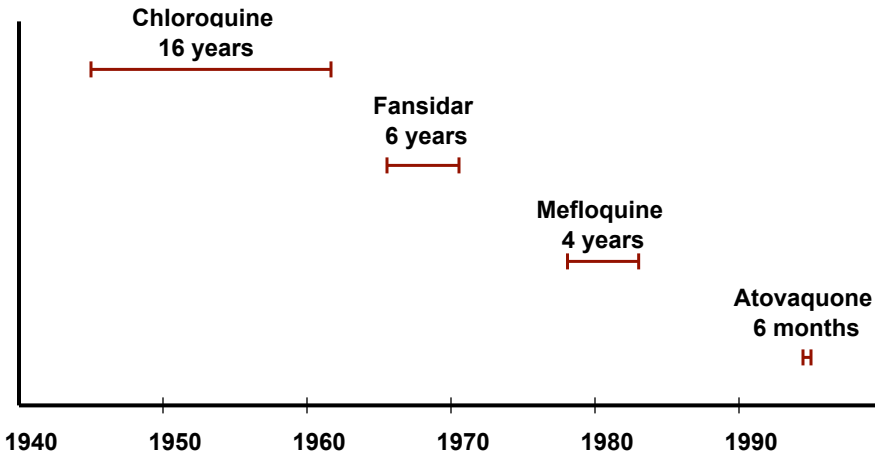
Time to Development of Resistance to Antimalarial Drugs



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

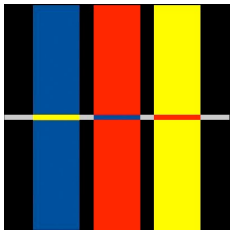
Chemotherapeutic index:
maximum does tolerated by host
minimum curative dose

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 is specific ways
Idea of specific affinity (selectivity)



Methylene blue (1891)

Trypan red (1904)

Compound 606 (1910)

Idea of empirical screening of new drugs
1909 - 1st treatment for syphilis - cmp 606

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
    - Crytosporidiosis
    - 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

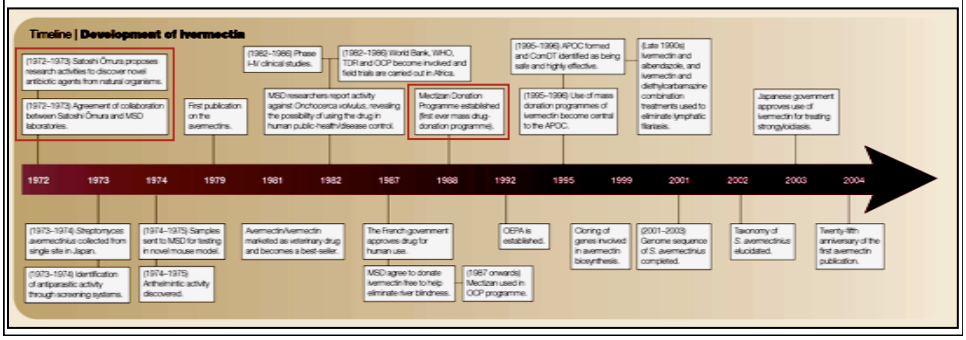
Discovery of Ivermectin

June 20, 2005
Vol. 83, Iss. 25

- Top 40 Pharmaceuticals
  - Drugs that changed our world - Aspirin - Viagra
- Ivermectin - A Wonder Drug
  - ~28 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



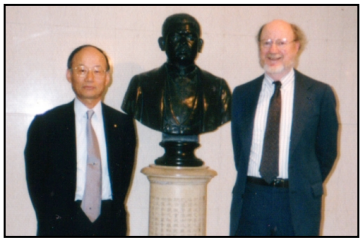
Professor Satoshi Omura

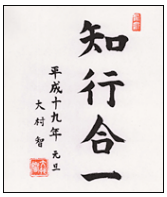
- President of Kitasato Institute
- More than 40 years of studying bioactive compounds from microbes
- Successful Philosophy
  - Unlimited supply of novel compounds
  - Produce gold-standard screening
  - Screening is not just an exercise
  - Contribution of basic research
  - Keep the human connection
- 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

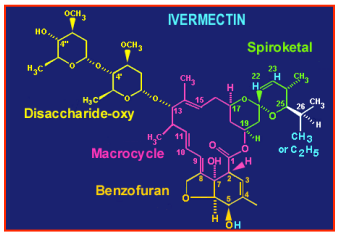
- Screened 40,000 samples - basic curiosity are there compounds that microbes make that can inhibit the worms?
- Found one that worked! (*in vitro* studies)
  - Japanese golf course - Dr. Satoshi Omura
  - Surprisingly powerful against the worms
  - *Streptomyces avermitilis* produced compounds they 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

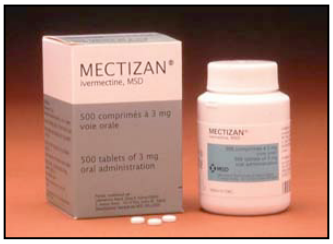
Dr. William Campbell

Dr. Mohammed Aziz

Ivermectin - Broad Spectrum

- Class of compounds called Avermectins - macrocylic lactones
- Lacked antibacterial and antifungal properties
- Ivermectin is the most widely used in 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.**



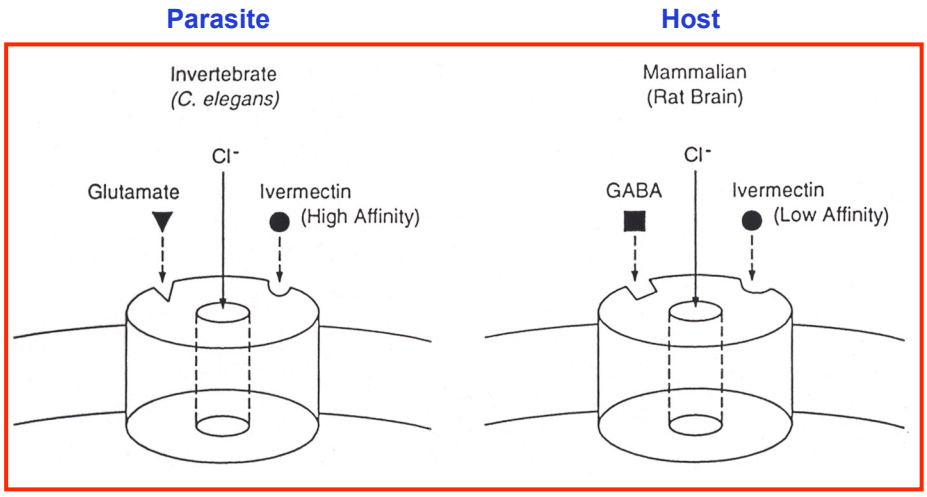


"Medicine is for the people. It is not for profits" - George W. Merck

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



Prevents closure of ion channel
Hyperpolarization of neurons

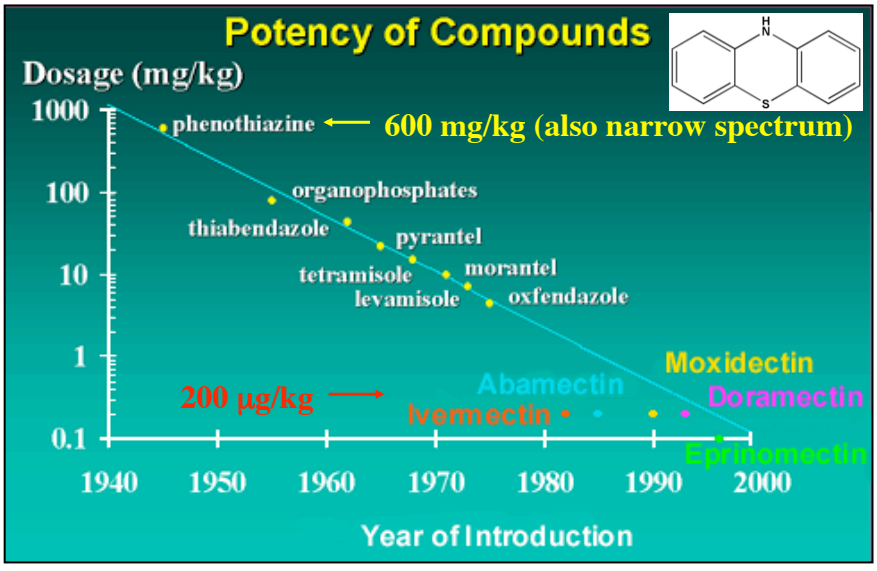
Ivermectin and Oncocerciasis

- 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 microfiliaria!
  - **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

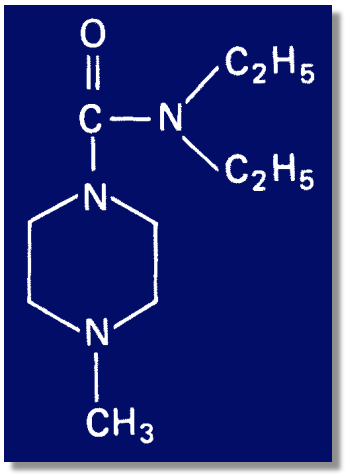
- Good News - Bad News
- OCP ended in 2002
- Conclusion - Onchocerciasis cannot be eradicated in most endemic areas with the current tools
- What is needed:
  - New drug regimens
  - Macrofilaricides
  - Better diagnostics
- Overlap with another program African Program for Onchocerciasis Control (1995)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

Analogues of Known Drugs



Diethylcarbamazine

- Synthetic organic compound with no toxic metallic components
- Tissue and blood nematodes (filarial worms)
- Hyperpolarizing neuro-muscular 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

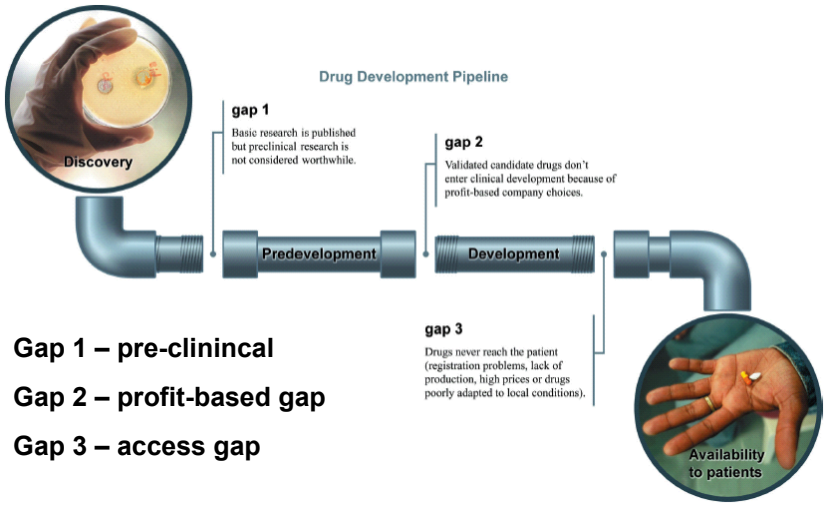
2. Drugs affecting energy generation

- Enteric helminths exist in an anaerobic environment, and have developed special mechanisms for generating energy.

Cofactor synthesis	Nucleic acid synthesis	Protein synthesis	Membrane function	Microtubule function	Energy metabolism	Neuromuscular function
Antiprotozoals						
Amprolium Dapsone Ethopabate Proguanil Pyrimethamine Sulphadiazine Sulphadoxine Sulphamethoxazole Sulphaquinazoline Trimethoprim	Amodiaquine Benznidazole Chloroquine Diminazine aceturate Halofantrine Homodium bromide Isometamidium Mefloquine Metronidazole Nifurtimox Pentamidine Quinine Satranidazole Tinidazole	Eflornithine Quinapyramine Tetracycline	Amphotericin B Lasalocid Monensin Salinomycin		Buparvaquone Clopidol Decoquinate Meglumine antimonate Melarsaprol Parvaquone Primaquine Robenidine Sodium stibogluconate Suramin	
Anthelmintics						
				Albendazole Fenbendazole Mebendazole Oxfendazole Thiabendazole Triclabendazole	Dichlorophen Niclosamide Nitroxynil Oxyclozanide Resorantel	Bephenium Bromophos Dichloros Diethylcarbamazine Haloxon Ivermectin Levamisole Metrifonate Morantel Naphthalophos Oxamniquine Piperazine Praziquantel Pyrantel

Differential uptake/ secretion	Drug activation only in parasite	Unique target in parasite	Drug discriminates between target in host and parasite	Pathway blocked more important in parasite than in host
Antiprotozoals				
Amodiaquine Chloroquine Diminazine aceturate Halofantrine Homidium bromide Isometamidium Mefloquine Pentamidine Quinapyramine Quinine Tetracycline	Benznidazole Metronidazole Nifurtimox Satranidazole Tinidazole	Dapsone Ethopabate Sulphadiazine Sulphadoxine Sulphamethoxazole Sulphaquinazoline Suramin	Amphotericin B Amprolium Buparvaquone Clopidol Decoquinate Eflornithine Parvaquone Primaquine Proguanil Pyrimethamine Robenidine Trimethoprim	Meglumine antimonate Melarsaprol Sodium stibogluconate
Anthelmintics				
			Albendazole Bephenium Bromophos Dichlorvos Diethylcarbamazine Fenbendazole Haloxon Levamisole Mebendazole Metrifonate Morantel Naphthalophos Oxfendazole Piperazine Pyrantel Thiabendazole Triclabendazole	

The research gap – 10/90



Gap 1 – pre-clinincal

Gap 2 – profit-based gap

Gap 3 – access gap

DOI: 10.1371/journal.pmed.0010006.g001

Pecoul, PLoS Med. 2004

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

↓

Year ~6

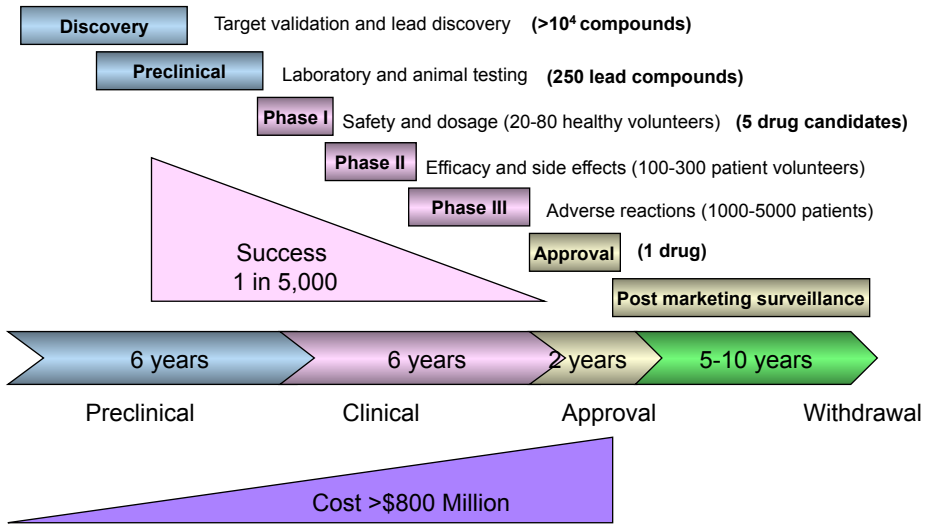
Validate target in vivo: animal models & trials

↓

Year 20

Approval & launch of new drug

Compound Success Rate by Stages



Source: Pharmaceutical Industry Profile 2001

Discovery Research & Drug Discovery Needs

Target identification - bioinformatics & chemical genomics

Target validation - inducible (conditional) gene knockout and RNAi / antisense

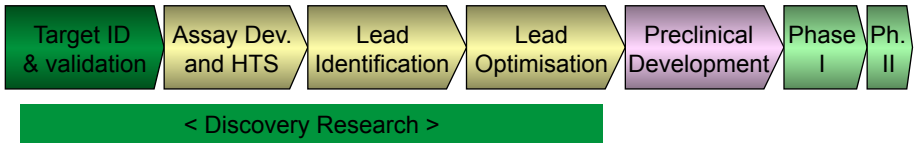
Target characterisation - function, mechanism and structure

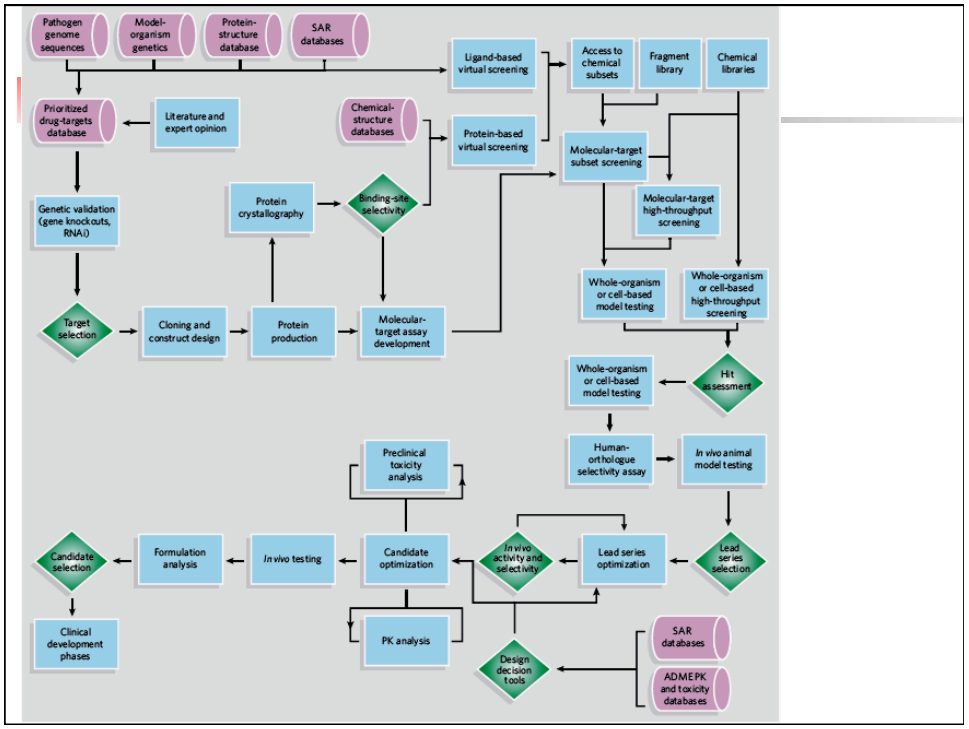
Compound evaluation - reporter systems for screening in vitro and in vivo

Assay development - high throughput screening (identify lead compounds)

Mechanistic studies - modes of drug action and drug resistance mechanisms

Molecular diagnostics - prediction of therapeutic efficacy and drug toxicity, especially non-invasive methods





www.drugdiscovery.dundee.ac.uk/initiatives.html/

Drug Discovery at Dundee

HOMEPAGE

Tropical Disease Initiative

Tropical Disease Initiative - Overview

Review *TRENDS in Parasitology* Vol.23 No.12

Full text provided by www.sciencedirect.com ScienceDirect

Target assessment for antiparasitic drug discovery

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Red/Yellow/Green Approach

Table 1. Traffic-light definitions for target assessment

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

Validation Approaches

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

Method	Strengths	Weaknesses
Chemical validation	• 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) • Identifies non-protein targets • Identifies prodrugs and compounds acting by lethal synthesis	• Highly specific inhibitors not available frequently • Lack of specificity or variable cellular pharmacokinetics might lead to poor structure–activity relationships • Correlation between target inhibition and predicted molecular or biochemical phenotype sometimes difficult to demonstrate *in vitro* or *in vivo*
Genetic validation	• Complete genomes available • Suitable for genes of unknown or uncertain function	• Cannot identify non-gene targets (e.g. haemozoin) • Does not address key druggability issues • Does not identify drugs acting by lethal synthesis
Knockout methods	• Definitive, 'clean' phenotype • Few or no off-target effects	• Laborious (usually requires multiple transfections in diploid organisms) • Null mutants for essential genes require genetic or nutritional rescue • Multicopy genes can be problematic • Compensatory (suppressor) mutations can occur
RNA interference (RNAi)	• Rapid and easy to perform • Suitable for multicopy gene families	• Not possible in many parasite species • No phenotype owing to insufficient silencing • Off-target effects owing to unintentional silencing • 'Escape' mutants with essential genes

Target Selection, Validation and Lead Discovery – Genomic Approach

Genome Transcriptome Proteome Metabolome

(target identification)

Comparative genomics & metabolomics

Comparative biochemistry & molecular biology

Potential targets

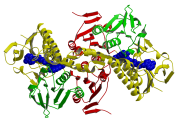
Genetic validation (essentiality)

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

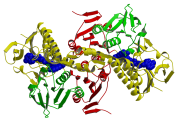
Mode of action studies (target identification)

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

Selected antiparasitic drug discovery projects involving collaboration between academics, not-for-profit research organisations and pharmaceutical companies

Disease	Target / Molecule	Stage	Academic site	Partners and Funders
Malaria	OZ277/RBx11160 & next-generation analog OZ439	Phase II and preclinical	University of Nebraska	Ranbaxy, MMV
Malaria	*N*-*t*-butyl isoquine	Phase I	University of Liverpool	GSK, MMV
Leishmaniasis	8-aminoquinoline NPC1161B	Preclinical	University of Mississippi	DNDi
Malaria	falcipain inhibitors	lead optimisation	University of California, San Francisco	GSK, MMV
Trypanosomiasis	kinase inhibitors	hit ID through lead optimisation	University of Dundee (DDU)	Wellcome Trust
Trypanosomiasis, Leishmaniasis	diamidines, others	hit ID through preclinical	University of North Carolina (CPDD)	DNDi, MMV
Malaria	artemisinin analogs	lead optimisation	Johns Hopkins University	Novartis (NITD), Wellcome Trust
Trypanosomiasis, Schistosomiasis, Malaria	cysteine protease inhibitors	lead ID, lead optimisation	University of California, San Francisco	Celera Genomics, Sandler Foundation

Table 1