Anti-Microbial Photodynamic Therapy (aPDT)

A New Treatment Option for Infectious Diseases

Robert Weber, MSc / Martin Junggebauer, MSc (Germany)
Structure:

1. Background
2. Basic mechanisms of aPDT
3. Photosensitizers for aPDT
4. In vitro research
5. Clinical application and latest studies
   1. Malaria
   2. Hepatitis B/C
   3. Lyme Disease
6. Outlook and Conclusion
Background:

- Development Economics/Global Health + Medical Laser Therapy
  → Special interest in research related to infectious diseases

- Idea: Can Laser Therapy work against diseases such as Malaria, Hepatitis or Lyme?
  → Several well-proven in-vitro studies available but no clinical data due to technical limitations

- Foundation of ISLA Research Group in 2013 in Germany

- Key area of research: **Antimicrobial Photodynamic Therapy**
Background / Aims:

- Basic research (in-vitro) to find best suitable and most (cost-) effective photosensitizers for different diseases
- Clinical research on infectious diseases (Malaria, HIV, Hepatitis, Tuberculosis, Lyme Disease)
- Improvement of treatment designs and extension of clinical applications of aPDT
- Establishment of a global research network
Scientific Partnerships:
Background / History of aPDT:

- Discovered at beginning of 20th century
- Neglected due to the discovery of antibiotics and lacking technology for clinical application
- Today: Continuous onset of multi-drug-resistant pathogens
- 2005: Approval of first laser machine for systemic (i.v.) light application
- aPDT as a new treatment option for infectious diseases with many favourable features:
  - Efficiency against multi-drug-resistant pathogens
  - No new resistances emerge
  - Little side-effects
  - Cost-efficiency
Advantages of PDT

- **Practical**
  - Safe for human tissue
  - Inexpensive, Instant results
  - No patient compliance
  - Versatile
  - Systemic antibiotics cannot get into dead or damaged tissue
  - Even if antibiotics work they take several days

- **Effective**
  - Broad therapeutic window
  - Eradicates pathogens in biofilms
  - Eliminates development of resistance
  - Destroys secreted virulence factors
aPDT: Mechanisms of Action

- Photosensitizer binds to microbes
- “Light Activation“: **Photosensitizer absorbs photons**
- Excitation of Photosensitizer to highly reactive states
- Reaction with ambient oxygen:
  - Type I Photochemical Pathway: Generation of reactive oxygen species
  - Type II Photochemical Pathway: Generation of singlet oxygen
- Both species induce **irreparable oxidative damages** to microbes as they interact with numerous enzymes
  → leading e.g. to the inhibition of protein synthesis and molecular alteration of DNA strands
- **Microbial death**
Desirable properties of anti-microbial photosensitizers:

• Selectivity for microbial cells over host mammalian cells (selective accumulation at target cells): Cationic charge

• Low toxicity

• Good quantum yields of ROS

• Action spectrum on a broad range of pathogens
aPDT: Overview Photosensitizers

List of photosensitizers for aPDT:

- Hypericin
- Curcumin
- Riboflavin
- Porphyrins
- Chlorins
- Methylene Blue
- Toluidine Blue
- Crystal Violet
- ALA
- Benzophenoxazine
- Haematoporphyrins
- Rose Bengal
aPDT: Overview Photosensitizers

Hypericin:
- Extract from St. John’s Wort
- Excitation peak at 589nm (yellow)
Curcumin:

- Derived from Curcuma Longa
- Excitation peak at 447nm (blue)
Riboflavin:

- Vitamin B2
- Activated by Blue Light with a peak at 447nm

\[
\text{Riboflavin} \\
\lambda_{\text{max}} = 450 \text{ nm}
\]
In vitro Research:

Mirasol Pathogen Reduction Technology (PRT):
Combination of Riboflavin and ultraviolet light for reduction of pathogen loads in blood products
In vitro Research: Safety

- There is a strong history (*in vivo*) and additional Terumo BCT Biotechnologies safety testing (*in vivo* and *in vitro*, summarized below) supporting the safety of riboflavin and its use in the Mirasol System

<table>
<thead>
<tr>
<th>Test</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute Toxicity</td>
<td>Negative</td>
</tr>
<tr>
<td>Subchronic Toxicity</td>
<td>Negative</td>
</tr>
<tr>
<td>Neoantigenicity</td>
<td>Negative</td>
</tr>
<tr>
<td>Ames</td>
<td>Negative</td>
</tr>
<tr>
<td>Chromosomal Aberration</td>
<td>Negative</td>
</tr>
<tr>
<td>Mouse Erthrocyte Micronucleus</td>
<td>Negative</td>
</tr>
<tr>
<td>Embryo-Fetal Development</td>
<td>Negative</td>
</tr>
<tr>
<td>Hemocompatibility</td>
<td>Passed</td>
</tr>
<tr>
<td>Leachables and Extractables</td>
<td>Passed</td>
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</table>
### In vitro Research:

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Model used</th>
<th>Log Reduction</th>
<th>Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV, active</td>
<td>Intracellular human HIV</td>
<td>5.9</td>
<td>Enveloped</td>
</tr>
<tr>
<td>HIV, latent</td>
<td>Cell-associated human HIV</td>
<td>4.5</td>
<td>Enveloped</td>
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<tr>
<td>Hepatitis C Virus</td>
<td>West Nile Virus</td>
<td>≥5.1</td>
<td>Enveloped</td>
</tr>
<tr>
<td>West Nile Virus</td>
<td>Sindbis Virus</td>
<td>3.2</td>
<td>Enveloped</td>
</tr>
<tr>
<td>Hepatitis B Virus</td>
<td>Human Hepatitis B</td>
<td>≤4.5*</td>
<td>Enveloped</td>
</tr>
<tr>
<td></td>
<td>Pseudorabies Virus</td>
<td>2.5</td>
<td>Enveloped</td>
</tr>
<tr>
<td>Rabies Virus</td>
<td>Vesicular Stomatitis Virus</td>
<td>≥6.3</td>
<td>Enveloped</td>
</tr>
<tr>
<td>Influenza Virus</td>
<td>Influenza A Virus</td>
<td>≥5.3</td>
<td>Enveloped</td>
</tr>
<tr>
<td>Avian Flu Virus</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cytomegalovirus</td>
<td>Human CMV</td>
<td>≥6.0**</td>
<td>Enveloped</td>
</tr>
<tr>
<td></td>
<td>Inf. Bov. Rhinotracheitis Virus</td>
<td>2.1</td>
<td>Enveloped</td>
</tr>
<tr>
<td>Human B-19 Virus</td>
<td>Porcine Parvovirus</td>
<td>≥5.0</td>
<td>Non-Enveloped</td>
</tr>
<tr>
<td>Hepatitis A Virus</td>
<td>Human Hepatitis A</td>
<td>1.6</td>
<td>Non-Enveloped</td>
</tr>
<tr>
<td></td>
<td>Encephalomyocarditis virus</td>
<td>3.2</td>
<td>Non-Enveloped</td>
</tr>
<tr>
<td>Chikungunya Virus</td>
<td>La Reunion Clinical Isolate</td>
<td>2.1 (Plasma)</td>
<td>Enveloped</td>
</tr>
<tr>
<td></td>
<td></td>
<td>≥4.0 (Media)</td>
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</table>
In vitro Research:

Parasite Study Data (Infectivity Studies)\textsuperscript{1-5}

<table>
<thead>
<tr>
<th>Parasite</th>
<th>Log Reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{Plasmodium falciparum}</td>
<td>\geq 3.2</td>
</tr>
<tr>
<td>\textit{Trypanosoma cruzi}</td>
<td>\geq 5.0</td>
</tr>
<tr>
<td>\textit{Leishmania major}</td>
<td>\geq 4.0</td>
</tr>
<tr>
<td>\textit{Babesia microti}</td>
<td>\geq 4.0 to \geq 5.0\textsuperscript{1}</td>
</tr>
<tr>
<td>\textit{Orientia tsutsugamushi}</td>
<td>\geq 5.0\textsuperscript{1}</td>
</tr>
</tbody>
</table>

The \geq symbol is used to indicate inactivation to the limits of detection. Levels of inactivation could be higher but the ability to quantify the full extent of pathogen reduction is limited by the assay sensitivity limits.

\textsuperscript{1} Tested in an animal infectivity model. No disease transmission observed with treated products

\textbf{All validated under standard use conditions as per IFU}

\textsuperscript{1} Cardo et al. 2006; \textsuperscript{2} Sullivan et al. 2008; \textsuperscript{3} Cardo et al. 2007; \textsuperscript{4} Tonnetti et al. 2010; \textsuperscript{5} Rentas et al. 2007
**In vitro Research:**

**Parasite Study Data**
**Whole Blood System**

<table>
<thead>
<tr>
<th>Parasite</th>
<th>Disease</th>
<th>Reduction Levels with Mirasol System</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Babesia microti</em></td>
<td>Babesiosis</td>
<td>≥ 5.0</td>
</tr>
<tr>
<td><em>Babesia divergens</em></td>
<td>Babesiosis</td>
<td>≥ 6.0</td>
</tr>
<tr>
<td><em>Trypanosoma cruzi</em></td>
<td>Chagas</td>
<td>≥ 3.5</td>
</tr>
</tbody>
</table>

*Studies conducted by Dr. David Leiby -ARC, Dr. Cheryl Lobo -NYBC*
In vitro Research:

<table>
<thead>
<tr>
<th>Item</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Products Tested</td>
<td>Whole Blood With ACH-2 Cells</td>
</tr>
<tr>
<td>Assay System Used</td>
<td>TCID\textsubscript{50}</td>
</tr>
<tr>
<td>Assay Reporter</td>
<td>MT-2 Cells</td>
</tr>
<tr>
<td>Average Initial Product Titer (log TCID\textsubscript{50}/mL)</td>
<td>6.9 ± 0.5</td>
</tr>
<tr>
<td>Average Treated Product Titer (log TCID\textsubscript{50}/mL)</td>
<td>2.4 ± 0.1</td>
</tr>
<tr>
<td>Average Log\textsubscript{10} Reduction of HIV\textsubscript{i}</td>
<td>4.5 ± 0.5</td>
</tr>
</tbody>
</table>
In vitro Research:


In Germany, Hypericin was even tested by the famous Robert-Koch-Institut: The researchers found an anti-HIV effect in-vitro, **BUT only in combination with light.**

**Following effects on HIV have been observed:**

- PDT inhibits HIV attachment and entry to human cells  
  → early blockage of HIV replication
- PDT can inactivate free viral particles
- selective destruction of infected white cells
In vitro Research:

Two pathogen reduction technologies - methylene blue plus light and shortwave ultraviolet light - effectively inactivate hepatitis C virus in blood products

Eike Steinmann, Ute Gravemann, Martina Friesland, Juliane Doerrbecker, Thomas H. Müller, Thomas Pietschmann and Axel Seltsam*

RESULTS: HCV was sensitive to inactivation by both pathogen reduction procedures. HCV in plasma was efficiently inactivated by MB plus light below the detection limit already by 1/12 of the full light dose. HCV in PCs was inactivated by UVC irradiation with a reduction factor of more than 5 log.

CONCLUSIONS: Pathogen reduction technologies such as MB plus light treatment and UVC irradiation have the potential to significantly reduce transfusion-transmitted HCV infections.
In vitro Research:

Sensitization of Mycobacteria strains with Chlorin e6

- high and rapid accumulation of Chlorin e6
In vitro Research:

survival fraction after PDI with chlorin e6 *in vitro*

Reduction of about 97%
Multi-drug-resistant MRSA:

Maisch et al. examined penetration and antibacterial efficacy of XF73 (a cationic porphyrin PS) against MRSA using an ex vivo model. Photoinactivation of pre-incubated *S. aureus* demonstrated >3 log₁₀ reduction, while illumination after XF73 was delivered to the bacteria on the skin resulted in a approximately 1 log₁₀ growth reduction independently of the antibiotic resistance pattern of used *S. aureus* strains.
Anti-microbial Photodynamic Therapy: A new treatment option for Malaria?

Michael Weber, MD
Robert Weber, MSc
Martin Junggebauer, MSc
Habeeb Ali, MD

Ondo, Nigeria
Abstract: Anti-microbial Photodynamic Therapy: A new treatment option for Malaria?

Michael Weber, MD / Robert Weber, MSc / Martin Junggebauer, MSc / Habeeb Ali, MD

Objectives: Evaluation of a treatment protocol consisting of anti-microbial photodynamic therapy (aPDT) and additional intravenous low-level-laser-therapy as a new treatment option for Malaria.

Methods: 20 patients suffering from Plasmodium Falciparum were separated in one treatment group and one control group, both consisting of 10 patients. Patients in the treatment group received aPDT as well as intravenous low-level laser therapy. Patients in the control group received conventional therapy only.

Results: After 9 days, 88.9% of treatment group patients were completely parasite-free whereas the same holds true for only 50% of control group patients. Almost all symptoms could be alleviated more rapidly in the treatment group.

Conclusion: The results indicate that the applied protocol can be an effective treatment option to treat Malaria caused by Plasmodium Falciparum. They strongly encourage further studies with bigger sample sizes.
aPDT: A new treatment option for Malaria?

**Protocol Treatment Group:**

- 5 treatment sessions in 9 days
- Intravenous administration of Riboflavin: Infusion over 30 minutes
- 30 minutes after infusion: Intravenous Blue Laser Application (447nm, 100mW, 75%, 45 min) to photoactivate the Riboflavin
- Subsequently, Green (532nm, 50mW, 50%), Yellow (589nm, 50mW, 50%) and Red Laser Light (635nm, 100mW, 35%) was applied through the same catheter system for 10 minutes each
- Parasite counts were conducted 5 times, each on the day after a treatment session
- Patients also provided information on various symptoms on those days
# Malaria parasite screening

<table>
<thead>
<tr>
<th>Time of test</th>
<th>Test result</th>
<th>Control</th>
<th>Study</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>10(100.0)</td>
<td>10(100.0)</td>
</tr>
<tr>
<td><strong>Baseline</strong></td>
<td>Positive (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Negative (%)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>After T2</strong></td>
<td>Positive (%)</td>
<td>-</td>
<td>10(100.0)</td>
</tr>
<tr>
<td></td>
<td>Negative (%)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>After T3</strong></td>
<td>Positive (%)</td>
<td>-</td>
<td>8(80.0)</td>
</tr>
<tr>
<td></td>
<td>Negative (%)</td>
<td>-</td>
<td>2(20.0)</td>
</tr>
<tr>
<td><strong>After T4</strong></td>
<td>Positive (%)</td>
<td>-</td>
<td>6(60.0)</td>
</tr>
<tr>
<td></td>
<td>Negative (%)</td>
<td>-</td>
<td>4(40.0)</td>
</tr>
<tr>
<td><strong>Final/after T5</strong></td>
<td>Positive (%)</td>
<td>5(50.0)</td>
<td>1(11.1)</td>
</tr>
<tr>
<td></td>
<td>Negative (%)</td>
<td>5(50.0)</td>
<td>8(88.9)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>Total (%)</td>
<td>10(100.0)</td>
<td>9(100.0)</td>
</tr>
</tbody>
</table>
## Malaria symptoms presented

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>Time of observation</th>
<th>Control group (%)</th>
<th>Study group (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Shaking chills</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
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<td>6(60.0)</td>
<td>7(70.0)</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>4(40.0)</td>
<td>3(30.0)</td>
</tr>
<tr>
<td>After T2</td>
<td>-</td>
<td>-</td>
<td>2(20.0)</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>No</td>
<td>8(80.0)</td>
</tr>
<tr>
<td>After T3</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>-</td>
<td>10(100.0)</td>
</tr>
<tr>
<td>After T4</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>-</td>
<td>10(100.0)</td>
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<tr>
<td>Final</td>
<td>Yes</td>
<td>0(0.0)</td>
<td>9(100.0)</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>10(100.0)</td>
<td>-</td>
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<tr>
<td><strong>Fever</strong></td>
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<tr>
<td>Baseline</td>
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<td>10(100.0)</td>
<td>10(100.0)</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>0(0.0)</td>
<td>-</td>
</tr>
<tr>
<td>After T2</td>
<td>-</td>
<td>-</td>
<td>6(60.0)</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>No</td>
<td>4(40.0)</td>
</tr>
<tr>
<td>After T3</td>
<td>-</td>
<td>-</td>
<td>2(20.0)</td>
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<td>8(80.0)</td>
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<tr>
<td>After T4</td>
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<td>1(10.0)</td>
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<td></td>
<td>-</td>
<td>-</td>
<td>9(90.0)</td>
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<tr>
<td>Final</td>
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<td>4(40.0)</td>
<td>9(100.0)</td>
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<tr>
<td><strong>Fever severity</strong></td>
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<tr>
<td>Baseline</td>
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<td>Mild</td>
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<tr>
<td></td>
<td>Moderate</td>
<td>7(70.0)</td>
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<td>Severe</td>
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<td>After T2</td>
<td>Mild</td>
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<td>Severe</td>
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<tr>
<td>After T3</td>
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<td>Moderate</td>
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<tr>
<td></td>
<td>Severe</td>
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<td>-</td>
</tr>
<tr>
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<tr>
<td><strong>Profuse sweating</strong></td>
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<td>Baseline</td>
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<td>10(100.0)</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>9(10.0)</td>
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</tr>
<tr>
<td>Final</td>
<td>No</td>
<td>10(100.0)</td>
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</tbody>
</table>
## Malaria symptoms presented

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>Time of observation</th>
<th>Control group (%)</th>
<th>Study group (%)</th>
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<tbody>
<tr>
<td><strong>Headache</strong></td>
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</tr>
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<td>Yes</td>
<td>10(100.0)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>After T2</td>
<td>Yes</td>
<td>10(100.0)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>After T3</td>
<td>Yes</td>
<td>8(80.0)</td>
</tr>
<tr>
<td></td>
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<td>No</td>
<td>2(20.0)</td>
</tr>
<tr>
<td></td>
<td>After T4</td>
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<td></td>
<td>Final</td>
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<td>5(50.0)</td>
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<td>No</td>
<td>9(100.0)</td>
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<tr>
<td><strong>Headache severity</strong></td>
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<tr>
<td></td>
<td>Baseline Mild</td>
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<tr>
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<td>Moderate</td>
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<tr>
<td></td>
<td>Severe</td>
<td>3(30.0)</td>
<td>Severe</td>
</tr>
<tr>
<td></td>
<td>After T2 Mild</td>
<td>4(40.0)</td>
<td>-</td>
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<tr>
<td></td>
<td>Moderate</td>
<td>5(50.0)</td>
<td>-</td>
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<tr>
<td></td>
<td>Severe</td>
<td>1(10.0)</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>After T3 Mild</td>
<td>8(100.0)</td>
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<td>Moderate</td>
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<td></td>
<td>Severe</td>
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<td>-</td>
</tr>
<tr>
<td></td>
<td>After T4 Mild</td>
<td>3(100.0)</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Moderate</td>
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<td>-</td>
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### Malaria symptoms presented

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Malaria symptoms presented

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<td>No 7(70.0)</td>
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Latest Studies: Hepatitis B/C

- Infectious diseases caused by the hepatitis B virus (HBV) / hepatitis C virus (HCV) affecting the liver
- For HCV, the virus persists in the liver in about 75% to 85% of those initially infected
- Chronic infections can lead to cirrhosis and liver cancer
- Over 750,000 people die of hepatitis B each year (300,000 due to liver cancer)
- About 167,000 deaths due to liver cancer and 326,000 deaths due to cirrhosis occurred in 2015 due to hepatitis C
Latest Studies: Hepatitis B/C

First pilot data (Weber Medical Clinic, Germany):

- 5 patients with HCV treated with Riboflavin and 447nm blue laser
- Results: Noticeable decreases of viral loads in all treated patients
- The effects became significant after 3-5 treatments

- Even patients that had been treated with conventional methods for many years without noticeable effects reacted very positive to aPDT. After five treatments the viral load decreased by 70% in average
Latest Studies: Hepatitis B/C

Dr. Laura Ailioaie, Iasi/Romania:

- 10 patients (5 HCV, 5 HBV) treated with oral Curcumin (2 capsules of highly bioavailable Ultracur+) followed by 30 min. i.v. 447nm blue laser (30 min after intake)

- Result: Average decrease of viral load from 43999 UI/ml to 1394 UI/ml

- Concusion: ILBI with the new 447 nm blue laser, synergistically combined with UltraBioavailable Curcumin has increased anti-microbial effects and the ability to modulate the immune system, with beneficial effects in infectious and age-related diseases.
Latest Studies: Lyme Disease

- Infectious disease caused by *Borrelia* bacteria which is spread by ticks
- Early symptoms (stadium 1) may include fever, headache and tiredness
- If untreated, chronic disease may develop with symptoms including chronic fatigue, facial paresis, joint pains, depression, severe headaches with neck stiffness, myocardial problems and co-infections due to weakened immune system (stadium 2 and 3)
- It is estimated to affect 300,000 people a year in the United States and 65,000 people a year in Europe
What do borrelia bacteria do?

- They screw into collagen fibers in connecting tissue, leading to inflammation and acidity.
- Structure of connecting tissue is affected and immune cells are disabled.
- Vascular processes lead to circulatory disorders, nutrient deficiency in the affected tissue and loss of functions.
- Main problem: Resistance against antibiotics (especially intra-cellular bacteria* and cell wall-deficient (CWD) borrelia).
- CWD borrelia: Antibiotics (i.e. Penecellin) lead to changes of form, properties and markers of the bacteria.
- Development of chronic lyme disease as antibiotics and immune system cannot fight intra-cellular and CWD borrelia (bacteria can survive for several years).
- Often connected to co-infections due to weakened immune system.

*Borrelia can hide in neuronal cells, fibroblasts, lymphocytes, macrophages etc.
Study Dr. I. Zuern, Germany (2016):

- 3 groups with 10 chronic lyme patients each

- Patients in all 3 groups received the following therapies:
  - Physical vascular therapy (Bemer)
  - Immunotherapy
  - Vitamins and additional natural supplements
  - Neural Therapy
  - Procain bases infusions
  - Oxygen therapy

Group B:
Additional Yellow Laser 589nm + Hypericin (10-15 treatments, 2-3 times per week)

Group C:
Additional 447 nm Blue Laser + Riboflavin (10-15 treatments, 2-3 times per week)
Diagnostics: Lymphocyte Transformation Test (LTT)

- Detection of the activity of chronic, persistent infections based on pathogen-specific T cell response (Borrelia, Chlamydia, Yersinia, Giardia lamblia, Herpes viruses etc.)

- The in-vitro test is based on the principle of antigen/allergen-specific induction of cell division in lymphocytes following contact with their «fitting» antigen

- A positive reaction in the LTT indicates the presence of antigen-specific lymphocytes (memory cells) in the patient’s blood (→ immune response to different borrelia antigens)

- Activity of infection can be measured
1. Gewinnung der Lymphozyten und Monocyten aus Patientenblut durch Dichtegradientenzentrifugation

2. Nach Aufnahme im Zellkulturmedium, Überführung der Zellen in eine sterile Zellkulturplatte


4. Erhöhung der Testspezifität durch Zugabe von Interferon-α, Glutamin und autologem Serum

5. Am fünften Tag Zugabe von [3H]-Thymidin (T) zu den Zellen für 12h, welches sich in den Tochterstrang aktivierter Zellen einbaut

6. Quantifizierung der antigen-induzierten DNA-Neusynthese durch Bestimmung des [3H]-Thymidin ([H]) Einbaus


8. Darstellung des Ergebnisses als Stimulationsindex

5 Tage Kultur
37°C / 5% CO₂

Am fünften Tag: Klonale proliferierte spezifische T-Lymphozyten, z. B. Gekoppelte T-Zellen

Ruhende Lymphozyten, keine Sensibilisierung
Stimulation Index  > 3,0
Treatment necessary
Results after successful treatment
Tested antigens:
- Borr. sensu stricto
- Borr. afzelii
- Borr. garinii
- Borr. OspC

Measurement of antigen-specific t-cells in patient’s blood:
- Stimulation index (SI) >3,0 positive
- Stimulation index (SI) 2,0-3.0 borderline
- Stimulation index (SI) <2,0 negative

Study Dr. I. Zuern, Germany (2016):
Study Dr. I. Zuern, Germany (2016):

 Results:

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<th>SI 3-6 months later</th>
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Study Dr. I. Zuern, Germany (2016):

Results:

Before Therapy vs. 3-6 months later
Outlook and Conclusion:

- Very promising results from first in-vivo studies on aPDT
- Seems to work against bacterial, parasitic and viral infections
- Larger patient populations necessary
- Development of ultraviolet diode should bring additional benefit
- Promising future due to increasing drug resistances
Application and Protocols:
References:

References:

• Zuern, I. (2016): Pilot Study on Treatment of Chronic Lyme Disease with Yellow and Blue Laser

• Goodrich, Raymond et al, 2011: Pathogen Reduction Technology Treatment of Platelets,

• Plasma and Whole Blood Using Riboflavin and UV Light. Published in: Transfusion medicine and Hemotherapy: 2011;38:8–18


Thank you!

Questions:

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Martin Junggebauer: junggebauer-research@isla-laser.org