Actively targeting colorectal cancer using nanoparticles co-loaded with small molecule inhibitors

Ramkrishna Sen, Mohammad Al-Natour, Leela Sai Lokesh Janardhanam, Sean M. Geary and Aliasger K. Salem*

Department of Pharmaceutical Sciences and Experimental Therapeutics, College of Pharmacy, Iowa City, IA 52242

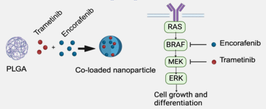
Contact Information: aliasger-salem@uiowa.edu, rksen012@gmail.com

379

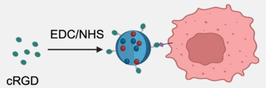
PURPOSE

To improve treatment outcomes for colorectal cancer patients with the BRAF V600E mutation, we developed PLGA nanoparticles (NPs) co-loaded with encorafenib and trametinib, targeting the RAS-RAF-MAPK signaling pathway, and functionalized with cRGD for targeted delivery. This formulation aims to enhance therapeutic efficacy compared to conventional small molecule inhibitors delivered orally.

OBJECTIVE(S)



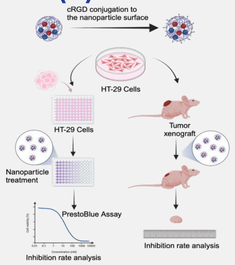
1. To develop co-loaded NPs for targeting the RAS-RAF-MAPK pathway



2. To enhance the uptake of co-loaded NPs through active targeting by decorating the NPs with cRGD

3. To evaluate the therapeutic impact of the cRGD-coated co-loaded NPs (vs non-coated co-loaded NPs vs soluble drug (oral)) in a murine xenograft CRC model at maximum tolerated doses.

METHOD(S)



RESULT(S)

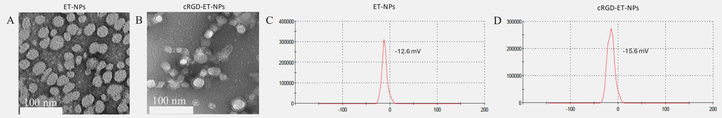


Figure 1: Physicochemical characterization of ET-NPs and cRGD-ET-NPs co-loaded NPs. Representative figures showing transmission electron microscopy images of **(A)** ET-NPs and **(B)** cRGD-ET-NPs. Zeta potential distribution of **(C)** ET-NPs and **(D)** cRGD-ET-NPs.

Code	PLGA Type	Drug amount	Drug Loading (µg/mg)	PDI	E: T Ratio
ET-NPs	PEG 50:50	E= 5 mg T= 25 µg	86.40 ± 17.47 0.68 ± 0.08	0.232 ± 0.003	127.05
cRGD-ET-NPs	PEG-COOH 50:50	E= 5 mg T= 25 µg	69.17 ± 15.63 0.58 ± 0.06	0.196 ± 0.015	119.25

Table 1: Physicochemical Evaluation of Formulations. Data from the three independent experiments denote mean ± standard deviation (n = 3). E = encorafenib, T = trametinib

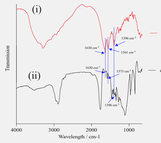


Figure 2: Surface analysis by FTIR spectroscopy.

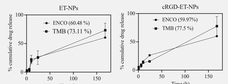


Figure 3: Cumulative *in-vitro* drug release profile of ET from ET-NPs and cRGD-ET-NPs in release medium containing PBS and 0.01% Tween 80 over a period for 7 days (n = 2).

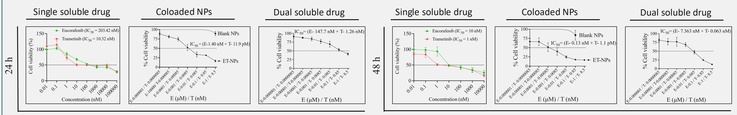


Figure 4: Both free drug alone and ET-NPs exhibited concentration and time-dependent cytotoxic behavior. Calculated IC50 values of free encorafenib and trametinib alone were significantly higher compared to those of ET-NPs at different incubation times.

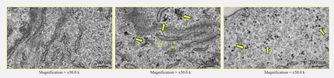


Figure 5: TEM analysis of HT-29 cells (2D) treated with ET-NPs and cRGD-ET-NPs, showing extensive NP internalization (spherical black dots) within the cytoplasm region. Nanoparticle uptake was observed as clustered aggregates for ET-NPs and as dispersed single particles for cRGD-ET-NPs.

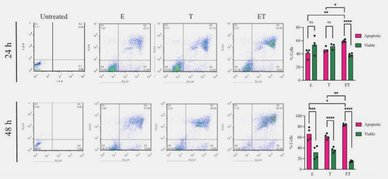


Figure 6: Flow cytometric analysis of apoptosis in HT-29 cells using FITC-Annexin V / PI staining.

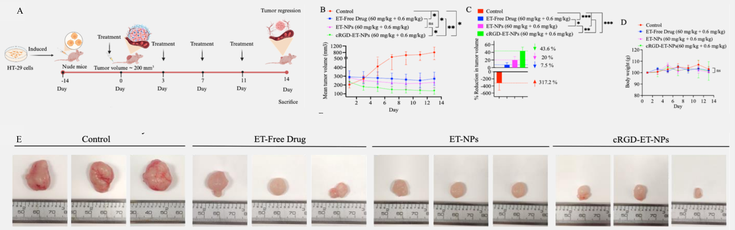


Figure 7: *In vivo* antitumor efficacy of ET-Free drug, ET-NPs, and cRGD-ET-NPs. **(A)** A schematic timeline of the efficacy study. **(B)** Mean tumor volume recorded during, and two days after, the treatment period. **(C)** Percent reduction in tumor volume comparing the tumor volume on day 1 and day 13 of treatment. **(D)** Normalized body weight recorded during the treatment period. **(E)** Macroscopic images of tumors collected from mice (day 13) demonstrating the therapeutic efficacy of ET-Free drug, ET-NPs, and cRGD-ET-NPs compared to the control group.

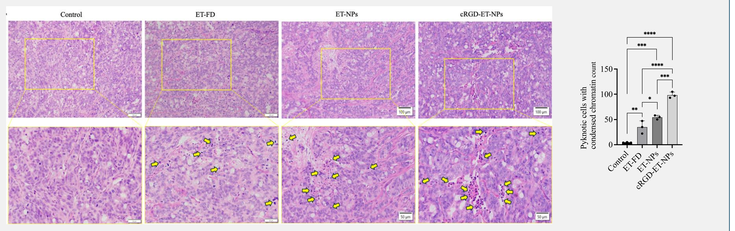


Figure 8: Histopathological analysis of tumor tissue sections collected from control and treated experimental animal groups. Pyknotic cells with condensed chromatin are indicated by yellow arrowheads. The upper panel shows images at x10.0 k magnification with a 100 µm scale bar, while the lower panel presents images at x20.0 k magnification with a 50 nm scale bar.

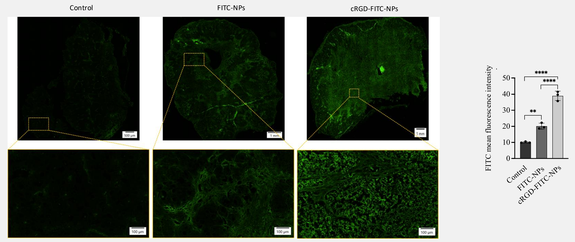


Figure 9: Fluorescence images (confocal microscope) of tumor tissue sections excised at 24 h after IV administration of FITC-loaded NPs (passive targeting) and FITC-loaded cRGD-NPs (active targeting); tumor tissue section without FITC-NPs were used as a control. The green color indicates the distribution of FITC-loadedd NPs.

CONCLUSION(S)

In this study, we developed cRGD-conjugated NPs co-loaded with encorafenib and trametinib for the treatment of colorectal cancer. These NPs (delivered IV) demonstrated superior anti-tumor activity in a HT-29 (BRAF V600E) xenograft model compared to the soluble form of the drugs (delivered orally) as well as compared to the passively targeting NPs (delivered IV). These findings could lead to more effective therapies for CRC patients, improving outcomes and quality of life. This combination is currently not used in patient treatment; thus, it deserves an opportunity to be included in clinical trials.

ACKNOWLEDGEMENTS

The author would like to acknowledge use of The University of Iowa Central Microscopy Research Facility. We are grateful to all members of the research group for their continuous support and encouragement.

Funding: This work was supported by the Gastro-Intestinal Research Foundation (GIRF).

The University of Iowa College of PHARMACY
REAL WORLD | REAL EXCELLENCE | REAL IMPACT