

# **New Biomarkers Help Monitor Patients Deficient in CA125 and Move Diagnosis from Late Stage Toward Stage I and II**

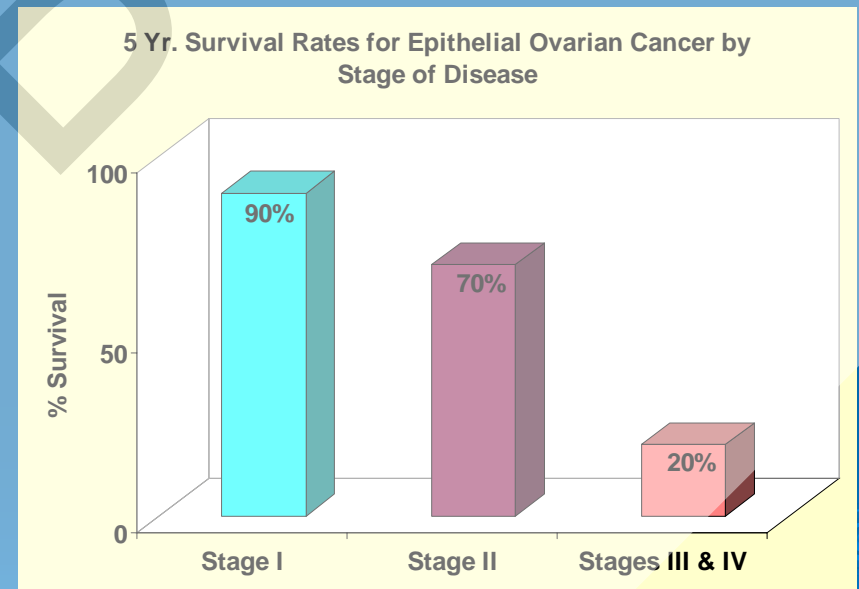
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# Ovarian Cancer Facts

- Ovarian cancer is very treatable when detected early; the vast majority of cases are not diagnosed until too late.
- When diagnosed in Stage I, 90+% will survive 5 years.
- Only 24% of ovarian cancer is detected early.
- When diagnosed after the disease has spread the chance of five-year survival drops to less than 25%.



# Criteria for Valuable Markers

- Highly over-expressed in tumor cells
- Secreted or released from tumor cells
- Relatively low molecular weight (<50,000)
- Low or no expression in normal adult tissue
- Reasonable half life in serum/bodily fluids
- Expressed & secreted in early stage disease

# Research and Development Process

- 30,000 Genes – Overexpression/Secretion
- PCR quantitative – 16 Candidates
- Cloning
- Expression Immunohistochemistry using Peptide Antibodies on Cell Lines
- Recombinant Products – E. Coli and Insect Cells
- Antibodies – Monoclonal
- Assays
- Validation
- Diversity of Antigen Expression

# The Antigens

- TADG-14 (KLK8)
- SCCE (KLK7)
- MMP-7 (Pump1)
- ALP (SLPI)
- TADG-15 (Matriptase)
- Hepsin
- TADG-12 (TMPRSS3)
- TADG-12D
- TADG-12V
- TADG-14V
- Hepsin V
- CA125 Repeat Variants

# Validation of Proposed Markers

- PCR on Tumors
- Immunohistochemistry
- Assessment of Stage I & II vs. Stage III & IV
- Markers Complementary to CA125

# Serum Assays

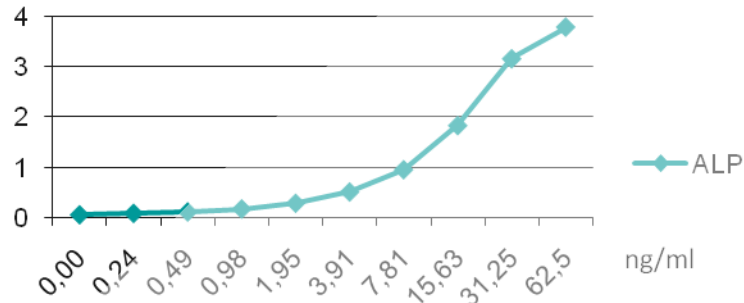
## ■ Current Markers

- CA125
- TADG-15 (Matriptase)
- SCCE (hK7)
- ALP
- MMP-7
- TADG-14 (hK8)

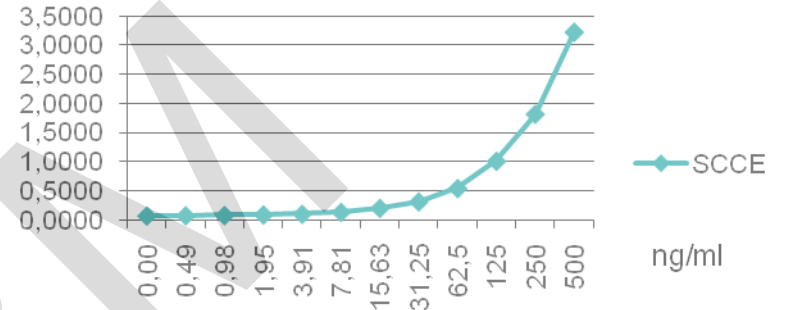
## ■ In Development

- Hepsin
- TADG-12/12D (TMPRSS3)

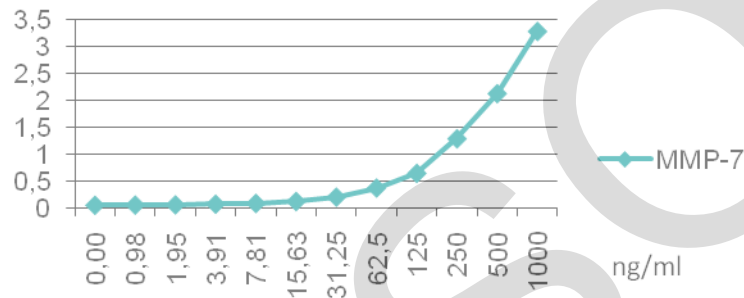
### ALP 2-Antibody Assay



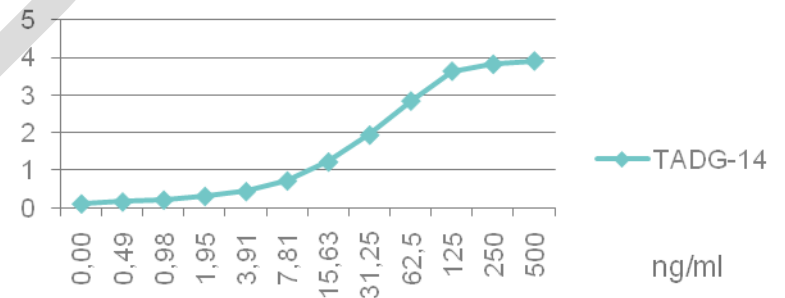
### SCCE 2-Antibody Assay



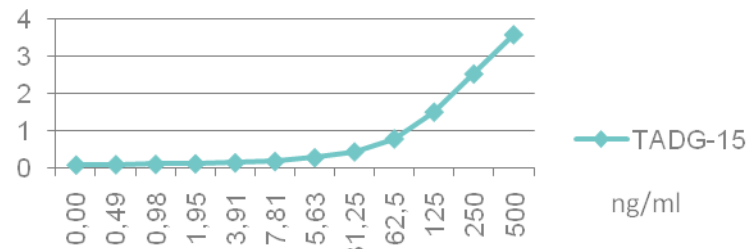
### MMP-7 2-Antibody Assay



### TADG-14 3-Antibody Assay



### TADG-15 2-Antibody Assay



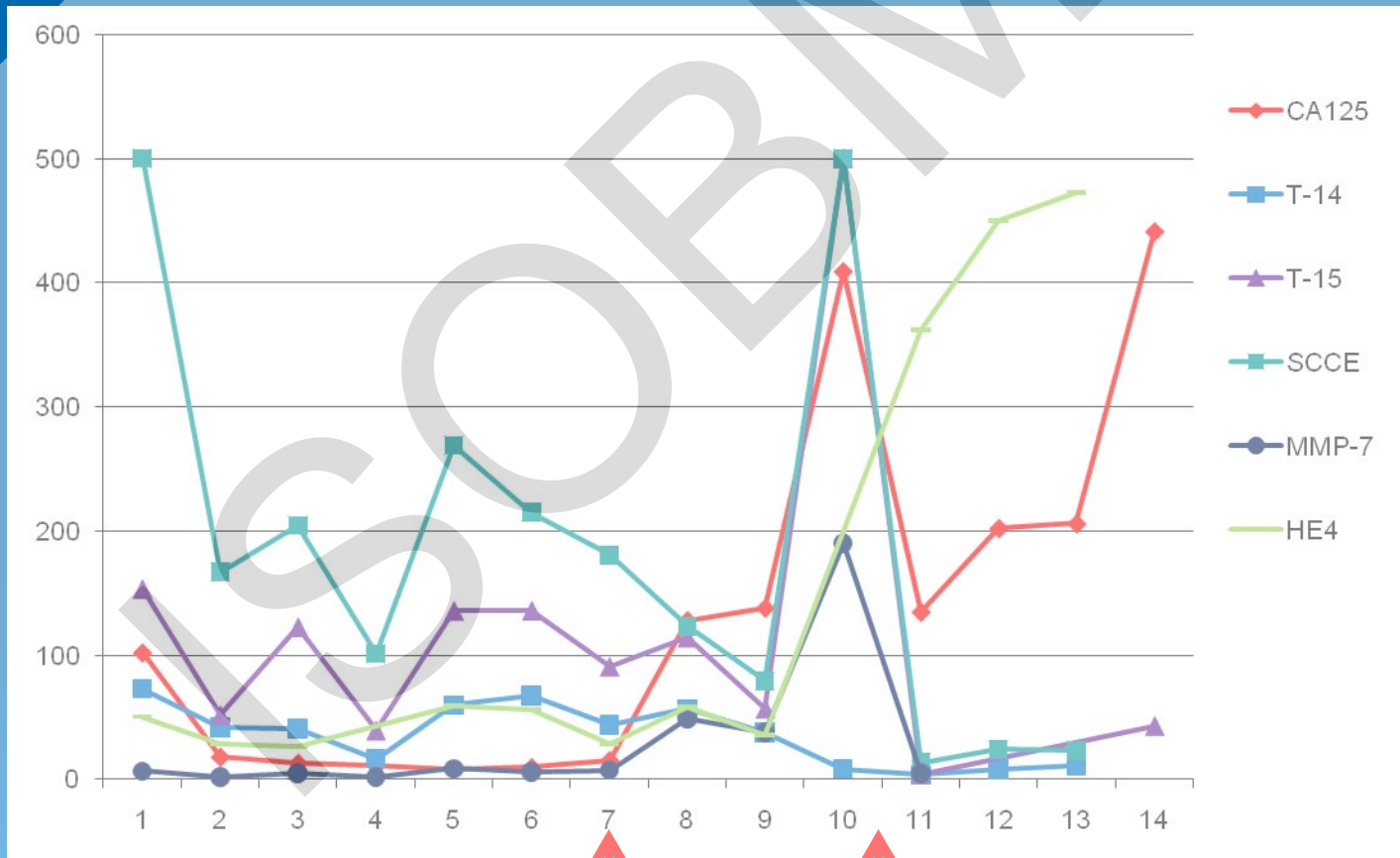
# Post-Menopausal Normal Serum Samples

| Post-Menopausal Normals | TADG-14<br>ng/ml | TADG-15<br>ng/ml | SCCE<br>ng/ml   | MMP-7<br>ng/ml  | ALP<br>ng/ml  | CA125<br>U/ml |
|-------------------------|------------------|------------------|-----------------|-----------------|---------------|---------------|
| 1                       | < 0.5            | 69.88            | < 0.5           | < 2.0           | 50.86         | 2.48          |
| 2                       | < 0.5            | 148.50           | < 0.5           | 4.08            | 75.41         | 21.62         |
| 3                       | < 0.5            | 24.60            | < 0.5           | 39.39           | 41.43         | 13.83         |
| 4                       | < 0.5            | 10.27            | 4.56            | < 2.0           | 89.59         | ND            |
| 5                       | < 0.5            | 19.55            | 2.28            | 24.47           | 47.35         | ND            |
| 6                       | < 0.5            | 17.10            | < 0.5           | < 2.0           | < 10.0        | 8.88          |
| 7                       | < 0.5            | 22.69            | 3.58            | < 2.0           | 40.21         | ND            |
| 8                       | < 0.5            | 21.48            | < 0.5           | 4.56            | 16.11         | 7.43          |
| 9                       | < 0.5            | 24.78            | < 2.0           | < 2.0           | 110.93        | ND            |
| 10                      | 6.67             | 7.33             | 0.6             | 5.35            | < 10.0        | 6.26          |
| 11                      | 5.76             | 4.13             | 1.4             | 4.87            | 71.32         | ND            |
| 12                      | < 0.5            | 51.39            | < 2.0           | < 2.0           | 137.74        | ND            |
| 13                      | < 0.5            | 83.43            | < 0.5           | < 2.0           | < 10.0        | ND            |
| 14                      | 1.49             | 88.87            | < 0.5           | < 2.0           | 102.39        | ND            |
| 15                      | < 1.0            | 1.21             | ND              | < 2.0           | 90.04         | ND            |
| 16                      | 3.19             | 117.53           | ND              | < 2.0           | 102.16        | ND            |
| 17                      | ND               | 5.04             | ND              | 2.55            | < 10.0        | ND            |
| 18                      | < 0.5            | 5.69             | 4.04            | 3.67            | ND            | ND            |
| <b>Mean</b>             | <b>1.42</b>      | <b>40.20</b>     | <b>1.40</b>     | <b>6.05</b>     | <b>59.74</b>  | <b>10.08</b>  |
| <b>Median</b>           | <b>&lt; 0.5</b>  | <b>22.08</b>     | <b>&lt; 0.5</b> | <b>&lt; 2.0</b> | <b>50.86</b>  | <b>8.15</b>   |
| <b>95%</b>              | <b>4.59</b>      | <b>111.67</b>    | <b>3.81</b>     | <b>22.16</b>    | <b>127.43</b> | <b>35.00</b>  |

# Monitoring

- CA125 Negative Patients
- Overall Expression
  - High Stage Disease

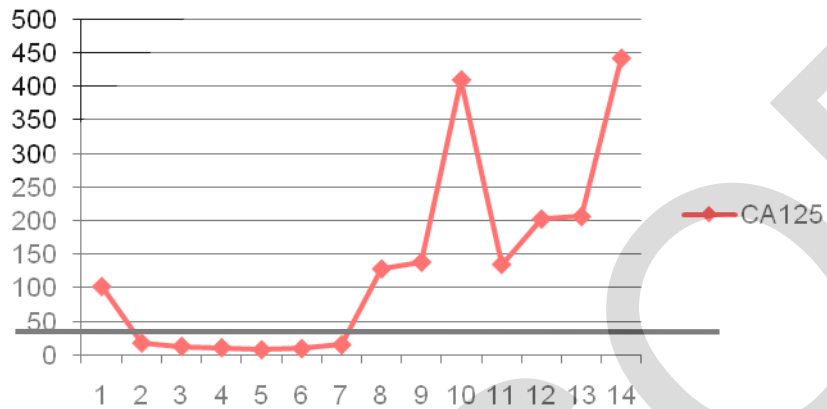
# Stage III/IV Monitoring Patient 1-SB



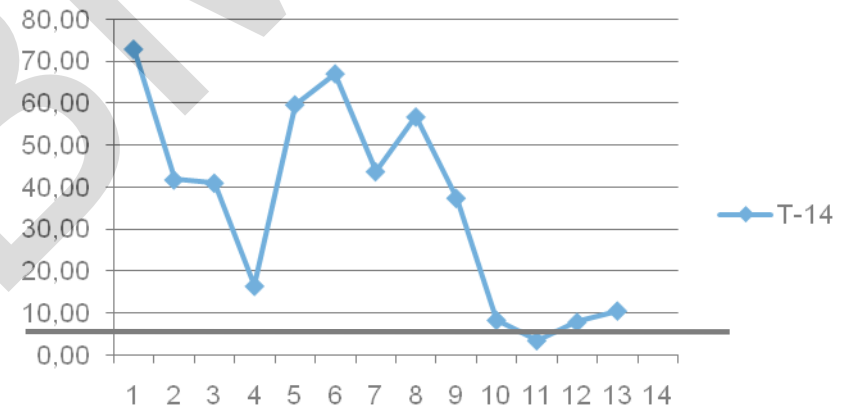
# Stage III/IV SCCE & T-14

## Patient 1-SB

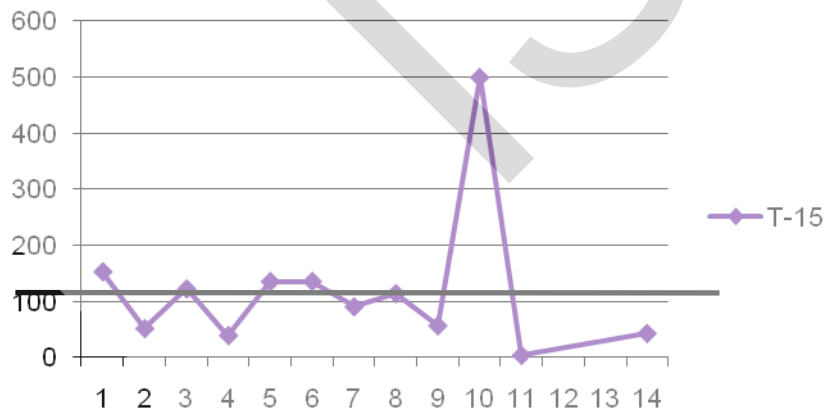
### CA125



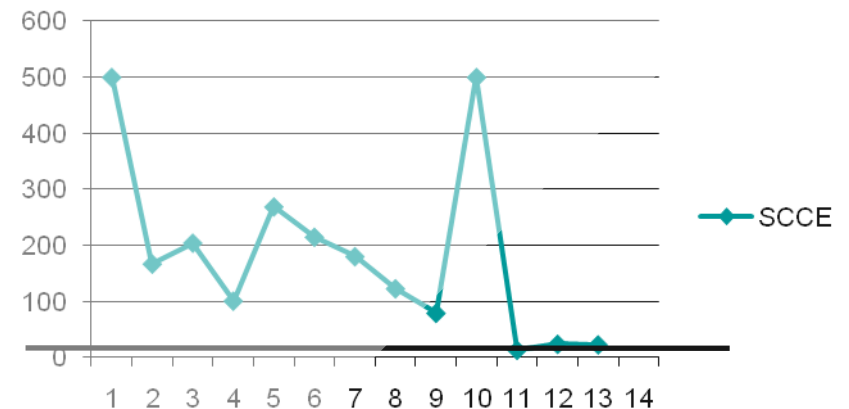
### T-14



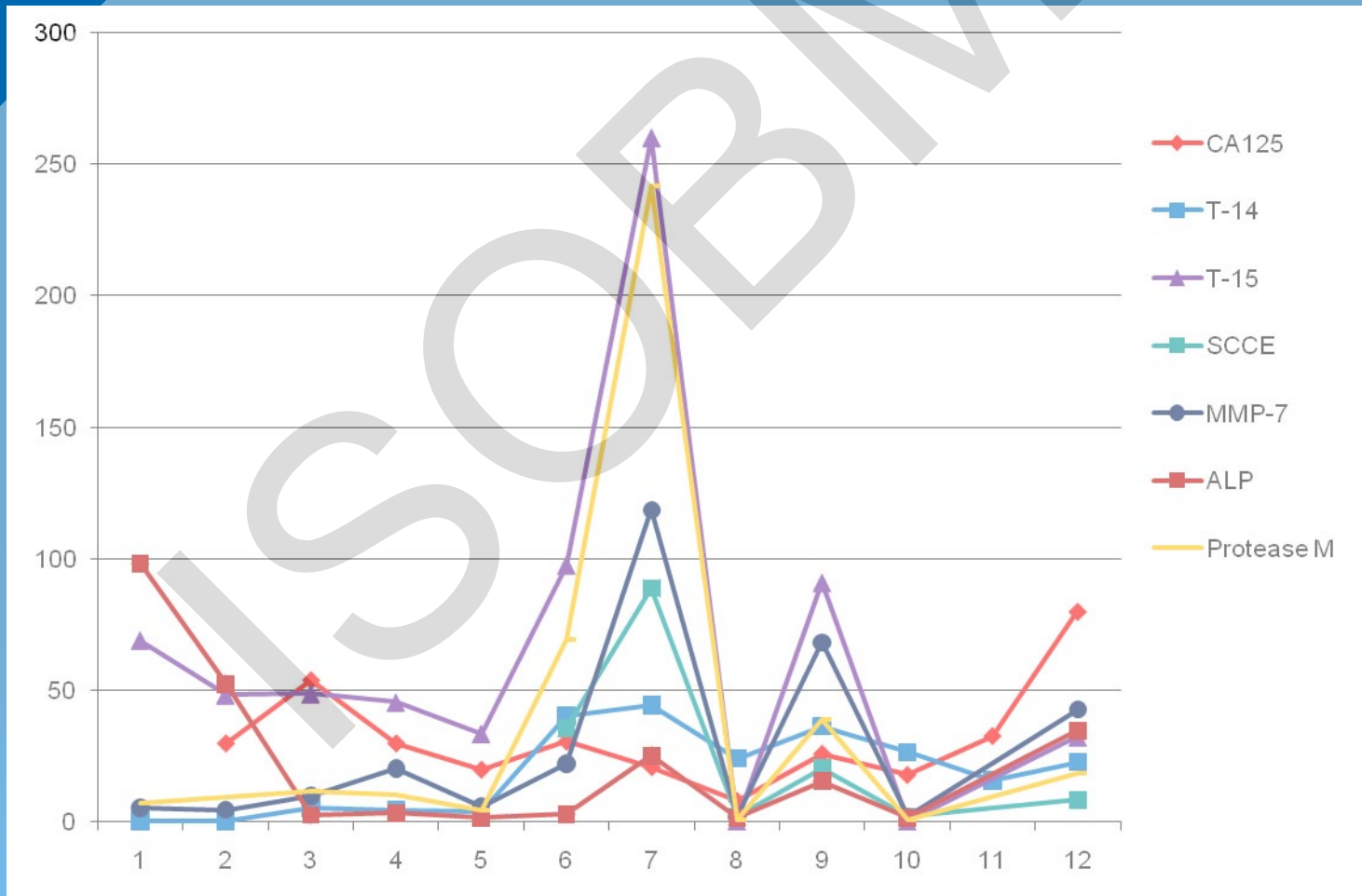
### T-15



### SCCE



# Stage III/IV T-15 & SCCE Patient 2-GR

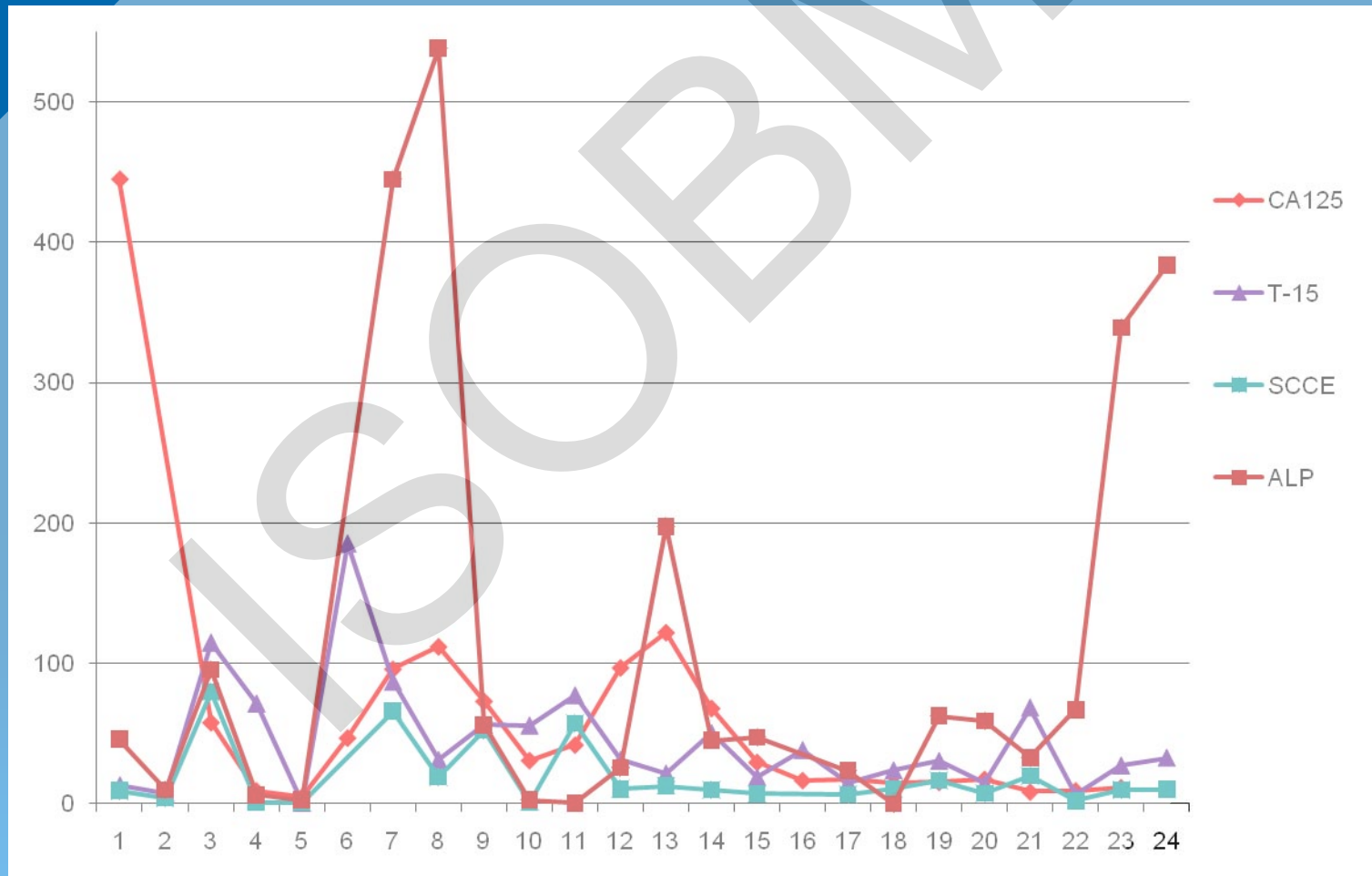


# Stage III/IV SCCE & ALP

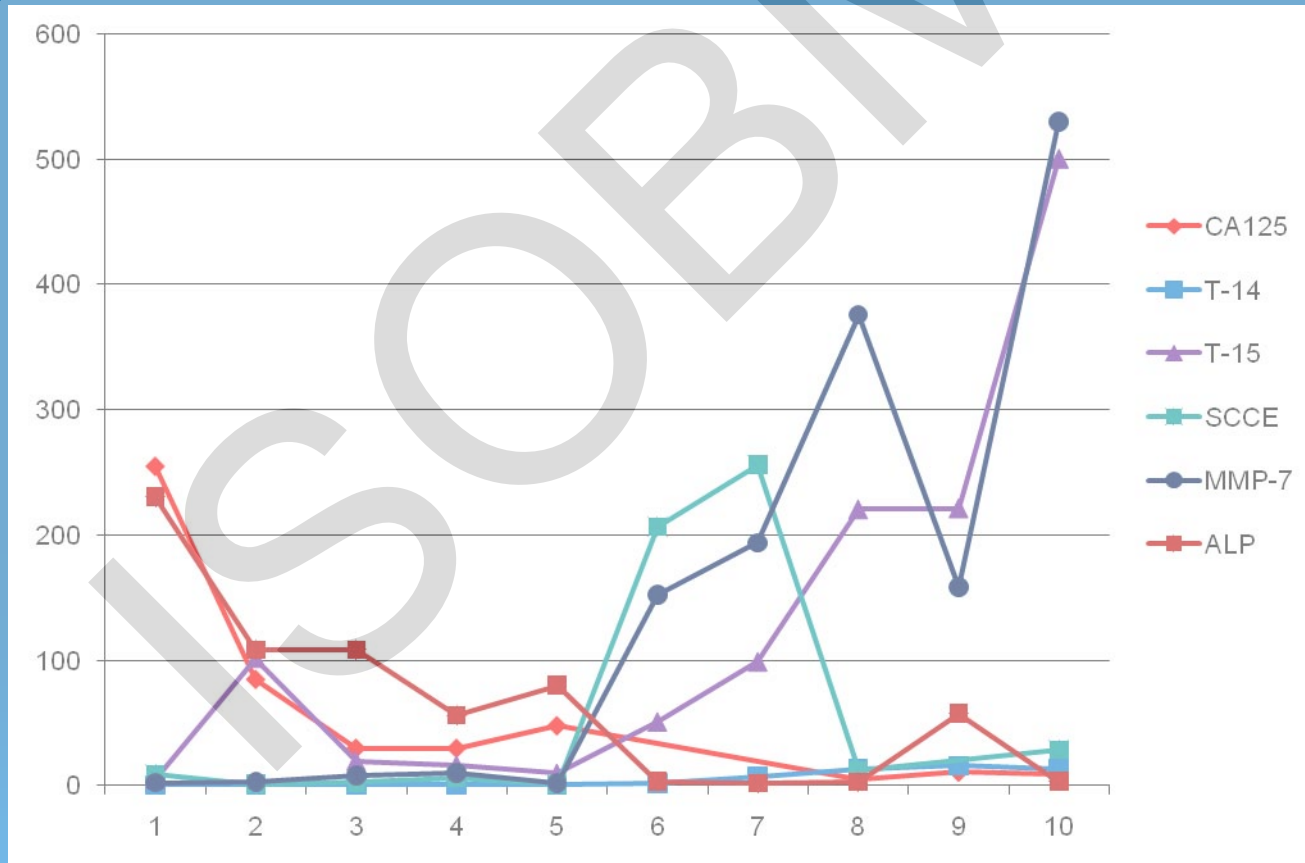
## Patient 3-MS



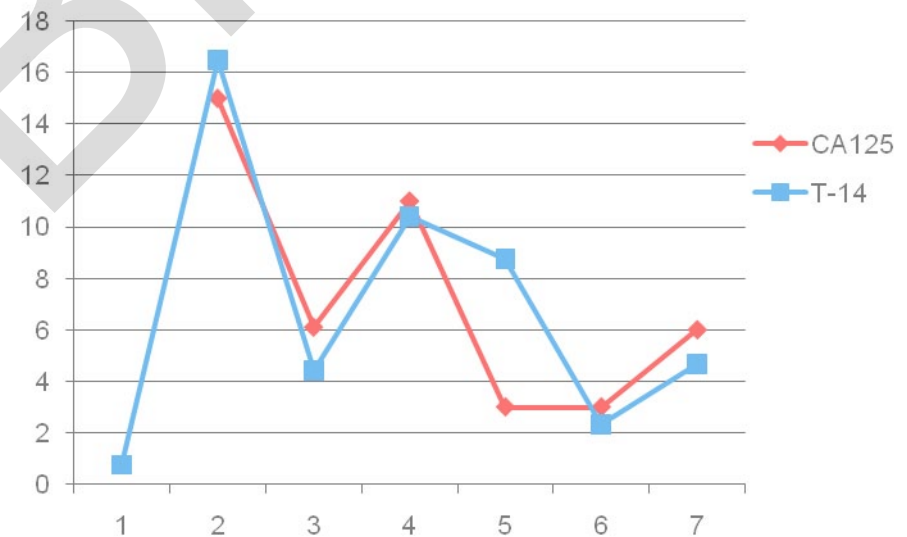
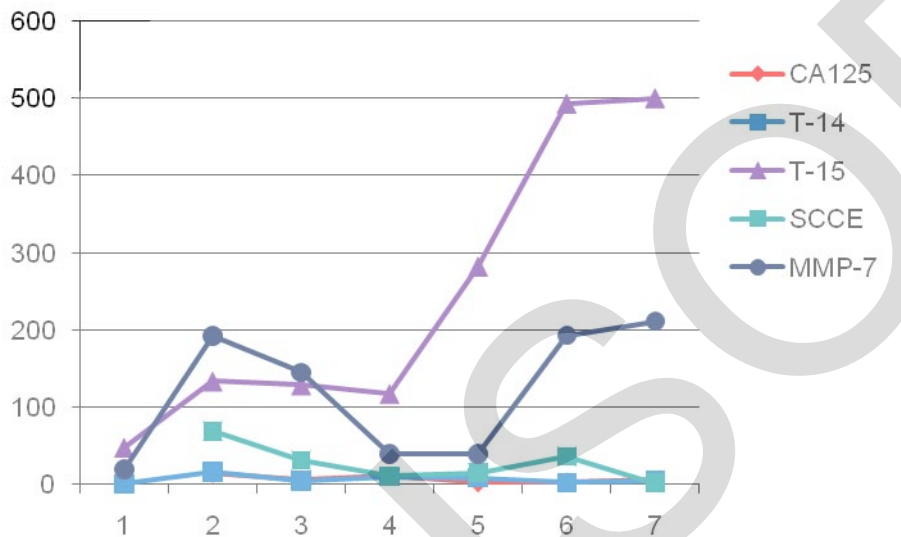
# Low Grade Serous ALP & CA125 Patient 4-AP



# Stage III/IV MMP-7 & T-15 Patient 5-DR



# Stage I Serous Patient 6-AF



# Diagnosis

- Screening
- Early Stage Disease
  - Stage I & II

# Screening Early Stage Tumors

| Sample     | Stage | Type              | TADG-14    | TADG-15      | SCCE       | MMP7        | ALP          | CA125     |
|------------|-------|-------------------|------------|--------------|------------|-------------|--------------|-----------|
| 1          | II    | Unknown           | < 0.5      | 3.5          | < 2.0      | < 2.0       | 418.4        | 7.4       |
| 2          | IA    | Endo-metrioid     | 2.9        | 4.0          | < 2.0      | < 2.0       | 8.4          | 6.4       |
| 3          | II    | Unknown           | < 0.5      | 425.9        | < 0.5      | 30.1        | ND           | ND        |
| 4          | IIB   | Endo-metrioid     | 0.6        | 180.9        | < 2.0      | ND          | 3.7          | 12        |
| 5          | IIC   | Serous            | 2.2        | 87.0         | < 1.0      | 7.8         | < 0.3        | 16        |
| 6          | II    | Pap. Serous       | 8.0        | 145.1        | 7.8        | 47.5        | 3.3          | 16        |
| 7          | II    | Serous            | 2.9        | 31.4         | 8.9        | 5.9         | 117.9        | 207       |
| 8          | II    | Unknown           | 0.8        | 793.4        | 95.0       | 31.3        | 172.9        | 202       |
| 9          | IC    | Granulosa         | < 0.5      | > 500        | 5.48       | 7.21        | 30.9         | ND        |
| 10         | IB    | Borderline Serous | 0.5        | 58.9         | 11.3       | 14.0        | 5.5          | 30        |
| 11         | IIB   | Endo-metrioid     | < 0.5      | 119.45       | < 2.0      | < 4.0       | 10.9         | 29        |
| 12         | II    | Serous            | < 0.5      | 908.4        | < 2.0      | 7.1         | ND           | 13        |
| 13         | IA    | Serous            | 11.2       | 167.9        | 69.0       | 13.4        | 267.0        | 15        |
| 14         | I     | Serous            | 2.0        | 496.8        | 48.5       | 18.0        | 22.4         | 20        |
| 15         | I     | Serous            | < 0.5      | 13.8         | < 2.0      | < 2.0       | 130.1        | ND        |
| <b>95%</b> |       |                   | <b>4.6</b> | <b>111.7</b> | <b>3.8</b> | <b>22.2</b> | <b>127.4</b> | <b>35</b> |

# Screening Early Stage Tumors 2

| Sample     | Stage | Type                | TADG-14    | TADG-15      | SCCE       | MMP7        | ALP          | CA125     |
|------------|-------|---------------------|------------|--------------|------------|-------------|--------------|-----------|
| 16         | IIB   | Clear Cell          | 7.8        | 28.3         | ND         | 157.6       | 1.6          | 8.3       |
| 17         | II    | Unknown             | < 0.5      | 1.0          | 43.0       | < 2.0       | 469.0        | ND        |
| 18         | II    | Endo-metrioid       | < 0.5      | ND           | < 2.0      | ND          | 87.6         | 5         |
| 19         | II    | Unknown             | 4.5        | 103.0        | 286.0      | 18.5        | 208.9        | 67        |
| 20         | I     | Borderline Serous   | 0.7        | 10.3         | < 2.0      | < 2.0       | 2.8          | 9         |
| 21         | I     | Mucinous            | 2.7        | 248.2        | 19.1       | ND          | 24.6         | 23        |
| 22         | II    | Unknown             | < 0.5      | 16.6         | < 2.0      | 2.8         | 162.0        | 120       |
| 23         | IIC   | Undiff.             | < 0.5      | 547.5        | 2.0        | 35.6        | ND           | 14        |
| 24         | IIC   | Endo-metrioid       | < 0.5      | 6.3          | < 2.0      | < 2.0       | 143.7        | 500       |
| 25         | IC    | Germ Cell           | 1.8        | 18.9         | < 2.0      | ND          | 29.9         | 179       |
| 26         | I     | Borderline Mucinous | ND         | 470.8        | < 2.0      | 4.0         | 18.6         | ND        |
| 27         | IC    | Mucinous            | < 0.5      | 441.7        | < 2.0      | 10.8        | ND           | 8         |
| 28         | II    | Unknown             | < 0.5      | 39.8         | < 2.0      | < 2.0       | 296.4        | 5         |
| 29         | IIB   | Endo-metrioid       | < 0.5      | 13.1         | < 2.0      | < 2.0       | 335.7        | ND        |
| 30         | II    | Unknown             | < 0.5      | 17.7         | < 2.0      | < 2.0       | 155.9        | 5         |
| <b>95%</b> |       |                     | <b>4.6</b> | <b>111.7</b> | <b>3.8</b> | <b>22.2</b> | <b>127.4</b> | <b>35</b> |

# Early Stage Tumor Serum Assay Summary

| Antigen         | Number Positive | Percentage |
|-----------------|-----------------|------------|
| CA125           | 6/25            | 24%        |
| TADG-14         | 3/28            | 11%        |
| TADG-15         | 13/29           | 45%        |
| SCCE            | 10/29           | 34%        |
| MMP-7           | 5/25            | 20%        |
| ALP             | 11/26           | 42%        |
| CA125 + TADG-14 | 9/29            | 31%        |
| CA125 + TADG-15 | 18/30           | 60%        |
| CA125 + SCCE    | 13/30           | 46%        |
| CA125 + MMP-7   | 10/30           | 33%        |
| CA125 + ALP     | 13/29           | 45%        |
| CA125 + All     | 26/30           | 87%        |

# Serum Assays Summary

| Assay        | Stage I&II   | Stage III&IV | Pap.Serous<br>+ Serous Ca. | Mucinous<br>Ca. | Endo-<br>metrioid<br>Ca. | Clear Cell<br>Ca. | Other<br>(granulosa,<br>germ cell,<br>etc.) |
|--------------|--------------|--------------|----------------------------|-----------------|--------------------------|-------------------|---|
| CA125        | 6            | 39           | 17                         | 4               | 4                        | 2                 | 6   |
| TADG-14      | 3            | 12           | 6                          | 0               | 3                        | 3                 | 2   |
| SCCE         | 10           | 28           | 16                         | 2               | 1                        | 3                 | 4   |
| ALP          | 11           | 16           | 7                          | 0               | 5                        | 2                 | 2   |
| MMP-7        | 5            | 11           | 7                          | 1               | 2                        | 1                 | 0   |
| TADG-15      | 13           | 24           | 12                         | 7               | 3                        | 1                 | 5   |
| <b>Total</b> | <b>26/30</b> | <b>66/70</b> | <b>34/39</b>               | <b>7/7</b>      | <b>12/15</b>             | <b>8/9</b>        | <b>8/8</b>                                  |

# Serum Assays

## ■ Current Markers

- CA125
- TADG-15 (Matriptase)
- SCCE (hK7)
- ALP (SLPI)
- MMP-7 (Pump-1)
- TADG-14 (hK8)

## ■ In Development

- Hepsin (TMPRSS1)
- TADG-12/12D (TMPRSS3)

ISOBIM



# Benign Disease Serum Assay Summary

| Antigen     | Number Positive | Percentage |
|-------------|-----------------|------------|
| CA125       | 5/18            | 28%        |
| TADG-14     | 1/19            | 5%         |
| TADG-15     | 5/20            | 25%        |
| SCCE        | 6/20            | 30%        |
| MMP-7       | 4/17            | 24%        |
| ALP         | 1/11            | 9%         |
| All – CA125 | 9/22            | 41%        |
| All + CA125 | 14/22           | 64%        |