MTX; Biochemistry and Metabolism: Clinical Significance Osteorheumatology,Genoa, 21 OCT, 2016

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Methotrexate

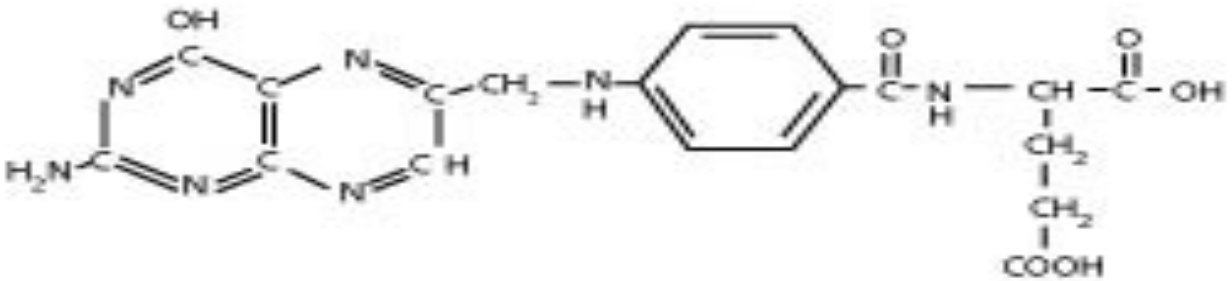
Biochemistry
How Biochemistry and
    Metabolism affect Function
Take-home Clinical Insights….

I have received research support from Lederle Labs.

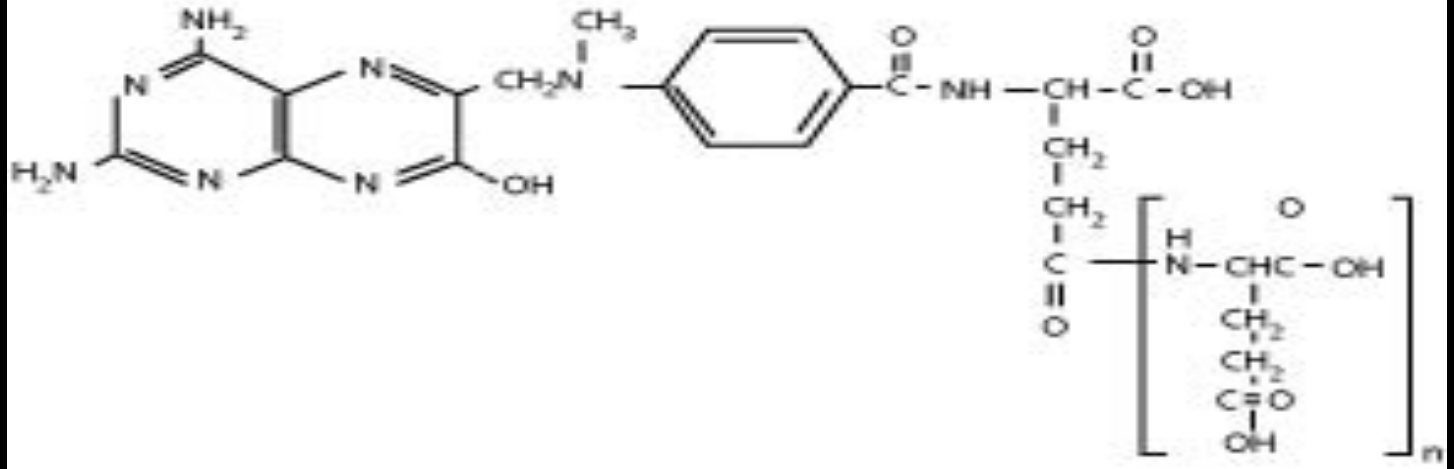
I have been a consultant to Cypress

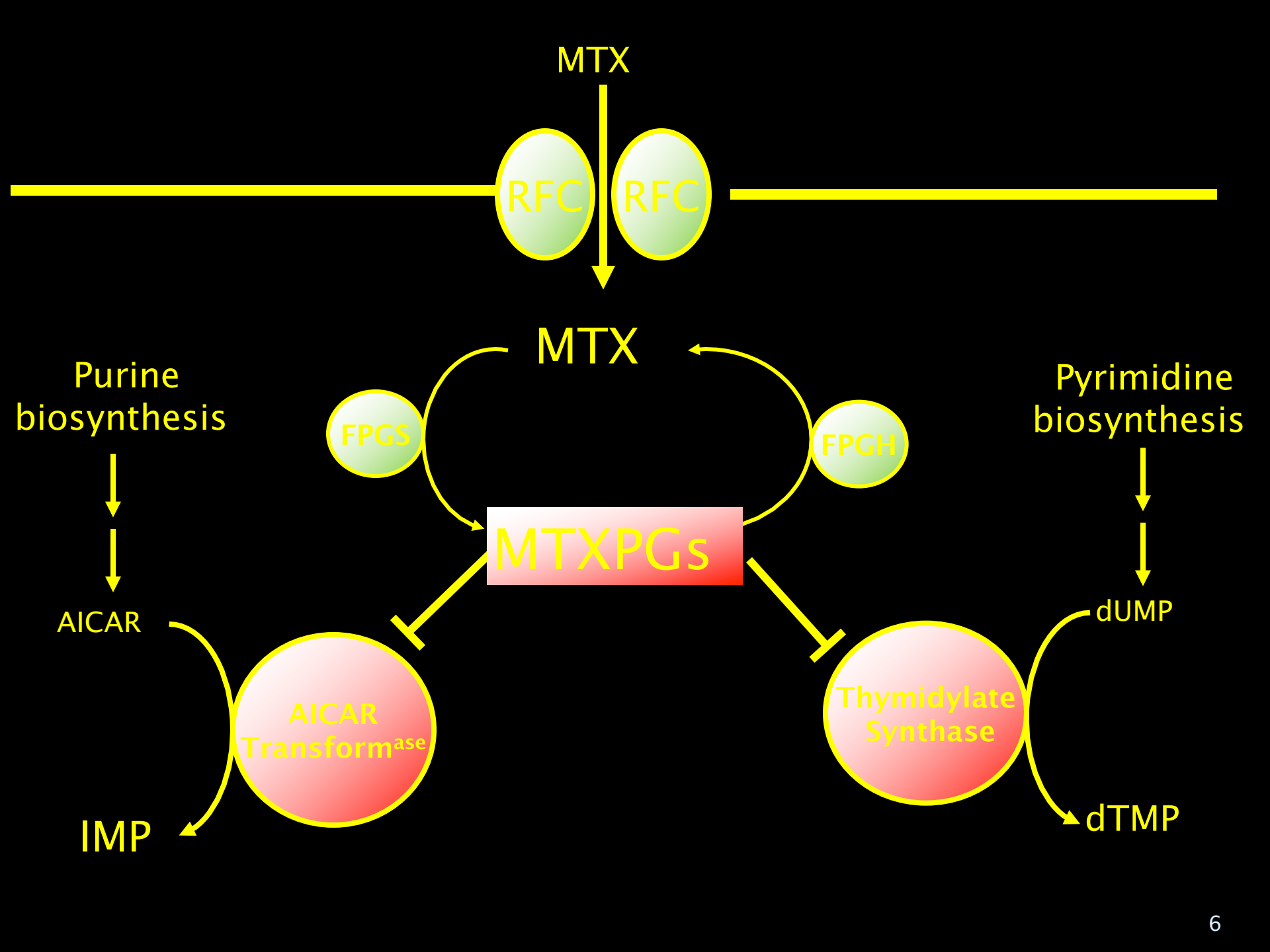
I won't 'derive'
everything I tell you.
(But there will be refs.)

Dihydrofolic Acid



Methotrexate




MTX
RFC
RFC
MTX
Purine biosynthesis
FPGS
FPGH
Pyrimidine biosynthesis
MTXPGs
AICAR
dUMP
AICAR Transformase
Thymidylate Synthase
IMP
dTMP

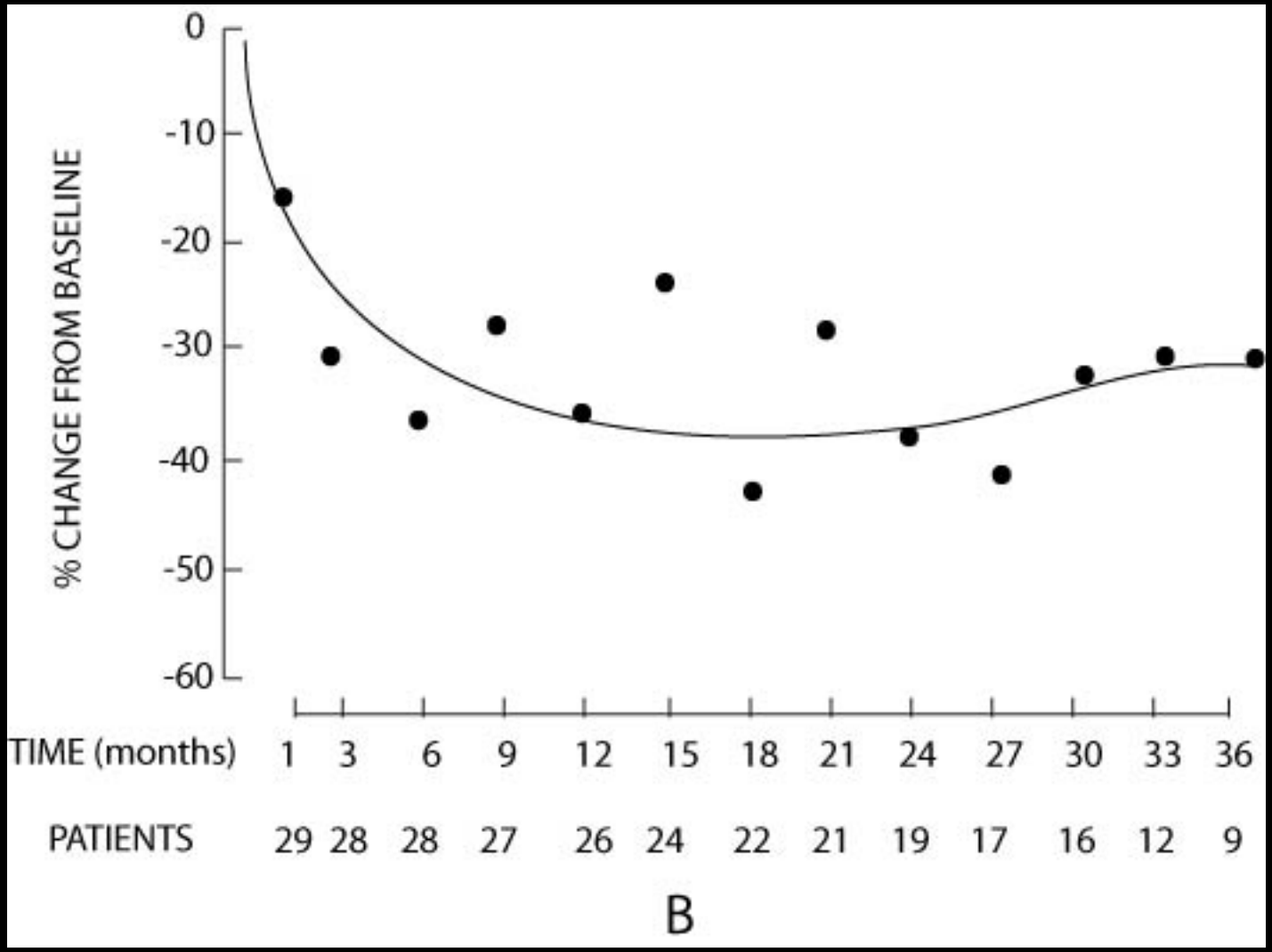
MTX Polyglutamates (MTX PGs)
What is the evidence?

- Dalrymple et al studied the effect of accumulation of PG moieties weekly in RBCs after starting and stopping MTX (Dalrymple et al, Arthritis Rheum 2008;58:3299–3308).
- MTX PG species were sequentially added and lost, but the process was slow.

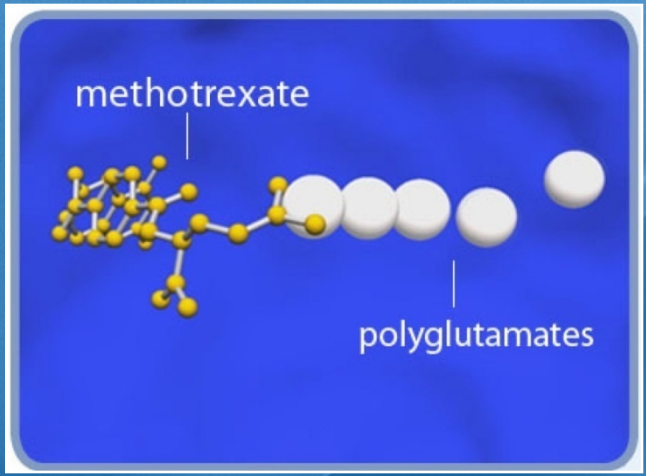
MTX PGs, cont'd

- The median time until 90% of the steady state concentration of MTX PG was reached was 27.5 weeks!
- The predominant MTX species was MTX PG_3 (37%), with PG_4 and PG_5 accounting for 11% and 6%

MTX Clinical plateau from (Kremer 1986, A&R)



Long-Chain MTX PGs Are the True Mediators of MTX Efficacy in RA



	Thymidylate Synthase	AICAR Transformylase
MTX	13.0	143
MTX PG_3	0.14	0.6
Difference	92-fold	238-fold

- Long-chain MTX PGs (i.e., MTX PG_3) are much more potent at inhibiting pyrimidine (thymidylate synthase) and purine (AICAR transformylase) synthesis than the parent drug, MTX
- The half-life of MTX PG_3 in RBCs is 60 days, vs. 6 to 8 hours in plasma

Abbreviation: AICAR, aminoimidazole carboxamide ribonucleotide.

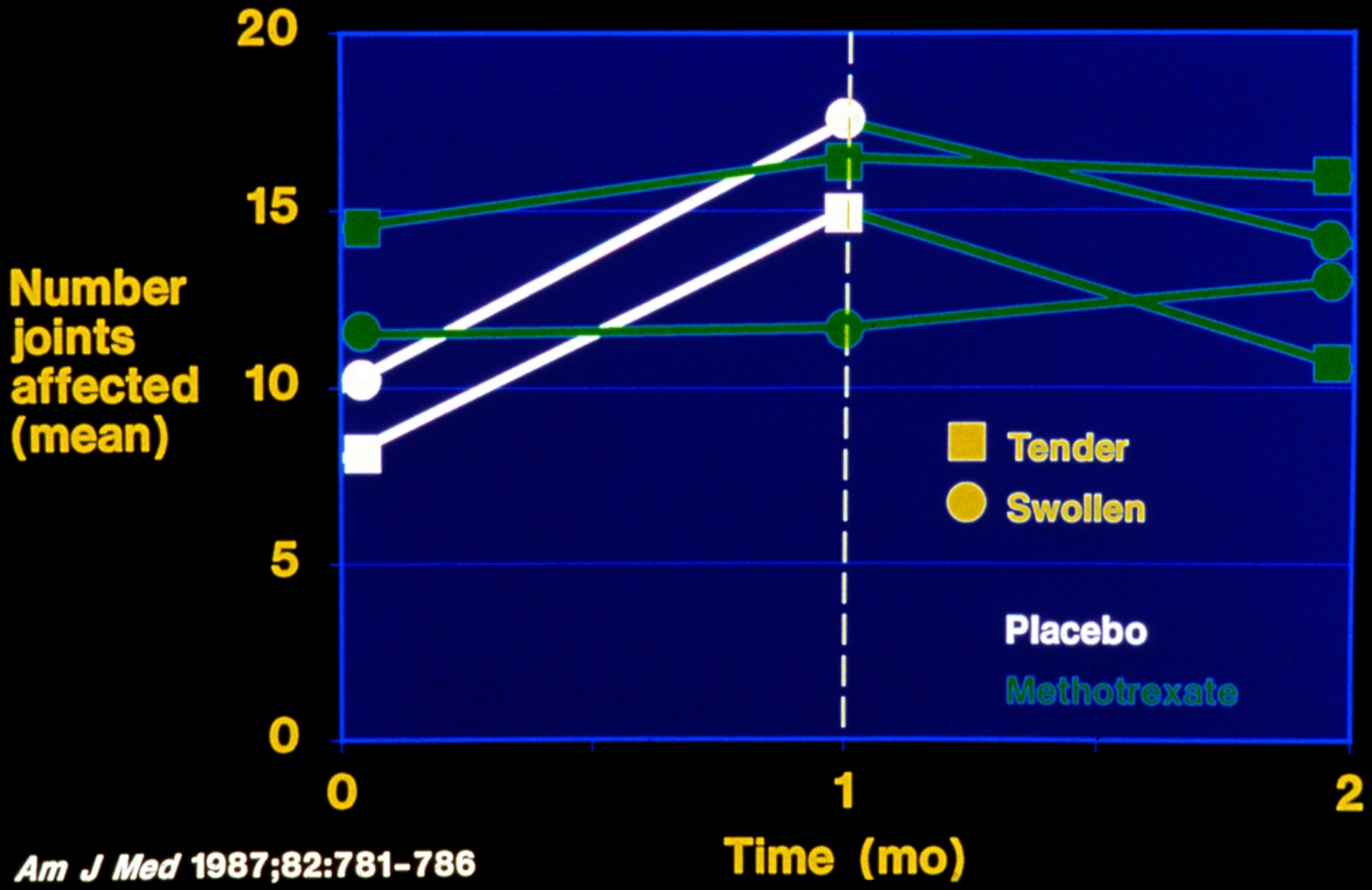
Allegra CJ et al. Proc Natl Acad Sci USA. 1985;82(15): 4881-4885.

Cypress BIOSCIENCE, INC.

MTX PGs, cont'd

Dalrymple et al:The elimination half-life of MTX PG_3 was 4.3 weeks, with a mean elimination half-life of 3.1 weeks for all MTX PG species

MTX *vs* PLACEBO AFTER LONG-TERM THERAPY



Am J Med 1987;82:781-786

MTX PGs, Conclusions

MTX (and folate) PGs are consistently associated with clinical efficacy of MTX.

The time course of MTX PG_{3-5} accumulation, and decrease, corresponds well with previously reported time course of MTX r*esponse*, and *time to flare*.

Clinical Take-away MTX-PGs

Don't Expect "Instant" Results When the Dose of MTX is Changed!

"Doc, I feel good and I haven't taken my MTX for three weeks. It must not be working" OR,

Call to the Office: "Doctor, you increased my weekly MTX three weeks ago and I don't feel any differently. It hasn't worked"

MTX PGs, Clinical, cont'd

The INTENSITY of MTX administration (dose,duration and route of admin) determines the effect of accumulation of MTX PGs.

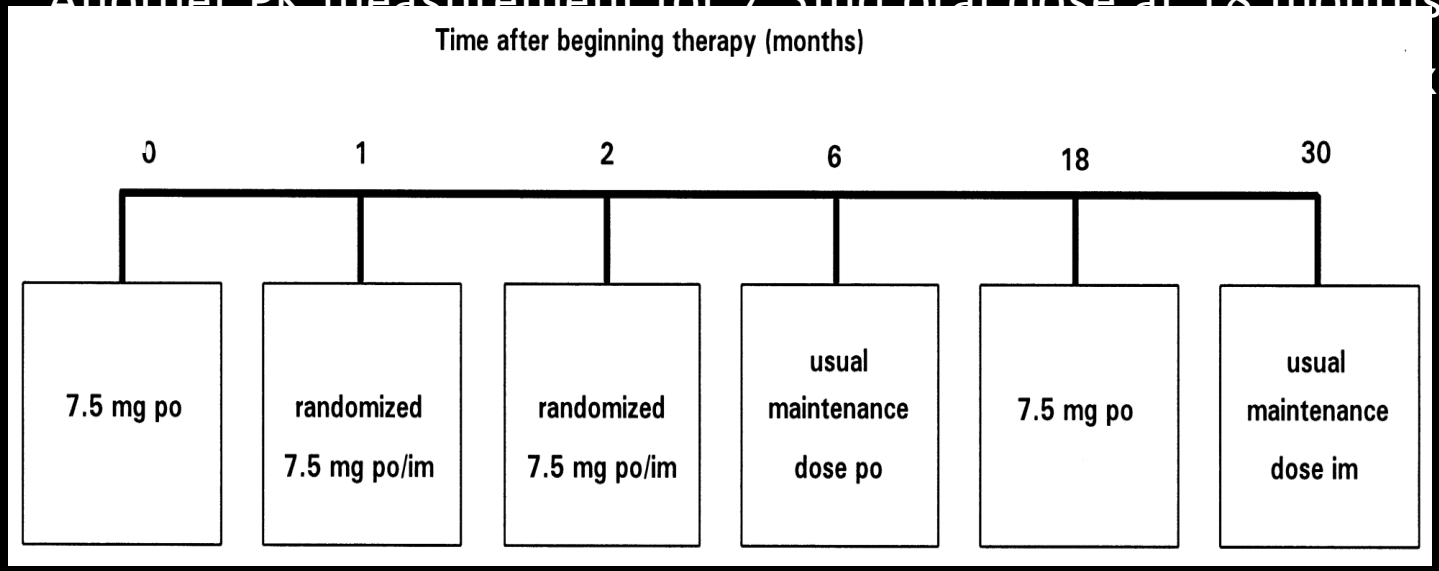
Therefore, it makes mechanistic sense to start with a higher dose, administer it SC, and rapidly advance while making certain that the pt is folate replete.

Oral vs. Subcutaneous MTX

- Oral absorption of MTX is *highly variable,* and may drop off by as much as 30% when increasing from 7.5 to 15 mg or more (Hamilton HR, Kremer JM, Br J Rheum 1997;36:86-90)
- Switching to Parenteral administration can be highly effective as it may greatly improve bioavailability (f)

Why IM MTX… Study Design

- 21 patients initiated on 7.5mg oral and i.m. MTX (randomized crossover)
- PK measurements for 7.5mg oral and i.m. MTX during first 2 months
- Patients titrated to an oral maintenance dose over next 6 months
- PK measurements for maintenance dose given oral (approx. 6 months)
- Another PK measurement for 7.5mg oral dose at 18 months
- [illegible]. 30



Source: Hamilton & Kremer, Br J Rheumatol 1997

Why IM MTX: PK Measurements

	Initiation	6 months	18 months	30 months
Oral MTX	7.5mg	Maint. Dose	7.5mg	Maint. Dose
AUC (µmol·h/l)	2.631	4.827		
IM MTX	7.5mg			Main. Dose
AUC (µmol·h/l)	2.617			5.500

Mean maintenance dose: 17 ± 3.8mg

Oral MTX bioavailability is reduced as the dose is increased.

Relative Bioavailability	7.5mg	Maintenance Dose
Oral vs. IM MTX	100%	88%

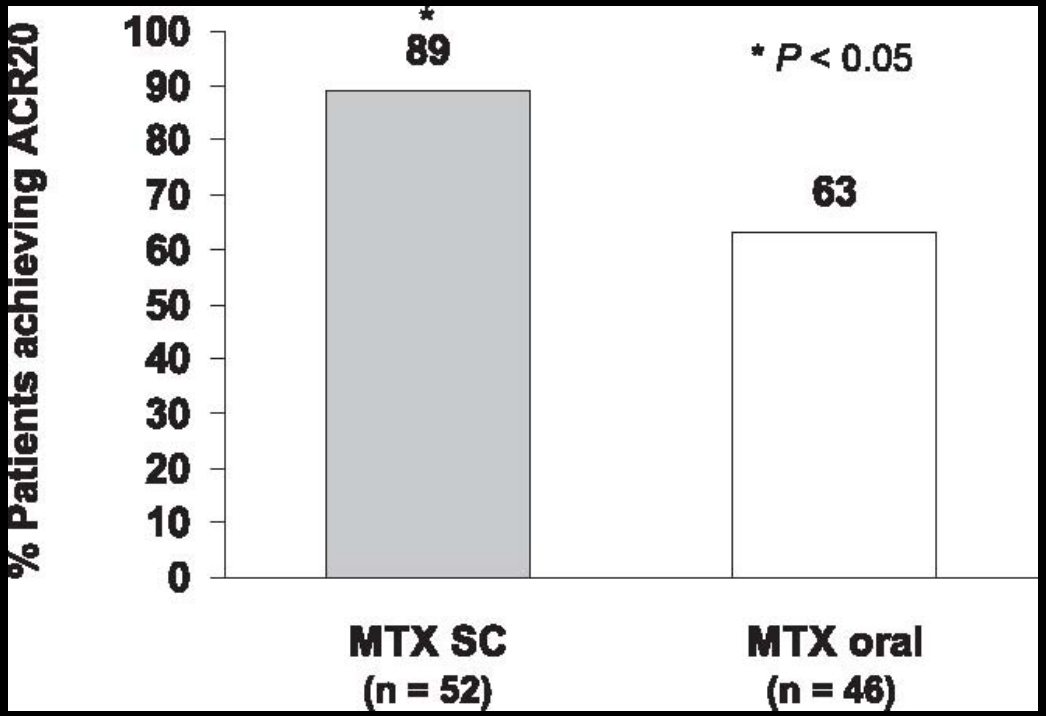
Source: Hamilton & Kremer, Br J Rheumatol 1997

Efficacy and Safety of Subcutaneous vs. Oral MTX in RA

- A large (N=375) 6-month, randomized, double-blind, controlled trial examining clinical efficacy and safety following oral and SC MTX (15 mg/week) administration.
- If ACR20 was not met at Week 16, patients receiving 15 mg oral MTX were switched to 15 mg SC MTX and patients receiving 15 mg SC MTX were switched to 20 mg SC MTX.

Source: Braun et al, Arthritis Rheum 2008

Subcutaneous vs. Oral MTX: ACR Results



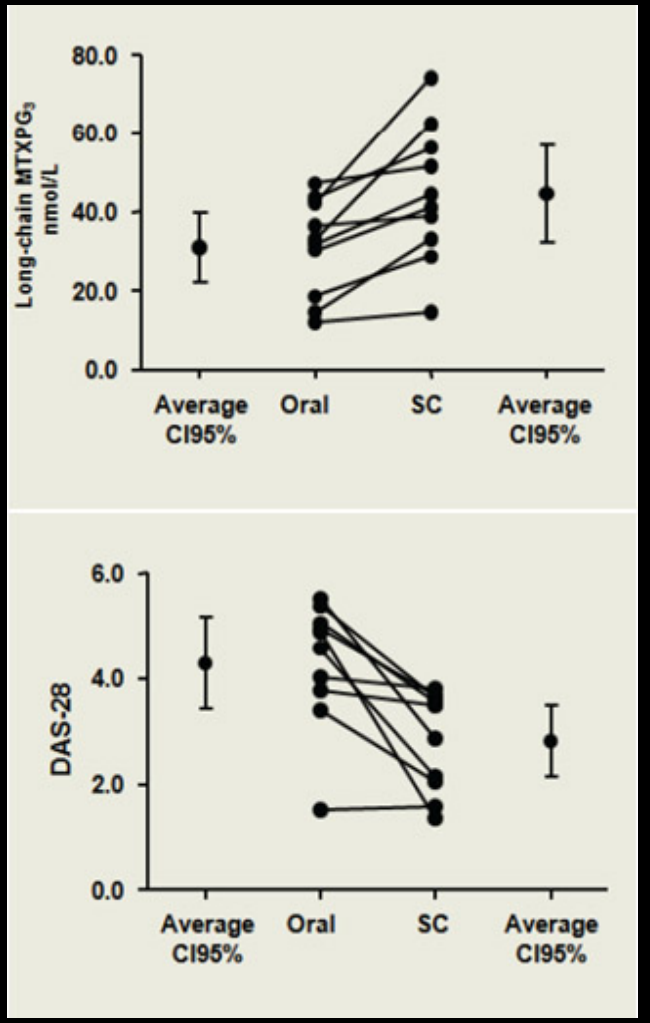
- Percentages of patients with ≥1 year between diagnosis and study entry who achieved an ACR20 response.
- The percentage of patients with an ACR20 response was significantly higher for the SC MTX group than for the oral MTX group.

Source: Braun et al, Arthritis Rheum 2008

Increased MTX PGs and Decreased DAS with Switch from Oral to Parenteral MTX

- 10 RA patients on oral MTX for avg. 27 weeks, with mean DAS-28 of 4.3 were switched from oral to parenteral MTX
- Switching to parenteral MTX resulted in significant reduction in DAS-28 (median 31%; range 5% to 72%; p=0.004)
- Median % increase in long-chain MTX PG_3 after switch to parenteral was 37% (range 6% to 123%) (p=0.002)
- % change in the DAS-28 was associated with the absolute change in MTX PG_3 (R^2=0.50; p=0.022) thereby indicating that parenteral MTX produced greater antiarthritic effects through greater accumulation of MTX PGs

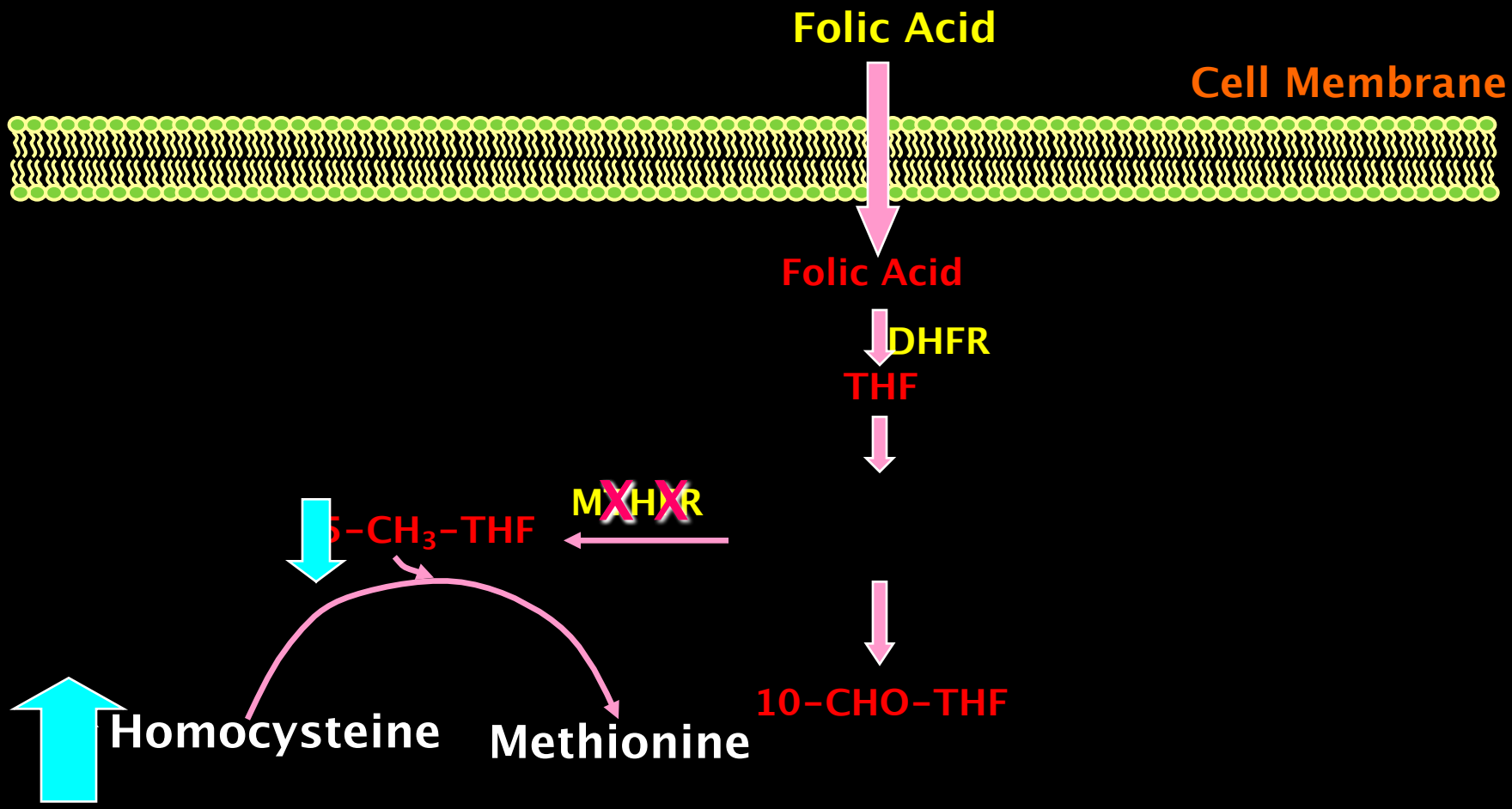
Dervieux, et al, Rheumatology, 2010



Clinical Take-Away;SC vs. po MTX

- If a patient experiences less than adequate response to oral MTX, switch to SC.
- OR, split the oral MTX dose as the lesser oral doses are better absorbed (Hamilton & Kremer Br J Rheum 1997)

Effect of C677T polymorphism on Folate Pathways

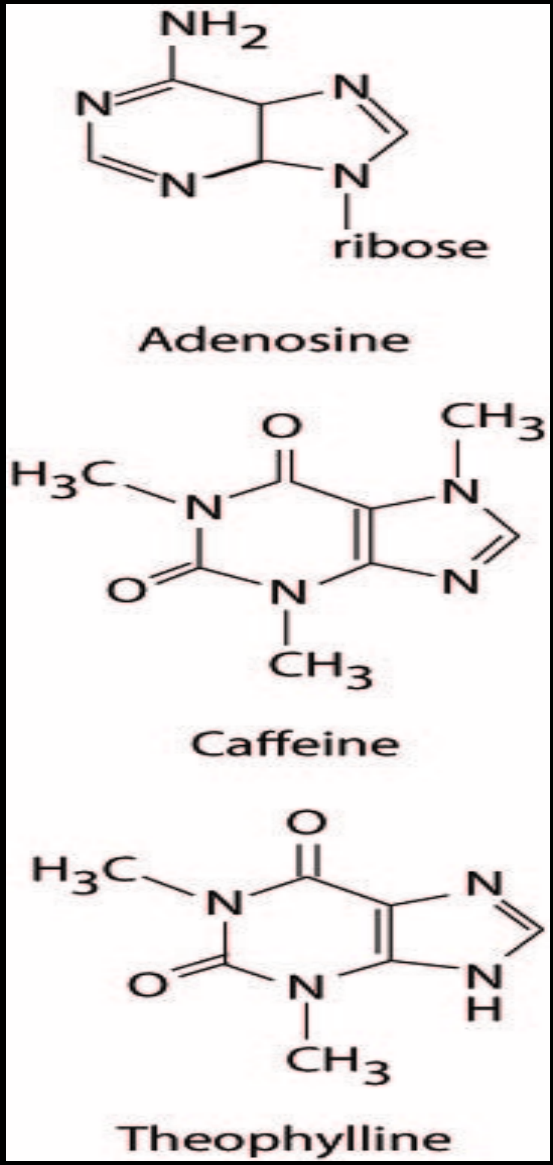


Clinical Take-Away:MTX Induction of Hyperhomocysteinemia Is Reversible

Folic acid supplementation will reverse these effects and restore elevated levels of homocysteine to baseline (Morgan SL, et al. J Rheumatol 1998;25:441-6)

Clinical Take-Away; MTX and Caffeine

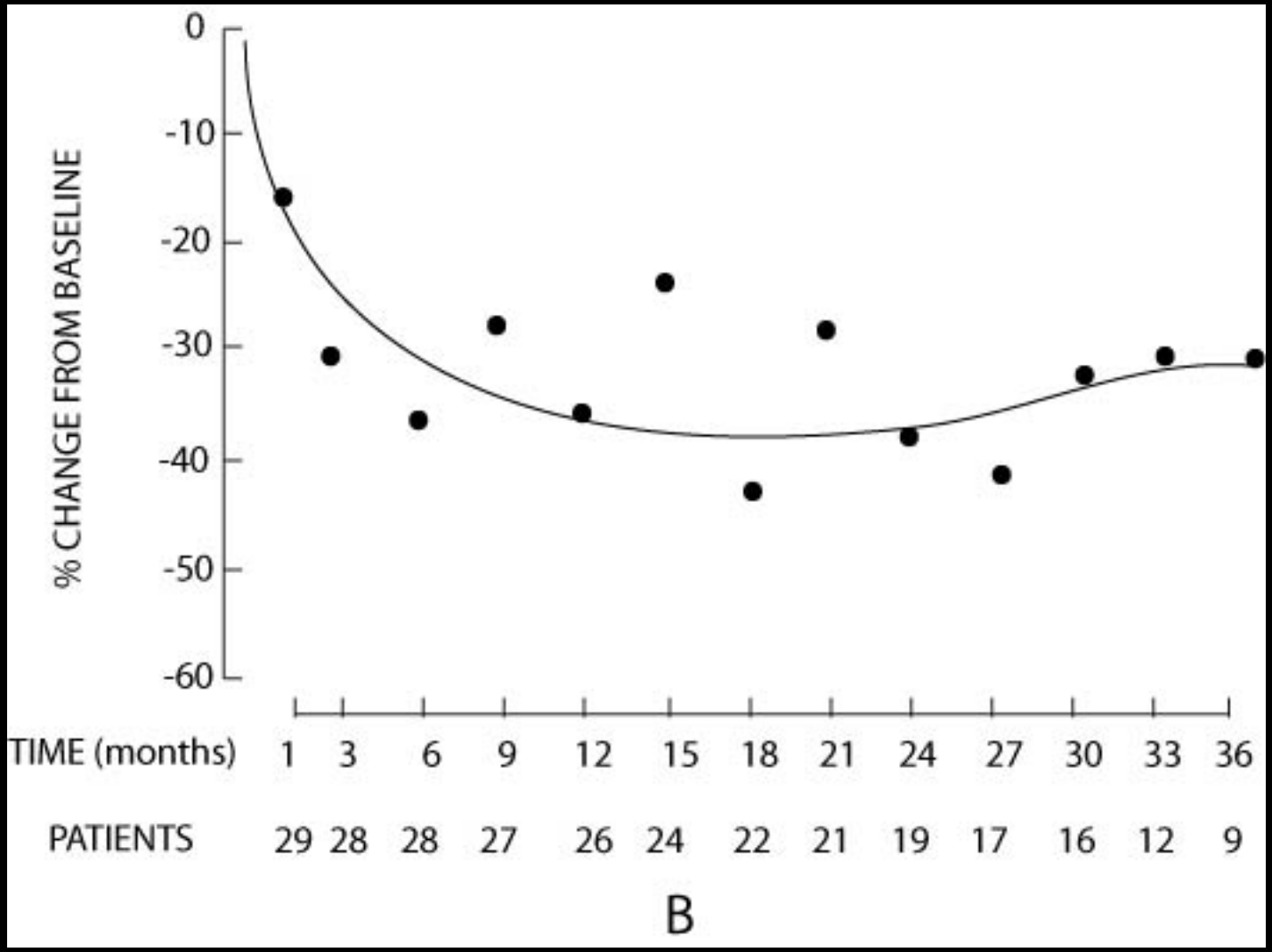
- If your patient has a significant intake of caffeinated beverages and has responded inadequately to MTX, try asking them to significantly decrease the caffeine intake.



Can RA Pts Become Resistant to MTX?

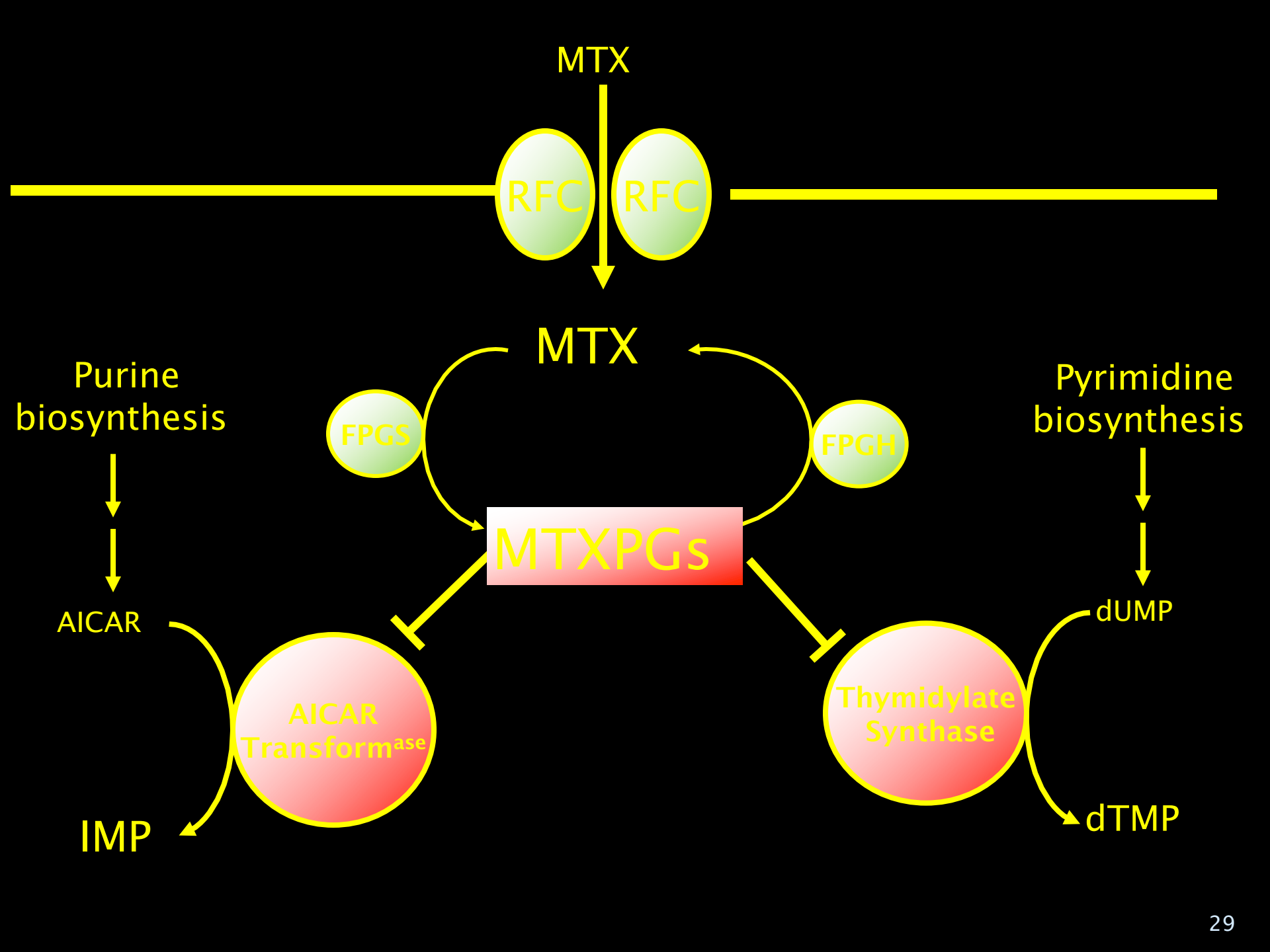
1. What is responsible for the plateau in response to MTX (Kremer, Lee. Arthritis Rheum 1986;29:822-31.) after 6 mos ?

MTX Clinical plateau from (Kremer 1986, A&R)



Resistance to MTX?

- Rapidly dividing cells take up MTX at the RFC more readily with a decreased rate of efflux. (Chello et al. Mol Pharmacol 1980;18:274-80)
- Activated synovial macrophages from RA tissue exhibit upregulated RFCs which enhance MTX transport. (Nakashima-Matsushita et al. Arthritis Rheum 1999;42:1609-16).
- The (MTX) resultant decreased cellular activity results in decreased uptake of MTX at the RFC. Thus, long-term, clinically effective MTX could lead to diminution of its own uptake and the well described plateau of response!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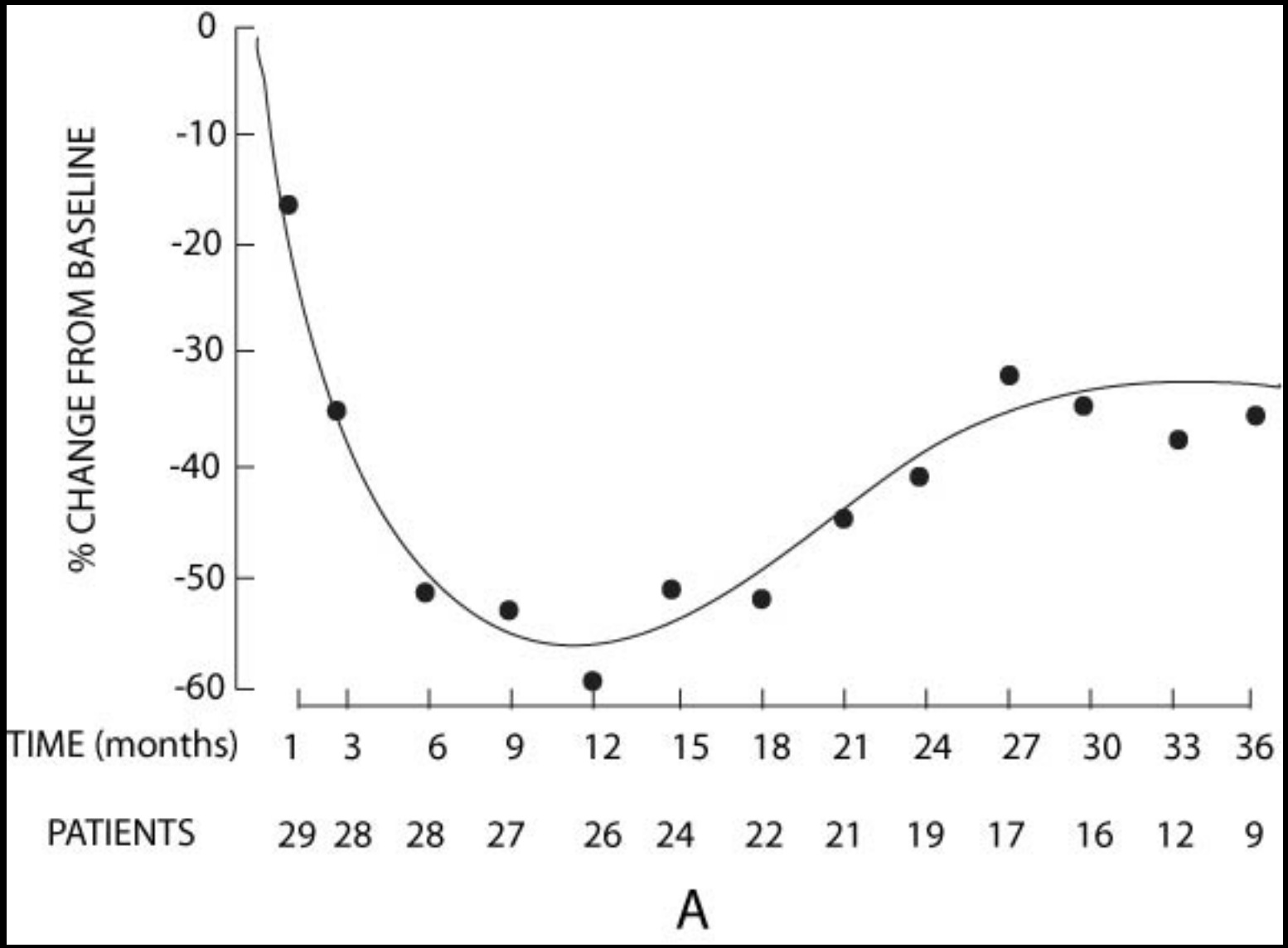

0
-10
-20
-30
-40
-50
-60
% CHANGE FROM BASELINE
TIME (months) 1 3 6 9 12 15 18 21 24 27 30 33 36
PATIENTS 29 28 28 27 26 24 22 21 19 17 16 12 9
A

Resistance to MTX, cont'd

There are additional mechanisms of induced biochemical resistance to MTX which are beyond the scope of this lecture.
(Bertino J. J Clin Oncol 1993;11:5–14.)
(Zhao, Goldman Oncogene 2003;22:7431–57).

Why doesn't the Addition of Folates Abrogate the Effects of MTX?

- Cells with a higher rate of metabolic activity will also polyglutamate MTX more readily than normally dividing cells. (Fabre I, et al. Cancer Res 1984;44:3190-50)

- The selective salvage of normal cells after addition of folic, or folinic acid, is possible because of the different rates of metabolic activity in different cell pops. Normal cells will respond more readily to salvage, as they have less MTX PG than more metabolically active cells.

The overall maintenance of therapeutic effect with the addition of folates is therefore made possible because of the *differential metabolic activity of cellular populations.* That is, resting cells require less folate to block MTX activity.

MTX Improves CV Mortality

- The beneficial effect of MTX on CV mortality are better than those of other traditional DMARDs

- Choi HK, et al. Lancet 2002;359:1173-7

MTX and CV Risk, cont'd

MTX potentiates A2A receptor mediated effects.
(Riksen NP, et al. Ann Rheum Dis 2006;65:465–470)

MTX and CV Risk

1. Direct biochemical potentiation of pathways associated with a decreased risk of CV disease
2. Anti-inflammatory effect on disease activity

DMARDs, MTX, BRMs and Cardiovascular Risk:

	Hazard Ratio	(95% CI)
TNF blocker	0.36	0.18-0.74
MTX	0.83	0.44-1.57
Nonbiologic DMARD	1.00	Reference
Steroid dose:		
none	1.00	Reference
1-2.5mg	1.90	0.93-3.88
3-7mg	1.89	1.05-3.40
>7mg	3.00	1.44-6.25

Greeenberg JD, Annals Rheum Dis, 2009

*Outcomes include MI and CVA

MTX and CV Disease: Clinical Take-Away

- Many of the drugs rheumatologists use to treat arthritis including corticosteroids and NSAIDs are potentially deleterious to the CV system. MTX is not in this category, and may indeed help!

Interactions of MTX with other DMARDs

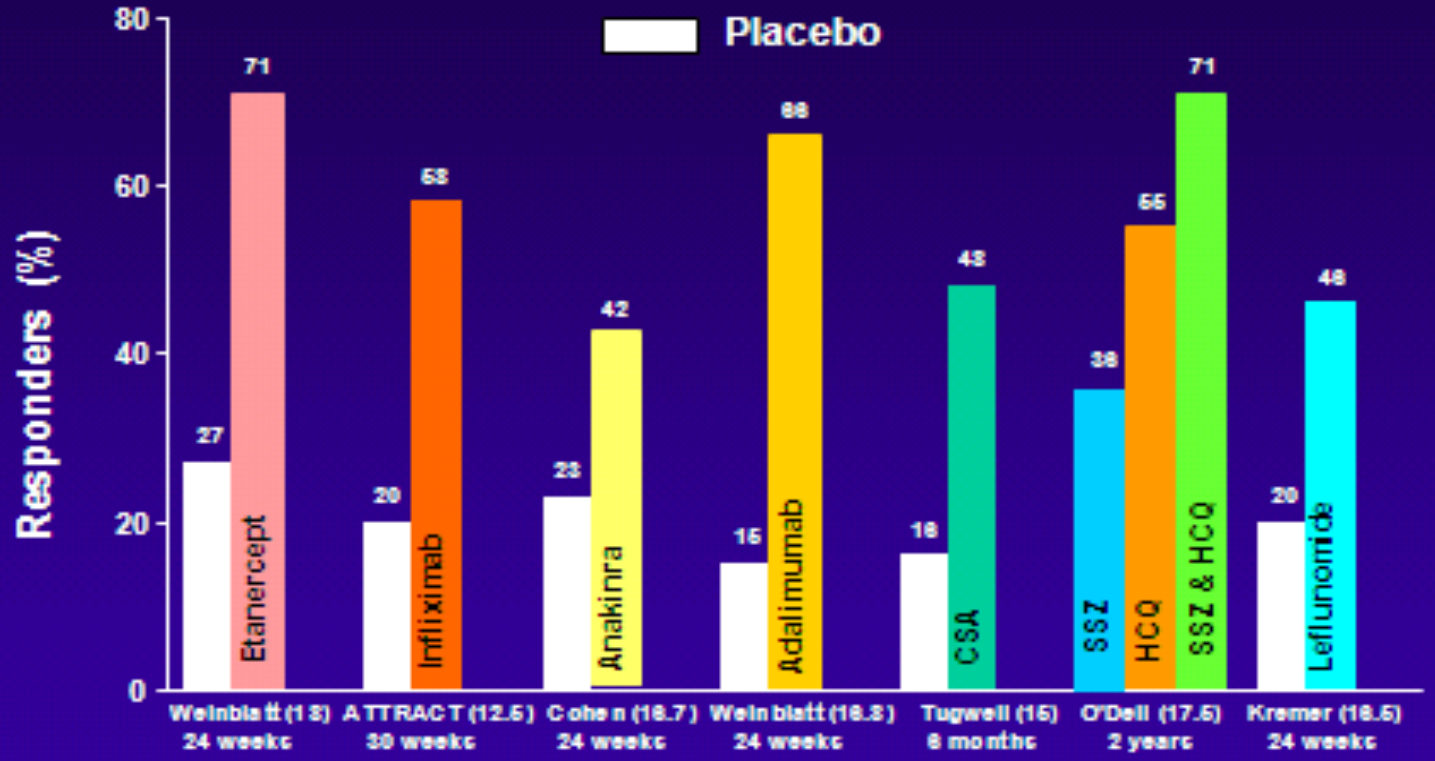
Sulfasalazine
Hydroxychloroquine

MTX and SSZ: Clinical Effects

Shouldn't MTX and SSZ be better than MTX/HCQ as the former represents a combination of antifolates which could be synergistic?

However this was not observed (O'Dell et al, Arthritis Rheum, 2002;46:1164-70), as the clinical results favored MTX/HCQ

MTX Failures: ACR20

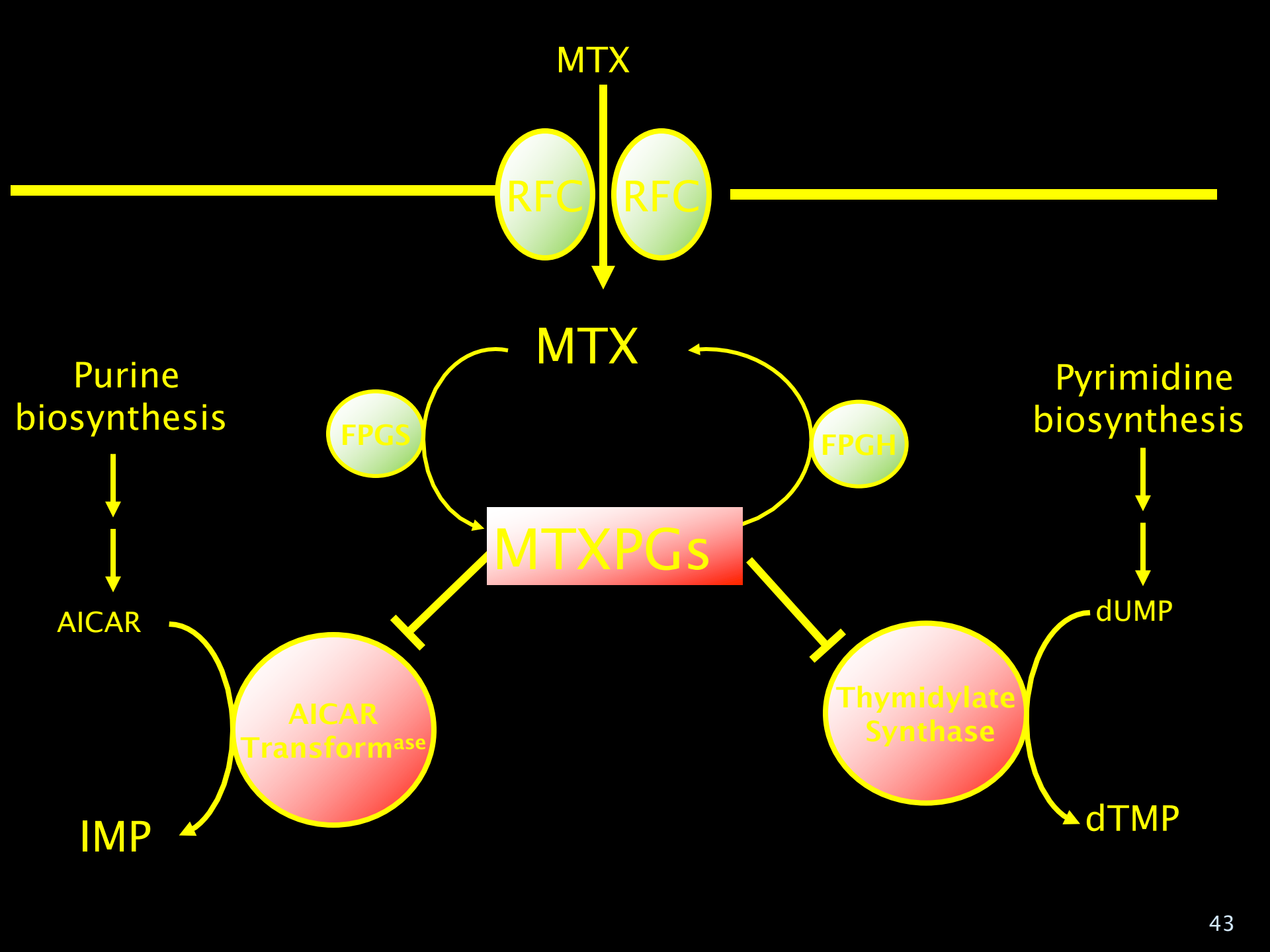


*All Patients Continue to Receive Baseline Methotrexate

Gold, Abatacept, Tocilizumab, Rituximab, Golimumab, and Certizumab as well as a number of non approved agents could all be added also

MTX-SSZ

SSZ is a potent, non-competitive inhibitor of the Reduced Folate Carrier (RFC) (Jansen et al. Arthritis Rheum 2004;50:2130-39)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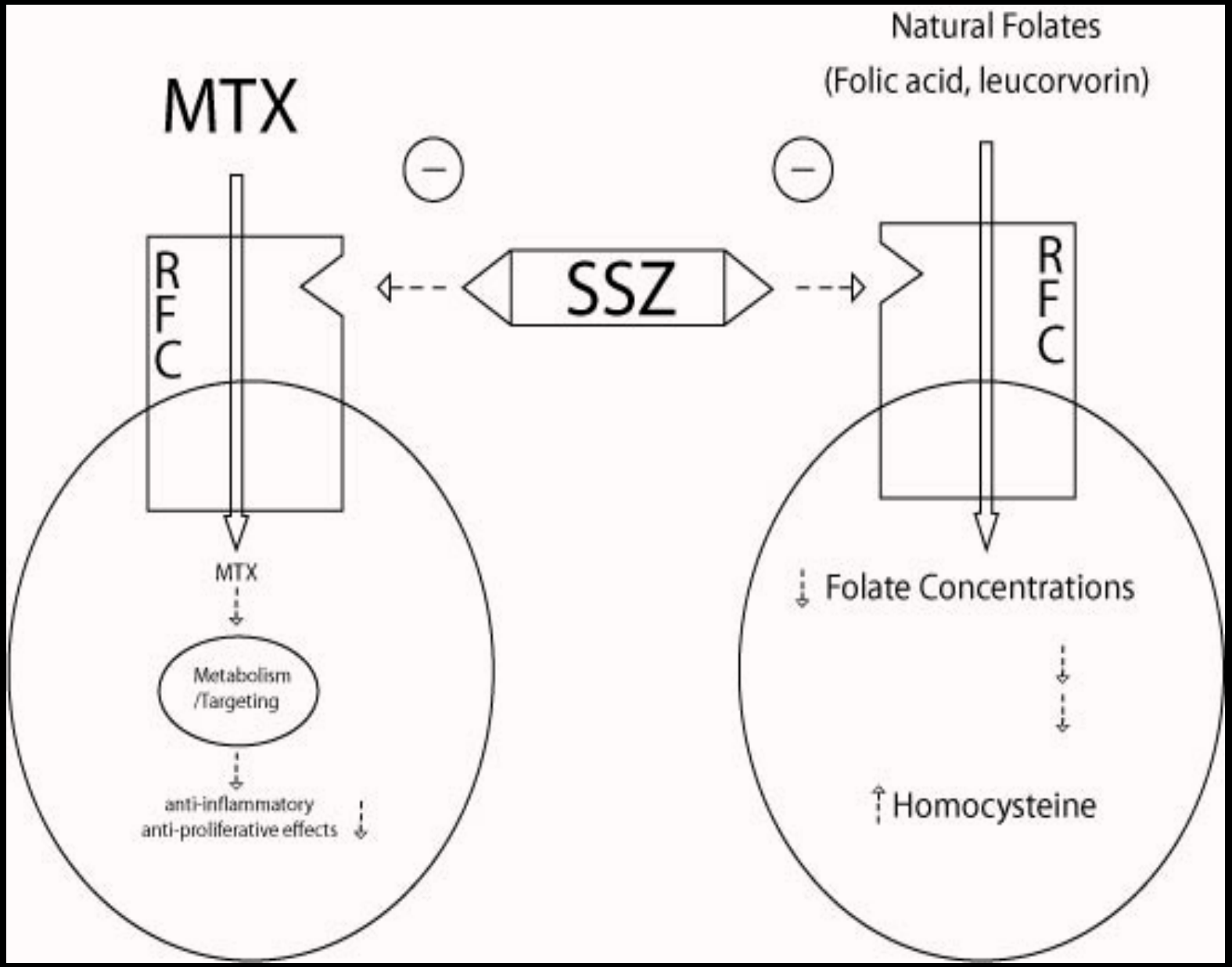

Natural Folates
(Folic acid, leucorvorin)
MTX
SSZ
RFC
RFC
MTX
Metabolism
/Targeting
anti-inflammatory
anti-proliferative effects
Folate Concentrations
Homocysteine

MTX and SSZ; Clinical Take-Away

Further study is warranted, but both biochemical and clinical evidence to date would indicate that this may not be an ideal biochemical cellular combination.

MTX-HCQ

1. HCQ sustains the AUC of MTX, although not vice versa (Carmichael SJ et al. J Rheumatol 2002;29:2077-83.)
2. HCQ increases active tubular reabsorption of MTX (Carmichael)

MTX and HCQ; Clinical Take-away

Independent of any therapeutic value of HCQ, it is likely that HCQ actually potentiates the effects of MTX.

Determinants of MTX Toxicity

Any prolonged exposure to a serum level of MTX >0.05μM for >24 hrs will result in a cytotoxic effect.

This is, the duration of exposure of rapidly dividing cells to MTX will determine whether they become toxic (as more cells enter S phase).

As MTX is excreted entirely via the kidneys, any compromise in renal function which prolongs the terminal half-life of MTX beyond 24 hrs at these levels will result in toxicity to rapidly turning over cells in the gut and bone marrow.

MTX Toxicity; Clinical Take-Away

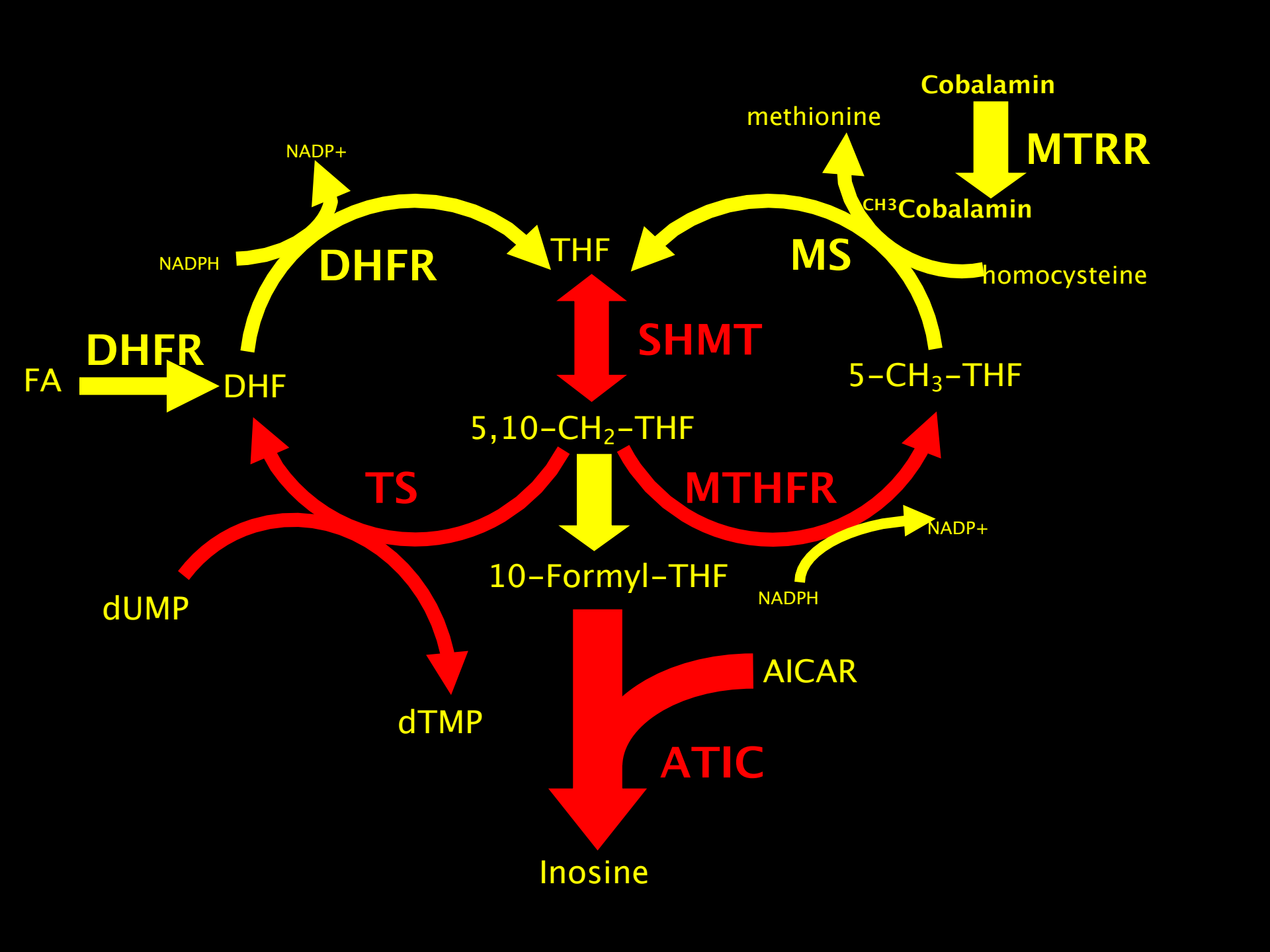
The reasons for toxicity to MTX are both simple (compromised renal function) and complex (all of the possible variations in folate pathway SNPs).

BE CAREFUL when using MTX in pts with renal insufficiency, OR when changing NSAIDs with different possible renal effects.

THANK YOU! GRAZI!

Determinants of MTX Toxicity, cont'd

In addition, the multiple well described SNPs in folate pathway enzymes will determine an individual's relative susceptibility to MTX toxicity (Ranganathan et al. Ann Rheum Dis 2003;62:4-9)


Cobalamin
methionine
MTRR
NADP+
CH3Cobalamin
NADPH
DHFR
THF
MS
homocysteine
SHMT
DHFR
FA
DHF
5-CH3-THF
5,10-CH2-THF
TS
MTHFR
NADP+
10-Formyl-THF
dUMP
NADPH
AICAR
dTMP
ATIC
Inosine

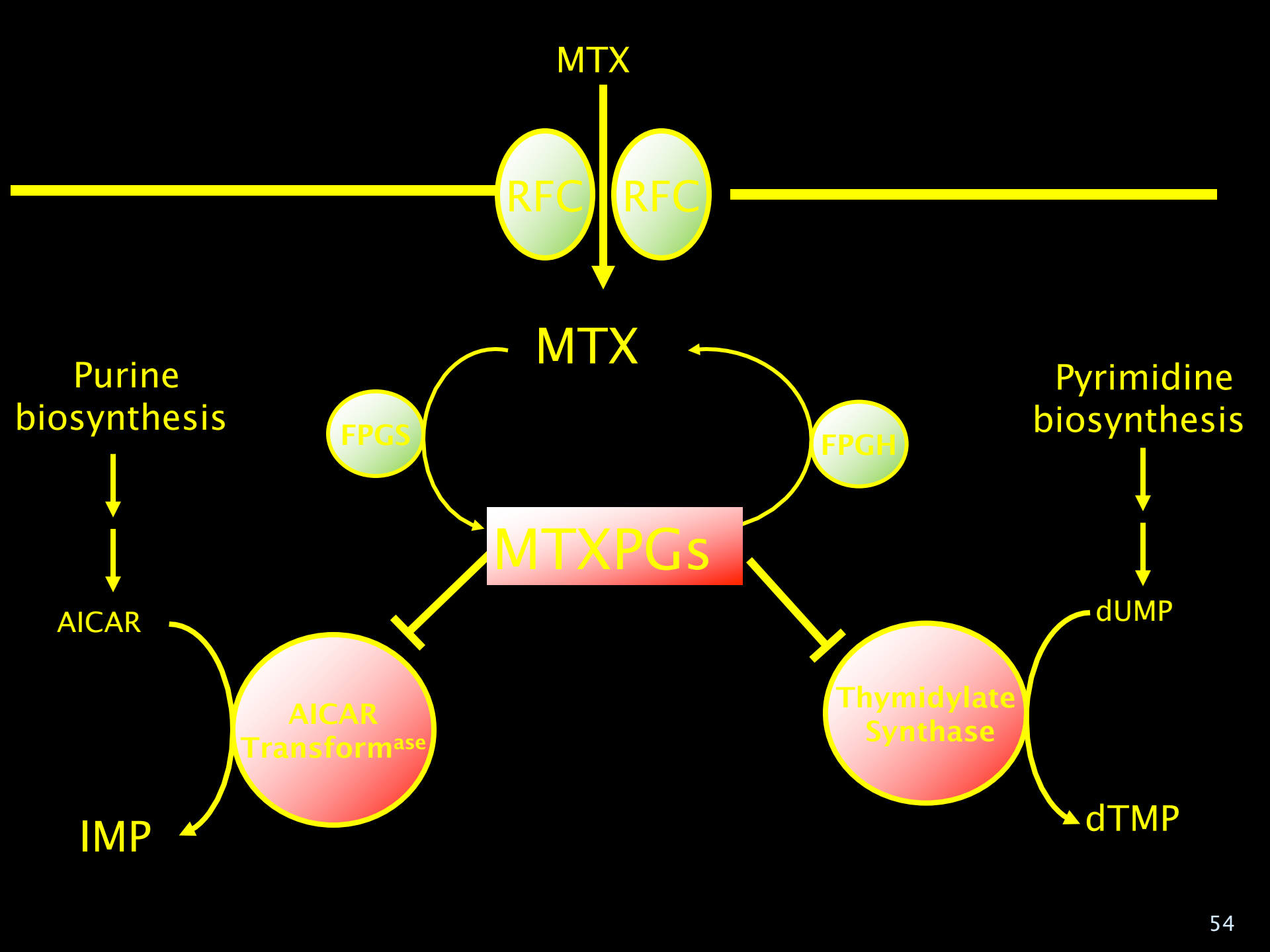
MTX Toxicity and Leucovorin

In Pts with compromised renal function, cytotoxic blood levels of the drug may persist for 3-5 days.

Leucovorin (folinic acid) should ideally be administered in the first 24 hrs after a MTX dose (when it will inhibit both cellular uptake [at RFC] and polyglutamation [FPGS]).

However even after periods of > 24 hrs, leucovorin is known to "chase" MTX from the cell (increased efflux) and can still be administered, although with less potential for full salvage.

(Chu, Allegra. Antifolates. In: Chabner BA, Longo DL, Eds. Cancer Chemotherapy and biotherapy. Lippincott-Raven;1996:109-47).


MTX
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Clinical Take-Away; MTX Toxicity

- The potential for MTX toxicity will be very different among individuals reflecting the complexity of all folate pathway SNPs
- Understanding the differential cellular sensitivities to MTX will allow for understanding of the sites of common toxicity and facilitate treatment (add'l folate or folinic acid supplementation) to alleviate them.

End of Lecture

Back-up follows

MTX Effects on CV System

1.Potentiation of adenosine and dipyrimadole -induced vasodilatation.
2. Adenosine associated negative inotropic and chronotropic effects.
3. Presynaptic inhibition of sympathetic neurotransmitter release.
4. Inhibition of vascular smooth muscle cell proliferation.
5. Inhibition of thrombocyte aggregation.
6. Enhanced myocardial resistance to ischemia and reperfusion.