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Intratumoral Exposure Levels of Pentaglutamated Pemetrexed following Treatment with LEAF-1401 and Pemetrexed

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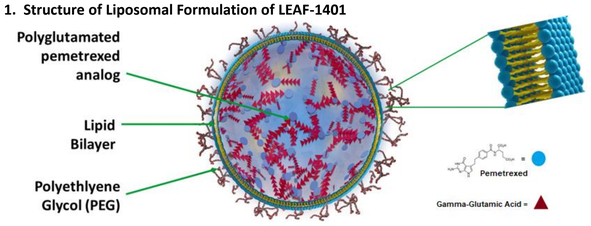
Background

- Antifolates including pemetrexed are well established antimetabolites used to treat many different cancers
- Pentaglutamated pemetrexed is over 80 times more potent than pemetrexed in inhibiting thymidylate synthase. However, to the best of our knowledge, prior to our present work, it has never been delivered directly into the tumor cell until now. Pemetrexed requires glutamation by intracellular enzyme activity of folylpolyglutamate synthase (FPGS). Resistance has been linked to downregulation of FPGS and increase of breakdown of polyglutamate by gamma-glutamyl hydrolase (GGH).[1,2]
- Key challenge of antifolate therapy is to ensure sufficient intratumoral concentration of these polyglutamate forms.
  - Pentaglutamate form does not cross cell membranes because of high negative charge.
  - Overcome resistance driven by FPGS and GGH.
- LEAF-1401 is a liposomal formulation of gamma L-pentaglutamated pemetrexed and the first of a new class of antifolates designed to overcome the challenges described above and be more potent, and safe.

Figure 1. Structure of Liposomal Formulation of LEAF-1401



Methods

- To demonstrate the increase in level of pentaglutamated pemetrexed in a CT-26 murine colorectal tumor xenograft following treatment of LEAF-1401 (with a single dose of either LEAF-1401 (80mg/kg; equivalent to 32 mg/kg pemetrexed) or pemetrexed (118mg/kg)) the following was performed:
  - Biodistribution analysis of pentaglutamated pemetrexed and pemetrexed in various tissues in 5 mice per several time points measured using quantitative LC/MS/MS
  - Tumor volume assessment and clinical monitoring

Main Findings

LEAF-1401 treatment vs. Pemetrexed treatment

- LEAF-1401 treatment resulted in a 20-fold higher exposure of pentaglutamated pemetrexed in the tumor. (Fig 2-A and Table 1)
- LEAF-1401 treatment resulted in a 30-fold higher exposure of pemetrexed in the tumor due to conversion by GGH function. (Fig 2-B and Table 1)
- Both treatments resulted in comparable plasma pemetrexed level (Fig 2-D).
- LEAF-1401 significantly inhibited tumor growth.
- Liver exposure levels of pentaglutamated pemetrexed and pemetrexed were also high with LEAF-1401 treatment as shown in Figs 2-E and 2-F
- LEAF-1401 at 80 mg/kg caused reversible weight loss (Fig 3-B).

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Results

Figure 2. Concentration of Pentaglutamated Pemetrexed and Pemetrexed in Tumor, Plasma and Liver

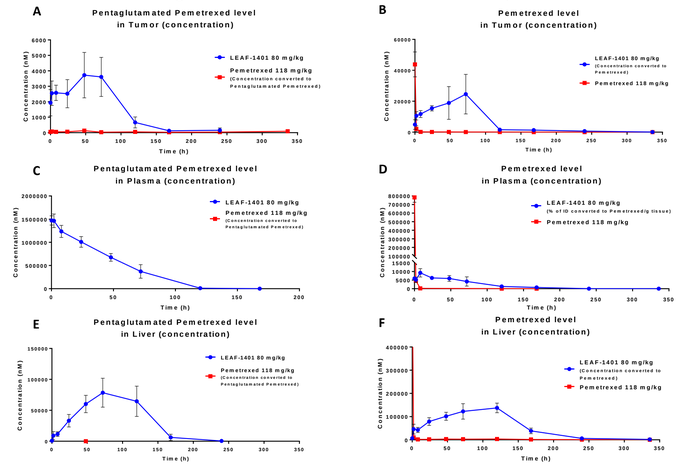
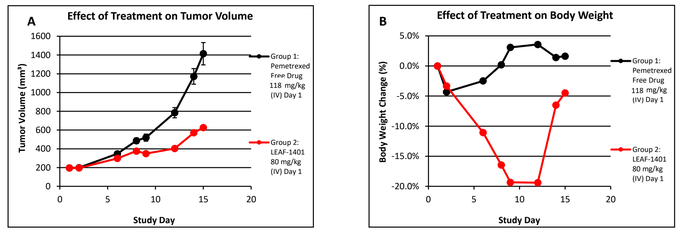


Table 1. Exposure Levels (AUC) of Pentaglutamated Pemetrexed and Pemetrexed

AUC calculation	LEAF-1401 dosed group		Pemetrexed dosed group	
(h*mM)	Pentaglutamated Pemetrexed	Pemetrexed	Pentaglutamated Pemetrexed	Pemetrexed
Tumor	0.355	2.035	0.018	0.065
Blood	71.153	0.661	<LLOQ	0.782
Liver	8.586	18.584	<LLOQ	1.324
Spleen	1.259	5.606	0.0004	0.115
Colon	0.782	0.326	0.685	0.103

Figure 3. Tumor Volume and Body Weight Following Treatment with LEAF-1401 and Pemetrexed



Future Direction

- Lower therapeutically relevant doses are being studied because of enhanced intratumoral delivery and high potency of LEAF-1401.
- GLP Toxicology studies are underway in preparation for first in human studies.

References

1. Chattopadhyay S, Moran RG, Goldman ID. Pemetrexed: biochemical and cellular pharmacology, mechanisms, and clinical applications. Molecular Cancer Therapeutics 6:404-417, 2007
2. Alimta® prescription information (https://www.accessdata.fda.gov/drugsatfda_docs/label/2008/021462s015lbl.pdf)