Supplementary Methods

Radiation Promotes Inflammation-Driven Targeting of Chemoradiotherapy Enhancing Nanoparticles

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Preparation of Hf-BDC nMOF

Hf-BDC nMOFs were synthesized as previously reported through reaction of hafnium chloride, BDC, acetic acid, and water.(1) Briefly, 1.5 g $HfCl_4$, 1.07 g BDC, 7.5 mL 18 MΩ water, and 44 mL acetic acid were combined with 100 mL DMF in a round bottom flask. The flask was heated to 120°C in an oil bath while under constant stirring for 15 minutes, until the solution changed color. Upon this change, particles were cooled to room temperature and centrifuged 2 times in DMF to remove excess materials. At this point, the nMOFs were centrifuged 2 additional times in ethanol to remove excess DMF and dried overnight to remove any residual solvent.

Characterization of Hf-BDC nMOFs

Particles were characterized by dynamic light scattering (DLS), transmission electron microscopy (TEM), and high-performance liquid chromatography (HPLC). DLS was performed using a Malvern Nano ZSP (Malvern Panalytical, Malvern, UK) to determine the size, zeta potential, and polydispersity. TEM images were obtained with a Technai with iCORR (FEI, Hillsboro, OR). The TEM samples were prepared by drop casting a colloidal nMOF solution onto the copper surface of Ted Pella Formvar/carbon-backed grids (Redding, CA, USA). Grids were submitted to the OHSU Multiscale Microscopy Core for imaging.

Drug loading was quantified by running the collected supernatant on a Shimadzu SPD-20A high performance liquid chromatography (HPLC) instrument (Torrance, CA, USA) equipped with a UV/Vis detector, a fluorescence detector, and an Agilent Zorbax Rapid Resolution SBC-8 column (4.6 x 100 mm 3.5 μm; Santa Clara, CA, USA). Samples were run with a 0% to 70% acetonitrile in water (0.1% TFA) gradient over 3 mins with a flow rate of 1 mL/min. TMZ eluted first (RT = 1 min) and was quantified using an absorbance of 328 nm. Tal eluted later (RT = 3.5

min) and was quantified using an excitation of 328 nm and an emission of 634 nm. A combined standard curve for Tal and TMZ was created by running samples at various concentrations and fitting a linear regression. By subtracting the amount free drug from the amount of drug loaded, the loading and encapsulation efficiency were determined.

Cellular uptake

8×10^3 CT26.wt or 5×10^3 bEnd.3 cells per well were plated in two, 8-well chamber slides and allowed to settle for 48 hrs. Next, one chamber slide was irradiated with 2 Gy and the other was left unirradiated. Six hours after irradiation, cyanine7.5-loaded nMOFs were added to wells and allowed to incubate for overnight. nMOFs were loaded with 0.1 mg/mL cyanine 7.5 and left uncoated or coated with dextran or Fuco and dosed at a 100-fold dilution. After overnight incubation, cells were fixed, DAPI stained, and imaged on an EVOS Fl Auto 2 at 20× magnification. Three images of each treatment group were analyzed in FIJI to determine the average pixel intensity for each treatment group. Fold change was determined by dividing the average pixel intensity for each treatment by the average pixel intensity of the no radiation, non-coated nanoparticle control.

In Vitro Cytotoxicity

Using the chosen TMZ:Tal ratio of 30:1, full viability curves for individual and combination drugs (30:1) were developed. CT26.wt cells were plated in 96-well black wall, clear bottom plates at 4 x 10^3 cells per well and allowed to settle overnight. Cells were then treated with free Tal, free TMZ or free Tal/TMZ at a 30:1 ratio at various concentrations and incubated at 37°C for 72 hrs. After 72 hrs, cells were washed with PBS, media containing 10% alamarBlue was added, and cells were

incubated for 3 hrs. Sample fluorescence at excitation and emission wavelengths of 530 and 590 nm, respectively, was evaluated using a Tecan Infinite M200 Pro plate reader (Morrisville, NC, USA). Cell viability was normalized to untreated cells and IC_{50} values were determined by fitting the data to a four-parameter dose-response variable slope model in GraphPad Prism 8. Cell viability measurements were performed in duplicate technical replicates and triplicate biologic replicates and averaged. CIs were determined for each concentration of the 1:30 ratio.

Toxicity of fucoidan and Hf-BDC-Fuco was also investigated in CT26.wt cells. 4×10^3 cells were plated per well and allowed to settle overnight. After settling, cells were dosed with various concentrations of fucoidan or Hf-BDC-Fuco and allowed to incubate at 37°C for 24 hrs. At 24 hrs, cells were irradiated with 2 Gy (or control plate was left unirradiated) and incubated for an additional 24 hrs before running the alamarBlue assay. To evaluate the ability of dual-drug loading to potentiate the radiosensitivity of CT26.wt cells, nMOF with various coatings (uncoated, dextran, fucoidan) were loaded with 1:1 TMZ:Tal. Cells were plated at 4×10^3 cells per well in two 96-well plates and allowed to settle for 24 hrs. To understand the impact of the drug combination, nMOF concentration was maintained across all treatments (15 μg/mL) while total drug concentration was evaluated across a range (9.57 μM - 0.07 μM total drug in half dilutions) 24 hrs after plating. Drug-loaded nMOF was spiked into each well and empty nMOF was added at the correct concentration so all wells had the same final nMOF concentration. One day later, one of the plates was irradiated with 2 Gy. Finally, after another 24 hrs later, cells were incubated with alamarBlue to determine viability.

Drug Release Profile

Drug release from the fucoidan coated Gen 1 MOFs was determined by loading nanoparticles into Slide-A-Lyzer MINI dialysis microtubes with a molecular weight cutoff of 10 kDa. The nanoparticles were dialyzed against a 39-fold excess of buffer (pH 7.4 PBS) with gentle stirring in a water bath at 37°C (total of 1.6 mg MOF per replicate). 6 mL samples were collected at designated time points between 0 and 168 hours and concentrations determined by HPLC. Talazoparib was quantified as previously described above, with the exception of a SBC-18 column instead of SBC-8. As a prodrug, temozolomide is known to degrade under physiological conditions, resulting in the need for an altered detection method.(2) Degraded temozolomide was detected on the SBC-18 column by the method described by Michels *et al*. with the exception of an extended run time of 10 minutes, and detection by fluorescence.(3) Temozolomide in PBS was degraded overnight at 100C to create a stock used to make a standard curve.(4) Drug release profiles were plotted as a cumulative percentage of total drug release versus time. All drug release measurements were performed in triplicate.

Fluorescence biodistribution assay

To understand the biodistribution of Hf-BDC-Fuco, BALB/c mice bearing hind flank CT26.wt tumors were treated with or without a focal dose of 6 Gy. Four hours after irradiation, mice were injected intravenously with Cy7.5@Hf-BDC-Fuco (43 mg/kg Hf-BDC, 8.7 mg/kg fucoidan, 0.87 mg/kg Cy7.5). 20 hours after *i.v.* injection, mice were euthanized and organs were collected for *ex vivo* imaging using an IVIS Lumina XRMS (Perkin-Elmer, Waltham, MA) with excitation and emission filters set to 780 and 845, respectively. Fluorescence-based organ biodistribution was

determined utilizing Living Image Software (Perkin-Elmer, Waltham, MA) by summing flux for all organs and dividing the flux of each organ by the summed flux.

To determine if a fucoidan coating increases tumoral nMOF accumulation, the above study was repeated with an additional control (Hf-BDC-Dex), slightly different timing, and slightly altered concentrations. Mice were administered a 6 Gy focal irradiation and particles were not administered until 24 hours later (37.5 mg/kg Hf-BDC, 7.5 mg/kg fucoidan, 3.75 mg/kg Cy7.5). Then, an additional 24 hours later, mice were euthanized, and organs collected to repeat IVIS imaging and analysis.

TT@Hf-BDC-Fuco proof of concept and dosing frequency

Ten days after tumor inoculation, 12 mice were randomized evenly into 4 treatment groups (n=3). Treatment groups were as follows: (1) Hf-BDC-Fuco b.i.d. 5 days, (2) TT@Hf-BDC-Fuco q.d. 5 days, (3) TT@Hf-BDC-Fuco b.i.d. every other day 5 days, or (4) TT@Hf-BDC-Fuco b.i.d. 5 days. Particles were administered via tail vein injection on the aforementioned schedule starting on day 0. A second round of treatment began one week (7 days) later but was not continued as the control group had reached the ethical endpoint and therefore, the study was ended. For all treatment groups, the nMOF and fucoidan concentrations were 20.8 and 4.2 mg/kg each administration. The corresponding TMZ and Tal concentrations were 4.2 and 0.3 mg/kg for mice. In this study, free drug was not removed, and it was assumed that all drug loaded was dosed upon treatment.

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