Complex Diseases, Success and Failure

Finding the „right“ target → valid targets

which constrains are limiting factors?

Dosage, bioavailability, Actual drug concentration in the respective compartment (cell, organelles)

Are we competing against a natural substrate, e.g. ATP? (concentration in the cell: ca. 4 mMol)
Antifungals

Ketoconazole, Fluconazole, Itraconazole, Clotrimazole, ...

Mechanism of action: Inhibition of 14-α-demethylase (CYP51) that is part of the biosynthesis pathway of ergosterol, which is an essential component of the fungal cell membrane (in mammals: cholesterol).

Cons: Inhibition of Cytochromes causes hepatotoxicity (Ketoconazole). Other conazoles are more specific. Development of resistances (overexpression of efflux proteins).
Failure (so far) (I)

Antibacterial agents targeting enzymes of the Shikimate pathway (responsible for the synthesis of the amino acids Phe, Tyr, and Trp).

Pro: Those enzymes are only found in plants, fungi, algae, and bacteria but not in mammals. Thus interference can be ruled out.
Failure (so far) (II)

Chorismate mutase

Prephenate

Phenylalanine

Tryosine

Anthranylate

Tyrosine

Pathogens such as *Oxoplasma gondii, Plasmodium falciparum*, and *Cryptosporidium parvum* contain the Shikimate pathway and the seven enzymes involved.


Con: Obviously the necessary inhibitor concentration in the respective compartment could not be achieved.

For comparison:

The cellular level of phosphoenolpyruvate (PEP) is ca. 4 mMol
Complex Diseases

malaria is the tropical disease no.1

300-500 millionen infections per year causing 1-3 million fatalities

clinical symptoms:

Strong fever, anemia, acidosis, multiple failure of organs

Due to the life cycle of the pathogen *Plasmodium flaciparum* and the transmission by the *anoph eles* fly, there are several starting points for control and therapy.

Malaria pathogens cause degradation of hemoglobin.

*Plasmodium falciparum* trophozoite

Further pathogens in human:
- *P. vivax*
- *P. malariae*
- *P. ovale*

and about 56 more species of *Plasmodium*.
Lifecyle of the malaria pathogens

source: http://www.dpd.cdc.gov/.../body_Malaria_page1.htm
Approaches to controlling (I)

1960-1980 exhaustive use of insecticides against the Anopheles fly with very good results by the use of DDT (dichloro-diphenyl-trichloroethane)

Disadvantages:

- Accumulation of DDT in the adipose tissue [Fettgewebe] of all creatures (mammals, birds, fish)
- DDT is biologically (almost) undegradable
- Metabolismus leads to a neurotransmitter-like substance (acts as contact insecticide !)
- Increasing resistance to DDT has been observed

\[
\begin{align*}
\text{DDT} & \quad \text{Cl} \quad \text{Cl} \\
& \quad \text{CCl}_3 \\
& \quad \text{Cl} \quad \text{Cl}
\end{align*}
\quad \rightarrow \quad
\begin{align*}
\text{DDE (antiandrogen)} & \quad \text{Cl} \quad \text{Cl} \\
& \quad \text{CCl}_2 \\
& \quad \text{Cl} \quad \text{Cl}
\end{align*}
\]
Distribution of Malaria (I)

Malaria Endemic Countries, 2003

Areas with risk of malaria

Note: This map shows countries with endemic malaria. In most of these countries, malaria risk is limited to certain areas.
Distribution of malaria (II)

Distribution of actual and potential malaria transmission stability

Distribution of the Anopheles fly

Approaches to controlling (II)

chloroquine: since the late 1940’s worldwide application at very low costs (0.2 US$ per dose)

mode of action (still partly unclear):
binds to HEM groups
inhibition of the glutathion-S-transferase

sulfadoxine
antibacterial

pyrimethamine
blocks the dihydrofolate reductase respectively the dihydropterate synthetase
Resistance of the Anopheles fly

red: areas with malaria
Approaches to controlling (III)

Alternatives to chloroquine and sulfadoxine/pyrimethamine
amodiaquine respectively chlorproguanil/dapsone

Disadvantage: expected build up of resistances due to identical targets
Approaches to controlling (IV)

Profile for new drugs and chemoprophylaxis

- efficient, cheap
- effective against the more rare, but lethal *Plasmodium vivax*
- Avoiding of resistances by the use of combinations drugs (several targets at the same time)

Example for chemoprophylaxis: mefloquine (Lariam®)

![Chemical structure of mefloquine](image)

Mode of action due to interaction with phospholipids (cell membrane, fatty acid synthesis)

Only very few adverse effects
Approaches to controlling (V)

Example for combination drugs:
atovaquone (antiparasitic) together with an antibiotic

Drugs derived from natural compounds:
artemisinin $\rightarrow$ artemether and artesunate (form cytotoxic radicals in the presence of HEM iron)

Disadvantage: metabolisms and thus short half life
## New malaria targets (I)

### Table 2 | Targets for antimalarial chemotherapy

<table>
<thead>
<tr>
<th>Target location</th>
<th>Pathway/mechanism</th>
<th>Target molecule</th>
<th>Existing therapies</th>
<th>New compounds</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytosol</td>
<td>Folate metabolism</td>
<td>Dihydrofolate reductase</td>
<td>Pyrimethamine, proguanil</td>
<td>Chlorproguanil</td>
<td>82,83</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dihydropteroate synthase</td>
<td>Sulphadoxine, dapsone</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Glycolysis</td>
<td>Thymidine synthase</td>
<td></td>
<td></td>
<td></td>
</tr>
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<td></td>
<td></td>
<td>Lactate dehydrogenase</td>
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<td></td>
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<td>Peptide deformylase</td>
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<tr>
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<td>Protein synthesis</td>
<td>Heat-shock protein 90</td>
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<tr>
<td></td>
<td>Glutathione metabolism</td>
<td>Glutathione reductase</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Signal transduction</td>
<td>Protein kinases</td>
<td></td>
<td></td>
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<td></td>
<td>Unknown</td>
<td>Ca^2+ - ATPase</td>
<td>Artemisinins</td>
<td></td>
<td>90</td>
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<tr>
<td>Parasite</td>
<td>Phospholipid synthesis</td>
<td>C nucleotide transporter</td>
<td>Quinolines</td>
<td>G25</td>
<td>71</td>
</tr>
<tr>
<td>membrane</td>
<td></td>
<td>Unique channels</td>
<td></td>
<td>Dinucleoside dimers</td>
<td>91</td>
</tr>
<tr>
<td></td>
<td>Membrane transport</td>
<td>Hexose transporter</td>
<td></td>
<td>Hexose derivatives</td>
<td>92</td>
</tr>
<tr>
<td>Food vacuole</td>
<td>Haem polymerization</td>
<td>Haemoglobin</td>
<td>Chloroquine</td>
<td></td>
<td></td>
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<td></td>
<td>Haemoglobin hydrolysis</td>
<td>Plasmodins</td>
<td></td>
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<td></td>
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<td></td>
<td>Free radical generation</td>
<td>Unknown</td>
<td>Artemisinins</td>
<td></td>
<td></td>
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<tr>
<td>Mitochondrion</td>
<td>Electron transport</td>
<td>Cytochrome c oxidoreductase</td>
<td>Atovaquone</td>
<td></td>
<td>101</td>
</tr>
<tr>
<td>Apicoplast</td>
<td>Protein synthesis</td>
<td>Apicoplast ribosome</td>
<td>Tetracyclines, clindamycin</td>
<td></td>
<td>102</td>
</tr>
<tr>
<td></td>
<td>DNA synthesis</td>
<td>DNA gyrase</td>
<td>Quinolones</td>
<td></td>
<td></td>
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<td>Transcription</td>
<td>RNA polymerase</td>
<td>Rifampin</td>
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<td>Type II fatty acid</td>
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<td>Thioactomyacin</td>
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<td>Triobion</td>
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<td>Isoprenoid synthetase</td>
<td>Fab/PiEPR</td>
<td></td>
<td>Foamidomycin</td>
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<td></td>
<td>Protein farnesylation</td>
<td>DOXP reductoisomerase</td>
<td></td>
<td>Peptidomimetics</td>
<td>25,104</td>
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<tr>
<td>Extracellular</td>
<td>Erythrocyte invasion</td>
<td>Subtilisin serine proteases</td>
<td>Protease inhibitors</td>
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<td>97,105</td>
</tr>
</tbody>
</table>

DOXP: 1-deoxy-D-xylulose 5-phosphate; PiEPR, Plasmodium falciparum enoyl-ACP reductase.

New malaria targets (II)

→ Target identification on the gene level
  homolog enzymes of known diseases

→ Improvement of drugs that are already in use against other (infective) diseases:
  dihydrofolate reductase → cancer
  cysteine protease → osteoporosis
  protein farnesyl transferase → cancer
  protein synthesis → other parasites

vaccines: proteins that are expressed on the cell surface
→ sequencing of the *Plasmodium falciparum* genome
New malaria targets (III)

Sequencing of *Plasmodium falciparum*

25 Mb on 14 chromosomes, ca. 5000 genes
  6 Kb genome of the mitochondrion
35 Kb circular DNA of the Apicoplast

Similar dimensions are also to be expected for *P. yoelii* and *P. vivax*.

http://www.ncbi.nlm.nih.gov/Malaria/

http://plasmodb.org (annotated Plasmodium genome)

Metabolic paths of *P. falciparum*:

http://sites.huji.ac.il/malaria/ (contains EC numbers)

Neglected Tropical Diseases (I)

Infections with pathogens prevalent in developing regions around the tropical belt of Africa, Asia, and America.

- ascariasis, trichuriasis, necatoriasis, ancylostomiasis
  - infection by soil transmitted helmintics (worms)

- Schistosomiasis (snail fever, bilharzia)

- Trachoma and onchoceriasis (river blindness)

- Leishmanias

- Chagas disease

- Leprosy

- African Trypanosomiasis
  - (sleeping sickness)

The impact of this diseases in numbers is similar to that of malaria and tuberculosis
Neglected Tropical Diseases (II)

The World Health Organisation lists further diseases, such as

Cysticerosis (infection by the pork tapeworm)

Dengue / dengue haemorrhagic fever
  (virus transmitted by mosquitos)

Rabis [Tollwut] (viral)

Yaws (bacterial) a similar treponemal disease is syphilis

Snake bites

Tropical diseases with outbreaks in other areas due to transmission by mosquitos:

West Nile virus

Ross River fever
Complex diseases

**obesity** [Fettleibigkeit]

typical symptoms:

- excess weight
- increased levels of cholesterol → arteriosclerosis
- hypertension

The connection to obesity was established by the genetic lack of cholesterol receptors (hypercholesterolaemia) and especially cholesterol-rich nutrition in animal studies.
Regulation of the cholesterol pool

Bile acids [Gallensäuren] -> intestine -> cholesterol from the nutrition -> NPC1L1 transporter -> HDL receptor, LDL receptor

Statins -> Acetyl-CoA -> HMG-CoA -> HMG-CoA reductase -> mevalonate -> squalene -> other steroids

Endogenic biosynthesis: CoA

Cholesterol -> LDL 70% HDL 30%

Cell membrane (flexibility)

Lit. F. Rinninger & H. Greten *Dtsch. Ärztebl.* **102** (2005) A516
Inhibition of HMG-CoA reductase (I)

compactin (from *Penicillium citrinum*) and mevinolin (=lovastatin) (from *Aspergillus terreus*) were first found as inhibitors.

Inhibition of HMG-CoA reductase (II)

The actually effective substance is the metabolite

\[ \text{lovastatin} \xrightarrow{\text{ester cleavage}} \text{mevinolin} \]

\[ K_i = 1 \text{ nM} \]
Sales potential of Statins

Market volume of cholesterol reducing agents

Turnover in billion US$ for USA, France, Germany, Italy, Spain, England and Japan, (market volume in %)
CEPT= cholesteryl ester transferase protein

Further statins

cerivastatin (Bayer)
simvastatin (Merck & Co)
pravastatin (Sankyo)
fluvastatin (Sandoz)
rosuvastatin (Astra-Zeneca)
atorvastatin (Warner-Lambert)
Further lipid lowering agents (I)

ezetimib inhibits the cholesterol transporter

Further lipid lowering agents (II)

avasimibe inhibits the acetyl-coenzyme-A-cholesterol-acetyltransferase (ACAT-inhibitor)
Further lipid lowering agents (III)

competitive cholesterol analogs

- cholesterol
- furazabol
- clomestrone
Further lipid lowering agents (IV)

Bile acid sequestrants

Polymers that are not absorbed from the intestine

- cholestyramine $\text{MW}>10^6$
- colestipol

absorb cholesterol and bile acid and therefore prevent uptake of cholesterol
Opinion drugs vs. life style modification

„obesity is a form of depression in which the eating is an antidepressant“

Fat storage is most efficient to preserve energy
Anorexic drugs (I)

Due to their complex affinity profile regarding a whole series of receptors ("dirty drugs") psychoactive drugs also modify the eating behaviour.

Anorexic drugs (II)

Prominent examples of psychoactive drugs with appetite suppressant (side-) effect:

- methylphenidate (Ritalin®) ADHD
- atomoxetine (Strattera®) [Aufmerksamkeitsdefizitsyndrome]
- fluoxetine (Prozac®)

Market volume of ADHD pharmacca in million US $