Time to Development of Resistance to Antimalarial Drugs

- Chloroquine: 16 years
- Fansidar: 6 years
- Mefloquine: 4 years
- Atovaquone: 6 months

Parasite Chemotherapy

- Why so important?
  - Absence of vaccines
  - Vector control is difficult
  - What to do when immunotherapy fails

- What is the basis of selectivity?
  - Uptake of drug
  - Activation of drug
  - Detoxification of drug
  - Importance of drug target
  - Binding to drug target
  - Unique drug target

Paul Ehrlich (1854-1915)
“chemotherapy”
Magic bullet

Idea of selectivity
Selective dyes

Chemotherapeutic index:
maximum does tolerated by host
minimum curative dose
Idea of a Magic Bullet

“If we picture an organism as infected by a certain species of bacterium, it will ... be easy to effect a cure if substances have been discovered which have a specific affinity for these bacteria and act ... on these alone ... while they possess no affinity for the normal constituents of the body ... such substances would then be ... magic bullets.”

Paul Erhlich

Observation: Chemical dyes interacted with particular cells or tissues in specific ways. Idea of specific affinity (selectivity)

Methylene blue (1891)
Trypan red (1904)
Compound 606 (1910)

Parasite Chemotherapy

- Is chemotherapy the perfect solution?
  - Re-infection in endemic areas
  - Few Drugs are 100% effective
  - Drug is active against only a few stages
  - Parasite clones resistant to Drug
  - Drug cannot reach migrating parasite
  - Some (most) drugs are expensive
  - Serious side effects
  - Many cannot be given orally
Properties of an Ideal Anti-parasitic

- Information based on the huge interest of veterinary market - driving force especially for anthelminthics!
  - Should possess a wide margin of SAFETY (men, women, children, fetus).
  - MINIMAL TOXICITY (tolerable side effects)
  - FEW CONTRAINDICATIONS (drug-drug interactions)
  - BROAD SPECTRUM of activity (all disease species including resistant lines)
  - EFFICACIOUS (relatively short treatment, <14 days)
  - RESISTANCE (low potential for drug resistance)
  - EASY Administration (orally active, avoid needles or hospitalization)
  - AFFORDABLE (diseases of the poor; ~$1 per patient)
  - STABLE (2 years shelf life at 40 °C, 75 % humidity)

Current State of Parasite Drug Development

- Protozoa
  - Military Interest
    - Malaria
    - Leishmaniasis
  - Immunocompromised
    - Cryptosporidiosis
    - Toxoplasmosis
  - Bioterrorism
    - Select agents
      - Cryptosporidium
      - Cyclospora
      - Giardia
      - Entamoeba
      - Toxoplasma

- Helminths
  - Veterinary Market - Huge
    - Cattle Industry
    - Companion animals
    - Sheep Industry
    - Equine Industry
How to Find New Drugs

1. Random screening  
   no design or biological insight
2. Analogues of known drugs  
   not a new target
3. Rational lead discovery  
   long time & expensive

Discovery of Ivermectin

- Top 40 Pharmaceuticals
  - Drugs that changed our world - Aspirin - Viagra

- Ivermectin - A Wonder Drug
  - ~20 years and still going strong
  - High potency (as low as 1 nM)
  - Extremely safe
  - Broad spectrum use
  - Various formulations
  - Single annual dose (slow release)
  - Few reports of resistance
  - ~$1 billion USA industry - the most highly effective antiparasitic drug ever introduced
Wonder Drug Highlights

- 1972 - Dr. Satoshi Omura, proposed important chemicals exist in fermentation products of microbes.
- 1972 - Formed partnership with Merck, Sharp and Dohme (MSD)
- 1974 - Second year of collaboration - isolated organism that produced compound with high antihelminth activity.
- 1988 - first mass drug donation program

Professor Satoshi Omura

- President of Kitasato Institute
- More than 40 years of studying bioactive compounds from microbes

Successful Philosophy

- Unlimited supply of novel compounds
- Produce gold-standard screening
- Screening is not just an exercise
- Contribution of basic research
- Keep the human connection

Huge success

- ~1 in 3 soil isolates have produced antimicrobial substances!

"...success by being able to stand on the shoulders of giants" - Sir Isaac Newton
Serendipity or not?

- Early 1970’s - empirical testing of synthetic compounds had diminishing returns
- Looking for something radically different - not just incrementally better than current compounds
- Find a compound with a truly novel structure
- Bioassay
  - Tandem assay - two parasites - 1 coccidian, 1 nematode
  - Feed microbial fermentation cultures
    - Small amounts of compounds produced
  - Why? Believed in medicated food
- Agreement: send unusual isolates to Merck.

Knowledge and practice united
Satoshi Ōmura (2007)

Discovery of Ivermectin

- Screened 40,000 samples - basic curiosity are there compounds that microbes make that can inhibit the worms?
- Found one that worked! (in vitro studies)
  - Japanese golf course - Dr. Satoshi Omura
  - Surprisingly powerful against the worms
  - *Streptomyces avermitilis* produced compounds they called avermectins
- Division of Merck (MSD) - drugs to treat parasitic worms in animals (in vivo studies)
- Simple screen
  - Infected mice with worms
  - Fed worms cultures of microbes from soil
  - Soil samples from around the world

Dr. William Campbell

Dr. Mohammed Aziz
Ivermectin - Broad Spectrum

- Class of compounds called Avermectins - macrocyclic lactones
- Lacked antibacterial and antifungal properties
- Ivermectin is the most widely used drug in veterinary medicine
  - Control nematodes
  - Control arthropod infestations
- Led to the most important contribution of the pharmaceutical industry
- Mectizan Donation Program
  - Mectizan would be provided free of charge for as long as needed.

"Medicine is for the people. It is not for profits" - George W. Merck

Ivermectin - Mechanism of Action

- Ivermectin works by acting as a potent agonist at Glutamate-gated chloride ion channels.
- In nematodes and arthropods, γ-aminobutyric acid (GABA) sends inhibitory signals to motor neurons.
- Ivermectin potentiates these inhibitory signals and this results in the paralysis of the parasite.
- In mammals, GABA receptors are confined to the CNS.
  - Ivermectin does not cross the blood brain barrier, so it does not cause paralysis.
Simplified schematic representation of the invertebrate and vertebrate chloride ion channels under avermectin control

Parasite                  Host

<table>
<thead>
<tr>
<th>Invertebrate (C. elegans)</th>
<th>Mammalian (Rat Brain)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glutamate</td>
<td>Cl^-</td>
</tr>
<tr>
<td>Ivermectin (High Affinity)</td>
<td>Cl^-</td>
</tr>
<tr>
<td></td>
<td>GABA</td>
</tr>
<tr>
<td></td>
<td>Ivermectin (Low Affinity)</td>
</tr>
</tbody>
</table>

Prevents closure of ion channel
Hyperpolarization of neurons

Ivermectin and Oncocerciasis

- Mid 1970’s - avermectins did not work against hookworms or tapeworms
- 1980 Dr. Mohammed Aziz - observation that ivermectin killed a horse parasite
- Simple clinical study
  - Skin snips prior to treatment - high numbers of microfilaria
  - Single dose of Mectizan - skin snip in 1 month - NO microfilaria
  - Skin snips 3 months later - still NO microfilaria!
  - First evidence for a single annual dose of Mectizan
- 1987 French government approved use in humans
- Eradication was a possibility - Mectizan donation program was initiated.
Onchocerciasis Control Program

- Good News - Bad News
- OCP ended in 2002
- Conclusion - Onchocerciasis cannot be eradicated in most endemic areas with the current tools
- What is needed:
  - New drug regimens
  - Macrofilaricides
  - Better diagnostics
- Overlap with another program African Program for Onchocerciasis Control (1995)
  - Aim: by 2007 create a sustainable community-directed distribution system for ivermectin in over 17 African nations

Analogues of Known Drugs

- Potency of Compounds
  - Dosage (mg/kg)
  - 1000 mg/kg
  - 600 mg/kg (also narrow spectrum)
  - 200 µg/kg
  - 100 µg/kg
  - 10 µg/kg
  - 1 µg/kg
  - 0.1 µg/kg

Year of Introduction:
- 1940
- 1950
- 1960
- 1970
- 1980
- 1990
- 2000

Drugs:
- Moxidectin
- Doramectin
- Eprinomectin
- Ivermectin
- Abamectin
- Pyrantel
- Morantel
- Oxendazole
- Tetramisole
- Levamisole
- Thiamazole
- Phenothiazine

Analogue of Known Drugs
Diethylcarbamazine

- Synthetic organic compound with no toxic metallic components
- Tissue and blood nematodes (filarial worms)
- Hyperpolarizing neuromuscular blockade
  - Paralysis of worm
- Headache, malaise, nausea, inflammation
- Most useful in a combined treatment regimen

Treatment of helminth infections

Adult worms do not multiply in the mammalian host.
The most effective chemotherapeutic targets have been:

Motility
Energy Metabolism

1. Drugs affecting motility
   - Worms have complex nervous systems
   - Active motility is essential for the worms to resist expulsion by bowel peristalsis

2. Drugs affecting energy generation
   - Enteric helminths exist in an anaerobic environment, and have developed special mechanisms for generating energy.
<table>
<thead>
<tr>
<th>Cofactor synthesis</th>
<th>Nucleic acid synthesis</th>
<th>Protein synthesis</th>
<th>Membrane function</th>
<th>Membrane kinase</th>
<th>Energy metabolism</th>
<th>Neurotransmitter kinase</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Antiparasitics</strong></td>
<td></td>
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<tr>
<td>Pyrimethamine</td>
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<tr>
<td>Sulphonamide</td>
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<tr>
<td>Sulphaizinamide</td>
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<td>Trimethoprim</td>
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<tr>
<td><strong>Anthelminitics</strong></td>
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<tr>
<td>Albendazole</td>
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<tr>
<td>Mebendazole</td>
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<td>Oxendazole</td>
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<tr>
<td>Thiabendazole</td>
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<tr>
<td>Triclabendazole</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Differential uptake/secretion</th>
<th>Drug activation only in parasite</th>
<th>Unique target in parasite</th>
<th>Drug discriminates between target in host and parasite</th>
<th>Pathway blocked more important in parasite than in host</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Antiparasitics</strong></td>
<td></td>
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<tr>
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<tr>
<td>Albendazole</td>
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<tr>
<td>Bephenium</td>
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<tr>
<td>Dichlorvos</td>
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<tr>
<td>Diethylcarbamazine</td>
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<tr>
<td>Haloxon</td>
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<td>Levamisole</td>
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<td>Mebendazole</td>
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<td>Metrifonate</td>
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<td>Morantel</td>
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<tr>
<td>Naphthalophos</td>
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<td>Oxendazole</td>
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<tr>
<td>Piperazine</td>
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<td>Pyrantel</td>
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</tr>
</tbody>
</table>
The research gap – 10/90

Gap 1 – pre-clinical gap
Gap 2 – profit-based gap
Gap 3 – access gap

Scheme for Rationale Drug Design

Year 1
- Identify key biochemical feature
  - Show feature important to parasite survival
    - Purify/clone & resolve 3D structure of target
      - Design & synthesis inhibitors
        - Screening of target in vitro
          - Validate target in vivo: animal models & trials
            - Approval & launch of new drug

Year ~6
Year 20
**Compound Success Rate by Stages**

<table>
<thead>
<tr>
<th>Discovery Research &amp; Drug Discovery Needs</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Target identification</strong> - bioinformatics &amp; chemical genomics</td>
</tr>
<tr>
<td><strong>Target validation</strong> - inducible (conditional) gene knockout and RNAi / antisense</td>
</tr>
<tr>
<td><strong>Target characterisation</strong> - function, mechanism and structure</td>
</tr>
<tr>
<td><strong>Compound evaluation</strong> - reporter systems for screening in vitro and in vivo</td>
</tr>
<tr>
<td><strong>Assay development</strong> - high throughput screening (identify lead compounds)</td>
</tr>
<tr>
<td><strong>Mechanistic studies</strong> - modes of drug action and drug resistance mechanisms</td>
</tr>
<tr>
<td><strong>Molecular diagnostics</strong> - prediction of therapeutic efficacy and drug toxicity, especially non-invasive methods</td>
</tr>
</tbody>
</table>

**Target ID & validation**

< Discovery Research >
## Red/Yellow/Green Approach

<table>
<thead>
<tr>
<th>Criterion</th>
<th>Red/Yellow/Green Approach</th>
<th>Validation Approaches</th>
</tr>
</thead>
<tbody>
<tr>
<td>Target validation</td>
<td>Either genetic or chemical evidence that target is essential for growth or survival</td>
<td>Genetic and chemical evidence that target is essential for growth or survival</td>
</tr>
<tr>
<td>Druggability</td>
<td>Drug-like inhibitors are known and active site is druggable</td>
<td>Drug-like inhibitors are known and druggable active site (i.e., clinical precedent within the target family)</td>
</tr>
<tr>
<td>Assay feasibility</td>
<td>In vitro assay exists, development into plate format feasible but not achieved</td>
<td>Assay ready in plate format and protein supply assured within appropriate timelines</td>
</tr>
<tr>
<td>Toxicity</td>
<td>Human homologue present but some structural or chemical evidence that selective inhibition is possible</td>
<td>No human homologue present or human homologue known to be non-essential</td>
</tr>
<tr>
<td>Resistance potential</td>
<td>Target has isoforms within the same species or might be subject to escape from inhibition</td>
<td>Target has no known isoforms within the same species and is not subject to escape from inhibition</td>
</tr>
<tr>
<td>Structural information</td>
<td>Structure without ligand available and/or poor resolution (&gt;2.3 Å) or opportunity to build a good homology model</td>
<td>Ligand-bound structure of target (or ligand in closely related homologue) available at high resolution (&gt;2.3 Å)</td>
</tr>
</tbody>
</table>

### Table 1. Traffic-light definitions for target assessment

<table>
<thead>
<tr>
<th>Criterion</th>
<th>Red</th>
<th>Amber</th>
<th>Green</th>
</tr>
</thead>
<tbody>
<tr>
<td>Target validation</td>
<td>No or weak evidence that the target is essential for growth or survival</td>
<td>Either genetic or chemical evidence that target is essential for growth or survival</td>
<td>Genetic and chemical evidence that target is essential for growth or survival</td>
</tr>
<tr>
<td>Druggability</td>
<td>No drug-like inhibitors are known and active site is not druggable</td>
<td>Drug-like inhibitors are known and active site is druggable</td>
<td>Drug-like inhibitors are known and druggable active site (i.e., clinical precedent within the target family)</td>
</tr>
<tr>
<td>Assay feasibility</td>
<td>In vitro assay developed and significant problems with reagents (cost or supply)</td>
<td>In vitro assay exists, development into plate format feasible but not achieved</td>
<td>Assay ready in plate format and protein supply assured within appropriate timelines</td>
</tr>
<tr>
<td>Toxicity</td>
<td>Human homologue present and little or no structural or chemical evidence that selective inhibition is possible</td>
<td>Human homologue present but some structural or chemical evidence that selective inhibition is possible</td>
<td>No human homologue present or human homologue known to be non-essential</td>
</tr>
<tr>
<td>Resistance potential</td>
<td>Target has multiple gene copies or isoforms within the same species and is subject to escape from inhibition</td>
<td>Target has isoforms within the same species or might be subject to escape from inhibition</td>
<td>Target has no known isoforms within the same species and is not subject to escape from inhibition</td>
</tr>
<tr>
<td>Structural information</td>
<td>No structure of target or closely related homologue</td>
<td>Structure without ligand available and/or poor resolution (&gt;2.3 Å) or opportunity to build a good homology model</td>
<td>Ligand-bound structure of target (or ligand in closely related homologue) available at high resolution (&gt;2.3 Å)</td>
</tr>
</tbody>
</table>

### Table 2. Strengths and weaknesses of different target-validation methods

<table>
<thead>
<tr>
<th>Method</th>
<th>Strengths</th>
<th>Weaknesses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemical validation</td>
<td>• Addresses the key druggability issues of cell permeability (in vitro whole-cell assays); selective toxicity and drug metabolism (in vivo animal models); safety and efficacy (clinical)</td>
<td>• Highly specific inhibitors not available frequently</td>
</tr>
<tr>
<td></td>
<td>• Identifies non-protein targets</td>
<td>• Lack of specificity or variable cellular pharmacokinetics might lead to poor structure-activity relationships</td>
</tr>
<tr>
<td></td>
<td>• Identifies prodrugs and compounds acting by lethal synthesis</td>
<td>• Correlation between target inhibition and predicted molecular or biochemical phenotype sometimes difficult to demonstrate in vitro or in vivo</td>
</tr>
<tr>
<td>Genetic validation</td>
<td>• Complete genomes available</td>
<td>• Cannot identify non-gene targets (e.g., haemoglobin)</td>
</tr>
<tr>
<td>Knockout methods</td>
<td>• Definitive, ‘clean’ phenotype</td>
<td>• Does not address key druggability issues</td>
</tr>
<tr>
<td></td>
<td>• Few or no off-target effects</td>
<td>• Does not identify drugs acting by lethal synthesis</td>
</tr>
<tr>
<td>RNA interference</td>
<td>• Rapid and easy to perform</td>
<td>• Laborious (usually requires multiple transfections in diploid organisms)</td>
</tr>
<tr>
<td>(RNAi)</td>
<td>• Suitable for multi-copy gene families</td>
<td>• Null mutants for essential genes require genetic or nutritional rescue</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Multi-copy genes can be problematic</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Compensatory (suppressor) mutations can occur</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Not possible in many parasite species</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Off-target effects owing to unintentional silencing</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• ‘Escape’ mutants with essential genes</td>
</tr>
</tbody>
</table>

15
**Target Selection, Validation and Lead Discovery – Genomic Approach**

- **Genome**
- **Transcriptome**
- **Proteome**
- **Metabolome**

(target identification)

**Comparative genomics & metabolomics** → **Comparative biochemistry & molecular biology**

Potential targets

- **Genetic validation** (essentiality)

Validated targets

- **Chemical validation** (selectivity/druggability)

Druggable targets + Lead

(added value)

**Lead Discovery & Target Validation – Chemical Genomics**

- **Parasite**
- **Mammalian cell**

Whole cell screens with chemical libraries / natural products

(selectivity/druggability)

Unknown potential targets & cellular poisons

- **Mode of action studies** (target identification)

Potential druggable targets

- **Genetic validation** (essentiality)

Validated druggable targets + lead

(added value)
Criteria for Target Selection

✓ Essential for survival in appropriate life cycle stage
  Generally not molecules involved in virulence or invasion

✓ Absent, significantly different or non-essential to host
  Examples: trypanothione, Type II Fab, DHFR-TS, ODC

✓ Selective inhibition possible with drug-like molecules
  Avoid enzyme co-factors and phosphorylated substrates

✓ Easy to isolate, overproduce and assay
  Ideally soluble recombinant enzyme, with known structure and chemical mechanism. Generally not multi-component, membrane or structural proteins

✓ Assay modifiable for HTS format
  Ideally “mix and measure” methods. Avoid separation methods / radioisotopes

<table>
<thead>
<tr>
<th>Disease</th>
<th>Target / Molecule</th>
<th>Stage</th>
<th>Academic</th>
<th>Partners and Funders</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malaria</td>
<td>02277/NDX1100 &amp; next-generation analog (2440)</td>
<td>Phase II and proclinical</td>
<td>University of Nebraska</td>
<td>Ramcoy, MMV</td>
</tr>
<tr>
<td>Malaria</td>
<td>2-4-butyloxime</td>
<td>Phase I</td>
<td>University of Liverpool</td>
<td>GSK, MMV</td>
</tr>
<tr>
<td>Leishmaniasis</td>
<td>8-aminoquinoline NPC1191B</td>
<td>Preclinical</td>
<td>University of Mississippi</td>
<td>DNDi</td>
</tr>
<tr>
<td>Malaria</td>
<td>Halopren inhibitors</td>
<td>Lead optimisation</td>
<td>University of California, San Francisco</td>
<td>GSK, MMV</td>
</tr>
<tr>
<td>Trypanosomias</td>
<td>Nucleoside inhibitors</td>
<td>HTD through lead optimisation</td>
<td>University of Oxford (OUI)</td>
<td>Wellcome Trust</td>
</tr>
<tr>
<td>Trypanosomias, Leishmaniasis</td>
<td>diamidines, others</td>
<td>HTD through preclinical</td>
<td>University of North Carolina (UNC)</td>
<td>DNDi, MMV</td>
</tr>
<tr>
<td>Malaria</td>
<td>Antimalarial analogs</td>
<td>Lead optimisation</td>
<td>Johns Hopkins University</td>
<td>Malaria Ts (MTD), Wellcome Trust</td>
</tr>
<tr>
<td>Trypanosomias, Schistosomiasis, Malaria</td>
<td>cyclaine protease inhibitors</td>
<td>Lead ID, lead optimisation</td>
<td>University of California, San Francisco</td>
<td>Calima Genomics, Sandhill Foundation</td>
</tr>
</tbody>
</table>

Table 1