Use of methionine gamma-lyase-loaded erythrocytes to induce effective methionine depletion in cancer therapy

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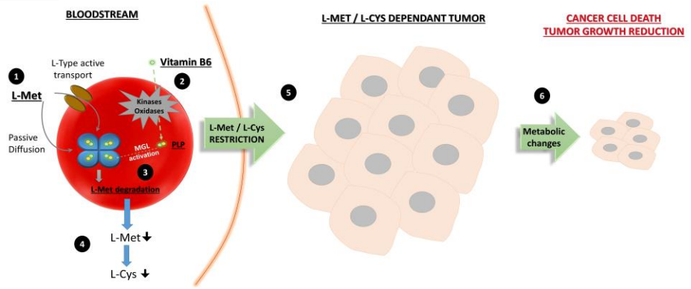
Abstract #2134

BACKGROUND

Methionine dependence is now widely accepted as the only known general metabolic defect in cancer (Hoffman, 2015). ERYTECH Pharma is developing a new therapeutic product, ERY-MET (erymethionase), inducing partial systemic L-methionine (Met) depletion and overcoming the short *in vivo* half-life of free methionine gamma-lyase (MGL) by its encapsulation into Red Blood Cells (RBCs).

CONCEPT :

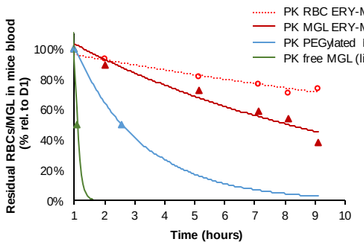


1- Plasma Met entry into RBC.
2- Conversion of vitamin B6 (pyridoxine) into PLP.
3- degradation by PLP-activated MGL entrapped in RBC.
4- Met and L-cysteine (Cys) partial depletion in plasma.
5- Reduced availability of Met / Cys in the tumor microenvironment.
6- Metabolic changes and induction of cancer cell death.

PHARMACOKINETICS OF ERYMET PRODUCT *IN VIVO*

In vivo, free MGL has a short half-life (~ 2 hours; Yang, 2004) that does not exceed 38 hours when PEGylated (Sun, 2003; Takakura, 2006). To overcome this limitation, MGL was encapsulated into RBCs. Pharmacokinetics (PK) of MGL-encapsulated RBCs and MGL enzyme was assessed in CD1 mice after intravenous administration of ERY-MET and PN oral supplementation from day 5 to day 8.

Method : PK of RBCs were determined by flow cytometry (FC500 cytometer, Beckman Coulter) follow-up of CFDA/SE-labelled RBCs.
PK of MGL was determined by MGL quantification in whole blood and plasma samples using Ammonia Assay Kit from Roche.



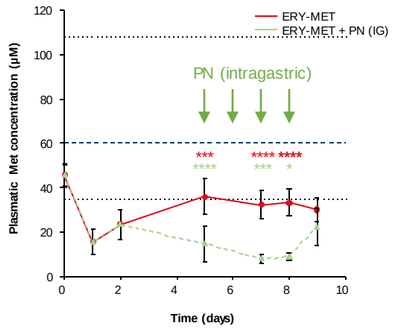
Product	Half-life of MGL-loaded RBCs	Half-life of MGL enzyme
Free MGL	NA	2 hours
PEGylated MGL	NA	38 hours
ERY-MET + oral PN	19 days	9 days

PK of encapsulated MGL is significantly improved as compared to the free form and half-life of RBCs encapsulating MGL is similar to normal transfused mouse erythrocytes (~17 days)

PHARMACODYNAMICS OF ERYMET PRODUCT *IN VIVO*

Pharmacodynamics (PD) of MGL-encapsulated RBCs alone and with PN administration was assessed in CD1 mice after intravenous administration of ERY-MET and PN oral supplementation from day 5 to day 8.

Method: plasmatic Met concentrations were determined by RPLC-ESI-MS/MS method (Piraud, 2005)



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.

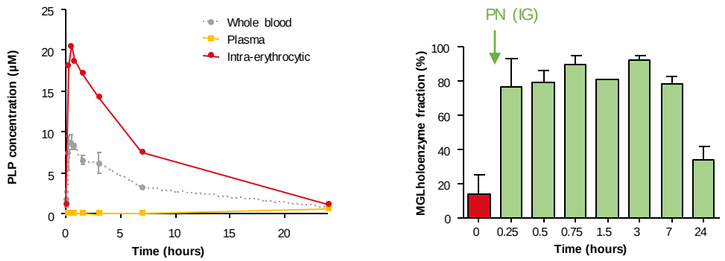
Association of PN to ERY-MET maintains the encapsulated MGL enzyme under its active form.

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

ROLE OF RBCS IN PROVIDING MGL COFACTOR PLP

In vivo Conversion of PN into biologically active PLP cofactor by RBCs was assessed in CD1 mice by injecting MGL-encapsulated RBCs followed by a single PN administration (IG) to the animals.

Method: quantitative determination of PLP concentration in plasma & whole blood was assessed by an enzymatic assay (Bühlmann). MGL holoenzyme fraction was determined by Ammonia Assay Kit.



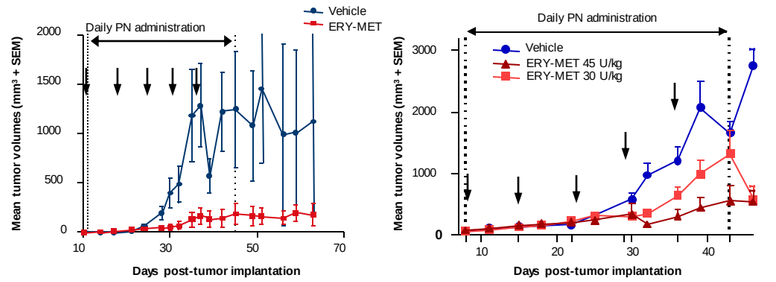
Intra-erythrocytic concentration rapidly increased and was maximal (20.5 µM) 30 minutes after uptake and then slowly decrease to return to initial concentration (1.2 µM) at 24 hours.
At day 5, without PN administration, RBCs contained only 15% of MGL holoenzyme. After PN supplementation, encapsulated MGL is found predominantly (70–80%) under its active form, only 15 minutes after uptake. Proportion of holoenzyme remained stable for 7 hours and declined only after 24 hours.

PN is rapidly converted in active cofactor PLP by RBCs, which contributes to sustain a high holoenzyme fraction over time

ERY-MET ANTI-TUMORAL EFFECT IN TWO XENOGRAFT MOUSE MODELS

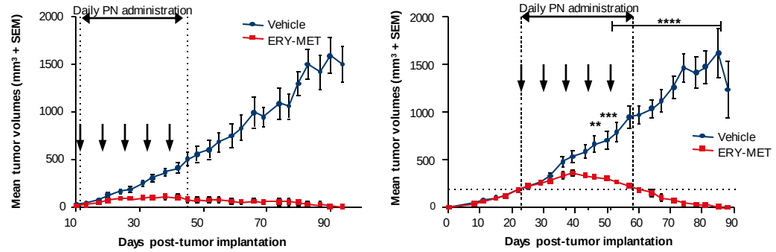
>50% of glioblastoma grade IV are not well treated with current Stupp therapy. More particularly, unmethylated MGMT subpopulation (resistant to Temozolomide).

Method: U-87 MG-luc2 human glioblastoma (GBM) cells (5.10^6) were subcutaneously xenografted on female NMRI *nude* mice. Animals received weekly single injection of ERY-MET for 5 consecutive weeks (*arrows*), and were supplemented with daily administrations of PN. ERY-MET administered doses were 76 U/kg (*left*) or with 30 and 45 U/kg (*right*).



Amplification of human epidermal growth factor receptor 2 (HER2) is associated with resistance to EGFR inhibitor in metastatic CRC patients.

Method: HER2-expressing NCI-N87 human gastric cancer cells (5.10^6) were subcutaneously xenografted on female NMRI *nude* mice. Animals received weekly single injection of ERY-MET for 5 consecutive weeks (*arrows*), and were supplemented with daily administrations of PN. In preventive model (*left*), ERY-MET treatments (116 U/kg) started at day 11. In curative model (*right*), treatments (80 U/kg) started at day 23. At this time, mean tumor volume was 183 ± 55 mm³ in vehicle arm and 184 ± 48 mm³ in ERY-MET arm.



ERY-MET treatment inhibits glioblastoma and gastric tumor growth in athymic *nude* mice

CONCLUSION

This study clearly demonstrated that encapsulation of MGL in RBCs both strongly improved the half-life and contributed to provide active cofactor. RBCs intrinsic properties can supply encapsulated MGL with PLP and maintain its enzyme activity over time. In parallel, a single injection harbored a good safety profile (data not shown), while repeated injections of ERY-MET were effective against tumor growth in two different mouse models. Thus, due to the RBC intrinsic characteristics, ERY-MET represents a new promising treatment against a broad scope of cancers that rely on Met metabolism.

Disclosure: Philip Lorenzi is member of the scientific board of ERYTECH Pharma.
All other authors are employees of ERYTECH Pharma.

AACR American Association for Cancer Research ANNUAL MEETING 2017 WASHINGTON, DC