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TITLE: In Vivo 18-FDG/18-Choline-Mediated Cerenkov Radiation Energy Transfer  
(CRET) Multiplexed Optical Imaging for Human Prostate Carcinoma Detection  
and Staging

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<b>14. ABSTRACT</b> Prostate cancer is treatable in its earliest stages, although treatment options for advanced forms are limited. Therefore, more sensitive means of early prostate cancer detection and new prostate cancer therapies are needed. Two novel biomarkers are proposed to associate with prostate cancer progression: the Thomsen-Friedenreich disaccharide (TF) antigen and the ErbB-2/ErbB-3 heterodimer (ErbB2/3). The objective of this proposal is to examine whether internal illumination via <sup>18</sup> F-fluorocholine Cerenkov radiation energy transfer (CRET) coupled with TF- and ErbB2/3- molecularly targeted near-infrared (NIR) QDs can be used to detect prostate cancer. We have shown that ErbB2/ErbB3 dimerization is heregulin mediated and upregulated in castrated mice bearing MDA-PCa-2b human prostate cancer xenografts. We have selected peptides from bacteriophage display libraries that target TF and ErbB2/ErbB3. The peptides have been attached to QDs and are being used to detect prostate cancer cell lines that express TF, ErbB2/ErbB3 and various stages of cancer.					
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**In Vivo 18-FDG/18-Choline-Mediated Cerenkov Radiation Energy Transfer (CRET) Multiplexed  
Optical Imaging for Human Prostate Carcinoma Detection and Staging**

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## 1. Introduction

Prostate cancer is treatable in its earliest stages, although treatment options for advanced forms are limited. Therefore, more sensitive means of early prostate cancer detection and new prostate cancer therapies are needed. Unquestionably, better biomarkers of prostate cancer would assist in diagnosis, predicting disease course, and therapy. Two novel biomarkers are proposed to associate with prostate cancer progression: the Thomsen-Friedenreich disaccharide (TF) antigen and the ErbB-2/ErbB-3 heterodimer (ErbB2/3). TF is a pan-carcinoma antigen expressed on 90% of carcinomas, including early and late stage prostate carcinomas. The ErbB2/3 heterodimer is thought to occur in ~85% of prostate cancers and is a biomarker for progression, aggressiveness, and recurrence in castration resistant prostate cancer. These biomarkers may serve as a foundation for new prostate cancer diagnostic imaging and therapeutic agents. Both molecular and functional imaging have gained attention as potential cancer diagnostics. Quantum dots (QDs) are being employed for in vivo molecular imaging because of their broad spectrum excitation, high fluorescence quantum yields, and large effective Stokes shifts; however, they are not ideal for use in vivo due to external visible light requirements and the resulting autofluorescence. Functional imaging utilizing positron emission tomography (PET) is currently in the clinic, and uptake of the PET tracers  $^{18}\text{F}$ -fluorodeoxyglucose (FDG) and  $^{18}\text{F}$ -fluorocholine in prostate tumors has been demonstrated. Unfortunately, distinction between benign and cancerous tissues with these PET tracers is not possible. A solution to this problem would be to couple QD based molecular imaging with  $^{18}\text{F}$ -fluorocholine (or  $^{18}\text{F}$ -FDG) radiation-luminescence (i.e. Cerenkov radiation) resulting from the  $^{18}\text{F}$  positron emission as an internal source of illumination. The objective of this proposal is to examine whether internal illumination via  $^{18}\text{F}$ -fluorocholine Cerenkov radiation energy transfer (CRET) coupled with TF- and ErbB2/3- molecularly targeted near-infrared (NIR) QDs can be used to detect prostate cancer.  $^{18}\text{F}$ -fluorocholine PET imaging will be utilized to define the metabolically active tumor tissue, while molecularly targeted QDs will facilitate biomarker-specific diagnosis.

The specific aims of the proposal are to: 1) select peptides that target the ErbB2/3 heterodimer using novel parallel in vitro/in vivo phage display techniques; 2) generate NIR-QDs decorated with TF- and ErbB2/3-avid peptides for in vivo molecular targeting; and 3) employ multimodal, multiplexed in vivo imaging of choline uptake, and ErbB2/3- and TF- expression in various stages of prostate cancer in mouse models of human cancer.

**2. Keywords:** Cerenkov radiation energy transfer (CRET), ErbB2, ErbB3, molecular imaging, PET, phage display, prostate cancer, quantum dots (QDs), Thomsen-Friedenreich disaccharide (TF).

## 3. Overall Project Summary

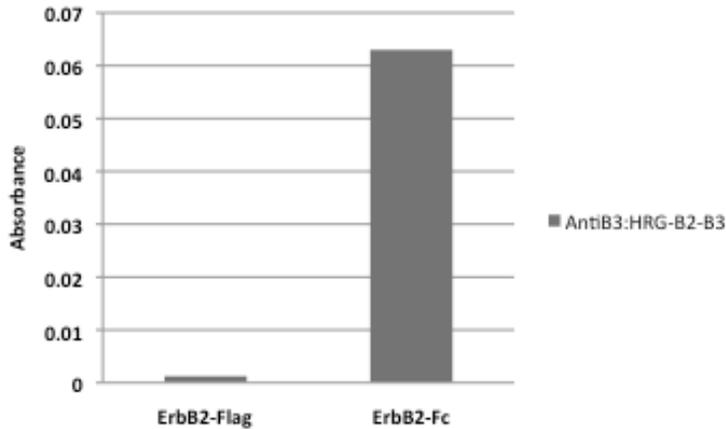
Task 1. Parallel In Vitro Phage Display Selection and In Vivo Phage Display Selection (months 1-10)

a. Purify ErbB2 ECD and characterize purified ErbB2/ErbB3 ECD heterodimer in preparation for in vitro selections (month 1-2).

Progress: Human embryonic kidney (HEK) cells were transfected with ErbB2 expressing vector containing Flag epitope (plasmid c-erbB-2-pRc/ CMVFLAG) encoding ErbB-2-ECD tagged with a FLAG sequence for purification (1). Cells were grown in RPMI, gentamicin and G418 for selection. Cultured supernatant was sterile filtered through 0.22  $\mu\text{m}$  filter and applied to an anti-FLAG affinity column equilibrated with 20 mM of Tris (pH 8.0) and 150 mM of NaCl. Bound protein was eluted with 0.1 M of glycine (pH 3.0) and neutralized with 1M of Tris HCl (pH 8.0). The protein was concentrated and dialyzed against PBS. So far, 25 mg of protein has been purified. The protein is stable in PBS in the refrigerator for at least one month and in PBS/40% glycerol for at least one year.

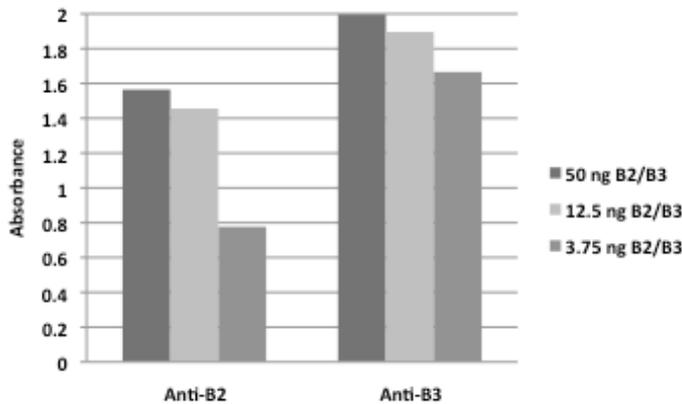
To facilitate dimerization, ErbB2 and ErbB3 with antibody Fc portions were utilized. Both purified ErbB2 and ErbB3 with Fc tags were purchased from Sino Inc. The proteins are stable for one year at -20°C.

A heterodimer of ErbB2 and ErbB3 was formed by treatment of ErbB2 with heregulin (HRG), addition of ErbB3 and dimer formation was observed and monitored by ELISA (Figure 1). As shown, the ErbB2 with the Fc tag formed much more heterodimer than flag tagged ErbB2.



**Figure 1. ErbB2/ErbB3 Heterodimer Heterodimer Formation.** Anti- ErbB3 ErbB3 antibody was coated overnight on a 96 on a 96 well plate. Wells were blocked and blocked and then pre-incubated HRG/ErbB2/ErbB3 was captured in the wells. the wells. The plate was washed and incubated with Anti-ErbB2 antibody. Wells Wells were washed and incubated with HRP with HRP conjugated secondary, washed again washed again and the plate was developed. developed. Absorbance was read, with with background subtracted.

A complex of ErbB2 and ErbB3 without addition of HRG was also formed as conformed by ELISA (Figure 2).



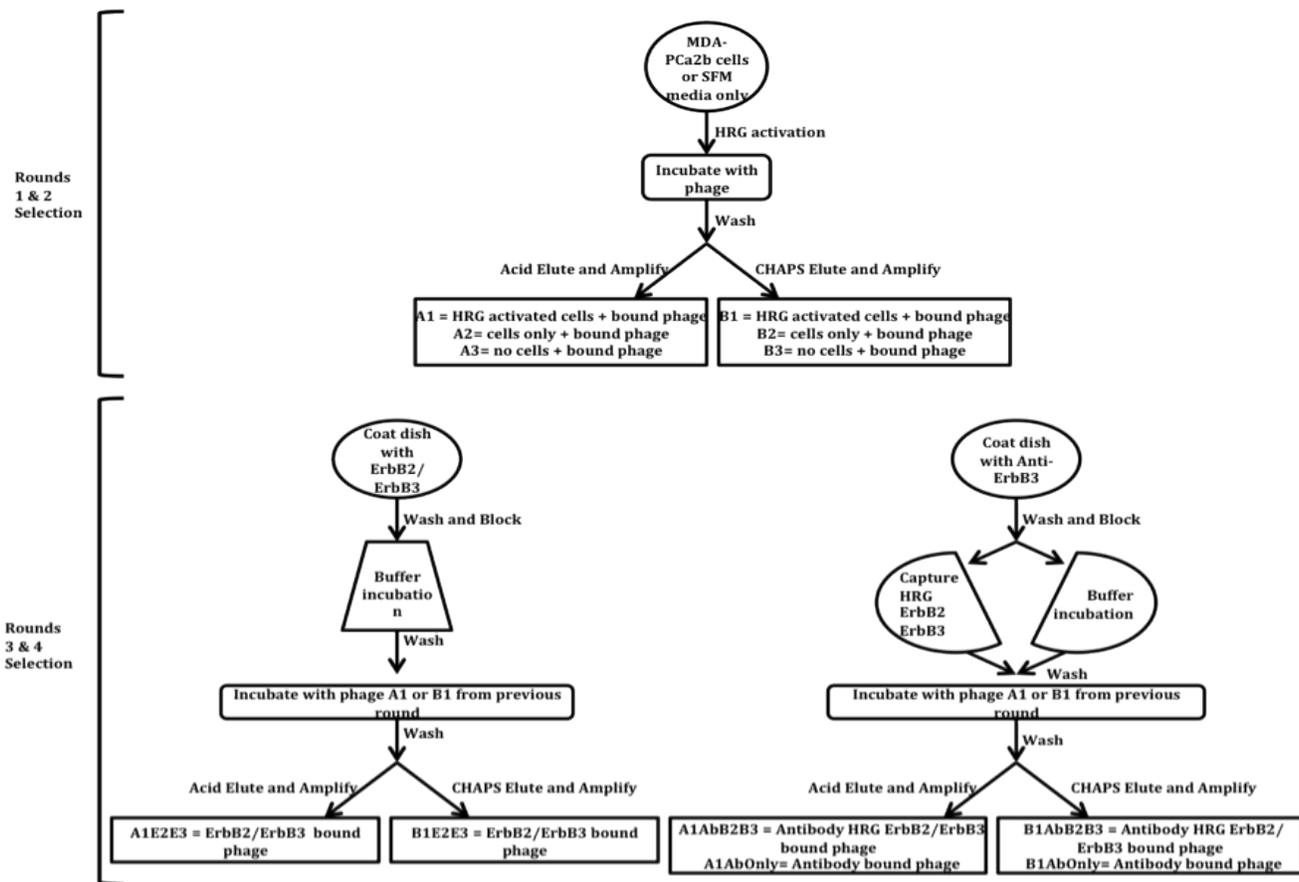
**Figure 2. ErbB2/ErbB3 ELISA.** ErbB2 and ErbB2 and ErbB3 were incubated together in a 96 in a 96 well plate, at various concentrations. Plates concentrations. Plates were blocked and incubated incubated with either Anti-ErbB2 antibody or Anti-antibody or Anti-ErbB3 antibody. Wells were washed and incubated with appropriate HRP appropriate HRP conjugated secondary, washed washed again and developed. Absorbance was was read and background subtracted.

b. Inoculate sixteen mice with MDA-PCa-2b human prostate carcinoma cell line in preparation for in vivo selections (month 3).

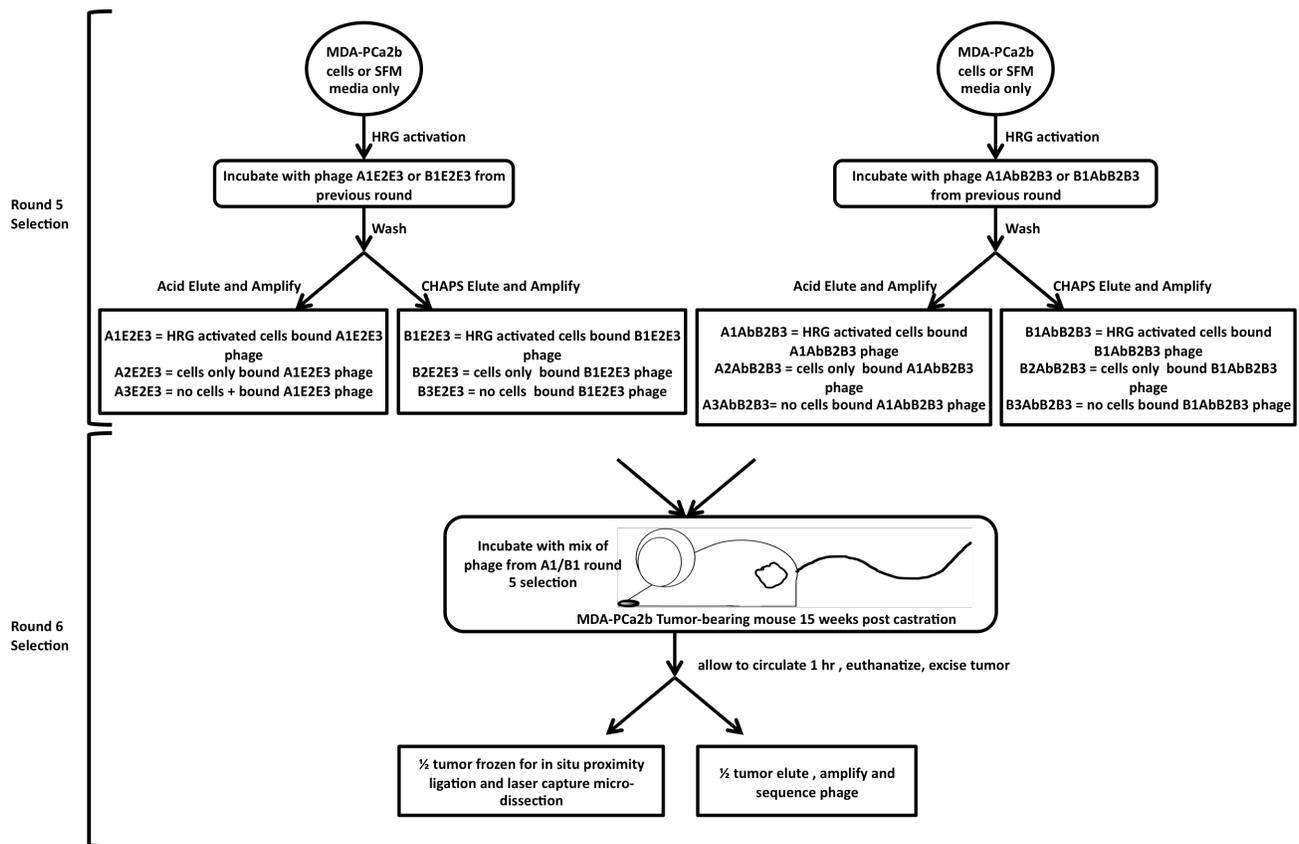
Progress: Sixteen nude mice were inoculated with MDA-PCa-2b cells in two batches of eight mice. Approximately 75% of the mice grew tumors and in 20% of these, tumors would regress after one month. We are performing histopathology studies on large and small (regressed) tumors to grade tumors. The in vivo selections are being performed in mice with non-regressing tumors.

c. Perform parallel in vitro phage display selections against ErbB2 ECD, ErbB3 ECD, and ErbB2/ErbB3 ECD heterodimer (months 3-6).

Progress: The selections were performed as outlined in Figure 3 and Figure 4. Parallel ErbB2 and ErbB3 selections were also performed.



**Figure 3. Selection Strategy Rounds 1-4.** 1st and 2nd round selection: In a 6 well plate (Row A and Row B), MDA-PCa2b cells plated in first 2 wells each row and last well each row had media only. Twenty four hours before selection, replace growth media with serum free media (SFM) in all six wells. In first wells of each row add HRG 100ng/mL for 10 minutes at 37°C. Remove all media from wells and replace with SFM containing 1013V/mL. Incubate 30 minutes at 37°C. Wash 1X (round 2 wash 3X) and elute phage. Acid elute top row in 800µL 0.1 N HCl, pH 2.2 with glycine for 10 min and neutralize. CHAPS elute bottom row in 1mL 2.5% CHAPS for 10 min. keep each well eluate separate and amplify and purify phage, saving cell pellets for RF DNA extraction. Each subsequent round of selection will use the amplified, eluted phage from the previous round as input. 3rd and 4th round selection: Two parallel selections for each round. One uses ErbB2 and ErbB3 coated directly on the plate. The other selection uses antibody to capture the HRG/ErbB2/ErbB3. In a 96 well plate coat 6 wells with 50 µL ErbB2 + 50 µL ErbB3 (50ng R3 and 25ng R4) in Na Carb pH9 buffer and 12 wells with 100 µL anti ErbB3 antibody (1µg/mL) in Na Carb pH9 buffer, overnight at 4°C. Wash plate and block wells with 400µL 5%BSA in TBS, 2 hours at RT. Aspirate blocking buffer. Add 100µL TBS to 6 ErbB2/ErbB3 wells and 6 antibody wells. Add 100µL of HRGErbB2/ErbB3 mixture in TBS in 6 of the antibody wells (premixed 30 minutes in advance: 3µg/mL ErbB2, 1µg/mL ErbB3-ECD (Sino), 1µg/mL HRG) and incubate 30 min at RT. Wash plate and add 100µL of 1012 V/mL phage in TBS to the appropriate wells (A1 phage for acid elute wells and B1 phage for the CHAPS elute wells). Incubate at 4°C for 4 hours. Wash plate (2X Round 3 and 4X Round 4) and elute bound phage. Acid elute appropriate wells in 200µL 0.1 N HCl, pH 2.2 with glycine for 10 min and neutralize. CHAPS elute appropriate wells in 200µL 2.5% CHAPS for 10 min. Amplify and purify phage as input for next round, saving cell pellets for RF DNA extraction.



**Figure 4. Fifth Round of Selection and In Vivo Selection.** 5th round selection: In two 6 well plates (Row A and Row B on each plate, one plate for E2E3 selection and one for AbB2B3 selection), MDA-PCa2b cells plated in first 2 wells each row and last well each row had media only. Twenty four hours before selection, replace growth media with serum free media (SFM) in all six wells. In first wells of each row add HRG 100ng/mL for 10 minutes at 37°C. Remove all media from wells and replace with SFM containing 1012V/mL : in one plate A1 or B1E2E3 and in the other plate A1 or B1AbB2B3. Incubate 30 minutes at 37°C. Wash 3X and elute phage. Acid elute top row in 800µL 0.1 N HCl, pH 2.2 with glycine for 10 min and neutralize. CHAPS elute bottom row in 1mL 2.5% CHAPS for 10 min. Keep each well eluate separate and amplify and purify phage as input for next round, saving cell pellets for RF DNA Extraction. Each subsequent round of selection will use the amplified, eluted phage from the previous round as input. 6th round selection: A1 and B1 phage from the 5th round, from both selection schemes, was mixed and injected into MDA-PCa2b tumor-bearing mice (n=2) 15 weeks post castration. The phage were allowed to circulate for 1 hour. Following euthanization, tumors were excised and ½ tumor frozen for in situ proximity ligation and laser capture micro-dissection. The other ½ tumor was processed to elute amplify and sequence phage.

d. Perform in vivo phage display to isolate phage that posses both appropriate pharmacokinetics and affinity for ErbB2/ErbB3 heterodimer (months 4-7). The in vivo selection occurred in MDA-PCa-2b xenografted male nude mice. There was not enough phage in the tumor to perform laser capture dissection to isolate phage. The phage were obtained, rather, from tumor tissue homogenates.

Progress: In vivo selection was performed as diagrammed in Figure 4.

e. Identification and analysis of selected peptide sequences using Next Generation sequencing – looking for sequence homology/motifs between the different selected phage populations (months 8-10).

Progress: Next Generation sequencing protocol was designed. After phage amplification the resultant E. coli pellet was processed for isolation of phagemid (phage plasmid) via midiprep. The purified

phage DNA was then quantified and enzyme digested with NciI and EagI restriction enzymes. The digested phage DNA was then run on a 2.2% agarose gel and the approximately 350 base pair band of interest was excised and gel purified. After which time, the samples were submitted to the DNA core for DNA blunting and ligation of appropriate sequencing adapters. Select sequence output from the first five rounds of selection are shown in Table 1. As seen, several multiple hit sequences were found (color highlights). Underlined sequences contained shared amino acid motifs found in other sequences from these selections. The highlighted phage are being further analyzed for binding ErbB2/ErbB3. We are waiting on the DNA sequencing results of the in vivo selection.

**Table 1. ErbB2/ErbB3 Dimer Sequencing Results**

selection	selection	selection	selection
R5A1E23 1LVLWPGRAYVVGSGV	R5B1E23 2ADAKIITGTAAAYLG	R5A1Ab 2LLATLSHLAYPSRAL	R5B1Ab 1PDHVLPEFAHLGPG
R5A1E23 4SFWRVAVNYAYAPGA	R5B1E23 4PAVASTSSLIIDGFF	R5A1Ab 3LLSSGFLYPLYSTSS	R5B1Ab 2AGPVADAVDCGGILC
R5A1E23 5VGRVSPWIYRMTGV	R5B1E23 5NAVRVAFWSVPLYPF	R5A1Ab 4AFLGSHWFPSVASR	R5B1Ab 3RCVAHLSRHRGDCG
R5A1E23 6GRRDFFPWFPLYDY	R5B1E23 6AISRATPLSVIHGVH	R5A1Ab 5RFWDYDMLRLVLRPL	R5B1Ab 4GCWELDFTRSWSVHG
R5A1E23 7GILLSEVFRSLTP	R5B1E23 7AAHVSEHYVSGSLRP	R5A1Ab 6RFWDYDMLRLVLRPL	R5B1Ab 5PLRPLRCCDVGSGY
R5A1E23 9HFAGPLKYPSYSISS	R5B1E23 8RSCPVALPMCRGWGS	R5A1Ab 7AVVRGVFLRSPISV	R5B1Ab 6TALFFPRDIGKGLYS
R5A1E23 10SLYPLFVTRWSAGSG	R5B1E23 9GGWRSSFSDRVPPAF	R5A1Ab 8HSLFGAPWLEFSDHL	R5B1Ab 7SRFPASTLSWVPAWR
R5A1E23 12TKLFPVWRPAPGGVP	R5B1E23 10DPVCCFTNADDAPT	R5A1Ab 9TKLFPVWRPAPGGVP	R5B1Ab 8ARHWGLRPS
R5A1E23 13RSYSSFVFSFQGAFS	R5B1E23 11HGSLGLGWPGHTSVR	R5A1Ab 10LGFPIVSAHGPHIRS	R5B1Ab 9TAFVLGWSAFGRPPR
R5A1E23 14APNSAVLMFGTAYPS	R5B1E23 12GSSALPRNRTPSGII	R5A1Ab 11RFWDYDMLRLVLRPL	R5B1Ab 10SLTSLIPSPSPAAR
R5A1E23 16RGVAPFRWPSFSLSS	R5B1E23 13ASSTGVPGYGYSGSD	R5A1Ab 12LLYSPGSWAAARQYM	R5B1Ab 12PSVLVRFVGLRLVTP
R5A1E23 17TKLFPVWRPAPGGVP	R5B1E23 14YVPASGLSGASWVLP	R5A1Ab 13RFWDYDMLRLVLRPL	R5B1Ab 13GLLPGSFVGQAYWLP
R5A1E23 18GTLFLLRSLHASGLP	R5B1E23 17IMILLIFSLWFGGA	R5A1Ab 15PWGLFGPGATKFGLL	R5B1Ab 14NAVRVAFWSVPLYPF
R5A1E23 19VFGTSLGLPRAAGDV	R5B1E23 18IKPGSSATHTFSPYR	R5A1Ab 16FHRVSPLLGREFAHD	R5B1Ab 15SFRYPRIISFDPSAT
R5A1E23 21VVLFPALYHSSVYGS	R5B1E23 19RLSHSIFELAPVSTP	R5A1Ab 17REFAHFHGSRSAPFP	R5B1Ab 16LKSYYVDFPHDDPVR
R5A1E23 23RWLARYWAGWHLPGF	R5B1E23 20PFVPTASSWALDLP	R5A1Ab 18VFPLRVDCFCVSGGL	R5B1Ab 17TGFESVSGGMRFGG
R5A1E23 24HGSLGLGWPGHTSVR	R5B1E23 22ILGSPAGFFRYPLTL	R5A1Ab 20CVDCYPWSRDLTRDS	R5B1Ab 18GLLPGSFVGQAYWLP
	R5B1E23 23PLDHFGAHSRGRSRV	R5A1Ab 21SFFGVWPFARHLGAS	R5B1Ab 20TFTRVTVDVYRGRLS
	R5B1E23 24RGGFSDTSRTGWVSV	R5A1Ab 22FLGPTLAKMVARARM	R5B1Ab 21RMRLSPIGFFGSRVP
			R5B1Ab 22ARFLSSTRSPSVSVS
			R5B1Ab 23SVYDVFTTRGTNSRGV

Sequences from 5th round of fUSE5 selection with PCa2b cells. Highlighted were multiple hits in these selections. 5 rounds of selections: 2 against PCa2b cells, 2 against either ErbB2/ErbB3 (E2) or AbB2B3HRG (Ab), 1 selection against PCa2b cells.

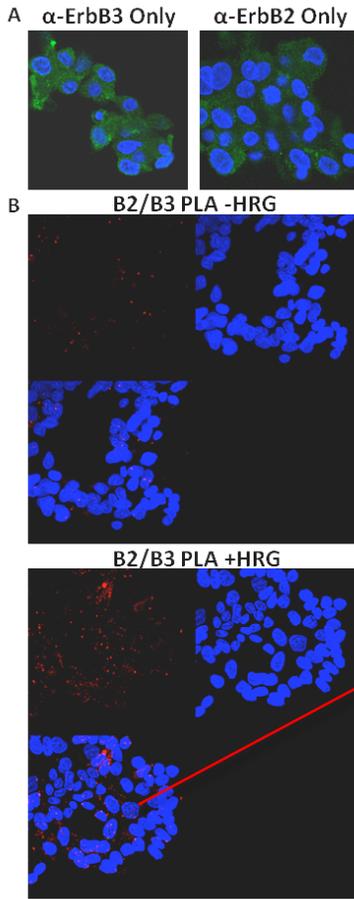
R5A1E2 = 4th round E2/E3 immobilized protein phage selected against HRG activated cells and eluted with acid;  
R5B1E2 = 4th round E2/E3 immobilized protein phage selected against HRG activated cells and eluted with CHAPs;  
R5A1Ab = 4th round Ab captured HRG E2/E3 protein phage selected against cells and eluted with acid;  
R5B1Ab = 4th round Ab captured HRG E2/E3 protein phage selected against cells and eluted with CHAPs.

**Task 2. In Vitro Characterization of Selected Phage Clones and QDs Conjugated to Selected Peptides (months 11-19)**

- a. The binding affinity and specificity of individual selected phage clones for purified, recombinant ErbB2/ErbB3 ECD heterodimer will be determined (month 11-12).  
Progress: We have large scale purified phage clones for TF (P-30-1), ErbB2 (KCCYSL), and ErbB3 (MP3) as well as putative heterodimer clones R5A1Ab #5, #9, #16; R5B1E23 #6, #11; R5B1Ab #12, #13.

b. The binding specificity of individual selected phage clones for native ErbB2/ErbB3 heterodimer within live prostate carcinoma cell lines will be determined. Followed by analysis of affinity for prostate carcinoma cells displaying native ErbB2/ErbB3 heterodimer (month 13-14).

Progress: Proximity ligation assays were performed to examine ErbB2/ErbB3 heterodimer formation. As shown in Figure 5 there was marked increase in ErbB2/ErbB3 heterodimer with addition of HRG (as shown in red in lower left panel).

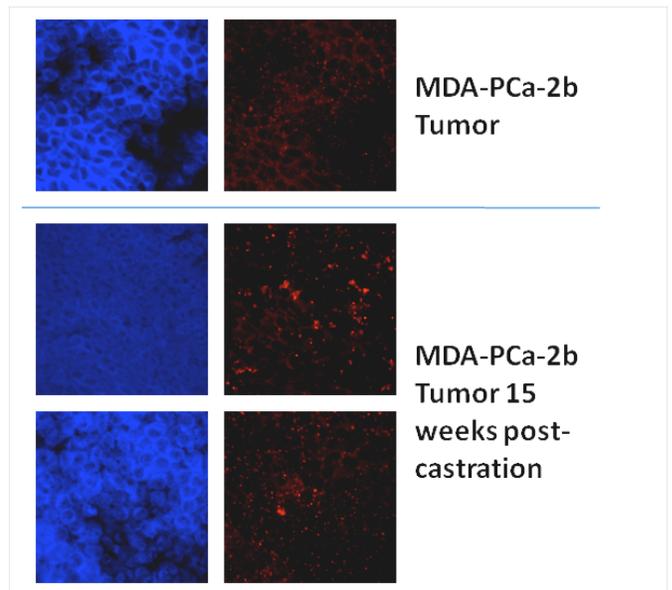


**Figure 5. ErbB2, ErbB3, and ErbB2/B3 heterodimer on MDA-PCa-MDA-PCa-2b cells.** MDA-PCa-2b cells were grown on tissue culture treated glass slides. The media was removed, cells gently washed, and then fixed then fixed in 10% buffered formalin for 30 minutes at 37°C. (A) Presence of ErbB2 and ErbB3 on the cell surface of MDA-PCa-2b cells was confirmed using antibodies specific for each receptor and an appropriate FITC labeled secondary antibody (green). (B) MDA-PCa-2b cells were serum starved for 24 hours then received a 15 minute treatment of 100ng/mL of HRG (bottom panel) or PBS only (middle panel) prior to washing and fixation. Matched pair antibodies specific for ErbB2 for ErbB2 and ErbB3 were utilized in a PLA signal amplification protocol (red). Cells were then imaged using a z-stacking technique in which 25 which 25 slices (images) were merged into 1 image. The resulting image contains B2/B3 PLA signal from many focal planes. Lower left image is DNA DNA staining (blue) and merged ErbB2/ErbB3 heterodimer (red).

Heterodimer

The existence of ErbB2/ErbB3 heterodimer was also verified in MDA-PCa-2b prostate tumor tissue from xenografted castrated mice (Figure 6). These studies show ErbB2/ErbB3 heterodimer formation is increased upon HRG treatment and in hormone resistant tumors.

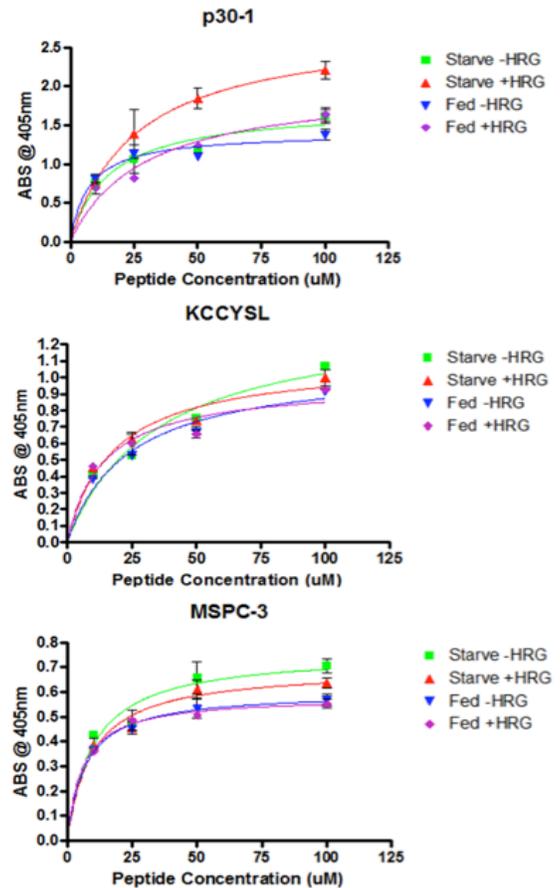
**Figure 6. B2/B3 heterodimer occurrence in MDA-PCa-2b xenografted tumors.** MDA-PCa-2b tumors resected from non-castrated mice at 14 weeks post-inoculation and tumors from castrated mice 15 weeks post-castration were probed for B2/B3 heterodimers (top panel) using the PLA technique. PLA signal from the B2/B3 heterodimer is shown as red.



c. Synthesize the anti-TF peptide (P30-1) and ErbB2/ErbB3 heterodimer targeting peptides for covalent conjugation to QDs (month 15).

Progress: TF targeting P30-1, ErbB2 targeting KCCYSL, and ErbB3 targeting MSP3 have been synthesized for conjugation to QDs. We will soon synthesize heterodimer peptides. Binding of TF targeting P30-1, ErbB2 targeting KCCYSL, and ErbB3 targeting MSP3 peptides to MDA-PCa-2b prostate cancer cells was analyzed with and without the presence of HRG (Figure 7). As shown, ErbB-2 and ErbB3 peptides alone do not exhibit increased binding to the prostate cancer cells in the presence of HRG, whereas the TF-binding peptide does. Thus, KCCYSL and MP3 serve as negative controls for ErbB2/ErbB3 heterodimerization.

**Figure 7. Binding of biotinylated peptide to MDA-PCa-2b cells.** MDA-PCa-2b cells were grown to 80% confluence in 96 well plates. The cells were then serum starved for 24 hours followed by a 15 minute treatment of either 100ng/mL HRG or PBS only. The media was removed and biotinylated peptide in PBS added to the cells for a 30 minute incubation. After which time the cells were washed and fixed in 10% buffered formalin for 30 minutes. The fixative was then washed away and presence of biotinylated peptide probed with streptavidin-HRP.

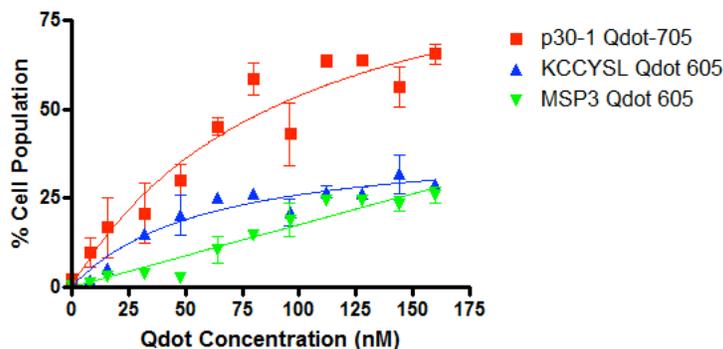


d. In vitro characterize ErbB2/ErbB3 heterodimer binding of five different phage display selected peptide-QD conjugates and five scrambled peptide-QD conjugates (month 16-17).

Progress: To be done.

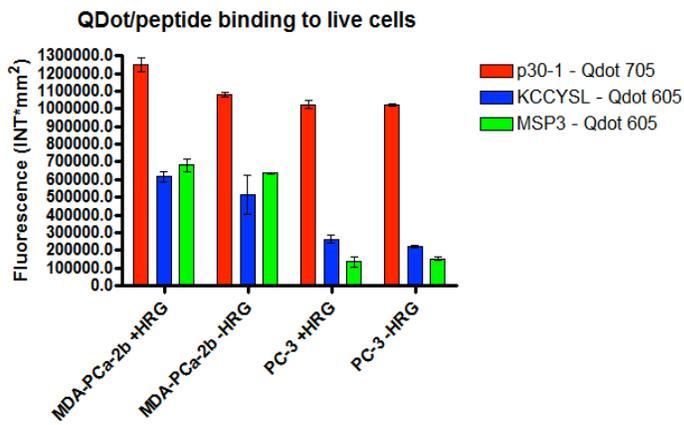
e. In vitro characterize TF binding of the p30-1-QD conjugate and scrambled p30-1-QD conjugate (month 16-17).

Progress: TF targeting P30-1, ErbB2 targeting KCCYSL, and ErbB3 targeting MSP3 peptides were conjugated to QDs and their binding to MDA-PCa-2b prostate cancer cells was analyzed by flow cytometry (Figure 8) and fluorescent optical imaging (Figure 9).



**Figure 8. Peptide/Qdot complex binding to MDA-PCa-2b cells via flow cytometry.** Peptide/Qdot complexes were incubated with a suspension of MDA-PCa-2b cells in PBS at 37°C for 30 minutes. Cells were then washed and fixed in 10% buffered formalin. Presence of Qdot was quantified via cell flow cytometry.

F



**Figure 9. Peptide/Qdot complex binding to binding to human prostate carcinoma cell lines.** Human prostate carcinoma cell lines, MDA-PCa-2b and PC-3 were grown in 24 well plates. The cells were then serum starved for 24 hours followed by a 15 minute treatment of 100ng/mL HRG. The media was removed and peptide/Qdot complexes were incubated with cells in PBS at 37°C for 30 minutes. Cells were then washed and presence of Qdot was quantified via fluorescent optical imaging.

f. Characterize binding patterns of QDs conjugated to anti-TF p30-1 peptide, scrambled p30-1, anti-ErbB2/ErbB3 heterodimer peptides, and corresponding scrambled peptides to prostate carcinoma cell lines as well as benign immortalized prostatic cell lines (month 18).

Progress: To be completed

g. Perform CRET imaging in vitro with molecularly targeted QDs bound to live human prostate carcinoma cell lines in the presence of <sup>18</sup>F-fluorocholine (month 19).

Progress: To be completed.

Task 3. Optimize Components of CRET Imaging in the Prostate Carcinoma Tumor Model MDA-PCa-2b (months 21-23). To be completed

a. Inoculation and castration of 32 nude mice to generate the MDA-PCa-2b human xenograft model of prostate carcinoma progression (month 17)

b. Optimize live <sup>18</sup>F-choline PET/CT imaging of nude mice bearing MDA-PCa-2b human prostate carcinoma xenograft model at 15 weeks post-castration (month 21)

c. Fluorescent optical imaging of molecularly targeted QDs in nude mice bearing MDA-PCa-2b human prostate carcinomas at 15 weeks post-castration (month 22-23)

d. Pharmacokinetic analysis of fluorescent imaging of QDs molecularly targeted to TF and ErbB2/ErbB3 heterodimer in nude mice (intact, 1 week post-castration, and 15 weeks post-castration) bearing MDA-PCa-2b human prostate carcinomas (month 22-23)

Task 4. Standardize Protocol for In Vivo Multiplexed CRET Imaging of Nude Mice (Castrated and Intact) Bearing MDA-PCa-2b Human Prostate Carcinoma Xenografts (months 24-29). To be completed

a. Inoculation and castration of 9 nude mice to generate the MDA-PCa-2b human xenograft model of prostate carcinoma progression (month 24)

b. Standardize <sup>18</sup>F-fluorocholine mediated CRET multiplexed optical imaging in nude mice (intact, 1 week post-castration, and 15 weeks post-castration) bearing MDA-PCa-2b human prostate carcinoma xenografts (months 28-29)

Task 5. Evaluate Diagnostic/Staging Potential of Multiplexed CRET Imaging within TRAMP Mice (months 30-36). To be completed

- a. TRAMP mice (ages 8, 12, 16, or 20 weeks old) with and without castrations will be imaged utilizing the standardized multiplexed CRET imaging protocol (30 mice) (months 31 – 36)
- d. Histopathologic analysis of imaged TRAMP mice to validate diagnosis of stage of tumor development and/or progression (months 31-36)

#### **4. Key Research Accomplishments**

- Purify large quantities of ErbB2 ECD.
- Perform stability studies in vitro of ErbB2 and ErbB3, and heterodimer.
- Perform ELISA showing ErbB2 and ErbB3 react with anti- ErbB2 and ErbB3 antibodies and ErbB2/ErbB3 can be captured in microtiter plate well and heregulin promotes ErbB2/ErbB3 heterodimer.
- Perform in vitro phage display selections with ErbB2, ErbB3, ErbB2/ErbB3, and heregulin treated ErbB2/ErbB3.
- Perform in vivo phage selections in MDA-PCa-2b human prostate cancer cell xenografted male mice.
- Sequence outputs of multiple in vitro and in vivo selections. Develop protocol for high throughput Next Gen sequencing.
- Analyze DNA sequencing data and displayed peptide sequences.
- Synthesize the TF, ErbB2 and ErbB3 avid peptides with a biotin for coupling to streptavidin coated QDs.
- Characterize binding of TF, ErbB2 and ErbB3 avid QDs to human prostate MDA-PCa-2b carcinoma cells using flow cytometry and fluorescent imaging.

#### **5. Conclusions**

In summary, we have shown that we can form ErbB2/ErbB3 heterodimers in vitro and we can promote heterodimer formation in human prostate cancer MDA-PCa-2b cells. Heregulin treatment stimulates dimer formation and is increased in castrated mice. Phage display selections against ErbB2/ErbB3 complexes numerous peptide sequences of interest. Many shared common motifs that may be important in binding the heterodimer. Several phage clones were found in different elution conditions and may represent promising candidates for further study. ErbB2 and ErbB3 –targeting peptides and QDots alone do not bind well to low expressing PC3 prostate cancer cells (as expected) and serve as a negative control. They bind to ErbB2/ErbB2 expressing MDA-PCa-2b cells. The TF-binding peptide attached to Qdots binds well to both PC3 and MDA-PCa-2b cells, as was proposed.

**6. Publications, Abstracts, Presentations.** Nothing to report

**7. Inventions, Patents, Licenses.** Nothing to report

#### **8. Reportable Outcomes**

- Invited Speaker “Phage Display for Tumor Imaging Agents” University of Missouri Research Reactor, November 2013.
- A proximity assay to demonstrate ErbB2/ErbB3 dimerization has been developed.
- Peptides that bind ErbB2/ErbB3 have been selected and may be used to detect these antigens in cancer samples.

**9. Other Achievements.** Nothing to report

**10. References**

1. Karasseva NG, Glinsky VV, Chen NX, Komatireddy R, Quinn TP. Identification and characterization of peptides that bind human ErbB-2 selected from a bacteriophage display library. *J Protein Chem*, 21:287-296, 2002.

**11. Appendices.** None