**Introduction**

The highly selective, ATP competitive PLK2 inhibitor GBO-006 was previously shown by us to arrest the interaction chamber at higher concentrations (50 mg/mL).

**Results:** A crystalline lipid nanoparticle of GBO-006 was feasible by bead milling and a high shear microfluidics processor. Initial trials, lower strength formulations (5 to 25 mg/mL) were nanosized and stabilized using bead milling with non-ionic surfactants. 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).

**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 mg), 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 non-aqueous media including lipids & oils, even in the presence of complexing agents. Degradation at however particle size of 260 nm accentuated RES uptake. Ongoing studies are focused on decreasing particle size, minimizing tissue distribution.

**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 (60.9) particle size was feasible by top-down technology using bead milling and a high shear microfluidics processor. Initial trials, lower strength formulations (5 to 25 mg/mL) were nanosized and stabilized using bead milling with non-ionic surfactants (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 mg of GBO-006 after qo 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. In vivo PK Cl (mL/min/kg) 36.32

**Conclusion:** A liposomal nanosuspension with an initial dose of 1 mg/kg showed a significant increase in AUC in mice and no significant increase in AUC in rats. A liposomal formulation was found to be safe in rats and mice with no deaths observed at any dose level.

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

**Introduction**

Vijaya G. Tirunagaru, Amab Roy Chowdhury, Jayaraj Athisayamani Duraliwamy, Srinivasa Rao Maddi, Sayan Mitra, Chandra Deb, Ram Sudheer Adluri, Jang B. Gupta. GVK BIOSCIENCES PVT. LTD, India

**Nanosuspension based Formulation**

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

**Duration**

- **GI50, uM**
- **Solubility (pH 7.4 ug/mL)**
- **CYP 3A4, 2D6, 2C9, 2C19,**
- **hERG (uM)**
- **Vd (L/kg)**
- **PPB (%)**
- **CL (mL/min/Kg)**
- **RLM/HLM (t1/2 min)**
- **Safety Pharmacology Clean**
- **Coverage (time above CC50) obtained at 8, 22, 79 mg/kg is 7, 9 and 24 hrs respectively**
- **Extravasation distribution increases significantly as the dose increases**
- **IV PK of GBO-006 Nano suspension in Rat and Mouse**
- **GBO-006 Nanosuspension reduced Vibs and improved systems Cl. in rat and mouse**
- **After IV administration, nanosuspension showed ~ 5 and 2 fold higher exposure than solvent in rat and mouse, respectively**

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

- **GBO-006 showed 50% reduction in tumor growth at 1.5 mg/kg dose**
- **Compound accumulation observed in liver and spleen**

**Conclusion**

- **A lipid nanosuspension based formulation of GBO-006 showed linear dose-related exposure in mice.**
- **No observable toxicity 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.