Methionine gamma-lyase-encapsulated into red blood cells (ERY-MET) shows profound anti-tumor activity in gastric carcinoma

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1 – BACKGROUND

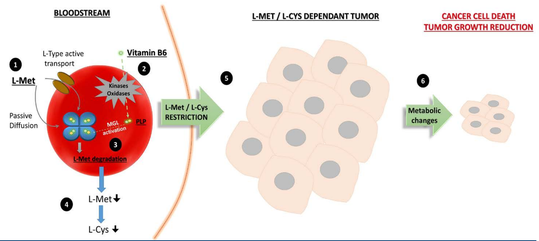
L-Methionine (Met) requirement is a cancer specific-metabolic defect especially important in gastric cancers *(Tagushi, 1995; Xiao, 2001; Graziosi, 2013)*. Methionine gamma-lyase (MGL; EC number 4.4.1.11) is a pyridoxal 5'-phosphate (PLP)-dependent enzyme that is able to degrade Met through the following reaction:

L-methionine + H2O ⇌ methanethiol + NH3 + 2-oxobutanoate.

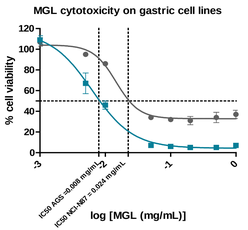
ERYTECH Pharma is developing a new therapeutic product, ERY-MET, inducing partial systemic Met depletion and overcoming the short *in vivo* half-life of free MGL by its encapsulation into Red Blood Cells (RBCs).

ERY-MET IN THE BLOODSTREAM ACTS AS A « BIOREACTOR »

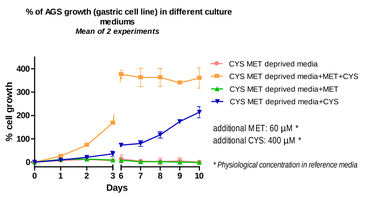
1. Plasma L-Met pumping by ERY-MET
2. Conversion of Vitamin B6 into PLP
3. L-Met degradation by PLP-activated MGL entrapped in RBCs
4. L-Met/L-Cys partial depletion in plasma
5. Reduced availibility of L-Met/L-Cys in tumor microenvironment
6. Metabolic changes and cancer cell death

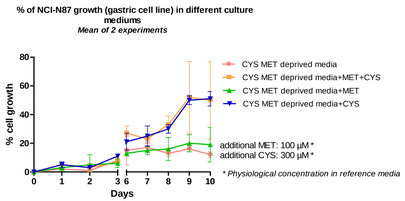


2 – *In vitro* sensitivity of gastric cancer cell lines to Methionine gamma-Lyase (MGL) enzyme



- Free MGL cytotoxicity was determined by inhibitory concentration displaying 50% of inhibition (IC50) in two human gastric cancer cell lines (AGS and NCI-N87) using a colorimetric assay Cell Counting Kit-8 (CCK-8) from Sigma Aldrich.
- Met and/or Cys requirements were then evaluated in these two cancer cell lines by proliferation assay in different culture media using CCK-8 reagent.





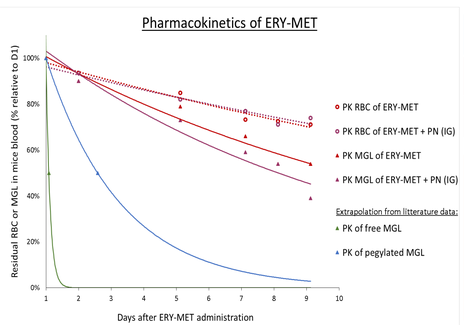
Human gastric cell line	IC50 (mg/mL)	IC50 (IU/mL)	Sensitivity to MGL	Met / Cys requirements
AGS	0,008 ± 0,001	0,12 ± 0,02	Highly (+++)	Both (++ / +++)
NCI-N87	0,024 ± 0,001	0,35 ± 0,01	Moderately (++)	Predominantly Cys (+ / +++)

- **NCI-N87 cells were chosen for further *in vivo* proof of concept studies as this gastric cancer cell line** :
  - displays a sensitivity to MGL enzyme *in vitro*
  - Is a tumorigenic cell line

3 – PK-PD parameters of ERY-MET product *in vivo* with or without PN supplementation

In vivo, free MGL has a short half-life (~2h; *Tan, 1997*) that does not exceed 38h when pegylated *(Sun, 2003; Takakura, 2006)*. To overcome this limitation, MGL was encapsulated into RBCs.

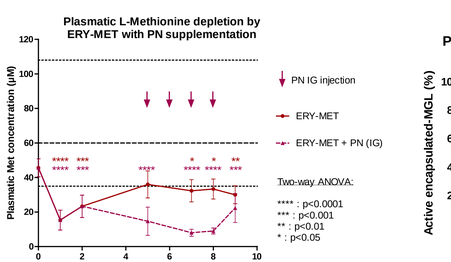
- ERY-MET product (1.05 mg of MGL/mL – 15.1 IU/mL) was injected intravenously (IV) to CD1 mice. Five days after ERY-MET injection, vitamin B6 (PN) was administered by intragastric (IG) route.
- Pharmacokinetics (PK) parameters of ERY-MET product were evaluated:
  - PK of RBCs were determined by follow-up of CFDA/SE-labelled RBCs
  - PK of MGL were determined by MGL quantification in whole blood and plasma samples using Ammonia assay kit from Roche.
- Plasmatic Met concentrations were determined by RPLC-ESI-MS/MS method *(Piraud, 2005)*.

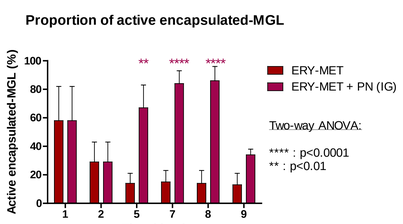


Product	24h RBC recovery	RBC *in vivo* half-life	MGL *in vivo* half-life
ERY-MET	79%	17 days	12 days
ERY-MET + PN (IG)	79%	19 days	9 days

- RBCs half-life of ERY-MET product was not modified compared to normal transfused mouse erythrocytes (~ 17 days).
- Encapsulation of MGL into RBCs extends MGL half-life to ~ 9 days.

PK of encapsulated MGL is significantly improved as compared to the free form.





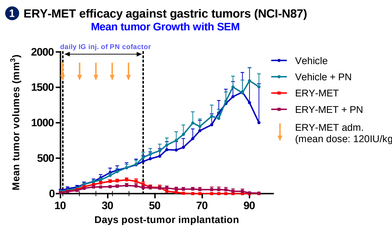
- ERY-MET treatment induces a strong plasmatic Met depletion during the first day (74%) that stabilize to ~ 40-50% between Day 5 and Day 9.
- PN supplementation (IG injected) between Day 5 and Day 8 induces a rebound on ERY-MET treatment efficacy reaching ~ 82% of Met depletion. Discontinuation of PN uptake on Day 9 results in Met levels increasing back to levels observed with ERY-MET treatment alone.
- Association of PN to ERY-MET maintains the encapsulated MGL enzyme under its active form.

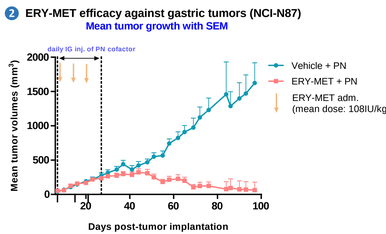
Daily PN supplementation is needed for an optimal activity of ERY-MET.

4 – *In vivo* anti-tumor activity of ERY-MET on subcutaneous gastric tumor model in NMRI mice

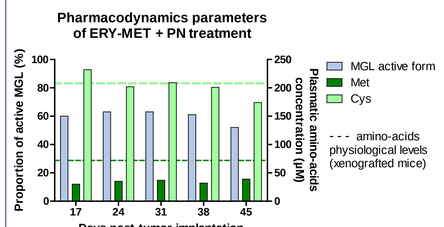
NCI-N87 cells (5.10^6) were subcutaneously xenografted on female NMRI *nude* mice.
Mice received weekly ERY-MET injections at 116 IU/kg ± 25% as described below:

- (1) ERY-MET (x5) with or without daily PN supplementation from day 11 to day 45 post-tumor xenograft.
- (2) ERY-MET (x3) with daily PN supplementation from day 7 to day 27 post-tumor xenograft.

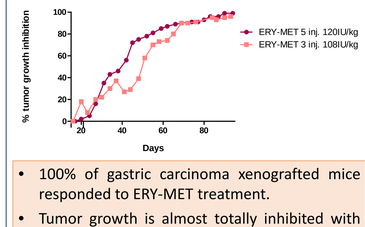




(1) Pharmacodynamics evaluation of ERY-MET treatment



Days post-tumor implantation	ERY-MET + PN				
	D17	D24	D31	D38	D45
Total ERY-MET injection received	1	2	3	4	5
% active MGL	60%	63%	63%	61%	52%
% Met depletion	59%	51%	48%	56%	46%
% Cys depletion	13%	12%	19%	12%	25%



- 100% of gastric carcinoma xenografted mice responded to ERY-MET treatment.
- Tumor growth is almost totally inhibited with ERY-MET + PN treatment with tumor growth inhibitions of 99% at Day 94 following 5 administrations ((1)) and 96% at Day 93 ((2)) following 3 administrations.
- From Day 65, tumor growth inhibition is >90% with either 3 or 5 injections of ERY-MET.

5 – CONCLUSION

By using erythrocytes as bioreactors – which can supply PLP cofactor to encapsulated-MGL from oral PN supplementation – *in vivo* MGL half-life and activity are greatly improved. ERY-MET product is able to induce partial systemic Met depletion that seems to cause a lowering of Cys plasma levels and most likely an impoverishment of both Met and Cys in the tumor micro-environment. Repeated injections of ERY-MET associated with daily PN oral administration displayed very high anti-tumor activity in our gastric carcinoma xenografted mouse model with an almost complete tumor growth inhibition. This work strengthens the rationale for developing the product in oncology and more particularly in gastric and other recalcitrant cancers. The safety profile of a single administration of ERY-MET product was demonstrated in mice (data not shown) making it possible to launch a first-in-man trial in 2017.