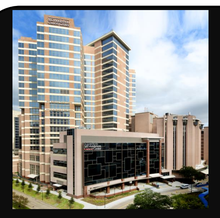

# Does TP53 increase the sensitivity of CA125 in early detection of ovarian cancer?

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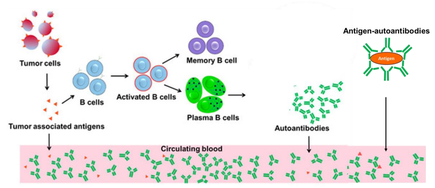
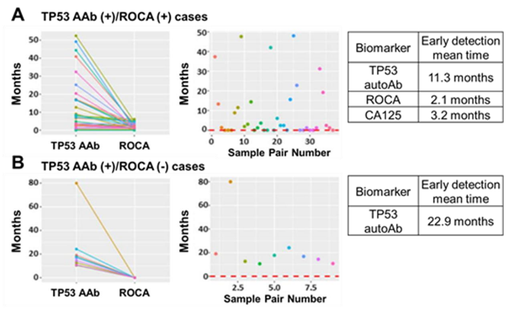
## BACKGROUND

- Computer simulation suggests that early detection of ovarian cancer could reduce mortality by as much as 43%.
- The TP53 tumor-suppressor gene is mutated in > 95% of high grade serous ovarian cancers. Detecting an autologous antibody response to TP53 might improve early detection.
- We have found elevated levels of anti-TP53 autoantibodies (AAb) in 20-25% of patients with the disease. Titers of anti-TP53 rise 12 months prior to CA125 and 22 months prior to diagnosis in patients where CA125 is absent.
- We have evaluated a panel of autoantibodies (AAb) including TP53 and antigens (Ag) as potential biomarkers to augment the sensitivity of CA125 for early detection in ovarian cancer.

### Autoantibodies, antigen-autoantibody complexes, and antigens in human blood




### TP53 Autoantibody shows lead time




| Biomarker | Early detection mean time |
|---|---|
| TP53 autoAb | 11.3 months |
| ROCA | 2.1 months |
| CA125 | 3.2 months |

| Biomarker | Early detection mean time |
|---|---|
| TP53 autoAb | 22.9 months |

**HYPOTHESIS:** Circulating autoantibodies including TP53 autoantibodies and antigens against ovarian cancer are potential biomarkers to augment the sensitivity of CA125

**AIMS:**

1) To develop immune serogical assay for TP53 autoantibody and other early detection biomarkers for ovarian cancer with high sensitivity and specificity of 90%

2) To examine if early detection biomarkers and TP53 autoantibody complement CA125 by detecting cancer cases missed by CA125

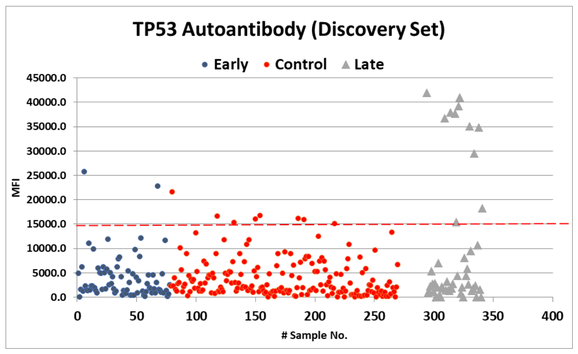
### Characteristics of patient population

| Patient and Tumor Characteristics | Discovery Set | | Validation Set | |
|---|---|---|---|---|
| | Cases | Normal Healthy Control | Cases | Normal Healthy Control |
| Number of Subjects, N | 121 | 216 | 116 | 133 |
| Age (Mean) | 58 | 63 | 58 | 66 |
| Stage, N (%) | | | | |
| Stage I and II (Early) | 75 (62.0%) | - | 69 (59.5%) | - |
| Stage III and IV (Late) | 46 (38.0%) | - | 47 (40.5%) | - |
| Histological Subtyp, N (%) | | | | |
| Serous | 69 (57.0%) | - | 87 (75.0%) | - |
| Non-Serous | 52 (43.0%) | - | 29 (25.0%) | - |

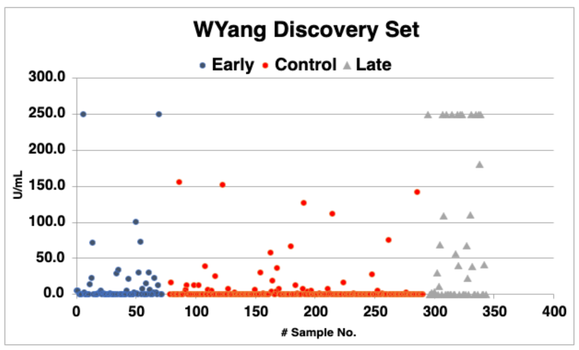
**Table 1. Characteristics of patient population** Number of patient, age, stage and subtype in Discovery sera set and Validation sera set were described respectively.

## RESULTS

### TP53 AAb assay in Discovery set




| 98% | Early | Late | Total | Ctrl |
|---|---|---|---|---|
| (+) Case | 2/75 | 11/46 | 13/121 | 5/216 |
| (%) | 2.7 | 23.9 | 10.7 | 2.3 |

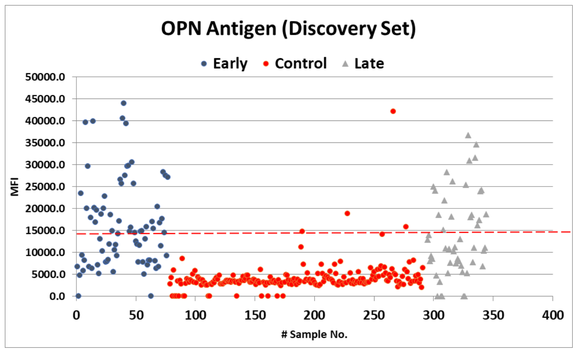



| 98% | Early | Late | Total | Ctrl |
|---|---|---|---|---|
| (+) Case | 3/78 | 15/47 | 18/125 | 5/216 |
| (%) | 3.84 | 31.9 | 14.4 | 2.3 |

**Fig 1. Serum levels of TP53 AAb are elevated in sera from early and late cases of ovarian cancer.** TP53 autoantibody were measured using Luminex Magpix in Discovery set. Each dot represents values for sera from early stage (I/II: blue dots), or late stage (III/IV: grey dots) ovarian cancer, and healthy controls (red dots). The red dashed lines represent the cutoff value at 98% specificity. The table below panel displays the fraction and percent of cases detected at 98% specificity (TP53 Cut off: 16634 Discovery set ).

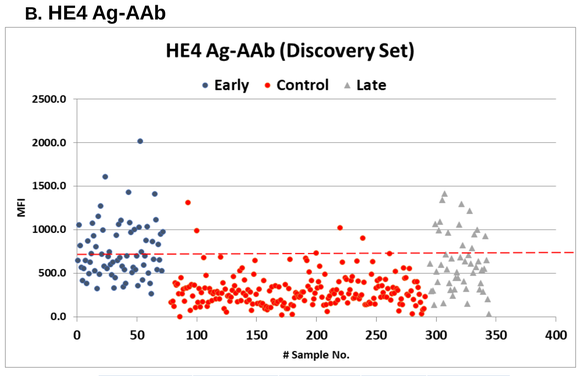
### Autoantibody and antigen markers are elevated in discovery set

**A. Osteopontin (OPN)**



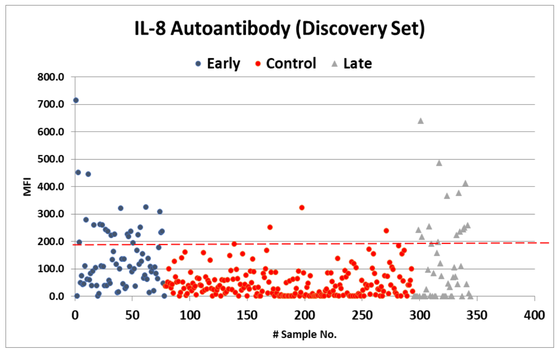

| 98% | Early | Late | Total | Ctrl |
|---|---|---|---|---|
| (+) Case | 38/75 | 20/46 | 58/121 | 5/216 |
| (%) | 50.7 | 43.5 | 47.9 | 2.3 |

**B. HE4 Ag-AAb**




| 98% | Early | Late | Total | Ctrl |
|---|---|---|---|---|
| (+) Case | 29/75 | 14/46 | 43/121 | 5/216 |
| (%) | 38.7 | 30.4 | 35.5 | 2.3 |

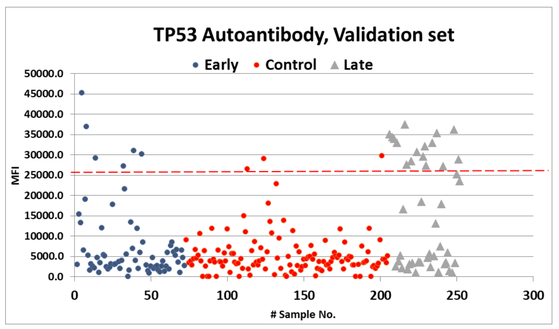
**C. IL-8**




| 98% | Early | Late | Total | Ctrl |
|---|---|---|---|---|
| (+) Case | 22/75 | 15/46 | 37/121 | 5/216 |
| (%) | 29.3 | 32.6 | 30.6 | 2.3 |

**Fig 2. Serum levels of multiple autoantibody and antigens are elevated in sera from early and late cases of ovarian cancer. (A) Osteopontin, (B) HE4 Ag-AAb (C) IL-8** were measured using Luminex Magpix in Discovery set. The table below panel displays the fraction and percent of cases detected at 98% specificity.

### TP53 AAb assay in Validation set




| 98% | Early | Late | Total | Ctrl |
|---|---|---|---|---|
| (+) Case | 6/69 | 16/47 | 22/116 | 3/131 |
| (%) | 8.7 | 34.0 | 18.9 | 2.2 |

**Fig 3. Serum levels of TP53 AAb are elevated in sera from early and late cases of ovarian cancer.** TP53 autoantibody were measured using Luminex Magpix in Validation set. Each dot represents values for sera from early stage (I/II: blue dots), or late stage (III/IV: grey dots) ovarian cancer, and healthy controls (red dots). The red dashed lines represent the cutoff value at 98% specificity. The table below panel displays the fraction and percent of cases detected at 98% specificity (TP53 Cut off: 26443.13 Validation set ).

### Multi-marker panel complements CA125 (-) cases

**Discovery Set**

| Total early case: 75 | CA125 (+) (n=46) | CA125 (-) cases Add up in each assay (n=29) | | |
|---|---|---|---|---|
| | CA125 (+) | CA125(-) +OPN | CA125 +OPN +HE4 Ag-AAb | CA125+ +OPN +HE4 Ag-AAb +IL-8 |
| Added Cases (98% ) | 46/75 61.3% | 46+13 59/75 78.6% | 46+13+5 64/75 85.3% | 46+13+5+3 67/75 81.2% |

**Table 2. Multi-marker panel complements CA125 by detecting cases missed by CA125 (-) cases.** Table displays the sensitivity (% and fraction) of early cancer cases with high CA125 (+) (≥35U/mL) and early cancer cases missed by CA125 (-) (< 35U/mL), respectively. As panel of tested markers were added, cases missed by CA125 were detected in (A) Discovery Set, achieving greater sensitivity compared with CA125 alone.

## CONCLUSIONS

- Anti-TP53 autoantibody assays in the discovery set detected 3-4% of early stage cases and greater late stage cases at 24-32% at 98% specificity, but could not detect cases missed by CA125.
- Among other autoantibodies, antigen, and antigen-autoantibody tested in discovery set, Osteopontin (OPN) showed the greatest sensitivity of 51% for early stage cases and 43.5% in late cases, detecting 13 of 29 (44.8%) of early stage cases missed by CA125, suggesting it can function as surrogate marker for CA125.
- While CA125 alone detected 61% of early stage cases at 98% specificity in discovery set, a combination of CA125, OPN, HE4 Ag-AAb, and IL-8 detected 81.2% of early stage cases.
- Anti-TP53 autoantibody assays in the validation set detected 9% of early stage cases and greater late stage cases at 34% at 98% specificity, but could only detect one case missed by CA125.

## FUTURE PLANS

- We will continue to identify and test other potential autoantibody and antigen markers for achieving greater sensitivity and specificity, complementing CA125.
- We will examine if potential markers show lead time with earlier interval using pre-diagnostic serial samples.
- We will examine if TP53 autoantibody serological assay can be applied to detection of cancers associated with Li-Fraumeni syndrome which shows prevalent of TP53 mutation.
- We will optimize TP53 autoantibody serological assay for achieving greater sensitivity in early cases.

## ACKNOWLEDGMENTS

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