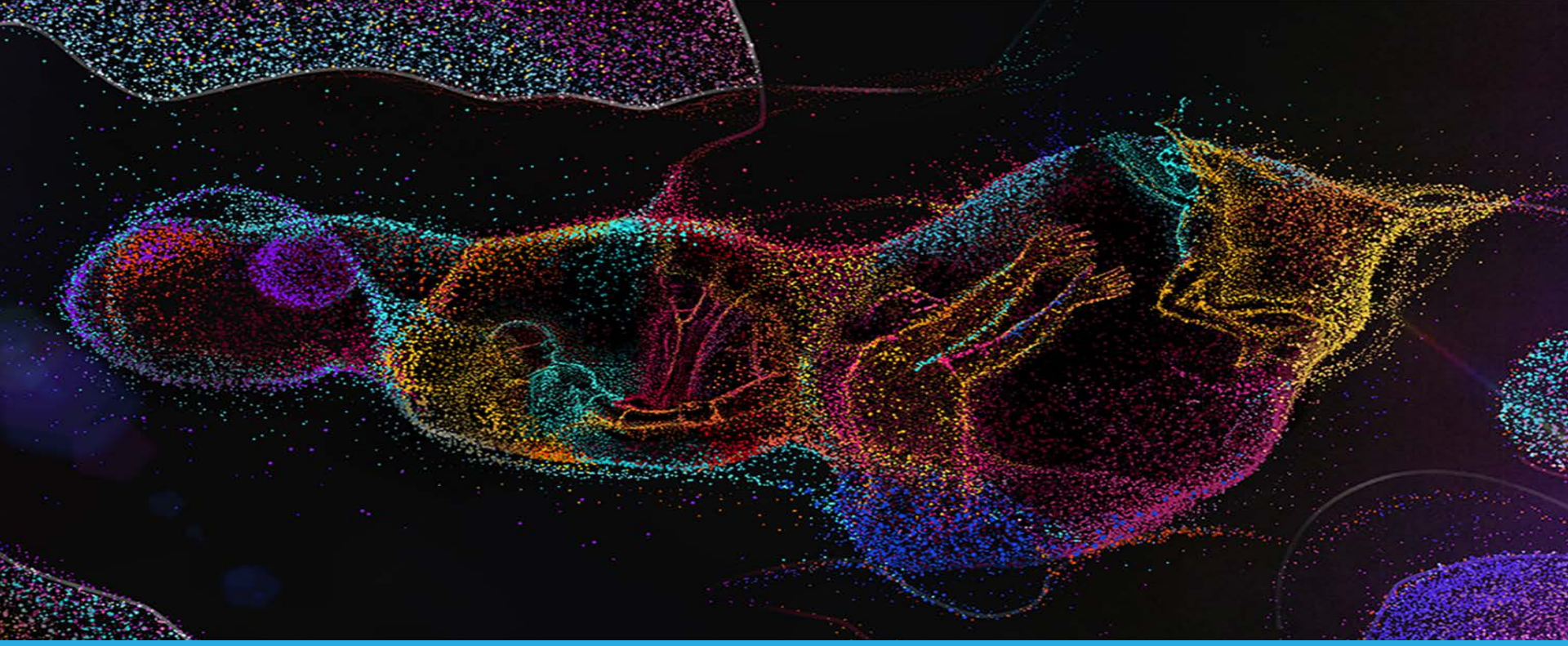

Emerging Importance of Nutrient Transporter-Mediated DDIs

-- focusing on the thiamine transporters

International Transporter Consortium Workshop 3
Washington DC
March 13, 2017

Yan Zhang, Ph.D
Incyte Corporation
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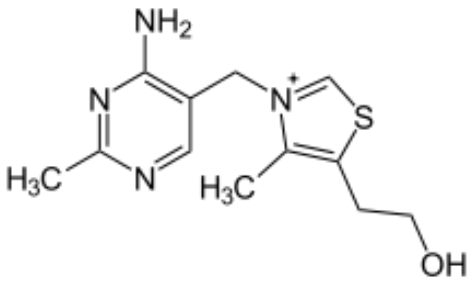
Mar-13-2017

Thiamine



- **Vitamin B1**
  - -Essential nutrient
    - oRecommended daily intake of 1.1 to 1.2 mg thiamine for adults
  - -Present in three forms: thiamine, thiamine monophosphate (TMP), and thiamine pyrophosphate (TPP)
  - -TPP is the coenzyme for cellular metabolism
  - -Pentose phosphate pathway and energy production

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Thiamine Deficiency

- **Poor dietary intake**
- **Increased metabolic requirement**
- **Reduced GI absorption**
- **Chronic medical conditions**
- **Chronic alcohol use**
  - -reduction of the THTR expression
  - -inhibition of thiamine absorption through intestine and the reabsorption by kidney
- **Clinical symptoms**
  - -Beriberi – cardiovascular
  - -Wernicke's encephalopathy (WE) – neurological

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Wernicke's encephalopathy (WE)

- **An acute neurological disorder**
  - -characterized by the triad ophthalmoplegia, ataxia, and mental confusion
  - -the most important encephalopathy due to a single vitamin deficiency
- **The main cause of WE is due to an inadequate supply of thiamine to the brain**
  - Absorption in GI epithelium - active and saturable transport at the intestine: THTR-1 and THTR-2
  - Export into the blood – basolaterol transporters
  - Distribution to extracellular fluid of brain- Cross the blood-brain barrier to reach the neurons
  - Enter into mitochondria of neurons
- **WE normally treated with high doses thiamine through parenteral route (IV/IM)**

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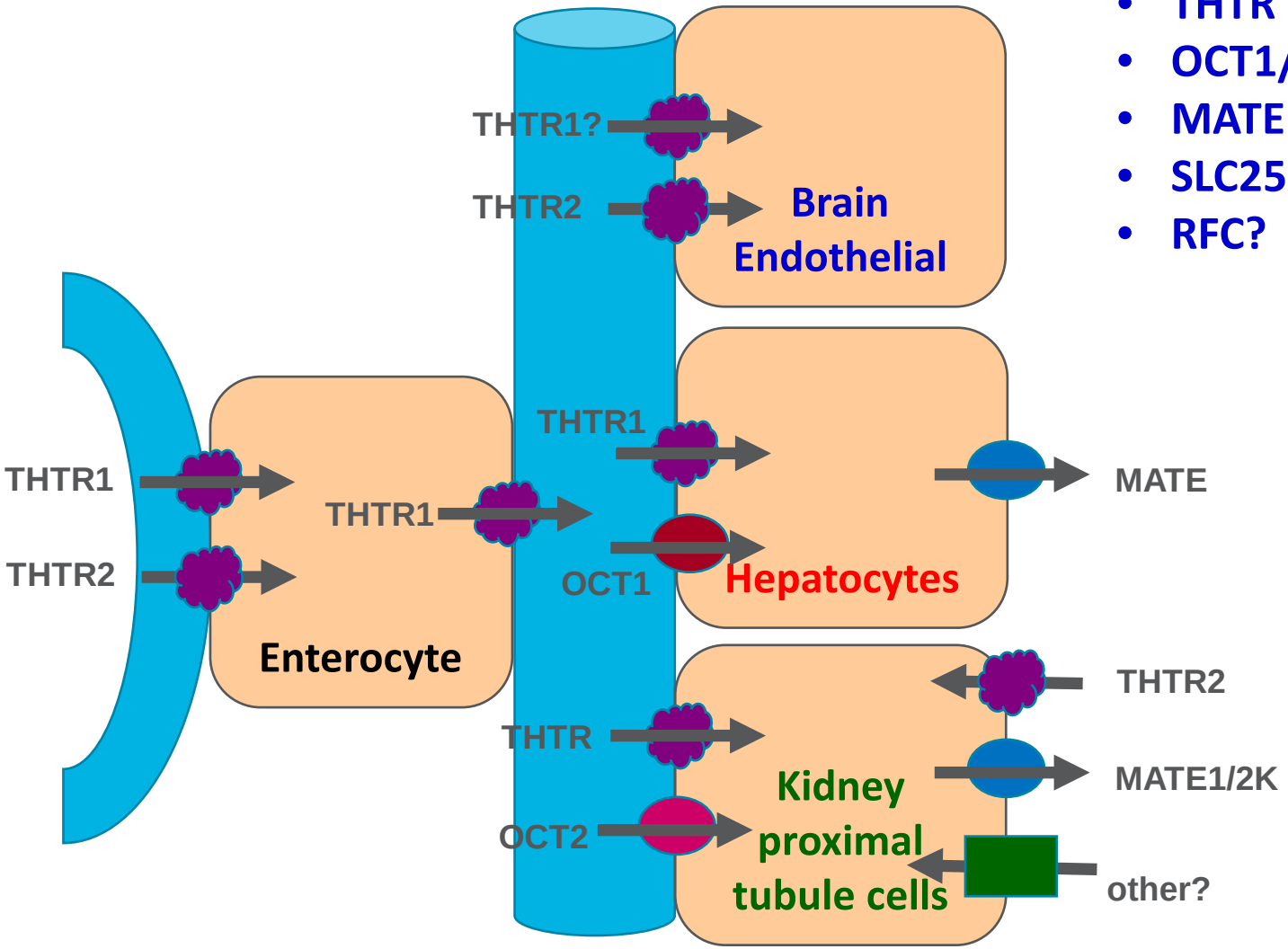
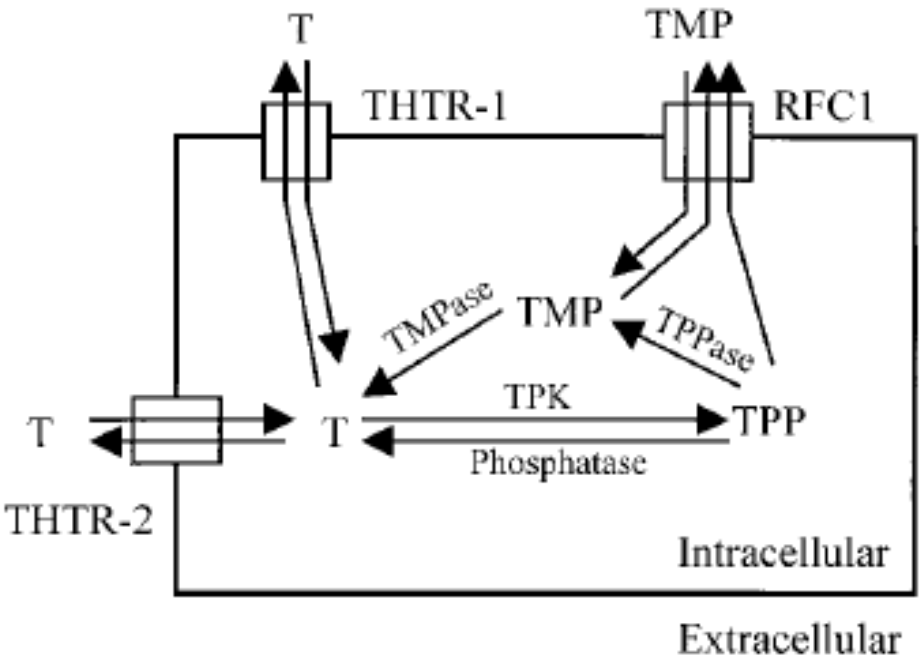
Transporters that are Responsible for Thiamine Transport

- THTR
- OCT1/2
- MATE1/2K
- SLC25A19
- RFC?



Liang et al, Mol Pharmaceu 12, 4301 – 4310, 2015
Giacomini M et al, Drug Metab Disp 45, 76 – 85, 2017
Zhao and Goldman, Mol Asp Med 34, 373 – 385, 2013
Chen et al, PNAS 111, 9983 – 9988, 2014

Interplay between Folate and Thiamine Transporters



- **Thiamine (T) is transported by THTR1/2**
- **TMP and TPP are transported by RFC**
- **Transport of TMP and TPP can be inhibited by folate**

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Reprinted with permission from Zhao and Goldman, Mol Asp Med 34, 373 – 385, 2013

Thiamine Transporters

- **THTR-1 (SLC19A2) and THTR-2 (SLC19A3)**
  - THTR-1 is widely expressed while THTR-2 is expressed at the highest level in the duodenum
  - THTR-1 localized on both the BBM and BLM of enterocytes, while THTR-2 restricted to the BBM localization.
  - Share considerable similarity to one another (48%) and to RFC (~40%)
    - THTR-1/2 do not transport folate and RFC does not transport thiamine
  - THTR-2 may be more important in intestinal thiamine uptake
    - Uptake was significantly reduced in THTR-2 knockout mice while normal uptake was observed in THTR-1 KO mice
  - Intestinal thiamine uptake is adaptively regulated by the thiamine level in the diet
    - Expression of THTR-2 was upregulated in transgenic mice fed with a thiamine deficient diet

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1521-009X/42/10/1656–1662$25.00
Drug Metabolism and Disposition

http://dx.doi.org/10.1124/dmd.114.058883
Drug Metab Dispos 42:1656–1662, October 2014

The Janus Kinase 2 Inhibitor Fedratinib Inhibits Thiamine Uptake: A Putative Mechanism for the Onset of Wernicke's Encephalopathy

Qiang Zhang, Yan Zhang, Sharon Diamond, Jason Boer, Jennifer J. Harris, Yu Li, Mark Rupar, Elham Behshad, Christine Gardiner, Paul Collier, Phillip Liu, Timothy Burn, Richard Wynn, Gregory Hollis, and Swamy Yeleswaram

Incyte Corporation, Wilmington, Delaware

Received April 30, 2014; accepted July 25, 2014

ABSTRACT

The clinical development of fedratinib, a Janus kinase (JAK2) inhibitor, was terminated after reports of Wernicke's encephalopathy in myelofibrosis patients. Since Wernicke's encephalopathy is induced by thiamine deficiency, investigations were conducted to probe possible mechanisms through which fedratinib may lead to a thiamine-deficient state. In vitro studies indicate that fedratinib potently inhibits the carrier-mediated uptake and transcellular flux of thiamine in Caco-2 cells, suggesting that oral absorption of dietary thiamine is significantly compromised by fedratinib dosing. Transport studies with recombinant human thiamine transporters identified the individual human thiamine transporter (hTHTR2) that is inhibited by fedratinib. Inhibition of thiamine uptake appears unique to fedratinib and is not shared by marketed JAK inhibitors, and this observation is consistent with the known structure-activity relationship for the binding of thiamine to its transporters. The results from these studies provide a molecular basis for the development of Wernicke's encephalopathy upon fedratinib treatment and highlight the need to evaluate interactions of investigational drugs with nutrient transporters in addition to classic xenobiotic transporters.

- **Fedratinib is a Janus kinase (JAK) inhibitor**
- **Terminated in 2013 due to the observations of WE in several patients in Phase III study**
  - WE reported with fedratinib use is likely due to thiamine deficiency

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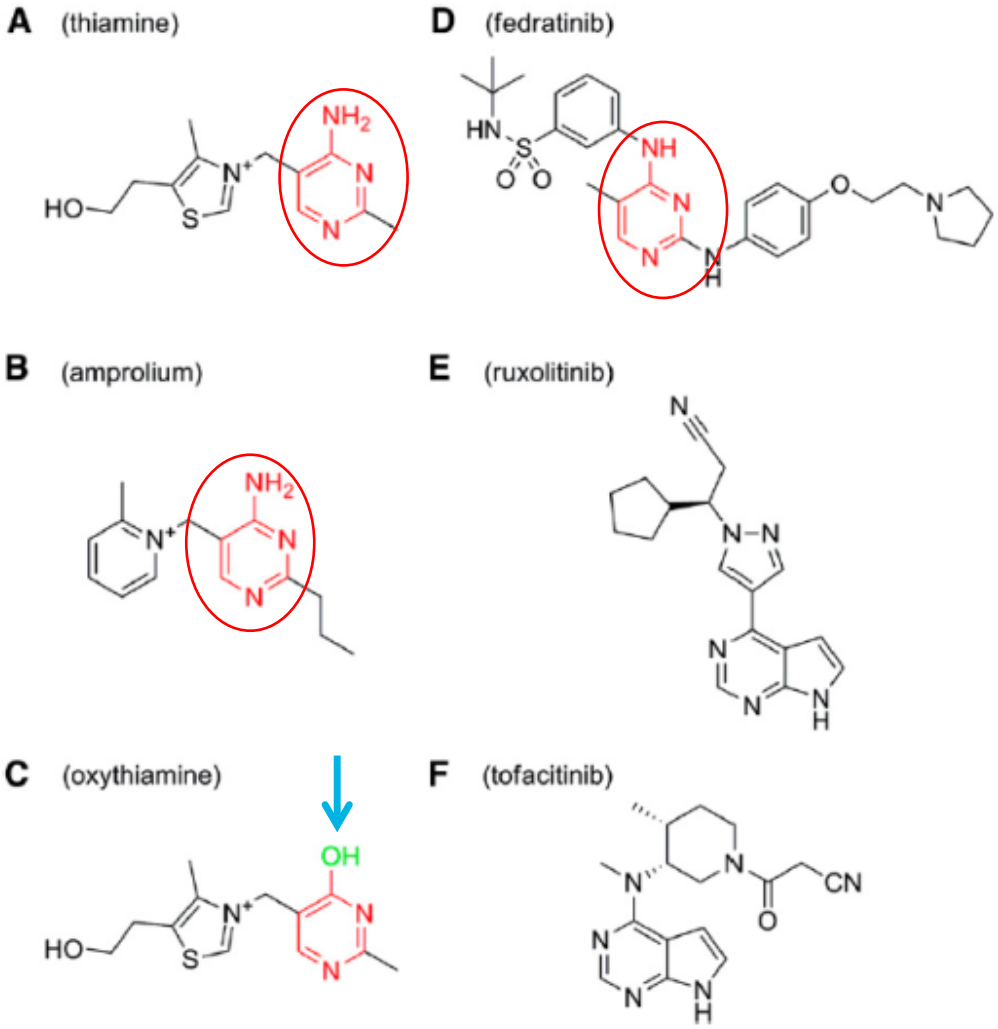

- **Fedratinib shares a common moiety, 4-aminopyrimidine, to thiamine, which is important for binding to thiamine transporters.**
- **Fedratinib interferes with the oral absorption of thiamine via inhibition of thiamine transporter.**

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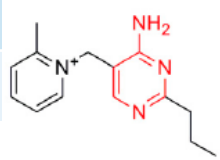
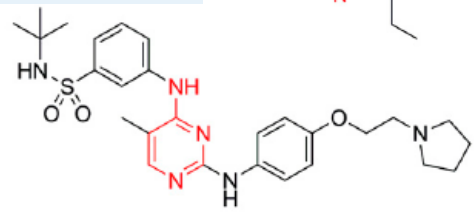
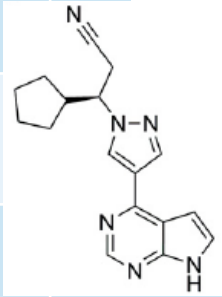
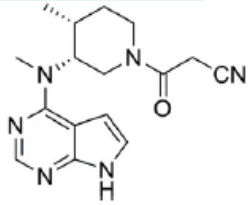
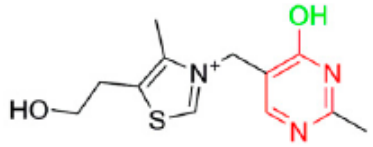
Adapted from *Drug Metab Disp,* Zhang, Q., et al, 2014, 42(10), pp. 1656–1662.

Summary from Fedratinib/THTR Study

- The uptake of thiamine in Caco-2 and THTR1/2 cells is saturable (Km ~ 2 to 3 uM);
- Fedratinib is a potent inhibitor of thiamine uptake

Compounds	IC50 (uM)			Structure
	Caco-2	HEK-THTR1	HEK-THTR2	
Amprolium	0.8	ND	ND	
Fedratinib	2.1	NA	1.2	
Ruxolitinib	NA	NA	NA	
Tofacitinib	NA	NA	NA	
Oxythiamine	> 200	ND	ND	

- No significant impact on thiamine metabolism pathway
- The oral absorption and brain uptake of thiamine may be compromised

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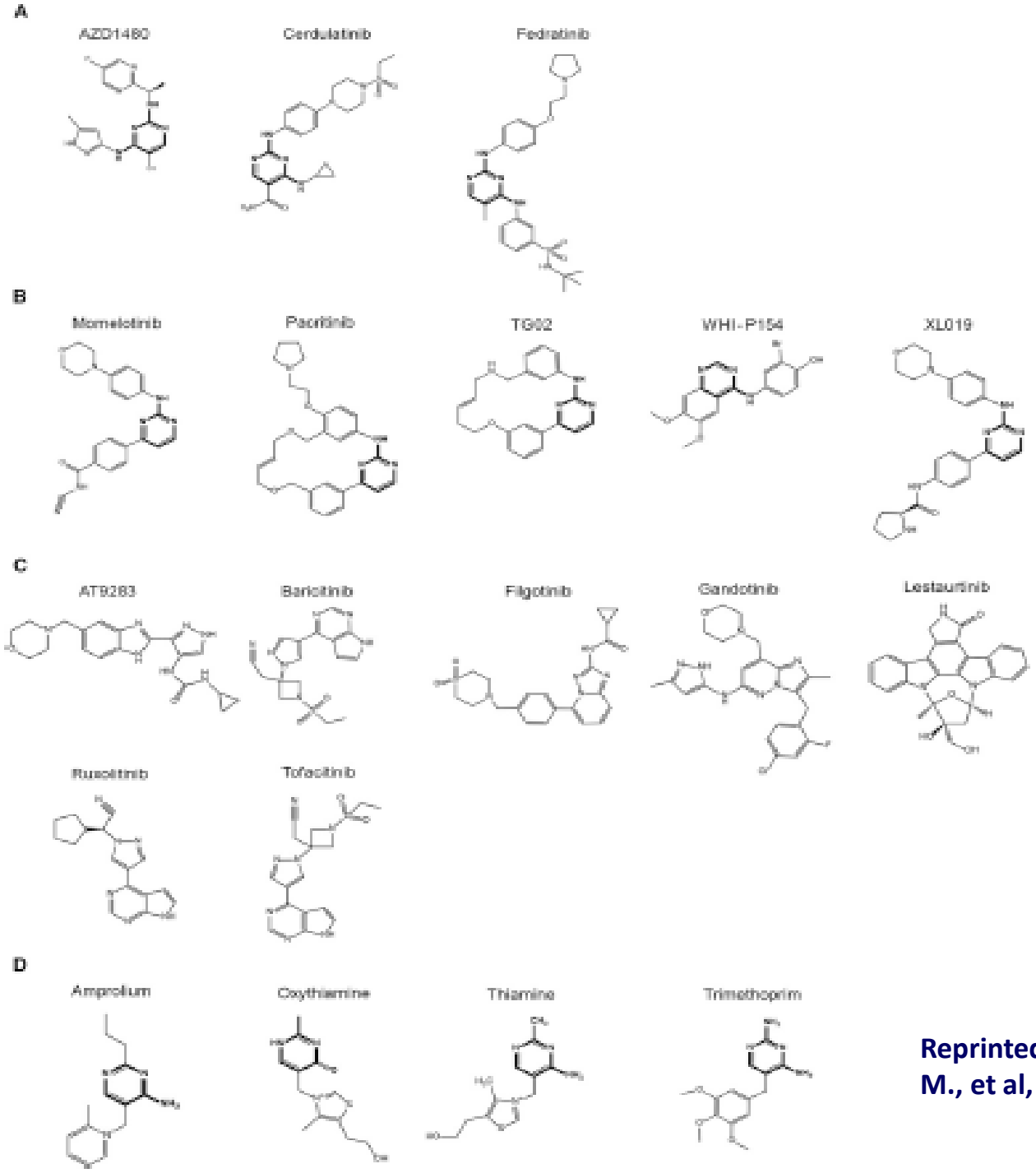
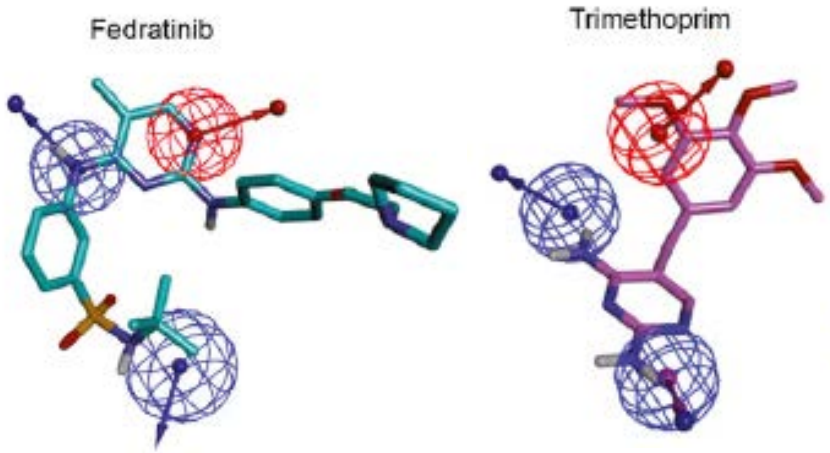


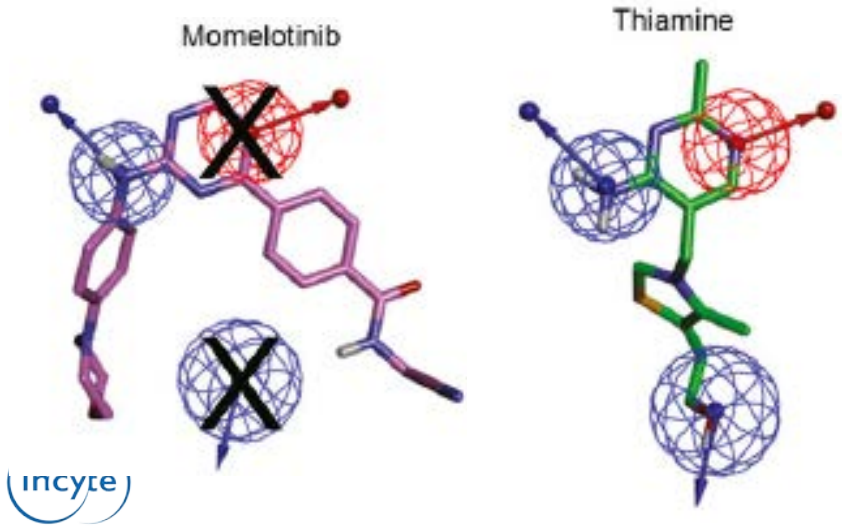
Reprinted from *Drug Metab Disp,* Giacomini, M., et al, 2017, 45(1), pp. 76–85.

Pharmacophore

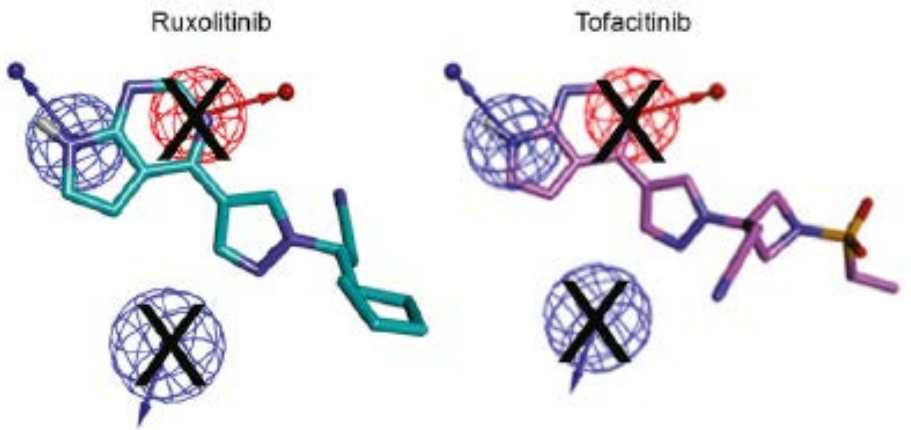
2,4-Diaminopyrimidine



2 or 4-Aminopyrimidine



Nonaminopyrimidine



Reprinted from *Drug Metab Disp,* Giacomini, M., et al, 2017, 45(1), pp. 76–85.

Inhibition of Thiamine Uptake by JAKi and Other Xenobiotics

Compound	IC_{50} Value for Thiamine Uptake		
	Caco-2	THTR-1	THTR-2
	μM	μM	μM
Fedratinib	0.940 ± 0.080	7.10 ± 1.26	1.36 ± 0.59
AZD1480	183 ± 68.9	22.2 ± 6.20	15.3 ± 2.90
Cerdulatinib	>300	276 ± 117	>300
Momelotinib	>30.0	>30.0	>30.0
Trimethoprim	154 ± 22.4	6.84 ± 1.68	5.56 ± 0.65
Oxythiamine	198 ± 16.0	67.3 ± 7.50	66.4 ± 21.4
Amprolium	9.40 ± 2.80	2.60 ± 0.93	0.620 ± 0.270

2,4-Diaminopyrimidine

3-Aminopyrimidine

2,4-Diaminopyrimidine

2-Aminopyrimidine

Reprinted from *Drug Metab Disp,* Giacomini, M., et al, 2017, 45(1), pp. 76–85.

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Are We Ready for a THTR2 Inhibition Decision Tree?

1. **What is the clinical significance of THTR2 inhibition in toxicity and drug-vitamin interactions?**
2. **Is there an established SAR for the THTR2 inhibition?**
3. **What are the current in vitro and in vivo models?**
4. **Are there generally available selective substrates and/or inhibitors to evaluate drug interactions in humans?**
5. **Is there an IVIVE? Can we extrapolate data from one drug to another?**

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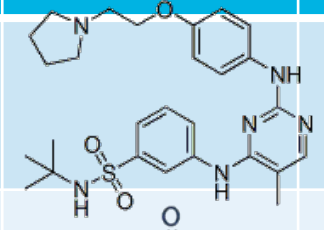
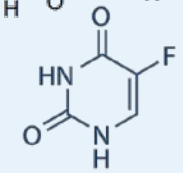
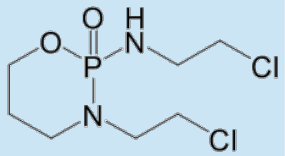
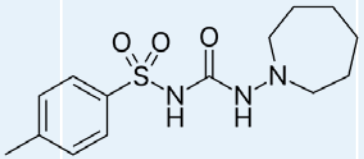
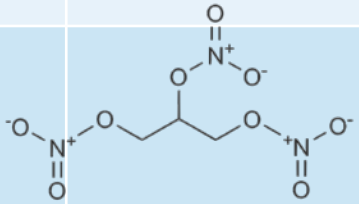
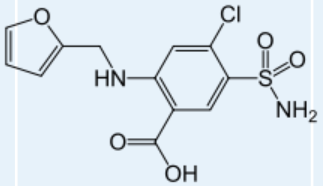
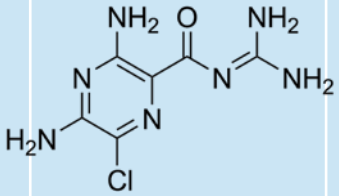
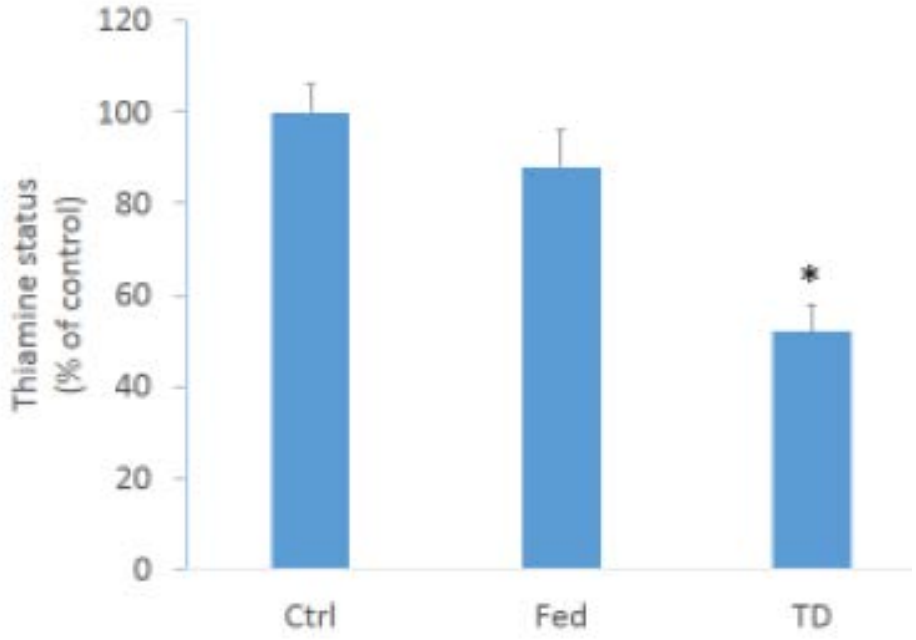
Drug	Structure	Indication	Mechanism	References
Fedratinib		cancer	THTR inhibition	Zhang et al, DMD 2014; 42:1656-1662
5-Fluorouracil		Cancer	inhibits the formation of TPP from Thiamine	Cho et al, J Korean Med Sci 2009; 24: 747-50
Ifosfamide		Cancer	Interfere the conversion from thiamine to TPP	Buesa et al, Clin Cancer Res 2003; 9: 4636-37
Tolazamide		Type II diabetes	Lowering thiamine levels by increase glucose metabolism	Kwee et al, NEJM 1983; 309: 599-600
nitroglycerine		Heart disease	Ethyl alcohol and propylene glycol on thiamine metabolism	Shorey et al, Ann Intern Med 1984; 101:500
furosemide		Heart disease	Interfere with magnesium absorption	Jain 2011, Drug induced WE
Co-amilofruse (Amiloride/furosemide)		Heart disease	Magnesium depletion And THTR inhibition??	McLean and Manchip Lancet 1999; 353: 1768

In vitro and In vivo Models for THTR2

- **In vitro models:**
  - Caco-2 (expression and function of THTR1/2)
  - Transfected cell lines
- **In vivo models:**
  - Thiamine depleted chow
  - Long term injection with pyrithiamine
  - KO mouse model



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Substrates & Inhibitors of THTR2

- **Substrates:**
  - -Drugs
    - oMetformin
    - oFedratinib
    - oFamotidine
    - oTrimethoprim
  - -Endogenous compounds
    - oThiamine
- **Inhibitors:**
  - -Fedratinib
  - -Metformin
  - -Trimethoprim
  - -Amiloride
  - -Amprolium
  - -Pyrithiamine
  - -Phenformin
  - -Chloroquine
  - -Verapamil
  - -Famotidine

Extensive overlapping on substrates with MATE, OCT1, and OCT2.

Giacomini M et al, Drug Metab Disp 45, 76 – 85, 2017
Liang et al, Mol Pharmaceu 12, 4301 – 4310, 2015
Zhang Q et al, Drug Metab Disp 42, 1656 – 1662, 2014
Kato K et al, Pharm Res 32, 2192-2204, 2015

What Is the Relevant in vitro Cut-off?

Drugs	Dose (mg)	Conc (uM)		IC_{50} [a] (uM)	$[I_2]/IC_{50}$	$[I_1]/IC_{50}$	WE
		$[I_2]$	$[I_1]$				
Fedratinib	400-500	>3000	5-10	~ 2	>1500	>2.5	+
Metformin	>500	>15000	2-4	680 [b]	~20	<0.01	-
Momelotinib	150-300	1500-3000	~ 1	>30 [c]	>50	0.03	-
Amiloride	5-10	100	~0.1	200 [d]	0.5	<0.001	+? Mg depletion

[a], IC50 values from Caco-2 or THTR2 cells
[b], Liang et al, Mol Pharmaceu 12, 4301 – 4310, 2015
[c], Giacomini M et al, Drug Metab Disp 45, 76 – 85, 2017
[d], Said et al, Am J Physio 277, C645-C651, 1999

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THTR2 Summary

- **THTR2 is important in the absorption and the distribution of thiamine, various drugs and xenobiotics.**
- **Further data are needed to establish a solid SAR between drugs/NCEs and THTR2**
- **Additional probe substrates are needed to predict THTR2 inhibition**
  - No firm IVIVC data to support an establishment of $[I_2]/IC_{50}$ and $[I_1]/IC_{50}$ value
  - Further studies to define thiamine as the appropriate probe substrate for THTR2
- **A combination of THTR2 transfected cell line (or Caco-2) and the THTR2 KO mouse model is helpful to define THTR2 substrates and inhibitors**

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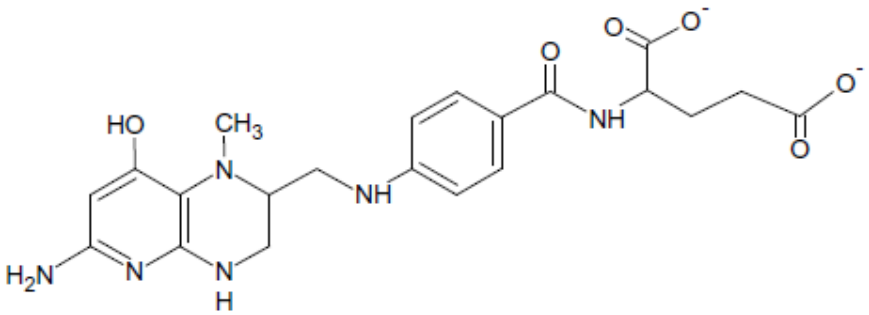
Acknowledgement

- Incyte
- ITCW3

Backup slide

Folate



- **Vitamin B9**
  - Essential nutrient
  - The major physiological folate is 5-methyltetrahydrofolate
  - Coenzyme for cellular one-carbon metabolism
  - Synthesis of thymidine and purine
  - Metabolism of amino acids
- **Factors affecting folate absorption:**
  - Congenital defect (gene mutation) in the uptake system
  - Intestinal disease
  - Chronic alcohol use
  - Drug interaction
- **Folate deficiency:**
  - Megaloblastic anemia
  - Growth retardation
  - Congenital neural tube defects

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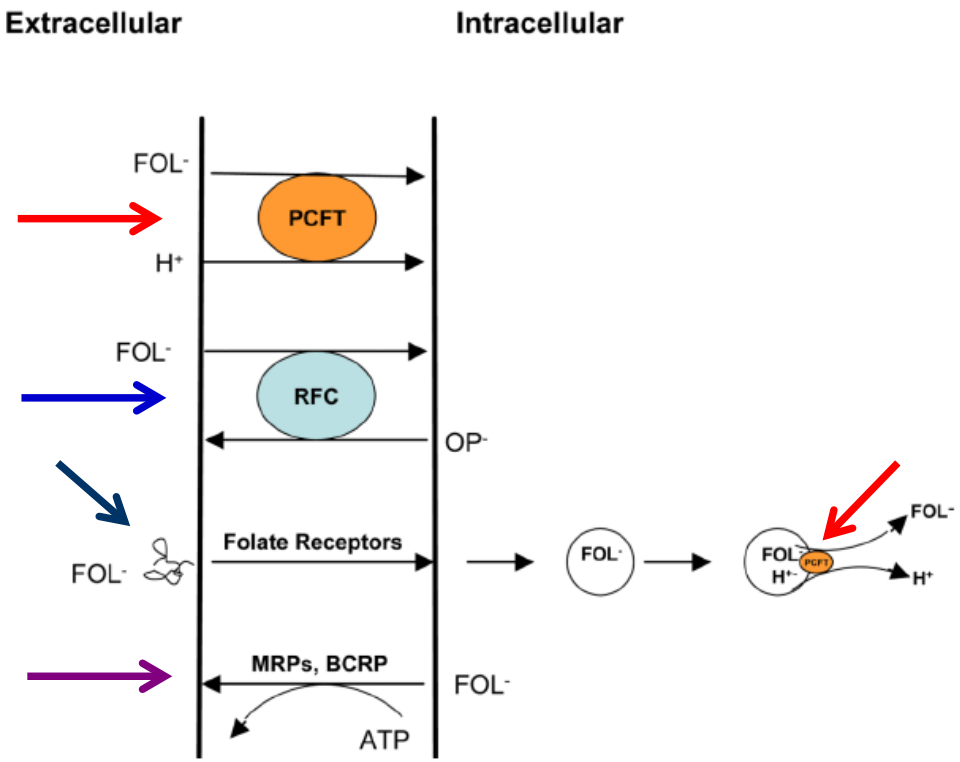
Folate Transporters -- RFC, PCFT, and FR



Reprinted with permission from Zhao and et al, Expert Rev Mol Med. 11, E4, 2009

- **Reduced folate carrier (SLC19A1)**
  - Functions at neutral pH for folate absorption in distal GI
  - Substrates include 5-methyltetrahydrofolate, leucovorin, folic acid, and methotrexate
- **Proton-coupled folate transporter (SLC46A1)**
  - Functions at acidic pH for folate absorption in GI
  - High affinity with both folic acid and reduced folate
- **Folate receptor (FOLR1/2)**
  - Play in concert with PCFT in acidified endosomes
- **Efflux transporters**
  - MRP1, MRP3 and BCRP

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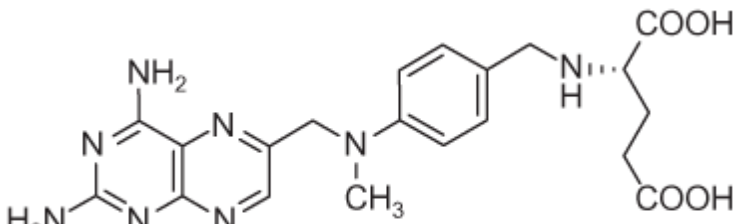
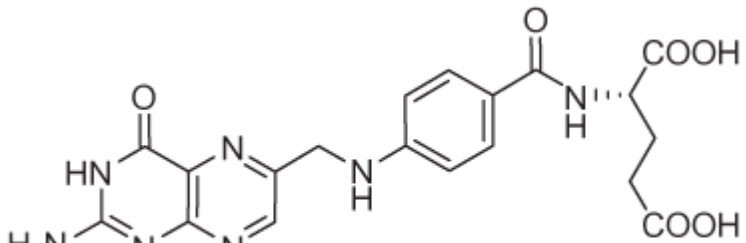
Drug Interactions involving Folate Transporter



Methotrexate (MTX)



Folic acid

Methotrexate

- Rheumatoid arthritis, Crohn's disease, and cancer
- High affinity substrate of PCFT and RFC
- Potent inhibitor of PCFT
  - competitive inhibition of folic acid uptake
- Adverse effect: folate deficiency
- Supplementation with folate during methotrexate treatment

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Encephalopathy

- **Encephalopathy:**
  - Disorder or disease of a brain.
  - The name is preceded by various terms that describe the reason, cause, or special conditions of the patient that leads to brain malfunction.
  - There are over 150 different terms that modify or precede "encephalopathy" in the medical literature

- **Examples:**
  - Anoxic encephalopathy (brain damage due to lack of oxygen)
  - Hepatic encephalopathy (brain malfunction due to liver disease)
  - Diabetic encephalopathy
  - Metabolic encephalopathy
  - Bovine spongiform encephalopathy (BSE) or "mad cow disease“
  - **Drug-induced encephalopathy**
  - **Wernicke's encephalopathy (Wernicke's syndrome)**

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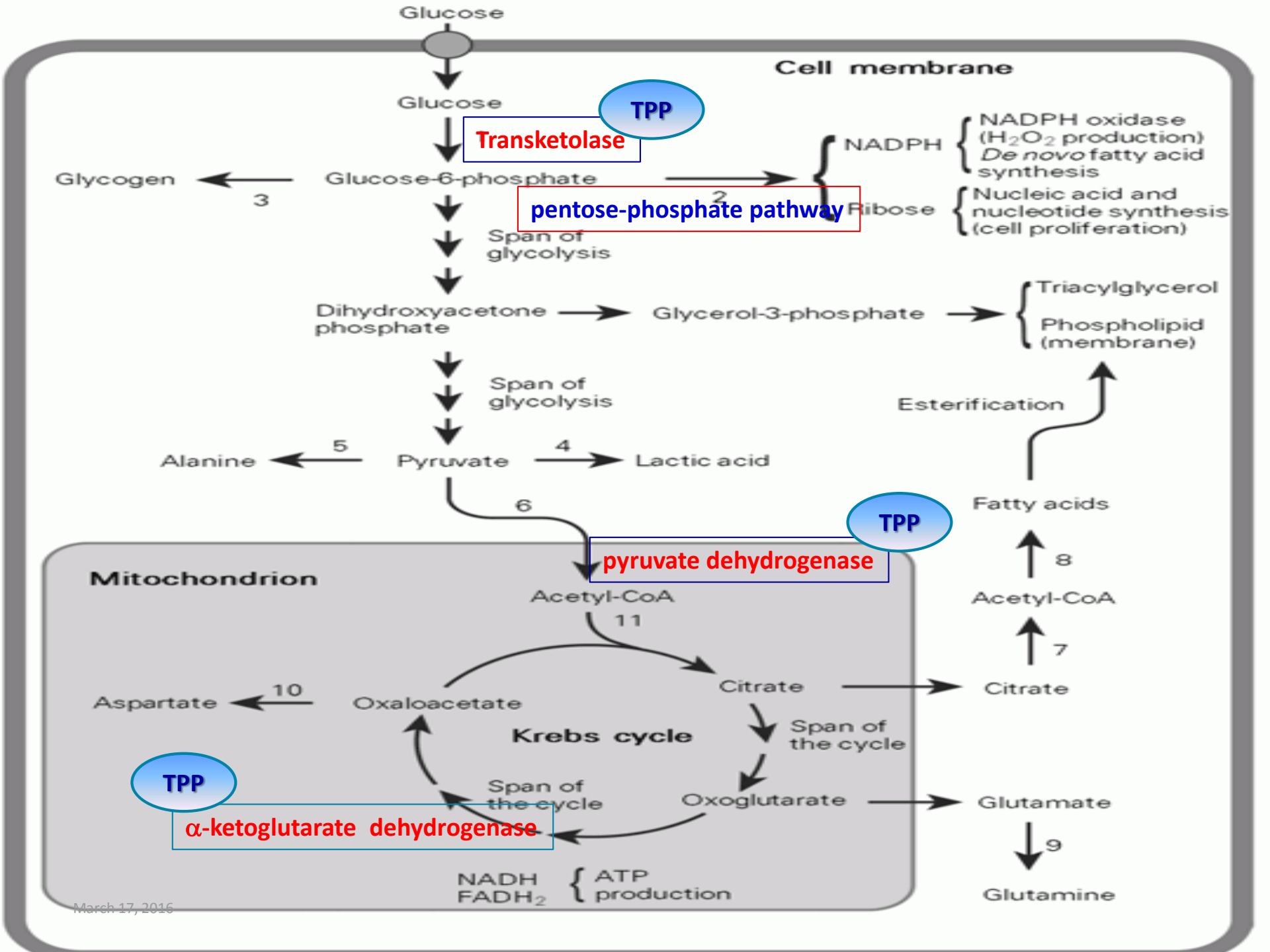
Glucose
Cell membrane
Glucose
TPP
Transketolase
Glycogen
3
Glucose-6-phosphate
2
NADPH
NADPH oxidase
(H_2O_2 production)
De novo fatty acid synthesis
Ribose
Nucleic acid and nucleotide synthesis (cell proliferation)
pentose-phosphate pathway
Span of glycolysis
Dihydroxyacetone phosphate
Glycerol-3-phosphate
Triacylglycerol
Phospholipid (membrane)
Esterification
Span of glycolysis
5
Alanine
Pyruvate
4
Lactic acid
6
Fatty acids
TPP
pyruvate dehydrogenase
Mitochondrion
Acetyl-CoA
11
8
Acetyl-CoA
7
Citrate
Citrate
10
Aspartate
Oxaloacetate
Krebs cycle
Span of the cycle
TPP
Span of the cycle
Oxoglutarate
Glutamate
α-ketoglutarate dehydrogenase
9
Glutamine
NADH
$FADH_2$
ATP production