Anti-Microbial Photodynamic Therapy (aPDT)

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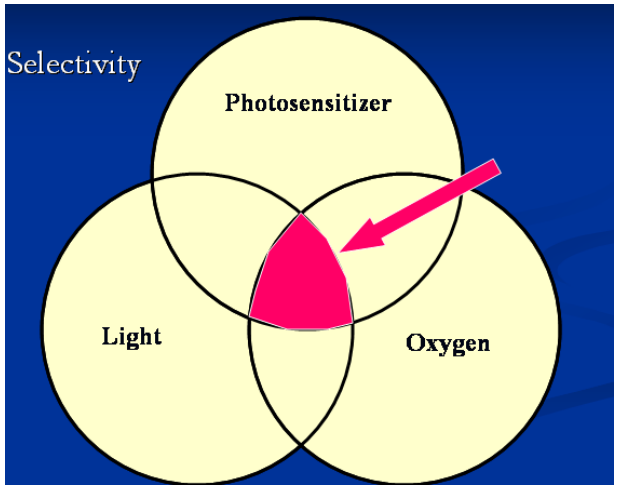

A New Treatment Option for Infectious Diseases

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

Structure:

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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:

GLOBAL HEALTH
IS OUR HEALTH

- 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:

GEORG-AUGUST-UNIVERSITÄT GÖTTINGEN

Philipps Universität Marburg

NAALT
North American Association for PhotobiomoduLation Therapy

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ONDO STATE, NIGERIA

FACULTY OF MEDICINE 1972
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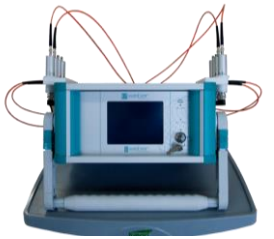
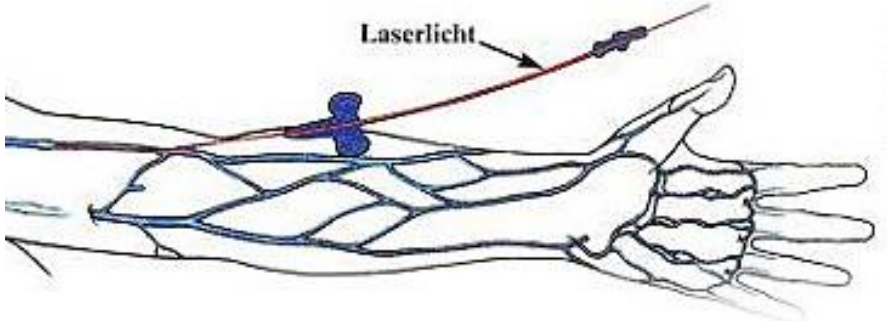
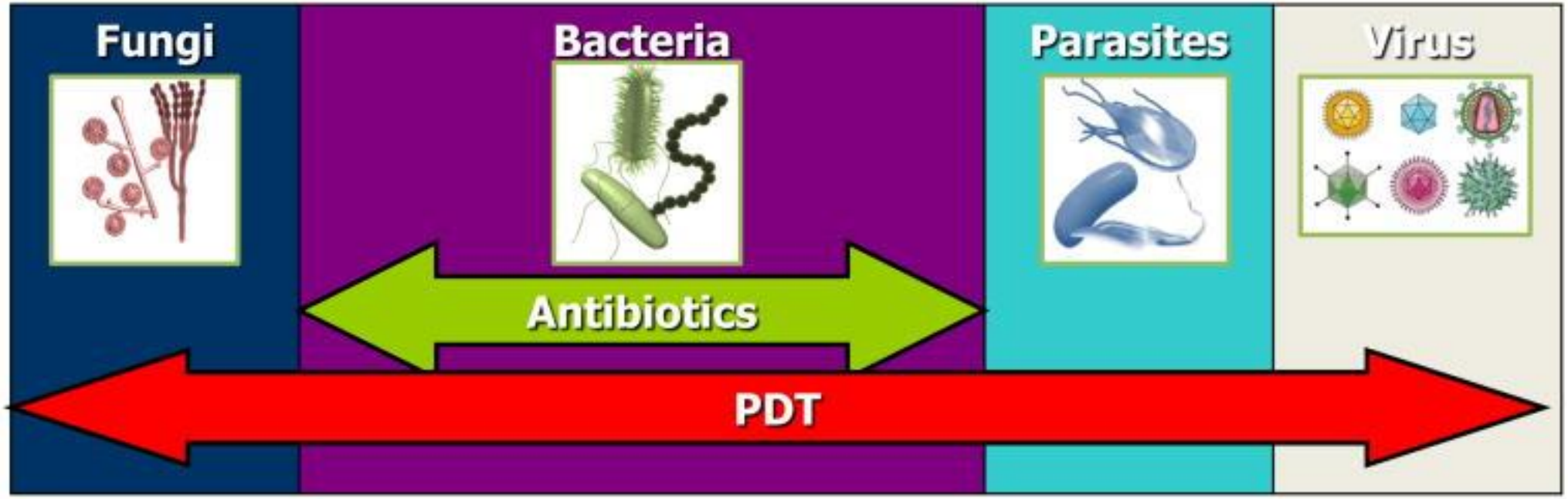
Background / History of aPDT:



- 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



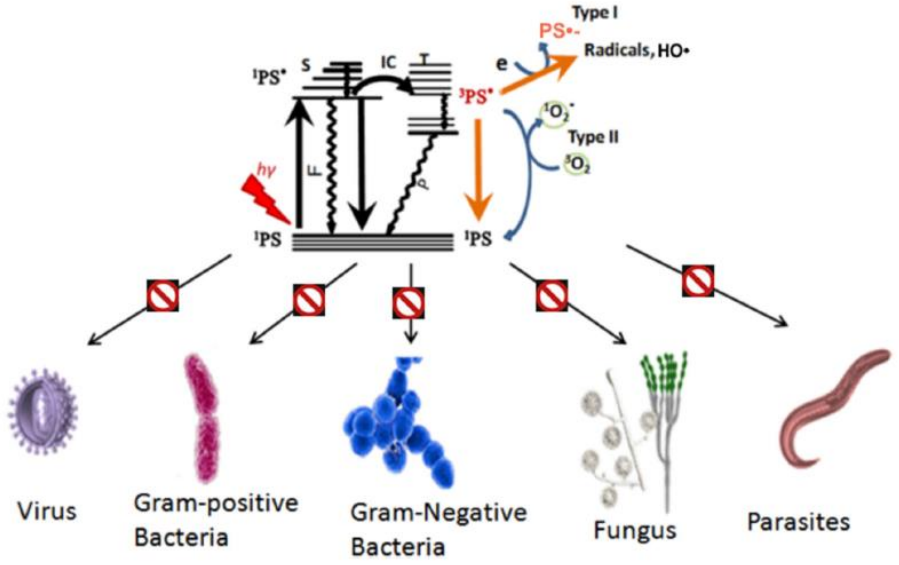
Advantages of PDT



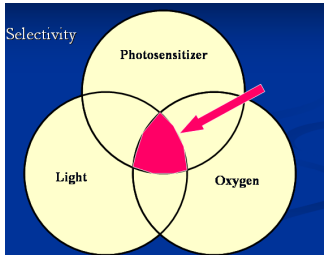
- Practical
  - Safe for human tissue
  - Inexpensive, Instant results
  - No patient compliance
  - Versatile
  - Systemic antibiotics cannot get into dead or damaged tissue
  - Even if antibiotics work they take several days
- Effective
  - Broad therapeutic window
  - Eradicates pathogens in biofilms
  - 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**



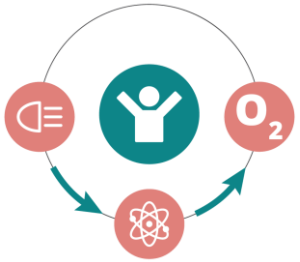
aPDT: Overview Photosensitizers



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



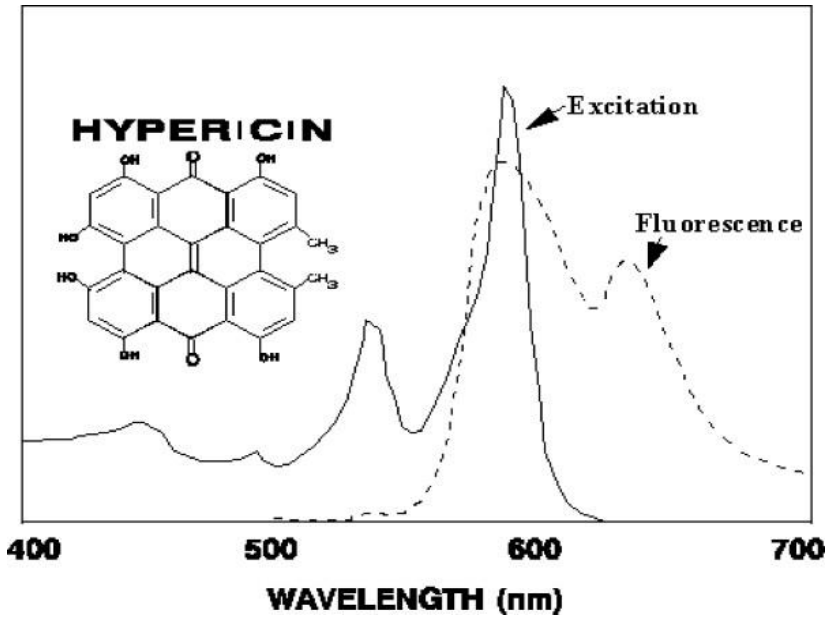
List of photosensitizers for aPDT:

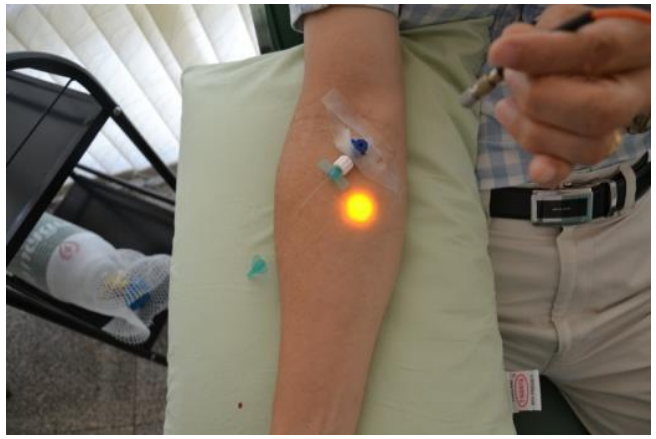
- Hypericin
- Curcumin
- Riboflavin
- Porphyrins
- Chlorins
- Methylene Blue
- Toluidine Blue
- Crystal Violet
- ALA
- Benzophenoxazine
- Haematoporphyrins
- Rose Bengal

aPDT: Overview Photosensitizers

Hypericin:

- Extract from St. John´s Wort
- Excitation peak at 589nm (yellow)

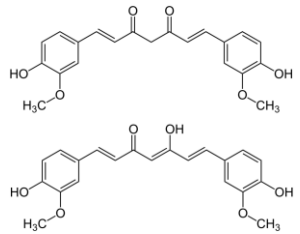


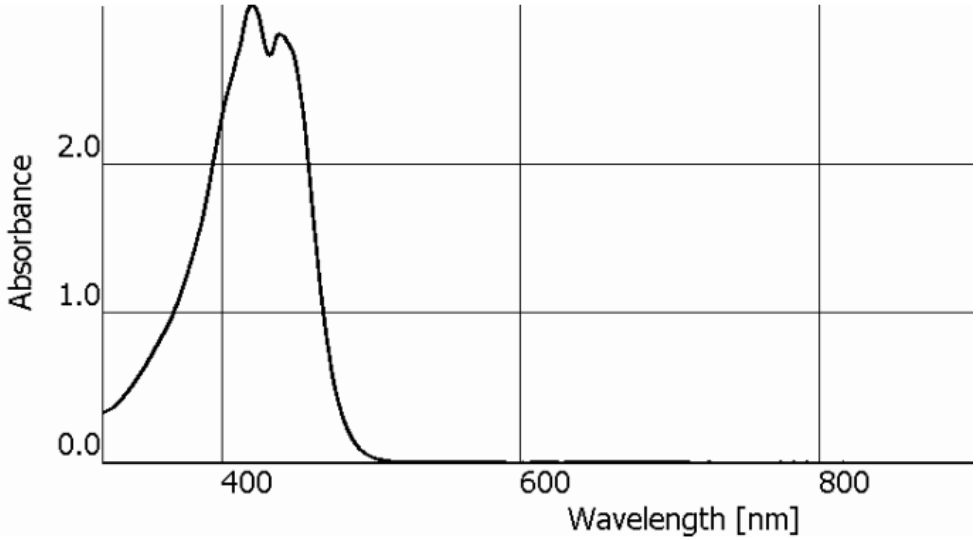


aPDT: Overview Photosensitizers

Curcumin:

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

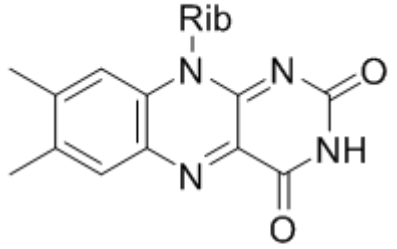




aPDT: Overview Photosensitizers

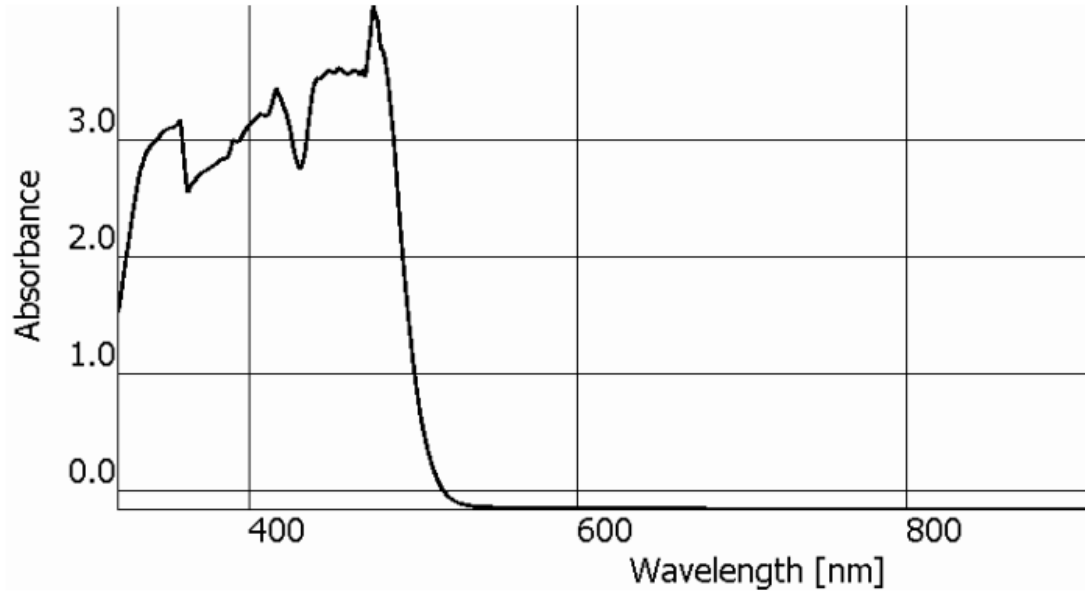
Riboflavin:

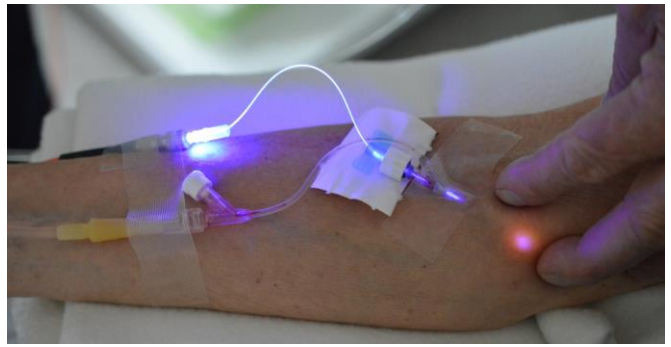
- Vitamin B2
- Activated by Blue Light with a peak at 447nm



Riboflavin

λ_{max} = 450 nm

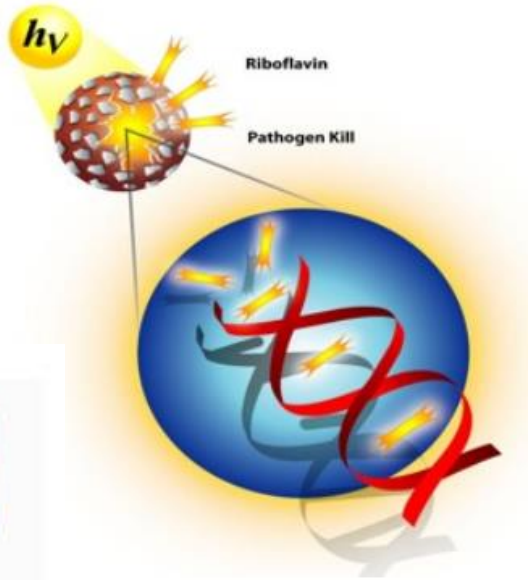


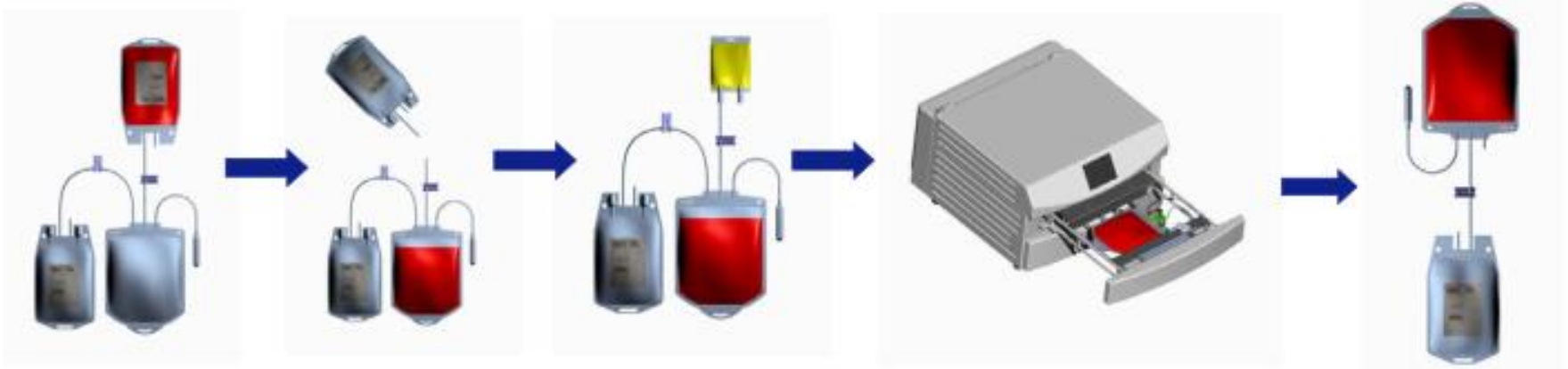


In vitro Research:

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
  - Reddy et al. Transfus Med Rev. 2008 Apr;22(2):133-53.

Test	Result
Acute Toxicity	Negative
Subchronic Toxicity	Negative
Neoantigenicity	Negative
Ames	Negative
Chromosomal Aberration	Negative
Mouse Erthrocyte Micronucleus	Negative
Embryo-Fetal Development	Negative
Hemocompatibility	Passed
Leachables and Extractables	Passed

In vitro Research:

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

In vitro Research:

Parasite Study Data (Infectivity Studies)[1-5]

Parasite	Log Reduction
Plasmodium falciparum	≥ 3.2
Trypanosoma cruzi	≥ 5.0
Leishmania major	≥ 4.0
Babesia microti	≥4.0 to ≥5.0[1]
Orientia tsutsugamushi	≥5.0[1]

The ≥ 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.

[1] Tested in an animal infectivity model. No disease transmission observed with treated products

All validated under standard use conditions as per IFU

[1] Cardo et al. 2006; [2] Sullivan et al. 2008; [3] Cardo et al. 2007; [4] Tonnetti et al. 2010; [5] Rentas et al. 2007

In vitro Research:

Parasite Study Data
Whole Blood System

Parasite	Disease	Reduction Levels with Mirasol System
Babesia microti	Babesiosis	≥ 5.0
Babesia divergens	Babesiosis	≥ 6.0
Trypanosoma cruzi	Chagas	≥ 3.5

Studies conducted by Dr. David Leiby -ARC, Dr. Cheryl Lobo -NYBC

In vitro Research:

Intracellular HIV	
Item	**Value**
Products Tested	Whole Blood With ACH-2 Cells
Assay System Used	$TCID_{50}$
Assay Reporter	MT-2 Cells
Average Initial Product Titer (log $TCID_{50}$/mL)	6.9 ± 0.5
Average Treated Product Titer (log $TCID_{50}$/mL)	2.4 ± 0.1
Average Log $_{10}$ Reduction of HIV_i	4.5 ± 0.5

In vitro Research:

In HIV research, several studies show a positive effect of PDT on HIV (Degar 1992, Hudson 1993, Lenard 1993, North 1993, Lavie 1995, Ben Hur 1997, Li 2011 etc.).

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

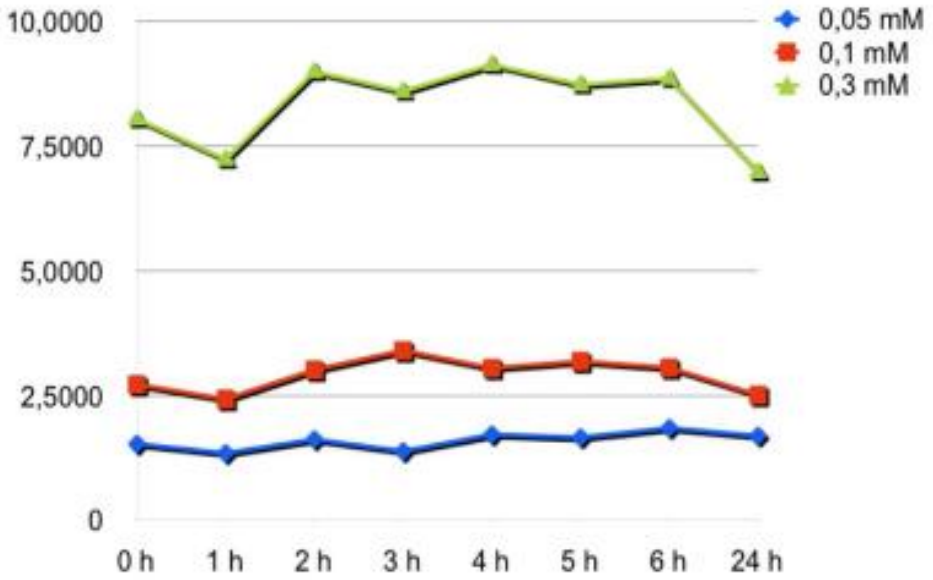
Eike Steinmann, Ute Gravemann, Martina Friesland, Juliane Doerrbecker, Thomas H. Müller, Thomas Pietschmann and Axel Seltsam*

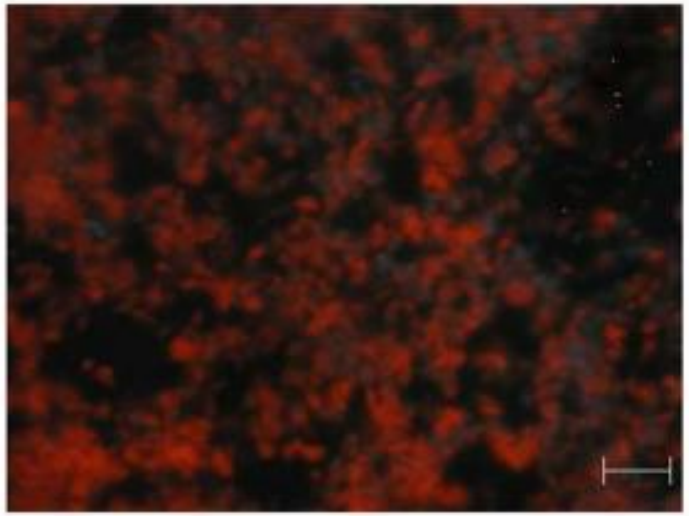
RESULTS: HCV was sensitive to inactivation by both pathogen reduction procedures. HCV in plasma was **efficiently inactivated by MB plus light below the detection limit already by 1/12 of the full light dose**. HCV in PCs was inactivated by UVC irradiation with a **reduction factor of more than 5 log**.

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



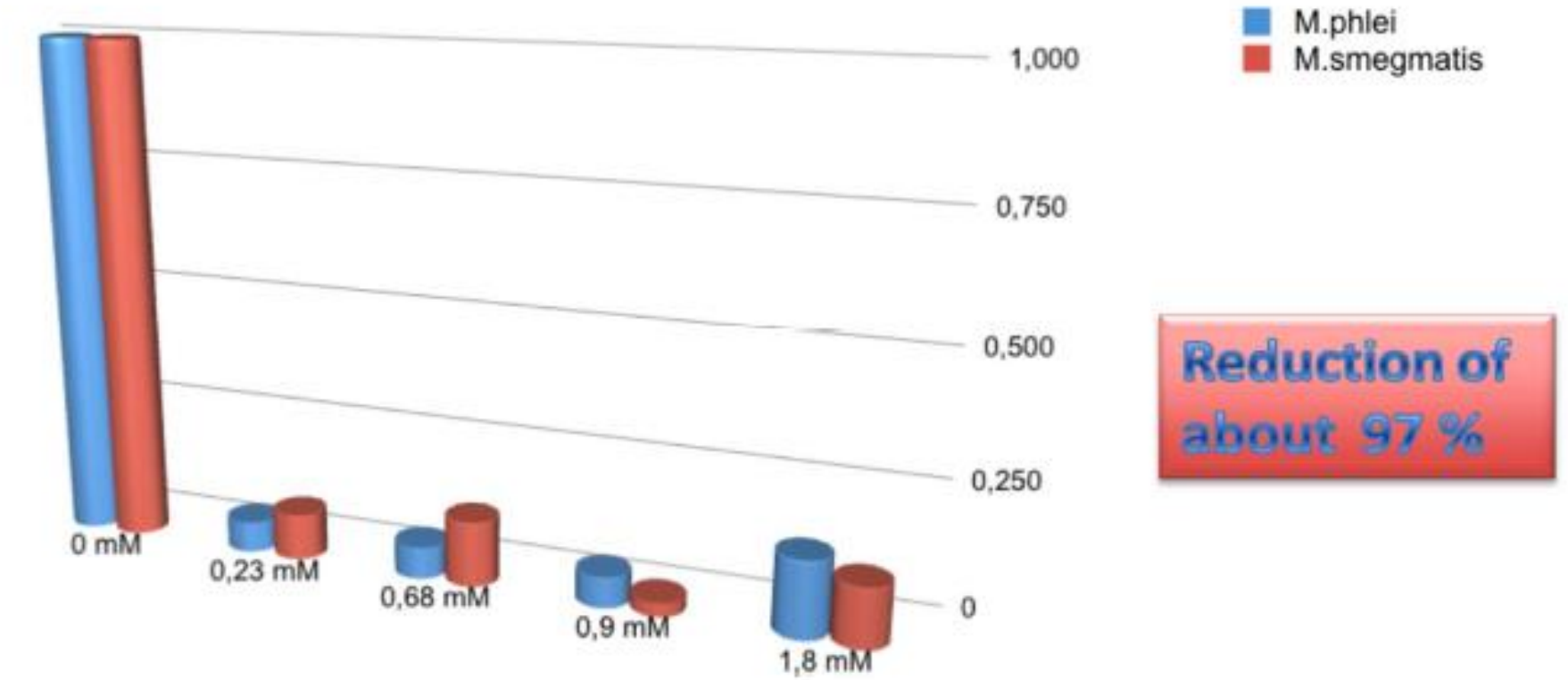


Fluorescence of Mycobacterium after Chlorine e6 application

- high and rapid accumulation of Chlorin e6

In vitro Research:

survival fraction after PDI with chlorin e6 *in vitro*



In vitro Research:

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

ISLA RESEARCH GROUP
International Society for Medical Laser Applications

UNIVERSITY OF MEDICAL SCIENCES
ONDO STATE, NIGERIA

aPDT: A new treatment option for Malaria?

Abstract: Anti- microbial Photodynamic Therapy: A new treatment option for Malaria?
Michael Weber, MD / Robert Weber, MSc / Martin Junggebauer, MSc / Habeeb Ali, MD

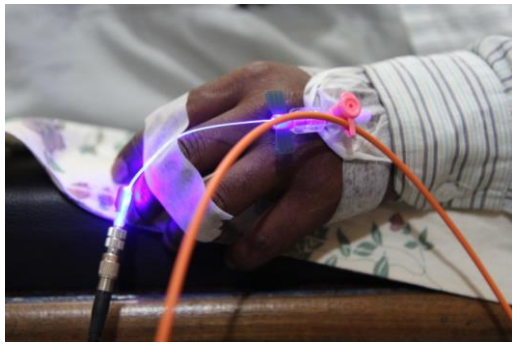
Objectives: Evaluation of a treatment protocol consisting of anti- microbial photodynamic therapy (aPDT) and additional intravenous low-level-laser-therapy as a new treatment option for Malaria.

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

Time of test	Test result	Control	Study
Baseline	Positive (%)	10(100.0)	10(100.0)
	Negative (%)	-	-
After T2	Positive (%)	-	10(100.0)
	Negative (%)	-	-
After T3	Positive (%)	-	8(80.0)
	Negative (%)	-	2(20.0)
After T4	Positive (%)	-	6(60.0)
	Negative (%)	-	4(40.0)
Final/after T5	Positive (%)	5(50.0)	1(11.1)
	Negative (%)	5(50.0)	8(88.9)
	Total (%)	10(100.0)	9(100.0)

Malaria symptoms presented

Symptoms	Time of observation	Control group (%)		Study group (%)	
Shaking chills	Baseline	Yes	6(60.0)	Yes	7(70.0)
		No	4(40.0)	No	3(30)
	After T2	-	-	Yes	2(20.0)
				No	8(80.0)
	After T3	-	-	Yes	-
		-	-	No	10(100.0)
	After T4	-	-	Yes	-
		-	-	No	10(100.0)
	Final	Yes	0(0.0)	No	9(100.0)
		No	10(100.0)	-	-
Fever	Baseline	Yes	10(10.0)	Yes	10(100.0)
		No	0(0.0)	-	-
	After T2	-	-	Yes	6(60.0)
		-	-	No	4(40.0)
	After T3	-	-	Yes	2(20.0)
		-	-	No	8(80.0)
	After T4	-	-	Yes	1(10.0)
		-	-	No	9(90.0)
	Final	Yes	4(40.0)	No	9(100.0)
		No	6(60.0)		
Fever severity	Baseline	Mild	2(40.0)	Mild	-
		Moderate	7(70.0)	Moderate	8(80.0)
		Severe	1(10.0)	Severe	2(20.0)
	After T2			Mild	3(30.0)
				Moderate	2(20.0)
				Severe	1(10.0)
	After T3			Mild	-
				Moderate	-
				Severe	-
	Final	Mild	4(100.0)		
Profuse sweating	Baseline	Yes	1(10.0)	No	10(100.0)
		No	9(10.0)		
	Final	No	10(100.0)		

Malaria symptoms presented

Symptoms	Time of observation	Control group (%)		Study group (%)	
Headache	Baseline	Yes	10(100.0	Yes	10(100.0)
	After T2			Yes	10(100.0)
				No	-
	After T3			Yes	8(80.0)
				No	2(20.0)
	After T4			Yes	2(80.0)
				No	2(20.0)
	Final	Yes	5(50.0)		-
		No	5(50.0)	No	9(100.0)
Headache severity	Baseline	Mild	2(20.0)	Mild	-
		Moderate	5(50.0)	Moderate	6(60.0)
		Severe	3(30.0)	Severe	4(40.0)
	After T2			Mild	4(40.0)
				Moderate	5(50.0)
				Severe	1(10.0)
	After T3			Mild	8(100.0)
				Moderate	-
				Severe	-
	After T4			Mild	3(100.0)
				Moderate	-
				Severe	-
	Final	Mild	5(83.3)		-
		Moderate	1(16.7)		-
Nausea	Baseline	Yes	2(20.0)	Yes	5(50.0)
		No	8(80.0)	No	5(50.0)
	After T2		-	Yes	1(10.0)
			-	No	9(90.0)
	After T3		-	Yes	1(10.0)
			-	No	9(90.0)
	After T4		-	Yes	1(10.0)
			-	No	9(90.0)
	Final	No	10(100.0)	No	9(100.0)

Malaria symptoms presented

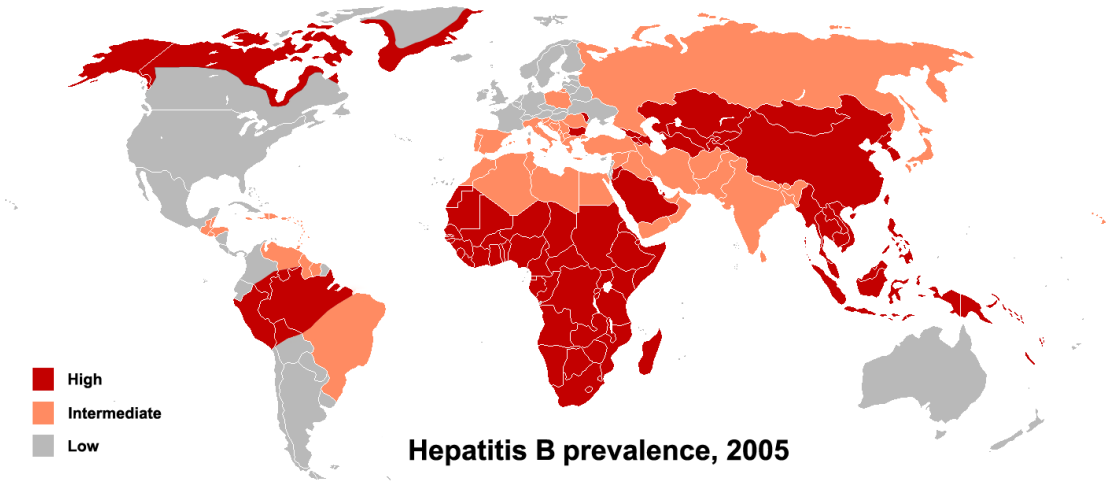
Symptoms	Time of observation	Control group (%)		Study group (%)	
Vomiting	Baseline	Yes	2(20.0)	Yes	1(10.0)
		No	8(80.0)	No	9(90.0)
	After T2			No	10(100.0)
	After T3			Yes	1(10.0)
				No	9(90.0)
	After T4			No	10(100.0)
	Final	No	10(100.0)	No	9(100.0)
Diarrhoea	Baseline	No	10(100.0)	Yes	3(30.0)
				No	7(70.0)
	After T2			No	10(100.0)
	After T3			No	10(100.0)
	After T4			No	10(100.0)
	Final	No	10(100.0)	No	9(100.0)
Muscle pain	Baseline	Yes	6(60.0)	Yes	9(90.0)
		No	4(40.0)	No	1(10.0)
	After T2			Yes	4(40.0)
				No	6(60.0)
	After T3			Yes	1(10.0)
				No	9(90.0)
	After T4			Yes	-
				No	10(100.0
	Final	No	10(100.0)	No	9(100.0)
Pain severity	Baseline	Mild	1(10.0)	Mild	6(66.7)
		Moderate	3(30.0)	Moderate	2(22.2)
		Severe	2(20.0)	Severe	1(11.1)
	After T2			Mild	3(60.0)
				Moderate	2(40.0)
	After T3			Mild	1(100.0)
	After T4			-	-
	Final	-	-	-	-
Convulsion	Baseline	Yes	1(10.0)	No	10(100.0)
		No	9(90.0)		
	Final	No	10(100.0)	No	9(100.0)
Bloody stool	Baseline	Yes	1(10.0)	No	10(100.0)
		No	9(90.0)		
	Final	No	10(100.0)	No	10(100.0)

Malaria symptoms presented

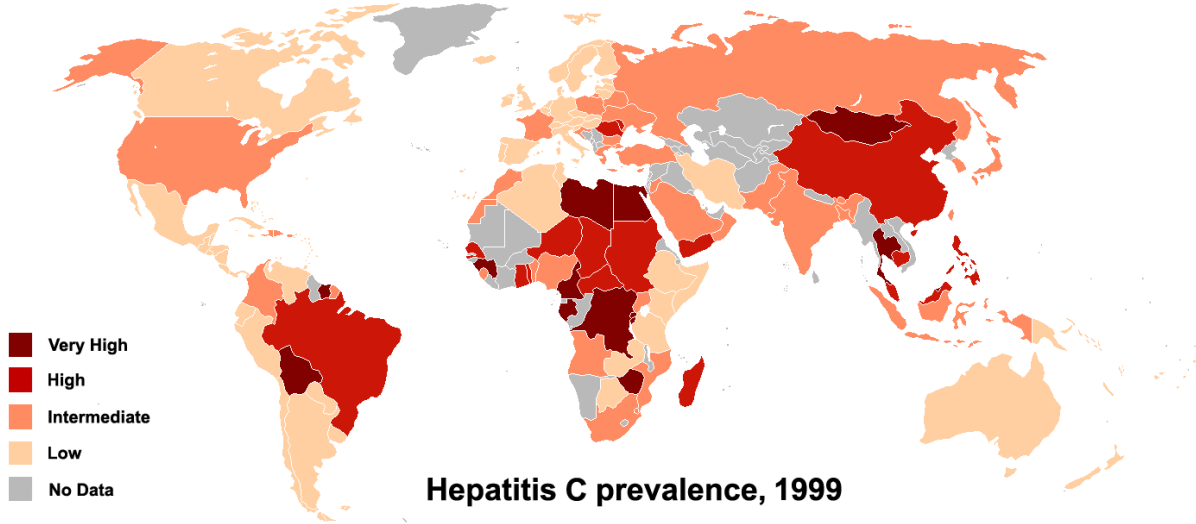
Symptoms	Time of observation	Control group (%)		Study group (%)	
Fatigue	Baseline	Yes	10(100.0)	Yes	10(100.0)
	After T2			Yes	5(150.0)
				No	5(50.0)
	After T3			Yes	1(10.0)
				No	9(90.0)
	After T4			Yes	1(10.0)
				No	9(90.0)
	Final	Yes	2(20.0)		
		No	8(80.0)	No	9(100.0)
Malaise	Baseline	Yes	10(100.0)	Yes	10(100.0)
	After T2			Yes	7(70.00
				No	3(30.0)
	After T3			Yes	4(40.0)
				No	6(60.0)
	After T4			Yes	3(30.0)
				No	7(70.0)
	Final	Yes	4(40.0)		
		No	6(60.0)	No	9(100.0)
Body aches	Baseline	Yes	10(100.0)	Yes	9(90.0)
				No	1(10.0)
	After T2			Yes	6(60.0)
				No	4(40.0)
	After T3			Yes	2(20.0)
				No	8(80.0)
	After T4			Yes	1(10.0)
				No	9(90.0)
	Final	Yes	2(20.0)		
		No	8(80.0)	No	9(100.0)

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



Hepatitis B prevalence, 2005



Hepatitis C prevalence, 1999

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

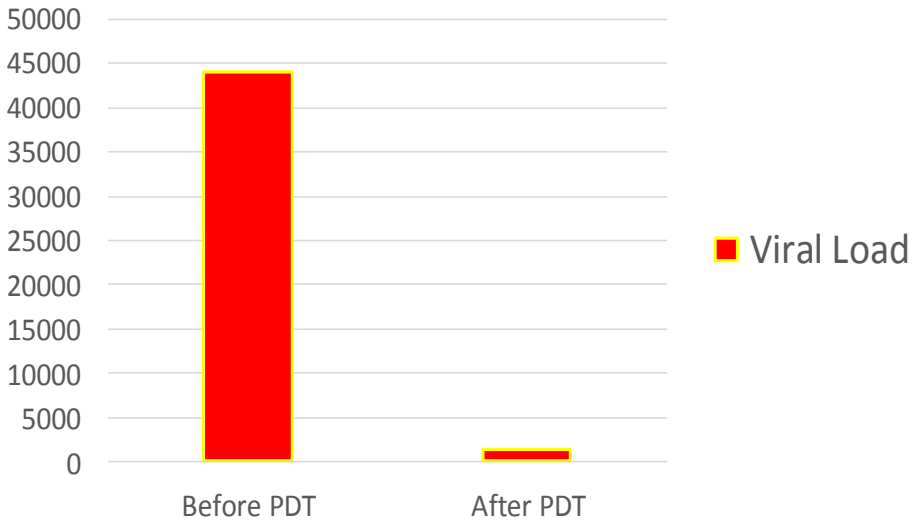
- **Even patients that had been treated with conventional methods for many years without noticeable effects reacted very positive to aPDT. After five treatments the viral load decreased by 70% in average**

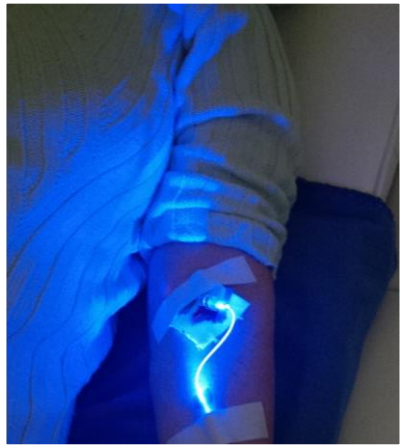
Latest Studies: Hepatitis B/C

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)

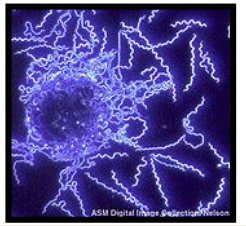
- **Result: Average decrease of viral load from 43999 UI/ml to 1394 UI/ml**



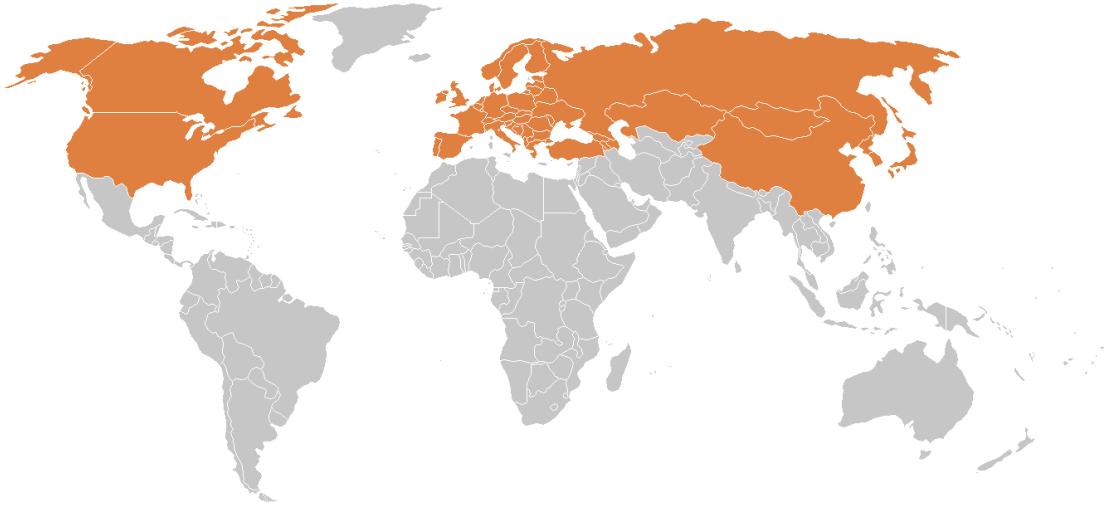


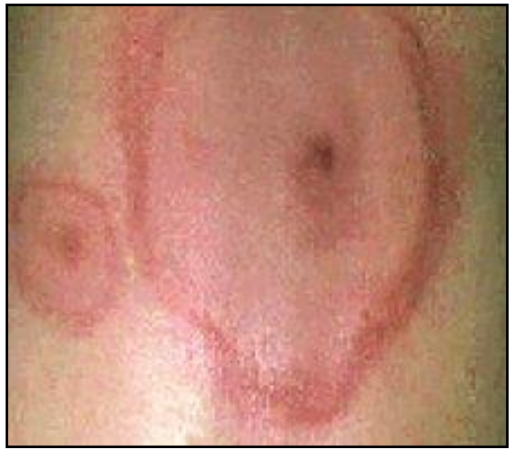
- **Concusion: ILBI with the new 447 nm blue laser, synergistically combined with UltraBioavailable Curcumin has increased anti-microbial effects and the ability to modulate the immune system, with beneficial effects in infectious and age-related diseases.**

Latest Studies: Lyme Disease

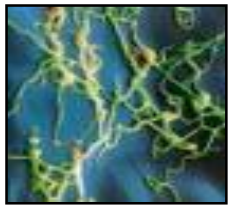


- Infectious disease caused by *Borrelia* bacteria which is spread by ticks
- Early symptoms (stadium 1) may include fever, headache and tiredness
- If untreated, chronic disease may develop with symptoms including chronic fatigue, facial paresis, joint pains, depression, severe headaches with neck stiffness, 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



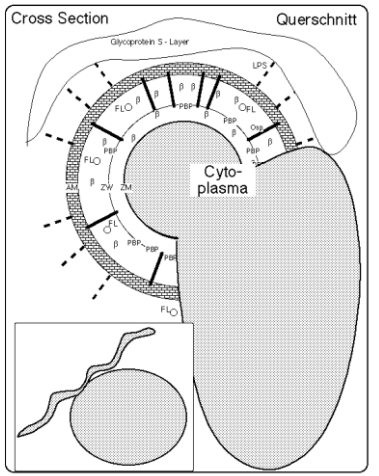


Latest Studies: Lyme Disease



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



Defective Cell Wall → Development of CWD borrelia

*Borrelia can hide in neuronal cells, fibroblasts, lymphocytes, macrophages etc.

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

- 3 groups with 10 chronic lyme patients each

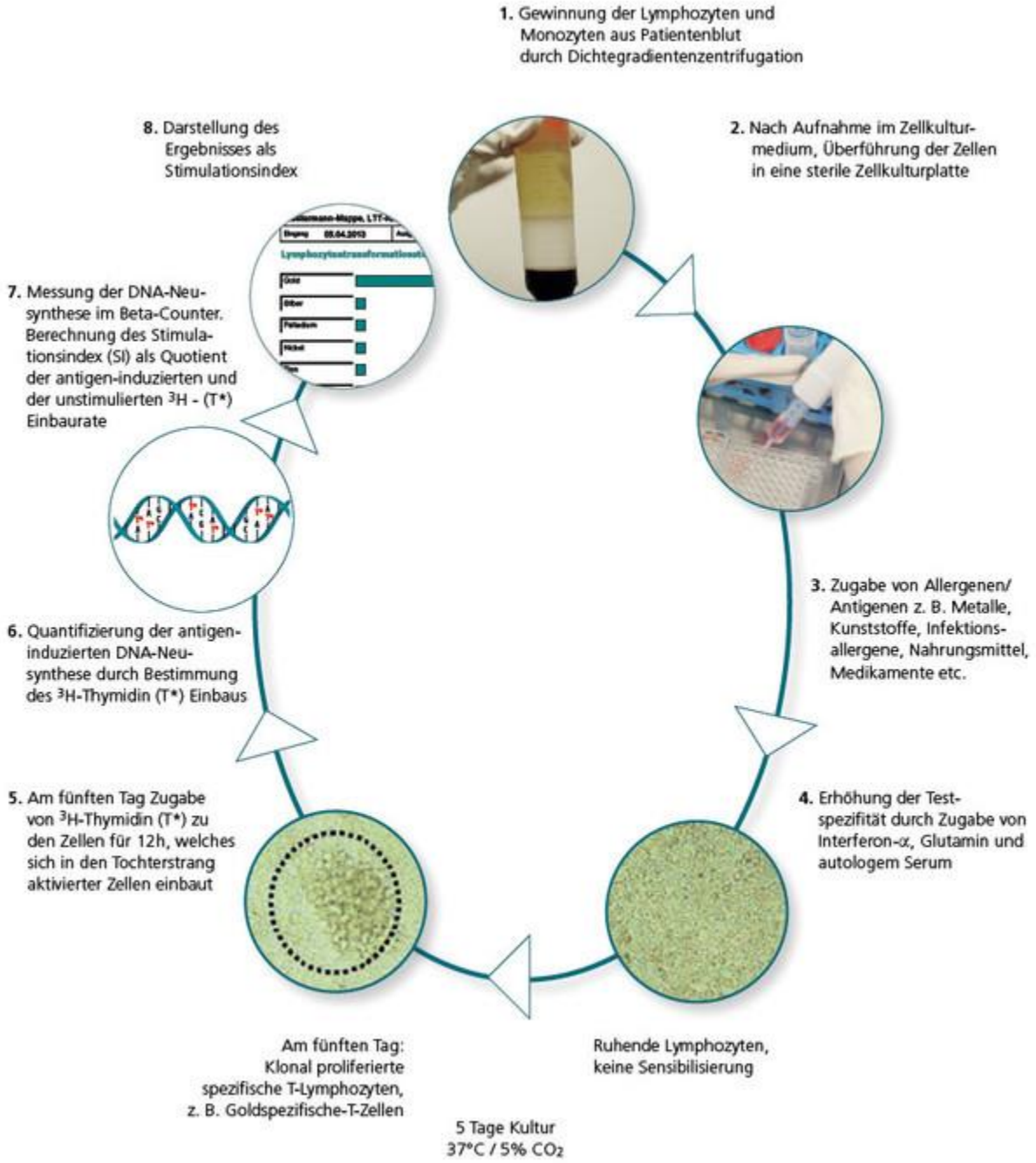
- Patients in all 3 groups received the following therapies:
  - Physical vascular therapy (Bemer)
  - Immunotherapy
  - Vitamins and additional natural supplements
  - Neural Therapy
  - Procain bases infusions
  - Oxygen therapy

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 Monozyten aus Patientenblut durch Dichtegradientenzentrifugation
2. Nach Aufnahme im Zellkulturmedium, Überführung der Zellen in eine sterile Zellkulturplatte
3. Zugabe von Allergenen/ Antigenen z. B. Metalle, Kunststoffe, Infektionsallergene, Nahrungsmittel, Medikamente etc.
4. Erhöhung der Testspezifität durch Zugabe von Interferon-α, Glutamin und autologem Serum
Ruhende Lymphozyten, keine Sensibilisierung
5. Am fünften Tag Zugabe von ^{3}H-Thymidin (T*) zu den Zellen für 12h, welches sich in den Tochterstrang aktivierter Zellen einbaut
Am fünften Tag: Klonal proliferierte spezifische T-Lymphozyten, z. B. Goldspezifische-T-Zellen
6. Quantifizierung der antigeninduzierten DNA-Neusynthese durch Bestimmung des ^{3}H-Thymidin (T*) Einbaus
7. Messung der DNA-Neusynthese im Beta-Counter. Berechnung des Stimulationsindex (SI) als Quotient der antigen-induzierten und der unstimulierten ^{3}H - (T*) Einbaurate
8. Darstellung des Ergebnisses als Stimulationsindex
5 Tage Kultur
37°C / 5% CO_2

Vielen Dank für Ihre Überweisung.
Wir haben folgenden Befund erhoben:

Ärztlicher Befundbericht

Patient	Tagebuch-Nr.	Geburtsdatum
[redacted]	2512543	[redacted]

Eingang	21.02.06	Ausgang	28.02.06

Untersuchung/Material: **Lymphozytentransformationstest - LTT-Borrelien** (Heparinblut)

Borrelien-Lysatantigene

	SI
B.garinii	8,8
B.afzelii	9,8
B.sens.strictu	9,4

Borrelienantigen rekombinant (allen 3 Spezies gemeinsam)

OspC-Antigen	7,9

Positivkontrollen	Antigenkontrolle	17,9
	PWM (Mitogen)	34,7

Leerwert (Negativkontrolle)	1236	Normalwert < 4000 cpm

Stimulationsindizes von > 8 bei der Mitogenkontrolle PWM und > 3 bei der Antigenkontrolle PPD sichern die Auswertbarkeit der Untersuchung.

Hinweise zur Untersuchungsmethode:
Die Werte rechts neben der Balkengraphik sind die Stimulationsindizes (SI) für das jeweilige Borrelienantigen, das den Patientenzellen zugesetzt wird (3-fach-Ansätzen). Der Stimulationsindex der Aktivierung durch das Antigen im Vergleich zum Leerwert (Thymidineinbaurate (Leerwert in cpm, angegeben ist der Mittelwert von 3 Paralleluntersuchungen). Ein SI > 3 bedeutet eine mehr als dreifache zelluläre Aktivierung durch das Antigen im Vergleich zum Leerwert und beweist die Existenz von zirkulierenden Borrelien-spezifischen T-Zellen im Patientenblut (positives Ergebnis). Ein SI < 2 gilt als sicher negativ. Ergebisse zwischen 2 und 3 sind als grenzwertig anzusehen (schwache bzw. fragliche Sensibilisierung), die ggf. kontrolliert werden sollten.

Die Positivkontrollen dienen ausschließlich zum Nachweis der Reaktionsfähigkeit der Lymphozyten. Hier wwird eine Tetanus/Influenza/CMV-Mischantigenprobe verwendet, bei der eine T-zelluläre Sensibilisierung immer vorhanden ist. PWM ist als Mitogen Indikator für die Vitalität der Immunzellen bei Probeneingang im Labor.

Befund: Es zeigen sich deutlich positive Reaktionen auf Borrelienantigene. Unter Berücksichtigung der ebenfalls deutlich positiven Serologie und des klinischen Bildes spricht der Befund für eine aktive Borrelieninfektion.
Wichtig: Besonders entscheidend für die Therapieindikation ist das gegenwärtige klinische Bild.
Empfehlung: Kontrolluntersuchung 6 Wochen nach Beendigung der antibiotischen Behandlung.

Stimulation Index
> 3,0

Treatment
necessary

Vielen Dank für Ihre Überweisung.
Wir haben folgenden Befund erhoben:

Ärztlicher Befundbericht

Patient		Tagebuch-Nr.	Geburtsdatum	
[redacted]		2525664	[redacted]	
Eingang 03.04.06	Ausgang 10.04.06			

Untersuchung/Material: **Lymphozytentransformationstest - LTT-Borrelien** (Heparinblut)

Borrelien-Lysatantigene

		SI
B.garinii	[redacted]	1,8
B.afzelii	[redacted]	2,4
B.sens.strictu	[redacted]	3,4

Borrelienantigen rekombinant (allen 3 Spezies gemeinsam)

OspC-Antigen	[redacted]	2,3

Positivkontrollen	Antigenkontrolle	21,7
	PWM (Mitogen)	42,6

Leerwert (Negativkontrolle)	1236	Normalwert < 4000 cpm

Stimulationsindizes von > 8 bei der Mitogenkontrolle PWM und > 3 bei der Antigenkontrolle PPD sichern die Auswertbarkeit der Untersuchung.

Hinweise zur Untersuchungsmethode:
Die Werte rechts neben der Balkengraphik sind die Stimulationsindizes (SI) für das jeweilige Borrelienantigen [illegible] der Antigen-induzierten- und der [illegible] Thymidineinbaurate (Leerwert in cpm, angegeben ist der Mittelwert von 3 Paralleluntersuchungen). Ein SI > 3 bedeutet eine mehr als dreifache zelluläre Aktivierung durch das Antigen im Vergleich zum Leerwert und beweist die Existenz von zirkulierenden Borrelien-spezifischen T-Zellen im Patientenblut (positives Ergebnis). Ein SI < 2 gilt als sicher negativ. Ergebisse zwischen 2 und 3 sind als grenzwertig anzusehen (schwache bzw. fragliche Sensibilisierung), die ggf. kontrolliert werden sollten.

Die Positivkontrollen dienen ausschließlich zum Nachweis der Reaktionsfähigkeit der Lymphozyten. Hier wwird eine Tetanus/Influenza/CMV-Mischantigenprobe verwendet, bei der eine T-zelluläre Sensibilisierung immer vorhanden ist. PWM ist als Mitogen Indikator für die Vitalität der Immunzellen bei Probeneingang im Labor.

Befund: Im Vergleich zum Vorbefund zeigt sich ein deutlicher Rückgang der Borrelien-spezifischen Reaktivität.
Dieser Befund spricht für eine effektiv durchgeführte antibiotische Therapie.
Kontrolluntersuchungen sind lediglich bei Verdacht auf persistierende (aktive) Infektion indiziert.

Results after successful treatment

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

Tested antigens:

- Borr. sensu stricto
- Borr. afzelii
- Borr. garinii
- Borr. OspC

Measurement of antigen-specific t-cells in patient‘s blood:

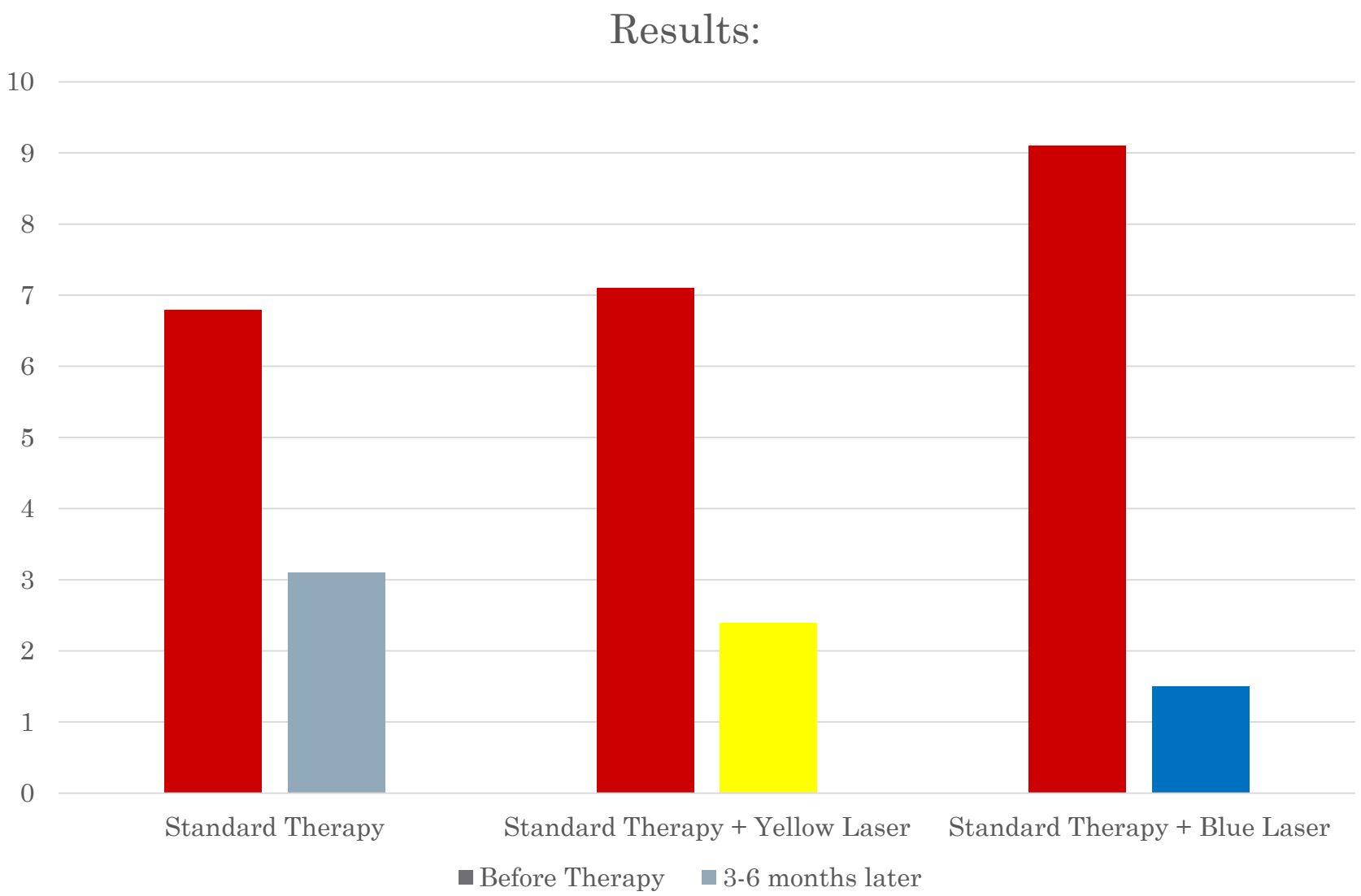
- Stimulation index (SI) >3,0 positive
- Stimulation index (SI) 2,0-3.0 boarderline
- Stimulation index (SI) <2,0 negative

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

Results:

	SI Before Therapy	**SI 3-6 months later**
Group 1 (n=10) Standard Therapy	6,8	3,1
Group 2 (n=10) Standard Therapy + Yellow Laser 589nm	7,1	2,4
Group 3 (n = 10) Standard Therapy + Blue Laser 447nm	9,1	1,5

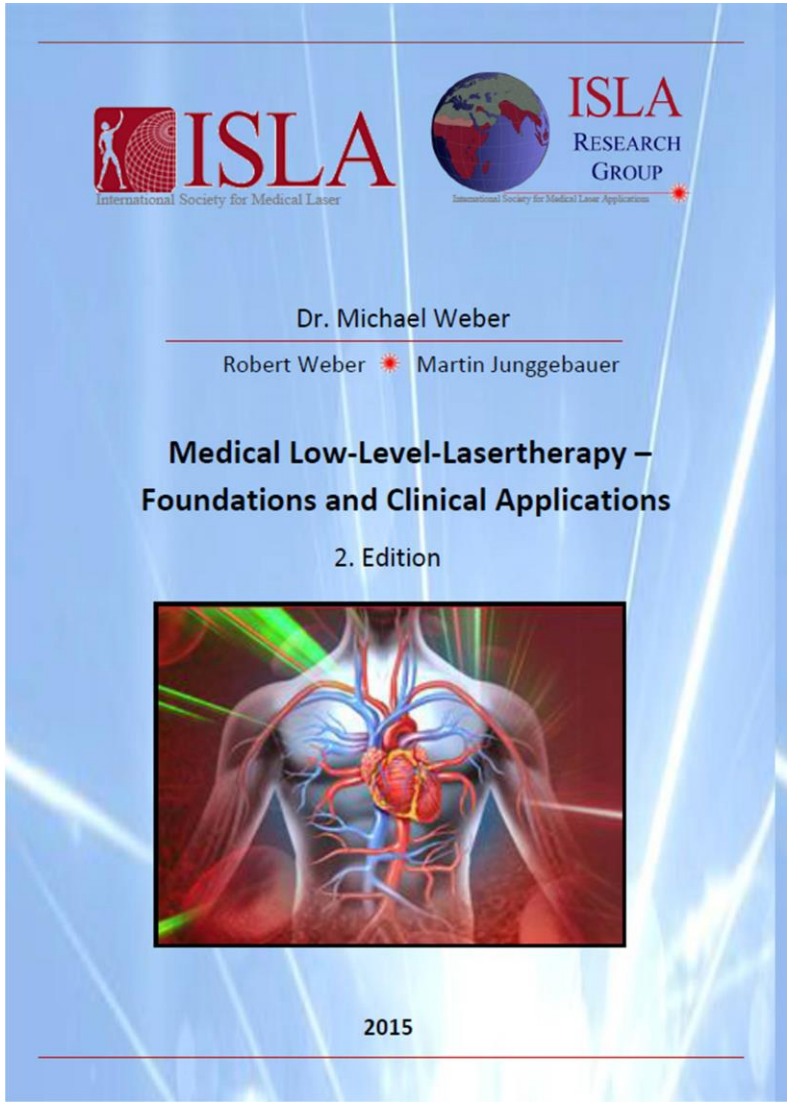
Study Dr. I. Zuern, Germany (2016):



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:



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Thank you!

ISLA
RESEARCH
GROUP
International Society for Medical Laser Applications

Questions:

Robert Weber: weber-research@isla-laser.org

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