

Sorafenib-loaded in Microsphere Beads to Minimize Toxicity in HCC treatment

Introduction

Hepatocellular carcinoma (HCC), the most common type of liver cancer, is one of the leading causes of cancer deaths around the world (1). Because of the difficulty in diagnosing liver cancer since the disease is mostly asymptomatic in the early stages, many patients find themselves having advanced HCC by the time they get diagnosed and thus, unable to undergo surgery to remove the tumors (2). Currently, one of the treatments for patients with advanced HCC (unresectable) is targeted therapy with sorafenib (SF) - a tyrosine kinase inhibitor (TKI) - that has been approved by the Food and Drug Administration (FDA). Once sorafenib enters the bloodstream, it will target two specific receptors: Rapidly accelerated fibrosarcoma (RAF) kinase and vascular endothelial growth factor (VEGF) receptors (figure 1). By blocking the RAF kinase, cell proliferation and cell survival in tumor cells will be inhibited. VEGF receptors are responsible for tumor angiogenesis - the development of new blood vessels that provide nutrients for the tumors. Therefore, once sorafenib inhibits VEGF receptors, tumors will no longer be able to receive the nutrient supplies they need from the blood vessels (3). However, some negative aspects of oral sorafenib are lacking tumor specificity, compared to immunotherapy treatment, as well as demonstrating strong adverse effects on the patients who are taking it. Thus, often results in either dosage reduction or discontinuation of taking oral sorafenib, which leads to ineffective tumor treatments and can only prolong patients' overall survival (OS) by about 3 months. (4)

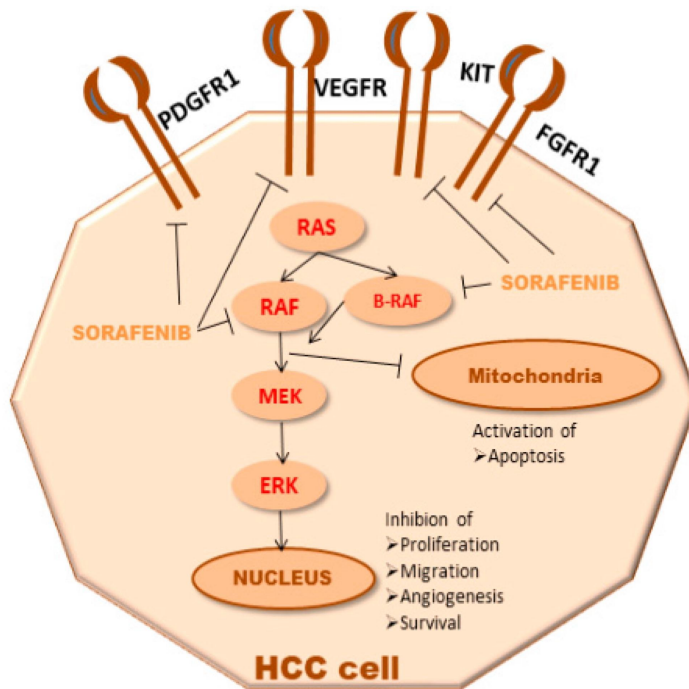


Figure 1: Sorafenib Targeting Different Receptors in HCC Cell and Show Different Inhibition Effects (6)

This summer project will aim to observe sorafenib's effects, with and without beads, on different HCC cell tumors. The overall aim of this project (beyond the summer) is to enhance therapeutic efficacy of sorafenib treatment in HCC while lessening the adverse effects caused by sorafenib. We propose to compare two different in-vitro treatments for tumor cells: (I) sorafenib-loaded in LC Beads LUMI, a micro-size carrier that is biodegradable, (II) sorafenib alone. **We hypothesize that sorafenib-loaded in microsphere beads will decrease HCC cell proliferation more in all three different cell lines compared to treatment with sorafenib alone.** For future in-vivo experiment (beyond the summer), we plan to deliver this sorafenib-loaded beads through transcatheter intrahepatic arterial (IHA) to rats' liver and expect it to target tumors more specifically while greatly reducing the systemic toxicity caused by sorafenib.

Specific Aims

- 1) To successfully load Sorafenib in microsphere beads and observe its loading and releasing rates over time.
- 2) Culture 3 different cell lines and treat them with Sorafenib alone versus Sorafenib-loaded in microsphere beads (same drug concentration) and compare their therapeutic effects on HCC tumor cells.
- 3) Beyond the summer timeline, I hope to continue working in vivo with rats by locally delivering sorafenib-loaded beads into their liver tumor and observe the adverse effects.

Materials and Methods

Drug loading: Dissolve 9.1 mg of Sorafenib + 100 mg DOTAP (1,2-Dioleoyl-3-trimethylammonium propane chloride salt) + 101.1 mg DOPC (1,2-Dioleoyl-sn-Glycero-3-Phosphocholine) in 0.5 ml dehydrated ethanol. Next, add this solution dropwise to a 9 mL 10% trehalose solution under stirring. The resulting emulsion formed from the last step will be stirred at room temperature for 5 minutes. Then, filter the emulsion solution with a manual liposomal extruder using a 200 nm polycarbonate membrane. Liposomal solution is then lyophilized overnight to yield a white solid. Solid is then treated with 1.82 mL of deionized (DI) water to yield a final Sorafenib concentration of 5 mg/ mL. Drain 2 mL of LC beads of as much liquid as possible and treat it with the liposomal solution.

To determine the drug loading content (LC) and the encapsulation efficiency (EE) of sorafenib-loaded beads, will use the following formulas: (5)

$LC\% = (\text{weight of SF in the microsphere beads}) / (\text{weight of the microsphere beads}) \times 100\%$

$EE\% = (\text{weight of SF in the microsphere beads}) / (\text{weight of feeding SF}) \times 100\%$

Drug releasing: The liposomal solution with the LC beads was passed through a cotton filter, leaving the LC beads on the filter. Phosphate buffer (PBS) (1.0 M, pH 7.4) heated to 40°C was

passed over the beads at 4 mL/min for 60 mins. Obtain six 40 mL vials of the result solution and send them to Quintara or perform UV-spectroscopy to measure Sorafenib concentration.

Cell culture: First is to remove the spent cell culture media from the culture vessel. Then wash the vessel with either PBS or solution that does not contain FBS serum and make sure to rock the vessel back and forth several times. Next, discard the washed solution from the culture vessel. Then, add a dissociation reagent called Trypsin to dissociate the cells. Gently rock the container to cover all the surface of the culture vessel. Incubate the vessel at room temperature for about 2 minutes and then place the cells under the microscope to observe detachment. When most of the cells have been detached, add a new complete medium (about twice the amount of added trypsin) into the culture vessel. Make sure to pipette the complete medium up and down several times for even distribution on the vessel surface. Transfer the cells to a tube and centrifuge at about 1.2 rpm for 5-8 minutes. After centrifuge, take out the tube and use a pipette to remove the cell pellet. Add an amount of complete medium to the cells and transfer some of the cells onto the new culture vessels.

Project Timeline (10-week period) June 21 - September 1, 2022

June 21 → July 12 (3 weeks)	July 13 → August 3 (3 weeks)	August 4 → September 1 (4 weeks)
Doing experiments to load Sorafenib in the microsphere beads and observe its releasing rate.	Culture three different HCC cell lines: rats (McA-RH7777), rabbits (VX2), and humans (Hep G2).	Set up two different experiments: one with treating the different cell lines with the prepared Sorafenib-loaded in beads and the other with treating with sorafenib alone. Goal is to observe their effects on the tumor cells.

Student Level of Participation

For this project, I will work under the supervision of my PI and work closely with a postdoctoral researcher and a visiting scholar, from which I will learn how to replicate the protocol needed for drug-loading and releasing, and also learn how to culture cells that would undergo in-vitro treatment. There are two main parts of the project:

1) Drug loading in beads and releasing

For this first part of the project, I will experiment loading Sorafenib in the LC Beads LUMI and use UV-spectroscopy to measure the loading rate of how much drug has been loaded

inside the beads. Then, I will go on to measure the drug releasing rate by following the chosen protocol. Once the drug has been observed to be effectively loaded (with an appropriate amount of drug concentration within beads) and released, I will do experimental treatment on different HCC cell lines.

2) *Culture tumor cells to undergo treatment*

Culture 3 different HCC cell lines: rat (McA-RH7777), human (Hep G2), rabbit (VX2) and once the cell levels reach the optimal state, they will undergo two different experimental treatments.

References

1. Yang, Ju Dong, and Lewis R Roberts. "Epidemiology and management of hepatocellular carcinoma." *Infectious disease clinics of North America* vol. 24,4 (2010): 899-919, viii. doi:10.1016/j.idc.2010.07.004
2. Elsayed, Mahmoud Ma et al. "Design And Characterisation Of Novel Sorafenib-Loaded Carbon Nanotubes With Distinct Tumour-Suppressive Activity In Hepatocellular Carcinoma." *International journal of nanomedicine* vol. 14 8445-8467. 29 Oct. 2019, doi:10.2147/IJN.S223920
3. Poursaid, Azadeh et al. "Silk-Elastinlike Protein Polymer Liquid Chemoembolic for Localized Release of Doxorubicin and Sorafenib." *Molecular pharmaceutics* vol. 13,8 (2016): 2736-48. doi:10.1021/acs.molpharmaceut.6b00325
4. Xiao, Yanan et al. "Sorafenib and gadolinium co-loaded liposomes for drug delivery and MRI-guided HCC treatment." *Colloids and surfaces. B, Biointerfaces* vol. 141 (2016): 83-92. doi:10.1016/j.colsurfb.2016.01.016
5. Tang, Xiaolong et al. "Anti-GPC3 antibody-modified sorafenib-loaded nanoparticles significantly inhibited HepG2 hepatocellular carcinoma." *Drug delivery* vol. 25,1 (2018): 1484-1494. doi:10.1080/10717544.2018.1477859
6. Brunetti, Oronzo et al. "Predictive and Prognostic Factors in HCC Patients Treated with Sorafenib." *Medicina (Kaunas, Lithuania)* vol. 55,10 707. 21 Oct. 2019, doi:10.3390/medicina55100707