Abstract # 160

In vitro and *in vivo* evaluation of pegaspargase (Oncaspar®) for the treatment of solid tumors and lymphomas

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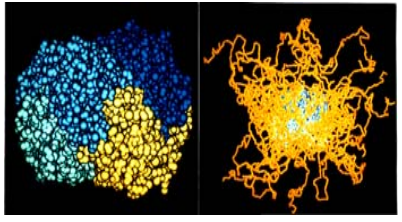
Background

Asparagine (ASN) is a nonessential amino acid synthesized from aspartic acid and glutamine by the enzyme, asparagine synthetase (ASNS). Certain tissue culture cell lines of acute lymphocytic leukemic origin have low levels of ASNS and are very dependent on exogenous ASN for survival [1]. This may explain, in part, why sensitivity or resistance to L-asparaginase (depending upon the ASNS levels) has been observed in patients with ALL [1, 2]. Although studies done decades ago suggest that L-asparaginase was not effective in a variety of solid tumors [3], no consideration of the level of ASNS was done. In new studies with tissue culture cell lines, it has been found the sensitivity of L-asparaginase is correlated with low expression of ASNS of ovarian origin [4, 5]

Oncaspar® (Pegaspargase) is PEGylated version of L-Asparaginase. It is approved for use in patients with ALL as a first line therapy or as a second line therapy in patients who require L-asparaginase as part of a treatment regimen, but have developed hypersensitivity to the native forms of L-asparaginase. In this study, we evaluate the utility of Oncaspar® in solid tumors and lymphomas and attempt to correlate the activity with the cellular levels of asparagine synthetase (ASNS). In particular, we evaluated the in vitro and in vivo efficacy of pegaspargase in pancreatic, ovarian and lymphoma cells with varying expression of ASNS. .



Crystal structure of L-asparaginase tetramer

Model of pegylated L-asparaginase

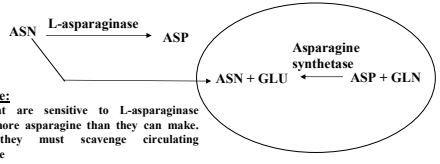
Limitations of L-Asparaginase

- Immunogenicity mediates hypersensitive reaction
  - 13%-30% of patients; lethal in 1% of patients
  - 60% of patients complete therapy
  - Risk greater with more frequent injections
- Antibodies may neutralize enzymatic activity
- Frequent dosing
  - 3x / week for 3-4 weeks or qd 10-20 days

Advantages of Oncaspar®

- Reduced immunogenicity
  - 10% of pts; 32% who had previous allergic reaction to L-asparaginase
  - 80% of patients complete therapy
- Dramatic increase in half-life of enzyme in plasma
- Less frequent dosing
  - Not more than once every 2 weeks

L-Asparaginase Therapy & the Role of Asparagine Synthetase



Rationale:
Cells that are sensitive to L-asparaginase require more asparagine than they can make. Hence, they must scavenge circulating asparagine

Prediction:
Cells with low levels of ASNS should be more responsive to L-asparaginase.
Elevation in ASNS level may mediate resistance to L-asparaginase therapy.

In vitro cytotoxicity (IC_{50} IU/mL) and ASNS levels (RT-PCR)

Pancreatic	PANC-1	MiaPaCa-2	CFPAC-2	PANC 10.05	AsPC-1
Oncaspar®	0.27	0.66	>20	0.43	>20
ASNS mRNA	+	+	++	++	++++

Ovarian	OV90	TOV21G	SKOV3	SW626	OVCAR3
Oncaspar®	0.66	1.3	>10	>20	>20
ASNS mRNA	++	++	++	++	++

Lymphoma	Daudi	Raji	Ramos	Molt 4
Oncaspar®	0.83	0.40	1.09	0.23
ASNS mRNA	+	+	++	+

ASNS mRNA
+ … ++++

The in vitro cytotoxicity of Oncaspar was determined using the MTS dye reduction assay. Cells were incubated with drugs for 72-96 h at 37°C. Following incubation, MTS dye was added and formation of a colored product (formazan) was measured at 490nm.The levels of ASNS were measured by a quantitative RT-PCR.

Therapeutic efficacy

In vivo efficacy of Oncaspar® as a single agent and in combination with gemcitabine was evaluated in xenograft models of low ASNS-expressing human pancreatic cancer (MiaPaCa-2) and high ASNS-expressing human pancreatic cancer (ASPC-1). Tumors were established by injecting 2.5 million MiaPaCa-2 or 2 million ASPC-1 cells per mouse in a single subcutaneous site into the right axillary flank of nude mice. When tumors reached the average volume of approximately 75 mm^3, the mice were divided into their experimental groups and treatment with Oncaspar® and /or gemcitabine was initiated.

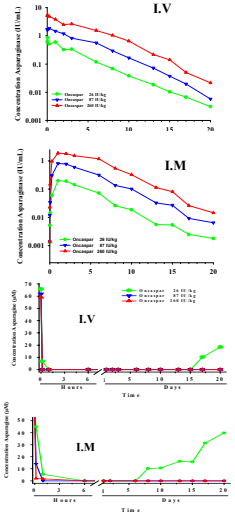
Low-ASNS-expressing model (MiaPaCa-2)

Group	Therapeutic	Final tumor volume (Means ± SD)	%Tumor Volume Change[1]	%Tumor Growth Inhibition
1	Saline	345.4 ± 135.6	340.4 ± 167.4	N/A
2	Oncaspar® (12.5KIU/kg)	187.4 ± 171.3	136.4 ± 195.4	46
3	Oncaspar® (0.8KIU/kg)	298.7 ± 101.0	328.3 ± 153.0	14
4	Gemcitabine (80mg/kg)	244.3 ± 117.1	198.8 ± 133.5	29
5	Oncaspar® (12.5KIU/kg) +Gemcitabine (80mg/kg)	218.6 ± 160.3	174.1 ± 162.1	37
6	Oncaspar® (0.8KIU/kg) +Gemcitabine (80mg/kg)	178.9 ± 138.9	145.3 ± 148.6	48
7	Gemcitabine (80mg/kg) +Oncaspar® (12.5KIU/kg)	186.0 ± 76.1	166.8 ± 119.2	46

[1] Compared to tumor size at the onset of dosing

In a low ASNS-expressing model, MiaPaCa-2, treatment with a single dose of 12.5 IU/g pegaspargase resulted in 46% tumor growth inhibition (TGI). Further, although treatment with gemcitabine alone (80 mg/kg q3d x 4) or with low dose pegaspargase (0.8 IU/kg, single dose) alone was not significantly better than controls, treatment with the combination of the two resulted in improved efficacy compared to controls (P<0.05) and a TGI of 48%. In contrast, in a high ASNS-expressing pancreatic model, ASPC-1, treatment with pegaspargase at various doses was ineffective (data not shown).

Pharmacokinetics and Pharmacodynamics



Pharmacokinetic Parameter	Route					
	IV			IM		
	Dose (IU/kg)					
	26	87	260	26	87	260
t_{max} (h)	-	-	-	37.1 (7.6)	35.4 (5.9)	34.9 (8.8)
C_{max} (IU/mL)	0.74 (0.24)	1.99 (0.28)	5.18 (0.51)	0.20 (0.05)	0.81 (0.05)	1.97 (0.24)
Vss (mL/kg)	39.3 (17.7)	44.6 (6.6)	50.6 (5.1)	-	-	-
CL (mL/h/kg)	0.49 (0.10)	0.48 (0.08)	0.56 (0.16)	-	-	-
Elimination Half-life [t½] (h)	55.4 (19.3)	65.8 (12.1)	67.3 (16.7)	47.2 (14.5)	56.6 (8.4)	79.3 (17.7)
$AUC_{0-\infty}$ (h*IU/mL)	55.5 (14.3)	186 (28)	502 (140)	23.9 (7.5)	103 (8)	306 (50)
Bioavailability (%)	-	-	-	43.0 (13.6)	55.1 (4.3)	61.0 (9.9)

Pharmacodynamic Parameter	Route					
	IV			IM		
	Dose (IU/kg)					
	26	87	260	26	87	260
T_{max} (h)	0.17-1	<0.17	<0.17	1-6	0.17-1	1-6
E_{max} (µM)	>65.9	>61.8	>59.2	>57.0	>55.3	>54.6
Time to Recovery (Days)	15-17	>20	>20	6-8	>20	>20

Female Sprague-Dawley rats were single dosed by intravenous bolus or intramuscular administration with Oncaspar ® at dose levels of 26, 87, or 260 IU/kg. Pharmaco-kinetic parameters were assessed by plasma asparaginase activity in a colorimetric mixed enzyme reaction. Pharmacodynamic parameters were determined using HPLC to examine plasma asparagine concentrations.

Conclusions

In vitro, Oncaspar® displays potent cytotoxicity against several pancreatic, ovarian, and lymphoma cell lines. In vivo, combination of Oncaspar® and Gemzar® are additive in MiaPaCa-2 xenograft model (low ASNS expressor). In contrast, in a high ASNS-expressing pancreatic model, ASPC-1, treatment with Oncaspar® at various doses was ineffective. Efficacy of Oncaspar® correlates with cellular ASNS in some cell lines. Therefore, estimation of the level of ASNS in solid tumors may help guide therapy in the future. However, a more complex signature for sensitivity to L-asparaginase may exist. In PK/PD studies, the Cmax and AUC0-∞ of pegaspargase increased and ASN levels decreased in a dose-proportional manner when Oncaspar® was dosed via either intramuscular (IM) or intravenous (IV) routes. The elimination half-lives by IM or IV routes were comparable. ASN levels depleted rapidly following Oncaspar® treatment and recovered with low dose but not with high dose treatment. Oncaspar® either as a single agent or in combination with gemcitabine should be evaluated clinically for the treatment of solid tumors and lymphomas.

References

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