Ternary Complex Nanoparticles Enable Sustained Release of Bortezomib for Local Chemotherapy of Hepatocellular Carcinoma

Yicheng Zhang [a,b], Yizong Hu [a,c], Ling Li [d], Florin M. Selaru [d], and Hai-Quan Mao [a,b,c,e,*]

[a]Institute for NanoBioTechnology, Johns Hopkins University, Baltimore, MD, USA; [b]Department of Materials Science and Engineering, Whiting School of Engineering, Johns Hopkins University, Baltimore, MD, USA; [c]Department of Biomedical Engineering, Johns Hopkins University School of Medicine, Baltimore, MD, USA; [d]Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, MD [e]Translational Tissue Engineering Center, Johns Hopkins University School of Medicine, Baltimore, MD, USA.

Statement of Purpose: Bortezomib (BTZ) is a reversible proteasome inhibitor that is FDA-approved for multiple myeloma and some forms of chronic myelogenous leukemia. We have shown its high potency for patient derived hepatocellular carcinoma (HCC)[1]. However, in a clinical trial, the toxicity of BTZ limited its utilization as an HCC agent. Here, we tested the hypothesis that a nanoparticle formulation capable of local and sustained release of BTZ can avoid high level systemic exposure and improve treatment outcomes.

Methods: A confined impinging jet (CIJ) mixer was used to generate turbulence for uniform mixing of BTZ and tannic acid (TA) through a flash nanocomplexation (FNC) process, where TA and BTZ form complexes through hydrogen bonding (**Fig. 1A**, Step 1). The formed BTZ/TA complexes were then encapsulated into poly(ethylene glycol-*b*-poly(L-lactide-*co*-glycolic acid) (PEG-*b*-PLGA) nanoparticles by a flash nanoprecipitation (FNP) process (**Fig. 1A**, Step 2). Releasing profile of free drug from BTZ nanoparticles (BTZ-NPs) of different formulations were tested by incubating the NPs in PBS (pH 7.4) at 37°C for 4 weeks. To study tumor retention of BTZ-NPs, Cy7.5-labeled NPs were intratumorally injected to a PDX mouse model of HCC. For therapeutic effect, BTZ-NPs was given to mice intratumorally at a dose of 1 mg kg^{-1} every 6 days, in comparison with intratumorally injection of free BTZ 0.5 mg kg^{-1} every 3 days.

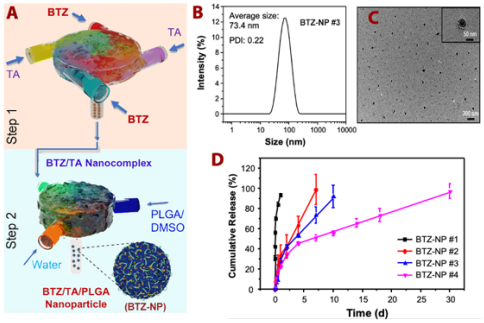

Fig. 1. (A) Assembly scheme for BTZ/TA/PEG-*b*-PLGA NPs using a two-step FNC/FNP process; **(B)** Size distribution of BTZ/TA/PEG-*b*-PLGA NPs by DLS assay; **(C)** TEM image showing the morphology of BTZ/TA/PEG-*b*-PLGA NPs; **(D)** *In vitro* releasing profiles of BTZ from different NP formulations.

Results: This new 2-step FNC/FNP process successfully generated ternary complex nanoparticles that improved uniformity, scalability, encapsulation efficiency of BTZ, and drug release profile in comparison with the traditional emulsion-based batch processes[2]. In this nanoparticle formulation, BTZ was loaded at a high payload capacity (>10 w/w%) in the form of BTZ/TA nanocomplexes mediated by hydrogen bonds between BTZ and TA. By varying the formulation parameters, including flow rate, pH condition, BTZ, TA, PLGA feeding concentration; and solvent composition, we can effectively tune the nanoparticle size from 30 nm to 400 nm with a high degree of uniformity (**Fig. 1B, C**). More importantly, BTZ releasing duration can be adjusted from 30 h to 30 days with a sustained release profile (**Fig. 1D**). Local retention of BTZ-NPs was confirmed by a whole-body imaging analysis following a single injection of the BTZ-NPs in a xenograft model (**Fig. 2A, B**), showing that the majority of injected BTZ-NPs were retained at tumor site for more than 10 days. BTZ-NPs intratumorally injected to mice showed an effective reduction of tumor size in the absence of noted toxicity (**Fig. 2C**).

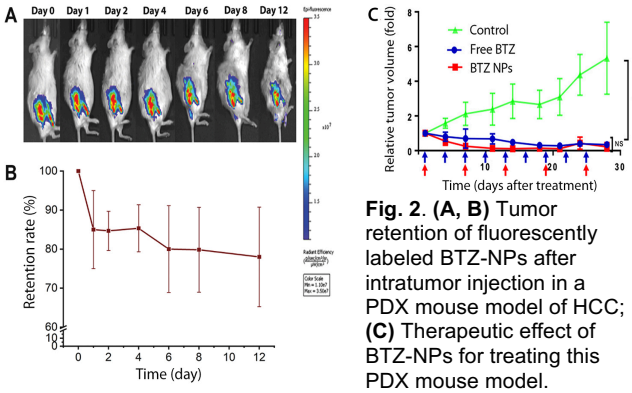

Fig. 2. (A, B) Tumor retention of fluorescently labeled BTZ-NPs after intratumor injection in a PDX mouse model of HCC; **(C)** Therapeutic effect of BTZ-NPs for treating this PDX mouse model.

Conclusions: By introducing TA into BTZ encapsulation process and using the new FNC/FNP platform, we have successfully developed a ternary complex nanoparticle formulation that is capable of controlling the nanoparticle size, composition, and BTZ release characteristics. The BTZ release can be extended from 30 h to 4 weeks *in vitro*. These nanoparticles were effectively retained in the tumor tissue following intratumor injection, indicating strong potential for local treatment of HCC via sustained delivery of BTZ.

Acknowledgement: This study is partially supported by a Maryland Innovation Initiative (MII) grant.

References:

[1] A. G. Singal, H. B. El-Serag, Hepatocellular Carcinoma from Epidemiology to Prevention: Translating Knowledge into Practice. *Clin Gastroenterol Hepatol* **13**, 2140-2151 (2015).

[2] S. Shen *et al.*, Delivery of bortezomib with nanoparticles for basal-like triple-negative breast cancer therapy. *Journal of Controlled Release* **208**, 14-24 (2015).