Antimalarial drug development through product development partnerships

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The University of Tokyo

GHIT-PDPs Webinar Series:
Advancing innovations for neglected disease
during and beyond the pandemic
September 3, 2021
Screening platform: 2018-2020
"High throughput screening of DDI compound library against the asexual blood stage of *Plasmodium falciparum*"

Hit-to-Lead platform: 2021-2023
"Hit-to-Lead development of DDI series for antimalarial development"

Jeremy Burrows
Norio Shibata
Paul Willis
James Duffy
Tomo NOZAKI
Who am I?

Professor
Department of Biomedical Chemistry
Vice Dean
Graduate School of Medicine,
The University of Tokyo

M.D., Ph.D. (Keio University School of Medicine)
Specialty: Molecular parasitology (*Entamoeba, Plasmodia, Trypanosoma*, and *Leishmania*)
Major interests: Metabolism, Pathogenesis, Evolution, and Drug development

My dream when I was a medical student: a doctor in Amazon
Tomo NOZAKI
Who am I?

Major achievements

Metabolism
Cysteine/methionine, FeS, NADH, CoA

Pathogenesis
Vesicular traffic, proteases, phago(& trogo)cytosis, lipid transport

Evolution
Mitochondria under anaerobic environment

Drug development
Target identification and anti-amebic drug lead identification

When we wanted to start our drug discovery campaign, ..

- A majority of screening projects were conducted by pharmaceutical companies, using their own libraries.

- There were very few projects from Japanese academia. Only a limited set of their institution-owned natural compounds were tested against malaria and other parasitic diseases.

Why not use academia-owned chemical libraries for malaria drug discovery.
How we started MMV-UTokyo collaboration

✓ We have expertise in molecular parasitology, and we are keen to develop medicines for malaria and other parasitic diseases.
✓ We have excellent target (enzyme)-based and cell-based (phenotypic) assays.
✓ Structurally-defined chemical and natural microbial culture broth libraries are readily available.

So, ........
✓ We need external help for chemistry, preclinical development including safety testing, PK/PD, and ADME.
✓ We need a compass for directions and good collaborative network for the above.

Then, ........
✓ We started searching potential collaborators and funding sources.
Our MMV-UTokyo collaboration

We aim at:

Discovery and optimization of new compounds that are efficacious against *Plasmodium falciparum* and have new scaffold and novel mechanism of action

Desired properties include:

- Fast acting for treatment
- Long duration for prophylaxis
- Oral dose
- Efficacious against drug resistant field strains
- Targeting multiple lifecycle stages
- Low propensity of raising drug resistance
- Cheap
- Applicable for vulnerable populations (children and women of child-bearing potential)

from a Japan-made structurally defined chemical library

Objective

Discover new compounds (with novel scaffold and mechanism of action) against *Plasmodium falciparum* from a Japan-made structurally defined library.

**Compound Library**

The compounds are obtained from Drug Discovery Initiative (DDI), The University of Tokyo - 210,150 compounds.

**Assay**

*P. falciparum* erythrocytic stage 3D7 386 well plates LDH/SYBR Green assays.

Haruka Ohno

Natsuki Watanabe

Yulia Rahmawati
Assay evaluation and validation

Z' value: 0.76 ± 0.07
Excellent assay

S/B value: mostly > 3
Inhibition profile and hit rate

Inhibition profile of DDI full library

Number of actives

<table>
<thead>
<tr>
<th>% Inhibition (2 µM)</th>
<th>Number of compounds</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥30</td>
<td>5,831</td>
</tr>
<tr>
<td>≥50</td>
<td>2,505</td>
</tr>
<tr>
<td>≥70</td>
<td>1,580</td>
</tr>
<tr>
<td>≥80</td>
<td>1,307</td>
</tr>
<tr>
<td>≥90</td>
<td>974</td>
</tr>
<tr>
<td>Total number</td>
<td>210,150</td>
</tr>
</tbody>
</table>

Active rate (>90% inhibition at 2 µM): 0.5%
Active rate (>50%): 1.2%
The University of Tokyo

Screening cascade

210,150 compounds (DDI)

P. falciparum 3D7 asexual stage (phenotypic) assay (LDH assay)
Cut off: 90% inhibition at 2 µM at 72 h

974

Secondary (confirmation) assay

P. falciparum 3D7 asexual stage
2 µM; 72 h, 50% cut-off

LDH Assay  SYBR Green Assay

648

Tertiary assay

yeast DHODH-expressing 3D7 strain
2 µM; 72 h, 70% cut-off

482 actives

Quaternary assay

Dd2 chloroquine resistant strain
2 µM; 72 h, 70% cut-off
Screening cascade

Exclusion by:
- No malaria drug fragment (overlaps with known antimalarials excluded)
- Structural novelty (Similarity < 0.7 with compounds in MMV databases)

Quaternary assay

Dd2 chloroquine resistant strain
2 µM; 72 h, 70% cut-off

IC₅₀ / EC₅₀ determination

Cytotoxicity against human cells (HepG2) (Safety index > 10)

Prioritization by:
- Potency, physicochemical properties, safety index, structural alerts
  (StarDrop multiparameter optimization > 0.1)

482 actives

106

82

44
(11 commercial available & 33 non-commercial available)

3 CA + 1 NCA actives selected

8 actives
(4 commercial available)

'Chemist's eye' selection based on chemical attractiveness

End of screening project
Screening cascade (after active discovery)

Clustering + Prioritization
1) StarDrop MPO (potency, phys. chem., SI, structural alerts)
2) Structural novelty (cf. MMV database, ChEMBL, etc.)
3) “Chemical attractiveness”

Screening active reconfirmation (purchase or re-supply/re-synthesis)

Profiling (Confirmed active)

Confirmed actives

Prioritization
1) Life-stage activity
2) Rate of kill
3) MoA
4) ADME
5) Synthetic tractability

Array design/synthesis or purchase
Max. 30 compounds (per active, 2 cycles), 10 mg, > 90% purity

Profiling (Array compounds)

Profiling (Validated hit)

Validated hit

Active and Hit profiling

<table>
<thead>
<tr>
<th>Assay</th>
<th>Confirmed active</th>
<th>Array compounds</th>
<th>Validated hit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pf Asexual blood stage</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Lifecycle profile(^1)</td>
<td>x</td>
<td>-</td>
<td>x</td>
</tr>
<tr>
<td>Rate of kill(^2)</td>
<td>x</td>
<td>-</td>
<td>x</td>
</tr>
<tr>
<td>Hit deconvolution(^3)</td>
<td>x</td>
<td>-</td>
<td>x</td>
</tr>
<tr>
<td>ADME I(^4)</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>ADME II(^5)</td>
<td>-</td>
<td>-</td>
<td>x</td>
</tr>
<tr>
<td>Cytotoxicity</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>hERG</td>
<td>x</td>
<td>-</td>
<td>x</td>
</tr>
<tr>
<td>Resistance panel(^6)</td>
<td>-</td>
<td>-</td>
<td>x</td>
</tr>
</tbody>
</table>

\(^1\) = Liver stage (Pb, Pf, Pv), Transmission blocking (gametocytes, DGFA)

\(^2\) = Confirmed active: High throughput rate of kill assay (e.g. 12 h vs 72 h incubation); Validated Hit: Full time course PRR

\(^3\) = Drug resistant strain (Dd2 or K1), yDHODH (mTETC inhibitors), Dd2-mutants (Carl/Pi4K/ACL), pH-based assays (PfATP4, etc.)

\(^4\) = LogD, kinetic sol., human liver microsomes, rat hepatocytes

\(^5\) = Albumax binding, human plasma protein binding, Caco-2 permeability, human plasma stability, CYP inhibition (5 isoforms)

\(^6\) = Lab-adapted field isolates and drug-resistant mutants
Three hit-to-lead candidates

Series 1

Series 2

Series 3

• Good lipophilicity (eLogD: 2.72-3.53) as starting actives
• Reasonable kinetic solubility (46~190 µM)
• Series 1-3 inhibit none of the following known targets: DHODH, electron transport chain including bc1 complex, ACS, CARL, PI4K, and ATP4
## Profiling of three hit-to-lead candidates

<table>
<thead>
<tr>
<th></th>
<th>Series 1</th>
<th>Series 2</th>
<th>Series 3</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>IC50 3D7 asexual stages (LDH 72 hr)</strong></td>
<td>0.75 µM</td>
<td>1.3 µM</td>
<td>0.23 µM</td>
</tr>
<tr>
<td><strong>3D7 rate of kill</strong></td>
<td>Slow</td>
<td><strong>Fast</strong></td>
<td><strong>Fast</strong></td>
</tr>
<tr>
<td><strong>IC50 drug resistant Dd2 (SYBR Green 72 hr)</strong></td>
<td>1.58 µM</td>
<td>0.52 µM</td>
<td>0.16 µM</td>
</tr>
<tr>
<td><strong>IC50 P. berghei liver activity</strong></td>
<td><strong>0.11 µM</strong></td>
<td>9.77 µM</td>
<td><strong>0.041 µM</strong></td>
</tr>
<tr>
<td><strong>% inhibition @ 1 µM</strong></td>
<td>NA</td>
<td><strong>43-96%</strong></td>
<td>0-3.9%</td>
</tr>
<tr>
<td><strong>IC50 human primary hepatocytes 96 hr</strong></td>
<td>&gt;10 µM</td>
<td>&gt;10 µM</td>
<td>&gt;10 µM</td>
</tr>
<tr>
<td><strong>hERG K⁺ CHO (patch clamp)</strong></td>
<td>&gt;30 µM</td>
<td>1.26 µM</td>
<td>&gt;30 µM</td>
</tr>
<tr>
<td><strong>Metabolism by human microsomes CLint</strong></td>
<td><strong>17 µL/min/mg</strong></td>
<td>270</td>
<td>116</td>
</tr>
<tr>
<td><strong>Metabolism by rat hepatocytes CLint</strong></td>
<td>44.5 µL/min/10⁶ cells</td>
<td>95.6</td>
<td>9</td>
</tr>
</tbody>
</table>
Advantages and issues of three hit-to-lead candidates

<table>
<thead>
<tr>
<th>Series 1</th>
<th>Series 2</th>
<th>Series 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>T-117040 (MMV1804220)</td>
<td>T-132522 (MMV1804245)</td>
<td>T-210671 (MMV1804223)</td>
</tr>
</tbody>
</table>

### Strengths

<table>
<thead>
<tr>
<th>Series 1</th>
<th>Series 2</th>
<th>Series 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver stage activity</td>
<td>Fast kill</td>
<td>Fast kill</td>
</tr>
<tr>
<td>Metabolic stability</td>
<td>Sexual stage activity</td>
<td>Liver stage activity</td>
</tr>
</tbody>
</table>

### Issues

<table>
<thead>
<tr>
<th>Series 1</th>
<th>Series 2</th>
<th>Series 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Improve potency</td>
<td>Need to identify minimal pharmacophore</td>
<td>Peptide like (Oral absorption?)</td>
</tr>
<tr>
<td>Define SAR</td>
<td></td>
<td>Complex synthesis (3 chiral centers)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Address liabilities in new molecules</td>
</tr>
</tbody>
</table>

### Number of derivatives synthesized

<table>
<thead>
<tr>
<th>Series 1</th>
<th>Series 2</th>
<th>Series 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>3</td>
<td>13</td>
</tr>
</tbody>
</table>
U Tokyo/MMV screened DDI library, Japanese academia-owned chemical library of 210k compounds, and identified 3-4 candidates to advance to "hit-to-lead".

Structural optimization (based on potency, physicochemical properties, stability, and SAR) and profiling of the improved actives (the rate of kill, multiple life cycle stages, mechanism of action, and ADME) are under way.
Future perspective and requests from academia

- We have directly gained and will gain knowledge and expertise on antimalarial (& other IDs) drug development from MMV and also indirectly via liaison with experts in pharmaceutical industries and other international partners.
- We, parasitologists in academia, need to create a broader network covering natural and synthetic chemistry, safety, and even regulatory sciences.
- MMV bridges a gap between academia and industries (also within academia) by promoting networking and helping us be connected with experts with necessary disciplines.
- MMV also provides young researchers an opportunity for exposure to the outside of the lab, and lots of experience, encouragement, and excitement for realization of their research.

- We are looking forward to good progress of our structurally optimized initial leads toward Lead Optimization in 1.5 years, and Preclinical/Clinical platforms in 3-4 years.

- It would be nice if GHIT Fund also aids in connecting us with pharmaceutical industries and even contract research organizations (CROs).
Acknowledgements

Drug Discovery Initiative
Hirotatsu Kojima
Riyo Imamura

Tokyo Medical Dental U
Tomoko Ishino
Naoki Shinzawa

Juntendo U
Makoto Hirai

National Institute of Infectious Diseases
Yumiko Saito-Nakano
Takeshi Annoura

Kyoto Inst Technolog
Tomoo Shiba

Nagasaki U
Daniel Inaoka
Takaya Sakura
Kiyoshi Kita

Keio U
Kiyotake Suenaga
Naoaki Kurisawa
Arihiro Iwasaki

Shizuoka Pref U
Yuta Tsunematsu
Ryota Shizu

MMV
Paul Willis
James Duffy
Jeremy Burrows

Nagoya Institute of Technology
Norio Shibata
Yuji Sumii

U Tokyo
Yulia Rahmawati
Haruka Ohno
Natsuki Watanabe
Ghulam Jeelani
Arif Nurkanto

NITE
Mihoko Mori

Bikaken
Masayuki Igarashi
Hideyuki Muramatsu
Kazuro Shiomi