Screening of an injectable formulation of stapled peptide RO6836101 for oncology preclinical study
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ABSTRACT SUMMARY
RO6836101 is a stapled, cell-penetrating α-helical peptide that binds both MDM2 and MDMX proteins that are responsible for many types of cancers. Early progenitor compounds of RO6836101 demonstrated limited solubility at high dose levels. Conventional solubilization approaches did not yield suitable injection formulations for these early compounds, so alternative methods were tested for these hit Stapled Peptides including the use of co-solvents, polymeric micelles, biopolymer coated polymeric micelles, and lipid dispersions. This research highlights the development of these parenteral formulations, including solubility and stability profiles and subsequent in-vivo screening to assess pharmacokinetics, distribution, and efficacy. The polymeric micelle formulation was found to provide solubility in excess of 15 mg/mL and over 9 month stability under refrigerated condition. This formulation solution also provided high plasma exposure and distribution to tumors.
INTRODUCTION
Stapled α-helical peptides are a unique family of peptides with better proteolytic stability, high potency, and better cell penetrability. With these physico-chemical features, the opportunity to target extracellular and intracellular targets is significant. These peptides are very helpful therapeutic modality for many disease indications. RO6836101 is currently being explored for oncology indications. This research provides a summary of some of the formulation development activities for this particular hit series of stapled α-helical peptides. The impact of the formulation type on the pharmacokinetics, biodistribution, and efficacy is explored to optimize formulation selection.
EXPERIMENTAL METHODS
Preparation of Polymeric Micelles: Polymeric Micelles were prepared by thin film method using mPEG2K-DSPE (Corden Pharma, Switzerland). The thin film formed was rehydrated with an aqueous buffer at pH 7 resulting in polymeric micelle solution containing RO6836101 at 3.75 mg/mL. This solution was further aseptically sterile filtered through a 0.22 µm Millex GV PVDF filter (Millipore, Billerica, MA).
Preparation of Polymer coated Polymeric Micelles: This procedure is similar to the one described above for polymeric micelles with the exception of additional biopolymer Sodium Hyaluronate (Genzyme, 110 KDa).
Preparation of Solution using cosolvent: A solution formulation of RO6836101 was prepared (3.75 mg/mL) by dissolving in DMA and mixing with isotonic aqueous buffer at Histidine buffer at pH 7 and aseptically sterile filtering the solution prior to dosing.
Preparation of Lipid Dispersion of RO6836101: A lipid dispersion of RO6836101 was prepared by thin film method using DOPG (1, 2-dioleoyl-Sn-glycero-3-phospho-(1’-rac-glycerol) (sodium salt), Avanti Polar Lipids). The thin film formed was rehydrated with aqueous buffer at pH 7 to yield a lipid dispersion of RO6836101 at 3.75 mg/mL. This solution was further aseptically sterile filtered through a 0.22 µm Millex GV PVDF filter (Millipore, Billerica, MA).
Solution characterization
The particle size of the individual solutions was determined using Dynamic Laser Light Scattering unit (Wyatt Dynapro) and the surface charge was determined using Zetameter (Brookhaven instruments).
Content and Stability determination
A UPLC system (Waters ACQUITY) with a BEH130, C18, 150 x 2.1 mm, 1.7 µm reversed phase column was used for content determination (label claim) as well as for stability assessment. Solvent system A (aqueous) and B (acetonitrile) contained TFA as ion pairing reagent. A 20 minute gradient was used from 35 to 90 % B at 0.2 mL/minute.
In-Vivo PK and efficacy Study
Each formulation (RO6836101, 3.75 mg/mL) was dosed to 4 groups of nude mice (9 mice each group) implanted with SJSA cell line based tumor model. Each animal was dosed 30 mg/kg of RO6836101 by IV route of administration through tail vein. The concentration of RO6836101 was determined in the plasma, liver homogenate, and tumor homogenate and the formulations compared. The polymeric micelle based formulation was also dosed in SJSA cell line based nude mice model to assess the efficacy of RO6836101.
RESULTS AND DISCUSSION

Figure 1: RO6836101 Distribution Coefficient profile

The four lead formulations selected for PK and efficacy were optimal from dosing perspective.

Figure 2: Ready to use Polymeric micelle formulation

Figure 3: Particle size distribution of Polymeric micelle formulation (left panel) and its cryo TEM image (right panel)

The 30 mg/kg dose was also found to be very efficacious in SJSA tumor bearing mice model.

Figure 4: Plasma concentration of RO6836101 in mice

Figure 5: Tumor concentration of RO6836101 in mice

Figure 6: Liver concentration of RO6836101 in mice

CONCLUSION

A polymeric micelle formulation using mPEG (2K)-DSPE provided very good solubility and stability (>9 months in refrigerated storage condition) for RO6836101. Further enhanced stability is also demonstrated with a lyophilized formulation.

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