



The activity of the anticancer antifolates methotrexate (MTX) and aminopterin (AMP) in *Plasmodium falciparum*.

Alexis Nzila, Eunice Nduati, Leah Mwai, Gilbert Kokwaro

Background:

MTX, AMP:

- * use in cancer and rheumatoid arthritis**
- * reference drug to study folate pathway**

Aim:

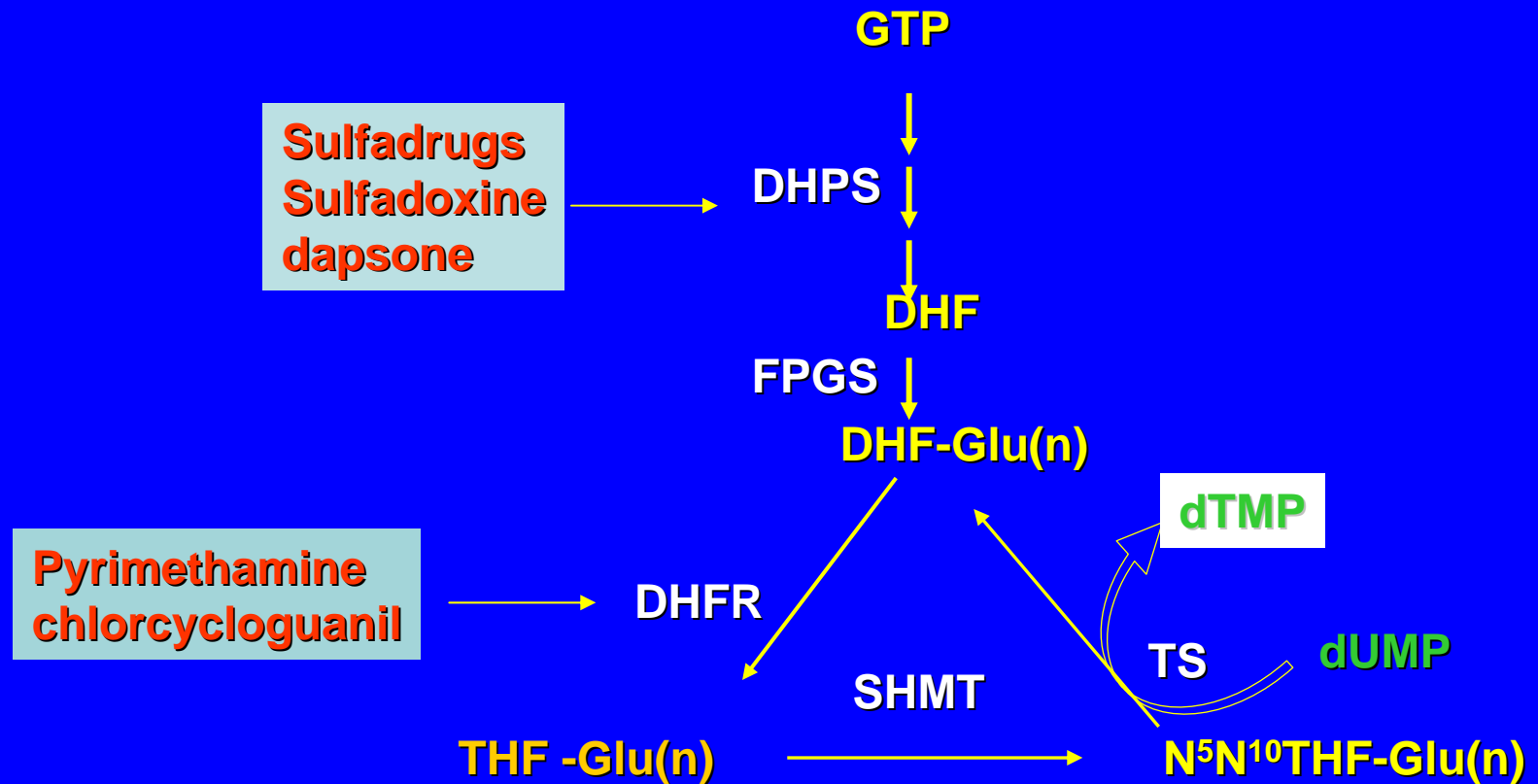
MTX, AMP as probe to understand malaria folate pathway

Result:

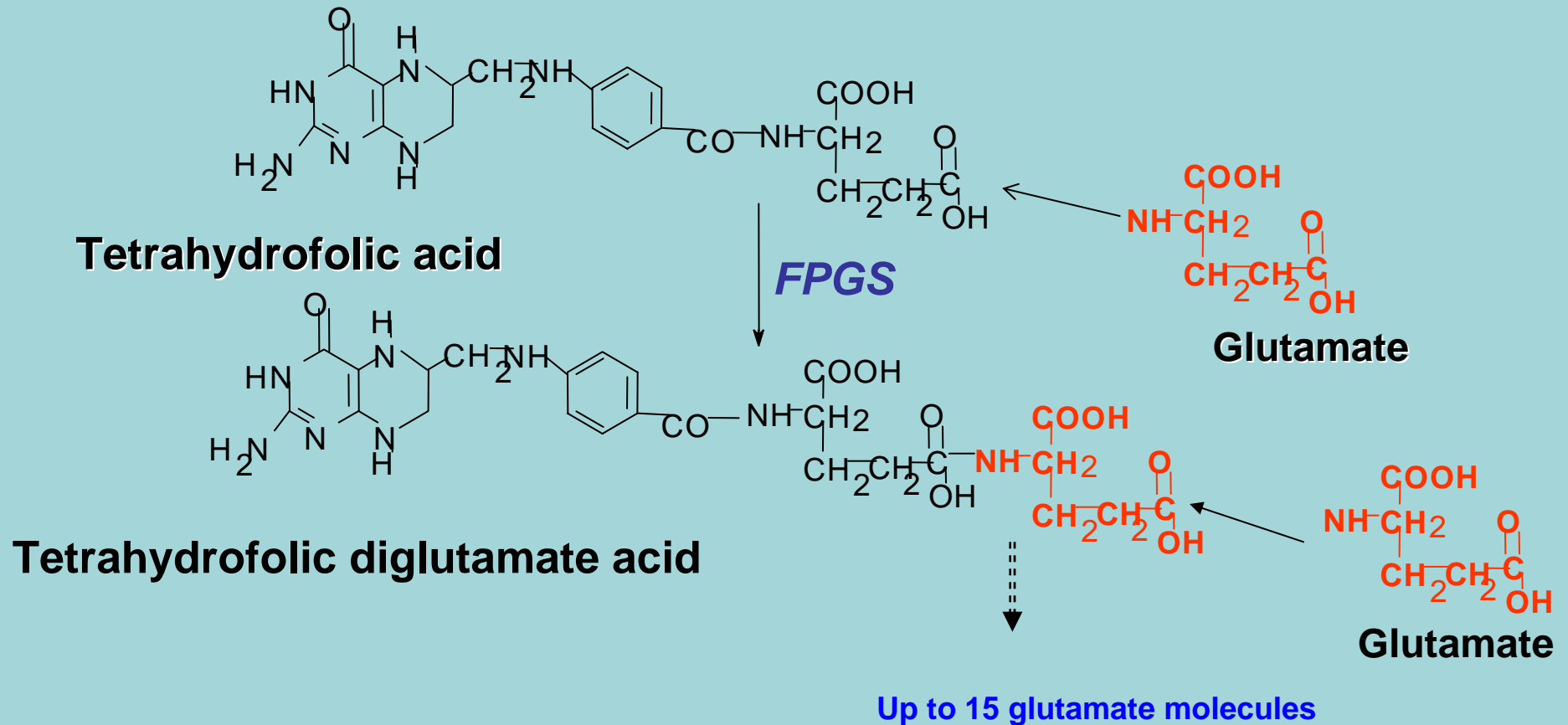
- * Discovery by serendipity of interested effects**

Folate in *P. falciparum*:

* Rely on *de novo* synthesis of pyrimidine (dTMP)



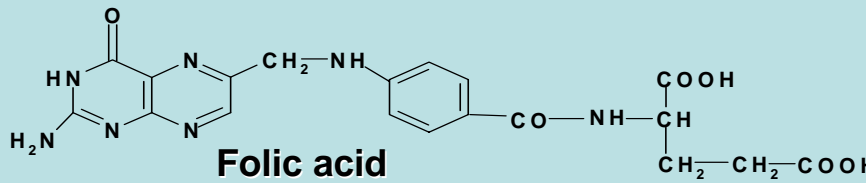
Polyglutamation reaction



Role of polyglutamation:

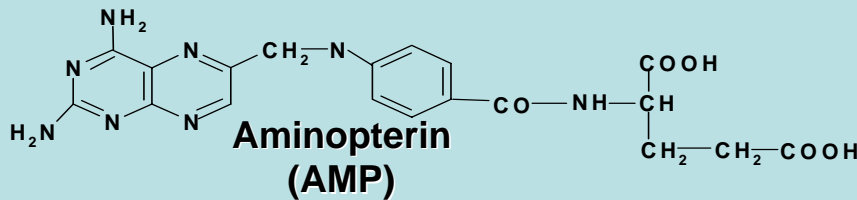
- **prevents efflux** of folate cofactors from the cell
- **increases binding of folate** to folate enzymes

Folate and antifolate

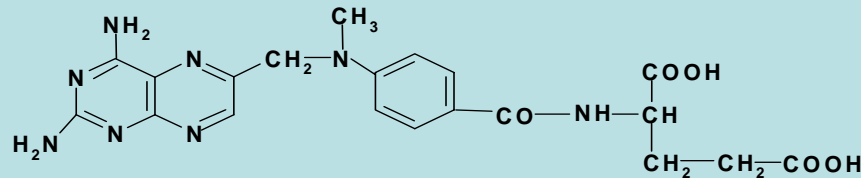


+ Glutamine

Fol-
Polyglutamate
(Fol-GI_n)

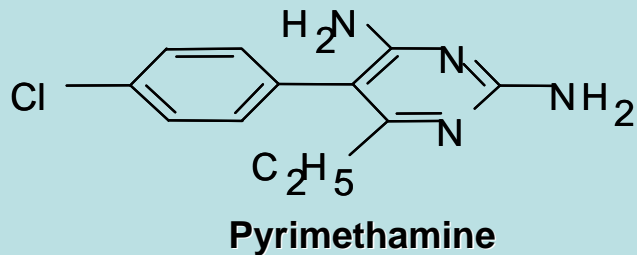


AMP-GI_n

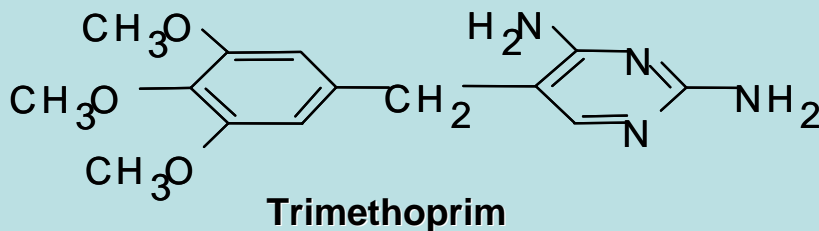


MTX-GI_n

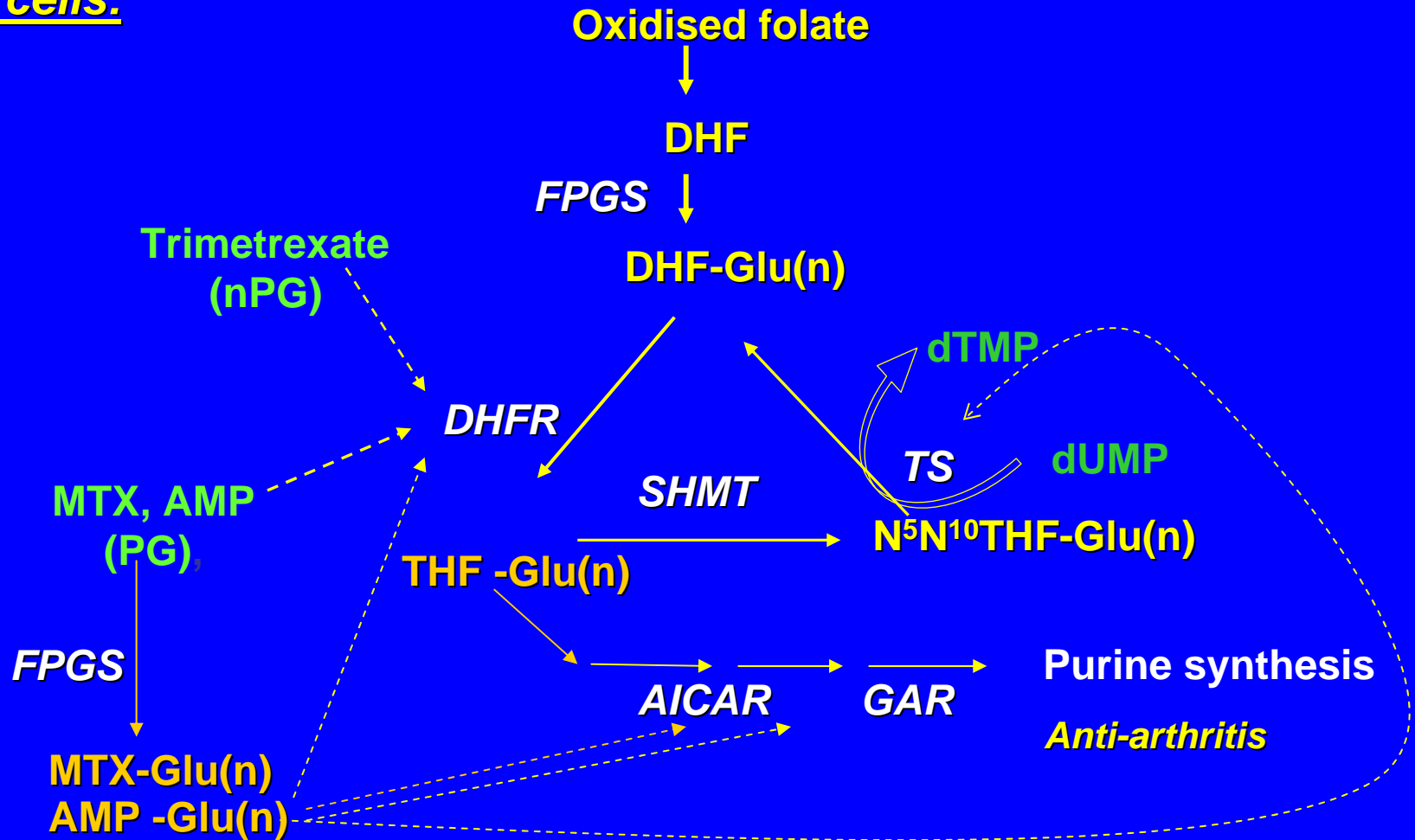
**Polyglutamatable
antifol
(PG)**



X → **Non-Polyglutamatable
antifol
(nPG)**



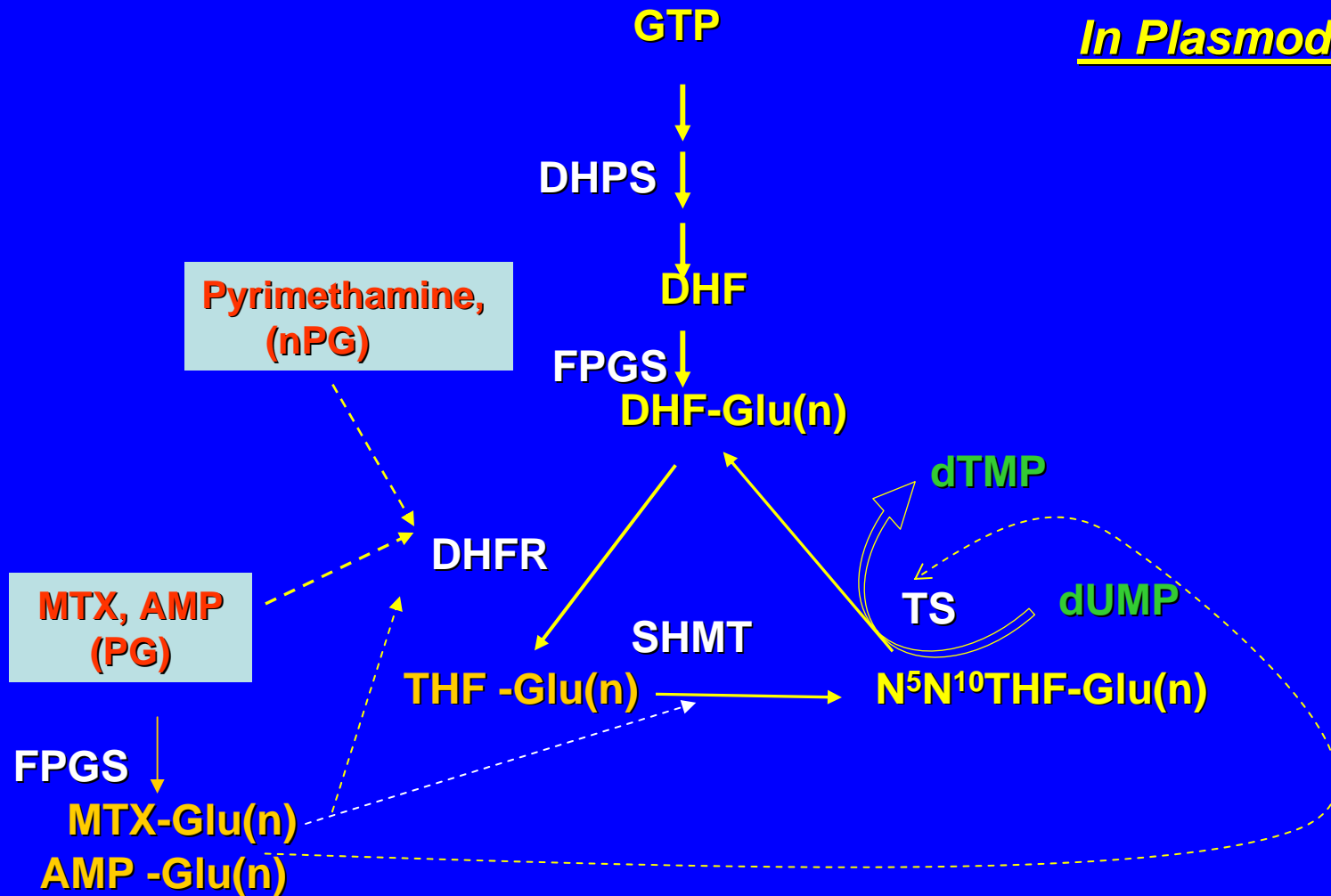
In Cancer cells:



Kisliuk effect:

- * nPG (DHFR) and PG (other enzymes)= sequential blockage=
PG and nPG are synergistic
- * Has been proven *in vivo*, in Monkey model

In Plasmodium



MTX and AMP: very potent against malaria:

nPG-PG: Sequential blockage: Kisliuk effect in P. falciparum?

Does “Kilsiuk effect” exist in malaria?

In vitro Study:

Using the multidrug resistant isolate ViS

Results:

No synergy between PG (MTX, AMP) with nPG (CCG, PM)

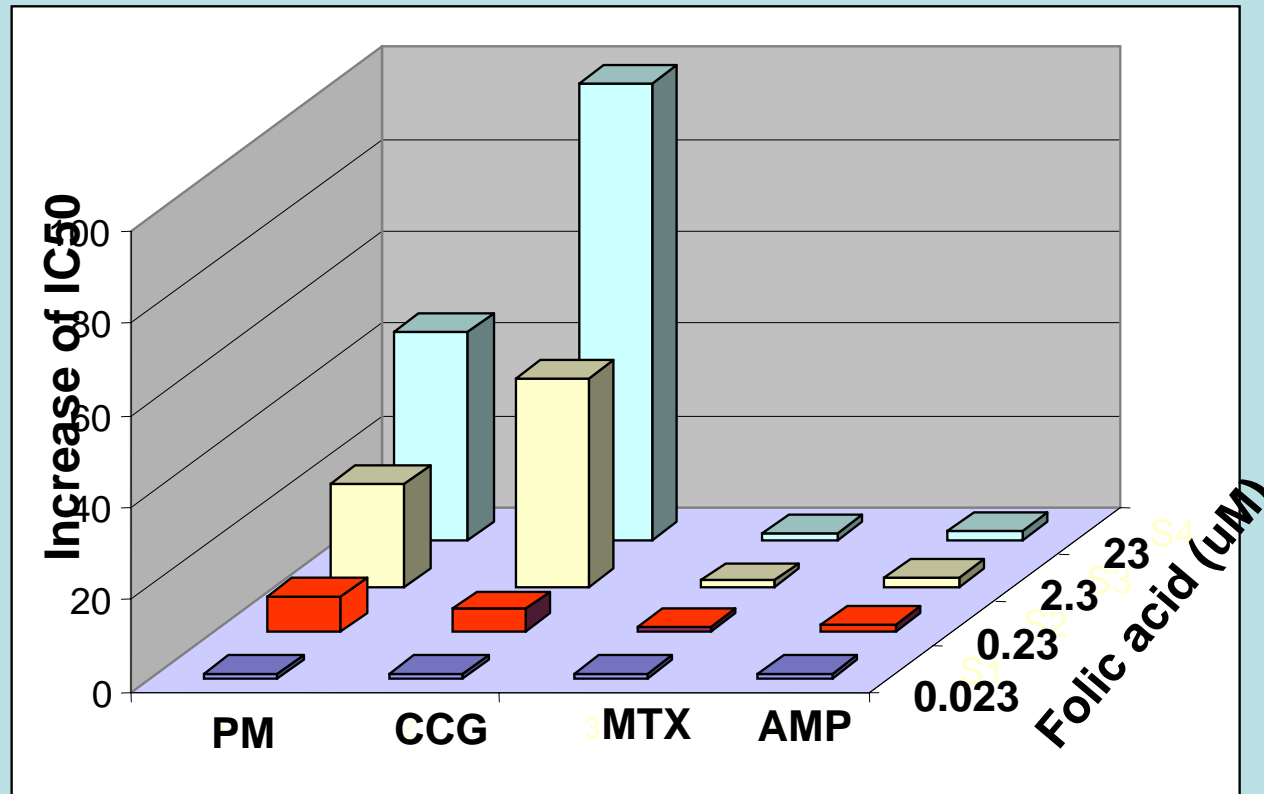
In cancer:

folic and folinic acid increase the “Kilsiuk effect”

In Plasmodium: * Non folate effect

* MTX, AMP: **Activity is not reduced by folate**

Effect of folic acid on the activity of PM, CCG, MTX and AMP against the multidrug resistant isolate V1S *in vitro*.



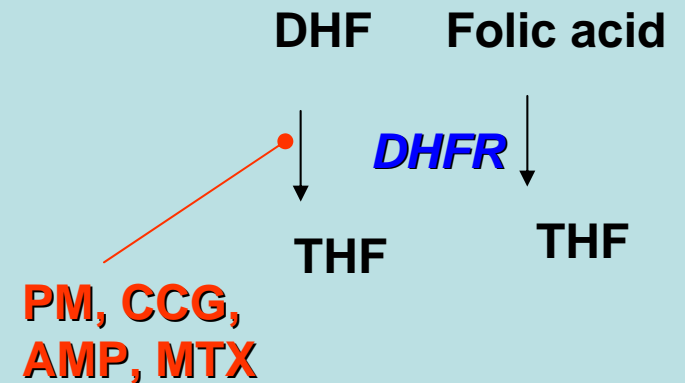
IC50 (nM) physiological folate (0.023 uM)

PM: 860

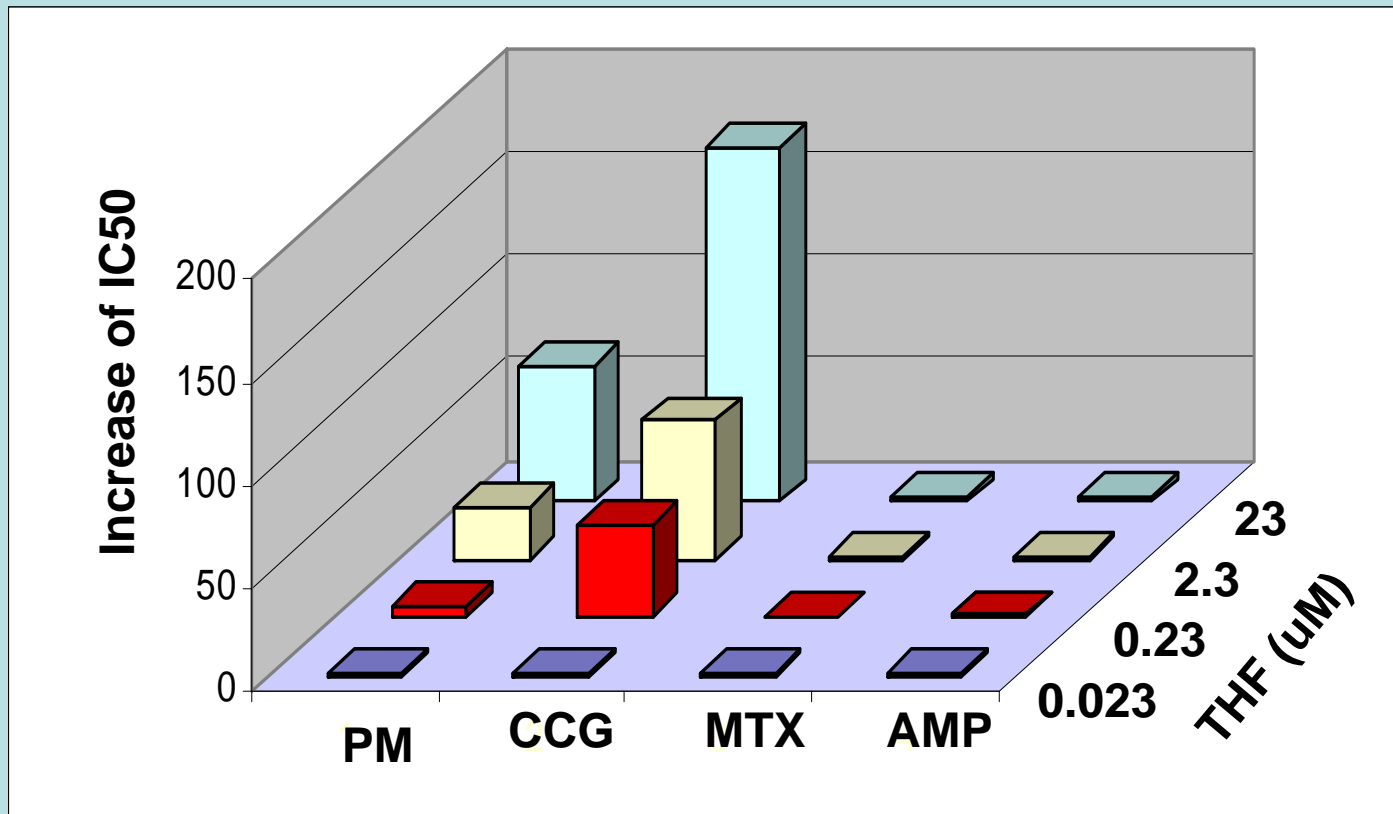
CCG: 12

MTX: 31

AMP: 60



Effect of THF on the activity of PM, CCG, MTX and AMP against the multidrugs resistant isolate V1S *in vitro*.



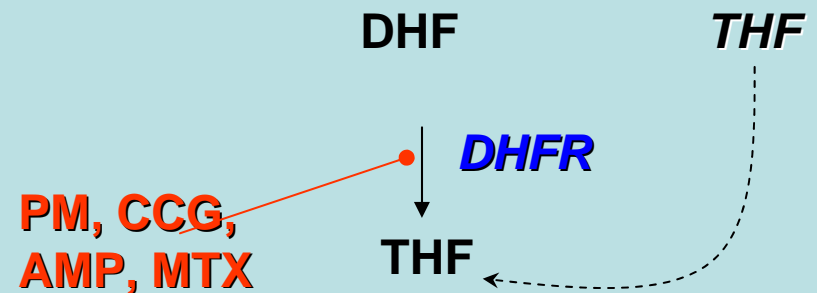
IC50 (nM) physiological folate (0.023 μM)

PM: 790

CCG: 10

MTX: 24

AMP: 67



Conclusion: MTX and AMP do not target DHFR

What are the possible mechanisms of action of MTX and AMP?

Source of information: **Use of MTX and AMP in cancer:**

- 1. MTX: can be metabolized *in vivo*
target other enzymes**
- 2. MTX: Pro-oxidative properties
P. falciparum: sensitive to oxidative stress,**



We are currently testing these hypotheses.

Initial aim:

Use of MTX as a probe.

Since [folate + MTX] as potent as MTX:

[MTX+ Folate] in the treatment of malaria?

MTX is toxic:

But this toxicity has to be relativised.

MTX in cancer (high dose):

X

X

5000 mg/week: effective concentration (Ceff): 1,000 uM
[life threatening toxicity.

X

MTX in Rhum. Arthritis (RA) [Low dose]:

* 25 mg/week, on a chronic basis, for several years (up to 5 years)

* Not toxic, safe

YES

* Ceff = 1 uM, far beyond values to kill the parasite [IC50 < 50 nM].

MTX Activity/Toxicity

*MTX toxicity = GI symptoms and bone marrow suppression=
inhibition of folate synthesis

* Addition folate= reverses toxicity and decreases activity

Chronic use of MTX in RA:

Addition of folate (low dose, < 10mg) **several hours (10 to 24 hours) after**
MTX administration:

if **Concomitant use of MTX+ folate** :

in human: **No toxicity but no efficacy**

In malaria: **Efficacy as good as MTX alone.**



MTX (low dose) + folate (even high dose): **malaria treatment**

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

* Clinically tested, in the 1970s

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

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

Not in Widespread use:

May be toxic

Addition of **folate to protect the host** will negate antimalarial activity.

Our data: No folate effect



[MTX + folate]: efficacy and toxicity in animal model

Acknowledgments

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