What is Malaria?

- Malaria is a parasitic disease caused by a protozoon of the genus Plasmodium. The four subspecies active in humans are *P. falciparum*, *P. vivax*, *P. malariae* and *P. ovale*, with *P. falciparum* being responsible for most malaria-related deaths.

- Symptoms include cyclical bouts of fever, with muscle stiffness, shaking and sweating. The most severe manifestations are cerebral malaria, anemia and kidney, liver and lung dysfunctions.

- Malaria is transmitted by the bites of the female mosquitoes of the genus *Anopheles*, especially *Anopheles gambiae*.

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The Life Cycle of Plasmodium Falciparum

- **Asexual phase - in human**
  1. Mosquito injects sporozoites into the human
  2. Sporozoites enter liver cells (30 min) and reproduce asexually - hepatocytes eventually burst, releasing parasites into blood stream (the pre-patent period, 8-26 days)
  3. The parasites attach to the red blood cell membrane and then enter the cell through a process of invagination
  4. Asexual division starts and the parasites develop through stages of rings, trophozoites, schizonts containing thousands of merozoites

- **Sexual phase - in mosquito**
  1. Gametocytes enter mosquito and continue development
  2. Male and female gametes fuse to form a zygote
  3. Zygote transforms into oocyste which penetrates the gut wall and becomes an oocyst
  4. Oocyst divides asexually into sporozoites which are ready to be injected into human.
  5. Blood cells burst, releasing the merozoites, and the cycle repeats every 48hrs. This phase stimulates production of TNF-α, resulting in the characteristic clinical manifestations of the disease
  6. Some merozoites undergo transformation into gametocytes - male and female.
The Life Cycle of Plasmodium Falciparum

Asexual phase - in human

1. Mosquito injects sporozoites into the human
2. Sporozoites enter liver cells (30 min) and reproduce asexually - hepatocytes eventually burst, releasing parasites into blood stream (the pre-patent period, 8-25 days)
3. The parasites attach to the red blood cell membrane and then enter the cell through a process of invagination
4. Asexual division starts and the parasites develop through stages of rings, trophozoites, schizonts containing thousands of merozoites

Sexual phase - in mosquito

1. Gametocytes enter mosquito and continue development
2. Male and female gametes fuse to form a zygote
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Artemisinin-a Rediscovered Ancient Remedy

Artemisinin is a natural sesquiterpene isolated from the leafy portions of Artemisia annua (qinghao) (~2%)

- First mentioned by Chinese herbalists in 168 B.C as a treatment for hemorrhoids, then in 340 A.D. as a fever reducing agent.
- Isolated and characterized by Chinese scientists in 1972, reisolated in the US in 1984
- Artemisinin is one of the very few naturally occuring endoperoxides. It is structurally related to the cadinane or amorphone class of sesquiterpene characterized by their cis-decalin skeleton.

Klayman J. Nat. Prod. 1984, 47, 715
**Artemisinin and Derivatives as Active Antimalarials**

- Artemisinin and related compounds are the fastest acting antimalarials on the market.

- Artemisinin and derivatives are active at nanomolar concentrations in vitro, even against multi-drug resistant strains of *P. falciparum*.

- Parasitocidal activity is manifested against the erythrocytic phase of plasmodium. The compounds are effective at the ring, trophozoite and gametocyte stage of the blood phase, and inactive on liver-stage parasite.

- The onset of action is very rapid, with clearance of parasites from the blood within 48 hours in most cases. However, because of the very short half-life of the drug (1-3hrs) a high rate of recrudescence was observed if treatment was not continued for at least five-seven days, or combined with a longer-acting antimalarial.

- No neurotoxicity or other serious side effects have been observed in humans at therapeutic doses.

Robert Pure Appl. Chem. 2001, 73, 1173
Meshnick Microbiol. Rev. 1996, 60, 301

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**Artemisinin - an Elusive Mode of Action**

- Reactive radical or electrophilic species are involved in the killing mechanism.

- Erythrocytes infected with *P. falciparum* take up and concentrate $^{14}$C- Artemisinin at 100-fold higher concentrations than uninfected cells, resulting in highly selective cytotoxicity.

- Peroxide bridge is essential for biological activity, and activation by Fe(II) is necessary.

- Generally accepted molecular mechanism of action.

Posner JACS 1998, 117, 5688; JACS 1992, 114, 8328
Artemisinin - Searching for a Cellular Target

- Where does the cytotoxic process take place?

- P. falciparum obtains the amino acids needed for growth by ingesting and degrading up to 80% of the host's hemoglobin in a compartment called the food vacuole. Fe^{2+}-heme is released and oxidized to Fe^{3+}-hematin (toxic for the parasite). Detoxification occurs by aggregation of hematin into a crystalline pigment called hemozoin.

- Because artemisinin is activated by Fe^{3+} it was believed that the specific antimalarial effect was due to the drug's entry in the food vacuole followed by interaction with Fe^{3+}-heme. This would set off a "cluster bomb" of free radicals, inhibiting several key parasite components and leading to parasite death.

Meshnick Microbiol. Rev 1996, 60, 301
Olindo Parasitol. Today 1999, 11, 234

Artemisinin - Searching for a Cellular Target

- Free radicals seem to be part of the cytotoxic pathway
  - In vitro antimalarial activity is enhanced by high oxygen tension and addition of other free-radical generating compounds, such as doxorubicin, micronazole, castanos.
  - Antioxidants such as α-tocopherol, catalase, dithiothreitol, ascorbate, reduced glutathione inhibit activity.
  - Unlike other free-radical-generating drugs, artemisinin does not cause "oxidant damage" through generation of oxygen free radicals such as superoxide. Very high concentrations are necessary to induce lipid peroxidation and membrane protein thiol oxidation and there is no selectivity between parasite and unaffected erythrocytes.

- Contradicting data about the importance of the artemisinin-Fe^{3+}-heme interaction for molecular activation:
  - Chloroquine (known antimalarial that binds tightly to heme) is antagonistic to artemisinin in vitro. BUT iron chelators also antagonize the antiparasitic effect of artemisinin, suggesting involvement of free Fe^{3+}.
  - In vivo activation could occur by a different mechanism involving malarial hemoproteins outside of the food vacuole.

- Heme seems to be not only an activator but also a target for artemisinin:
  - A covalent artemisinin-heme adduct has been isolated and potential mechanisms cytotoxic pathways were proposed:
    - The artemisinin-heme adduct could be intrinsically toxic to the parasite (similar to chloroquine-heme) BUT addition of the preformed adduct to cell cultures yields no antimalarial activity.
    - The iron released upon artemisinin-heme aggregation could be toxic for Plasmodium BUT no detectable increase in free iron has been observed in artemisinin-treated parasites.
    - The artemisinin-heme adduct might inhibit detoxification by blocking hemozoin formation (similar to the chloroquine mechanism of action) or by hemozoin degradation. BUT no decrease in hemozoin content has been observed in artemisinin-treated parasites.
    - Formation of the artemisinin-heme adduct might be completely unrelated to the mechanism of action.

- Artemisinin is capable of alkylating specific proteins at therapeutic doses:
  - Treatment of malaria-infected erythrocytes with radioactive artemisinin specifically labels 6 proteins with masses of 25, 32, 65, 125 and >200 kDa.
  - No labeling is observed in intact erythrocytes.
  - The 25 kDa protein was identified as the malarial translational allosteric protein (TCTP), but since the physiological role of this protein is unknown it is not clear this binding is relevant for the antimalarial activity of artemisinin.

Meshnick Microbiol. Rev 1996, 60, 301
Meshnick Int. J. Parasitol. 2002, 32, 1655
Recent Insights into the Mechanism of Action

Artemisinin DOES NOT in fact concentrate in the food vacuole, but in the membranous structures and in the endoplasmic reticulum.

Structural similarities to known mammalian Sarco/endoplasmic reticulum calcium-dependent ATPase (SERCA) inhibitor thapsigargin strengthen the hypothesis.

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The genome for Plasmodium falciparum encodes for only one SERCA-type Ca\(^{2+}\) ATPase - PiATP6, which proved to be very similar to mammalian SERCA.

Artemisinin and thapsigargin have identical PiATP6 inhibition profiles in vitro.

Artemisinin and thapsigargin are competitive inhibitors of PiATP6 in vivo.

At cellular level, exposure to artemisinins causes rapid swelling of endoplasmic reticulum in parasites, providing a morphological correlate for the proposed site of action.
The First Total Synthesis of Artemisinin - Zhou et al.

- **Retroynthetic analysis**

- **Synthesis of an advanced decaline intermediate**


The First Total Synthesis of Artemisinin - Zhou et al.

- **Synthesis of the key ketone methyl enol ether**

- **Completion of the synthesis through a stereoselective singlet oxygen cycloaddition**

The Schmid and Hofheinz synthesis of Artemisinin

- The same intermediate enol-ether is used, accessed from (-)-isopulegol

\[
\begin{align*}
\text{HO}^- & \quad \xrightarrow{1. \text{ MOMCl, PhNMMe}_2} \quad \text{BnO}^- \\
& \quad \xrightarrow{2. \text{ B}_{2}\text{H}_6, \text{H}_2\text{O}_2/\text{NaOH}} \quad \text{BrO}^- \\
& \quad \xrightarrow{3. \text{ PhCH}_2\text{Br, KH}} \quad \text{PCC} \\
& \quad \xrightarrow{4. \text{ LDA, 0°C}} \quad \text{TMS}^- \\
& \quad \xrightarrow{\text{TMS-Li}} \quad \text{MeO}^- \\
& \quad \xrightarrow{\text{TMS-Cl}} \quad \text{MeC} \\
& \quad \xrightarrow{\text{1. Li/NH}_2(f)} \quad \text{PCC} \\
& \quad \xrightarrow{2. \text{ mCPBA}} \quad \text{TBAF} \\
& \quad \xrightarrow{8:1 \text{ mixture of diastereomers}} \quad \text{TBAF} \\
& \quad \xrightarrow{\text{O}_2, \text{methylene Blue}} \quad \text{Artemisinin} \\
\end{align*}
\]

Hofheinz, J. Am. Chem. Soc. 1983, 105, 624

The Avery Synthesis of Artemisinin

- Endo-peroxide unit is installed via the abnormal "ozonolysis" of a vinyl silane

\[
\begin{align*}
\text{H}_2\text{O}_2, \text{NaOH}, \text{THF} & \quad \xrightarrow{74\% \text{ yield}} \\
& \quad \xrightarrow{1. \text{ NaSPh} \quad 2. \text{ mCPBA}} \\
& \quad \xrightarrow{95\% \text{ yield}} \\
& \quad \xrightarrow{1. \text{ LDA, HMPPT} \quad 2. \text{ Al(Hg), wet}} \\
& \quad \xrightarrow{40-50\% \text{ yield}} \\
& \quad \xrightarrow{1. \text{TsnH/NH}_2 \quad 2. \text{ BuLi, TMEDA, DMF}} \\
& \quad \xrightarrow{70\% \text{ yield}} \\
& \quad \xrightarrow{\text{TMS}_2\text{AlOEt}_2, \text{Et}_2\text{O}, -78°C} \\
& \quad \xrightarrow{88\% \text{ yield}} \\
& \quad \xrightarrow{1. \text{ O}_2\text{O}_2, -78°C} \\
& \quad \xrightarrow{2. \text{ SiO}_2} \\
& \quad \xrightarrow{3. \text{ aq H}_2\text{SO}_4} \\
& \quad \xrightarrow{88\% \text{ yield}} \\
& \quad \xrightarrow{1. \text{ LDEA, THF, -78°C} \quad 2. \text{ LDA, THF, 50°C/CMe, -78°C}} \\
& \quad \xrightarrow{88\% \text{ yield}} \\
& \quad \xrightarrow{88\% \text{ yield}} \\
& \quad \xrightarrow{\text{Artemisinin}} \\
\end{align*}
\]

Avery, Tetrahedron Lett. 1987, 28, 4629
Avery, J. Am. Chem. Soc. 1992, 114, 974
**Ravindranathan's Formal Synthesis of Artemisinin**

- Intramolecular Diels-Alder reaction furnishes the cis-decalin skeleton

**Liu, Yeh and Chew - Total Synthesis of Artemisinin**

- Intermolecular Diels-Alder affords a trans decaline

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Liu *Tetrahedron Lett.* 1993, 34, 4435

Liu *Heterocycles* 1996, 42, 493
Conclusions and Future Directions

- Artemisinin and its more bioavailable congeners are currently the state of the art treatment of uncomplicated multidrug resistant malaria. To delay the onset of parasite resistance, caution should be exerted in using artemisinins as a first line of intervention in areas where more traditional drugs are still active.

- The molecular mechanism of action against P. falciparum has been investigated and the role of active radical or electrophilic species generated through oxidation by iron (II) is well established. More work needs to be done to elucidate the exact source of iron used for activation (free iron, iron-heme, iron bound to cytoplasmic metalloproteines).

- A potential target for the parasitocide activity has been identified to be the malarial PIATP6 enzyme. More proof is required to establish if PIATP6 inhibition is actually part of the killing mechanism. The sequencing of parasite genome should provide more insights into other potential artemisinin targets, and shed light on the mechanism of resistance to antimalarials.

- A number of syntheses have been reported to date, involving mostly ene reactions or singlet oxygen additions to an olefin, followed by a rearrangement cascade to furnish the 1, 2, 4 trioxane ring.