*pharmaceutics*

MDPI

# Supplementary Materials: Nanotechnology driven cancer chemoradiation: Exploiting the full potential of radiotherapy with a unique combination of gold nanoparticles and bleomycin

**Ocean Han, Kyle Bromma, Nicholas Palmerley, Ariadne T. Bido, Mesa Monica, Abdulaziz Alhussan, Perry L. Howard, Alexandre G. Brolo, Wayne Beckham, Abraham S. Alexander and Devika B. Chithrani**

**Supplement section S1:** Summary of GNP characterization data
**Supplement section S1.1**

| | UV peak wavelength (nm) | Hydrodynamic diameter (nm) | Zeta potential (mV) |
|---|---|---|---|
| GNP | 517 | 24.9±12.6 | -20.5±1.6 |
| GNP-PEG | 519 | 27.0±8.5 | -16.8±0.9 |
| GNP-PEG-RGD (GNP complex) | 519 | 27.0±5.4 | -5.2±1.0 |
| GNP-PEG-RGD-BLM (GNP-BLM complex) | 519 | 29.3±5.7 | -0.8±0.6 |

**Figure S1.1.** Nanoparticles were characterized using UV Visible absorption spectroscopy, dynamic light scattering, and zeta potential measurements. The table below summarizes the data for as-made GNP, GNP-PEG, GNP-PEG-RGD (GNP complex) and GNP-BLM complex.

**Supplement section S1.2**
Polarization modulation infrared reflection absorption spectroscopy data

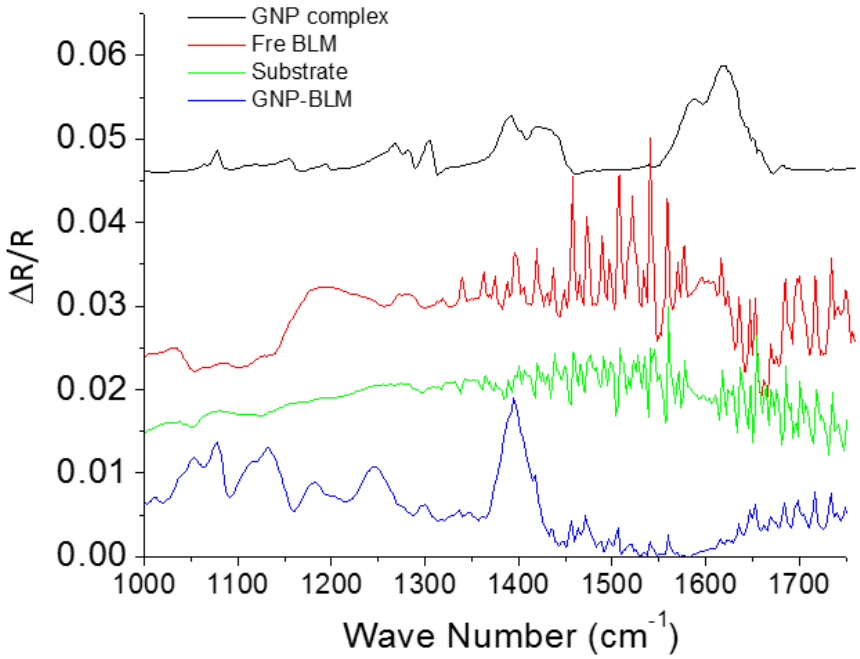


**Figure S1.2.** Polarization modulation infrared reflection absorption spectroscopy data for substrate, free BLM, GNP complex, and GNP-BLM.

**Supplement section S1.3:** Effect of GNP complex, GNP-BLM and free BLM on cell cycle

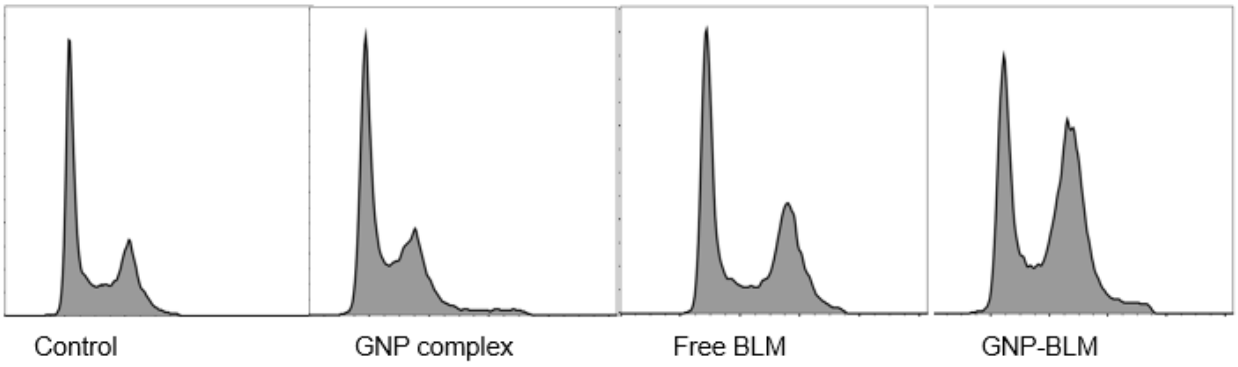


**Figure S1.3.** Comparison of cell cycle for control cells, cell treated with GNP complex (GNP-PEG-RGD), Free BLM and GNP-BLM for PC-3 cells.

**Supplement section S2:** Intracellular distribution of GNP-BLM complex and GNP complex. Darkfield and hyperspectral imaging were used to map the NP distribution.

*S2.1 Imaging of GNPs using darkfield and hyperspectral feature*

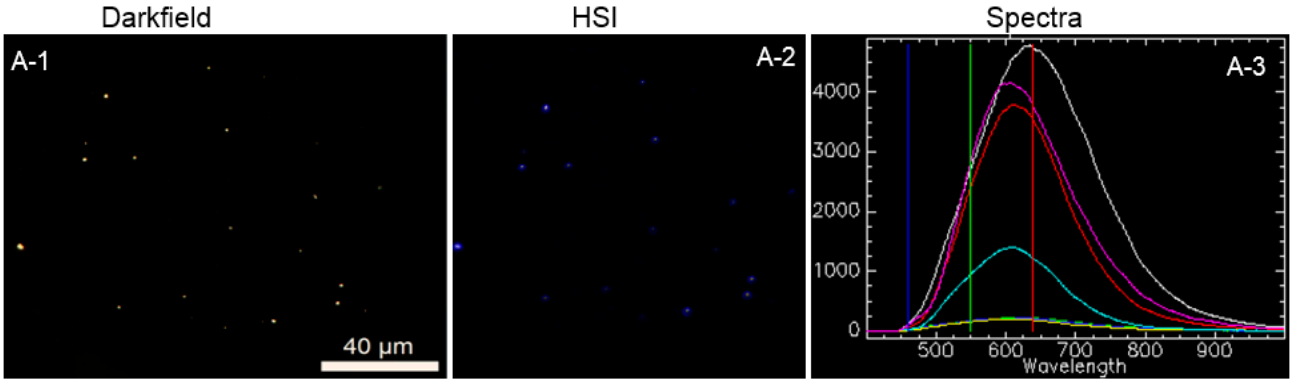


**Figure S2.1** Darkfield imaging of GNPs. A-1) Darkfield image (DFI): Yellow bright specs in the image indicates the presence of NP clusters. A-2) Hyperspectral image (HSI): Spectral map of NPs. A-3) A collection of reflectance spectra collected from few GNPs in A-2.

*S2.2 Imaging of GNPs in MIA PaCa-2 cells using darkfield and hyperspectral feature*

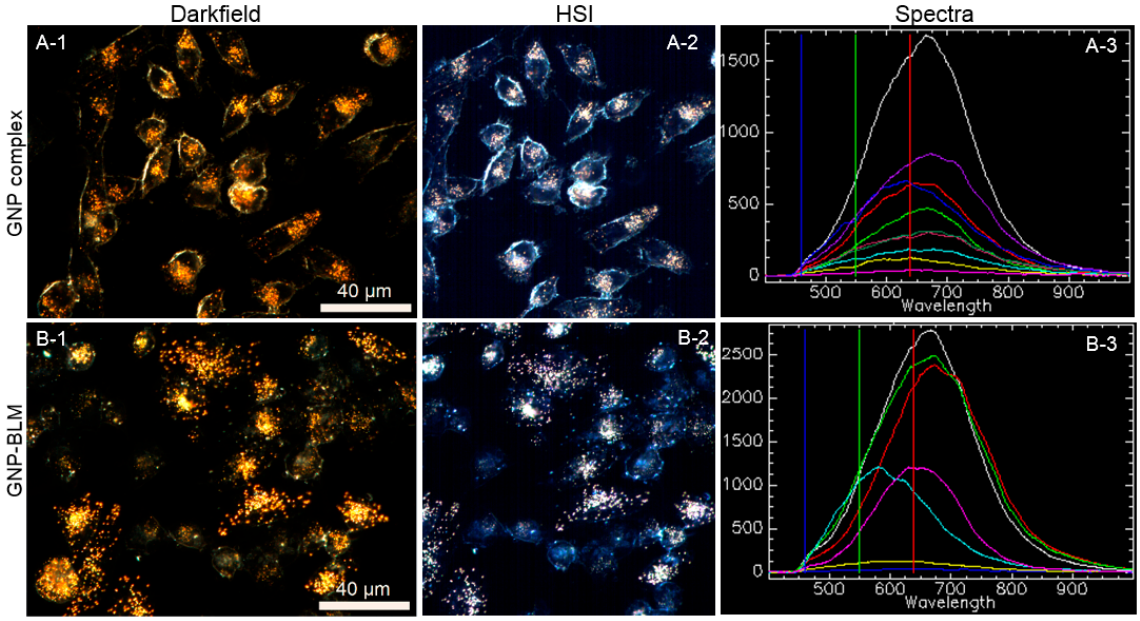


**Figure S2.2** Darkfield imaging of NPs localized within MIA PaCa-2 cells. A-1, B-1) Darkfield images (DFI): Yellow bright specs in the image indicates the presence of NP clusters. A-2, B-2) Hyperspectral images (HSI): Spectral map of NPs. A-3, B-3) A collection of reflectance spectra collected from few nanoclusters in A-2 and B-2.

*S2.3 Imaging of GNPs in PC-3 cells using darkfield and hyperspectral feature*

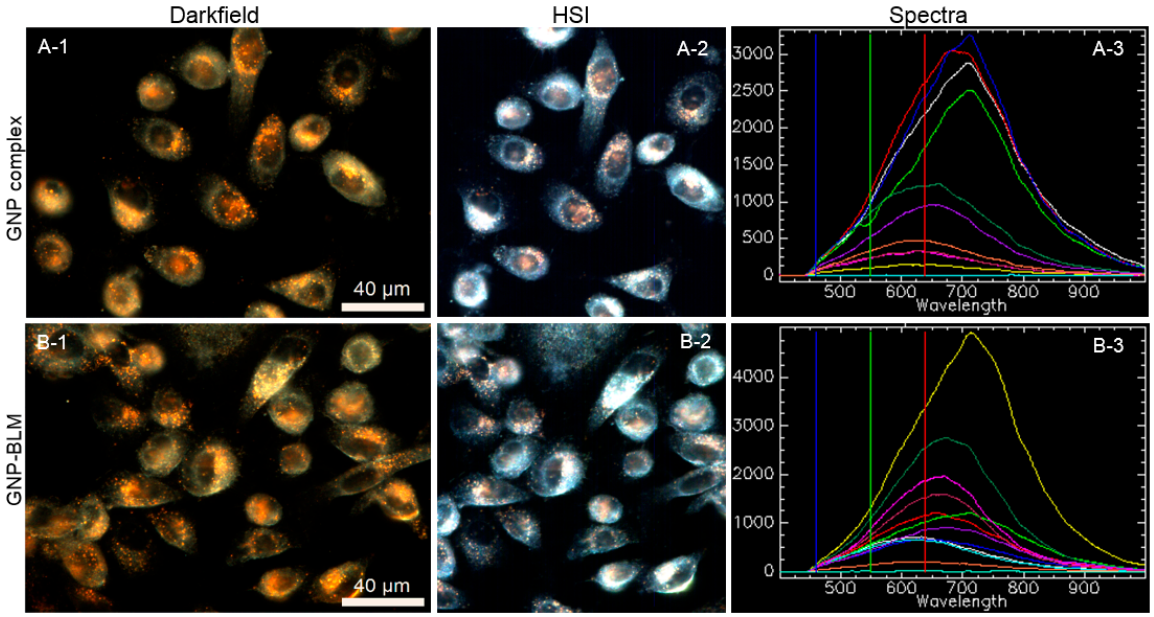


**Figure S2.3** Darkfield imaging of NPs localized within PC-3 cells. A-1, B-1) Darkfield images (DFI): Yellow bright specs in the image indicates the presence of NP clusters. A-2, B-2) Hyperspectral images (HSI): Spectral map of NPs. A-3, B-3) A collection of reflectance spectra collected from few nanoclusters in A-2 and B-2.

**Supplement section S3:** Cell cycle variation as a function of time for cells treated with GNP- BLM vs. free BLM

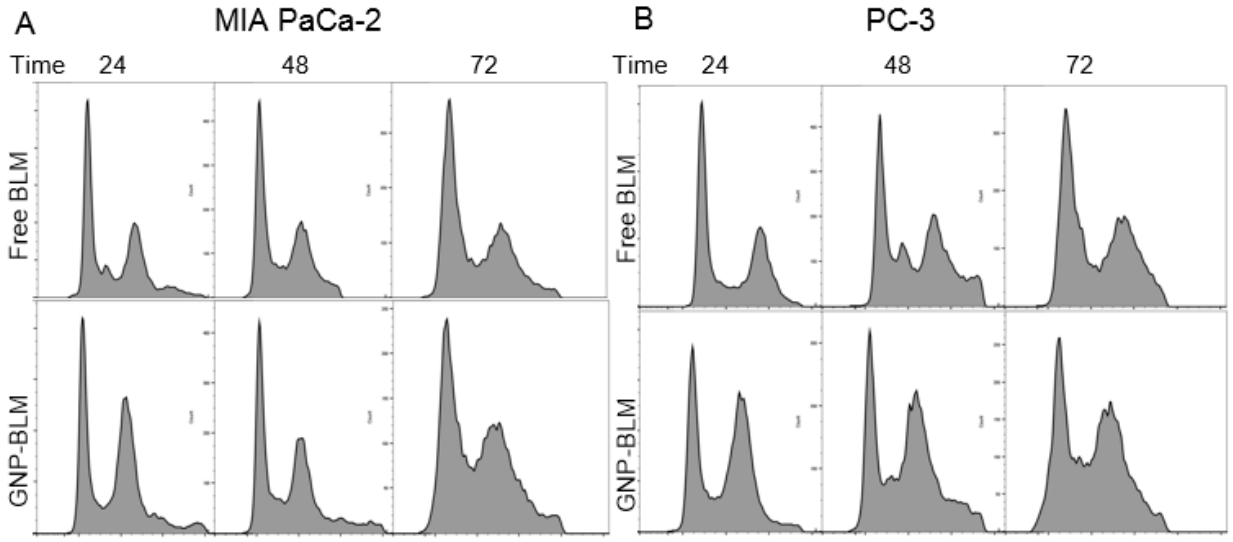


**Figure S3.** Distribution of cell cycle phase after treatment with 1 nM concentration of GNP-BLM vs 250 nM free BLM in MIA PaCa-2 and PC-3. A, B) Dynamics of cell cycle phase distribution for MIA PaCa-2 and PC-3 cells after 24 hr treatment with GNP-BLM and free BLM, respectively.

**Supplement section S4:** Imaging of DNA DSBs through tracking of two DNA DSBs repair proteins: γH2AX and 53BP1

*S4.1 MIA PaCa-2 cell line*

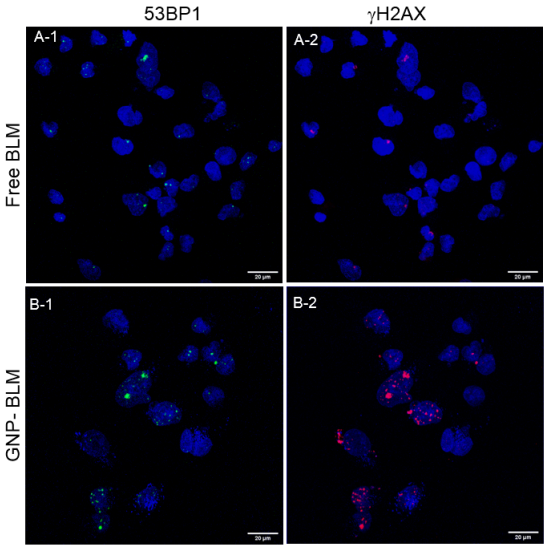


**Figure S4.1** Imaging of DNA DSBs through mapping of two DNA DSBs repair proteins: 53BP1 and γH2AX**.** A-1, A-2) 53BP1 and γH2AX foci for cells treated with free BLM, respectively. B-1, B-2) 53BP1 and γH2AX foci for cells treated with GNP-BLM, respectively. Scale bar is 20 μm.

*S4.2 PC-3 cell line*

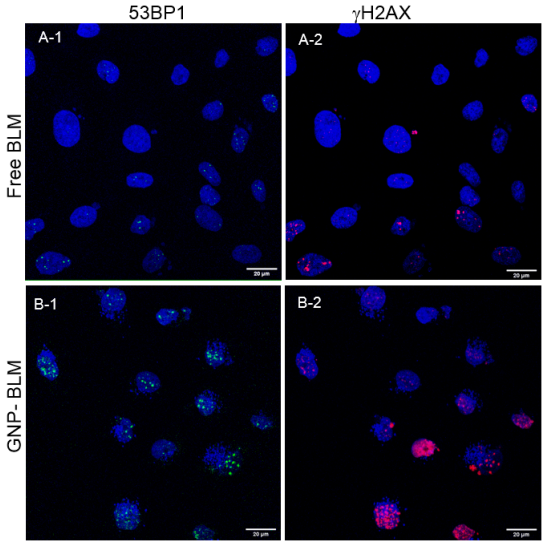


**Figure S4.2** Imaging of DNA DSBs through mapping of two DNA DSBs repair proteins: 53BP1 and γH2AX**.** A-1, A-2) 53BP1 and γH2AX foci for cells treated with free BLM, respectively. B-1, B-2) 53BP1 and γH2AX foci for cells treated with GNP-BLM, respectively. Scale bar is 20 μm.

**Supplement section S5:** GNP-mediated BLM delivery vs. free BLM

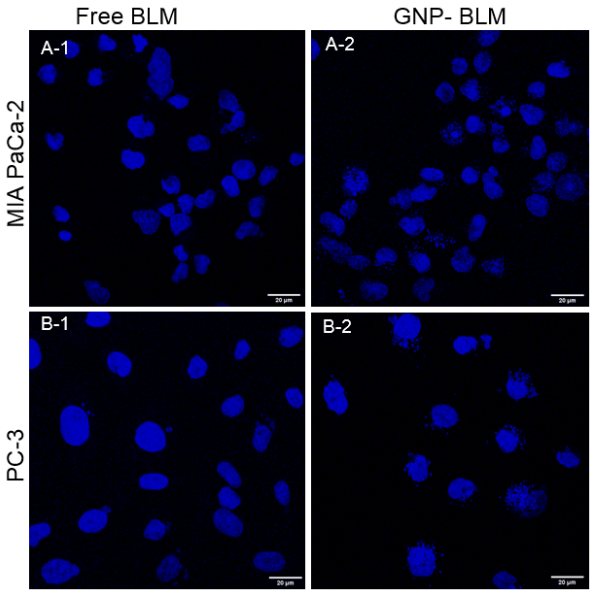


**Figure S5.** Comparison of the damage to the nucleus with GNP-mediated BLM delivery vs. free BLM. A-1, A-2) Nuclei of MIA PaCa-2 treated with GNP-BLM vs. free BLM, respectively. B-1, B-2) Nuclei of PC-3 cells treated with GNP-BLM vs. free BLM, respectively. Scale bar is 20 μm.

**Supplement section S6:** GNP-mediated chemoradiation

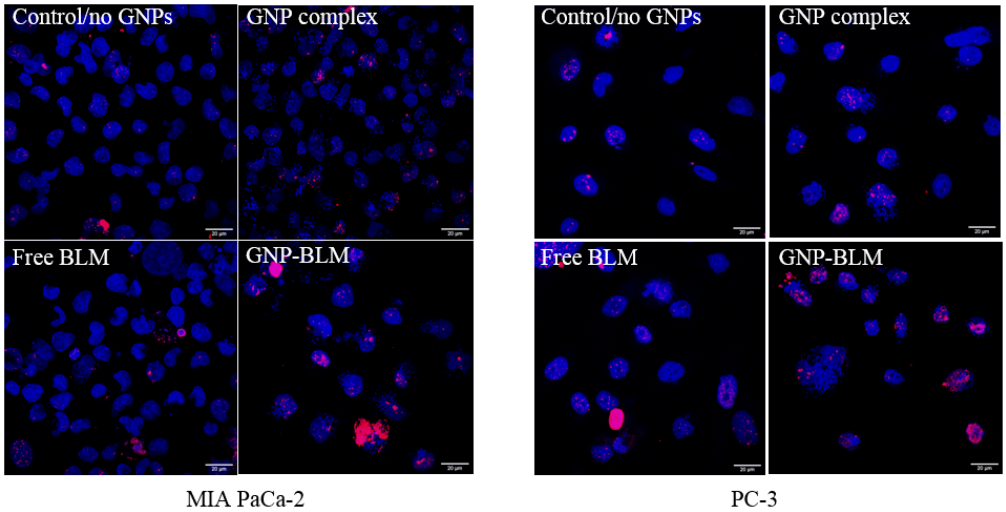


**Figure S6.** Comparison of the damage to the nucleus with radiation for control cells, cells treated with GNP complex, free BLM, and GNP-BLM for MIA PaCa-2 and PC-3. Scale bar is 20 μm.

**Supplement section S7:** DNA DSBs data for in PC-3 cells treated with a 2Gy radiation dose with a clinical linear accelerator

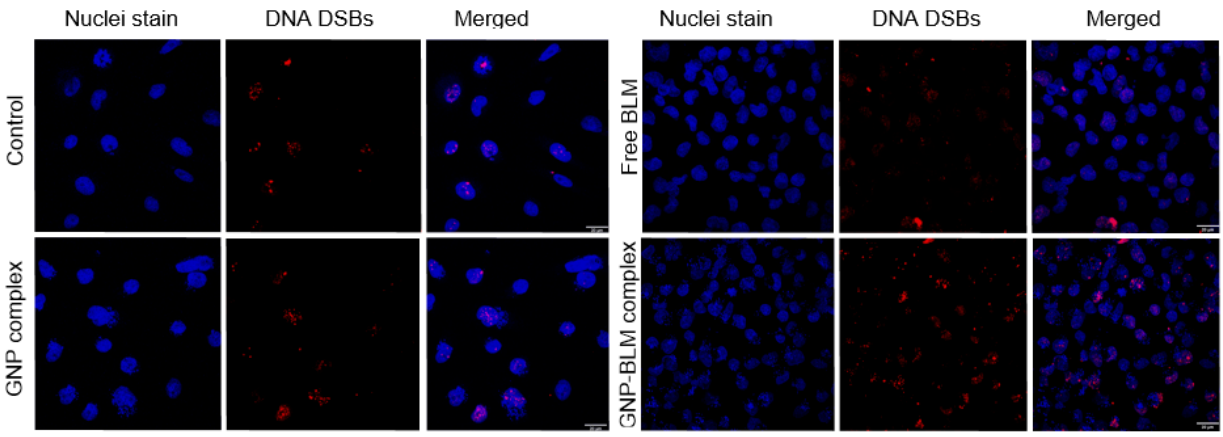


**Figure S7.** Detailed **c**omparison of the damage to the nucleus with radiation for control cells, cells treated with GNP complex, free BLM, and GNP-BLM for PC-3. Scale bar is 20 μm.