Clinical Research on Traditional Medicines

Dr Merlin Willcox
Clinical Researcher
Plan

- Why traditional medicine?
- Innovative methods for clinical research on TMs
- Research capacity strengthening
Why study traditional herbal medicine?

- Has been the source of many of our most effective medicines
- Africa has a “competitive advantage”
  - Very rich traditions of herbal medicine
- Available and affordable locally
- Can generate income for farmers and producers
- Preferred by many patients
- Is in widespread use
What is different about traditional medicine?

- People are using it already
  - observational studies can be done before any laboratory work
- History of traditional use, with no observed adverse effects, reduces the need for pre-clinical toxicology
- Less funding available
  - approaches need to be more cost-effective
- Multidisciplinary teams are essential:
  - Clinicians
  - Statisticians
  - Traditional healers
  - Botanists, anthropologists
  - Pharmacologists, Chemists
How to research and develop traditional medicines?
Conventional Drug Discovery

- **In vitro studies**
  - Inactive
  - Cytotoxic
  - Already known
  - Unstable
  - Synergic effects or « difficult » molecule

- **Animal studies**
  - Inactive
  - Toxic
  - Not absorbed
  - Metabolized

- **Clinical trials**
  - Inactive
  - Metabolized
  - Not absorbed
  - Serious Side effects
Conventional Drug Discovery

- Takes 15 years
- Costs up to $800m
- End product is often unaffordable and unavailable to the poor
But there are other approaches…
Reverse Pharmacology

- Took 6 years to develop an “improved traditional phytomedicine” in Mali
- Cost about 0.4m Euros
- End product is easily affordable and available
Aim

- To develop a new cost-effective antimalarial phytomedicine for Mali
- For production as an “Improved Traditional Medicine”
- And for local cultivation and production at the village level
Stage 1: Selection of a remedy
Retrospective Treatment Outcome Study

- Household heads interviewed at end of rainy season
- “Has anyone in your household had malaria / fever in the last 2 weeks?”
- “If so, what treatment(s) did they take?”
- “What was their outcome?”

Graz et al (2005), J Ethnopharm
RTO in Mali

- Studied case histories of 952 children with recent “malaria”
  - 87% treated at home
  - 40% with modern medicine alone
  - 33% with modern + trad medicine
  - 27% with trad medicine alone
- 66 plants used traditionally for treatment of malaria

RTO: analysis

- Adjust for confounding factors
- Select traditional medicines which
  - are systematically associated with rapid and complete cure,
  - with no failures

Table 1 Sample results from the RTO study for the three most promising plants (the full table included 66 plants in total)

<table>
<thead>
<tr>
<th>Plant</th>
<th>Preparation</th>
<th>No of cases reporting use</th>
<th>No of cases reporting clinical recovery</th>
<th>No of treatment failures</th>
<th>Correlation with clinical recovery</th>
<th>(95% CI)</th>
<th>P (Fisher)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Argemone mexicana</td>
<td>Aerial parts</td>
<td>30</td>
<td>30</td>
<td>0</td>
<td>100%</td>
<td>(88-100)</td>
<td>NA (best results)</td>
</tr>
<tr>
<td>(Papaveraceae)</td>
<td>decoction</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carica papaya</td>
<td>Leaves</td>
<td>33</td>
<td>28</td>
<td>5</td>
<td>85%</td>
<td>(68-95)</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>decoction</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anogeissus leiocarpus</td>
<td>Leaves</td>
<td>33</td>
<td>27</td>
<td>6</td>
<td>82%</td>
<td>(64-93)</td>
<td>0.03</td>
</tr>
</tbody>
</table>

*(Number with and without clinical recovery compared to the plant with best results.*
Selection of a plant

- *Argemone mexicana* associated with clinical recovery in all cases.
- Four crude extracts had IC$_{50}$ <5mcg/ml:
  - *Spondias mombin*,
  - *Opilia celtidifolia*,
  - *Securinega virosa*,
  - *Argemone mexicana*.

Stage 2: Dose-escalating clinical study

- Observation of patients who chose to take the remedy
- Diagnosis of malaria confirmed (microscopy)
- Starting at lowest dose traditionally used
- Increasing dose according to response
Dose Optimisation

Range of traditionally recommended doses: *Start with lowest dose*

Clinical results

Good effectiveness

Safe and well tolerated

NO

Decrease dose

YES

Optimal dose

Insufficient effectiveness

Safe and well tolerated

NO

Decrease dose

YES

Increase dose

Stop the trial (failure)
Inclusion criteria

- Traditional healer decided to treat with “AM”
- Clinical diagnosis of malaria
- Parasitaemia >2000 / mcl
- No other obvious cause of fever
- No signs of severe malaria
- Informed consent
Clinical follow-up

- Days 1, 2, 3, 7, 14, 28
- Additional consultations as necessary
- At each follow-up: history, temperature, clinical examination
- Laboratory analyses: parasitaemia, haematocrit, platelets, WCC, Creatinine, AST, ALT, ECGs.
Dose escalation

- Dose of remedy in 3 groups:
  - A: Low range = od for 3 days
  - B: Mid range = bd for 7 days
  - C: High range = qds for 4 days, then bd for 3 days
Baseline characteristics

<table>
<thead>
<tr>
<th>Group</th>
<th>A</th>
<th>B</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>23</td>
<td>40</td>
<td>17</td>
</tr>
<tr>
<td>% males</td>
<td>48%</td>
<td>50%</td>
<td>47%</td>
</tr>
<tr>
<td>Mean age (years)</td>
<td>3.69</td>
<td>4.74</td>
<td>3.25</td>
</tr>
</tbody>
</table>
D14 efficacy by intention to treat

<table>
<thead>
<tr>
<th>Dose group:</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>P=</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>23</td>
<td>40</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>Adequate Clinical Response</td>
<td>39%</td>
<td>72.5%</td>
<td>64.7%</td>
<td>0.03</td>
</tr>
<tr>
<td>Lost to Follow-Up</td>
<td>9%</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>
Parasitaemia in all patients during treatment with *Argemone mexicana* alone

<table>
<thead>
<tr>
<th>Day</th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>28</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Adverse effects

- Diarrhoea / cough in 17-25% of patients (all doses)
- Transitory elevation of AST/ALT in 7 patients
- At highest dose, prolonged QTc interval in 2 patients.
- No serious adverse effects
Further Toxicology

- Chemical analysis: no sanguinarine detected in decoction.
- Animal tests: no evidence of acute toxicity at doses up to 3.2g/kg of the freeze-dried tea (equivalent to 35g/kg of plant powder)
Conclusions:

- AM decoction appears effective in a dose-escalating study
- The optimal dosage is bd for 1 week
- This dosage is safe and well tolerated

Stage 3: Randomised Controlled Trial

- **P**: patients with presumed uncomplicated malaria, all ages
- **I**: *Argemone mexicana* decoction twice daily for 7-14 days
- **C**: Artesunate-amodiaquine
- **O**: outcomes:
  - Clinical recovery (no need for 2\(^{nd}\) line Rx)
  - Incidence of new clinical episodes of malaria;
  - Incidence of severe malaria
Consultation with village health worker
Informed Consent
RCT: AM vs ACT

313 patients consult healer with presumed malaria

301 patients included
Median age: 5 years
87% + P. falciparum
(Mean parasitaemia = 871)

12 excluded

4 excluded during FU

AM group (N=197)

ACT group (N=101)

0 lost to FU!!
Clinical recovery and new episodes

- **AM**
  - Clinical recovery: 89.3%
  - New episode 15-28: 12.8%

- **CTA**
  - Clinical recovery: 95%
  - New episode 15-28: 9.9%

The chart illustrates the percentage of clinical recovery and new episodes for the two treatment groups, AM and CTA.
## Follow-up of first 28 days

<table>
<thead>
<tr>
<th></th>
<th>AM group</th>
<th>ACT group</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mortality</strong></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>Severe mal &gt;5yo</strong></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>Severe mal (0-5 yo)</strong></td>
<td>1.0% (0.02 – 5.3)</td>
<td>1.9% (0.05 – 10.3)</td>
</tr>
<tr>
<td><strong>Coma / convulsions</strong></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>Adverse effects</strong></td>
<td>14% (cough, diarrhoea)</td>
<td>19% (vomiting)</td>
</tr>
</tbody>
</table>
Incidence of severe malaria over 3 months (% children aged 0-5y)

Coma/convulsions: 2% in each group

AM: 4.9
ACT: 3.8
Presence of parasites and incidence of new episodes of malaria

- Parasite positive, AM (n=174)
- Parasite positive, ACT (n=88)
- New clinical episodes, AM (n=174)
- New clinical episodes, ACT (n=88)

% of patients

Day of follow-up
Comparison of AM and ACT strategies: cost per episode (FCFA)
Stage 4: Isolation of “active compounds”

- For agricultural selection of best plants
- For standardisation
- For quality control
- What is an “active compound”?
LC-MS analysis of *Argemone* decoction

![Image of LC-MS analysis](image)

- **Base Peak**
  - 15.93 370.15

- **Allocryptopine**
  - 17.67 336.16

- **Berberine**
  - 15.93 370.15
  - 17.67 336.16
  - 18.68 348.13

- **Protopine**
  - 11.15 297.04
  - 12.47 317.17
  - 14.01 317.17
  - 15.28 354.13
  - 17.17 336.16
  - 17.67 336.16
  - 18.68 348.13
  - 26.42 415.03
  - 29.63 341.28
  - 33.97 282.05
## Argemone alkaloids - activity

<table>
<thead>
<tr>
<th></th>
<th>P. falciparum*</th>
<th>T. cruzi</th>
<th>T. b. brucei</th>
<th>Cytotoxicity**</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IC$_{50}$ (µg/mL)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>protopine (A1)</td>
<td>0.32</td>
<td>&gt;32.00</td>
<td>10.75</td>
<td>&gt;32.00</td>
</tr>
<tr>
<td>allocryptopine (A2)</td>
<td>1.46</td>
<td>&gt;32.00</td>
<td>10.49</td>
<td>&gt;32.00</td>
</tr>
<tr>
<td>berberine (A3)</td>
<td>0.32</td>
<td>0.32</td>
<td>1.66</td>
<td>3.20</td>
</tr>
<tr>
<td>A4</td>
<td>8.58</td>
<td>7.85</td>
<td>&gt;32.00</td>
<td>24.78</td>
</tr>
<tr>
<td>sanguinarine</td>
<td>7.02</td>
<td>7.42</td>
<td>&gt;32.00</td>
<td>16.26</td>
</tr>
</tbody>
</table>

*IC$_{50}$ < 2.0 µg/mL: highly active

** On human fibroblasts (MRC-5). IC$_{50}$ < 10 µg/mL: highly toxic
Pharmacokinetic study

- Healthy adult volunteers with / without asymptomatic malaria
- Samples of blood at different time intervals after ingestion of *A. mexicana* decoction
- Analysis of blood
Lessons Learned

- “Reverse pharmacology” is a viable method for the development of phytomedicines
- It is becoming the PREFERABLE method
- Phytomedicines can be developed in parallel with conventional drug development
- Dose escalation is a crucial step
What would have happened in a conventional approach?

- *Argemone mexicana* screened and active in vitro
- Bioguided fractionation reveals berberine as the “active compound”
- NOT effective in animal models (poor bioavailability)
- Dustbin
Conventional Drug Discovery

In vitro studies
- Inactive
- Cytotoxic
- Already known
- Unstable
- Synergic effects or "difficult" molecule

Animal studies
- Inactive
- Toxic
- Not absorbed
- Metabolized

Clinical trials
- Inactive
- Metabolized
- Not absorbed
- Serious Side effects

Diagram showing the process from in vitro studies, animal studies, to clinical trials, highlighting the failures and losses at each stage.
A “reverse pharmacology” approach for developing an anti-malarial phytomedicine

Merlin L Willcox, Bertrand Graz, Jacques Falquet, Chiaka Diakite, Sergio Giani, Drissa Diallo

Abstract
A “reverse pharmacology” approach to developing an anti-malarial phytomedicine was designed and implemented in Mali, resulting in a new standardized herbal anti-malarial after six years of research. The first step was to select a remedy for development, through a retrospective treatment-outcome study. The second step was a dose-escalating clinical trial that showed a dose-response phenomenon and helped select the safest and most efficacious dose. The third step was a randomized controlled trial to compare the phytomedicine to the standard first-line treatment. The last step was to identify active compounds which can be used as markers for standardization and quality control. This example of “reverse pharmacology” shows that a standardized phytomedicine can be developed faster and more cheaply than conventional drugs. Even if both approaches are not fully comparable, their efficiency in terms of public health and their complementarity should be thoroughly...
Multi-disciplinary University Traditional Health Initiative: Building Sustainable Research Capacity on Plants for Better Public Health in Africa

EU 7th Research Framework Programme – Theme HEALTH, Coordination and support action
Grant Agreement No.: 266005
MUTHI workpackages

- 1: Medical Anthropology and Ethnobotany
- 2: Quality Control of phytomedicines and nutraceuticals
- 3: Bioactivity and safety of phytomedicines and nutraceuticals
- 4: Observational and Clinical trials
- 5: Intellectual Property Rights
- 6: Management
Training needs assessment

- 58 responded by the deadline (response rate = 63%).

<table>
<thead>
<tr>
<th>Country</th>
<th>No of institutions</th>
<th>No of respondents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uganda</td>
<td>11</td>
<td>18</td>
</tr>
<tr>
<td>Nigeria</td>
<td>6</td>
<td>12</td>
</tr>
<tr>
<td>Cameroon</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Mali</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>Sudan</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>South Africa</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Burkina Faso</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Democratic Republic of Congo</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Tanzania</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Gabon</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Kenya</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Madagascar</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Mozambique</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Zambia</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>
Experience in clinical trials of herbal medicines

- Thirty-two (57%) of the respondents had already been involved in conducting a clinical trial of a herbal medicine.
  - 23 completed
  - 5 ongoing
- Commonest conditions studied were
  - malaria (9 trials)
  - HIV/AIDS (6 trials)
- Fifty-four (96%) of the researchers were planning future clinical trials of herbal medicines
5 trials (21%) had been published

- Three of these in a peer-reviewed, Medline-listed journal.
- One had resulted in a product with an official marketing authorisation: NIPRISAN for the prevention of crises in patients with sickle cell disorder.
- Another product (FARADIN, also for sickle cell disease) had been licensed although the trial had not been published.
### Difficulties encountered

<table>
<thead>
<tr>
<th>Broad category</th>
<th>Examples</th>
<th>Frequency in completed / ongoing trials (n=28)</th>
<th>Frequency anticipated in future trials (n=54)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resource constraints</td>
<td>Lack of funding; lack of equipment; lack of human resources</td>
<td>19</td>
<td>35</td>
</tr>
<tr>
<td>Social acceptance of the clinical trials</td>
<td>Rapport with traditional healers, willingness of healers to cooperate; difficulty involving biomedical doctors; patient recruitment; patient compliance</td>
<td>18</td>
<td>25</td>
</tr>
<tr>
<td>Logistical constraints</td>
<td>Loss to follow-up of patients; distance to study sites; trial management</td>
<td>9</td>
<td>8</td>
</tr>
<tr>
<td>Herbal medicine supply</td>
<td>Cultivation, sustainable harvesting and production of herbal medicine; formulation and quality control</td>
<td>9</td>
<td>19</td>
</tr>
<tr>
<td>Need for training or support</td>
<td>Lack of trained staff</td>
<td>6</td>
<td>12</td>
</tr>
<tr>
<td>Trial design</td>
<td>Protocol design, blinding, data management and analysis</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Ethics</td>
<td>Obtaining ethical approval</td>
<td>2</td>
<td>5</td>
</tr>
</tbody>
</table>
Global Health Trials e-Learning Short Courses

Our e-learning short courses are designed to cover every step, process, and issue that needs to be understood in order to conduct a high quality clinical study. These courses should take about 45 minutes to complete and a certificate is issued on completion. Every course is written to be globally applicable, so for all diseases and all regions. They are also highly pragmatic and adaptable. Each course is carefully researched to provide up to date and high quality material that is peer reviewed and regularly reviewed and updated.

These courses are built through the support and partnership of the Bill and Melinda Gates Foundation, the World-Wide Antimalarial Resistance Network (www.wwarn.org) and The East African Consortium for Clinical Research (www.eaccr.org).

Available courses:

1. Introduction to Clinical Research - Français, Español, 中文, Português, Việt
2. ICH Good Clinical Practice - Français, Español
3. Setting the Research Question - Français, Español, 中文, Việt
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4. The Research Protocol: Part one - Français
5. The Research Protocol: Part two - Français
6. Data Safety Monitoring Boards for Clinical Trials - Français
7. Introduction to Consent
8. Introduction to Data Management For Clinical Research Studies
9. Introduction to Collecting and Reporting Adverse Events in Clinical Research
10. Introduction to Reviewing Genomic Research (New!)
11. Basic Malaria Microscopy (New!)
12. The Retrospective Treatment Outcome Study (New!)

We will continue to extend this list of courses so tell us if you need to learn about something that you do not see listed here. Please also get in touch if you currently teach a course that we have not listed. All of our courses are continuously updated and are peer reviewed. Therefore please do let us know if you have comments or remarks as we can incorporate these into the courses.
The Retrospective Treatment Outcome Study (RTO)

This module is designed for researchers interested in selecting traditional medicinal plants for further research (including pre-clinical, and clinical trials).

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Funding:
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http://www.mn.ulo.no/farmas/english/research/projects/muthi/
It was funded by the European Union Research Directorate through the MUTHI project, FP7 Grant Agreement No : 266005.

Reviewers:
MUTHI clinical research course
Makerere University, 7-13 Feb 2014

- 25 participants (clinicians, pharmacologists, chemists, botanists, traditional healers)
- Syllabus:
  - Clinical trial design
  - Writing a protocol and ethical approval
  - Statistics: sample size and analysis
  - Data management and monitoring
  - Reporting and publication
  - Critical appraisal and systematic reviews
Plans for ongoing support after the workshop

- Mentoring to help participants
  - develop their protocols,
  - apply for funding and ethical approval,
  - carry out the research
  - publish it.
- Online training materials
- Cascading of training
Our goal: high quality, standardised, safe, effective, evidence-based “improved traditional medicines”