Update on systemic amyloidosis

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University College London
UK
Amyloidosis

Serum Amyloid A → Mis-folding → Amyloid fibrils

IL-6 mediated release

Light chains → Mis-folding → Amyloid fibrils

Transthyretin

SAP → Oligomers

GAG’s

Plasma cell clone

UCL
Clinical features of AL amyloidosis

- Heart (NT-proBNP): 81%
- Heart (Echo): 46.2%
- Heart (Echo and NT-proBNP): 44.8%
- Kidney: 72.1%
- Liver: 11.2%

Dominant organ
- Renal
- Cardiac
- Soft Tissue
- Liver
- PNS
- ANS
- GI
- Lung

- Third Organ Involved
- Second Organ Involved
- Dominant Organ Involved
Confirm the diagnosis of amyloidosis

Tissue biopsy is still needed in all cases

| Congo Red remains the gold standard | Other Thioflavin dyes also useful |

Biopsy confirms amyloid deposition

| Does not confirm type | Does not confirm extent |

Type the fibrils

| Immunohistochemistry (IHC) | Mass spectrometry (LCMS) |

What to biopsy?
Target organ
Fat
Rectal

IHC
Excellent in AA
Reliable in 75% AL
Experienced lab
False pos/neg

10% false positive and negatives
# Immunohistochemistry In AL

<table>
<thead>
<tr>
<th></th>
<th>2014</th>
<th>2013</th>
<th>2012</th>
<th>2011</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>cases</td>
<td>cases</td>
<td>cases</td>
<td>cases</td>
</tr>
<tr>
<td>NISS</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>150 (26%)</td>
<td>209 (36%)</td>
<td>248 (42%)</td>
<td>47%</td>
</tr>
<tr>
<td>Kappa</td>
<td>67 (12%)</td>
<td>64 (11%)</td>
<td>54 (11%)</td>
<td>10%</td>
</tr>
<tr>
<td>Lambda</td>
<td>359 (62%)</td>
<td>306 (53%)</td>
<td>227 (47%)</td>
<td>43%</td>
</tr>
</tbody>
</table>

![Pie chart showing the distribution of NISS, Kappa, and Lambda cases.](chart.png)
Amyloid proteomics

Capture

Database identification

Diagnosis

Amyloid proteomics
## Typing of samples inconclusive by IHC

<table>
<thead>
<tr>
<th>Protein</th>
<th>Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>ApoAI</td>
<td>1</td>
</tr>
<tr>
<td>ApoE</td>
<td>1</td>
</tr>
<tr>
<td>Fib (E526V)</td>
<td>1</td>
</tr>
<tr>
<td>Gelsolin</td>
<td>1</td>
</tr>
<tr>
<td>Inconclusive</td>
<td>11</td>
</tr>
<tr>
<td>Insufficient</td>
<td>17</td>
</tr>
<tr>
<td>Kappa</td>
<td>42</td>
</tr>
<tr>
<td>Lambda</td>
<td>24</td>
</tr>
<tr>
<td>No evidence of amyloid</td>
<td>13</td>
</tr>
<tr>
<td>Transthyretin</td>
<td>19</td>
</tr>
<tr>
<td>Heavy chains</td>
<td>2</td>
</tr>
</tbody>
</table>
Detecting clonality and clonal burden

- All patients have a detectable bone marrow clone
- Median plasma cell percentage ~7-8%
- t(11;14) is common
- High risk abnormalities rare

- Urine BJP - 61%
- Serum PP or FLC - 69%
- Abnormal FLC ratio - 79%
- Any detectable clonal marker – 96%

*UK NAC unpublished data*
Nature of mutations in AL on Exome Seq

- We compared the mutations present in AL to our previously sequenced MM and MGUS samples.
- Although there was an overlap, 30% of mutations seen in amyloidosis were not seen in MM.

Myeloma (n=463)
AL (n=25)
MGUS (n=4)

- 8217 total mutations
- 360 overlap
- 814 unique to AL
- 125 unique to Myeloma
- 106 unique to MGUS
- 29 unique to MGUS
- 3 unique to AL
- Walker et al, Blood 2013
- Boyle et al #637
Patients with higher clonal burden have worse outcomes

“One size fits all” approach in AL – time for revision
Impact of t(11;14) on outcomes of bortezomib treated patients

Tilmann Bochtler et al. JCO doi:10.1200/JCO.2014.57.4947
Figure 2 Characteristic Examples From CMR Scans CMR end-diastolic cine still (top), shortened modified look-locker inversion recovery native T1 map (middle), and late gadolinium enhancement (LGE) images (bottom) in (left to right) healthy volunteer...

Figure 3 Native T1 in Healthy Volunteers, Mutation Carriers, HCM, Definite AL, and Definite ATTR Mean native myocardial T1 ± 2 SE in healthy control subjects, gene carriers, patients with definite AL cardiac amyloidosis, and patients with definite ATTR ca.

Fontana M et al; JACC: Cardiovascular Imaging, Volume 7, Issue 2, 2014, 157 - 165
Potential therapeutic avenues in amyloidosis

- High flux dialysis
- Anti-SAP antibodies
- Doxycycline EGCG
- Anti-fibril antibodies
- Eprodisate
- SAP
- GAG’s
- Oligomers
- Amyloid fibrils
- Interfere with formation or accelerate removal

Plasma cell clone
Treatments to kill plasma cells
Reduce precursor protein
Light chains
Mis-folding
Current survival in AL amyloidosis

Median – 49 months
Poorer outcomes for advanced stages persist

Estimated 5 year survival

- Stage 1 - 82%
- Stage 2 – 52%
- Stage 3 – 22% (median 8.9 months)
How soon does a patient need to start treatment?

![Bar chart showing the distribution of delays in starting treatment.](image)
Impact of treatment delay in stage III AL

Stage IIIb

Survival from diagnosis in months

Cum Survival

Delay
Months

<1
1-2
2-3
>3
1-censored
2-censored
3-censored
4-censored

UCL
Single worse prognostic factor – high NT-proBNP

- NT-proBNP <1000 pMol/L; 60% at 5 years
- NT-proBNP >1000 pMol/L; Median - 4.4 m
OS by disease stage

Stage 1 – normal NT-proBNP/TNT
Stage 2 – Either NT-proBNP >35 pMol/L or TNT >0.05
Stage 3 – Both NT-proBNP and TNT above the thresholds
Stage 3b – additionally NT-proBNP >1000 pMol/L

Stage 1
Stage 2
Stage IIIa - median 24 m
Stage IIIb - median 4.4 m
+ SBP <100: median 3.3 m
+ dFLC >500mg/L: median 2.0 m
Goals of therapy AL chemotherapy

**Challenge:**
- Sicker patients
- Poorer treatment tolerance

Yet – very rapid and deep responses are needed

- CR (97 patients, 3.6 deaths/100 py)
- VGPR (233 patients, 9.6 deaths/100 py)
- PR (140 patients, 23.7 deaths/100 py)
- NR (179 patients, 47.2 deaths/100 py)

Palladini et al. Validation of Response Criteria in AL Amyloidosis. JCO 2012
Rapid response impacts survival in advanced AL (ALChemy data UK 2014)

Alive for response assessment

[Graph showing survival rates over months for different response categories: CR, VGPR, Switched before C3, PR, NR]
AL with Low risk disease (15-20%)
- Stage 1/Early stage 2
- Young
- Good performance status
- NT-proBNP <500 pMol/L
- Good renal function

AL with intermediate risk disease (~60%)
- Stage 2 and early stage 3
- ECOG 2
- SBP >100 mm Hg
- NT-proBNP <8500 ng/L
- Age ?

AL with high risk disease (15-20%)
- Advanced stage 3
- NT-proBNP >8500 ng/L
- SBP <100 mm of Hg systolic
- Marked coagulopathy
- ECOG 4
- NYHA 3-4

Risk stratified treatment is critical
Low risk patients – possible HDM-ASCT candidates

Main problem: Rate of CR still remains modest – novel agent based chemo – similar responses

<table>
<thead>
<tr>
<th>Induction chemo prior to ASCT</th>
<th>Consolidation chemo after ASCT</th>
</tr>
</thead>
<tbody>
<tr>
<td>35 patients</td>
<td>40 patients</td>
</tr>
<tr>
<td>VD x 2 followed by Vel-Mel-ASCT</td>
<td>Risk adapted Mel-ASCT</td>
</tr>
<tr>
<td>15% deteriorated prior to ASCT</td>
<td>10% TRM with ASCT</td>
</tr>
<tr>
<td>10% TRM with ASCT</td>
<td>HR-post ASCT – CR27%, &gt;PR 45%</td>
</tr>
<tr>
<td>On ITT: CR 55%, VGPR 16%</td>
<td>57% received consolidation VD</td>
</tr>
<tr>
<td>Evaluable: CR 74%, VGPR 22%</td>
<td>CR 58% at 12 m, 79% &gt;PR</td>
</tr>
</tbody>
</table>

Sanchorawala et al ISA 2014

Landau et al Leukermia 2013
Better Long term outcomes after ASCT

Cibeira et al. Blood; 2011; 118 (16);4646-52

Organ response - 78%
Organ response - 39%

Should we be considering ASCT In All stage I patients in UK?
Estimated transplant eligible patients – 50/year
No. Of transplants upfront – 4-5/year

Cibeira et al. Blood; 2011; 118 (16);4646-52
AL with Low risk disease (15-20%)
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- Good performance status
- NT-proBNP <500 pMol/L
- Good renal function

AL with intermediate risk disease (~60%)
- Stage 2 and early stage 3
- ECOG 2
- SBP >100 mm Hg
- NT-proBNP <8500 ng/L
- Age ?

Risk stratified treatment is critical

HDM-ASCT
Full dose chemo
Chemotherapy in AL amyloidosis

- Alkylator based
  - MDex
  - MP
  - Cyclo-Dex

- Proteasome inhibitor based
  - Bortz-D
  - B-Mdex
  - Cy-Bor-D
  - Ixa-D

- IMiD based
  - Thal-D
  - C-Thal-D
  - Len-D
  - Pom-D
• Treatment regimes:
  – CTDa – 35%
  – Velcade based 42%
  – Mel-Dex – 4%
  – ASCT – 0.8%
  – Others – 10%
  – Died before treatment – 4%
Response to CVD

• Hematologic response by intent-to-treat: 62% (CR 21%, VGPR 22%)

<table>
<thead>
<tr>
<th>Response category</th>
<th>Stage I (30 patients)</th>
<th>Stage II (67 patients)</th>
<th>Stage IIIa (61 patients)</th>
<th>Stage IIIb (43 patients)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall hem.</td>
<td>77%</td>
<td>64%</td>
<td>69%</td>
<td>42%*</td>
</tr>
<tr>
<td>CR</td>
<td>33%</td>
<td>18%</td>
<td>23%</td>
<td>14%</td>
</tr>
<tr>
<td>VGPR</td>
<td>23%</td>
<td>27%</td>
<td>26%</td>
<td>9%</td>
</tr>
<tr>
<td>PR</td>
<td>20%</td>
<td>19%</td>
<td>20%</td>
<td>19%</td>
</tr>
<tr>
<td>Cardiac</td>
<td>-</td>
<td>29%</td>
<td>17%</td>
<td>4%*</td>
</tr>
</tbody>
</table>

• Renal response: 27% (not affected by renal stage)

*P<0.05 compared to stages (I), II, and IIIa

Palladini et al Blood April 2015
Survival by cardiac stage

Survival probability (%)

Time (months)

Stage I
Stage II
Stage IIIa
Stage IIIb

P<0.001

median 7 months

P<0.001
### Lenalidomide for relapsed AL amyloidosis

<table>
<thead>
<tr>
<th>Regime</th>
<th>Response rate</th>
<th>OS</th>
<th>TRM</th>
<th>≥Grade toxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Len ± dex (Sanchorawala, et al 2007b)</td>
<td>67%</td>
<td>ns#</td>
<td>NIL</td>
<td>35%</td>
</tr>
<tr>
<td>Len ± dex (Dispenzieri, et al 2007)</td>
<td>75%</td>
<td>ns#</td>
<td>NIL</td>
<td>73%</td>
</tr>
<tr>
<td>Len+Dex (UK experience)</td>
<td>56%</td>
<td>84%@2 yrs</td>
<td>Nil</td>
<td>ns</td>
</tr>
<tr>
<td>CRD (Kumar et al 2012)</td>
<td>63%</td>
<td>37</td>
<td>9%</td>
<td>74%</td>
</tr>
<tr>
<td>CRD (Kastritis et al 2012)</td>
<td>55%</td>
<td>14</td>
<td>19%</td>
<td>24%</td>
</tr>
<tr>
<td>CRD (Palladini et al 2012)</td>
<td>62%</td>
<td>36</td>
<td>NIL</td>
<td>57%</td>
</tr>
<tr>
<td>Mel-RD (Moreau 2010)</td>
<td>53%</td>
<td>54% at 2 yrs</td>
<td>NIL</td>
<td>81%</td>
</tr>
<tr>
<td>Mel-RD (Sanchorawala 2012)</td>
<td>44%</td>
<td>24</td>
<td>13%</td>
<td>88%</td>
</tr>
<tr>
<td>Pomalidomide ± dexamethasone</td>
<td>48%</td>
<td>24</td>
<td>3%</td>
<td>30%</td>
</tr>
</tbody>
</table>

CR rate <15% in most series
High toxicity – although mainly haematological for alk combinations
Worsening renal function in some patients
### Pomalidomide in AL amyloidosis

<table>
<thead>
<tr>
<th></th>
<th>Dispenzieri et al (Low dose)</th>
<th>Palladini et al (higher dose)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haematologic response</td>
<td>38%</td>
<td>67%</td>
</tr>
<tr>
<td>Complete remission</td>
<td>8 PR and 3 VGPR (no CR)</td>
<td>18% VGPR</td>
</tr>
<tr>
<td>Discontinuation</td>
<td>18</td>
<td>18%</td>
</tr>
<tr>
<td>Organ response</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>SAE (≥ Grade 3)</td>
<td>21</td>
<td>67%</td>
</tr>
</tbody>
</table>

- Appears to be better tolerated than len
- No dose modification in renal failure
- Some patients get autonomic dysfunction

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Dispenzieri et al Blood (ASH Annual Meeting Abstracts) 2010
116: Abstract 987
Palladini et al (ASH annual meeting abstracts) 2013
**AL with Low risk disease (15-20%)**
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- Good performance status
- NT-proBNP <500 pMol/L
- Good renal function

**AL with intermediate risk disease (~60%)**
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- Advanced stage 3
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- SBP <100 mm of Hg systolic
- Marked coagulopathy
- ECOG 4
- NYHA 3-4

**Challange of the advanced stage III patient**
- HDM-ASCT
- Full dose chemo
- Dose attenuated chemo
RFH protocol for stage III patients

- Start chemotherapy as inpatient – weekly FU for outpatients
- Cardiac monitoring for 48 hrs to 2 weeks depending on clinical status
- Consider ICD or pacemaker for any arrhythmias
- Use amiodarone at same time
- Stop β-blockers or ACE inhibitors
- Loop diuretic + spironolactone (accept worsening renal function)
- Start midodrine if SBP <100 mm of Hg but not decrease in diuretics
- Oral doxycycline 100 mg BD
- Monitor FLC alternate weeks – consider change regime end of #1 if no FLC response
Impact of Haematological response on survival

- CR/VGPR - 38 months
- PR - 7.4 months
- NR - 2.6 months

Log rank p <0.0001
Potential therapeutic avenues in amyloidosis

- **SAP**
  - Anti-SAP antibodies
- **GAG’s**
  - Eprodisate
- **Oligomers**
  - Interfere with formation or accelerate removal
- **Amyloid fibrils**
  - Anti-fibril antibodies

**Potential therapeutic avenues**
- **High flux dialysis**
- **Reduce precursor protein**
- **Treatments to kill plasma cells**
- **Plasma cell clone**
- **Mis-folding**
- **Doxycycline**
- **Interfere with formation or accelerate removal**
Molecules to stabilise unstable protein

- Transthyretin amyloidosis is the main target.

- Tafamidis is a licenced TTR stabilising drug in FAP
- Diflunisal (an old NSAID) does the same
Basic principle of the mechanism of action of conventional ASO and siRNA. Both antisense molecules pair with their complementary target RNA and inhibit synthesis of target proteins on the transcript level. (RISC: RNA induced silencing complex)

Efficacy of ALN-TTR01 in Patients with Transthyretin Amyloidosis.

RNAi packaged in a lipid Nano-particle
AL-Light chains induces cardiomyocyte death in zebrafish.

EGCG inhibits oligomer toxicity.


Green tea halts progression of cardiac transthyretin amyloidosis: an observational report

Arnt V. Kristen · Stephanie Lehrke · Sebastian Buss · Derliz Mereles · Henning Steen · Philipp Ehlermann · Stefan Hardt · Evangelos Giannitsis · Rupert Schreiner · Uwe Haberkorn · Philipp A. Schnabel · Reinhold P. Linke · Christoph Röcken · Erich E. Wanker · Thomas J. Dengler · Klaus Altland · Hugo A. Katus

Protecting and disrupting fibrils in cardiac ATTR and AL – new role for an old drug (Doxycycline)
Protecting and disrupting fibrils in cardiac ATTR and AL – new role for an old drug (Doxycycline)

Overall Survival

Doxycycline treated group

Not treated with Doxycycline

Log rank p = 0.04

Survival

Months

0.0 0.2 0.4 0.6 0.8 1.0

0.0 10.0 20.0 30.0 40.0 50.0
Targeting GAG’s - Eprodisate

Amyloid + GAGs:
Amyloid fibril formation leads to progression of disease

Eprodisate should prevent amyloid fibril formation, deposition and associated toxicity in the organs.
Immunotherapy – Anti-SAP antibodies – the Concept

1. Deplete plasma of SAP using CPHPC
2. Some SAP still remains on amyloid deposits
3. Give anti-SAP antibody to target amyloid deposits
Response to Administration of CPHPC and Anti–Serum Amyloid P Component (SAP) Antibody.

Immunotherapy in AL - NEOD001

Potential NEOD001 MOAs: Neutralizes Soluble Amyloid and Clears Insoluble Amyloid
Immunotherapy in AL – NEOD001

Cardiac: NT-proBNP % Change from Baseline
Best Response Analysis

Cardiac Evaluable Patients
(N=14)

All Patients
(N=27)

50% Responders
50% Stable
Study of Chimeric Fibril-Reactive Monoclonal Antibody 11-1F4 in Patients With AL Amyloidosis

Primary Outcome Measures:
To establish the maximum tolerated dose of Ch mAb 11-1F4 [Time Frame: 2 years (approximately)] [Designated as safety issue: Yes]
Establish the maximum tolerated dose (up to 500 mg/m2) of Ch mAb 11-1F4

Prothena is developing NEOD001, a humanized novel monoclonal antibody designed to neutralize and clear soluble and insoluble amyloid aggregates in patients with AL. NEOD001 is being studied in an ongoing Phase 1 study.

The abstract accepted for poster presentation is as follows:

(Abstract #PB-48) Preliminary cardiac biomarker responses demonstrated in an ongoing phase 1 study of NEOD001 in patients with AL amyloidosis and persistent organ dysfunction

- Presenter: Dr. Michaela Liedtke, Stanford Cancer Institute, Stanford, California
- Date: Tuesday, April 29

A Study to Evaluate the Safety of GSK2398852 When Co-administered With GSK2315698 in Patients With Systemic Amyloidosis

This study is currently recruiting participants.
Verified February 2014 by GlaxoSmithKline
Sponsor:

ClinicalTrials.gov identifier:
NCT01777243
First received: January 24, 2013
Last updated: February 12, 2014
Current clinical trials in AL amyloidosis

- **Upfront**
  - VITAL – NEO001D with CyBorD
  - BMDex vs. Mdex (Phase III)
  - Radiolabelled CD66 for ASCT conditioning

- **Relapsed**
  - Ixa-Dex vs. Physicians choice
  - CATALYST – Car-Thal-Dex

- **Upfront/Relapsed**
  - CPHPC+anti-SAP Phase II