# ARMED FORCES RESEARCH INSTITUTE OF MEDICAL SCIENCES

( A F R I M S )

## Most patients in Cambodia with treatment failure post atovaquone-proguanil lack *cytb* mutations in Y268 locus by Sanger sequencing

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# Disclaimer

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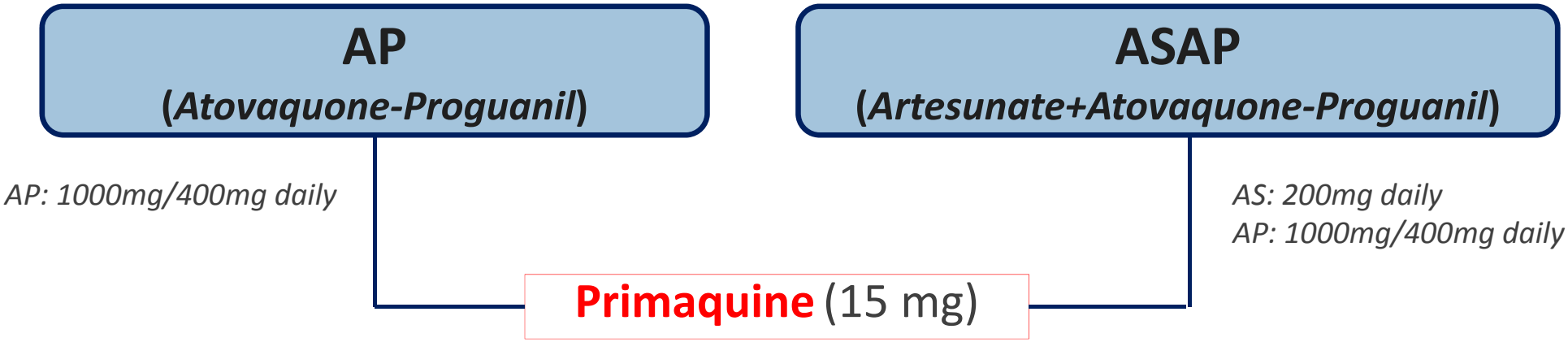
*The investigators have adhered to the policies for protection of human subjects as prescribed in AR 70–25.*

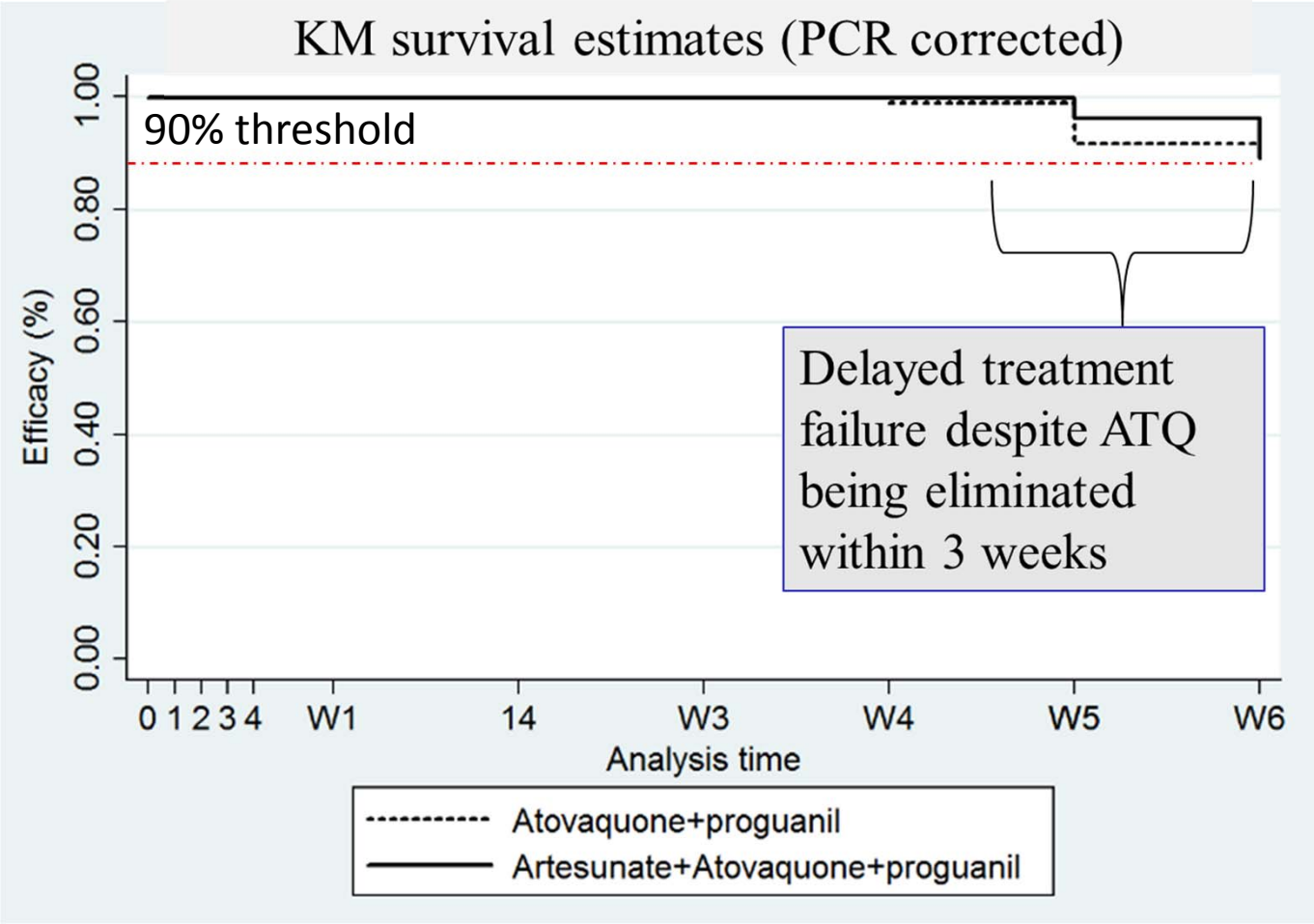
# Limited data on drug resistance to malarone in Cambodia

**TES in volunteers with uncomplicated *P. falciparum* or mixed *P. falciparum/P. vivax* infection**




**Weekly follow-up** and malaria smears/PCR correction until 42 days (6 weeks)

Analyzed atovoquone and cycloguanil **markers of resistance**



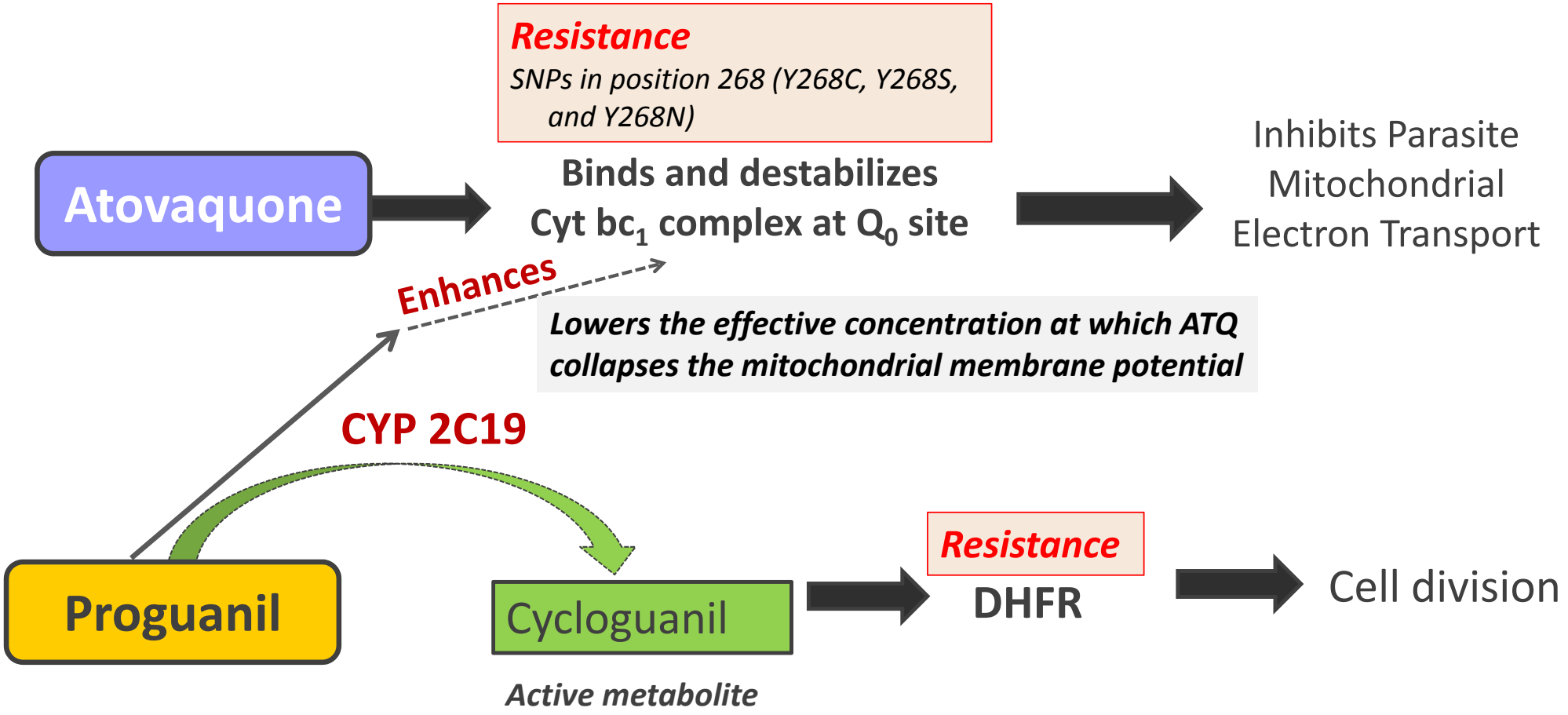

**205 volunteers (1:1)**

**AP vs. ASAP**

10% failure over 6 weeks:

- ❖8 (AP)
- ❖6 (ASAP)

# What is the cause of treatment failure with AP in Cambodia?



# Cause of treatment failure (n=14)

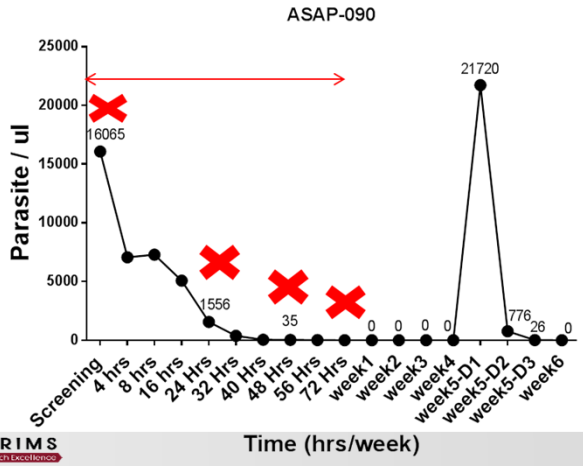
## 1 volunteer with *Pfcytb* mutation on DR

| Atovaquone and cycloguanil markers of resistance (n = 205) | |
|---|---|
| Northern Cambodia (n=157) Recrudescence n= 13 (8%) | Eastern Cambodia (n=48) Recrudescence n=1 (2%) |
| **1/13 Y268C mutation** | **0/1 *cytb* mutation** |

*Pf* cytochrome b (*cytb*) gene by ***Sanger sequencing***

↓

*ATQ resistance developed through treatment (n=1)*




Amplicon deep sequencing targeting *cytb* confirmed the presence of the Y268C mutation in 99.8% of the sequence reads at recrudescence, but did not detect the mutation at D0, or 24, 48, 72 hours into treatment, despite ~1.3 million read depth, even at a minor allele frequency down to 0.25%

# Conclusions

- The vast majority of treatment failures (13/14) could not be explained by atovaquone resistance in *Pfcytb*
- *De novo* mutation through treatment with AP was infrequent (0.5%) consistent with prior estimates
- "Monotherapy" with AP should be avoided in Cambodia (high resistance to cycloguanil, short half-life of proguanil, and variability in ATQ exposures leaves leaves ATQ unprotected during treatment with AP)

- ✓ **AP remains a reasonable rescue drug choice given its PCR-adjusted ACPR of approximately 90% but should not be used as a first-line agent on its own**
- ✓ **There may be a role for AP use in combination with other antimalarials, such as ACTs. This approach could become a viable option against MDR parasites in Cambodia but clinical efficacy studies are needed to identify safe and effective drug combinations with AP.**

# Acknowledgements

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