67 Pharmacodynamic Monitoring of Targeted Drug Therapy

The engaging session will cover the use of endogenous biomarkers to achieve personalized immunosuppression in transplant recipients. Pharmacokinetic monitoring of immunosuppressive drugs does not entirely predict pharmacological effects on immune cells and, ultimately, clinical outcome. Appropriate biomarker signatures could be helpful in identifying transplant recipients, who would benefit from immunosuppression minimization. Furthermore, TDM and biomarkers in targeted cancer therapy will be discussed, using tyrosine kinase inhibitors as an example. A further topic relates to the early detection of Alzheimer’s disease, trying to find an answer to the question, whether biochemical and imaging biomarkers are ready for clinical use. Session participants will leave with a better understanding of the best use of new biomarkers in therapeutic drug monitoring, and an increased understanding of anti-retroviral drug monitoring.

- Best use of new biomarkers in therapeutic drug monitoring (transplantation, cancer therapy).
- Better understanding of antiretroviral drug monitoring.
- Biomarkers for detection of Alzheimer's disease.

FACULTY:

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Les Shaw PhD
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Use of endogenous biomarkers to achieve personalized immunosuppression in transplant recipients

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Disclosure Information
I have nothing to disclose.

PK / PD concepts for monitoring drug therapy

Limitations of immunosuppressive drug level monitoring

- TDM does not precisely predict the effects of immunosuppressive drugs on immune cells (*over- or under-immunosuppression*)
- Primary value of TDM is to prevent toxicity
- Intersubject variability in the sensitivity to suppression of immune function
- Intersubject variability of intralymphocyte immunosuppressive drug levels
- Synergistic effects of immunosuppressive drugs
- Immunological risk assessment prior to transplantation
- Predicting tolerance before drug weaning

Intracellular CsA T-lymphocyte concentration has a potential of predicting rejection

Falck et al, Transplantation 2008; 85: 179-184

Influence of MDRI 3435 genotype on intralymphocyte trough CsA levels

<table>
<thead>
<tr>
<th>ABCB1 3435C&gt;T</th>
<th>Number*</th>
<th>Intracellular concentration (ng/10^6 cells)</th>
<th>Blood concentration (ng/ml)</th>
<th>Ratio of intracellular to blood concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>C/T/TT</td>
<td>P value</td>
<td>(mg/10^6 cells)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CC</td>
<td>CT/TT</td>
<td>P value</td>
</tr>
<tr>
<td>Number*</td>
<td>22</td>
<td>42</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data are given as geometric mean (95% confidence interval).

* transplant recipients (renal, liver, lung)

Crettol S et al, Pharmacogenet Genom 2008; 18: 307-315
Biomarkers desirable in addition to TDM?

Inability to measure effects of immunosuppressive drugs on immune cells in vivo has severely limited:

- preclinical drug development
- design and interpretation of clinical trials
- optimal clinical use in transplantation


Factors limiting long-term outcome in transplantation

- Irreversible chronic rejection
- Side effects of standard immunosuppression (e.g. nephrotoxicity, cardiovascular disease, opportunistic infection, malignancy)

> 50 % of transplanted kidneys fail within 10 years

→ numerous attempts to develop biomarkers that would complement TDM to achieve personalized immunosuppression

Sagoo et al, J Clin Invest 2010; 120: 1848-61
Wieland et al, Ther Drug Monit 2010; 32: 560-572
Schrüppel et al, J Clin Invest 2010; 120: 1803-1806

Cyclosporin and Sirolimus Inhibit Different Pathways in the Immune Response

Shaw et al Clinical Therapeutics 2000; 22 (Suppl. B) : B3
**Proposed peripheral blood biomarkers**

- **Drug target enzymes**
  - Calcineurin phosphatase (CN) → cyclosporin, tacrolimus
  - Inosine monophosphate dehydrogenase (IMPDH) → mycophenolic acid (MPA)
  - p70 S6 k phosphorylation → sirolimus, everolimus

- **Cytokines**
  - Cytokine mRNA expression (e.g. IL-2)
    NFAT-regulated gene expression (mRNA expression of IL-2, IFN-γ, GM-CSF)
  - Cytokine production by T-cells (e.g. IL-2, IFN-γ)
    T-cell alloreactivity, IFN-γ ELISpot → cyclosporin, tacrolimus

- **Markers of lymphocyte proliferation**
  - PCNA (proliferating cell nuclear antigen, auxiliary protein of DNA polymerase)

- **Markers of lymphocyte activation**
  - CD25, CD71, CD134 (T-cell surface growth factor receptors)
  - CD26 (T-cell signaling, co-stimulation)
  - CD8 (co-stimulation of T-cell proliferation) → calcineurin-, mTOR inhibitors, MPA

- **Marker of Th2 activation**
  - sCD30 (indicates increased global immunologic responsiveness, heightened rejection risk)

- **Markers of global immune cell response**
  - PHA – stimulated ATP production by CD4+ cells → calcineurin-, mTOR inhibitors, MPA

- **Potential predictors of tolerance**
  - Natural regulatory T-cells (CD4+CD25highFOXP3+)
  - Signature of B-cell genes (IGKV4-1, IGLLA, IGKV1D-13)
Calcineurin inhibition in patients after a first single dose of Neoral

Halloran et al, Transplantation 1999; 68: 1356

Association between pretransplant IMPDH, MMF exposure and acute rejection

IMPDH > cut-off */
Dose reduction
renal transplant recipients

| 0 %  | - / - | 0 | 4 | 8 |
| 37 % | - / + | 0 | 6 | 9 |
| 36 % | + / - | 0 | 5 | 6 |
| 82 % | + / + | 0 | 6 | 9 |

*8.53 nmol/mg protein/h

Adapted from Glander et al., Am J Transplant 2004; 4: 2045-2051

PK-PD of MMF in pediatric kidney transplant recipients

Inosine monophosphate dehydrogenase variability in renal transplant patients on long-term mycophenolate mofetil therapy


IMPDH activity in renal transplant patients mainly treated with MMF for 20-50 months increased from 5.9 to 9.0 nmol h⁻¹ mg⁻¹ with an intra- and interpatient variability of 28 % and 42 % respectively.

IMPDH activity increased during rejection vs. non-rejection.


Association between IMPDH inhibition and acute rejection in renal transplant recipients

IMPDH activity (AUECact0-4); EC-MPS therapy

Raggi MC et al, Transplantation 2010; 90: 1536-1541

IMPDH variant allozyme structural analysis

Wu TY et al, Br J Pharmacol 2010; 161: 1584-1598
**IMPDH type I and II as targets of MPA**

Polymorphisms in type I and II inosine monophosphate dehydrogenase genes and association with clinical outcome in patients on mycophenolate mofetil


Study design: DNA and clinical data of 456 renal transplant recipients from clinical trials (Apomygre, FDCC)

Results: **IMPDH I** rs2278294 SNP was associated with a lower risk of BPAR and a higher risk of leukopenia over the first year post-transplantation.

Conclusion: **IMPDH II** genotyping may not improve MPA treatment outcome over the first year post-transplantation, in contrast to MPA and calcineurine inhibitor therapeutic drug monitoring and **IMPDH I** genotyping.

Pharmacogenetics and Genomics 2010; 20: 537-543

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**Inhibition of lymphocyte proliferation and activation in renal transplant recipients**

![Graph showing inhibition of lymphocyte proliferation and activation in renal transplant recipients](image)

Adapted from Stalder et al, Ther Drug Monit 2003; 25:22-27

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**CD28 expression by PBMCs and risk of malignancy**

CD28 Expression by Peripheral Blood Lymphocytes as a Potential Predictor of the Development of De Novo Malignancies in Long-Term Survivors After Liver Transplantation

Stammarius Dieter,12 Sarria Per Oblinna,1,2 Leela Anjum,1,n Sandrine Chevaux-Rouss,1,n Olivier Scallion,1 Olivier Sourdaine,1,2 Yvon Colomes,1,2 Christin Behrem,1,2 and Flavio Mero Conti1,2

Study design: 134 stable long-term survivors of liver transplantation

CD28 expression by peripheral lymphocytes measured by flow cytometry

Results: Frequency of CD28+CD8+ cells significantly lower in cancer group vs. noncancer group (39 ± 22% vs. 51 ± 21%, P = 0.0088) Negative predictive value: 89.7%

Conclusion: Identification of patients at high risk of developing de novo malignancies

Liver Transpl 2011; 17: 299-305
NFAT-regulated gene expression to assess response to tacrolimus

**NFAT-regulated genes:**
- IL-2, IFN-γ, granulocyte-macrophage colony stimulating factor

**Patients with CMV disease**

**Patients with acute rejection**

*NFAT RE (%): 1.5 h post Tac dose; stable renal transplant recipients

<table>
<thead>
<tr>
<th></th>
<th>Tac</th>
<th>No Tac</th>
</tr>
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<tbody>
<tr>
<td>A</td>
<td>p &lt; 0.02</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>p &lt; 0.05</td>
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</tr>
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</table>

No difference in PK data

Sommerer C et al, Ther Drug Monit 2011; 33: 375-379

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Percentage of IL-2 producing CD8+ T-cells in liver recipients with and without acute rejection

Boleslawski et al, Transplantation 2004; 77: 1815 - 1820

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Intracellular IL-2 expression in CD8+ T-cells during ISPT withdrawal in stable liver recipients

Millán O et al, Clin Immunol 2010; 137: 337-346

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Global CD4+ cellular response measured by iATP synthesis

Sodium heparinate whole blood

Kowalski et al, Clin Transplant 2003; 17: 77-88

Immune Response vs. CD4 Count

Kowalski et al, J Immunotoxicol 2007; 4: 225-32

Comparison of ATP production in mitogen-stimulated CD4+ cells and immunosuppressive drug concentrations before and 2-hour postdose

Stable renal transplant recipients (n=46)

<table>
<thead>
<tr>
<th>Parameters Measured</th>
<th>Before Dose</th>
<th>2-Hour Postdose</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>ImmuKnow level (ng/mL ATP)</td>
<td>321 ± 134</td>
<td>332 ± 162</td>
<td>0.704</td>
</tr>
<tr>
<td>Tacrolimus concentration (ng/mL)</td>
<td>5.1 ± 2.3</td>
<td>10.7 ± 8.6</td>
<td>0.001</td>
</tr>
<tr>
<td>Mycophenolic acid concentration (mg/dL)</td>
<td>4.8 ± 5.3</td>
<td>7.5 ± 5.7</td>
<td>0.025</td>
</tr>
<tr>
<td>Prednisolone concentration (ng/mL)</td>
<td>6.8 ± 7.4</td>
<td>122.5 ± 62.5</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

ImmuKnow test is a marker for the overall level of immune function

Akhlaghi F, Gohh RY, Ther Drug Monit 2010; 32: 116-117
Correlation between immune response and CNI trough levels in stable pediatric liver transplant recipients

Ther. range

525
525

Strong
Strong

low
low

r = 0.172
p = 0.527

r = 0.024
p = 0.918

Adapted from Brandhorst et al, Clin Chem 2010; 56(suppl.): A241

Immune response of solid organ transplant recipients during periods of rejection, infection and stability

Rejection (n=39)

Infection (n=66)

Stable (n=504)

P < 0.001

Zone of minimal risk

Adapted from Kowalski et al, Transplantation 2006; 82: 663-668

Association between pretransplant iATP levels (ImmuKnow, Cylex) with kidney graft outcome

No clinical reason

Negative Ab-mediated AR

Positive Ab

Ab-mediated AR

P < 0.05

Adapted from Rentosemite et al, Transplantation 2000; 85: 462-470
Immune response in renal transplant recipients with infection or rejection

Single time point immune function assay (ImmuKnowTM) testing does not aid in the prediction of future opportunistic infections or acute rejection.

Huskey J, Gralla J, Wiseman AC.

Study design:
Retrospective analysis of 1330 ImmuKnow assay values in 583 renal transplant recipients and correlation with OI and AR episodes.

Conclusion:
No association between single time point ImmuKnow test results and adverse event in subsequent 90 days.


Immune response in heart transplant recipients with infection and rejection

Longitudinal ImmuKnow monitoring in a patient 4 years post-HTx

Israeli M et al. Transplantation 2010; 89: 968-976

Are baseline values for individual patients required?

Longitudinal ImmuKnow monitoring in a patient 4 years post-HTx

Israeli M et al. Transplantation 2010; 89: 968-976
IM scores in heart transplant recipients with infection or rejection

Kobashigawa et al, J Heart Lung Transplant 2010; 29: 504-508

ImmuKnow (iATP) values in lung transplant recipients with infections

Husain et al, Transplantation 2009; 87: 1852-1857

Progression of Aspergillus colonization to invasive pulmonary aspergillosis (iA)

Husain et al, Transplantation 2009; 87: 1852-1857
Immune response in adult liver transplant recipients classified with biopsy findings

Hashimoto et al, Clin Transplant 2010; 24: 701-708

Immune cell response in a liver transplant recipient (2 y, boy) with EBV infection

Brandhorst et al, Clin Chem 2010; 56(suppl.): A241

Immune cell response in a liver transplant recipient (15 y, girl) with acute rejection

Brandhorst et al, Clin Chem 2010; 56(suppl.): A241
Immune profiles of liver transplant recipients who developed rejection when IST was withdrawn

Relationship between immune cell response (Cylex) and acute rejection (AR)

<table>
<thead>
<tr>
<th>Significant results (p &lt; 0.05)</th>
<th>Author</th>
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<tbody>
<tr>
<td><strong>Renal transplantation</strong></td>
<td></td>
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<tr>
<td>↑ AR risk with higher iATP levels</td>
<td>Kowalski 2006; Reinsmoen 2008</td>
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<tr>
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<td>Heskey 2010; Serban 2009</td>
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<td><strong>Cardiac transplantation</strong></td>
<td></td>
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<tr>
<td>↑ AR risk with higher iATP levels</td>
<td>Israeli 2010; Kowalski 2006</td>
</tr>
<tr>
<td>No significant relationship</td>
<td>Gupta 2008; Kobashigawa 2010</td>
</tr>
<tr>
<td><strong>Hepatic transplantation</strong></td>
<td></td>
</tr>
<tr>
<td>↑ AR risk with higher iATP levels</td>
<td>Kowalski 2006; Cabrera 2009; Brandhorst 2010; Hashimoto 2010</td>
</tr>
<tr>
<td>No significant relationship</td>
<td>Millan 2009</td>
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Relationship between immune cell response (Cylex) and infection

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<tbody>
<tr>
<td><strong>Renal transplantation</strong></td>
<td></td>
</tr>
<tr>
<td>↑ Incidence of infection with lower iATP levels</td>
<td>Kowalski 2006; Serban 2009; Castillo-Chavez 2006; De Paula 2011</td>
</tr>
<tr>
<td>No significant relationship</td>
<td>Heskey 2010</td>
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<tr>
<td>↑ Incidence of infection with lower iATP levels</td>
<td>Xue 2010; Hashimoto 2010; Kowalski 2006; Cabrera 2009; Lee 2006</td>
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<tr>
<td>No significant relationship</td>
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<tr>
<td><strong>Lung transplantation</strong></td>
<td></td>
</tr>
<tr>
<td>↑ Incidence of infection with lower iATP levels</td>
<td>Bhorade 2008; Husain 2009</td>
</tr>
<tr>
<td>No significant relationship</td>
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</tr>
</tbody>
</table>
Association between immune response and mortality risk

Screening of mortality in transplant patients using an assay for immune function.
Berglund D, Bengtsson M, Biglarnia A, Berland E, Yamamoto S, von Zur-Mühlen R, Lorant T, Tufveson G.

Study design:
1031 ImmuKnow assays (iATP) in 362 patients (pts)
(kidney, pancreas, islet cells, liver allografts)

Results:
Mortality: 14.4 % iATP < 175 ng/ml (at least once)
5.2 % iATP > 175 ng/ml

Conclusion:
Potential usefulness of ImmuKnow assay for identification of pts with increased short-term mortality risk.

Transpl Immunol 2011, in press

Allograft tolerance in solid organ transplantation

Spontaneous operational tolerance:
- long-term maintenance of stable graft function without a clinically significant, detrimental response or immune deficit following discontinuation of conventional immunosuppression
- stable renal transplant recipients without immunosuppression ≥ 1 year (serum creatinine < 10 % increase)
"Almost tolerance":
- stable graft function in minimally immunosuppressed recipients (low dose monotherapy)

Estimated incidence of operational tolerance:
- liver transplantation: < 20%
- renal transplantation: low frequency

Roussey-Kerler et al, Am J Transplant 2006; 6: 756-746
Newell et al, J Clin Invest 2010; 120: 836-847
Sánchez-Fueyo A et al, Gut 2011; 60: 518-524
Sagoo P et al, J Clin Invest 2010; 120: 1848-1861

Biomarker signatures related to tolerance in transplantation

Goal: Identification of recipients who would benefit from immunosuppression withdrawal or minimization

Indices of tolerance:
- Signature of B-cell differentiation genes (IGKV4-1, IGLLA, IGKV1D-13) highly predictive of renal transplant tolerance
- Number of regulatory T-cells (Treg)
  FOX P3 demethylation as Treg signature useful for monitoring Treg in human peripheral blood

Newell et al, J Clin Invest 2010; 120: 1836-47
Sagoo et al, J Clin Invest 2010; 120: 1848-61
Wazni et al, Cancer Res 2008; 68: 399-408
**Gene expression signatures**

**Identification of a B cell signature associated with renal transplant tolerance in humans**

*Kenneth A. Newell et al*

**Study design:**
Identification of recipients who would benefit from IS withdrawal or minimization
Cohort of 25 tolerant renal transplant recipients (> 1 y, no IS)

**Results:**
Tolerant patients exhibited increased numbers of total and naive B cells and showed increased expression of multiple B cell differentiation genes. Signature of 3 genes (IGKV4-1, IGLLA, IGKV1D-13) was highly predictive of tolerance.

**Conclusion:**
Transitioning or maturing B cells involved in tolerance induction and/or maintenance

*J Clin Invest 2010; 120: 1836-47*

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**FOXP3 demethylation as Treg cell signature**

*Baron et al, Eur J Immunol 2007; 37: 2378-89*

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**Treg in total CD3⁺CD4⁺ cell population**

T-cell subsets in peripheral blood from liver transplant patients with renal dysfunction

<table>
<thead>
<tr>
<th></th>
<th>MMF group (n=22)*</th>
<th>Control group (n=14)**</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CD3+CD8+ cells/mL</td>
<td>CD3+CD4+ cells/mL</td>
</tr>
<tr>
<td>Baseline</td>
<td>0.613 ± 0.092</td>
<td>0.474 ± 0.085</td>
</tr>
<tr>
<td>Month 12</td>
<td>0.424 ± 0.055</td>
<td>0.374 ± 0.075</td>
</tr>
</tbody>
</table>

* P < 0.001 vs. baseline; † P < 0.05 vs. baseline; mean ± S.E.M.
* MMF 2x1 g; CsA 25-50 µg/l or TAC 2-4 µg/l
** CsA 80-120 µg/l or TAC 5-7 µg/l

Adapted from Cicinнатi et al, Aliment Pharmacol Ther 2007; 26: 1195-1208

Biomarker signature to detect renal transplant tolerance

Tolerance signature comprising:
- a set of 10 genes with significantly altered expression
- elevated numbers of peripheral blood B and NK cells
- diminished numbers of recently activated CD4+ T cells
- donor-specific hyporesponsiveness of CD4+ T cells (IFN-γ ELISpot)
- high ratio of FoxP3/α-1,2-mannosidase gene expression in peripheral blood

Sagoo et al, J Clin Invest 2010; 120: 1848-1861

Peripheral blood biomarker combinations

Overall immune function (Over-immunosuppression)
- CD4+ cellular response measured by iATP synthesis (mitochondrial metabolic competence)

Risk of rejection
- IL-2 expression by CD8+ T-lymphocytes (cytotoxic properties)

Indices of tolerance
- Regulatory T-cells (FOXP3 demethylation signature)
- Signature of B-cell differentiation genes
<table>
<thead>
<tr>
<th>Bio-markers for immunosuppressive drug effects</th>
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</thead>
<tbody>
<tr>
<td>Potential complementary tools in addition to TDM</td>
</tr>
<tr>
<td>- may identify candidates for minimization of immunosuppressive therapy</td>
</tr>
<tr>
<td>- may identify patients at risk for acute rejection or infection</td>
</tr>
<tr>
<td>- may be useful to guide immunosuppressant weaning</td>
</tr>
<tr>
<td>PD-monitoring using bio-markers is in its early stages</td>
</tr>
<tr>
<td>- optimal combinations of biomarkers may be necessary</td>
</tr>
<tr>
<td>- baseline values for individual patients may be required</td>
</tr>
<tr>
<td>- no prospectively validated target ranges available</td>
</tr>
<tr>
<td>Development of &quot;tolerance permissive&quot; immunosuppressive regimens would be desirable</td>
</tr>
</tbody>
</table>
Early detection of Alzheimer’s Disease: are CSF Ab42 and tau biomarker tests ready for the challenge?

Leslie M Shaw
Department of Pathology and Laboratory Medicine
Perelman School of Medicine
University of Pennsylvania

Alzheimer’s disease

- AD is one of the most disabling & burdensome health conditions worldwide
- An estimated 5.3 million people in the US and 35 million people have dementia today
- 4.6 million new cases diagnosed each year
- Number of people affected is expected to double every 20 yrs to reach ~81 million by 2040
- Dementia prevalence <1% at age 60-64, increases exponentially, thus by age >85 prevalence is 24-33%


Disclosures

Grant support: ADNI 1, ADNI GO, ADNI 2, NIH/NIA; Pfizer/UPenn rbm studies
Consultant to: Innogenetics/Fujirebio; Janssen Research & Development
Alzheimer’s Disease

- Alzheimer’s disease (AD) is an age-related progressive neurological disorder characterized by:
  - Loss of memory
  - Confusion
  - Disorganized thinking
  - Impaired judgment
  - Compromised expression
  - Disorientation.

- Affected individuals:
  - Incapable of managing their personal affairs
  - Eventually unable to tend to basic physical needs
  - Nursing home placement or similar intensive home care is eventually required
  - Enormous emotional and financial burden on families
  - Failure of bodily functions predispose to infection and death occurs on average 7–9 yrs following clinical diagnosis.

- Plaques are extracellular deposits of amyloid-β surrounded by dystrophic neurites, reactive astrocytes, & microglia.
- Tangles are intracellular aggregates composed of a hyperphosphorylated form of the microtubular stabilizing protein, Tau.
- Degeneration of neurons and synapses is a characteristic finding associated with plaques & tangles.
- Oxidative stress & neuronal/neuritic dysfunction (eg, impairment of acetylcholine transmitter activity) accompany these lesions.

Senile plaques & neurofibrillary tangles are characteristic lesions in the medial temporal lobe structures & cortical areas of AD brain.

- Major causes of death are cerebrovascular disease & cardiac disease.

Natural history of AD

- Majority of cases are sporadic with dominantly inherited forms accounting for <1%.
- Inheritance of ε4 allele of APO E is an established genetic risk factor.
- Age is a major risk factor, small numbers of plaques and tangles in most aged.
- Tau pathology appears 1st in transentorhinal region (entorhinal cortex & hippocampus) with some amyloid-β deposits in the neocortex.
- Amyloid-β deposits tend to appear first in the neocortex.
- Both types of pathology seem to form independently with tangles appearing first, followed at later stages by extensive amyloid-β deposition in the neocortex.
- Amyloid-β might exacerbate Tau pathology.
- Neuropathology features of mild cognitive impairment (MCI) are intermediate between normal aging and AD: Tau deposits abundant in the entorhinal cortex & hippocampus with some amyloid-β deposits in the neocortex.
Multiple amyloid-β peptides are produced from the normal metabolism of amyloid precursor protein (APP) by various peptidases with amyloid-β (1-40) the most prevalent and amyloid-β (1-42) the least soluble peptide. Overproduction of, and/or lowered elimination of amyloid-β (1-42), are presumed cause of senile plaque formation.
Loss of tau function leads to neuronal dysfunction


- Are pathological and insoluble tau isoforms that assemble into paired helical and/or straight filaments
- Form fibrillar amyloid inclusions in neurons > glia
- Approximately 95% of PHF tau deposits in neuronal processes while ~5% localizes to the perikarya of neurons
- Aberrantly phosphorylated at many serine and threonine residues
- Unable to bind to MTs, but enzymatic dephosphorylation can restore MT binding


PHFtau Amyloid In AD

Jean Marx, Science, 316:1416-1417, 2007
The Most Promising Biomarkers for AD Detection: CSF Aβ₁-₄₂, t-tau, pTau₁₈₁p

- **Benefits**
  - Most widely studied so far
  - Linked to AD pathology
  - May detect pathology before memory dysfunction or dementia appear clinically

- **Challenges**
  - Can be abnormal to varying degrees in non-AD neurodegenerative diseases
  - Analytical methods for measurement need standardization

**Overview**

- More than 30 studies (mostly single center small studies) have shown the diagnostic utility of CSF Aβ₁-₄₂ and tau measurement for AD detection—50% or more decrease in Aβ₁-₄₂ and ~2-3-fold increase in tau in comparison to age-matched normals.
- ELISA and Luminex xMAP multiplex immunoassays and Innogenetics reagents most commonly used methods; Mesoscale Diagnostics immunoassay and others.
- Several studies show the predictive performance for Aβ₁-₄₂ and t-tau/ Aβ₁-₄₂ as predictors of progression from Cog Norm→MCI or MCI→AD (Fagan, 2007; Hannes, 2006; ADNI, 2009; Mattson, 2010).
- Studies from UWash, WashU, ADNI show that about a third of normal elderly have these changes but requires many years of time to observe conversion to MCI or early AD but characterizes early AD pathology.
- It is possible to obtain reproducible results within one laboratory, using one lot of manufacturer’s reagents, such that pooled samples can be assayed with confidence in the results, provided that appropriate qc materials are used to check performance continuously.
- A primary reference material in CSF matrix is needed for accuracy assessments.
- High level standardization of sample collection, storage, handling, reagent manufacture, lab performance is needed to move toward use of reagents within a laboratory across different lots of manufacturer’s reagents—this requires considerable effort and expertise.
- An international CSF qc program sponsored by the Alz Association has been established that provides feedback to participating labs and should lead to improved practice worldwide (>60 participating labs).

**Clinical Diagnosis of AD is Imprecise**

- Early diagnosis of AD is a high priority need
  - Definitive diagnosis requires autopsy confirmation
  - Diagnostic accuracy rate of ~90% using consensus criteria for probable AD
  - Diagnosis is especially difficult at early pre-clinical stages of AD
  - Confusion with other dementias is common

- **Hypotheses:** CSF biochemical biomarkers can improve clinical diagnostic accuracy and predict conversion to AD from pre-clinical disease
Mild Cognitive Impairment

- 50% probability of progressing to symptomatic AD within 4 years
- Some progress to another dementia type
  - dementia with Lewy bodies (DLB)
  - frontotemporal lobe dementia (FTLD)
  - Parkinson’s disease (PD)
- Some will not progress to any significant extent

DeCarli, 2003

Revised criteria for AD diagnosis proposed in 2007

- Earlier diagnosis—at the MCI stage
- Use of biomarkers of AD a key
Alzheimer’s Disease Neuroimaging Initiative (ADNI)

- ADNI was created in an environment where there is:
  - Low power of clinical measures for disease progression and modification
  - Need for highly standardized biomarkers that provide direct evidence of disease and can monitor disease progression and modification
- Public funding drives public sharing of ADNI research results
  - $67 million total funding over 6 years, start date, 2004-2010
    - $40 million public funding from NIH, National Institute of Aging
    - $27 million private via the FNIH
  - $24 million public funding from NIH/NIA for ADNI GO, 2009-2011
  - $60+ million public/private funding applied for (ADNI 2): 2010-2017
- ADNI website: http://www.adni-info.org/

ADNI Cores

There are 10 ADNI cores (M. Weiner is overall PI):

<table>
<thead>
<tr>
<th>Core</th>
<th>PIs</th>
<th>Institution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Administrative</td>
<td>Mike Weiner</td>
<td>UCSF</td>
</tr>
<tr>
<td>Clinical</td>
<td>Ron Peterson Paul Eisen</td>
<td>Mayo Clinic UCSD</td>
</tr>
<tr>
<td>MRI</td>
<td>Cliff Jack</td>
<td>Mayo Clinic</td>
</tr>
<tr>
<td>PET</td>
<td>Wm Jagust</td>
<td>UC Berkeley</td>
</tr>
<tr>
<td>Biomarker</td>
<td>John Trojanowski Les Shaw</td>
<td>UPenn</td>
</tr>
<tr>
<td>Neuropath</td>
<td>John Morris</td>
<td>Wash U</td>
</tr>
<tr>
<td>Informatics</td>
<td>Art Toga</td>
<td>UCLA</td>
</tr>
<tr>
<td>Genetics</td>
<td>Andy Saykin</td>
<td>IUP</td>
</tr>
<tr>
<td>Biostatistics</td>
<td>Laurel Beckett</td>
<td>UC Davis</td>
</tr>
</tbody>
</table>
57 ADNI sites

Biofluid sampling & imaging schedule for the ADNI study

All subjects (age 55-90, well matched for age across mildAD, aMCI, NC cohorts):

- Clinical MRI (1.5 T) at all time points
- FDG PET at all time points in 50%
- 3 T MRI at all time points in 25%
- PIB add-on study underway
- Blood and urine at all time points from all subjects
- CSF from 51% of subjects at BL & 12 mos, but extended to 24 & 36 mos in an add-on study
- APO E in all subjects; Genome wide add-on study in all subjects
- ISAB sponsored add-on studies: assess plasma & CSF for new candidate biomarkers using RBM panels
- ADNI GO grant funded by NIH/NIA; add 200 new early MCI subjects
Overview of the Penn ADNI Biomarker Core

• Core Leaders: Les Shaw & John Trojanowski
• Co-Investigator: V.M.-Y. Lee
• Core staff: M Korecka, M Brylska, T Waligorska, M Figurski, R Patel
• Goals of the Penn Biomarker Core:
  1) Aliquot, label & bank CSF, plasma, serum & urine samples in the repository, storage at -80 °C with continuous temp monitoring
  2) Validate, standardize & assay these analytes in ADNI subjects:
     - ApoE genotype (blood) — Path & Lab Med Molecular Core
     - Excess blood — Pfizer, TGen for genome-wide genotyping
     - Homocysteine (plasma, CSF)
     - All Isoprostane species (plasma, urine, CSF)
     - All Tau (CSF) and all Aβ species (CSF & plasma)
  3) Create immortalized cell lines (blood) — NCRAD
  4) Utilize the Resource Allocation Review Committee (RARC) to distribute samples to other qualified investigators (including “add on studies”)

---

More than 12,000 Biofluid Samples Received

Qualification of the analytical and clinical performance of CSF Aβ1–42, tau and p-tau181 in the ADNI study

Selection of CSF Aβ1–42, tau, p-tau181 based on prior studies that showed their promise for AD detection & a consensus among experts in this field

2. Pre-analytical factors
   - Identify and control for pre-analytical variables
     - Time of day for lp-morning following overnight fast
     - Collection tube type
     - Transport temperature
     - # of freeze-thaw cycles
     - Time from collection to freezing

3. Analytical performance
   - Assure stability of reproducibility of test performance
     - Within each run
     - Day to day
     - Among expert laboratories
     - From batch to batch of immunoassay reagents
   - AA-sponsored international CSF external blinded quality control program

4. Clinical diagnostic performance
   - Using the qualified test method
     - Establish sensitivity and specificity in an ADNI-independent set of CSF samples from individuals with autopsy-confirmed AD
     - Use diagnostic cutoffs derived from the ADNI independent data to assess and characterize an AD CSF pathologic biomarker signature in the ADNI subjects
     - Characterize the longitudinal changes in CSF biomarker changes in a subset of ADNI CSF donors
CSF Tau, Aβ42, pTau181: measured using the Luminex multiplex platform and Innogenetics INNO-BIA AlzBio3 immunoassay reagents

Key characteristics of the xMAP system compared to ELISA:

<table>
<thead>
<tr>
<th>xMAP</th>
<th>ELISA</th>
</tr>
</thead>
<tbody>
<tr>
<td>format</td>
<td>Antibody covalently bound to beads</td>
</tr>
<tr>
<td>biomarker tests per run</td>
<td>3</td>
</tr>
<tr>
<td>volume</td>
<td>75 μL(x2)</td>
</tr>
<tr>
<td>precision</td>
<td>3-10%; excellent test-re-test precision and better dynamic range for xMAP calibration curves.</td>
</tr>
<tr>
<td>Analytical validation</td>
<td>Completed 7 lab interlab validation study</td>
</tr>
<tr>
<td>biomarker concentrations</td>
<td>Equivalent clinical correlation for xMAP vs ELISA</td>
</tr>
</tbody>
</table>

Analytical performance of the xMAP Innogenetics immunoassay

Test precision

Inter-laboratory study (with ISAB and academic collaborators) done achieving within-center variability of <10%.

CSF pools included for run-validation reproducibility confirmed in CSF testing in 2007 (n=14) and 2008 (n=28). Good test-re-test correlation

Result consistency

Deming regression analysis used to normalize 2007 BASELINE data to 2007 CSF biomarker testing, providing mechanism(s) to achieve in the future reagent lot-to-lot consistency

Analytical performance of Luminex platform with Innogenetics immunoassay reagents in 2007 and 2008

<table>
<thead>
<tr>
<th></th>
<th>2007</th>
<th>2008</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Precision for CSF pools</td>
<td>Test-re-test performance**</td>
</tr>
<tr>
<td></td>
<td>%CV</td>
<td>r²; slope</td>
</tr>
<tr>
<td>CSF pools</td>
<td>t-tau</td>
<td>Aβ42</td>
</tr>
<tr>
<td>14 days</td>
<td>#80</td>
<td>6.4</td>
</tr>
<tr>
<td>#12</td>
<td>4.8</td>
<td>3.3</td>
</tr>
<tr>
<td>28 days</td>
<td>#50</td>
<td>6.4</td>
</tr>
<tr>
<td>#52</td>
<td>7.7</td>
<td>8.6</td>
</tr>
</tbody>
</table>

**In the 2007 series of CSF analyses 24 samples were re-tested; in 2008 81 were re-tested.
CSF biomarkers:
- High level of within-lab reproducibility
- Interlaboratory reproducibility needs improvement
- Need harmonization across immunoassay methods

ADNI interlab study using Luminex xMAP/Innogenetics reagents - center study

CSF biomarkers: • High level of within-lab reproducibility • Interlaboratory reproducibility needs improvement • Need harmonization across immunoassay methods

The Alzheimer’s Association International QC program for CSF biomarkers

- Overview:
  1. Establish a standardized protocol for lumbar puncture and CSF processing
     - will minimize variation due to confounding factors (e.g., pain, stress, etc.)
  2. Establish an external QC program for CSF biomarkers
     - will allow comparisons of biomarker levels between labs (and future harmonization of levels)
     - will assess longitudinal deviation in biomarker (due to e.g., batch-to-batch variation in kit)
Cerebrospinal Fluid Biomarker Signature in Alzheimer’s Disease Neuroimaging Initiative Subjects

Edith A. Brown, PhD; Hugo Friedland MD, PhD; Michael Farlow MD, PhD; Christopher M. Clark, MD; Hui Xu, PhD; Michael G. Albert MD, MPH; Ronald C. Petersen MD, PhD; Eric Reiman MD, PhD; Josephine C. Thompson MD, PhD; John Q. Trojanowski MD, PhD; and the Alzheimer’s Disease Neuroimaging Initiative


Demographics of ADNI subjects who provided CSF at baseline

<table>
<thead>
<tr>
<th></th>
<th>AD</th>
<th>MCI</th>
<th>NC</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>150</td>
<td>150</td>
<td>150</td>
</tr>
<tr>
<td>%</td>
<td>55.42%</td>
<td>59.85%</td>
<td>53.33%</td>
</tr>
<tr>
<td>Age, y</td>
<td>Mean SD</td>
<td>Mean SD</td>
<td>Mean SD</td>
</tr>
<tr>
<td></td>
<td>75.8</td>
<td>75.7</td>
<td>76.6</td>
</tr>
<tr>
<td>MMSE</td>
<td>Mean SD</td>
<td>Mean SD</td>
<td>Mean SD</td>
</tr>
<tr>
<td></td>
<td>23.5±1.9</td>
<td>26.0±1.8</td>
<td>29.1±1.0</td>
</tr>
<tr>
<td>ADAS Cog</td>
<td>Mean SD</td>
<td>Mean SD</td>
<td>Mean SD</td>
</tr>
<tr>
<td></td>
<td>19±4.2</td>
<td>11±4.5</td>
<td>8±4.0</td>
</tr>
<tr>
<td>APOE ε4</td>
<td>Mean%</td>
<td>Mean%</td>
<td>Mean%</td>
</tr>
<tr>
<td></td>
<td>69.31%</td>
<td>58.69%</td>
<td>27.67%</td>
</tr>
</tbody>
</table>
ADNI BASELINE CSF biomarker concentrations show the expected average differences between AD and MCI and NC

<table>
<thead>
<tr>
<th></th>
<th>Tau</th>
<th>Aβ1-42</th>
<th>P-Tau/Aβ</th>
<th>Tau/Aβ</th>
<th>P-Tau/Aβ</th>
<th>Aβ1-42/Aβ</th>
<th>P-Tau/Aβ/Aβ</th>
</tr>
</thead>
<tbody>
<tr>
<td>AD (n=102)</td>
<td>122±58</td>
<td>143±41</td>
<td>42±20</td>
<td>0.9±0.5</td>
<td>0.3±0.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MCI (n=200)</td>
<td>103±61</td>
<td>164±55</td>
<td>35±18</td>
<td>0.8±0.6</td>
<td>0.3±0.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NC (n=114)</td>
<td>70±30</td>
<td>206±55</td>
<td>25±15</td>
<td>0.4±0.3</td>
<td>0.1±0.1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

p<0.001, for each of the 5 biomarker tests for AD vs NC and for MCI vs NC.
For AD vs MCI p<0.005, Tau p<0.01, Aβ p<0.01, P-Tau p<0.005, Tau/Aβ p<0.005, P-Tau/Aβ p<0.005.
Mann-Whitney test for statistical differences used for these non-normally distributed data sets.

CSF biomarkers for ADNI subjects stratified by # of APOE ε4 alleles

MCI = Mild cognitive Impairment; NC = no cognitive impairment.

Proposed CSF biomarker uses in research & clinical management

- Clinical diagnosis and management
  - Improve diagnostic accuracy, especially in early stages of AD
  - Combine with clinical exam results and further testing (blood tests, CT/MRI, PET)
- Predicting disease progression
- Enrichment of AD cases in treatment trials
  - ~45-70% of MCI patients have prodromal AD
  - CSF biomarkers can enrich the treatment cohort with subjects at greatest risk for progression to AD
- Assessing drug effects
  - Assessment of specific biochemical effects of a drug:
    eg. CSF Aβ1-42 in trials of anti-Aβ antibodies & -secretase inhibitors; CSF-tau in trials of tau kinase inhibitors; plasma Aβ1-40/1-42 in trials of γ-secretase inhibitors
  - Assessment of the effect of a drug on neurodegeneration:
    eg. CSF-tau in trials of Aβ vaccine
Qualification of the multiplex immunoassay system for AD detection using an ADNI-independent set of autopsy-based AD vs NC subjects’ CSF samples

<table>
<thead>
<tr>
<th></th>
<th>Tau</th>
<th>Aβ1-42</th>
<th>p-Tau181P</th>
<th>Tau/Aβ1-42</th>
<th>P-Tau181P/Aβ1-42</th>
</tr>
</thead>
<tbody>
<tr>
<td>AD (n=58)</td>
<td>135±95</td>
<td>132±34</td>
<td>39±29</td>
<td>1.1±1.0</td>
<td>0.3±0.2</td>
</tr>
<tr>
<td>NC (n=57)</td>
<td>57±30</td>
<td>233±58</td>
<td>18±16</td>
<td>0.3±0.2</td>
<td>0.1±0.1</td>
</tr>
</tbody>
</table>

Mann-Whitney test: p<0.0001 for each biomarker or ratio for Alzheimer’s Disease (AD) vs cognitively normal (NC).

58 autopsy-based AD cases and 57 NC subject CSF samples from the UPENN ADCR were included in this study.

AD mean age±SD; median age(range): 71±10; 75(44-86);
NC mean age±SD; median age(range): 70±11; 69(41-94);

Independent Autopsy Results Provide the Basis for the Diagnostic Utility of CSF Biomarkers Measured by ADNI

Operating Characteristic (ROC) curve analysis: established cut-points for use in ADNI study

ADNI-Independent Comparison of AD (autopsy-based) vs NC Subjects’ CSF samples

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Threshold values</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>Accuracy (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>t-tau</td>
<td>93 pg/mL</td>
<td>69.6</td>
<td>92.3</td>
<td>80.6</td>
</tr>
<tr>
<td>Aβ1-42</td>
<td>192 pg/mL</td>
<td>96.4</td>
<td>76.9</td>
<td>86.0</td>
</tr>
<tr>
<td>p-Tau181P</td>
<td>23 pg/mL</td>
<td>87.9</td>
<td>73.1</td>
<td>78.3</td>
</tr>
<tr>
<td>Tau/Aβ1-42</td>
<td>0.39</td>
<td>94.6</td>
<td>94.6</td>
<td>94.6</td>
</tr>
<tr>
<td>P-Tau181P/Aβ1-42</td>
<td>0.10</td>
<td>91.1</td>
<td>71.2</td>
<td>81.5</td>
</tr>
<tr>
<td>LRDAAX</td>
<td>0.34</td>
<td>98.2</td>
<td>79.5</td>
<td>99.7</td>
</tr>
</tbody>
</table>


Autopsy-Based CSF Biomarker Data Established AD Signature in the ADNI Study Cohorts

| % of ADNI patients in whom biomarker signature was detected using ROC cutpoints |
|-------------------------------|---|---|---|
| AD   | MCI | NC |
| Aβ1-42 | 91 | 78 | 34 |
| Tau/Aβ1-42 ratio | 89 | 69 | 34 |
| LRDAAX model | 89 | 70 | 31 |

LRDAAX = a logistic regression model that includes Tau, Aβ1-42, and ApoE ε4 allele number; ROC = receiver operating characteristic curve
Diagnosis-Independent Analysis of ADNI Data Reveals AD Biomarker Signature in Cognitively Normal Elderly People

• Applied statistical technique of "mixture-modeling" to the entire ADNI population without regard to diagnosis
• ADNI CSF Aβ1-42, t-tau, pTau data utilized

• Aβ1-42 is distributed bimodally in the ADNI total population as well as in each of the 3 cohorts, AD, MCI, NC
• Assume a mixture of 2 different normal distributions with 2 means and 2 SDs


A diagnosis-independent analysis of ADNI data reveals an AD biomarker signature in cognitively normal elderly people

• "decision boundary" at 188 pg/mL for Aβ1-42
• Best mixture model obtained with combination of Aβ1-42 & pTau or t-tau
• When applied to an independent group of 71 Belgian autopsy-based AD premortem CSF s 67 (94%) were classified as AD
• When applied to ADNI cohorts, AD, MCI & NC: 91%, 73% & 38% were classified as having the AD biomarker signature
• This independent statistical analysis confirms that there is an AD biomarker signature in more than 1/3rd of the elderly cognitively normal control group at BASELINE.

Baseline ADNI CSF biomarkers in 77 MCI→AD* converters (24-36 months) & in 5 MCI→cog normal “back-converters”

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Aβ1-42</th>
<th>t-tau</th>
<th>p-tau</th>
<th>t-tau/Aβ1-42</th>
<th>p-tau/Aβ1-42</th>
<th>LRT</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>AD</td>
<td>100</td>
<td>144</td>
<td>41</td>
<td>58</td>
<td>42</td>
<td>0.92</td>
<td>0.5</td>
<td>0.32</td>
</tr>
<tr>
<td>MCI→AD 12 mos</td>
<td>37</td>
<td>144</td>
<td>37</td>
<td>53</td>
<td>43</td>
<td>0.81</td>
<td>0.4</td>
<td>0.32</td>
</tr>
<tr>
<td>MCI→AD 24-36 mos</td>
<td>40</td>
<td>144±45</td>
<td>37±14</td>
<td>115±48</td>
<td>0.89±0.5</td>
<td>0.28±0.1</td>
<td>0.78±0.3</td>
<td></td>
</tr>
<tr>
<td>MCI→NL</td>
<td>115</td>
<td>163±55</td>
<td>104±61</td>
<td>36±18</td>
<td>0.76±0.6</td>
<td>0.26±0.2</td>
<td>0.62±0.4</td>
<td></td>
</tr>
<tr>
<td>NC</td>
<td>14</td>
<td>232±60</td>
<td>74±9</td>
<td>23±3</td>
<td>0.35±0.1</td>
<td>0.12±0.1</td>
<td>0.22±0.3</td>
<td></td>
</tr>
<tr>
<td>Remaining MCI</td>
<td>113</td>
<td>206±55</td>
<td>70±30</td>
<td>25±15</td>
<td>0.38±0.3</td>
<td>0.14±0.1</td>
<td>0.29±0.3</td>
<td></td>
</tr>
</tbody>
</table>

*as of 11/15/2009
Survival analyses for ADNI MCI subjects: Progression to AD for CSF biomarkers > or < cutpoints

As of June 28, 2010
ADNI study data
ADNI study data as of June 28, 2010

CSF Aβ1-42 is Strongly Correlated to Plaque Counts and Plaque Burden in Autopsied Brains

CSF Aβ1-42 vs plaque counts (neocortex and hippocampus)

CSF Aβ1-42 vs mean cortical PIB SUVR

Pittsburgh compound B labeled positron emission tomography; SUVR = standard uptake value ratio


CSF Aβ1-42 concentration decreases substantially with age, more so in APOE ε4 carriers, providing support for this as a preclinical biomarker of AD – Process starts as early as age 60

Hippocampal atrophy rates (L+R) – free surfer data – in ADNI subjects with CSF Aβ₁₋₄₂ >192 pg/mL or <192 pg/mL

<table>
<thead>
<tr>
<th>Condition</th>
<th>Aβ₁₋₄₂ &lt;192 pg/mL (%)</th>
<th>Aβ₁₋₄₂ &gt;192 pg/mL (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALL</td>
<td>-5.6±4.7</td>
<td>-2.6±4.1</td>
</tr>
<tr>
<td>AD</td>
<td>-8.0±5.9</td>
<td>-4.2±3.5</td>
</tr>
<tr>
<td>MCI</td>
<td>-4.8±3.6</td>
<td>-2.9±3.7</td>
</tr>
<tr>
<td>NC</td>
<td>-3.6±3.2</td>
<td>-2.2±4.3</td>
</tr>
</tbody>
</table>

These data show that in ADNI AD, MCI and NC subjects the rate of hippocampal atrophy increases at a significantly higher rate in subjects with Aβ₁₋₄₂ <192 pg/mL concentration compared to those >192 pg/mL.

Lateral ventricular volume increase rates (L+R) – ADNI free surfer data – in ADNI subjects are significantly greater when CSF Aβ₁₋₄₂ <192 pg/mL, the ADNI-independent autopsy-based cutoff

<table>
<thead>
<tr>
<th>Condition</th>
<th>Aβ₁₋₄₂ &lt;192 pg/mL (%)</th>
<th>Aβ₁₋₄₂ &gt;192 pg/mL (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALL</td>
<td>8.8±8.1</td>
<td>3.2±8.4</td>
</tr>
<tr>
<td>AD</td>
<td>9.9±9.8</td>
<td>4.9±4.8</td>
</tr>
<tr>
<td>MCI</td>
<td>9.2±7.4</td>
<td>4.2±5.9</td>
</tr>
<tr>
<td>NC</td>
<td>5.9±5.7</td>
<td>2.4±9.9</td>
</tr>
</tbody>
</table>

These data show that in ADNI AD, MCI and NC subjects the rate of lateral ventricular volume increases at a significantly higher rate in Subjects with Aβ₁₋₄₂ <192 pg/mL cutoff concentration compared to those >192 pg/mL.
Current Research on CSF Biomarkers is Making Strides in Addressing Biomarker Criteria

- Clinical diagnosis and management
  - Improving diagnostic accuracy, especially in early stages of AD
  - Combined with clinical exam results and further testing (blood tests, CT/MRI, PET)
- Monitoring disease progression
- Enrichment of AD cases in treatment trials
  - ~40-70% of MCI patients have prodromal AD
  - Enriching treatment cohorts with subjects at greatest risk for progression to AD
- Assessing drug effects
  - Assessing the specific biochemical effects of a drug:
    - CSF Aβ1-42 in trials of anti-Aβ antibodies; CSF p-tau in trials of tau kinase inhibitors; plasma Aβ1-40 in trials of γ-secretase inhibitors
  - Evaluating the effects of a drug on neurodegeneration:
    - CSF t-tau in trials of Aβ vaccine

Current Data Begins to Address Neurodegeneration and Specific Drug Effect

![Image](image.png)


Alzheimer’s Biomarker Initiative Hits Its Stride

An effort to develop biomarkers for Alzheimer’s disease is charting new data and making plans to expand
ADNI Data Integration is off and Running

The Future of Biomarker Research: Critical Goals

- Standardize CSF sampling / handling procedures
- Laboratory procedures
- Manufacture lot to lot consistency
- External control program
- Prospective studies:
  - Interrelationships between imaging and biochemical biomarkers in longitudinal studies
  - Longitudinal trajectories for transitions from cognitively normal—early MCI—late MCI—AD
  - Effect(s) of various treatment strategies on disease progression and biomarker measures
  - More biomarker study data on natural history of AD in the cognitively normal elderly population
- Approval for use in clinical practice
It takes a great team effort!

John Q. Trojanowski
Virginia M.-Y. Lee
Chris Clark
Steve Arnold
Hugo Vanderstichele
Magdalena Korcova
Margaret Knapik-Czajka
Magdalena Brylska
Teresa Waligorska
Michal Pilajuk
Ravi Patel
Leona Fields

Supported by the NIH/NIA and families of our patients

ADNI investigators include
(complete listing available at www.loni.ucla.edu/ADNI/Collaboration/ADNI Manuscript_Citations.pdf)

Acknowledgements
Drug Targeted Anticancer Therapy: Is there a role for TDM?

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  – Jeffrey Barrett, PhD, Philadelphia, PA

• Disclosures
  – None
21st Century Personalized Medicine

**Patient-Centered Healthcare**
- Respect for patients’ values, preferences, and expressed needs, including consideration of 1) coordination and integration of care, 2) information, communication, and education, 3) physical comfort, 4) emotional support, and 5) involvement of family and friends
  
  [Institute of Medicine (IOM) - “A New Health System for the 21st Century”]

**Individualized Therapeutics**
- Pharmacokinetic, pharmacogenetic and biomarker profiling and the adjustment of treatment to individual needs
- This is at the heart of Therapeutic Drug Management and now being ‘re-discovered’ in the era of pharmacogenomic medicine

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Diagnostic testing
- Genotype
- Gene expression
- Proteins
- Metabolites

Medication prescribed

Monitoring of effects

Optimization of treatment
- PK/PD/PG Model-based TDM

After P. Keown, IATDMCT Montreal 2009
Targeted Cancer Therapies

• Drugs that block the growth and spread of cancer by interfering with specific molecules involved in tumor growth and progression
• Interfere with cancer cell division (proliferation) and spread in different ways. Many of these therapies focus on proteins that are involved in cell signaling pathways.
• Stop cancer progression and may induce cancer cell death through a process known as apoptosis.
First targets for Targeted Cancer Therapies

• Cellular receptor for the female sex hormone estrogen, which many breast cancers require for growth

• Selective estrogen receptor modulators:
  – Tamoxifen, toremifene, fulvestrant

• Aromatase inhibitors interfere with estrogen’s ability to promote the growth of ER-positive breast cancers:
  – Anastrozole, exemestane, letrozole
## The Imatinib Success Story

### Discoveries Leading to FDA Approval of STI571/Gleevec for Treatment of Chronic Myelogenous Leukemia

<table>
<thead>
<tr>
<th>Year</th>
<th>Event</th>
</tr>
</thead>
<tbody>
<tr>
<td>1960</td>
<td>Abnormal chromosome 22 (Philadelphia Chromosome) observed in CML patients</td>
</tr>
<tr>
<td>1970</td>
<td>Chromosome 22 and 9 translocation observed by new staining techniques</td>
</tr>
<tr>
<td>1980</td>
<td><em>abl</em> Proto-oncogene identified in chromosome 22 translocation</td>
</tr>
<tr>
<td>1984-1987</td>
<td>BCR-ABL protein identified as possible cause of CML</td>
</tr>
<tr>
<td>1990</td>
<td><em>bcr-abl</em> Gene identified as cause of leukemia in mice</td>
</tr>
<tr>
<td>1993</td>
<td>First STI571/Gleevec laboratory studies begin</td>
</tr>
<tr>
<td>1998</td>
<td>First human tests begin</td>
</tr>
<tr>
<td>1999</td>
<td>First human results reported</td>
</tr>
<tr>
<td>2000</td>
<td>April: Larger study confirms earlier findings</td>
</tr>
<tr>
<td>2001</td>
<td>May: FDA approves STI571/Gleevec for treatment for CML</td>
</tr>
</tbody>
</table>

TIME magazine – May 28, 2001
Imatinib - Proof of concept therapeutic targeting of kinases

**Imatinib: HOW IT WORKS**

Bcr-abl kinase (green), which causes CML, inhibited by imatinib (red; small molecule)

*breakpoint cluster region - Abelson murine leukemia viral oncogene homolog*
# Approved Tyrosine-Kinase Inhibitors

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose (mg)</th>
<th>Tyrosinekinase Receptor</th>
<th>Indication</th>
<th>Metabolism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Imatinib (Gleevec)</td>
<td>100-400</td>
<td>Bcr-Abl, c-Kit, PDGF</td>
<td>ALL, CML, fibrosarcoma</td>
<td>CYP3A4, 2D6, 2C9, 2C19</td>
</tr>
<tr>
<td>Dasatinib (Sprycel)</td>
<td>20-50-70</td>
<td>Bcr-Abl, Scr</td>
<td>ALL, CML</td>
<td>CYP3A4</td>
</tr>
<tr>
<td>Erlotinib (Tarceva)</td>
<td>25-100 150</td>
<td>EGF</td>
<td>Non-small cell Lung CA</td>
<td>CYP3A4, 1A2</td>
</tr>
<tr>
<td>Sunitinib (Sutent)</td>
<td>12.5-25 50</td>
<td>VEGF, PDGF, c-KIT, FLT-3</td>
<td>GIST, kidney cell CA</td>
<td>CYP3A4</td>
</tr>
<tr>
<td>Sorafenib (Nexavar)</td>
<td>200</td>
<td>VEGF, PDGF, c-KIT, FLT-3, RET</td>
<td>Kidney cell CA</td>
<td>CYP3A4, 2C9, UGT1A1/9</td>
</tr>
<tr>
<td>Lapatinib (Tykerb)</td>
<td>250</td>
<td>EGF (HER1 &amp; HER2)</td>
<td>Kidney cell CA</td>
<td>CYP3A4/5</td>
</tr>
<tr>
<td>Crizotinib (Xalkori)</td>
<td>250</td>
<td>ALK, HGFR, RON</td>
<td>Non-small cell lung CA</td>
<td>CYP3A4/5</td>
</tr>
</tbody>
</table>
Rationale for Therapeutic Drug Management

• Despite the outstanding results generally obtained with imatinib in the treatment of chronic myeloid leukemia (CML), some patients show poor molecular response.

• Potential for drug interactions:
  – Increased clearance: St. John’s worth
  – Decreased clearance: ketokonazole

• Low intracellular concentration Drug does not get to target.
Targets for PK/PD and PG monitoring
Imatinib Clinical Pharmacology

• Large variability in drug disposition, especially total body clearance (Peng, 2005)
• Association exposure – clinical response:
  – Trough concentrations have been associated with complete cytogenetic and major molecular responses* in chronic myeloid leukemia (Picard, 2007)
  – Concentrations < 1100 ng/mL associated with lower response and development of progressive disease in patients with GIST (von Mehren, 2011)
• Imatinib toxicity are exposure-related:
  – Neutropenia associated with AUC (Delbaldo, 2006)
  • Hematologic (blood) response — Blood counts returned to normal.
  • Cytogenetic (cellular) response — number of cells with the Philadelphia chromosome reduced.
    A major response means at least 65% of cells normal; complete cytogenetic response - all cells test normal
  • Molecular response —no PCR-detected abnormal BCR/ABL genes by the Philadelphia chromosome.
## Imatinib Pharmacokinetics

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Adult (400 mg)</th>
<th>Pediatric (340mg/m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F (%)</td>
<td>&gt;90%</td>
<td>&gt;90%</td>
</tr>
<tr>
<td>$T_{\text{max}}$ (h)</td>
<td>3.3 (1.1)</td>
<td>2.5-3</td>
</tr>
<tr>
<td>$t_{1/2\beta}$</td>
<td>19.3 (4.4)</td>
<td>14-20</td>
</tr>
<tr>
<td>CL/F (L/h)</td>
<td>11.2 (4.0)</td>
<td>6.2 (2.0-11.5)</td>
</tr>
<tr>
<td>Vz/F (L)</td>
<td>295 (63)</td>
<td>202 (120)</td>
</tr>
<tr>
<td>Trough (μg/L)</td>
<td>1215 (750)</td>
<td>1800</td>
</tr>
<tr>
<td>$AUC_{0-24}$ (μg h/mL)</td>
<td>49.3 (17.1)</td>
<td>60-70</td>
</tr>
</tbody>
</table>

Quantification of Tyrosine Kinase Inhibitors

**Fig 1.** MRM chromatograms spiked with 10 ng/mL of imatinib (A), of blank plasma without IS and without analyte (B), and of a zero sample (blank plasma with IS) (C).

Standard dose results in large variability in exposure
Targets for Pharmacogenetic monitoring

- Drug metabolizing enzymes:
  - CYP3A5*1, CYP3A5*3
    - CYP3A5 expressor genotypes (CYP3A5*1/*1 and *1/*3) metabolize some CYP3A substrates more rapidly than CYP3A5 non-expressors
    - Allele frequency of CYP3A5*3 is 0.85 in Caucasians; African American, 0.55; Japanese, 0.85; Chinese, 0.65; Mexicans, 0.75; Pacific islanders, 0.65 and Southwest American Indians, 0.4

- Efflux Transporters
  - P-glycoprotein (MDR1, ABCB1)

- Influx Transporters
  - Solute carrier (SLC) membrane transport proteins (OATP; SLC22A1, SLCO1B1 and 1B3)

http://www.cypalleles.ki.se/; http://www.pharmgkb.org/index.jsp
Association of Genetic Polymorphisms with Imatinib PK

- 4.6-fold variability in individual clearance (3.4–15.5 L/h)
- Association of PGx of influx transporter SLCO1B3 and efflux transporter ABCB1 with imatinib PK
- Clearance increased in patients with the SLCO1B3 334 GG genotype (9.5 vs. 7.0 L/h)
- Patients with the ABCB1 3435 CC genotype had higher imatinib clearance (12.7 ± 3.0 L/h)

Yamakawa et al. Ther Drug Monit. 2011 Apr;33(2):244-50.
Combining Population PK/PD & TDM

• To study the interplay between genetics and the drug dose-exposure-effect relationship and its variability
• Collect informative PG data to use as Bayesian priors for PK/PD model-based individualized drug selection and dosing
• To Predict and therefore Control drug exposure and effects in individual patients
Requirements for Contemporary Dosing

Focus on interpretation rather than ‘numbers only’

Provided by experts trained in clinical pharmacology who practice direct patient care

Emphasis on the exposure–response and biomarkers rather then on dose-response

Implementation of software tools – with easy to use interphases

Implementation of control algorithms for dosing – such as Bayesian feedback

A Therapeutic Drug Monitoring Algorithm for Refining the Imatinib Trough Level Obtained at Different Sampling Times

Yanfeng Wang, PhD,* Yen Lin Chia, PhD,† Jerry Nedelman, PhD,† Horst Schran, PhD,* Francois-Xavier Mahon, MD, PhD,‡ and Mathieu Molimard, MD, PhD

\[ C_{min, std} = C(t) \exp(k_e \Delta t) \]

- **Dose:** 400 mg, SS Day 29
- **Steady-state imatinib conc. (ng/mL)**
- **Time since the last dose (hr)**

\[ CL = 9.98 \left( \frac{hL}{m} \right)^{0.277} \left( \frac{hL}{kg} \right)^{0.895} \exp(\eta_{CL}), \eta_{CL} \sim N(0, 0.104) \]

\[ V = 244.95 \left( \frac{hL}{m} \right)^{0.565} \left( \frac{hL}{kg} \right)^{0.662} \exp(\eta_V), \eta_V \sim N(0, 0.078) \]

Bayesian Estimation

Prior Probability

New Info

Objective Function

Posterior Probability

Goals

Control

Population Model

Concentration Biomarker

Consider Prior + New

Individual Model

Look at Patient Think

Select drug Calculate Dose

\[ \Phi_2 = \sum_{i=1}^{n} \left( \frac{C_i - E_i}{S_i} \right)^2 + \sum_{k=1}^{m} \left( \frac{\theta_k - \mu_k}{\sigma_k} \right)^2 \]

 Courtesy: Roger Jelliffe, MD, USC, Los Angeles

Thomas Bayes 1702 - 1761
Target-Controlled Model-Based Individualized Dosing Paradigm

- Patient data
- PK/PD/PG Population Model
- Check Target Attainment and Response
- Targeted Dosing
- Patient

Disease progression - improvement & Outcomes measures
Bayesian Guided Dosing

Yamakawa et al. Ther Drug Monit. 2011 Apr;33(2):244-50.
Real life application of M&S

Medical Centers

Patient visit
Sample collection
UPS shipment
Web/email notification

Centralized
LC-MS/MS Analysis

Confirmation
Dose change

Bayesian estimation
Dosing recommendation
Uploaded to web
Email notification

Results reported
On Web
Email notification
Sent out

http://clinicaltrials.gov/ct2/show/NCT00634270 term=sirolimus+NF1
Prototype Dashboard

Vinks & Barrett et al. 2011 Decision Support Tools to Improving Safety and Efficacy of Mycophenolate Therapy
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Update on Monitoring of Antiretroviral Drugs in HIV Therapy

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The George Washington University

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Learning Goals

- Understand the principles of the antiretroviral therapy
- Review current application of the therapeutic drug monitoring in the management of HIV
- Identify therapeutic targets of antiretroviral drugs
- Review the role of biomarkers in predicting the response and monitoring of HIV therapy
**HIV Life Cycle**

**Goals of Antiretroviral Therapy**
- Maximal suppression of HIV replication
- Maximal recovery and preservation of immune function
- Suppression of HIV-associated inflammation
- Prevention of opportunistic and other forms of infection
- Preservation of the quality and normal expectancy of life

**Classes of Antiretroviral Drugs**
- Nucleoside Reverse Transcriptase Inhibitors (NRTIs)
- Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs)
- Protease Inhibitors (PIs)
- Entry and Fusion Inhibitors (EIs and FIs)
- Integrase Inhibitors (IIs)
Measuring Therapeutic Effect of ART

Laboratory Efficacy:
- HIV RNA viral load
- CD4+ T lymphocyte count/percentage

Laboratory Toxicity:
- Liver enzymes (AST, ALT, bilirubin)
- Lipids (cholesterol, triglycerides) and glucose
- Hematologic parameters (WBC, RBC, Hb/Hct)
- Renal function parameters (BUN, Creatinine, UA)

Principles of TDM and ARV Therapy

TDM=Therapeutic Drug Monitoring

Major Barriers to TDM of ARV Drugs
- Lack of the data on therapeutic range of concentrations for all ARV drugs
- Limited number of clinical laboratories with quality assurance/quality control standards
- Shortage of experts with ARV drugs clinical pharmacological and virological expertise
- Lack of large prospective studies on ARV drugs TDM
Current Application of TDM in HIV Infection

- Evaluation of virologic failure with established adherence
- Optimization of the dose in treatment experienced patents
- Prevention of ART associated toxicity
- Management of drug-drug interactions
- Management of patients with significant physiologic changes (hepatic/renal impairment, pregnancy, pediatrics)

Pharmacokinetic Considerations for ART

- Maximal efficacy concentrations (EC) required to reduce viral replication of the wild type virus by minimum of 50% (EC50)
- The area under the time-concentration (AUC) and plasma trough concentration (Cmin) are linked to the exposure/efficacy and sustained virologic suppression
- AUC and peak plasma concentration (Cmax) are associated with the exposure/toxicity

Therapeutic Targets of ART

Efficacy trough
- NNRTIs: Efavirenz, Nevirapine
- PIs: Fosamprenavir, Atazanavir, Darunavir, Indinavir, Nelfinavir, Lopinavir, Saquinavir, Tipranavir

Toxicity trough
- NNRTIs: Efavirenz
- PIs: Indinavir
NRTIs=Nucleoside Reverse Transcriptase Inhibitors

**TDM of NRTIs**
- NRTIs are metabolized inside the cell to active triphosphate metabolites
- Only a few studies established a relationship between NRTIs plasma concentrations and virologic and immunologic outcomes
- It is not known whether the plasma concentrations reflect the real-time NRTIs exposure

NNRTIs=Non-Nucleoside Reverse Transcriptase Inhibitors

**TDM and NNRTIs**
- NNRTIs have a low plasma concentration resistance threshold
- Resistance to first-generation NRTIs is unlikely to overcome with increased drug exposure
- Second-generation NNRTIs have a different mechanism of viral resistance and may benefit from increased drug exposure

PIs=Protease Inhibitors

**TDM of PIs**
- PIs have a high plasma concentration threshold for HIV resistance
- Increasing plasma PIs exposure has been shown to overcome resistance and improve virologic outcome
- Combined use of virologic resistance tests with TDM results provide mechanisms for optimizing the PIs pharmacodynamics
Therapeutic Targets of PIs

- Inhibitory quotient (IQ) = the ratio of real-time plasma trough concentration ($C_{min}$) to the parameters of viral resistance/susceptibility to ARV drugs

- Phenotypic viral resistance is used to calculate phenotypic IQ (pIQ), virtual IQ (vIQ), or normalized IQ (nIQ)

- Phenotypic IQ ($pIQ = \frac{C_{min}}{IC_{50}}$)

- Virtual IQ ($vIQ = \frac{C_{min}}{fold \ change \ in \ virtual \ IC_{50}}$ from genotype x matched reference wild-type protein adjusted IC$_{50}$

- Normalized IQ ($nIQ = \frac{patient \ specific \ IQ}{reference \ IQ^*}$)

- * calculated as the ratio of typical $C_{min}$ for a given dose and wild type viral IC$_{50}$ which normalizes the IQ target across ARV to the ratio of >1

Genotypic tests are used to calculate a genotypic IQ (gIQ)

- Genotypic IQ ($gIQ = \frac{C_{min}}{number \ of \ ARV \ specific \ resistance-associated \ mutations}$)

- gIQ for PIs: Fosamprenavir, Atazanavir, Darunavir, Indinavir, Lopinavir, Saquinavir, Tipranavir

Pis = Protease Inhibitors

2011 ASCP Annual Meeting
The Role of Biomarkers in HIV Therapy

- Prediction of the progression of HIV disease and response to treatment
- Evaluation of chronic inflammation
- Prediction and evaluation of the immune reconstitution inflammatory syndrome (IRIS)
- Prediction of HIV-associated co-morbidity
- Prediction and evaluation of drug-associated toxicity

Chronic Inflammation in Patients on ART

- Higher levels of soluble inflammatory and endothelial dysfunction makers (plasminogen activator inhibitor type 1, soluble tumor necrosis factor (TNF) receptor type 1 and intercellular and vascular adhesion molecules)
- Two-fold increase in Framingham scores for cardiovascular disease (coronary heart diseases and stroke)

IRIS and HIV

- Restoration of immune system resulting in an exuberant host response to pathogens and/or antigens
- Frequently observed with Mycobacterium tuberculosis and Mycobacterium avium co-infections
- Significant challenge to initiation and continuation of ART and treatment of infections
Prediction and Evaluation of IRIS in HIV

- Higher levels of the interleukin (IL)-6 and soluble IL-6 in patients with IRIS

- Higher levels of the C-reactive protein (CRP), D-dimer, IL-6, IL-8, TNF-α, and interferon-γ associated with IRIS, AIDS and death

Prediction and Evaluation of the Progression of HIV Disease

- Significant decline in D-dimer (not IL-6 and high sensitivity CPR) in patients with immediate vs. delayed ART initiation

- Resumption of HIV replication following ART interruption is associated with increase in plasma cytokines and chemokines (TNF-α, IL-10, and CXCL10)

IRIS and HIV

- Restoration of immune system resulting in an exuberant host response to pathogens and/or antigens

- Frequently observed with Mycobacterium tuberculosis and Mycobacterium avium co-infections

- Significant challenge to initiation and continuation of ART and treatment of infections
Biomarkers of the Nevirapine Toxicity

- Phase II activation of the NVP metabolite 12-hydroxy-NVP mediates NVP binding to bionucleophiles NVP toxicity
- In vitro model using the synthetic electrophile 12-mesyloxy-NVP as a surrogate of the 12-sulfoxy-NVP
- LC-ESI-MS/MD and MALDI-TOF-TOF-MS
- Identification of cysteine, lysine, tryptophan, histidine, serine and N-terminal valine of Hb

Inflammatory and Thrombotic Markers in IL-2 Therapy

- IL-2 cycling in patients on ART has been shown to increase the long-term CD4 cell counts
- The clinical benefit is unclear
- The use of IL-2 cycling increased high-sensitivity C-reactive protein and D-dimer regardless of HIV RNA suppression
- Possible increased risk of thrombotic events

Conclusions

- Limited data from small prospective studies support role of TDM in improving virologic response and/or decreasing the ARV drugs concentration-related drug toxicities
- Studies of cytokines and chemokines support the use of biomarkers in predicting and evaluating response to ART and ART associated inflammation and/or toxicity
Conclusions

- Large prospective studies on TDM of ARV drugs are necessary to further investigate the application in therapy of HIV
- Earlier initiation of ART and growing evidence supporting universal ART in HIV-positive patient prompt further development of biomarkers evaluating the suppression of HIV-associated inflammation and ARV drugs response and/or toxicities