#5491 Development of lipid based nanosuspension formulation of first-in-class PLK2 inhibitor GBO-006* to treat triple negative breast cancer

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Introduction

The highly selective, ATP competitive PLK2 inhibitor GBO-006 was previously shown by us to arrest growth of triple negative breast cancer (TNBC) at 30mg/kg dose (mpk) in MDA-MB-231 xenograft model. However, the formulation was a challenge for further efficacy, toxicity studies and clinical development. GBO-006 was found to be crystalline and poorly soluble (<1 mg/mL) in organic solvents/co-solvents and non-aqueous media including lipids & oils, even in the presence of complexing agents. Degradation at very high temperatures (~346 °C) limited the use of amorphous based strategies. Efforts to dissolve GBO-006 using one solvation strategy (co-solvency, complexation, micellar solubilization) were unsuccessful. The study described herein focused on particle engineering efforts to develop a 50 mg/ml nanosuspension formulation of GBO-006 stabilized by ionic, non-ionic, and polymeric and lipid stabilizers alone or in combination.

Experimental procedures: Nanosizing of GBO-006 by 'bottom-up' and 'combination of bottom-up and top-down' technologies did not yield particles in the desirable nanosize range. Nanosizing GBO-006 to less than 400 nm (d0.9) particle sizes was feasible by top-down technology using bead milling and a high shear microfluidics processor. During initial trials, lower strength formulations (5 to 25 mg/mL) were nanosized and stabilized using bead milling with non-ionic surfactant(s), Tween 80 & poloxamer 188, in addition to polymers such as PVP K12. Microfluidization was not pursued further due to clogging of the interaction chamber at higher concentrations (50 mg/mL).

Results: A crystalline, lipid nanoparticle of GBO-006 was feasible by bead milling and further assessed for pharmacokinetic evaluation and efficacy studies. Intraperitoneal dose escalation studies in mice showed a dose-dependent linear increase in plasma exposure of GBO-006. Fifty percent reduction in MDA-MB-231 xenograft tumor volume was observed with 1.5 mpk of GBO-006 after qd dosing. Significant accumulation of GBO-006 was observed in spleen and liver upon chronic dosing (21 days). We hypothesized that accumulation was likely due to reticulo-endothelial system (RES) mediated uptake, which was further proven by in vitro experiments with differentiated macrophages.

Conclusion: We have successfully developed a nanosuspension formulation for GBO-006. Notably, this nanosuspension showed similar efficacy to previous formulations at much lower doses (1.5 mpk), however particle size of 260 nm accentuated RES uptake. Ongoing studies are focused on decreasing particle size below 150 nm and incorporating a negative zeta potential to bypass RES uptake and minimize tissue distribution.

GBO – 006 Profile & Formulation Strategies

	Parameters	GBO-006
Biology	PLK2 (IC50, uM)	0.3
	MDA-MB-231 (GI50, uM)	0.32
	DU145 (GI50, uM)	0.1
In vitro PK	LogD pH 7.4	3
	Solubility (pH 7.4 ug/mL)	<0.1
	PAMPA (10^{-6}cm/s)	5.2
	Caco-2 A-B (10^{-6}cm/s), efflux ratio	7.7, 0.89
	CYP 3A4, 2D6, 2C9, 2C19, 1A2 (IC50, uM)	> 10
	RLM/HLM (t1/2 min)	15.75 (r), 13.01(h)
	PPB (%)	99.7 (h), 99.5 (r)
In vivo PK	Cl (mL/min/kg)	36.32
	Vd (L/kg)	1.38
	F %	2
Safety profiles	Kinase Selectivity & other PLKs selectivity	Yes
	hERG (uM)	> 30
	Safety Pharmacology	Clean
	Mini - AMES	Clean
In vivo Efficacy	MDA-MB-231 Xenograft Tumor in Nude mice	52% Reduction at 30 mg/kg

Formulation efforts

- Crystalline based Nano suspension by Microfluidics → Feasibility attempt at 50 mg/mL using HSP process led to clogging of the interaction chamber repeatedly and was not pursued further
- Crystalline based Nano suspension by Bead milling (Crys-NS-BM) → Higher strength 50 mg/mL, separation of formulation into three layers was observed which could not be re-dispersed. Aggregation observed during autoclaving (at 121°C) might have been resulted due to temperature close to critical flocculation temperature of stabilizing polymers
- Lipid nanosuspension by High shear fluid processing (Lipid-NS-HSP) → Sterilization of this formulation by autoclaving process led to appreciable increase in particle size and therefore need alternative sterilization method
- Lipid nanosuspension by Bead milling (Lipid-NS-BM) → The nanosuspension formulations were found to be stable in terms of particle size (d0.9), % purity, % assay and pH at refrigerated and 25 °C/60% RH storage condition up to 14 days

The lipid based nanosuspension was taken forward for pharmacokinetic and pharmacodynamic studies.

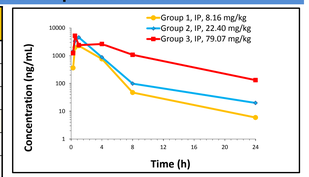
Nanosuspension based Formulation

GBO-006 strength (mg/ml)	Vehicle composition details	Storage time	particle size, d(0.9) µm	pH	% purity	% assay	particle size, d(0.9) µm	pH	% purity	% assay
50	1.5% w/v poloxamer 188 + 1.5% w/v lecithin + 0.5% w/v PVPK12 + 2% v/v ethanol + 0.075% w/v alpha-tocopherol + Dextrose q.s	Initial	0.249	5.98	99.26	101	-	-	-	-
		3 days	0.235	5.97	99.10	90	0.228	5.87	99.13	98
		7 days	0.249	5.95	98.57	111	0.240	5.81	98.45	91
		14 days	0.286	5.86	98.57	98	0.269	5.70	98.45	95

- 50 mg/mL GBO-006 lipid nanosuspension was feasible by bead milling process
- The nanosuspension formulations were found to be stable in terms of particle size (d0.9), % purity, % assay and pH at refrigerated and 25 °C/60% RH storage condition up to 14 days
- Standalone nanosuspension formulations at strengths 1 mg/mL and 5 mg/mL were found to be feasible using standalone bead milling process

Dose escalation IP PK of GBO-006 Nano suspension in Mouse

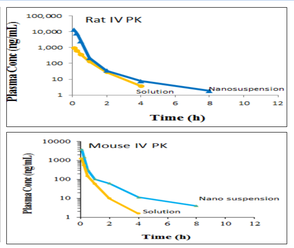
Dose (mg/kg)	10 DMSO	8.1 Nano	22.4 Nano	79.0 Nano
Cmax (ng/mL)	952	2272	4550	5222
Tmax (h)	0.25	1.0	1.0	0.5
thalf	1.6	3.3	4.2	4.8
Vdss (L/kg)	7	5.6	11.6	21.5
$AUC_{0\text{-}inf,}$ (ng*h/mL)	2451	6915	11775	25425
$DNAUC_{0\text{-}inf,}$ (ng*h/mL)	245	847	526	321



- Exposure escalation observed from 8 to 80 mg/kg
- Coverage (time above CC50) obtained at 8, 22, 79 mg/kg is 7, 9 and 24 hrs respectively
- Extravascular distribution increases significantly as the dose increases

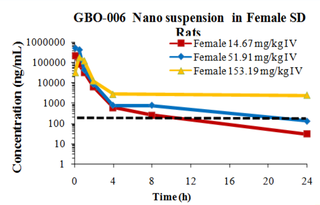
IV PK of GBO-006 Nano suspension in Rat and Mouse

	Rat		Mouse	
	Sol	Nano	Sol	Nano
Dose (mg/kg)	1	1.8	2	2.3
CL (mL/min/Kg)	30	6.1	77	29
t_{half} (h)	0.5	1.5	0.5	1.5
Vdss (L/kg)	1.5	0.8	3.4	1.0
$AUC_{0\text{-}inf,}$ (ng /mL*h)	558	4868	431	1273
*$DNAUC_{0\text{-}inf,}$ (ng /mL*h)	558	2714	215	556



- GBO-006 Nanosuspension reduced Vdss and improved systemic CL in rat and mouse
- After IV administration, nanosuspension showed ~ 5 and 2-fold higher exposure than solution in rat and mouse, respectively

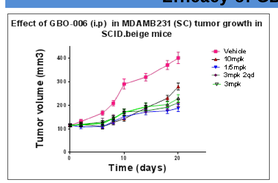
GBO-006 non-GLP Rat MTD Studies

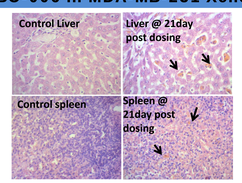


Dose (mg/kg)	14.7		51.9		153.2	
	Male	Female	Male	Female	Male	Female
Cmax (ng / mL)	204309	209860	416083	512107	241719	169694
AUC_{last} (ng / mL*h)	132654	125074	302755	352293	324998	228756
$DNAUC_{last}$ (ng /mL*h)	9083	8543	5841	6812	2582	1606
CL (mL/ min / Kg)	1.8	2.0	2.9	2.5	6.5	10.4
t_{half} (h)	5.2	4.8	6.0	7.3	7.0	5.2
Vd (L / kg)	0.84	0.81	1.5	1.6	4.0	5.0
$MRT_{0\text{-}last}$ (h)	0.7	0.8	0.7	0.7	5.3	3.7

- No clinical signs at 15, 50 & 150 mg/ Kg doses
- Mortality observed at 300 & 500 mg/Kg doses

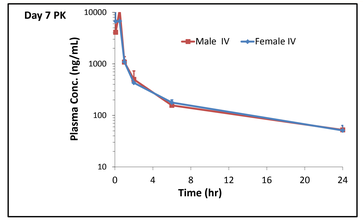
Efficacy of GBO-006 in MDA-MB-231 Xenograft





- GBO-006 showed 50% reduction in tumor growth at 1.5 mpk dose
- Compound accumulation observed in liver and spleen

Repeat Dose Toxicity- Plasma Exposure of GBO-006 on Day 1 & 7 & Tissue Distribution (SD Rat)



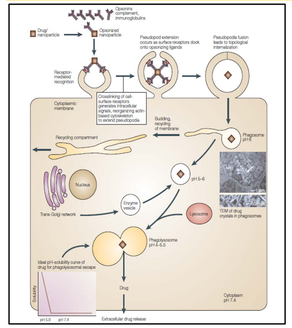
	Day 1		Day 7	
	Male	Female	Male	Female
Dose (mg/kg)	52	52	43	43
CL (mL/min/Kg)	2.9	2.5	70	75
t_{half} (h)	6.0	7.3	8	8
Vdss (L/kg)	1.5	1.6	21	26
$AUC_{0\text{-}inf}$ (ng /mL*h)	302755	352293	9992	9164

Tissue Concentration (ng/g) after completion of 7 days				
TISSUE	MALE	MALE	FEMALE	FEMALE
	50	150*	50	150*
SPLEEN	12,028,573	11,652,862	6,085,587	2,008,820
LIVER	11,154,449	2,469,027	2,687,360	682,413
LUNG	989,288	3,573,327	431,245	1,532,876

* Death on day 6

- Plasma exposure of GBO-006 significantly (~ 30-fold) decreased on day 7 with corresponding increase in Vdss (~16 fold)
- GBO-006 showed significant extravascular distribution on Day 7
- No clinical signs observed at 50 mg/kg dose

Reticuloendothelial system (RES) mediated uptake of nanosuspension particles



Differentiation of THP-1 cells (Monocytes)

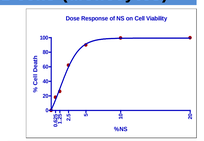
20000 cells/well (THP-1) in the log phase of growth (60-70% confluency) were plated in a 96-well plate.

100 nM PMA, 96 h

Media was removed and cells were treated with two-fold dilutions of Nanosuspension

After incubation with Nanosuspension, medium was removed and fresh 100µL medium was added followed by addition of 50µL cell-titer glo

Plate was vortexed for 5 minutes and luminescence was recorded in Envision



%NS	AVG RLU	% cell death	%Viability
20	49	99.79	0.21
10	132	99.44	0.56
5	2381	89.91	10.09
2.5	8952	62.05	37.95
1.25	17440	26.07	73.93
0.625	19335	18.04	81.96
0	23591	0.00	100.00

2.5 % of Nanosuspension vehicle causes about 60% cell death

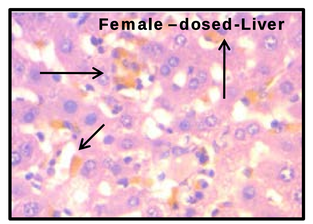
Nanosuspension drug particles, opsonized by proteins, dock onto receptors on the cell surface. This initiates phagocytosis or internalization of the particle. Membrane material is recycled back to cell surface via the recycling compartment, as enzyme vesicles from the trans-Golgi network fuse with the phagosomes, while the pH is progressively decreased. Fusion with lysosomes lowers pH further. Depending on the pH–solubility curve of drug, the drug can escape from depots in intracytoplasmic compartments, entering first the cytoplasm then extracellularly, providing sustained systemic drug release. However, particles will remain in the macrophages if they are too insoluble or if they cannot be metabolized to soluble particles (NATURE REVIEWS DRUG DISCOVERY, 2004, 3 , 785).

GBO-006 Phagocytosis by Differentiated THP-1 cells (macrophages)

%GBO-006 NS	Added Conc./well (mg/mL)	Added Conc./well Net (mg)	Recovered Conc./well (mg/mL)	Recovered Conc./well Net (mg)	% Phagocytosed
2.5	1.25	3.75	4.47	2.23	48.8
1.25	0.63	1.88	3.13	1.57	62.1
Blank	0.00	0.00	0.00	0.00	0.0
Wash	1.25	3.75	0.80	0.40	0.0

Corrected for cell viability (2.5% = 38% live cells; 1.25% = 74% live cells)

% GBO-006 NS	% Phagocytosis
2.5	**128.0**
1.25	**84**



Conclusion

- A lipid nanosuspension based formulation of GBO-006 showed linear dose-related exposure in mice.
- No observable toxicities at up to 150 mg/Kg dosage.
- Significant efficacy observed at dosage as low as 1.5 mg/Kg.
- However, macrophage mediated RES uptake was observed both in vivo and in vitro, which was likely due to the particle size that was greater than 200 nm.
- Current focus is to develop nanoparticles with a particle size <150 nm and with a negative charge, so as to bypass the RES mediated uptake, retaining the efficacy.

GBO-006 (ON 1231320) is being developed under a joint collaboration between GVK Biosciences and Onconova Therapeutics.