Panitumumab modified polymer-based nano-theranostics for non-small cell lung cancer imaging and therapy

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Introduction

- Lung cancer is the most common cancer diagnosed in Canada and it is the leading cause of death from cancer in Canada.This cancer has two different subtypes **Small Cell Lung Cancer** (SCLC) and **non-small cell lung cancer** (NSCLC). The first type (SCLC) is more aggressive(1); however, **NSCLC** is more common among lung cancer cases. 65-90% of NSCLC express Epithelial Growth Factor Receptors (EGFR) therefore, EGFR can be used as targeting sites in NSCLC. Panitumumab is a human IgG2 monoclonal antibody that can attach to EGFR(2).
- Our hypothesis is that a **radiolabeled** nano delivery system which is **Modified** with a monoclonal antibodies against **EGFR** are expected to: **Home** on EGFR+ solid tumor; provide means for **tracking**; and enhanced **therapy** of EGFR+ NSCLC tumor.

References
1) https://www.verywellhealth.com/small-cell-vs-non-small-cell-lung-cancer-5208050
2 Saltz L, Easley C, Kirkpatrick P. Panitumumab. Nat Rev Drug Discov. 2006 Dec;5(12):987-8. doi: 10.1038/nrd2204. PMID: 17201026

Method

For this study 3 main steps are followed:

1.Micelle Preparation

Maleimide-poly(ethylene oxide)-poly(α-benzyl carboxylate-ε-caprolactone) (maleimide-PEO-PBCL) polymers were prepared and mixed with methoxy PEO-PBCL at 3:7 weight ratio. Both block copolymers or their mixture were self assembled to nanostructures by a co-solvent evaporation method.

Micellar size and polydispercity index (PDI), and morphology were assessed.

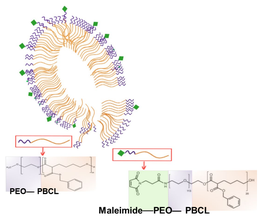

Figure 1.Micelles' formation from polymers

2. Radiolabeling Panitumumab

2,2',2"-(1,4,7-triazacyclononane-1,4,7-triyl) triacetic acid (NOTA)-NCS was reacted with panitumumab at pH 8.5 on thermoshaker at 22°C and 750rpm for 1 hour. Then, the sample was purified using a P10 size exclusion column. Using MALDI, the number of chelators per panitumumab was determined. Labeling with 64Cu was done in pH of 5.5 on thermoshaker at 37°C and 700rpm for 1 hour. The reaction efficiency was measured using radio-TLC

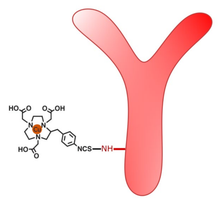

Figure 2.Radiolabeling Panitumumab

3. Panitumumab Attachment

Panitumumab was thiolated through reaction with 2-iminothiolane (Traut's reagent). Then thiolated panitumumab was reacted with malemide micelles. This was followed by reaction with 2-mecrcaptoethanol to neutralize remaining free thiol groups on the antibody. By using size exclusion chromatography, the obtained micelles were purified. Through elusion from Sepharose® CL-6B column by PBS and fraction were collected. The eluted fractions were characterized by dynamic light scattering and absorption spectroscopy at 280 nm

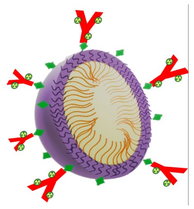

Figure 3.Attachment of radiolabeled panitumumab to micelles

Results

Type of micelle	Plain micelles	**Antibody modified** micelles
Size results	Z-Average (d.nm): 34.66 PdI: 0.181 Intercept: 0.962 Result quality : Good Peak 1: 42.04 (Size d.nm) 100.0 (% Intensity) 19.60 (Width d.nm) Peak 2: 0.000 0.0 0.000 Peak 3: 0.000 0.0 0.000 Size Distribution by Intensity Record 212: hood w/ Cent 15 Mar 1 Record 213: hood w/ Cent 15 Mar 2 Record 214: hood w/ Cent 15 Mar 3	Z-Average (d.nm): 35.58 PdI: 0.335 Intercept: 0.952 Result quality : Good Peak 1: 59.78 (Size d.nm) 98.5 (% Intensity) 58.88 (Width d.nm) Peak 2: 4258 1.5 987.2 Peak 3: 0.000 0.0 0.000 Size Distribution by Intensity Record 319: 5mg/ml 28mar after cen 1 Record 320: 5mg/ml 28mar after cen 2 Record 321: 5mg/ml 28mar after cen 3

Figure 4. Size distribution by dynamic light scattering; micellar size and polydispersity index (PdI) for plain micelles and antibody attached micelles are reported.

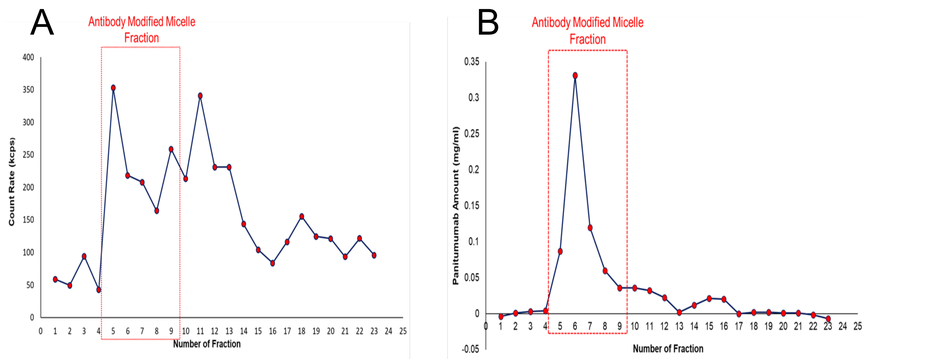

Figure 5. A) Count rate for each collected fraction from Sepharose® column by dynamic light scattering. B) Concentration of antibody detected in each collected fraction from Sepharose® column by measuring their absorption at 280nm.

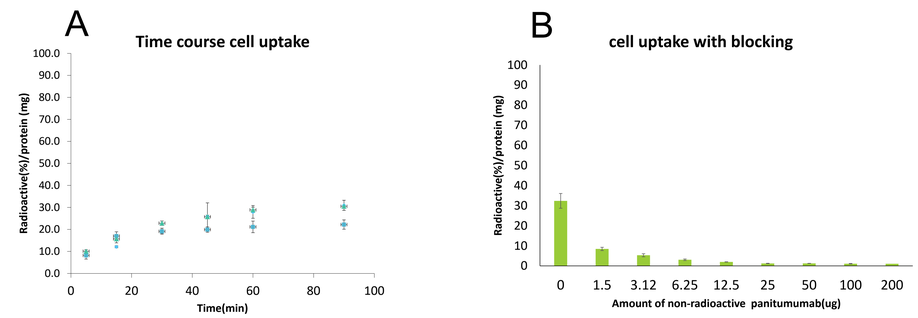

Figure 6. A) H1299 cell uptake of 64Cu-panitumumab at different time points. B) H1299 cell uptake of 64Cu-panitumumab in presents of different amounts of panitumumab after 60 minutes of incubation

Results

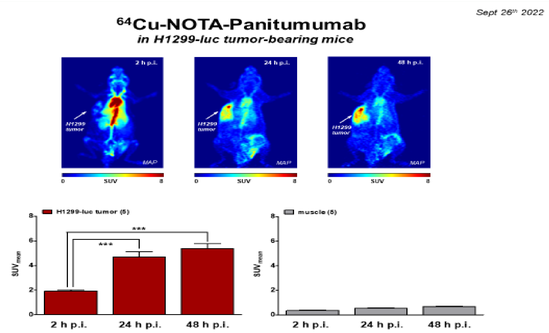

Figure7. 64Cu-panitumumab distribution in NSG-nod mice bearing EGFR+ tumor

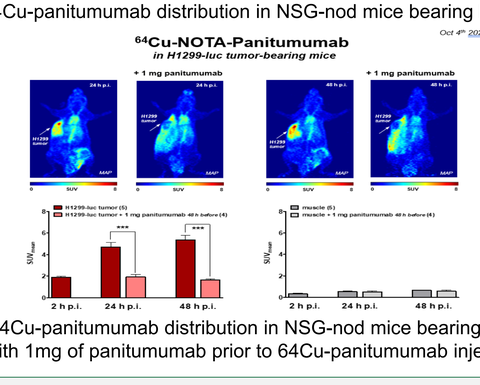

Figure8. 64Cu-panitumumab distribution in NSG-nod mice bearing EGFR+ tumor blocked with 1mg of panitumumab prior to 64Cu-panitumumab injection

Conclusion

The results show:

- Successful development of panitumumab attached polymeric micelles.
- Successful attachment of panitumumab on polymeric micelles surfaces
- Successful attachment of NOTA chelator to panitumumab and radiolabeling panitumumab.
- 64Cu labeled panitumumab homes on mice bearing EGFR+ tumors

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