International Health Lecture 2012

Health impacts of product development partnerships

Professor Janet Hemingway FRS FMedSci
Director of the Liverpool School of Tropical Medicine
The Impact of PDPs on Health
Malaria Focused PDPs

Medicines for Malaria Venture

Malaria Vaccine Initiative

Innovative Vector Control Consortium
• Why do we need Product Development Partnerships
• The role of PDPs in Vector Borne Disease
• Progress in new Vector Control Interventions
Market Rupture Needed

1956

Music: Vinyl Disc

Telephone: Wire Phone

Television: Cathode Ray Tube

IRS: Hudson Sprayer WP formulation 2000 mg DDT/m²

2006

Music: CD and MP3

Telephone: Mobile+Camera+

Television: Plasma/LCD HD

IRS: Hudson Sprayer WP formulation 2000 mg DDT/m²
BMGF supports IVCC as the leading Product Development Partnership in Vector Control

“Your promising research, and the rising concern of people around the world, represent an historic opportunity not just to treat malaria or to control it—but to chart a long-term course to eradicate it”

• BMGF / Boston Consulting Group Study
  – Eradication will cost $ 4-6 Bn per year until it is complete.
  – Vector Control is ~30 % of that.
The Ideal Portfolio; Synergy of Key Objectives

Formulation and Repurposing: For Resistance Management and Residuality

3 x Repurposed AI’s in LLIRS
1-2 x Agricultural AI’s Repurposed in LLIN
Cost $M 9-12 by 2010-2013

Novel Sustainable Public Health Products: New Actives

3 New AI’s without shared target sites or metabolic detoxification pathways
Cost $M 200-700 by 2020

Strategy & Best Practice: Information systems & Tools For Vector Control

5 Kits
Cost $57 M by 2020

“Casa Segura” Project Under way
Options for This sub portfolio under discussion
IVCC Public Health Insecticides Portfolio

New Active Ingredients
- Discovery Platform
  - Bayer (5 Classes)
  - AI Library Screening
    - Syngenta (3 Classes)
  - AI Library Screening
    - Dow
  - Screening Platform
    - Scynexis

New Formulations
- LLIRS Formulation
  - Dupont
  - BASF
  - Sumitomo (3 Classes)
- LLIN Combination
  - BASF
  - Sumitomo
  - Vestergaard
  - Agricultural AI Review
  - Fungal Biopesticide

Proof of Concept
- Data Mining
  - Lead Generation
  - Screening
- Lead Optimisation
- Development
  - Toxicology
  - AI Library Screening
    - Syngenta (3 Classes)
  - AI Library Screening
    - Dow
  - Screening Platform
    - Scynexis

Registration
- Proof of Concept
- Development
- WHOPES
  - Phase I
  - Phase II
  - Phase III
- Country Registration

Country Registration

Legend:
- Active
- Under Negotiation
- In Preparation
How do we assess value?

The long term role of competition

Cost of Goods Example

DDT → Malathion → Pyrethroids
Insecticide treated nets (ITNs) - indoor residual spraying (IRS): major control measures in the fight against malaria.

Vector Data and insecticide Quality Assurance: vital elements in planning successful - sustainable interventions

The IVCC is funding the development of diagnostic tools for these traits which can dramatically improve insecticide based malaria control
The IVCC is sponsoring the development of simple tests for measuring insecticides in situ.

As - Is

Days  Variation  Complicate  Expensive

To be

Quick  Accurate  Easy  Cheap

Uganda 2009
Detection technologies

**Pyrethroids (type II)**

- IVCC
- COMBATING INSECT BORNE DISEASE
- Add DDT extract to enzyme (Cl\(^-\) release) (Room temp., 1 hr)
- Evaporate (water bath, 1.5 - 2 hr)
- Reconstitute with water and measure Cl\(^-\) (DDT conc., Quantab strips, 5 - 10 min)

**OPs / Carbamates**

- Chemical Test
  - <5 min
  - Hydrolysis
  - Measure color intensity (Photometer)
  - Check yellow intensity against color chart

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**Fig 2. Basic components of the DQK**

- Kaur et al, MIM 2009
- Dowd et al 2009
- Morou, Vontas et al (in preparation)
Evaluation of the GST-based biosensor DDT “Kit” in the field: WHO – India & Africa

(Leishmaniasis - sandflies)
Insecticide (lambda cyhalothrin) content measured on small (R=1cm) pads (9/house) by IQK the day after spraying. Representative bad (House #1) and good (House #6) spraying is shown, as well as pads being removed and lambda cyhalothrin content measured. Heat map on right indicates some patchy spraying (i.e. varied concentrations at individual positions), however, 85% of averaged (pooled) concentrations of houses (N=30) were sprayed at or above required rate (20 mg/cm²) indicating effective coverage. Mop-up operations to include re-spraying House #1, identify spray teams responsible for sub-optimal spraying and consider retraining if poor quality consistently linked with a particular team(s).
The IQK for bendiocarb was integrated into the routine surveillance of the Bioko Island vector control program.

**WP 1: Quality control of IRS intervention using IQK, to inform the BIMCP-EGMCI about the effectiveness of the activity**

More than 1500 samples from both pre and post sprayed surfaces were taken and analyzed at Bioko.
**IQK vs HPLC for measuring insecticide from IRS**

* v. good correlation; IQK doesn’t have the ability to calculate the exact amount of insecticide, but it can detect the walls that have insecticide above certain thresholds

<table>
<thead>
<tr>
<th>House</th>
<th>IQK test (µg/cm²)</th>
<th>HPLC (µg/cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>House no 0097534 (b.s.)</td>
<td>Living room high</td>
<td>&lt;0,1</td>
</tr>
<tr>
<td></td>
<td>Living room medium</td>
<td>&lt;0,1</td>
</tr>
<tr>
<td></td>
<td>Living room low</td>
<td>&lt;0,1</td>
</tr>
<tr>
<td></td>
<td>Bedroom high</td>
<td>&lt;0,1</td>
</tr>
<tr>
<td></td>
<td>Bedroom medium</td>
<td>&lt;0,1</td>
</tr>
<tr>
<td></td>
<td>Bedroom low</td>
<td>&lt;0,1</td>
</tr>
<tr>
<td>House no 112898</td>
<td>Living room</td>
<td>1-5</td>
</tr>
<tr>
<td></td>
<td>Bedroom</td>
<td>1-5</td>
</tr>
<tr>
<td></td>
<td>living room</td>
<td>1-5</td>
</tr>
<tr>
<td></td>
<td>Bedroom</td>
<td>1-5</td>
</tr>
<tr>
<td>House no 162544</td>
<td>Living room high</td>
<td>&gt;10</td>
</tr>
<tr>
<td></td>
<td>Living room medium</td>
<td>&gt;10</td>
</tr>
<tr>
<td></td>
<td>Living room low</td>
<td>&gt;10</td>
</tr>
<tr>
<td></td>
<td>Bedroom high</td>
<td>&gt;10</td>
</tr>
<tr>
<td></td>
<td>Bedroom medium</td>
<td>&gt;10</td>
</tr>
<tr>
<td></td>
<td>Bedroom low</td>
<td>&gt;10</td>
</tr>
</tbody>
</table>
The IQK was found easy to use by spray supervisors

feedback for further simplification / optimisation obtained
(easy of use; most directly informative scale)
Vector Population Monitoring

- Species
- Infection status
- Insecticide Resistance status
Infection and insecticide resistance detection in *Anopheles gambiae* and *Aedes aegypti*

- **Phenotype populations for insecticide resistance**
- Identify candidate loci associated with insecticide resistance
  - Gene expression group: microarrays, qPCR
  - Genotyping group: association mapping
- **Validate** that the candidates are the likely cause of resistance
  - Enzyme characterization group
- Develop DEC appropriate technology for detecting the validated resistance locus
- **Field test** the kits in DEC setting Africa, Latin America and South East Asia
**TaqMan Plasmodium assay**

Assay tested with artificially infected mosquitoes (Bob Sinden) and field collected mosquitoes stored in Ethanol, Isopropanol, on Silica

- More sensitive than nested gold standard
- Not inhibited by three storage conditions
- Cheaper than nested PCR (US$ ~0.5)
- Much higher throughput than PCR
- Now main diagnostic used at MRC, Durban
Diagnostics for resistance management

**Anopheles gambiae**

**Knockdown-resistance**
Bass *et al* Malaria Journal 2007, 6:e111 (13 August 2007)
Ridl *et al* Malaria Journal 2008 7: e194 DEC trial (also *Plasmodium* detection)

**iAcetylcholinesterase**
Bass *et al* 2010 Pesticide Biochemistry and Physiology (in press)

**GABA receptors (Dieldrin/cyclodienes)**
Nikou *et al* (in prep)

**Species identification**
Bass *et al* Malaria Journal 2007, 6: e155 (22nd November 2007)

**Plasmodium infection**

**Anopheles funestus**

**Species identification**
Vezenegho *et al* Malaria Journal 2009 8:e 282
CYPs that detoxify deltamethrin

Mark Paine et al, unpublished
Experimental approach

1 h exposure deltamethrin
- Mortality recorded 24 hours after
- Survivors were stored in RNA later
- Deads were dried with silica gel.

Reared to adulthood
Tube bioassays on 3 day old non blood fed females

10-15 min exposure bendiocarb
Control tube = non exposed mosquitoes

Bioko

LSTM

RNA labeling (cy3/cy5) → RNA control quality → RNA extractions 3 pools of 16-17 females/treatment → DNA extractions Species ID (SINE PCR)

RNA extractions 3 pools of 16-17 females/treatment

Microarrays

Bioanalyser

Species ID (SINE PCR)

24
Bioassay results

Bioassays showed very low resistance to Bendiocarb, but resistance against deltamethrin (mortality after 1 hour exposure: 40%)

What is causing pyrethroid resistance?
Experimental approach: Microarrays

Use of the “Agilent Anopheles chip”

- Per slide: eight arrays containing 15,000 probes.

This approach allows to work on the whole transcriptome
Experimental approach: Microarrays

- Mortality after 10-15 min exposure to bendiocarb: 85% → Low resistance level

- Dye swaps were made for each comparison.

- Mortality after one hour exposure to deltamethrin: 40%

- BIOKO non exposed

- BIOKO Bendiocarb survivors

- BIOKO Deltamethrin survivors

- NGUSSO M form Cameroon
Microarray results

Gene expression was analyzed using microarrays. No significant differentially transcribed genes were identified in the BIOKO non-exposed group, while 553 differentially transcribed genes were observed in the BIOKO Bendiocarb survivors group and 608 differentially transcribed genes in the NGUSSO M form Cameroon group. Deltamethrin survivors also showed 512 differentially transcribed genes.

Genes are considered significantly over/under-transcribed with an absolute fold change > 2 and a corrected p-val < 0.01 (Benjamini Hochberg correction).
## Microarray results

<table>
<thead>
<tr>
<th>Transcript ID</th>
<th>Description (Blast2GO annotation)</th>
<th>Ngusso vs Non exposed</th>
<th>Ngusso vs Deltamethrin survivors</th>
<th>Ngusso vs Benocarb survivors</th>
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</thead>
<tbody>
<tr>
<td>AGAP012296-RA</td>
<td>CYP9J5 - Cytochrome P450 monoxygenase</td>
<td>17</td>
<td>7.61</td>
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<tr>
<td>AGAP002113-RB</td>
<td>cytochrome b5</td>
<td>4.00</td>
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<td>AGAP005599-RA</td>
<td>CYP302A1 - Cytochrome P450 monoxygenase</td>
<td>3.26</td>
<td>3.59</td>
<td>3.25</td>
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<td>AGAP002863-RA</td>
<td>COEA6O - Carboxylesterase</td>
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<td>AGAP000284-RA</td>
<td>cytochrome P450 (Novel?)</td>
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<td>AGAP002113-RC</td>
<td>cytochrome b5</td>
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<td>AGAP000458-RA</td>
<td>glutathione transferase GSTD12</td>
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<td>GSTF1 - Glutathione S-transferase</td>
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<td>COEF3C - Carboxylesterase</td>
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<td>AGAP000497-RA</td>
<td>CPR134 - cuticle protein</td>
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<td>AGAP005385-RA</td>
<td>CPR123 - cuticle protein</td>
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<td>AGAP003579-RA</td>
<td>CPR 117 - cuticle protein</td>
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<tr>
<td>AGAP012795-RA</td>
<td>cuticle protein patellae</td>
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<tr>
<td>AGAP019056-RA</td>
<td>CPR5 - cuticular protein 5 from CPF1 family</td>
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<td>AGAP019058-RA</td>
<td>CPR7 - cuticular protein 7 from CPF1 family</td>
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<td>AGAP006226-RA</td>
<td>aldehyde oxidase</td>
<td>46.13</td>
<td>41.74</td>
<td>35.40</td>
</tr>
</tbody>
</table>

### Cuticular genes

- AGAP000497-RA: CPR134 - cuticle protein
- AGAP005385-RA: CPR123 - cuticle protein
- AGAP003579-RA: CPR 117 - cuticle protein
- AGAP012795-RA: cuticle protein patellae
- AGAP019056-RA: CPR5 - cuticular protein 5 from CPF1 family
- AGAP019058-RA: CPR7 - cuticular protein 7 from CPF1 family

### Oxidative stress

- AGAP006226-RA: aldehyde oxidase
- AGAP011054-RA: TPX2 - Thioredoxin dependent peroxidase

### ABC transporters

- AGAP011518-RA: atp-binding cassette sub-family a member = ABCA1
- AGAP001416-RA: abc transporter
- AGAP003504-RA: atp-binding cassette sub-family a member
The common known pyrethroid metabolizers in *An gambiae* CYP6P3 and CYP6M2 are NOT significantly overexpressed in Anopheles populations from Bioko.

Up-regulations of some other putative detoxification enzymes, such as monooxygenases CYP9J5.
No AChE resistance mutations present (also no apparent metabolic resistance to Bendiocarb; it remains a good option)

Pyrethroid resistance mutations present at moderate frequency (F = 58% and L = 42%)
Sporozoite rate in *A. gambiae* relative to spray round, Bioko 2004
What Does this Mean for Insecticide Choice?
International Health Lecture 2012

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