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WHOLE -LEAF *ARTEMISIA ANNUA*-BASED ANTIMALARIAL DRUG:

REPORT ON PROOF-OF-CONCEPT STUDIES

A COLLABORATIVE PROJECT

BETWEEN

ICIPE, KEMRI AND N.U.S.AG

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1. INTRODUCTION

Malaria is the world's most important parasitic infection, endemic in over one third of the world, causing more than 2 million deaths and 500 million infections annually [1]. The exact extent of the direct and indirect costs of the burden of malaria on society cannot be accurately included in economic calculations. The bulk of malaria is caused by *Plasmodium falciparum*. Malaria is causally associated with kidney dysfunction, hyperactive malarial splenomegaly, Burkitt's lymphoma, anaemia and a myriad of other chronic health consequences. Perhaps most pointedly, endemic malaria has been so burdensome throughout the times to the extent that it has shaped the course human evolution in the tropics by influencing the selection of mutations that confer some resistance to the disease. Sickle cell anaemia and the thalasemias are the best known of such diseases arising from single base mutations in the human genome. Sickle cell disease occurs in close to 200,000 children in Sub-Saharan Africa each year.

Unfortunately many populations of this Plasmodium spp causing malaria have become multi-drug resistant. In both Asia and Africa, malaria patients do not respond to pyrimethamine-sulfadoxine, mefloquine, and chloroquine [2]. Chloroquine, a safe and efficacious drug, was the first-line treatment for malaria in numerous African countries. Pyrimethamine resistance was fast in developing, probably as a consequence of its long half-life [3].

The plant A*rtemisia annua* has been used for over 2000 years to treat various febrile illnesses including malaria in ancient Chinese cultures, in the form of herbal teas. Studies in China in 1970s led to the isolation and characterization of artemisinin as the principal antimalarial compound. Because of the problems relating to compositional variation of artemisinin (and other constituents) in traditional herbal preparations, interest in the exploitation of *A. annua* shifted to purified artemisinin and some of its derivatives. Malaria caused by resistant strains

of *Plasmodium falciparum* and other species of plasmodia have been found to respond well to artemisinin derived drugs. Moreover, still, artemisinin and its derivatives have demonstrated therapeutic potential against several important infectious diseases, besides malaria. *Schistosoma* spp. have been found to respond to artemether [4] [5]. Alpha-Arteether blocks the function of quinolone resistant DNA-gyrase in *Escherichia coli*, and some *Mycobacterium spp* [6] [7].

Artemisinin is a sesquiterpene lactone with an endoperoxide moiety unlike most anti-malarial drugs that contain a nitrogen-containing heterocyclic ring. The drug (and its dihydro derivatives) acts against the malaria parasite in several ways, mainly by i) disruption of the parasite's haemoglobin catabolism, ii) damaging the haem detoxification system of the plasmodium, iii) generation of free radicals from the sesquiterpene lactone which attack the parasite membranes, and iv) alkylation of intracellular proteins in the parasite either by free radicals or by the haem-artemisinin complex [8]. In the last few decades, artemisinin and its synthetic derivatives artemether and artesunate have been established as safe and effective antimalarial drugs [9-11] even against resistant strains of *Plasmodium falciparum*. Significant adverse effects or signs of toxicity have not been reported with therapeutic dosages [12]. The major problem has been relatively high rates of recrudescence observed in all clinical trials based on purified artemisinin or its dihydro derivatives [18]. Apart from artemisinin, thirteen closely related terpenoid lactones and a series of monoterpenoids and flavonoids have been identified in *A. annua* [13-17], some with probable synergistic effects on artemisinin against malaria parasites, when whole-leaf extracts or tablets are used to treat malaria. Could such constituents avoid or minimise recrudescence associated with monotherapy based exclusively on artemisinin or its derivatives?

Whilst drugs based on pure synthetic forms of artemisinin derivatives are already available in the African market, their cost is prohibitive to the very people suffering the major burden of malaria, the poor. The total chemical synthesis of

artemisinin is complex and therefore uneconomical [19-21]. Currently, the plant *Artemisia annua* of the family Asteraceae is the only viable source of artemisinin.

Here, we report the results of the clinical, parasitological and serum chemistry studies that allow us to conclude that whole-leaf *Artemisia annua* tablets produced under good manufacturing practices from high yielding plants is a safe, efficacious and tolerable treatment for uncomplicated *Plasmodium falciparum* malaria.

2. JUSTIFICATION

If safe, efficacious and tolerable medication could be produced from locally-grown medicinal plants; such preparations may offer an additional tool for malaria control, especially in socio-economic circumstances that preclude the availability or accessibility of the more expensive synthetic anti-malarial drugs [5]. There is rising resistance to first-line therapy in the eastern Africa region as a whole and this is coupled with high costs of alternative antimalarials. This innovative approach will be more significant if this herbal preparation is devoid of the threat of development of resistance by the malaria parasites. The product itself presents a form of probable synergistic treatment in its natural form since, in addition to artemisinin, it contains a series of other terpenoids (including sesquiterpenoid lactones) and flavonoids some of which have been shown to contribute to antiplasmodium activity of artemisinin.

Further, it is hoped that cultivation of this plant will spread into other suitable areas of eastern Africa countries and serve as a cash crop for economic empowerment of farming communities.

The present study was undertaken to provide 'proof-of-concept' relating to the special efficacy of the natural phytochemical blend of whole *A. annua* in malaria therapy and to the feasibility of a quality-control production process with

reproducible phytochemical content (and, therefore, reliable antimalarial effects) in whole-leaf drugs produced from selected hybrids of *A. annua* grown in eastern Africa.

3. GENERAL OBJECTIVE

To (i) demonstrate the feasibility of producing whole-leaf *A. annua* tablets with relatively uniform phytochemical composition,
(ii) assess the efficacy of different oral dosages of these tablets in patients with uncomplicated malaria, and
(iii) determine and tolerability of the unrefined leaf preparations.

3.1 SPECIFIC OBJECTIVES

1. To develop a chromatographic system to analyze quantitatively levels of artemisinin (and, in future, other relevant constituents) of *A. annua* during growing stages, post-harvest treatment, and key stages in tablet manufacture.
2. To analyze randomly picked groups of tablets from a batch prepared for clinical trials and to determine levels and degree of variation in artemisinin content.
3. To assess the efficacy, safety, tolerability of the *A. annua* tablets at dosages given over 6 days in uncomplicated malaria.
4. To determine the maximum tolerated dose of *A. annua* tablets.

4. STUDY DESIGN, MATERIALS AND METHODS

4.1 A. ANNUA TABLETS: QUALITY MONITORING.

4.1.1. **Source of *A. annua*.** Several successful plantations of *A. annua* hybrids adapted to tropical conditions have been initiated in the east

African countries. Plants growing in the Arusha Region (Tanzania) were found to consistently contain relatively high levels (0.62-0.82%) of artemisinin, and leaves from these were harvested when they were 8 months old, just before flowering. They were dried under shade for 3 weeks (which was found to be optimum) and the dried material transported by N.U.S.AG to Switzerland, where they were crushed to fine powder, mixed thoroughly to ensure batch homogeneity, and pressed into 500mg tablets under ambient thermal conditions.

4.1.1.Quality control. Two analytical techniques for quantifying constituents of *A. annua* were evaluated: gas chromatography (GC) and high performance liquid chromatography (HPLC). The latter, based on reverse-phase C18-silica analytical columns, was found to be sufficiently mild for quantification of artemisinin. The technique was used to quantify artemisinin in various *A. annua* leaf collections following different drying conditions as well as tablets provided by N.U.S.AG.

Groups of 5 tablets were picked randomly from the batch prepared for clinical trials, extracted with dichrolomethane and analysed by HPLC. **The tablets showed remarkable uniformity in the content of artemisinin (0.745 $\pm$ 0.016%), indicating the possibility of producing phytochemically relatively uniform tablets if guided by HPLC-based quality-control routine at each step in the production process**. The tablets were also tested on mice models at the Centre of Traditional Medicine and Drug Research in KEMRI and were found to give relatively uniform performance.

4.2 Clinical Studies: Site Description.

The study was conducted in the St. Jude's Clinic at ICIPE's Mbita Field Station, Suba District. The District is located between longitudes 34'E and 34' 20' E and latitudes 0' 20' S and 0' 52' S. It covers an area of 1048 km^2 excluding water surfaces. It comprises of five divisions namely, Central, Gwassi, Lambwe, Mufangano and Mbita (where the study is located). The altitude varies from 1143m to 2134m above sea level. It has an inland

equatorial type of climate that is modified by the effects of altitude and closeness to Lake Victoria, which lowers the temperature. The annual rainfall varies from 700mm to 1200mm, with 10% reliability. The long rains occur in March to May while short rains are experienced in August/November. The district experiences high temperatures throughout the year, which range from 17.10'C to 34.8'C. The hot months are between December and March, with February being the hottest. The minimum temperatures vary from 17.10C to 18.0'C and increase towards the lowland region of Mbita Division.

Mbita, in which the study was conducted, has the highest number of people accounting for about 69% of the total population estimated to be about 186,000, in the district. The members of the community belong to the Luo ethnic group and the predominant language is Dholuo.
Fishing and farming of crops such as maize, millet, and cassava are the main economic activities. These crops are grown for local consumption. Livestock keeping is carried out on small-scale basis and the animals reared include sheep, goats, and cattle. Other off farm activities, in addition to fishing, include weaving, selling of fish, baskets, vegetables and grain. Most families earn less than Ksh. 2000 (USD 27) per month. The district has a high infant mortality, 143/1000, compared to the national figure of 72/1000 live births. Malaria is the leading cause of morbidity constituting 42-48% of all illnesses clinically diagnosed. The level of malaria endemicity varies from moderate to intense transmission.

4.3 Clinical Study Design.

This collaborative study involved the Kenya Medical Research Institute (KEMRI), Natural Uwemba System for Health (N.U.S.AG), and the ICIPE. It was designed to provide the maximum potential therapeutic efficacy of the test drug while defining the maximum tolerated dose in the patients recruited. It was an open-label, dose-rising, non-randomized, single center study for the efficacy, safety and tolerance of increasing doses of *Artemisia annua* tablets in consenting,

informed individuals with uncomplicated malaria who attend treatment at the Ministry of Health's Mbita Health Centre and the St. Jude's Clinic in ICIPE Mbita Field Station, Suba District, Nyanza Province in Western Kenya.

4.3 PATIENT HANDLING AND SCREENING.

Sensitization of the community on the availability of free treatment for malaria was carried out with the assistance of the schools, churches and the provincial administration. All patients who turned up were counseled on the principle of the study and its consequences, those who were able to read were issued with a patients' information brochure. On accepting to be screened, they were screened for malaria parasites by Giemsa's staining of both thin and thick smears; these were then read microscopically to identify the presence of *P. falciparum* malaria parasites.

We recruited 48 patients into 4 cohorts of 12 each (the last was reduced to 11). Entry into the cohorts was sequential depending only on recruitment. If 50% of the individuals recruited into a cohort had failed to respond, the regimen would have been considered a failure and subsequent patients would have been recruited into the next cohort.

Patients presenting at the Mbita Health Center or the St. Jude's Clinic and found to have 2+ parasitaemia were educated about the study and given the patient's information brochure. Informed consent was then sought. Consenting informed patients were screened using history, physical examination and laboratory investigations to determine their eligibility for the study, to exclude those with complicated malaria or other diseases, and to determine the level of parasitaemia.

4.4.1 Inclusion Criteria. Patients were included into this study on a voluntary basis with clear understanding that the treatment they were receiving was

experimental and that they were free to leave the study at their convenience without any consequences to their health. Subjects giving written informed consent, with mild to moderate *p. Falciparum* malaria, aged between 15 and 65 years, with parasitaemia of between 0.02% to 4% (on Giemsa-stained blood smears counted against 200wbc), haemoglobin levels greater than or equal to 8mg/dl were recruited into the study. They lived within the defined study area, were willing to attend scheduled follow up for the entire 28 day period, and had no history of ingestion of known anti-malarial drugs in the 72 hours preceding recruitment. They also did not have any other febrile disease requiring specific treatment.

4.4.2 Exclusion Criteria. Patients were excluded, if they did not give consent, had severe malaria or any other severe illness requiring specific treatment. Anyone below 15 and above 65 years, with a haemoglobin level of less than 8gm/dl, or who was unable to complete follow up was not included in the study. Patients who had ingested known anti-malarial drugs in the previous 72 hours, or expectant mothers were not included.

4.5 **Screening Phase.** Once included, the patients underwent a complete history and physical examination, parasitologic examination of their blood smears by Giemsa-stained thin and thick films, haemoglobin levels, serum chemistry, and a baseline electrocardiogram were obtained. The serum chemistries included liver function tests (bilirubin, transaminases, and alkaline phosphatase), bun, creatinine and gamma-glutaryl transferase. The initial follow up during the 6 day treatment phase involved daily clinical review of the progression of the baseline symptoms and signs, checking of the patients' vital signs, a specific check for adverse events related to the study medication. All vital signs were taken using digital equipment to eliminate individual clinician errors.

4.5.1 Laboratory tests. The laboratory tests were carried out according to strict guidelines that were pinned on the appropriate desks at all times for the whole duration of the project. Thin and thick blood smears were taken on a daily basis for the first 7 days. The blood smears were air-dried, stained using Giemsa's technique (see Appendix 1) after which 200 fields are screened for sexual or asexual stages of the parasite. Parasitaemia was expressed as parasites per microlitre. Mixed infections were excluded. The serum chemistries were done on Day 0, 3, 7, 14, and 28 using the BTS 310 Automatic Spectrophotometer. The samples of 3ml whole blood were centrifuged at 2000 rpm for 5 minutes and the resulting serum used for the photometric analysis. Haemoglobin was checked using portable photometric machine (Haemocue®) on days 0, 7, 14 and 28.

4.5.2 Electrocardiogram. Electrocardiographs of each patient were performed on days 0, 2, 7, 14 and 28.

4.6 **Non-admissible patients.** Those patients with exclusion criteria or those who refused recruitment for whatever reason were offered appropriate alternative treatment as required by their clinical presentation and diagnosis.

4.7 **Drug Administration**

4.7.1 **Formulation:** The medicine was supplied as 500mg tablets prepared from dried crushed finely powdered whole leaf of *A. annua*, each containing 3.74 $\pm$ 0.08mg of artemisinin.

4.7.2 Observation of Treatment. The study was carried out as an outpatient study. Morning doses of the drug were given under direct Registered Nurse observation. The patient was observed for 10 minutes to ensure

no vomiting of the drug occurred, and to look out for acute reactions that may have occurred. The patient was then told what time exactly to swallow the tablets at home and this information is counterchecked on the patient's next visit.

4.7.3 Dosage of the study drug. The study drug was administered orally in progressively increasing doses on 4 cohorts (C1, C2, C3 and C4) as follows (level of artemisinin in the dosages given in brackets):

C1: 2 tablets (7.4mg) twice a day for day 1; 1 (3.7mg) tablet twice daily for the next 5 days.
C2: 3 tablets (11.1mg) twice a day for day 1; 2 tablets (7.4mg) twice daily for the next 5 days.
C3: 4 tablets (14.8mg) twice a day for day 1; 3 tablets (11.1mg) twice daily for the next 5 days.
C4: 5 tablets (18.5mg) twice a day for day 1; 4 tablets (14.8mg) twice daily for the next 5 days.

4.7.4 **Concomitant Treatment**. The use of other drugs during the study was strictly restricted. The use of drugs known to have potential anti-malarial effects, e.g. co-trimoxazole, erythromycin, azithromycin, and doxycycline was banned. All other medications taken during the time of the study were recorded.

5. RESULTS OF CLINICAL STUDIES

The clinical trial begun on 10th June 2004 and the last patient is due for his last day of follow-up on the 9th February 2005.

5.1. COHORT 1.

Demographic characteristics. Of our first cohort of 12 patients, 7 patients were females and 5 were males. The average age of the first cohort was 21.42 years. The ages ranged between 16 and 29 years.

Clinical response. Eleven (91.66%) of the 12 patients reported relief of clinical symptoms and signs by day 3 of treatment.

Parasitology. Of these 12 patients, 83.33% (10) had no malaria parasites by day 6.

Eleven patients (91.6%) had no parasitaemia or clinical complaints by day 7. On day 14, ten of the patients (83.33%) had negative blood smears. And on day 28, nine (75%) of the patients had negative blood smears on Giemsa staining for malaria parasites.

There was a case of recrudescence on day 14. This was a 29 year old lady whose parasitaemia had cleared on day 7 but she was symptomatic and parasitaemic on day 14.

We also had a further case of recrudescence or probable re-infection as occasioned by the reappearance of parasitaemia on day 28 in patients who had no parasitaemia or clinical features of malaria by day 7.

Follow-up. We had one patient, due for day 28 follow-up, came on day 42 with no clinical or parasitologic regression. In the results, we have considered her as being negative on day 28 given that she was asymptomatic in the intervening period and took no medicines.

Serum chemistry. The serum chemistries were run on a newly acquired BTS 310 Spectrophotometer. The suppliers of the equipment trained our laboratory technologist for 5 days. We have had a few inconsistent results attributable to technical errors of interpretation and equipment failure. However, it is encouraging that to date all the verified

readings of the serum chemistries show readings within normal limits and with no significant elevation in the course of treatment.

Electrocardiographs (ECGs). The main cardiac concern in dogs was the prolongation of the cardiac QT interval on the ECG. In humans, this ECG changes have been observed in a small percentage of patients [5]. In the on-going study however, the automated and manual interpretation of the QT interval has shown no significant changes during the course of treatment. All our ECG readings have been normal.

Haematology. The major haematologic concern in animal experiments with A. *annua* has been haemolysis of red blood cells. But this was not reproduced in controlled trials on human beings [5]. As a rough guide of the trend, the mean haemoglobin level on day 0 (n = 12) is 13.45g/dl, and the mean on day 7 (n = 11) is 12.94g/dl as compared to a mean (n=10) of 14.21g/dl on day 28.

Adverse events. Of our first 12 patients, 6 reported absolutely no adverse events related to the medication through out the 6 days they were on treatment and on the follow-up days. It should be noted that this was the case despite study clinicians specifically asking them whether they experienced any symptoms after taking the study medication at every visit to the clinic. The remaining six patients mentioned the following adverse events. The figure in the brackets denotes the number of times the symptom was mentioned: nausea (2), vomiting (1), abdominal pain (1), dizziness (2), backache (1), tiredness (1), and body itchiness (1). The only adverse event that bothered the patient who complained of it was the general body itchiness that made the 18-year-old female to swallow antihistamine tablets but still disturbed her sleep. This is in line with the conclusion of many studies

that the adverse events profile of this medicine has generally been reassuring [5].

Conclusion: Cohort 1. The *A. annua* tablets, given at the specified dose for 6 days, are effective in clinical and parasitological regression with minimal adverse events noted.

5.2 COHORT 2

Demographic characteristics. There were 9 females and 3 males with age ranging between 16 and 39 years. The mean age was 23.33 years.

Clinical response. Of the 12 patients, 11 (91.67%) were free of base-line clinical complaints by day 6. The one who remained symptomatic proved to be a case of early treatment failure on day 7. All other patients had relief of clinical symptoms for the rest of the 28-day follow up period.

Parasitology. Nine (75%) of the patients were aparasitaemic on day 5. One patient showed increasing parasitaemia, was declared a treatment failure and alternative treatment instituted. We had a further case of recrudescence on day 28.

Serum chemistry. The patients in the second cohort have shown no significant elevation in their serum chemistries through out treatment as compared to baseline readings. A 100% of the biochemical test results are within the normal limits.

Electrocardiographs. The ECGs have continued to show no significant abnormalities relative to baseline.

Haematology. The mean haemoglobin on day 0 (n = 11) is 12.68, ranging between 8.8g/dl and 16.8g/dl. The mean on day 7 (n = 9) is 11.8g/dl ranging between 9.8 and 13.7g/dl. The mean on day 14 is 13.28g/dl (n = 7) ranging between 10.1 and 15.6g/dl.

Adverse events. Four of the patients in this cohort reported absolutely no adverse events related to the study medication on all days of follow up. The patients reported the following adverse events. Body itchiness was mentioned 8 times by three different patients. One patient had significant body itchiness requiring treatment and mentioned it 5 out of the 8 times this adverse event was mentioned. A localized rash was mentioned once. Transient abdominal pain was mentioned at six different times by three patients. Bad taste /flavour of the study medication (mentioned twice), nausea (once), and an itchy tongue (once) were the other adverse events listed by the patients.

Conclusion: cohort 2. The study medication of *A.annua* tablets, given in the specified dose over 6 days appeared to be effective in clinical and parasitological cure of uncomplicated malaria.

5.3 COHORT 3.

Demographic characteristics. This cohort had 4 male and 8 female patients. Their ages ranged from 15 to 56 years with a mean age of 25.92 years.

Clinical response. There was a case of rising parasitaemia with worsening clinical picture in a patient who failed to respond at all to treatment, and was offered treatment for severe malaria by day 3. Of the remaining 11 patients, all were relieved of their clinical complaints

by day 4, albeit one later had recurrence of clinical complaints on day 6.

Parasitology. In this cohort, 75% of the patients had parasite clearance by day 3. We had two cases of early treatment failure and a further two cases of recrudescence as represented by reoccurrence of parasitaemia on day 28. Interestingly, in both these cases, there were no accompanying clinical features.

Follow up. We had one patient who was lost to follow up on day 14 due to family issues.

Serum Chemistry. All the serum chemistry results are within normal limits. Three of the samples were haemolysed and thus the tests were aborted.

Electrocardiographs (ECGs). One patient had an abnormal ECG reading right from the outset, despite clinically being found to have normal cardiac functions. No significant ECG changes developed in any of the patients.

Haematology. The mean haemoglobin on recruitment for this cohort was 12.58g/dL (n=12) compared to 12.2g/dL (n=11) on completion of treatment, and 12.8g/dL on the last day of follow up (n=8). This shows no significant changes in haemoglobin levels and probably rules out haemolysis.

Adverse events. Only one patient in this cohort reported an adverse event: nausea and vomiting.

Conclusion: cohort 3. The *Artemisia annua* tablets given at the specified dose over 6 days are safe efficacious and tolerable in uncomplicated malaria, with no significant adverse events.

5.4 COHORT 4.

Demographic characteristics. Four female and 7 male patients constituted this cohort. Their ages ranged between 16 and 31 years with a mean of 23.91 years.

Clinical response. Ten of the 11 (90.90%) patients were clinically relieved by day 3 of treatment. The other patient was lost on day 3.

Parasitology. We had one case of early failure to respond to treatment. She was given alternative treatment and discharged from follow-up. It turned out that she had been vomiting her study medication and was keeping this information away from the clinicians. All the other patients showed rapid response of parasite counts to treatment, with 81.82% being *Plasmodium falciparum* free by day 4. One case of recrudescecnce occurred at day 14. No recrudescence occurred on day 28.

Haematology. The mean hemoglobin for day 0 was 13.47g/dL n=11, and the mean immediately after treatment on day 7 was 12.9g/dL.

Adverse events. Only one patient answered in the affirmative when asked whether they had experienced any adverse events associated to the study medication. The patient experienced nausea and vomiting when taking the study medication.

Serum Chemistry. The results showed no significant changes in the serum levels of urea, serum proteins, creatinine, ϒ-glutaryl transferase, SGPT, SGOT, or Alkaline phosphatase levels.

Electrocardiographs (ECGs). There have been no significant ECG changes in any patient on follow up in this study.

Conclusion: Cohort 4. *Artemisia annua* tablets taken at the specified dose over six days is efficacious in treating uncomplicated malaria with a good safety profile with minimal adverse events.

6. SUMMARY & RECOMMENDATIONS

6.1 SUMMARY OF RESULTS

This 'proof-of-concept' studies has demonstrated that:

(i) It is possible to manufacture whole-leaf *A. annua* antimalarial tables with more or less comparable levels of artemisinin and other constituents if the key steps in the manufacturing process is guided by phytochemical analyses.

(ii) The results of the clinical study show impressive efficacy of these tablets in treating malaria with no significant side effects. Interestingly, there were no discernable differences between the results obtained from different dose regimes (Table 1). The performance of the lowest dose (with 14.8mg of artemisinin on day 1, followed by 7.4mg daily until day 6) is remarkable, suggesting the operation of synergistic effects of artemisinin and other constituents in the whole-leaf tablets.

(iii) Cases of recrudescence/re-infection represent about 10% and suggests that a different drug administration regime comprising an

initial intake for a period of say 5 days followed by another intake for the same period 5-6 days later (to counter slower maturing parasites that may emerge later into the blood stream) may reduce recrudescence further or eliminate it all together.

Table 1. Summary of *A. annua* drug dosage administered to malaria patients in different cohorts and results

Cohort	No. of Patients	Dosage[a] day 1; 2-6		Parastemia[b] Before	Parastemia[b] After[d]	Symptoms[c] Before	Symptoms[c] After[d]
1.	12[e]	2x2;	1x2	12(100%)	2(17%) 1(9%)[f]	12(100%)	1(8%) 1(9%)[f]
2.	12[g]	3x2;	2x2	11(100%)	1(9%)	11(100%)	1(9%)
3.	12[h]	4x2;	3x2	12(100%)	2(17%)[i]	12(100%)	1(8%)[j]
4.	11	5x2;	4x2	11(100%)	1(9%)[j,k]	11(100%)	1(9%)[k]

a - no. of 500mg tablets, each containing 3.7 $\pm$ 0.1mg of artemisinin, twice a day
b - based on Giesma-stained blood smears counted against 200WBC
c - standard subjective symptoms, including headache, fever, chills and others
d - 2 sets of results indicated, day 6/7 and day 14 following day 1 of administration
e - 1 subject migrated away after day 7
f - 1 case of recrudescence on day 14 and another recrudescence/re-infection on day 28
g - 1 subject lost before completing course of treatment
h - 1 subject not available by day 14
i - 2 cases of early treatment failure and 2 of recrudescence without clinical features
j - 1 case of recrudescence on day 14
k - 1 patient not available from day 3

6.2 RECOMMENDATIONS

The following follow-up activities are recommended:

(i) Characterization of key constituents that contribute to the antimalaria activity of *A. annua*, which would allow refinement of the quality control process.

(ii) Pharmacokinetic studies of the key constituents of the anti-malarial blend to help us optimize the most effective drug administration regime.

(iii) Follow-up large-scale clinical trials involving different drug administration regimes and other categories of malaria patients.

(iv) Select appropriate varieties/hybrids of *A. annua* on the basis of the optimum 'constituents profile'.

(v) Develop a detailed business plan for large-scale planting and commercial production of the *A. annua* drug for different age categories of malaria patients.

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