Enzymatic combination investigation in cancer therapy

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Abstract #5827

ERYTECH'S PRODUCTS

Tumor micro-environment and more specifically aminoacids play a major role in cancer cell proliferation. ERYTECH Pharma develops innovative anti-cancer therapies based on the encapsulation of aminoacid-degrading enzymes into red blood cells by using its proprietary ERYCAPS® technology.

The enzymes are encapsulated following a mechanical procedure based on successive hypotonic and hypertonic stresses inducing cell swelling, enzyme incorporation and pores resealing. ERYTECH Pharma products greatly improve the PK-PD properties of enzymes and act as bioreactors, the amino acid degradation directly occuring inside the red cell.

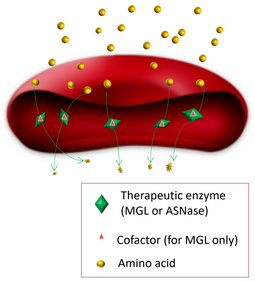

Figure 1 : erytech innovative products consist in therapeutic enzymes loaded in red blood cells

Eryaspase — Phase 3/MAA

Encapsulation of L-asparaginase (ASNase), an enzyme currently used in combination with chemotherapy for Acute Lymphoblastic Leukemia (ALL) treatment, hydrolyzes L-asparagine (Asn) - a non-essential amino acid - into aspartic acid (Asp) and ammonia, leading to Asn removal from the circulation.

Erymethionase — Preclinical / Phase 1

Encapsulation of methionine-γ-lyase (MGL), a pyridoxal 5'-phosphate (PLP)-dependent enzyme that is able to degrade plasma L-methionine (Met) an essential amino-acid. Exogenous Met restriction in Met-dependent cancer cells blocks cell division in the late S or G2 phase of the cell cycle. Thus, restriction of Met by MGL seems a promising approach to target Met-dependent cancers.

ENZYMATIC COMBINATION RATIONALE

Based on ERYTECH's previous work, and the knowledge in the field describing the potential role of MGL and ASNase as cytostatic or cytotoxic drug respectively, ERYTECH Pharma's strategy was to combine both enzymes whatever the form (free or encapsulated) *in vitro* and *in vivo*. The primary focus was on the sequential administration of MGL followed by ASNase. Indeed, the principle of combination was based on Met depletion, using MGL with then Asn depletion (*via* ASNase activity), to investigate the therapeutic benefit of those orthogonal mechanisms of action.

DECREASE OF TUMOR CELL VIABILITY ENHANCED BY ENZYMATIC COMBINATION *IN VITRO*

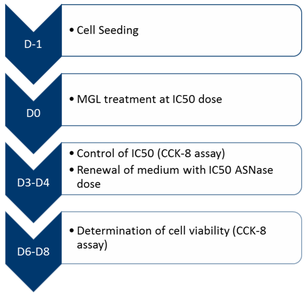

Figure 2 : Study design for evaluation of MGL and ASNase enzymatic combination *in vitro*

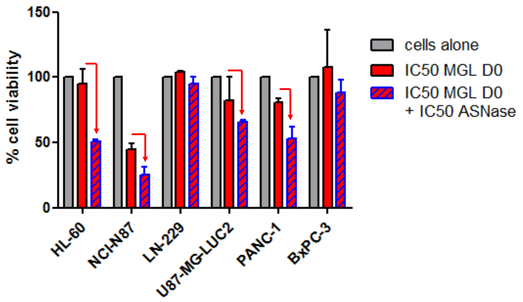

Figure 3 : *In vitro* CCK8 viability results of MGL and sequential combination with ASNase treatment at day 8

Cell lines	HL-60	NCI-N87	LN-229	U-87 MG-luc2	PANC-1	BxPC-3
Cancer type	AML	Gastric	Glioblastoma		Pancreatic	
MGL first, then ASNase	-39%	-50%	0%	-21%	-39%	0%

Table 1 : Reduction of cell viability after the combined therapy compared to MGL-only treatment

ERYMETHIONASE COMBINED WITH ERYASPASE POTENTIATES TUMOR GROWTH INHIBITION *IN VIVO*

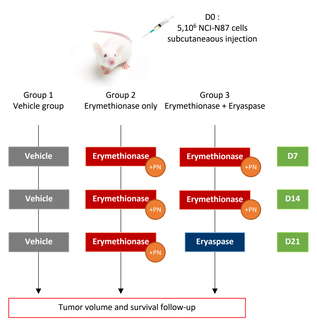

Figure 4 : Study design for evaluation of Erymethionase and Eryaspase® combination *in vivo*
Erymethionase mean dose per adm: 108 U/kg
Eryaspase adm. dose: 100 U/kg

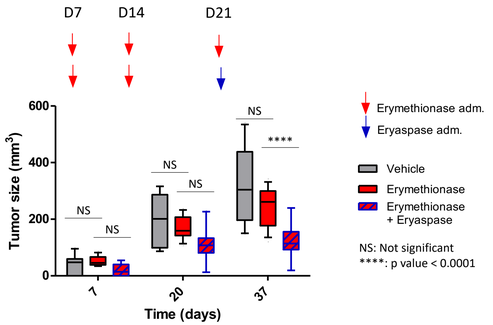

Figure 5 : *In vivo* anti-tumor effect of Erymethionase and Erymethionase/Eryaspase combination on gastric tumors (NCI-N87).
Tumor volumes are represented by box plot for each condition
(Line: median tumor volume ; Whiskers: 10-90 percentile)

MECHANISM OF ACTION INVESTIGATION BY TRANSCRIPTOMICS APPROACH

Transcriptional analysis were performed on RNA extracts from NCI-N87-treated cells.

RNA extracts from :
-Cells only
-MGL-treated cells
-ASNase-treated cells
-MGL+ASNase sequentially-treated cells
→ ARNc synthesis with incorporation of CTP-Cy3 → Microarray analysis using Human AMADID Gene expression Microarray (Agilent)

Table 2 : Microarray analysis

Conditions	Number of significant probes (T-test)	Geneset analysis using Genespring and KegArray softwares		
		Significant KEGG pathway	P-value	Extrapolated MoA from geneset expression analysis
MGL VS Control	5689+1	Ribosome Huntington'sdisease Alzheimer's disease Parkinson's disease Oxidative Phosphorylation Proteasome RNA transport Pathways in cancer Pyrimidine metabolism Ribosome biogenesis in eucaryotes	1.81e-33 1.73e-17 2.48e-14 8.40e-13 6.48e-12 8.43e-11 1.75e-09 1.19e-08 1.28e-08 1.28e-08	↗ RNA transport and ribosome biogenesis ↗ Oxydative phosphorylation ↗ Proteasome **Cell cycle arrest**
ASNase VS Control	4088	Huntington's disease Ribosome Parkinson's disease Oxidative phosphorylation Alzheimer's disease RNA transport Pathways in cancer Ribosome biogenesis in eukaryotes p53 signaling pathway Phagosome	2.68e-29 3.60e-29 9.42e-28 1.58e-25 2.39e-25 1.64e-16 1.95e-13 3.09e-12 3.02e-11 3.95e-11	↗ Oxydative phosphorylation **↗ p53 pathway and apoptosis** Inhibition of proliferation ↗ RNA transport and ribosome biogenesis ↗ mTOR pathway
MGL-ASNase VS Control	7112+4	Ribosome Cell cycle Pathways in cancer MAPK signaling pathway Systemic lupus erythematosus Endocytosis p53 signaling pathway Lysosome Spliceosome Toxoplasmosis	6.09e-21 3.05e-20 3.34e-18 3.34e-18 7.88e-14 3.38e-13 2.39e-12 2.70e-12 4.24e-12 1.29e-09	↗ Ribosome **Cell cycle inhibition/Cell cycle arrest** **↗ Apoptosis** ↗ Lysosome ↘ mTOR pathway ↘ Spliceosome
MGL-ASNase VS MGL only	7333+74	Cell cycle Spliceosome DNA replication RNA transport Purine metabolism Systemic lupus erythematosus Pathways in cancer Pyrimidine metabolism Protein processing in ER Proteasome	3.14e-33 2.02e-32 4.23e-20 4.77e-19 7.07e-18 1.13e-17 1.13e-17 1.13e-17 6.30e-17 4.58e-15	**Cell cycle inhibition/Cell cycle arrest** ↘ Spliceosome ↘ DNA replication ↘ RNA transport ↘Purine and pyrimidine metabolism ↘ Proteasome and protein processing in ER **↗ Apoptosis**
MGL-ASNase VS ASNase only	7256+2	Cell cycle Spliceosome RNA transport Huntington's disease Ribosome biogenesis in eukaryotes Purine metabolism Parkinson's disease DNA replication Systemic lupus erythematosus Pyrimidine metabolism	2.19e-32 1.02e-28 2.46e-21 2.78e-21 4.59e-21 3.36e-20 4.88e-19 4.88e-19 3.62e-17 4.60e-17	**Cell cycle inhibition** ↘ Spliceosome ↘ RNA transport ↘ Ribosome biogenesis ↘Purine and pyrimidine metabolism ↘ DNA replication **↗ Apoptosis**

CONCLUSION

In vitro results showed promising potential for enzymatic bi-therapy treatment on several cancer cell lines that were later confirmed by our *in vivo* investigation in a gastric cancer model. Interesting leads regarding cytostatic and cytotoxic effect of each enzyme were obtained by transcriptomic analysis. Using this data as a starting point, we are currently in the process of identifying the underlying Erymethionase/Eryaspase's mechanism of action (MoA). This identification should enable the optimization of the therapeutic design and help select best responders.

Disclosure : Karine AGUERA, Fanny GALLIX, Fabien GAY, Karine SENECHAL, Severine CIRE, Françoise HORAND, Alexander SCHEER and Vanessa BOURGEAUX are employees of ERYTECH Pharma