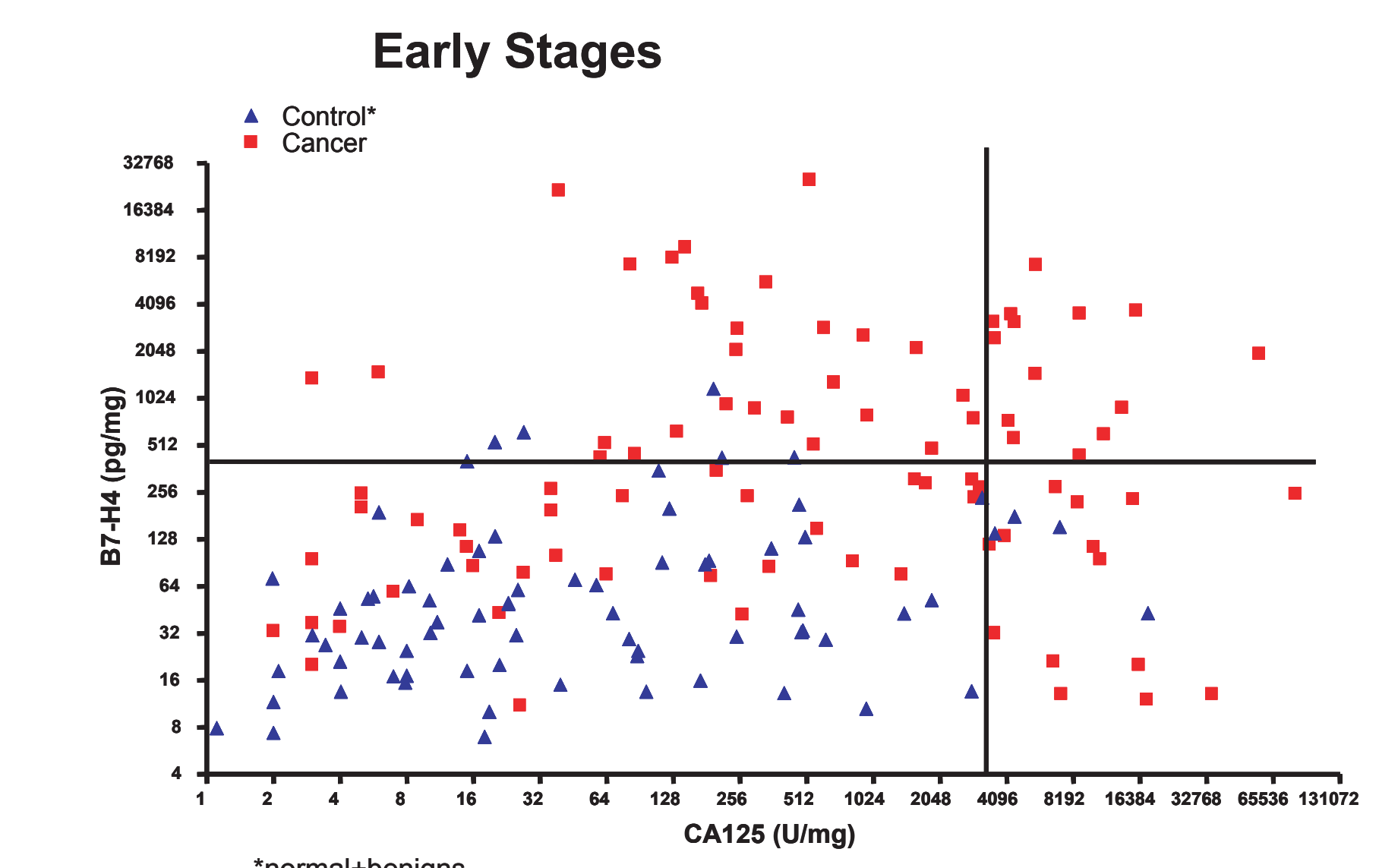
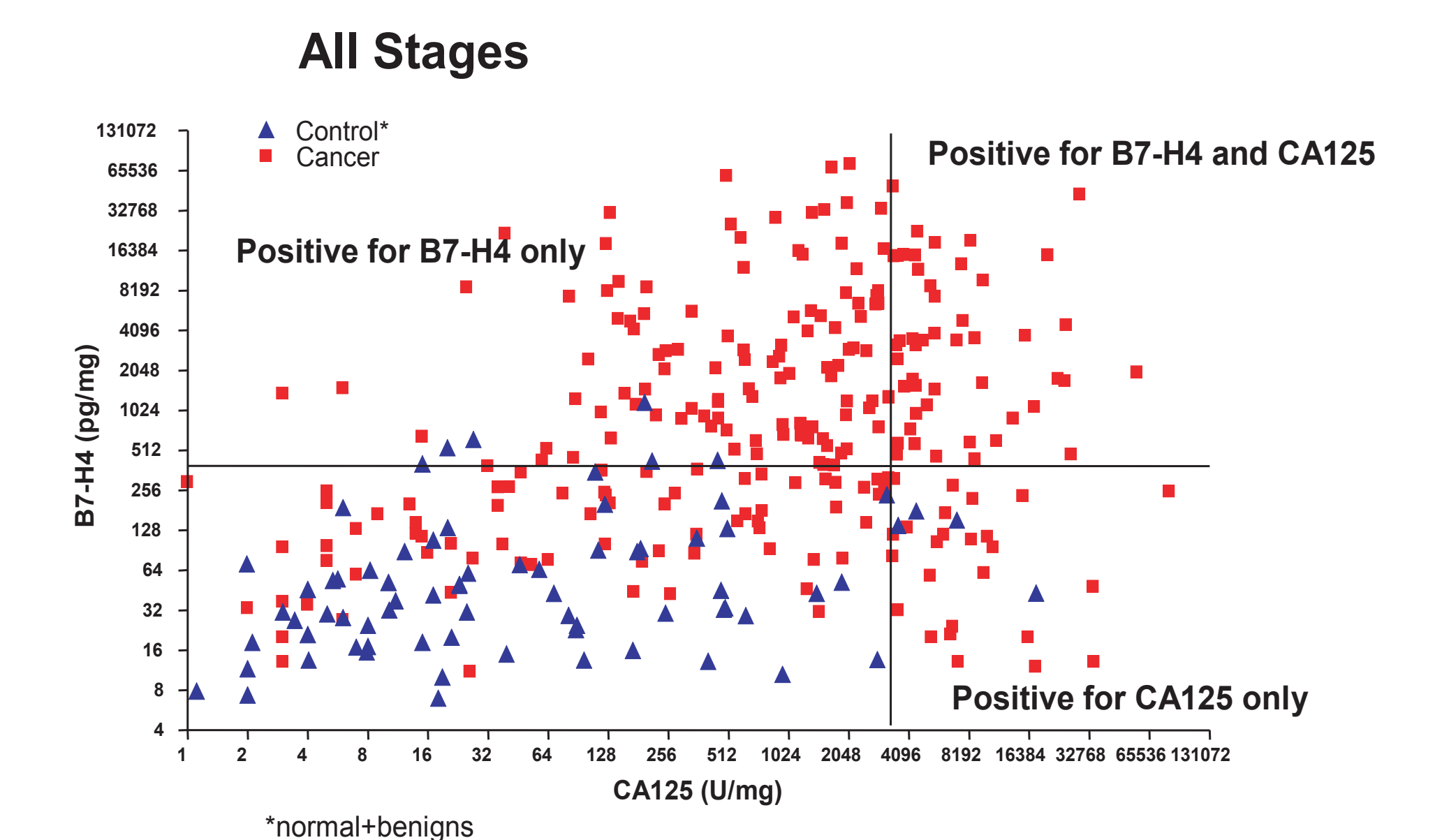
Expression by histological types



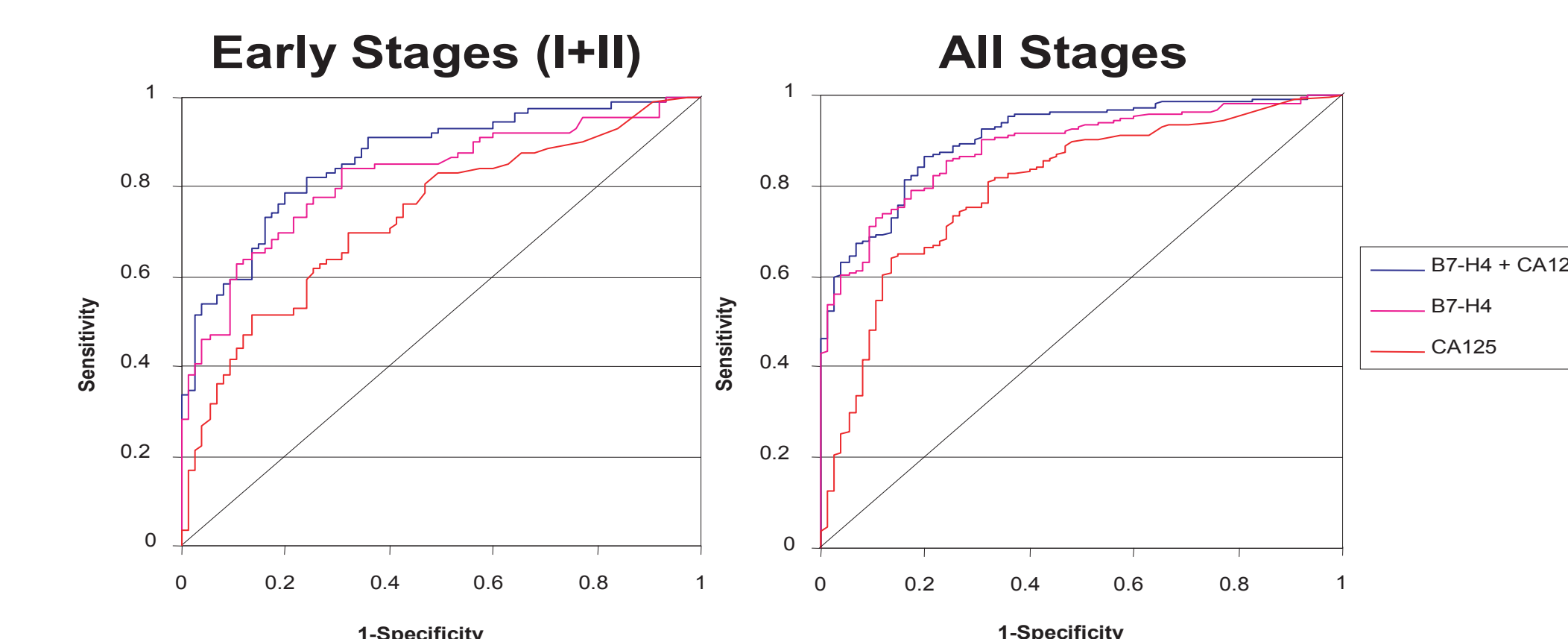
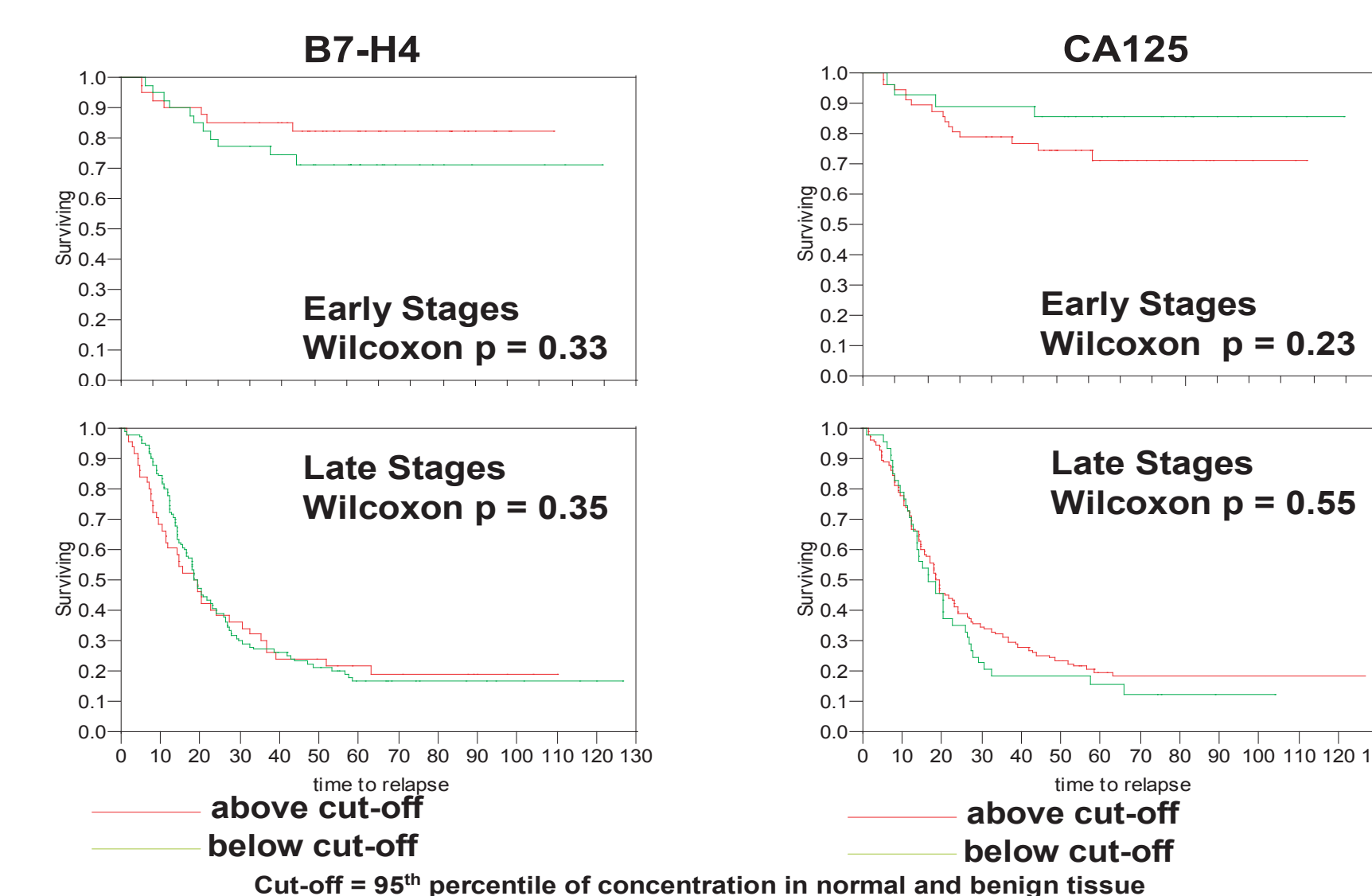
Expression by stage



Complementarity between B7-H4 and CA125



B7-H4 and CA125 expression and outcome



	AUC	Early Stages (n=89)	All Stages (n=254)
B7-H4	0.82	0.73	0.89
*P-value (vs. CA125)		(0.053)	(0.003)
CA125	0.73	0.80	0.80
B7-H4+CA125	0.86	0.86	0.91
*P-value (vs. B7-H4)		(0.028)	(0.007)
*P-value (vs. CA125)		(<0.001)	(0<.001)

*Hanley JA, McNeil BJ. Radiol 1983; 148:839-43

CA125	Controls (N+B)		Cases (Stage I+II)		Cases (All stages)	
	-	+	-	+	-	+
	+	4	0	14	14	26
-	67	4	33	28	74	104

*Cut-off values (95th percentile of normal and benign tissue lysate concentration): B7-H4 = 426.9 pg/mg, CA125 = 3220 U/mg

Methods & Materials

Patient Population

Ovarian tissue lysates from 256 patients (median age 56 years, range 22-99 years) with ovarian carcinoma were assessed for the levels of B7-H4 and CA-125. Eighty-seven patients were diagnosed with early stage cancer, 169 patients had late stage cancer. For comparison, ovarian tissue from patients with benign diseases (n=43) and from normal control patients (n=32) were tested (median age 49 years, range 24-78 years). The concentrations of B7-H4 and CA-125 in ovarian tissue extracts were correlated with clinicopathological variables documented at the time of surgical excision and with patient outcome.

ELISA

High binding polystyrene plates were coated overnight with 0.8 ug/well of anti-B7-H4 (DD-O110) Mab. After blocking with 300ul/well Superblock-TBS (Pierce) plus 10% Calf serum and washing, 75ul of Assay Buffer (TBS, 1% BSA, 1% Mouse Serum, 1% Calf Serum, 0.1% Tween-20) was added to each well. Then, 25ul of antigen was added for 90 minutes incubation. For each sandwich ELISA, standards of 25, 10, 2.5, 1, 0.5, 0.2 and 0 ng/ml B7-H4 were run in parallel with the test samples. For detection, 100ul of biotinylated Mab (1 ug/ml) were added to each well and incubated for 1 hour at room temperature, while shaking. After washing, 100ul of horseradish peroxidase conjugated streptavidin (1mg/ml, Jackson Laboratories) at a 1:20,000 dilution was added to each well and incubated for 30 minutes. The plate was then developed using DAKO TMB Plus substrate (DAKO, Denmark) for 30 minutes. After stopping the reaction with HCl, the plates were read at 450nm.

Statistical Analysis

The 95th percentile of B7-H4 and CA125 concentration in the control group (normal and benign) was selected to categorize patients as B7-H4/ CA125 positive or negative. Kaplan-Meier analyses were performed based on binary categorizations (above or below the cut-off of B7-H4 and CA125) and the results of log rank tests for time until relapse or death were determined. For Kaplan-Meier analyses, 101 patients still alive at the end of the study were censored. JMP software v5.0.1a (SAS Institute, Cary, NC) was used to assess distributions of values, to perform Wilcoxon Rank-Sum tests, univariate and multivariate logistic regressions based on continuous variables, and Kaplan-Meier survival analyses.

Summary

- B7-H4 (DD-O110) was over-expressed in 73% of undifferentiated cancers, 67% of serous adenocarcinomas, 63% of endometrioid cancer, 45% of mucinous cancer and 24% of non-epithelial cancers.
- B7-H4 expression was low in normal ovaries and ovaries from patients with benign gynecologic diseases. B7-H4 expression was also low or undetectable in other normal tissues.
- In early stage cancers, 48% of patients with stage I cancer, 57% of patients with stage II cancer, and 67% of patients with late stage cancer had B7-H4 values higher than normal controls or benign diseases.
- B7-H4 was elevated in 42 patients with early stage cancer, and CA125 was elevated in 28 patients. When the two markers were combined, 56 patients (62%) with early stage disease were positive for B7-H4 and/ or CA125.
- The survival analysis showed that neither B7-H4 nor CA125 were markers for the prognosis of relapse or survival of patients when the data were analyzed for stage subgroups. The multivariate Cox regression analysis confirmed that the risk of poor outcome increased with stage but not with expression of either B7-H4 or CA125.
- The data demonstrate elevation of B7-H4 expression in early stage ovarian cancer tissues, and support the potential utility of measuring B7-H4 levels in serum for the detection of early stage ovarian cancer.