

Novel Biomarkers (Kallikreins) for Prognosis and Therapy Response in Ovarian cancer

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The Samuel Lunenfeld
Research Institute



University Health Network

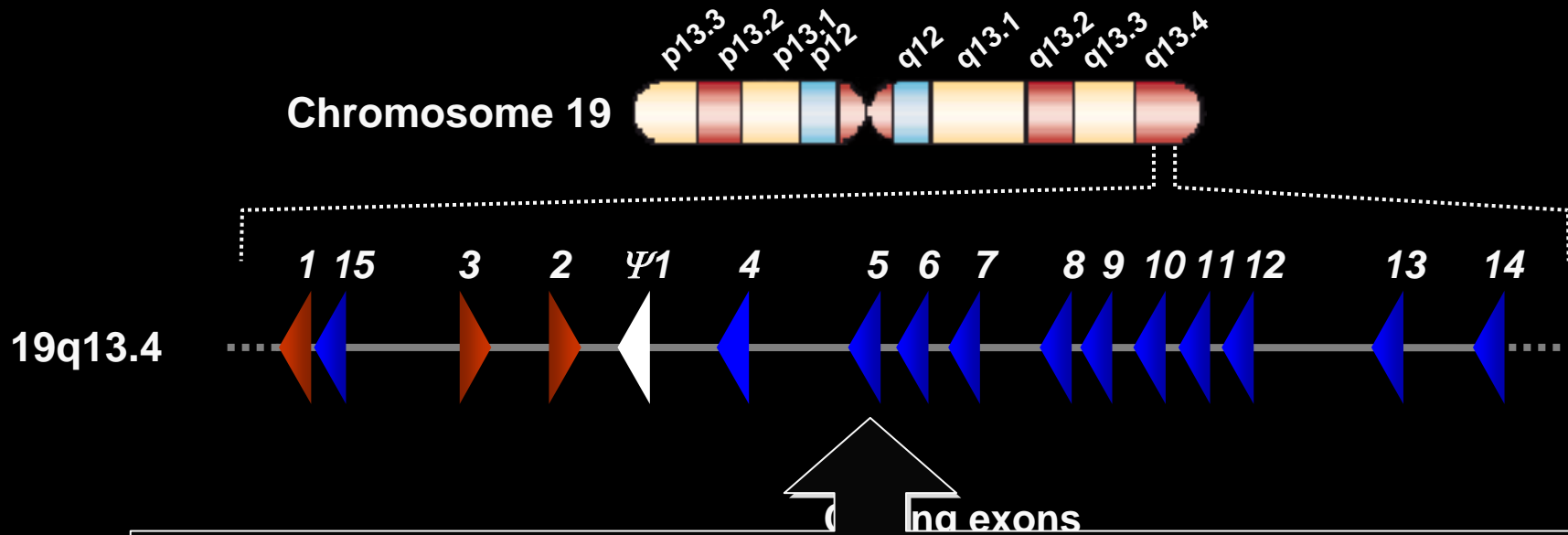
Achieving Global Impact



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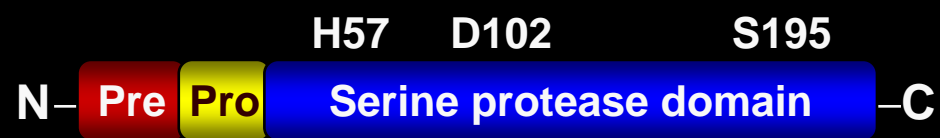


Human kallikrein gene cluster (KLK) locus



Within the entire human genome, **kallikrein genes** represent:

1. the *largest cluster* of contiguous protease genes of any kind
2. the *largest group* of serine proteases



Kallikreins as Cancer Biomarkers



Discovery of a New Biomarker

(classical method)

Discovery of the gene



Recombinant protein



Antibodies



Assays



Preliminary Studies



Detailed Studies



Clinical Acceptance

It took us 7 years to set-up
KLK ELISAS

Efforts to improve assays
are still ongoing

Kallikrein ELISAs

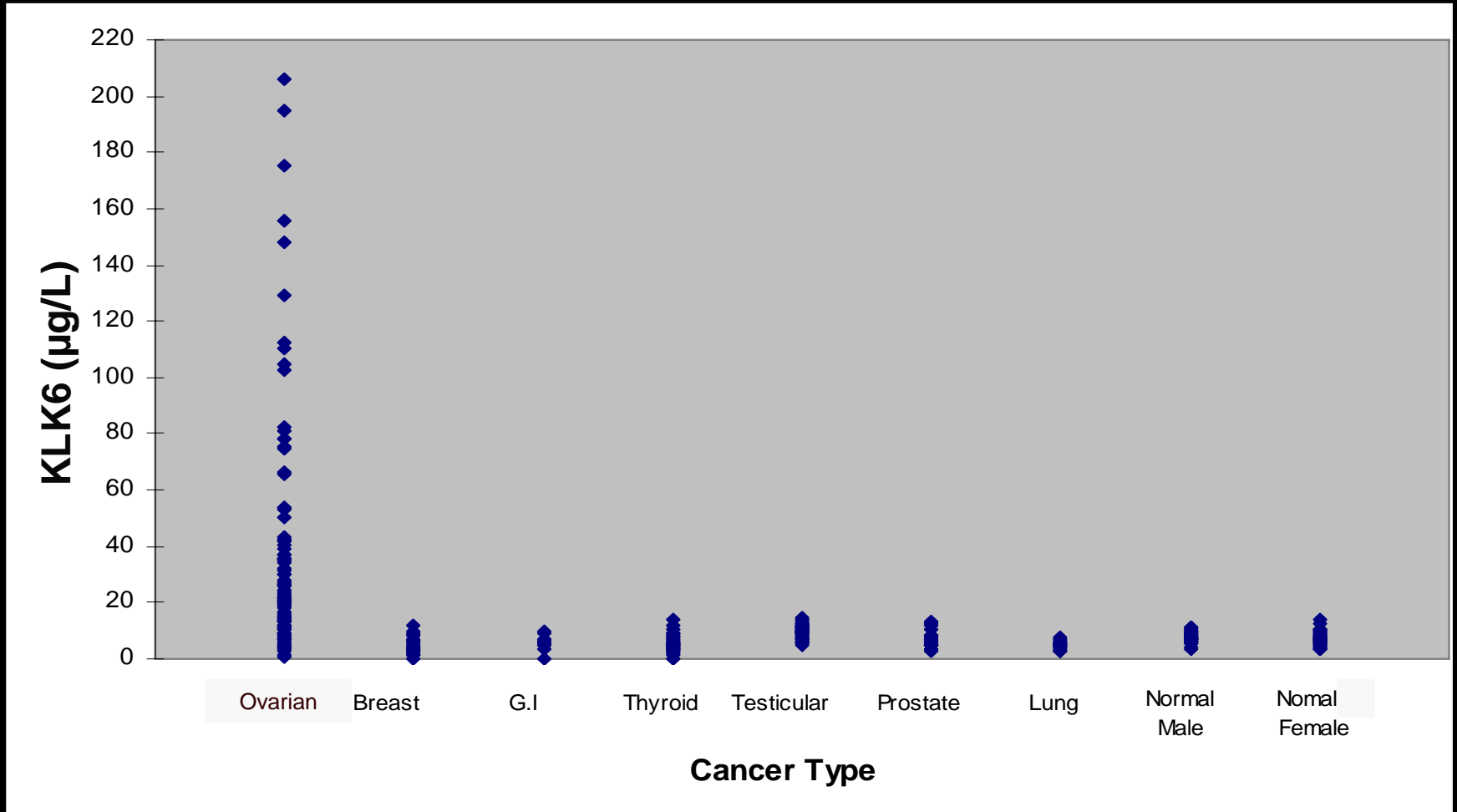
Developed/Published

- KLK4
- KLK5
- KLK6
- KLK7
- KLK8
- KLK9
- KLK10
- KLK11
- KLK12
- KLK13
- KLK14
- KLK15



Kallikrein 6
A Novel Ovarian Cancer
Biomarker

KLK6 in serum



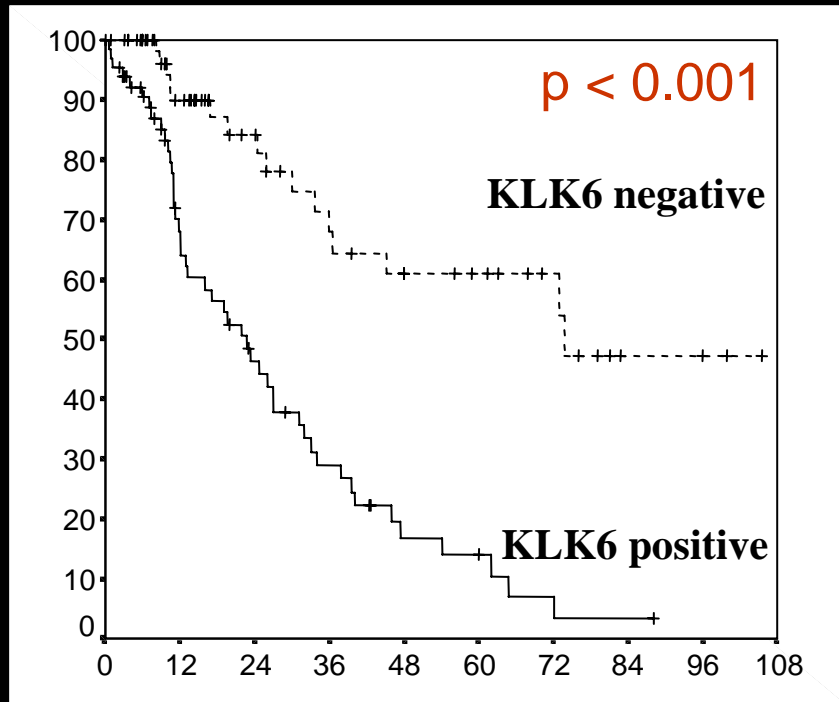
High Serum KLK6 in Ovarian Cancer

- Associated with: ($p < 0.001$)
 - Late stage
 - High grade
 - Serous histotype
 - No response to chemotherapy
 - Residual tumor
 - Decreased disease-free & overall survival

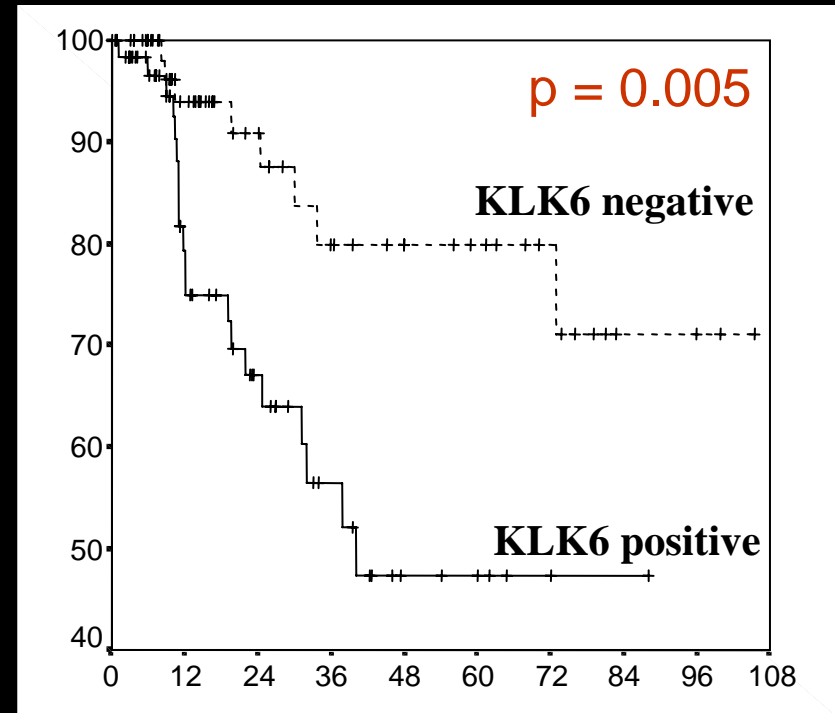
Independent & Unfavorable Prognostic Indicator

Survival Analysis and Serum KLK6

Survival probability (%)

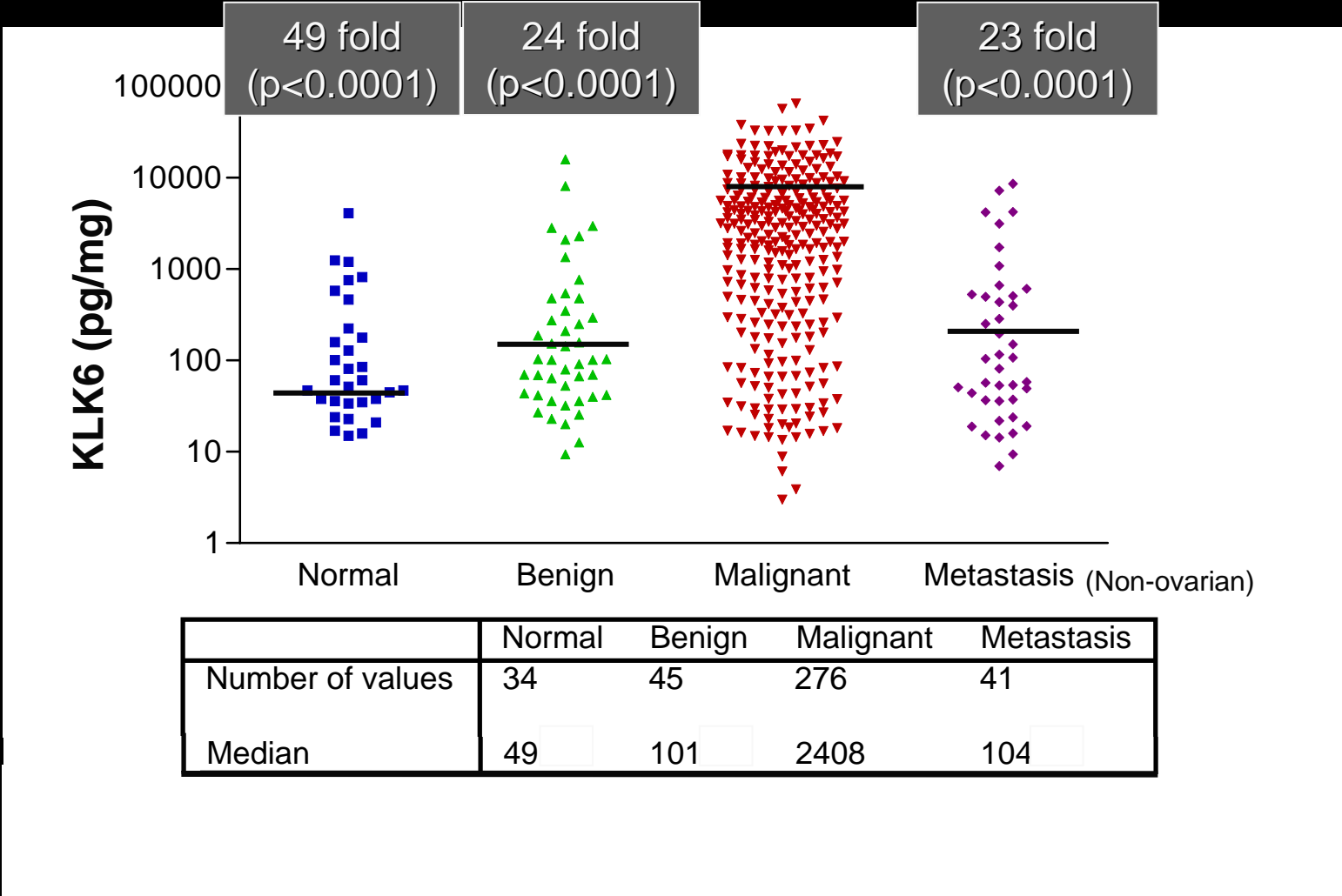


PFS (months)

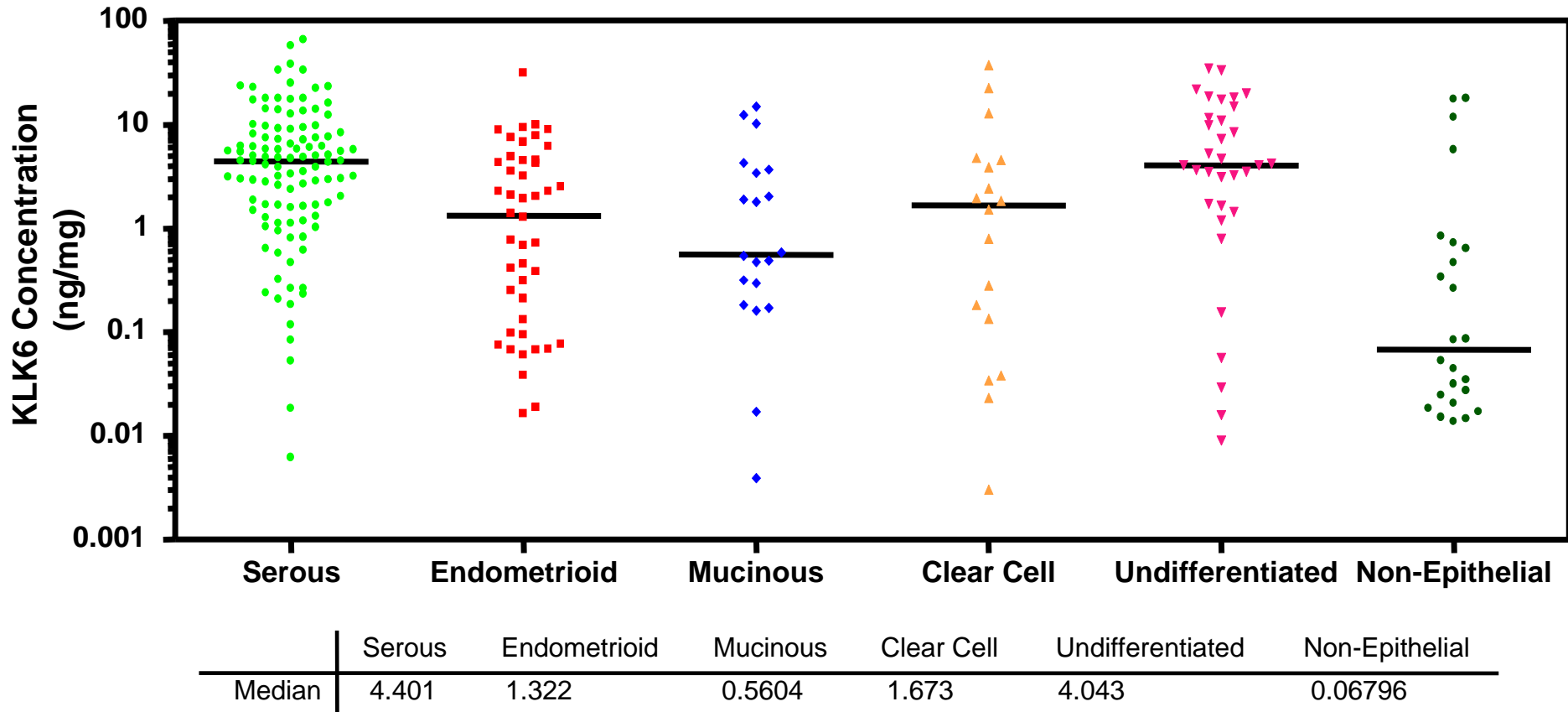


OS (months)

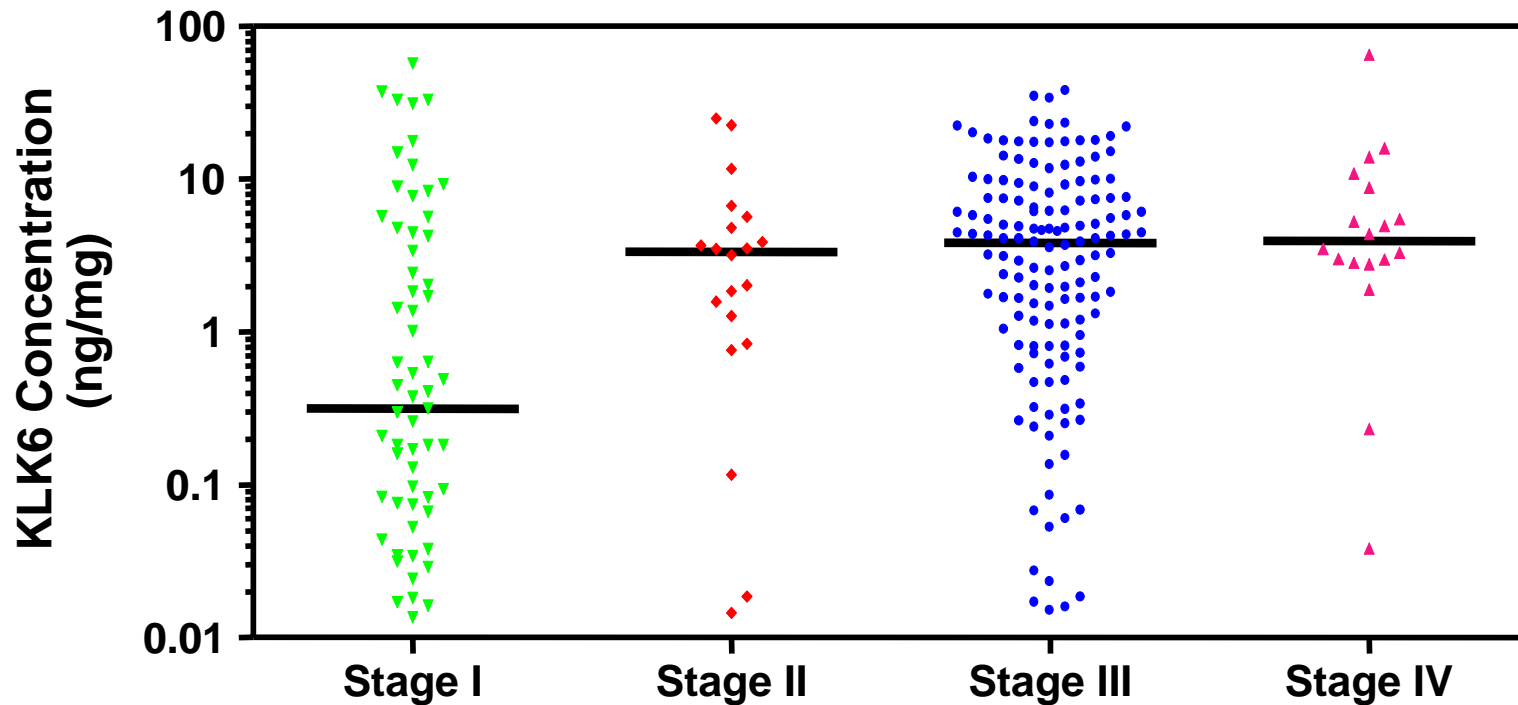
KLK6 Protein Expression in Ovarian Tissue Extracts



KLK6 and Histological Subtypes

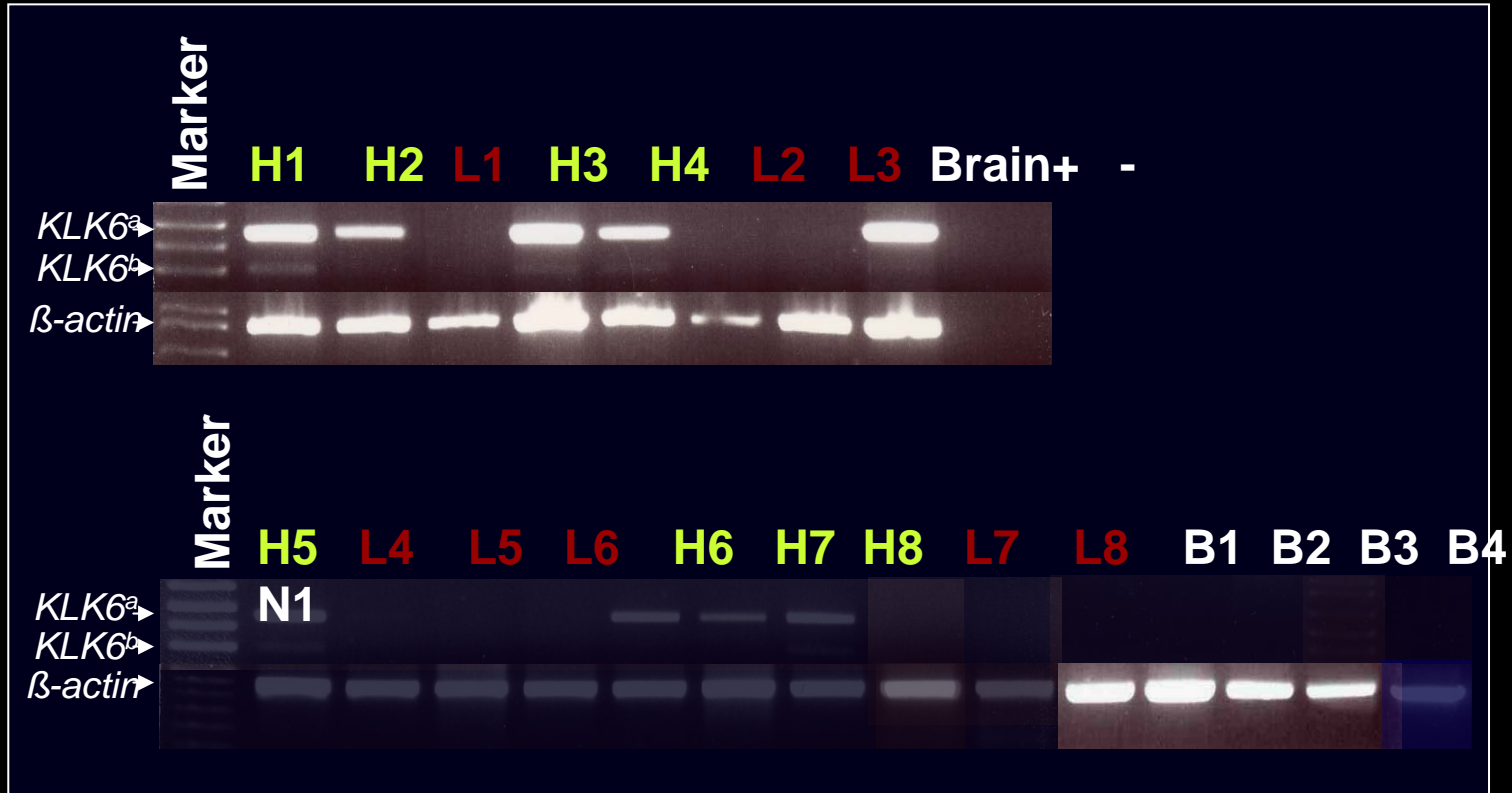


KLK6 and FIGO stage



	Stage I	Stage II	Stage III	Stage IV
Median	0.3158	3.333	3.838	3.936

KLK6 mRNA Expression in Ovarian Tissues



Complete concordance between KLK6 mRNA and protein expression points to transcriptional regulation.

Kallikreins Overexpressed in Ovarian Cancer

KLK5, KLK6, KLK7, KLK8, KLK10, KLK11 & KLK14

Verified by:

- Tissue, serum & ascites protein levels
- mRNA (quantitative RT-PCR)
- Bioinformatics (EST & SAGE databases)
 - Digital Differential Display
 - In-Silico Northern



Independent Validation

Early

Detection

Research

Network

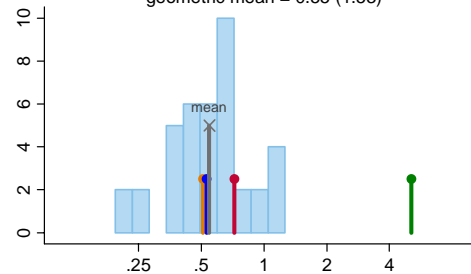


Ovarian Cancer-Blinded Study-Pools

marker: KLK 5

premenopausal controls

geometric mean = 0.55 (1.53)

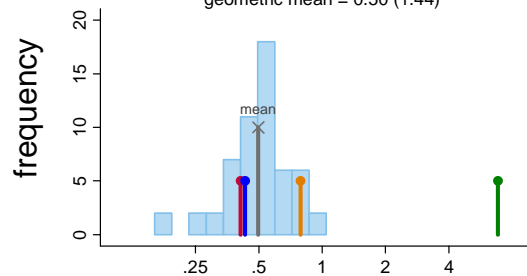


disease pool

- 1: Ov-Ca-1
z = 5.27
- 6: Ov-Ben-1
z = -0.16
- 8: Br-Ca-2
z = 0.66
- 11: Br-Ben-1
z = -0.07

postmenopausal controls

geometric mean = 0.50 (1.44)

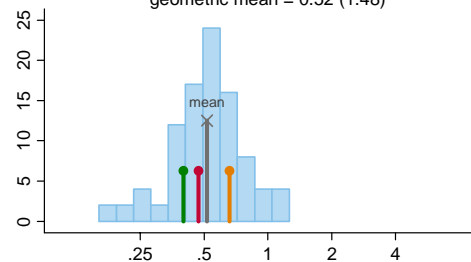


disease pool

- 2: Ov-Ca-2
z = 7.20
- 3: Ov-Ca-3
z = 1.28
- 7: Ov-Ben-2
z = -0.52
- 9: Br-Ca-3
z = -0.39

all controls

geometric mean = 0.52 (1.48)

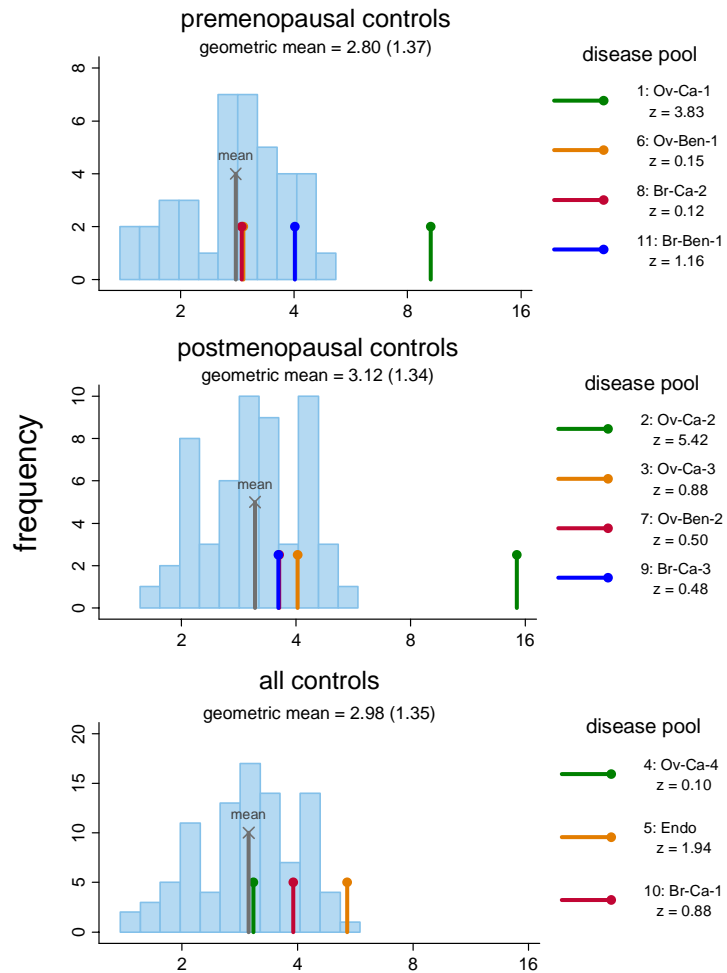


disease pool

- 4: Ov-Ca-4
z = -0.65
- 5: Endo
z = 0.63
- 10: Br-Ca-1
z = -0.24

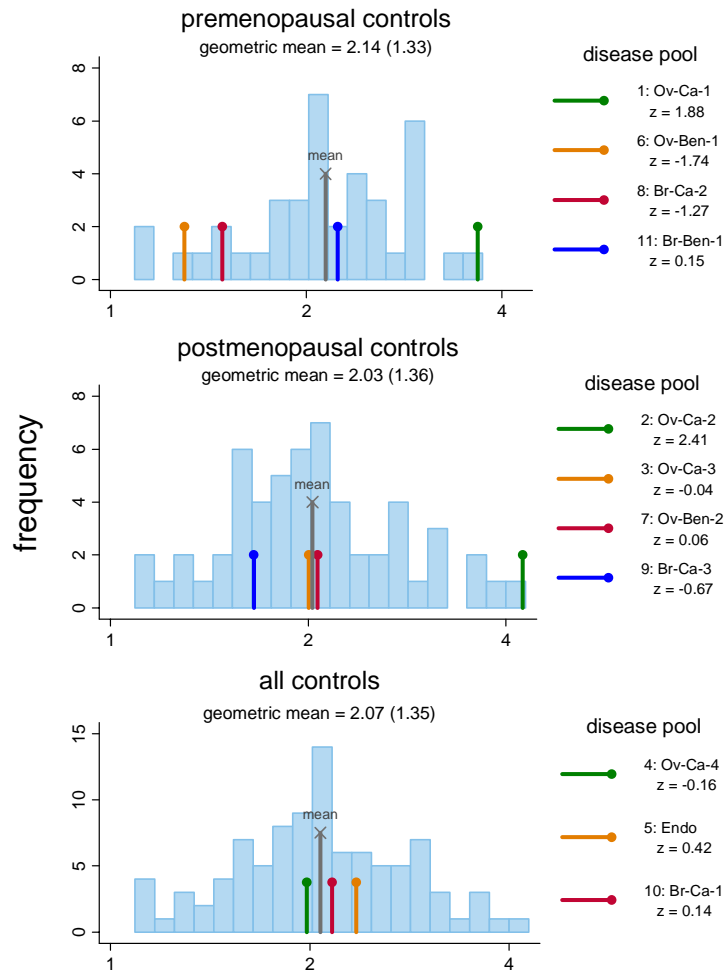
Ovarian cancer

marker: KLK 6



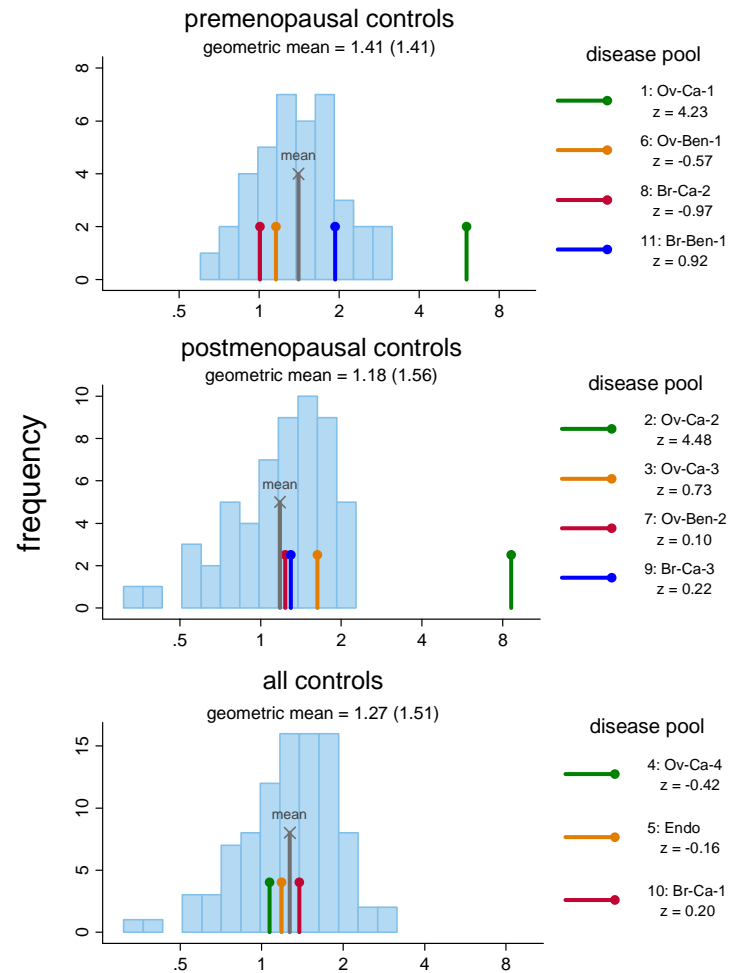
Ovarian cancer

marker: KLK 8



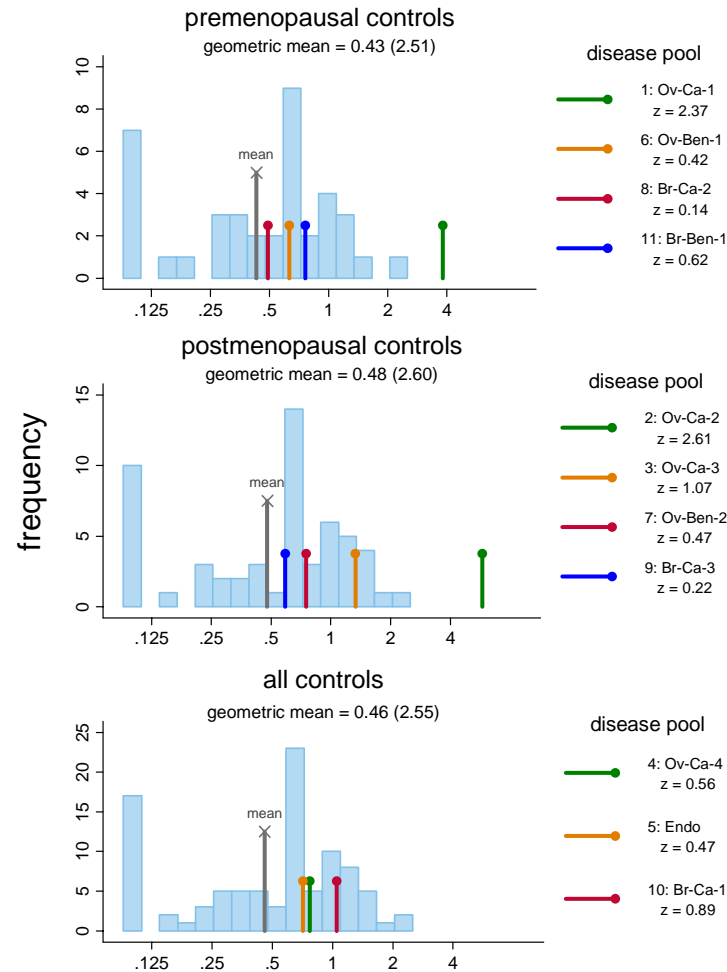
Ovarian cancer

marker: KLK 10



Ovarian cancer

marker: KLK 11



EDRN-Ovarian Study Validation (Serum)

- ❑ PLCO samples (approx. 1,000; mix normal/ benign/ early and late stage ovarian cancer)
- ❑ 30-40 biomarkers and proteomic patterns under evaluation
- ❑ Blinded
- ❑ Identify individual markers and panels

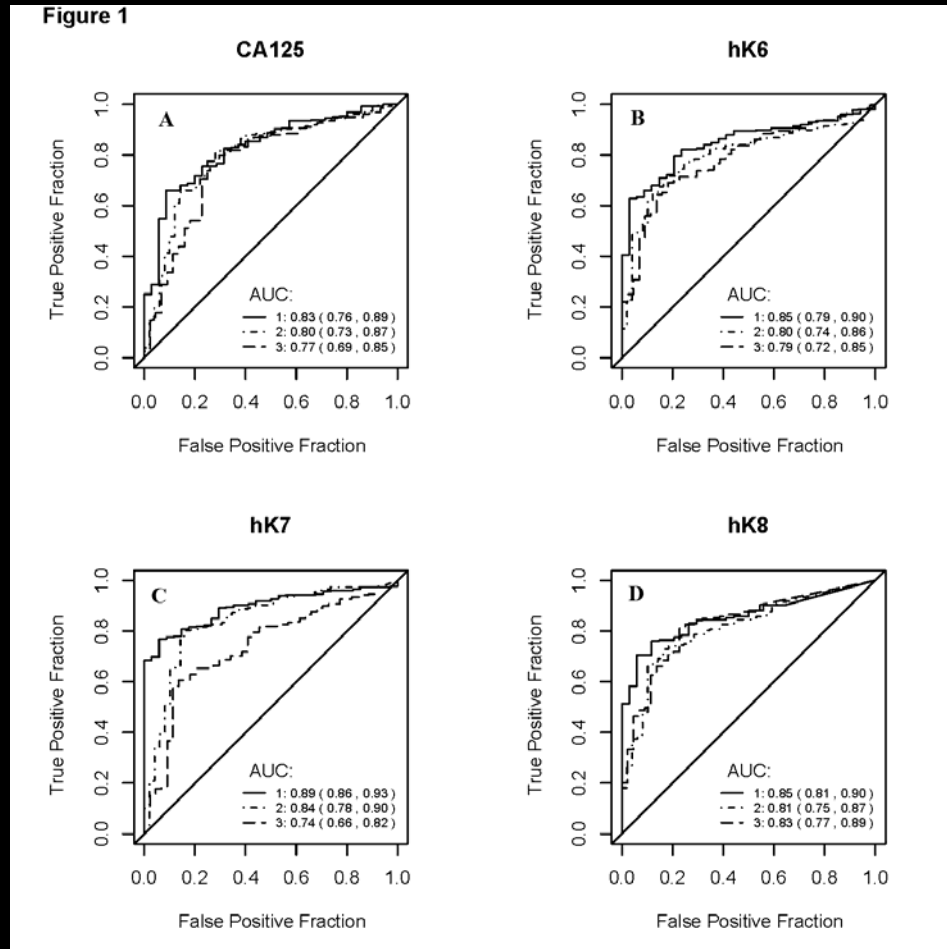
Results will be available early next year

Multiparametric KLK Prognostic Panel

- We measured nine KLKs in ovarian cancer cytosolic extracts
- Developed multiparametric models for prognosis and prediction of patient response to chemotherapy

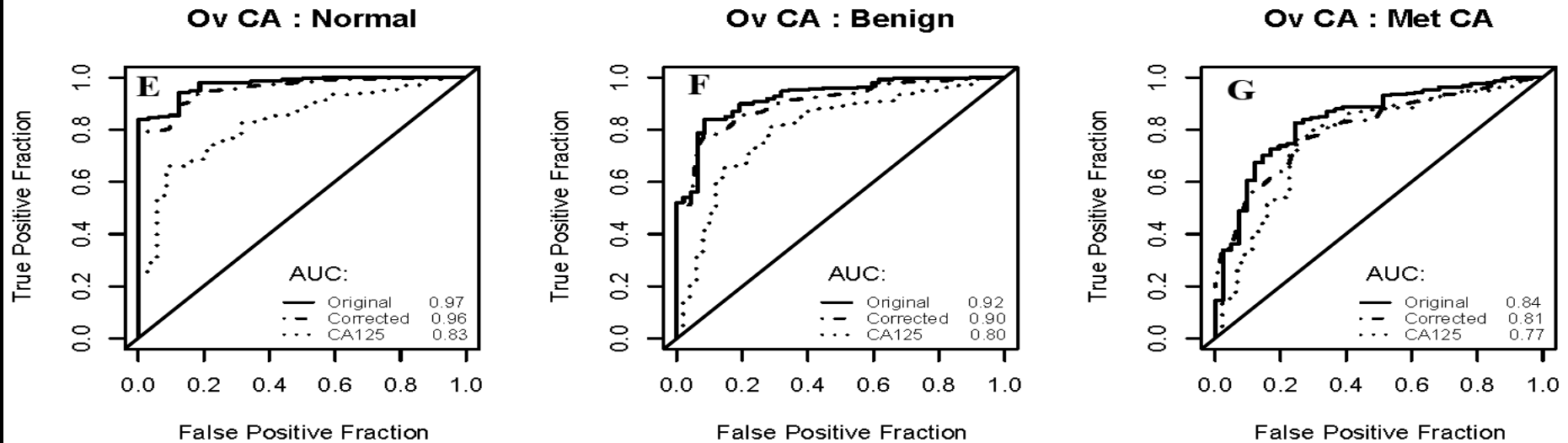
Multiparametric KLK Prognostic Panel

Individual Markers-Patient Classification



Multiparametric KLK Prognostic Panel

□ Biomarker Combinations-Patient Classification

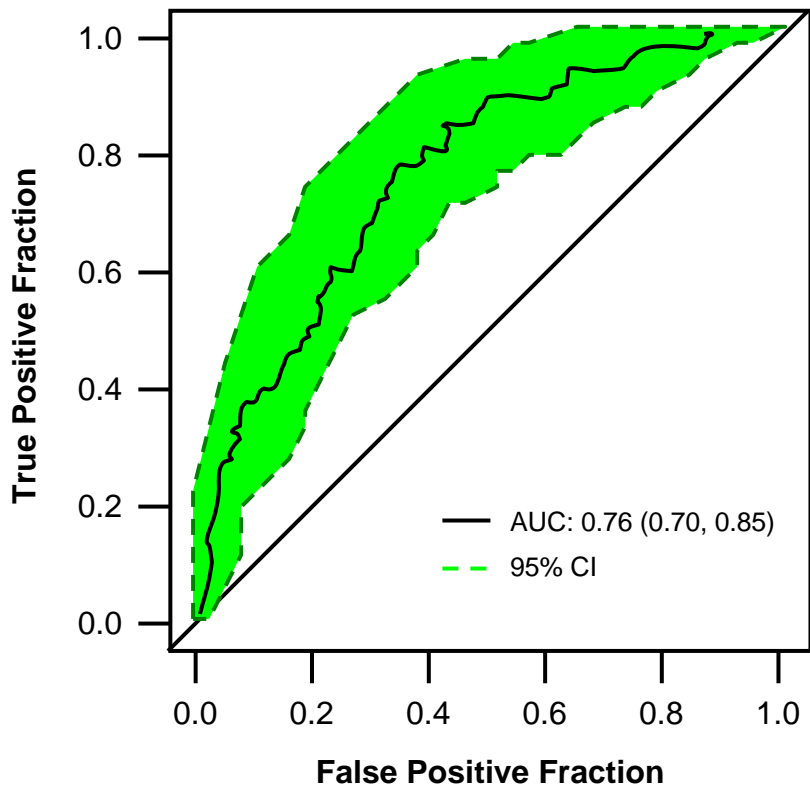


Zheng et al Clin Cancer Res (in press)

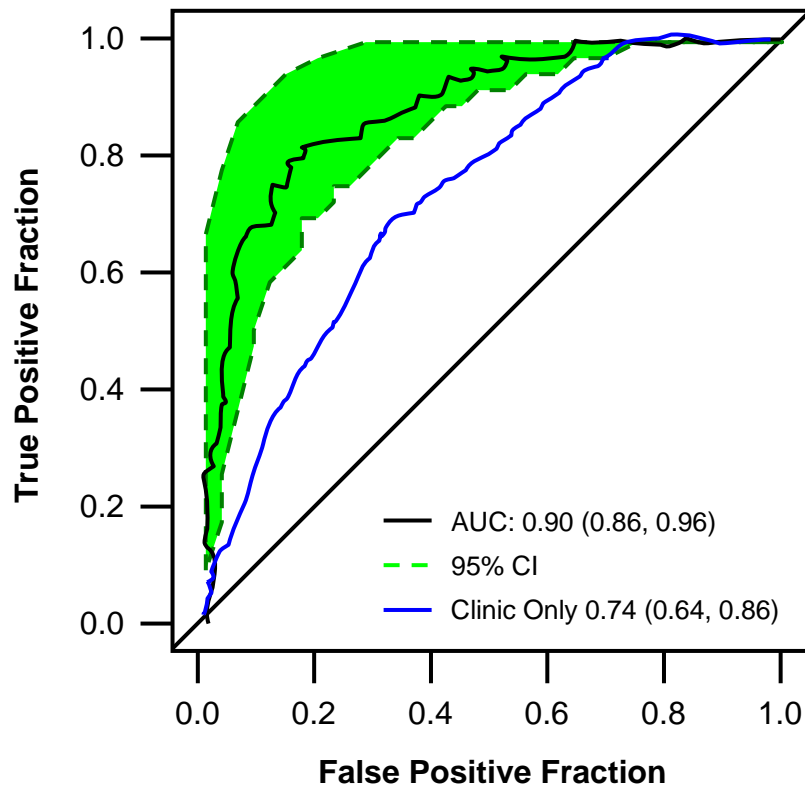
Multiparametric KLK Prognostic Panel

❖ Patient Outcome (PFS) at 1 year

Combined Marker (1 year)



Combined Marker + Clinical (1 year)

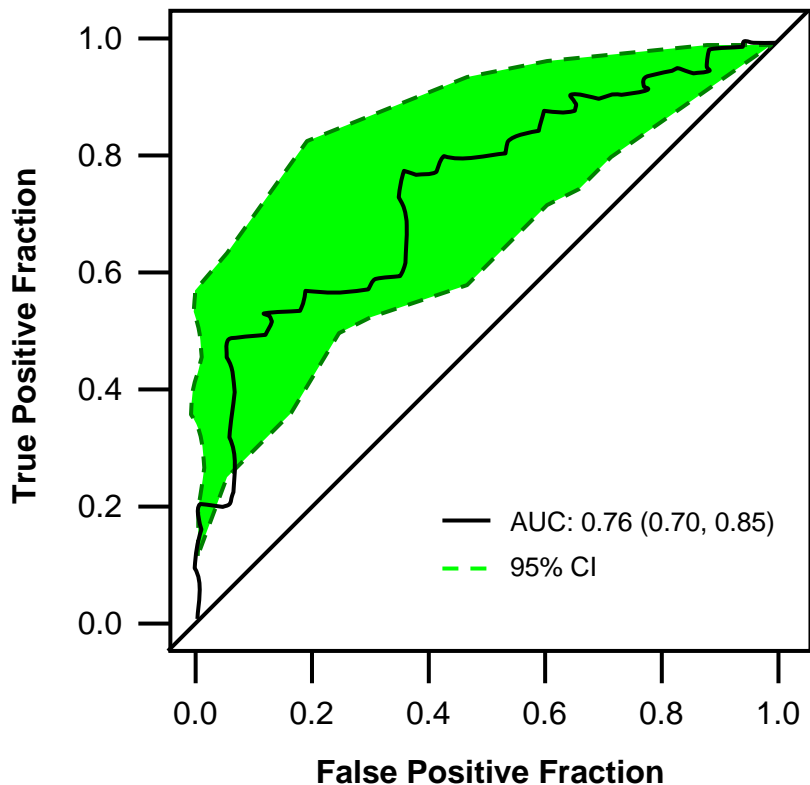


Combined marker for 1 year: KLK6, KLK8, KLK11 and KLK13

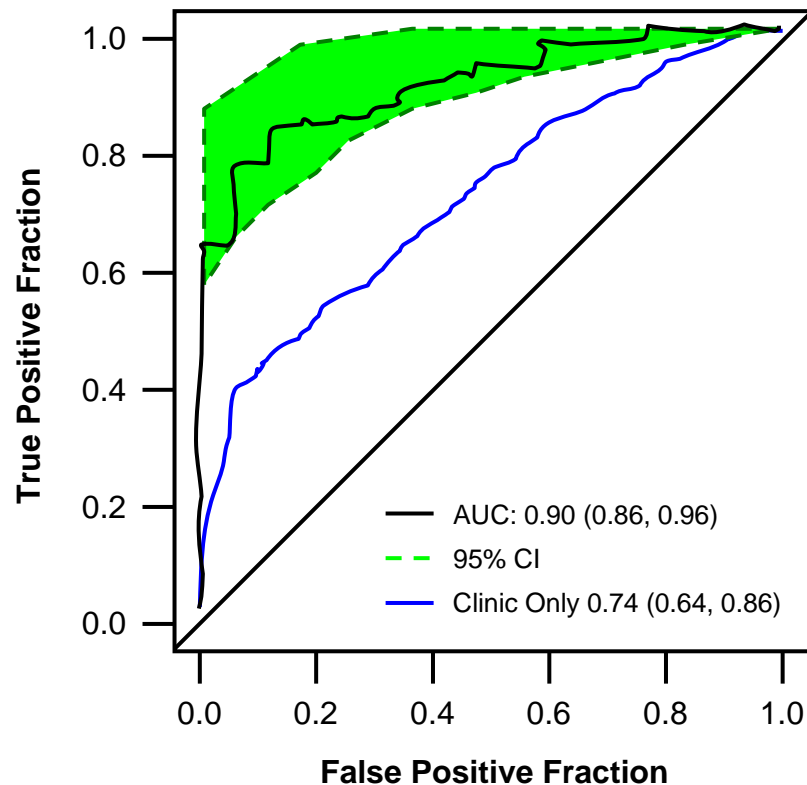
Multiparametric KLK Prognostic Panel

❖ Response to chemotherapy

Combined Marker
CR/PR : NC/PD



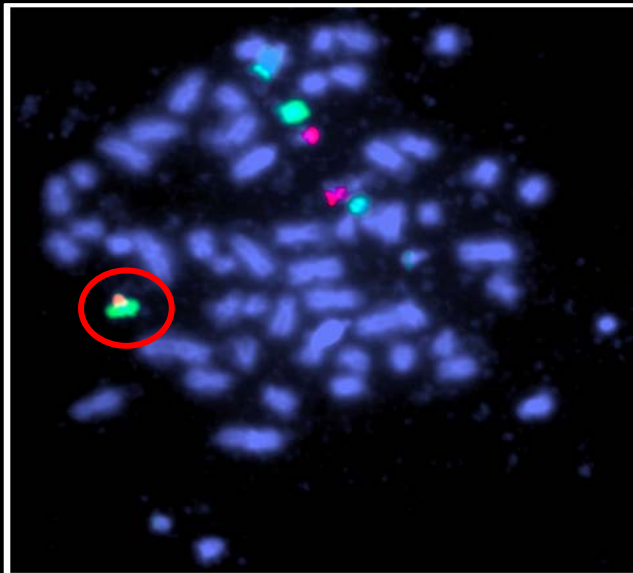
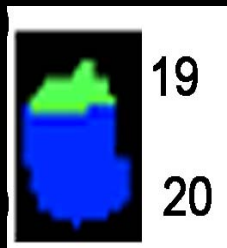
Combined Marker + Clinical
CR/PR : NC/PD



Group of Biomarkers (CA 125, KLK8, KLK13)

KLK Gene Dysregulation in Cancer

- Translocation of *KLK* locus in cancer
 - Breast cancer cell line: **MDA-MB-468**
 - FISH analysis



- FISH using the whole chromosome paint 20 (green) co-hybridizes with the 19q13.3/4 BACs (red) confirming previous SKY data suggesting that chromosome 19 material was translocated to chromosome 20.

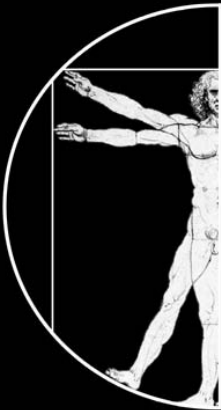
CONCLUSION: Low copy number gain in this hyper-diploid line (pseudotriploid). Two copies reside at the resident chromosome 19 and the other copy was translocated to chromosome 20.

Unbalanced Translocations of the KLK Locus in Ovarian Cancer

- Very frequent in cell lines (ovarian and breast)
- Translocations found in 8 out of 8 (100%) of ovarian cancer patients
- Relationship of translocations to KLK dysregulation (up or down- regulation is currently under investigation
- Role of translocations to prognosis is under investigation

Conclusions

- KLKs are among the most promising new cancer biomarkers but they need additional validation
- Diagnostic, prognostic, predictive
- Panels with other biomarkers may be they way to the future
- Understanding their physiology/pathobiology may lead to better clinical applications, including therapeutics



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