Mechanism of Piperaquine (PQ) and lumefantrine resistance

Leah Mwai (Phd student) and Alexis Nzila (Fellow)

and

Potential of the anticancer methotrexate for the treatment of malaria

Kenya Medical Research Institute/Wellcome Trust Supported Collaborative Programme

Research focus:

Piperaquine resistance (A. Nzila, fellowship)

Lumefantrine resistance (L. Mwai, PhD studentship) resistance

- **Interrelated projects**

- **Presented by Leah Mwai**

Other studies in our Unit (indirectly funded by EDCPT)

→ **“anticancer methotrexate for the malarai treatment”**

My gratitude to EDCTP

in 2005:

*** Non funds**

*** My lab was closing down**

staff leaving, I was left alone……..

*** EDCPT-fellowship (3 years) from 2005-2008**

**** Rebuilt all my lab..**

**** Put me back on track…**

**** EDCPT supported Leah Mwai….**

work has been recognised internationally

Pfizer-Royal Society Award (in 2006)

Not possible without EDCPT

Before 2005…..

work on antifolate/folate biochemistry…

but was stopped due to lack of funds

With the lab back on track…

has revisited some of my earlier discovery

……while working on antifolate in malaria

*interest in other uses of antifolate….

*A lot has been done on Methotrexate (MTX) in cancer…..

**MTX = first antifolate in cancer

- its success
- diseases of the rich

= more studies on antifolates/folates biochemistry

Exploitation of cancer information:

for new targets….. new drugs

Testing of Kisliuk effect (from cancer)

Discovery of methotrexate as an antimalarial

Methotrexate (MTX) in malaria…..

* MTX:

**potent antimalarial agent

**potent against multidrug resistant isolate

MTX IC_{50}= 25-35 nM

* Old drug: Pharmacokinetics/pharmacodynamics: well known

**Low and safe dose of MTX= in vivo effective concentration> 250 nM

→ [MTX (low and safe dose) for malaria

Methotrexate for malaria treatment…….

Problem with this drug

1. known to be toxic……will argue, it is not…..

2. Should not be used in pregnancy..

Limitation:

- cannot be developped as a tab

- not a

Alte

- fo…ndiation for children (sirop, …..)

- can restricted for IPTi, severe malaria……

Is it safe and efficacious?

…(2 year discussion in our group)

We have agreed for a clinical trial

Toxicity……..

That is what people know and are afraid of……

High dose of MTX in cancer

- =(5000-12000 mg (70 to 170 mg/Kg) /week/several months).
- = life threatening toxicity of MTX.

Low dose of MTX (LD-MTX)

*MTX= low and non toxic dose to treat rheumatoid arthritis (RA) in adult

7.5 mg- 30 mg adult dose (0.1- 0.4 mg/Kg) (up to 5 years).

*MTX= treatment of juvenile arthritis (JA) in children (< 1 year old)

0.2-0.8 mg/Kg (up to 2-5 years)

- LD-MTX (30 years of experience)
  - Safe
  - Well tolerated (better tolerated by children)

Unfortunately, this is not well known

Most common Side effect (SE)

High dose (HD) in cancer:

bone marrow suppression, liver and kidney ✗

LD-MTX in RA:

mucositis (oral ulcer)
GI truck disturbance (diarrhea)
liver and kidney (rare occurrence)

In RA, toxicity = chronic use (years), and increase dose

in Malaria= 3 day-course
no increase of dose

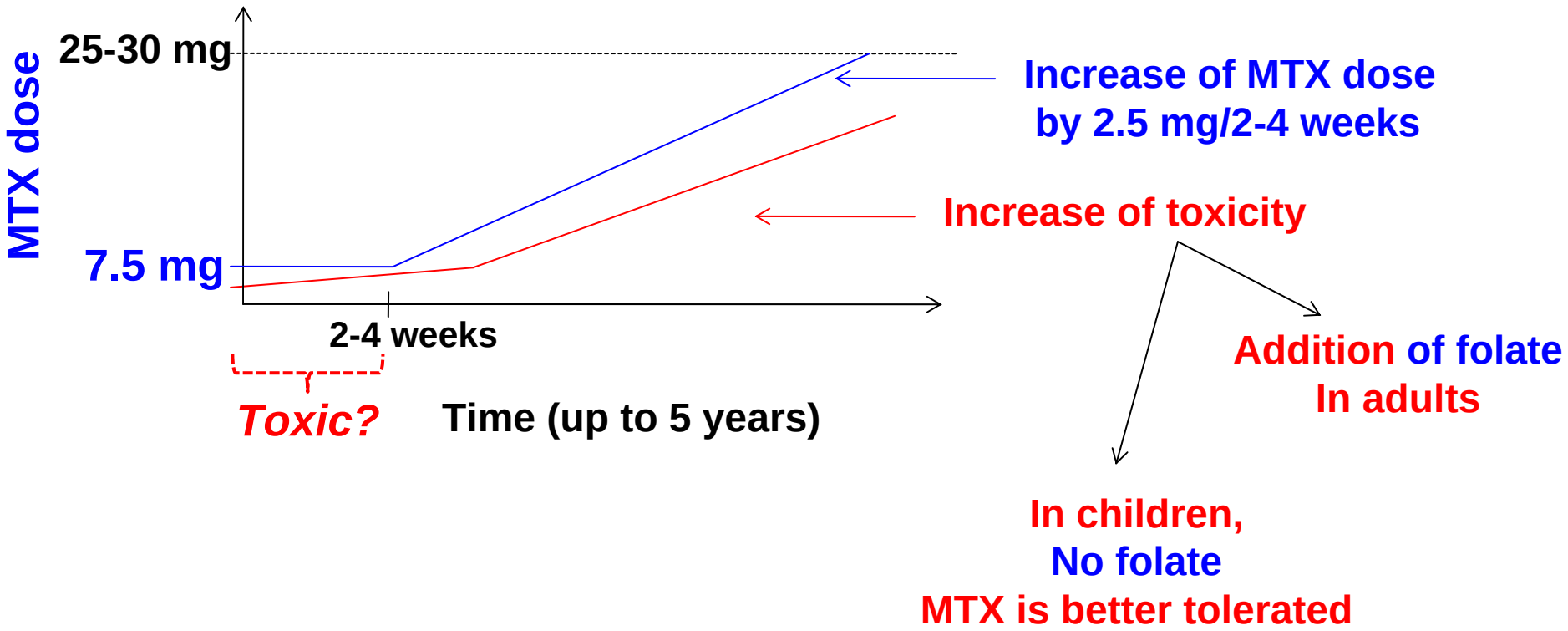
Will RA SE be common in malaria?

Discussion with rheumatologists…

* Prof Tim Nieheus :
  - Pediatrician-rheumatologist, University of Dusseldorf, Ge
  - Many publications on MTX in Juvenile arthritis…

* Dr Oyoo Omondi, the only rheumatologist Kenya

MTX toxicity pattern?



So could we conclude that 7.5 mg is safe?

Other use of LD- MTX:

MTX : increasing used in other immune diseases

- ***Inflammation bowel disease**
  - **Ulcerative colitis
  - ** Crohn's disease
- * Psoriasis
- * Urticaria
- * Ankylosing spondylitis
- * Multiple sclerosis (MS)

MS= 7.5 mg of MTX, fixed dose (without folic acid) up to 2 years

Cochrane analysis:

Follow up: 200 patients with 7.5 mg weekly up to 2 years

No side effect

Studies more patients are underway

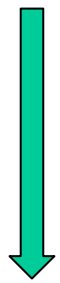
So… MTX = dose < 7.5 mg + chronicity= safe

So…7.5 mg

= lowest dose in RA
= Use in MS (2 years) with no need of folate supplementation
= much lower than in JA
= In vivo concentration that kill malaria parasite

In malaria:

We propose not to go beyond 7.5 mg



Dose to use: 2.5 to 5 mg

MTX for malaria in human (proof of concept already demonstrated):

Clinically tested:

in the 1970s (JAMA 1970 214, 109-14; Ther Umsch 1973 30, 218-22)

*** 3-5 day course (2.5 mg per day): Efficacious and safe**

But was not developed

*** because toxicity concerns**

*** Information of the safety of low dose of MTX was not available**

**** in 70s: MTX used only for cancer treatment= toxic**

**** in 80s: beginning of the use of low dose of MTX in arthritis**

→ **They abandoned MTX as antimalarial**

⇒ We now know a lot about MTX safely (30 years)

→ **Clinical trial of MTX: Jan 08 in adults in Kilifi (repeat the 70s' studies)**

From Tim Nieheus and Dr Oyoo Omondi

Every week….

0,5 -1 millions of adults receive LD-MTX for RA (world wide)

25-50,000 children treated with MTX for JA in Europe
(50 -100,000 world wide)

Number on MTX is increasing
(the safest and most tolerated anti-RA drug)

*Inflammation bowel disease,Psoriasis, Multiple sclerosis (MS)

* JA: commonly found in children> 1 year old
so data on MTX safely on these children

* What about less than 12 months?
Hemophagocytic lymphohistiocytosis (HLH): infant <12 months
**MTX is increasing being used…

Many other drugs exist (there are alternatives),
but LD-MTX is increasingly used= NOT TOXIC

Supportive for MTX in human

Collaborative work :

1. Prof O.K. Dumbo, Dr Djimde (Mali)/ Dr Sowumni (Nigeria)

*** Trials in Mali/Nigeria**

*** Recent meeting (Sept 07):**

To wait for the Kilifi trial first

2. Prof Nick White and Francois Nosten (Thailand)….

*** Already a proof of concept: 1 clinical study in P. vivax**

Recently: we sent them MTX for *in vitro* test against P. vivax

→ **May to lead a clinical trial in Thalaiand**

Conclusion:

A lot to learn from cancer research

* Probenecid (PROB)…already in clinic

**PROB increases Fansidar efficacy in vitro
Proven in vivo with by Dr Sowumni, Nigeria

** PROB= reverses chloroquine resistance in vitro
Discussion with Sowumni
on a trial of PROB on [Fansidar + Chloroquine]

*Methotrexate:

to go in clinic
* against falciparum in Africa
* against vivax in Asia

* We are testing other concepts…..

Acknowledgement:

- European-Union Developing countries clinical trials partnership (EDCTP)

- Pfizer-Royal Society

- Kenya Medical Research Institute (KEMRI)/ Wellcome Trust Program, [Kenya]

To Prof Nieheus and Dr Omondi

Is 2.5-5 mg/ 3 days will be toxic?

No risk of toxicity with one treatment course

Never have any toxicity the first week of treatment

Their concern:

2.5-5 mg: low, may not be efficacious

but… may be efficacious

Safe in Europe but what Africa?

But.. MTX is now used in Africa for RA..

in South Africa : MTX better than other drugs (Meta-analysis)
(from Publications)

- **Dr Oyoo Omondi, the only rheumatologist Kenya**

In Kenya: increase MTX prescription in adults
not published data yet

Currently:
Follow up of a cohort of 250 Kenyan
50 children (JA) [some of them more than 5 years]

In Kenya " MTX is the safest of all Anti-RA drugs"

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Reversal of drug resistance….

Not new….old concept… but not in widespread use…

Problem:

Verapamil (other):

[C] to reverse resistance higher than normal dose

= risk of toxicity

= not interest

Probenecid:

[C] to reverse resistance [50-100 uM] lower than normal dose [750 uM]

= not risk of toxicity

= has a potential

**** But PM/SD and CQ are failing drugs…….***

- Artemesinin (ART) combinations are effective…

Yes.. but (recent data from South East Asia)

Decrease of activity of ART is now a cause of concern in Asia

ART combination: now deployed in Africa…

Effective but widespread of drug= emergence of resistance

→ **Need of new approaches and new drugs**

Pharmacokinetics and in vitro activity of MTX

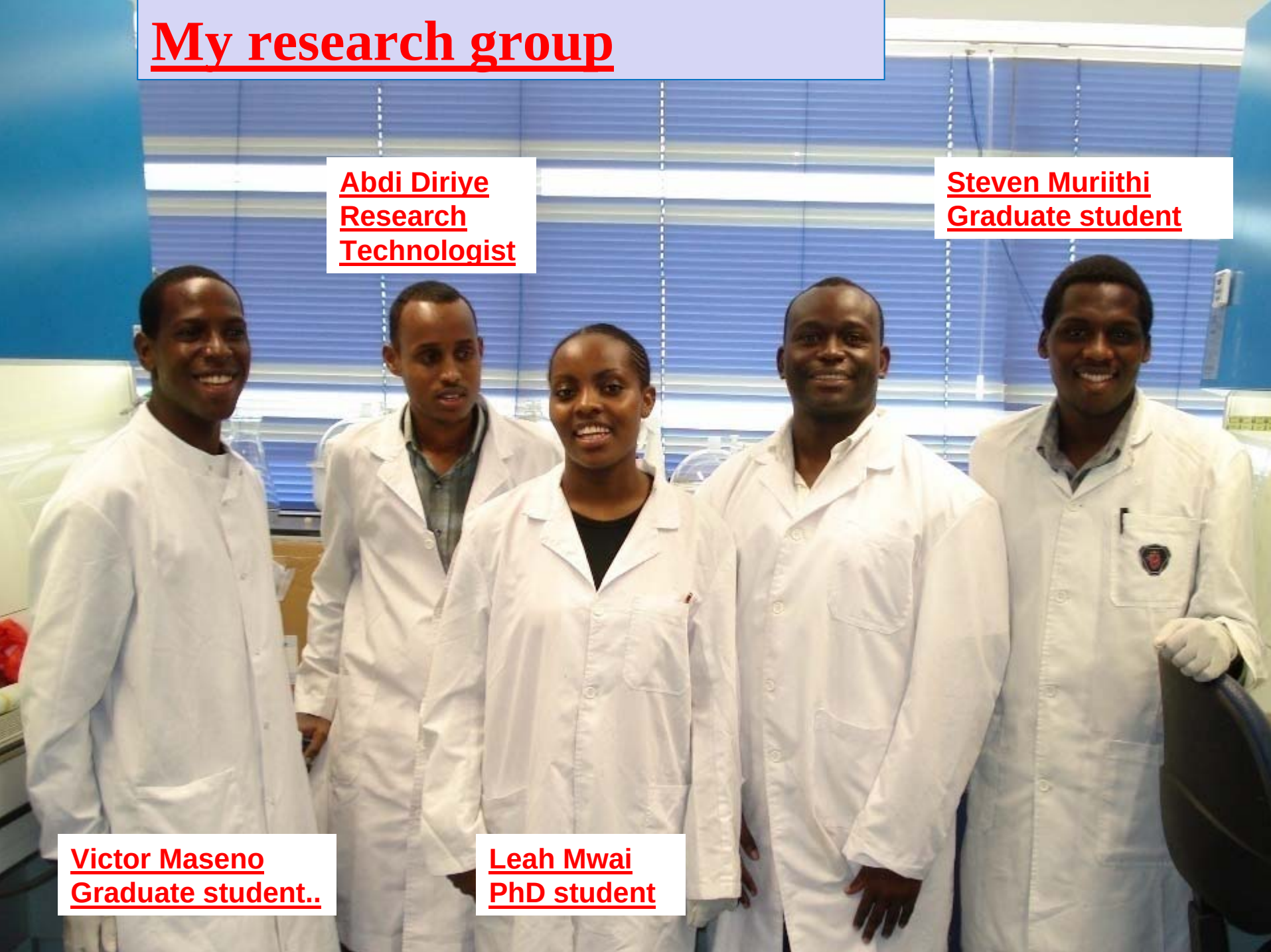
MTX IC_{90}-IC_{99} = *150 nM*

Cmax of single dose in children :

2.5 mg-7.5 mg = 150 to 700 nM

Cmax above MTX IC_{90-99}

My research group



Dissemination and commnunication (EDCTP fellowship)

* **Participate to MIM meeting in Yahounde, Nov 06**
  Preliminary data were presented

* **Workshop participation (LMwai)**

* **Completed manuscription writing from previous projects**

a. **Nzila Alexis.** The past, the present and the future of antifolate in the treatment of Plasmodium falciparum infection. ***J. Antimicrobial Agent, 2006***.

b. **Nzila Alexis.** Why are pteridin analogs not used as potentiator of anti-dihydrofolate reductase agents against malaria parasite. *Submitted for publication*.

c. Nduati E. W., **Leah Mwai**, S. Ommeh, Kokwaro G.O and **Nzila Alexis**. The antimalarial activity of the antifolate anticancer methotrexate and aminopterin in P. falciparum. ***Submitted for publication.***

C. Ochong E, **Alexis Nzila**, Eunice Nduati, Sera Kimanu, Isabelle Ochola and Carol Sibley. Longitudinal analyses of dihydrofolate folate reductase and dihydropteroate genotypes in *Plasmodium falcipar*um isolates from different sites in Kenya. ***Submitted for publication..***

EDCTP was acknowledged

THANKS