In-situ Protein Expression Analysis of Cancer Testis Antigen SSX2
Achim Jungbluth, Denise Frosina, Cecilia Lezcano and Klaus Busam

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European Society of Pathology
31st European Congress of Pathology
7-12/September/2019 – Nice, France
In-situ Protein Expression Analysis of Cancer Testis Antigen SSX2

Background – CT Antigens

Identified by autologous T-cell cloning (prototype MAGE-A1)!
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Limited knowledge about many CT antigens - lack of suitable reagents (‘antibodies’)
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Background – CT Antigens

Expression in testis
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Background – SSX

Identified as fusion partners of SYT in synovial sarcoma

Identification of novel genes, SYT and SSX, involved in the t(X;18)(p11.2;q11.2) translocation found in human synovial sarcoma

Jeremy Clark, Philippe J. Rosquet, I. Jayne Creel, Sandra Giff, Janet Shipley, Andrew M.-L. Chan, Barry A. Gaster and Colin S. Cooper

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Member of a multi-gene family, SSX1-SSX9
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Elicit autologous immune responses in melanoma/tumor patients (SEREX)
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Smith et al., Clin Dev Immunol. 2010
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Knowledge about in-situ expression limited
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Most common fusion partner in synovial sarcoma
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Interesting antigen as potential vaccine target
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Interesting antigen as potential vaccine target
Limited and contradictory data about expression, mostly rt-PCR based
In-situ Protein Expression Analysis of Cancer Testis Antigen SSX2

Background – SSX2 Expression

Published Expression Data
# In-situ Protein Expression Analysis of Cancer Testis Antigen SSX2

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<td>Tureci, 1998</td>
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In-situ Protein Expression Analysis of Cancer Testis Antigen SSX2

SSX2 Protein Expression – Available Antibodies

Identified in serum of synovial sarcoma patient by serological analysis of tumor expression library SEREX

Interesting antigen as potential vaccine target

Contradictory expression data
In-situ Protein Expression Analysis of Cancer Testis Antigen SSX2

Background – SSX2 Protein Expression

<table>
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<tr>
<th>Clone</th>
<th>Recognized Antigen</th>
<th>SXX Splice Variant</th>
<th>Comment</th>
<th>References</th>
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<td>E3AS</td>
<td>SSX2, SSX3, SSX4</td>
<td>SSX2v1 &amp; SSXv2</td>
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<td>Unspecific</td>
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<td>CL3202</td>
<td>SSX2;</td>
<td>SSX2v1</td>
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<td>Atlas Antibodies;</td>
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</table>
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SSX2 – CL3202 Immunohistochemical Analysis

SSX2-isoform 1

MNGDDAFARRPTVGAQIKEPIQKAFDDIAKYFSKEEWEKMKASEKIFYVYMKRKYAMTKLGFKATLPPFMCNKRAEDF
QGNDLDNDPNRGNQVERPQMTFGRLOQGISPKIMPKPAEEGNDEEVPESGPMQNDGKELCPPGKPTTSEKHERSGP
KRGEHAWTHRLRERKQLVIYEEISDPEEDDE
In-situ Protein Expression Analysis of Cancer Testis Antigen SSX2

SSX2 – CL3202 Immunohistochemical Analysis

BLAST

Filter by:

Reviewed (10)
Swiss-Prot
Unreviewed (184)
TREMBL
Proteomes (178)

Overview

Show all 194

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<tr>
<th>Entry</th>
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<th>Identity</th>
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<td>Protein SSX7</td>
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<td>Putative protein SSX6</td>
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<tr>
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<td>Protein SSX4</td>
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<td>Protein SSX5</td>
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Memorial Sloan Kettering Cancer Center
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SSX – CL3202 Immunohistochemical Analysis

CL3202 is not SSX2-specific but most likely reactive with most/all SSX proteins!
In-situ Protein Expression Analysis of Cancer Testis Antigen SSX2

SSX – CL3202 Immunohistochemical Analysis

30 normal tissues and 322 tumors were analyzed.

Grading:

Percentage of immunopositive:

- **Neg**: 0
- **Focal**: <5%
- **+**: 5-25%
- **++**: 26-50%
- **+++**: 51-75%
- **++++**: >75%

Carrier-Based Multi-Tissue Blocks
In-situ Protein Expression Analysis of Cancer Testis Antigen SSX2

SSX – Normal Tissues
In-situ Protein Expression Analysis of Cancer Testis Antigen SSX2

SSX – Normal Tissues
## In-situ Protein Expression Analysis of Cancer Testis Antigen SSX2

### SSX – CL3202 IHC Positive Tumors

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<th>Tumor</th>
<th>Total</th>
<th>%</th>
<th>Neg</th>
<th>Pos</th>
<th>foc</th>
<th>+</th>
<th>++</th>
<th>+++</th>
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<tbody>
<tr>
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<td>20</td>
<td>100%</td>
<td>0</td>
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<td>Melanoma</td>
<td>30</td>
<td>23%</td>
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<td>1</td>
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</tr>
<tr>
<td>Thyroid ca</td>
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<td>41%</td>
<td>10</td>
<td>7</td>
<td>7</td>
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<tr>
<td>Osteo SX</td>
<td>15</td>
<td>33%</td>
<td>10</td>
<td>5</td>
<td>3</td>
<td>1</td>
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<td>IDC (breast)</td>
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CL3202

SSX – Tumors, Synovial Sarcoma

20 cases

13 Female; 7 Male

14 monophasic, 7 biphasic

13 primaries, 4 metastasis, 3 recurrences
In-situ Protein Expression Analysis of Cancer Testis Antigen SSX2

CL3202

SSX – Tumors, Synovial Sarcoma, monophasic
In-situ Protein Expression Analysis of Cancer Testis Antigen SSX2

CL3202

SSX – Positive Tumors, Synovial Sarcoma, biphasic
In-situ Protein Expression Analysis of Cancer Testis Antigen SSX2

CL3202

SSX – Positive Tumors, Synovial Sarcoma, biphasic
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SSX – CL3202 IHC Positive Tumors

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CL3202
SSX – Positive Tumors, Melanoma (metast), homogeneous expression
In-situ Protein Expression Analysis of Cancer Testis Antigen SSX2

CL3202
SSX – Positive Tumors, Melanoma (metast), heterogeneous expression
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<tr>
<td>NSCLC -SQCC</td>
<td>20</td>
<td>5%</td>
<td>19</td>
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In-situ Protein Expression Analysis of Cancer Testis Antigen SSX2

CL3202
SSX – Positive Tumors, NSCLC, sqcc
# In-situ Protein Expression Analysis of Cancer Testis Antigen SSX2

**SSX – CL3202 IHC Positive Tumors**

<table>
<thead>
<tr>
<th>Tumor</th>
<th>Total</th>
<th>%</th>
<th>Neg</th>
<th>Pos</th>
<th>foc</th>
<th>+</th>
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<th>+++</th>
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<tbody>
<tr>
<td>Synovial SX</td>
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<td>Melanoma</td>
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<td>23%</td>
<td>23</td>
<td>7</td>
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<td>1</td>
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<tr>
<td>Thyroid ca</td>
<td>17</td>
<td>41%</td>
<td>10</td>
<td>7</td>
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<tr>
<td>Osteo SX</td>
<td>15</td>
<td>33%</td>
<td>10</td>
<td>5</td>
<td>3</td>
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<tr>
<td>Seminoma</td>
<td>15</td>
<td>27%</td>
<td>11</td>
<td>4</td>
<td>4</td>
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<tr>
<td>IDC (breast)</td>
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<td>7%</td>
<td>14</td>
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</tr>
<tr>
<td>NSCLC -SQCC</td>
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<td>5%</td>
<td>19</td>
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</table>
In-situ Protein Expression Analysis of Cancer Testis Antigen SSX2

CL3202
SSX – Positive Tumors

Thyroid ca; papillary

Seminoma
# In-situ Protein Expression Analysis of Cancer Testis Antigen SSX2

## SSX – CL3202 IHC Negative Tumors

<table>
<thead>
<tr>
<th>Tumor</th>
<th>Total</th>
<th>%</th>
<th>Neg</th>
<th>Pos</th>
<th>foc</th>
<th>+</th>
<th>++</th>
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<td>Pancreas, ductal ca.</td>
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</tbody>
</table>
In-situ Protein Expression Analysis of Cancer Testis Antigen SSX2

CL3202

SSX – Negative Tumors

Leiomyosarcoma

Serous ovarian carcinoma
In-situ Protein Expression Analysis of Cancer Testis Antigen SSX\textsubscript{2}

Conclusion

CL3202 (Atlas, AMAb 91141) reactive with various/all SSX proteins
In-situ Protein Expression Analysis of Cancer Testis Antigen SSX2

CL3202

Conclusion

CL3202 (Atlas, AMAb 91141) reactive with various/all SSX proteins

SSX is present in a high percentage (all?) of synovial sarcomas
In-situ Protein Expression Analysis of Cancer Testis Antigen SSX2

CL3202

Conclusion

CL3202 (Atlas, AMAb 91141) reactive with various/all SSX proteins

SSX is present in a high percentage (all?) of synovial sarcomas

SSX is homogeneously expressed in synovial sarcomas (vaccines, diagnostics)
In-situ Protein Expression Analysis of Cancer Testis Antigen SSX2

CL3202

Conclusion

CL3202 (Atlas, AMAb 91141) reactive with various/all SSX proteins

SSX is present in a high percentage (all?) of synovial sarcomas

SSX is homogeneously expressed in synovial sarcomas (vaccines, diagnostics)

SSX is present in a high percentage melanomas
In-situ Protein Expression Analysis of Cancer Testis Antigen SSX2

CL3202

Conclusion

CL3202 (Atlas, AMAb 91141) reactive with various/all SSX proteins

SSX is present in a high percentage (all?) of synovial sarcomas

SSX is homogeneously expressed in synovial sarcomas (vaccines, diagnostics)

SSX is present in a high percentage melanomas

SSX is only occasionally expressed in most other tumors