Biology and treatment of thymoma

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Brown Bag Seminar
April 13, 2011
Thymus and thymic tumors

Primary Mediastinal Tumors (<1% of all cancers)

- Thymic epithelial tumors: 30%
- Lymphomas: 25%
- Germ Cell Tumors: 20%
- Neurogenic tumors: 15%
- Miscellaneous: 10%

Muller-Hermelink et al. WHO classification 2004
Thymic Epithelial Tumors

• Tumors originating from epithelial cells of thymus
• Rare (600/year in US), but the most frequent tumors of the anterior mediastinum in adults
• Thymoma often associated with paraneoplastic syndromes (e.g. Myasthenia Gravis)
• Prognostic factors: Stage and Completeness of resection
• There are different histotypes with different outcome
WHO 2004 histological classification of Thymic Epithelial Tumors

- Reproduce medullary structure
- Reproduce Cortex structure
- Reproduce structure of carcinomas from organs other than thymus

Muller-Hermelink et al. WHO classification 2004
WHO classification: survival implications

10 years

10-year DRS

A  100%
AB 100%
B1  93%
B2  89%
B3  80%
C   60%

Petrini I. et al. JTO 2010, Jul 21
Thymomas: General Characteristics

- 17% of mediastinal enlargements
- Higher incidence at 40-60 years of age
- Equal gender distribution
- 30-65% associated with myasthenia gravis
- 10-15% cases of myasthenia gravis are associated with thymoma
- 70% well encapsulated
- Rare (1%) extrathoracic progression
681 thymomas and 2719 matched controls (1958-2004) - overall survival

Gadalla et al Int J Cancer 2010
Results – overall survival, age ≤40

Log rank p<0.0001

Thymoma pts

Controls
Paraneoplastic syndromes associated with thymoma (1 of 3)

- myasthenia gravis
- pure red cell aplasia
- acquired hypogamma-globulinemia
- Eaton-Lambert syndrome
- peripheral neuropathy
- limbic encephalitis
- myeloradiculopathy
- Isaac’s syndrome
- hyperthyroidism
- panhypopituitarism
- Addison’s disease

- macrogenitosomia precox
- rheumatoid arthritis
- systemic lupus erythematosus
- systemic sclerosis
- dermatomyositis
- Whipple’s disease
- inflammatory bowel disease
- nontropical sprue
- nephrotic syndrome

Lara PN Jr, Ca Tr Rev 26:127, 2000
Thymoma - surgery

- Surgery is the mainstay treatment of thymoma
- pre-operative biopsy is not considered necessary in well encapsulated tumors
- complete median sternotomy necessary
- cervical approach may lead to local recurrence
- total thymectomy necessary also in case of intrathymic thymomas (multifocal disease)
- Radical resection is the best prognostic factor
- debulking surgery indicated in case of invasive thymoma
- re-operation indicated in case of local recurrence
### Masaoka Staging System

<table>
<thead>
<tr>
<th>Stage</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage I</td>
<td>Macro and microscopically encapsulated (tumors invading into but not through the capsule)</td>
</tr>
</tbody>
</table>
| Stage II | A. Microscopic transcapsular invasion  
B. Macroscopic invasion into surrounding fatty tissue or grossly adherent to but not through mediastinal pleura or pericardium |
| Stage III | Macroscopic invasion into neighboring organs (i.e. pericardium, great vessels, lung)  
A. without invasion of great vessels  
B. with invasion of great vessels |
| Stage IV | A. Pleural or pericardial dissemination  
B. Lymphogenous or hematogenous metastases |

Masaoka et al, Cancer 1981
Thymoma - 5-year survival

- Stage I: 93%
- stage II: 86%
- stage III: 70%
- Stage IV: 50%
World Health Organization (WHO) classification (1)

- several types of thymomas are distinguished based on histological criteria:
  - type A thymomas (also called medullary or spindle-cell thymoma)
  - type AB thymomas (also called mixed thymoma)
World Health Organization (WHO) classification (2)

- **type B thymomas** which are subclassified as:
  - type B1 thymomas (also called lymphocyte-rich thymoma, lymphocytic thymoma, predominantly cortical thymoma, or organoid thymoma)
  - type B2 thymomas (also called cortical thymoma) and
  - type B3 thymomas (also called epithelial, atypical, or squamoid thymoma or well-differentiated thymic carcinoma, respectively))

- **4) type C thymomas** (thymic carcinomas)
Stage IVa Thymoma

Pericardial Metastasis

Pleural Metastasis
Thymoma - radiotherapy

• Post-operative radiotherapy: 30-60 Gy in 1.8-2.0 Gy/fraction in 3-6 weeks
• Recommended in Masaoka stage III
• may help in stage II (recurrence rate 30%)
• not indicated in stage I (recurrence rate 1.5%)
<table>
<thead>
<tr>
<th>Drug</th>
<th>Responses/No. Pts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cisplatin</td>
<td>7/28 (25%)</td>
</tr>
<tr>
<td>Ifosfamide</td>
<td>6/13 (46%)</td>
</tr>
<tr>
<td>Maytansine</td>
<td>5/7 (71%)</td>
</tr>
<tr>
<td>Interleukin-2</td>
<td>0/14</td>
</tr>
<tr>
<td>Octreotide</td>
<td>4/38 (11%)</td>
</tr>
<tr>
<td>- plus prednisone</td>
<td>9/38 (24%)*</td>
</tr>
<tr>
<td>Pemetrexed</td>
<td>5/27 (19%)</td>
</tr>
</tbody>
</table>

Thymoma: Single Agent Activity
**Combination Chemotherapy**

- **Anthracycline Regimens:**
<table>
<thead>
<tr>
<th>Regimen</th>
<th>Stage</th>
<th>No. Pts.</th>
<th>CR + PR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Loehrer et al PAC</td>
<td>IV</td>
<td>30</td>
<td>50%</td>
</tr>
<tr>
<td>Loehrer et al PAC*</td>
<td>III</td>
<td>23</td>
<td>70%</td>
</tr>
<tr>
<td>Fornasiero et al ADOC</td>
<td>III/IV</td>
<td>32</td>
<td>90%</td>
</tr>
<tr>
<td>Lucchi et al PEpiVP-16</td>
<td>III/IV</td>
<td>7</td>
<td>100%</td>
</tr>
<tr>
<td>Venuta et al PAC</td>
<td>III</td>
<td>15</td>
<td>69%</td>
</tr>
<tr>
<td>Kim et al PAC+Pred*</td>
<td>III/IV&lt;sub&gt;A&amp;B&lt;/sub&gt;</td>
<td>22</td>
<td>77%</td>
</tr>
</tbody>
</table>

- **Non-Anthracycline Regimens:**
<table>
<thead>
<tr>
<th>Regimen</th>
<th>Stage</th>
<th>No. Pts.</th>
<th>CR + PR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Giaccone et al PE</td>
<td>IV</td>
<td>16</td>
<td>56%</td>
</tr>
<tr>
<td>Loehrer et al VIP</td>
<td>III/IV</td>
<td>28</td>
<td>32%</td>
</tr>
<tr>
<td>Lemma et al:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>– Thymoma Carbo/Pac</td>
<td>III/IV</td>
<td>23</td>
<td>35%</td>
</tr>
<tr>
<td>– Thymic CA Carbo/Pac</td>
<td>III/IV</td>
<td>21</td>
<td>29%</td>
</tr>
</tbody>
</table>

*Radiotherapy to limited disease
Targeted therapy
Anectodal activities

- Erlotinib
- Cetuximab
- Sorafenib
- Imatinib
- Dasatinib
- ZD2171
- AMG706
- SU14813: 2 PR/4 in a phase I study
- etc..
Octreotide +/- prednisone

- 38 eligible patients
- 32 thymomas
- 5 thymic carcinomas and 1 carcinoid
- Octreotide: 4 PRs / 38 (10.5%)
- Octreotide + prednisone: 2 CR+6 PRs / 21 (31.6%)
- Overall: 2 CR + 10 PR / 38 (31.6%)
- No responses in non-thymomas

Loehrer et al. JCO 22, 293-299, 2004
Somatostatin + prednisone in a patient with metastatic thymoma

8 Dec 03

3 Feb 04
Phase II of Gefitinib in previously treated thymomas

- 26 enrolled
  - 19 thymomas
  - 7 thymic carcinomas
- 1PR (5m)
- 14 NC
- In 5 patients: no EGFR or K-Ras mutation

Kurup A et al. ASCO 2005, abstract 7068
Phase II of erlotinib and bevacizumab in previously treated thymomas

- 18 enrolled
  - 11 thymomas
  - 7 thymic carcinomas
- No responses; 11 NC, 7 PD

Bedano PM et al. ASCO 2008, abstract 19087
EGFR summary

<table>
<thead>
<tr>
<th>Tumor Type</th>
<th>IHC</th>
<th>Mutations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thymoma</td>
<td>135/176 (77%)</td>
<td>2/114 (2%)</td>
</tr>
<tr>
<td>Thymic carcinoma</td>
<td>23/36 (64%)</td>
<td>0/22 (0%)</td>
</tr>
</tbody>
</table>
Phase II imatinib in WHO thymoma B3 and C

• 400 - 800 mg/day
• 7 patients
  – 3 no prior chemotherapy
• Gender:
  – 6 man
  – 1 woman
• Performance status:
  – 3 PS 1
  – 4 PS 2

  • Histology:
    – 2 Thymoma B3
    – 5 Thymus carcinoma C
  • Stage:
    – 2 IVA
    – 5 IVB

• 2 NC, 5 PD
No mutations in cKit or PDGFRA in 3 patients tested

Giaccone G et al. JTO 4, 1270, 2009
Imatinib in thymic carcinomas

- 11 previously treated thymic carcinoma
  - IHC positive for C-KIT (9 cases)
  - or PDGFR (2 cases)
- 3 NC, 4 PD, 3 toxicity discontinuations

Salter et al. ASCO 2008, abstract 8116
C-Kit expression and survival (n=120)

Petrini et al JTO 5, 1447, 2010
## C-Kit summary

<table>
<thead>
<tr>
<th></th>
<th>IHC</th>
<th>Mutations</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Thymoma</strong></td>
<td>7/366 (2%)</td>
<td>0/67 (0%)</td>
</tr>
<tr>
<td><strong>Thymic carcinoma</strong></td>
<td>95/127 (75%)</td>
<td>5/59 (8%)</td>
</tr>
</tbody>
</table>

Petrini et al JTO 5, 1447, 2010
Phase II study of Belinostat

- **Background:**
  - No standard treatment for patients with advanced thymoma who progressed after platinum-based chemotherapy
  - One minor long lasting response in a phase I study of the pan-HDAC inhibitor belinostat

- **Eligibility:**
  - Recurrent thymoma or thymic carcinoma failing chemotherapy
  - Measurable disease
  - ECOG PS ≤ 2
  - Baseline QTc interval > 500 msec was an exclusion criterion

- **Schedule:**
  - 1 g/m² x 5 days q3w until progression or development of unacceptable adverse events
# Phase II of Belinostat Patient Characteristics

<table>
<thead>
<tr>
<th>Clinical Parameter</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Accrual:</strong> December 17, 2007 - January 5, 2010</td>
<td></td>
</tr>
<tr>
<td>Number of patients enrolled</td>
<td>41</td>
</tr>
<tr>
<td>Median Age (range), years</td>
<td>53 years (23 – 83 years)</td>
</tr>
<tr>
<td>Gender, M : F</td>
<td>20:21</td>
</tr>
<tr>
<td>Histology</td>
<td></td>
</tr>
<tr>
<td>Thymoma</td>
<td>25</td>
</tr>
<tr>
<td>Thymic Carcinoma</td>
<td>16</td>
</tr>
<tr>
<td>Paraneoplastic syndromes</td>
<td></td>
</tr>
<tr>
<td>Myasthenia Gravis</td>
<td>4</td>
</tr>
<tr>
<td>Schulman’s syndrome</td>
<td>1</td>
</tr>
<tr>
<td>Infections (Candida mucositis)</td>
<td>2</td>
</tr>
<tr>
<td>Median number of prior regimens (range)</td>
<td>2 (1 – 10)</td>
</tr>
</tbody>
</table>

Giaccone et al. JCO (in press)
Belinostat Study - outcome

- Patients evaluable for response: 40 (24 T; 16 TC)
  - Thymoma: 2 PR; 17 SD; 5 PD
  - Thymic Carcinoma: 0 PR; 8 SD; 8 PD

- Median Time To Progression (TTP)
  - Thymoma: 343 days
  - Thymic carcinoma: 82 days

- Median Survival
  - Thymoma: Not reached
  - Thymic carcinoma: 371 days
Thymoma vs thymic carcinoma

Time to progression

Survival Functions

Survival

Survival Functions

Cum Survival

TTP (d)

0.0 0.2 0.4 0.6 0.8 1.0

0 200 400 600 800 1000

Thymomas

Thymic Carcinomas

Cum Survival

survival (d)

0.0 0.2 0.4 0.6 0.8 1.0

0 200 400 600 800 1000
Belinostat Study

• Common toxicities:
  – Nausea
  – Flushing
  – Transient hypotension

• Uncommon but potentially serious toxicity:
  – Prolongation of QT interval
PBMCs

Acetylated Tubulin

Fold increase

C1D1 pre  C1D3 post  C2D1 pre

Time

J. Trepel
Rationale for combinations

Sim: PXD+Cis; PXD+VP: Synergistic at most doses
Phase I/II of B-PAC - Schema

Enrollment CT scan
FDG-PET scan

Re-staging CT scan
Re-staging FDG-PET scan

Up to 6 cycles or PD

Belinostat CIV
Day 1 Day 2 Day 3
Cycle 1
AP AC

Belinostat CIV
Day 1 Day 2 Day 3
Cycle 2
AP AC

Belinostat CIV
Day 1 Day 2 Day 3
Cycle 3
AP AC

Belinostat CIV
Day 1 Day 2 Day 3
Cycle 4
AP AC

cyclophosphamide(C), doxorubicin (A), cisplatin (P)
Phase II of IMC-A12, an IGF-1R MoAb in Thymic Malignancies

**Rationale:**
- IGF-1R identified in rat thymocytes
- Increased expression of IGF-1 and IGF-1R in thymic epithelial cells of patients with thymic-hyperplasia associated MG
- One thymoma patient with reduction of 10% in a phase I study figitumumab for over 1 year
- Increased IGF1R expression in higher grade and stage thymic malignancies
IGF-1R and p-Akt expression in resected thymomas (n=111)

* >5% cells, any intensity

Zucali et al. Cancer 2010
Phase II study of IMC-A12 in thymic malignancies

Single center study: open August 2009

<table>
<thead>
<tr>
<th></th>
<th>Thymoma</th>
<th>Thymic carcinoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Accrued</td>
<td>15</td>
<td>12</td>
</tr>
<tr>
<td>Male : female</td>
<td>8 : 7</td>
<td>5 : 7</td>
</tr>
<tr>
<td>Median age (range)</td>
<td>55 (36 – 69)</td>
<td>47 (26 – 71)</td>
</tr>
<tr>
<td>Response: PR</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>NC</td>
<td>11</td>
<td>4</td>
</tr>
<tr>
<td>PD</td>
<td>2</td>
<td>8</td>
</tr>
</tbody>
</table>
CT scans demonstrating a Partial Response in a patient with thymoma

Baseline – Oct 2009

May 2010
CT scans demonstrating a Partial Response in a patient with thymoma.
09-C-0212

• Common Toxicities:
  – Pain at the site of tumor(s)
  – Hyperglycemia
  – Hyperuricemia
  – Asymptomatic elevation of creatinine

• Uncommon but potentially serious toxicity
  – High frequency sensori-neural hearing loss (1 out of 18)
Genomic aberrations of TETs

- Karyotype studies
  - 13 patients evaluated
  - Several events of genomic imbalance reported
  - Only t(15;19) and Del chr6p25 were recurrent
  - t(15;19) was recurrent, resulting in the NUT-BRD4 fusion-gene


- CGH studies
  - TET histotypes present peculiar copy number CN imbalance
  - Type A: Rare chromosomal abnormalities
  - Type B3: chr1q CN gain chr6 CN loss and chr13 CN loss
  - Thymic carcinoma: chr1q CN gain, chr6, chr13, chr16q and chr17p CN loss

- 23 tumors

- Unsupervised clustering analysis:
  - cluster 1 (n=8): type B2 thymomas
  - cluster 2 (n=15): others
   
  \[ p = 0.023 \]

- Genes overexpressed in cluster 1:
  - immune system process \[ p = 5.3 \times 10^{-19} \]
  - immune response \[ p = 2.0 \times 10^{-15} \]
  - T cell activation \[ p = 6.5 \times 10^{-11} \]
  - lymphocyte activation \[ p = 6.5 \times 10^{-11} \]

- Cluster 2 was subdivided in:
  - cluster 2a (n=6): thymic carcinoma
  - cluster 2b (n=9): type A thymomas
   
  \[ p = 0.023 \]
EGFR and RAS

- 45 cases
- No *EGFR* mutation, but 3 (7%) *RAS* mutations:

**Type A thymoma**

*HRAS* $^{G13V}$ mutation

**Type B2 thymoma**

*KRAS* $^{G12A}$ mutation

**Thymic carcinoma**

*KRAS* $^{G12V}$ mutation

EGFR expression
- 45 cases
- 2 KIT mutations, both in thymic carcinomas:
  - The \( \text{KIT}^{\text{V560del}} \) mutation was previously reported in a case of thymic carcinoma sensitive to imatinib.
  - No correlation with KIT expression at IHC

Strobel et al. NEJM 2004;350;2625
Array CGH study

- Premise: obscure biology
- FFPE blocks from a series of 134 resected thymoma patients
- 59 samples with > 80% epithelial cancer cells were analyzed

Petrini, unpublished
Copy number variation (n=59)

Progression by histological type
Genes involved in development of thymus with frequent copy number imbalance
Regulation of TBX1

**Hypothesis:**
PBX1 and/or FOXC1 and/or NTRK1 are involved in development of thymomas

**Goals:**
Confirm results with alternative methods
Establish function in cells lines
Develop therapeutic strategies
FISH for **NTRK1** and **SNIP1** in T1889 cells
Thymic epithelial tumors FFPE
Phase II studies of oral PHA-848125AC in patients with Thymic Carcinoma

- PHA-848125AC – inhibits the kinase activity of the CDK2/Cyclin A complex, CDK1, CDK4, CDK5 and TRKA.
- Currently being evaluated in two phase II studies in patients with thymic carcinoma
  - Multicenter study in patients with WHO subtype C disease who have received only one prior line of systemic therapy:
    • Number of patients screened (as of 2/22/2011) – 13
    • Number of patients enrolled (as of 2/22/2011) – 11
  - Single institution study (NCI) in patients with WHO subtype B3 and C disease who have received more than one prior line of systemic therapy:
    • Number of patients screened and enrolled (as of 3/7/2011) – 1
Distribution of CN aberrations

Number of CN aberrations

Distribution of CN aberrations

Percentage of chromosome arm in the aberrations

\[ y = 6.5636x^{-2.545} \]

\( p < 0.001 \)
Survival implications

No-Chr13q Loss

Chr13q Loss

p=0.013

No-Chr16q Loss

Chr16q Loss

p=0.015

No-Chr13q Loss

Chr13q Loss

p=0.065

No-Chr16q Loss

Chr16q Loss

p=0.118

Time to progression (months)

Disease related survival (months)
Transcriptome/exome sequencing

Objectives

• To identify genomic imbalances in TETs
• To characterize genes or gene regions potentially important for tumor development, prognosis or potential targets for systemic therapies
Study design

20 Frozen TET samples with tumor and normal tissue available

Selection of TETs with more than 80% of cancer cells and high quality RNA

CGH in order to exclude normal cells contamination

RNA sequencing

Expression of mutated genes
Fusion genes

Exome sequencing of Tumor DNA
Exome sequencing of Normal DNA

Somatic SNVs
Somatic INDELs

Sanger sequencing confirmation

Functional studies
Sequencing technologies

- cDNA libraries were created from RNA using Illumina kit poly-A tail enrichment was used to select mRNA
- Enon libraries were built using SureSelect Human All Exon Kits (Agilent)
- Libraries were sequenced using Hiseq2000 (Illumina)
- Complete genome sequencing became an available tool and we integrated the analysis with this technique
Complete Genome sequencing of a B3 thymoma
Molecularly Targeted Treatment of Advanced Thoracic Malignancies

Patient selection:
Molecular + NSCLC, SCLC, thymic malignancies

Treatment:
Targeted therapies

FFPE Core 1
Raffel’s Lab
Pack’s Lab

FFPE Core 2
Meltzer’s Lab

Frozen Core 3
Giaccone’s Lab

EGFR, KRAS, BRAF
FISH: ERBB2, PIK3CA, PDGFRA, ALK

AKT1, AKT2, BRAF, EGFR, ERBB2, HRAS, KIT, KRAS, NRAS, PDGFRA, PIK3CA, PTEN

Full exome sequencing

Exploratory Analyses

Available study

Biopsy

NCI / CTEP protocol 8639

Other*
Acknowledgments

Clinic
A. Rajan
R. Kelly
C. Carter
A. Lopez-Chavez
P. Dennis
E. Szabo
B. Scepura
A. Berman
C. Keen
M. Manu
G. Chen

Lab
J. Voortman
T. Harada
H.S. Lee
Y. Wang
D. Voeller
T. Pham
I. Petrini
A. Lee
D.H. Lee
J. Luo

CCR
P. Meltzer’s group
T. Ried

CTEP
I. Espinoza
H. Chen

University of Pisa
M. Lucchi
Humanitas Institute
Milan
P. Zucali