Responses to Questions posed during TechTalk on "Surveillance for Antimalarial Drug Resistance"
September 30th 2021, 2 – 3.30 pm (Singapore time)

Q1 From Yannawan Wongchai
"In addition to surveillance, do patients with different K13 status get different treatment regimen?"

Prof. Arjen Dondorp (MORU):
*"*The WHO-defined threshold for changing the first line ACT to a different one is a drop in 28- or 48-day efficacy to below 90%. Only the presence of artemisinin resistance will currently not change the first line ACT treatment (since the only real alternative at the moment is another artemisinin-based combination)."

Dr. Keo Chindavongsa (CMPE, Lao PDR):
"No different treatment regimen."

Q2 From Victor S Koko
"Is it a good idea to use two first line ACTs? Where one is used as an alternative?"

Dr. Keo Chindavongsa (CMPE, Lao PDR):
"It is good to used Artemisinin Combination Therapy first line (Artemisinin based plus partner drug) and not recommendation from WHO to used mono therapy."

Q3 From Martha Suarez-Mutis
"Does Lumefantrine have resistance markers?"

Prof. Olivo Miotto (MORU):
"There is a lot of interest in genetic markers for lumefantrine resistance, particularly because this antimalarial is widely used in Africa. However, we have no definitive marker as yet. By "definitive marker" I mean one that is associated with large drops in sensitivity and clinical failures (such as the *crt* mutations associated with chloroquine resistance, or the *kelch13* mutations responsible for artemisinin resistance).

It is known that certain variants of the *mdr1* gene affect response to lumefantrine. In particular, it has been shown by more than one study that a duplication of this gene, which is known to cause resistance to mefloquine, also causes a loss in sensitivity to lumefantrine, but less pronounced. I believe it is worth monitoring *mdr1* amplification as a potential "stepping stone" to lumefantrine resistance.

One important thing about discovering resistance markers: you need resistance first. It is not clear whether significant clinical resistance to lumefantrine has emerged in the field yet, so it is possible that markers that have a marginal effect (such as the mdr1 amplification) are the most informative at present."

Q4 From Dr Susanta Kumar Ghosh for Prof Olivo
"Can we extract sample from used RDTs for such studies?"

Prof. Olivo Miotto (MORU):
"That would make life easier in many ways. However, our assessments show that it is unreliable- it often works, but has a much greater chance of failing than filter paper blood spots. There are several

important issues, such as the amount of blood, the spread over the RDT strip, the contamination with reagents. We believe the extra effort required to collect a dried blood spot sample is a worthwhile investment."

Q5 From Dr Khanh for Prof Olivo
"Could you please talk about the relation between the results of molecular maker surveillance and clinical trial in GMS?"

Prof. Olivo Miotto (MORU):
"My view is that clinical efficacy studies and genetic surveillance are complementary approaches, with some significant differences.

As you know, clinical efficacy studies are well established, and ***by definition*** they are the best at telling us about clinical efficacy. Clinical efficacy is a key parameter that NCMPs need to monitor: if efficacy is low, frontline therapies need changing. However, while they provide crucial knowledge, clinical efficacy studies have some inherent difficulties.

- They must be well controlled and therefore require planning, good clinical facilities, trained staff, microscopy expertise and patient monitoring- all of these things make them resource-intensive and costly, and impractical to conduct in many low-resource endemic locations. Therefore, only relatively few clinical efficacy studies can be conducted, at a limited choice of locations.
- Since they require advance planning and resource management, clinical studies are not well suited for fast response, e.g. to outbreaks.
- Unfortunately, loss of efficacy is an advanced stage of drug resistance: by the time efficacy studies trigger alarm bells, resistance has reached significant levels, and the battle is likely to be lost. The timing issue is an important one, because the prevalence of resistance in parasites can rise very rapidly, while changes in frontline therapy take time and resources.
- As Prof. Arjen mentioned in his talk, in the case of combination therapies, the efficacy of one drug can prevent you from seeing a loss of efficacy of its partner drug, further delaying the detection of clinical resistance.

Genetic surveillance requires the analysis of genetic markers in parasite DNA extracted from patient's blood. In their operationally simplest form they do not measure clinical outcome (e.g. current projects conducted by GenRe-Mekong with NMCPs in Laos, Vietnam and Cambodia). Therefore, the interpretation of the results relies on known associations between the markers and clinical efficacy. There are several large studies in this region where both clinical data and data from genetic markers were analyzed, and there is good agreement between them: we find failures primarily where genetic markers of resistance are also found. Prof Arjen has shown some graphs that show this strong relationship (see Ashley et al. NEJM 2014, and van der Pluijm et al. Lancet Infectious Diseases, 2019). This is not surprising, since this is often how we find markers in the first place (for example, see the discovery of piperaquine resistance markers in Amato et al., Lancet Infectious Diseases, 2016).

Although it does not tell us directly about clinical efficacy, genetic surveillance studies overcome some limitations of clinical efficacy studies.

- They do not require as much "bedside" effort. Thanks to advanced technologies, we are able to work with a few drops of blood from a fingerprick, dried on filter paper. This can be done when the patient presents for treatment, requires minimal training and can be implemented in the

simplest settings, which allows samples to be collected in the most endemic regions. This means that surveillance can be scaled up to hundreds of sites and thousands of cases.

- Once it is in place, a genetic surveillance project can deliver data with a relatively fast turnaround, and therefore can respond to outbreaks, and to changing epidemiology.
- Because markers are specific to the drugs, we can separate the levels of resistance to different drugs, and monitor levels of resistance even when this has not yet resulted in treatment failures. Therefore, genetic surveillance has the potential for delivering early warning signals to public health.

Of course, genetic surveillance has some limitations of its own:

- There are some drugs for which we do not have a reliable marker of resistance, so research must continue to identify potential markers
- Advanced laboratory and data processing techniques are required (GenRe-Mekong is working hard at making these available to endemic countries)

There is one important aspect of genetic surveillance that needs to be considered. We do not only monitor drug resistance markers: we also look at many genetic variants that characterize parasite strains. This allows us to identify unusual epidemiological patterns, such as strains that suddenly rise in frequency- even when we do not know that they are resistant to a specific drug, or they are resistant to a drug for which we have not marker. In other words, we are currently putting effort into making genetic surveillance more “predictive”, to use different approaches to generate knowledge that will avoid NMCPs being taken by surprise. We are still in early stages of development of these new methods, but initial results are very encouraging.”