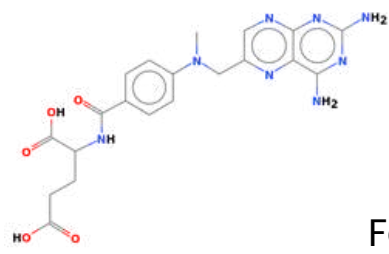


PROPOSED IMMUNOSUPPRESSANT FUNCTION—METHOTREXATE

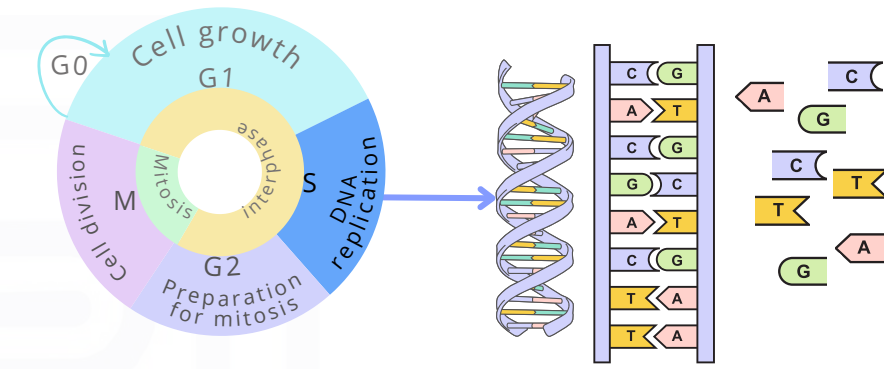


MTX molecule
4-amino-10-methylfolic acid

1

Folate antagonism

Via inhibition of
DHFR TYMS
ATIC



MTX
Glu

tetrahydrofolate
essential for purines &
thymidylate production

Cell-cycle arrest at S1.
Affecting mostly rapidly
dividing cells, including
T & B lymphocytes

2

Adenosine signalling
& anti-inflammatory
effect

Via inhibition of
ATIC

AICAR &
adenosine

Adenosine is an
important anti-
inflammatory mediator
acting on various cell
subtypes

3

Interference with
Methyl donors

Via inhibition of
DHFR

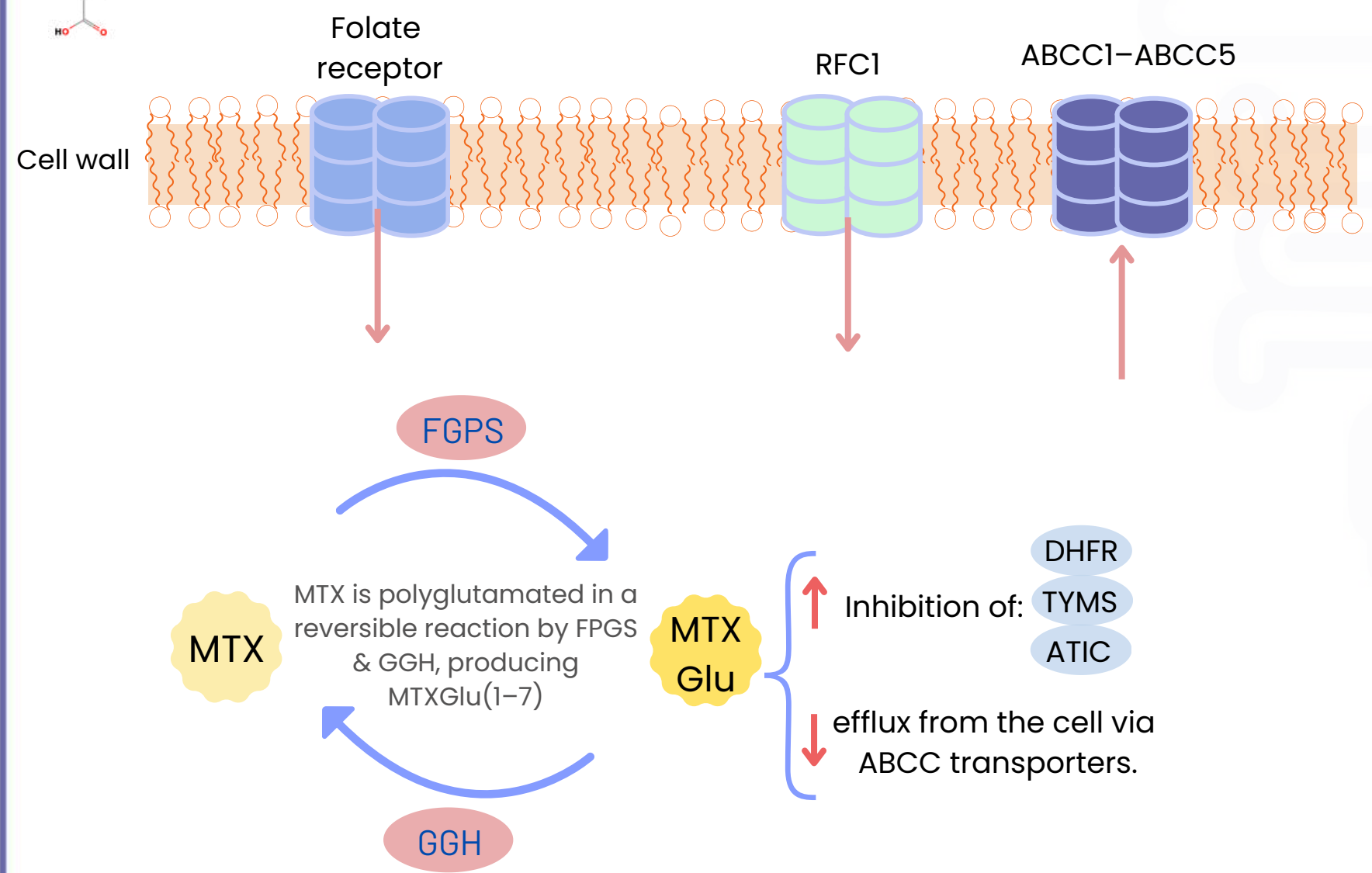
Methionine
depletion

Transmethylation-
dependent reactions,
including
immunoglobulin
production

4

OTHER PROPOSED MECHANISMS:

- Decrease in cytokine, eicosanoids and metalloproteinases (MMPs) production
- Adhesion molecules interference
- Reactive Oxygen Species (ROS) generation
- Antibacterial activity (in vivo studies)



Dihydrofolate reductase (DHFR)
Folypolyglutamate synthetase (FPGS)
γ-glutamyl hydrolase (GGH)
Thymidylate synthetase (TYMS)
5-aminoimidazole-4-carboxamide ribonucleotide (AICAR)
5-aminoimidazole-4-carboxamide ribonucleotide transformylase (ATIC)

Brown PM et al. Nat Rev Rheumatol. 2016;12(12):731-742
Egan LJ et al. Mayo Clin Proc. 1996;71(1):69-80
Yu, J et al. Inflammopharmacol.2020; 28, 1183-1193
Friedman B et al. one Spine. 2019;86(3):301-307

