**Abstract**

Imaging probes targeting type 2 cannabinoid receptor (CB$_2$R) overexpressed in pancreatic duct adenocarcinoma (PDAC) tissue have the potential to improve early detection and surgical outcome of PDAC. The aim of our study was to evaluate the molecular imaging potential of a CB$_2$R-targeted near-infrared (NIR) fluorescent probe (NIR760-XLP6) for PDAC. CB$_2$R overexpression was observed in both PDAC patient tissues and various pancreatic cancer cell lines. *In vitro* fluorescence imaging indicated specific binding of NIR760-XLP6 to CB$_2$R in human PDAC PANC-1 cells. In a xenograft mouse tumor model, NIR760-XLP6 showed remarkable 50- (ex *vivo*) and 3.2-fold (in *vivo*) tumor to normal contrast enhancement with minimal liver and kidney uptake. In a PDAC lymph node metastasis model, significant signal contrast was observed in bilateral axillary lymph nodes with PDAC metastasis after injection of the probe. In conclusion, NIR760-XLP6 exhibits promising characteristics for imaging PDAC, and CB$_2$R appears to be an attractive target for PDAC imaging.

**Introduction**

Pancreatic duct adenocarcinoma (PDAC) is the fourth leading cause of cancer-related death in the United States, with an extremely poor 5-year survival rate of less than 10% and a median survival of 5-8 months after diagnosis [1]. Due to the absence of early symptoms and a lack of accurate diagnostic tools for early-stage detection, only 20% of cases are candidates for surgical resection at the time of diagnosis [2]. Preoperative assessment of PDAC margin status is challenging using current technologies, so even if surgical resection can be performed, most patients have residual disease from margin and therefore recur quickly [3-5]. For patients with advanced disease, treatment options are limited to chemotherapy and radiotherapy, but the effectiveness is unsatisfactory. This is because PDAC is a heterogeneous disease, reflected in diverse clinical response patterns to therapy. Additionally, the tumor hypovascular nature and dense desmoplastic stroma barrier prevent drug delivery [6-8]. Molecular
agents that can specifically target PDAC to allow for early diagnosis and improved therapeutic outcome are therefore in urgent need. To date, though some targets have been explored for PDAC imaging and therapy, such as integrin αvβ3, claudin-4, epidermal growth factor receptor, vascular endothelial growth factor receptor, urokinase-type plasminogen activator receptor, carcinoembryonic antigen, and carbohydrate antigen 19-9 (CA19-9), more molecular probes with high PDAC imaging contrast still need to be developed [9–14].

The endogenous cannabinoids system composed of endocannabinoids and two major G protein–coupled receptors (GPCRs), cannabinoid receptor type 1 (CB1R) and type 2 (CB2R), plays an important role in various physiological and pathological conditions, making this system an attractive therapeutic target [15–17]. Under basal conditions, CB2R is highly expressed in the central nervous system and mediates the psychotropic effects of cannabinoids, whereas CB1R is predominantly found in the immune system with high expression only in the spleen and lymph nodes [18–20]. However, many types of cancer, including PDAC, overexpress CB2R, and the expression levels of CB2R appear to be associated with tumor aggressiveness [21–25]. Moreover, CB2R agonists potently inhibited viability, proliferation, adhesion, and migration of various types of cancer cells. Therefore, CB2R appears to be a promising target for PDAC imaging and therapy.

To date, only a limited number of CB2R-targeted imaging agents have been reported, which are mainly applied in position emission tomography (PET) imaging [26–28]. Although PET is a great imaging technique for clinical imaging with high sensitivity and deep tissue penetration, it has many limitations, such as relatively low spatial resolution, narrow time window, high instrument cost, and injection of radioactive agents. In contrast, fluorescence imaging is a low-cost imaging method with superior resolution and sensitivity, and is becoming more popularly used in the clinic for diagnostic and surgical navigation purposes. When accompanied with dyes in the near-infrared (NIR) spectrum (650–900 nm) where the psychotropic effects of cannabinoids, whereas CB2R is highly expressed in the central nervous system and mediates the psychotropic effects of cannabinoids, whereas CB1R is predominantly found in the immune system with high expression only in the spleen and lymph nodes [18–20]. However, many types of cancer, including PDAC, overexpress CB2R, and the expression levels of CB2R appear to be associated with tumor aggressiveness [21–25]. Moreover, CB2R agonists potently inhibited viability, proliferation, adhesion, and migration of various types of cancer cells. Therefore, CB2R appears to be a promising target for PDAC imaging and therapy.

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In Vivo Optical Imaging of NIR760-XLP6 in Xenograft Tumor Model

Experiments with tumor-bearing mice were performed 15 days after the injection of tumor cells. A total of 15 mice were divided into 3 groups, each of which was injected with the following agents (dissolved in 100 μl saline) via tail vein: (1) five mice received 10 nmol of NIR760-XLP6, (2) five mice received 100 nmol of 4Q3C followed by 10 nmol of NIR760-XLP6 after 1 hour, and (3) five mice received 10 nmol of NIR760. Mice were anesthetized with 2.5% isoflurane, and images were captured at preinjection and at 0.5, 1, 3, 6, 9, 24, 48, and 72 hours postinjection with a Xenogen IVIS Spectrum imaging system using the following parameters: excitation filter, 745 nm; emission filter, 800 nm; exposure time, 1 second; binning, small; field of view, 12; f/stop, 2; open filter. The signal intensity was expressed as radiant efficiency ([photons/s/cm²/sr]/[μW/cm²]). Images were analyzed using Living Image 4.5 software (PerkinElmer). To determine tumor contrast, regions of interest (ROIs) of tumor site at the right flank of the animal (T) and normal tissue at the left flank (N) were drawn. Dividing fluorescence intensity at T by that at N yielded the contrast between the tumor and normal tissue.

Ex Vivo Optical Imaging, Biodistribution, and Histological Study

After the last imaging time point (72 hours postinjection), all mice were sacrificed by cervical dislocation. Tumors and selected organs (heart, lung, liver, spleen, pancreas, kidneys, brain, muscle from left leg, and blood) were excised and imaged using the Xenogen IVIS Spectrum imaging system. The ROIs along the excised tumors and organs were drawn, and the fluorescence intensities were measured. The biodistribution was analyzed by comparing the average fluorescence intensities in the tumor or organs with those in the muscle of left leg from the same animal. For histological study, all excised tumor and organ specimens were fixed in 4% paraformaldehyde and embedded in paraffin for 5-μm tissue sections. After deparaffinization, all sections were stained with hematoxylin and eosin (H&E).

In Vivo and Ex Vivo Optical Imaging in PDAC Lymph Node Metastasis Model

The in vivo imaging of NIR760-XLP6 in the PDAC lymph node metastasis mice was performed 5 weeks after the inoculation. Three mice were injected with 10 nmol of NIR760-XLP6 (dissolved in 100 μl saline) via tail vein. Mice were anesthetized with 2.5% isoflurane; images were captured at 0.5, 1, 3, 6, 9, 24, 48 hours postinjection. To determine the lymph node uptake of NIR760-XLP6, the PDAC lymph node metastasis mice were euthanized 48 hours postinjection, and selected lymph nodes, including left proper axillary lymph node, right proper axillary lymph node, left superficial parotid lymph node, and right superficial parotid lymph node, were excised and imaged under Xenogen IVIS Spectrum imaging system. The imaging procedure and image processing method were as described above. To confirm lymph node metastasis, all excised lymph nodes were sectioned for H&E.

Statistical Analysis

All data were presented as mean ± SD (standard error of the mean) of n independent measurements. Statistical analysis was performed using Student’s t test (IBM SPSS Statistics version 23). A value of P < .05 was considered statistically significant.

Results

CB2R Expression in Human PDAC Tissues and PDAC Cell Lines

As initial evaluation of whether CB2R could serve as a potential PDAC target, we quantified the CB2R expression of human PDAC tissues and cells. Real-time PCR results indicate that the expression level of CB2R in PDAC tissues was significantly higher than that in normal pancreatic tissues (Figure 1A). All PDAC cell lines express both CB1R and CB2R, but the expressed levels vary among these cells. Figure 1B shows that PANC-1, CAPAN-1, and BxPC3 PDAC cell lines have higher CB2R expression level than Mia PaCa-2 and CFPAC-1 cell lines. To minimize the interference of CB1R, we selected PANC-1 cells for our experiments, which have the highest CB2R/CB1R expression ratio.

In Vitro Binding Assay of NIR760-XLP6

To evaluate the specificity and imaging potential of NIR760-XLP6 in vitro, PANC-1 cells were treated with 5 μM NIR760-XLP6 or NIR760 with or without 4Q3C as the blocking agent. As shown in Figure 2, we observed strong fluorescent signal from cells treated with NIR760-XLP6, which mainly localized in cytoplasm, whereas cells treated with the free dye (NIR760) control failed to show fluorescent
signal. In addition, when cells were pretreated with 4Q3C, NIR760-XLP6 showed a much lower degree of cellular uptake compared to unchallenged cells. These fluorescent imaging results indicate specific binding of NIR760-XLP6 to CB2R.

**In Vivo Optical Imaging Studies of NIR760-XLP6 in Xenograft Tumor Model**

To study the potential of NIR760-XLP6 for imaging PDAC in living system, we used a xenograft tumor mouse model. *In vivo* optical imaging was performed approximately 15 days postinoculation of PANC-1 cells subcutaneously. The tumor sizes and overall tumor growth in all mice showed no significant difference. A total of 15 mice were divided into 3 groups and administrated with NIR760-XLP6, NIR760-XLP6+ 4Q3C (blocking agent), and NIR760 (nontargeted free dye control), respectively. Figure 3A shows the time-dependent fluorescence images of one representative mouse from each group, and all images were displayed on the same scale. Right after the injection, NIR760-XLP6 dispersed rapidly in mice during the first 9 hours and then underwent slow clearance after 24 hours. The tumor of the mouse injected with NIR760-XLP6 showed a visible signal contrast, the T/N ratio gradually increased over time, and the fluorescent signal remained intense even at 72 hours after injection. Comparing the NIR760-XLP6 treatment only group, pretreatment of 4Q3C reduced the T/N ratio at nearly all time points, with statistical significance at 48 hours (2.47 ± 0.07 vs 1.87 ± 0.08, *P < .05, respectively) and 72 hours (3.22 ± 0.24 vs 2.25 ± 0.18, *P < .05, respectively) postinjection. In contrast, NIR760 exhibited rapid biodistribution within 1 hour postinjection followed by quick tissue clearance without obvious tumor contrast. The T/N ratio of NIR760 was significantly lower than that of NIR760-XLP6 at 48 hours (1.09 ± 0.05 vs 2.47 ± 0.11, ***P < .001, respectively) and 72 hours (1.09 ± 0.06 vs 3.22 ± 0.41, ***P < .001, respectively) (Figure 3B).

**Ex Vivo Imaging, Biodistribution, and Histological Study**

After the last imaging time point, *ex vivo* imaging was carried out to study the biodistribution and further evaluate the binding specificity of NIR760-XLP6. Tumor, heart, lung, liver, spleen, pancreas, kidneys, brain, muscle from left leg, and blood were excised and imaged (Figure 4A). Remarkably, mice treated with NIR760-XLP6 showed T/N ratio as high as 50, which is significantly higher than the blocking group (49.61 ± 3.92 vs 30.86 ± 6.51, *P < .05, respectively). Additionally, the NIR760 group showed much lower tumor contrast than the NIR760-XLP6 treatment group (1.20 ± 0.01 vs 49.61 ± 3.92, ***P < .001, respectively). Other than the tumors, liver, lung, and kidneys also exhibited comparable signal contrast in both probe and blocking group (liver = 12.28 ± 1.25 vs 11.07 ± 1.37, lung = 13.52 ± 1.24 vs 12.67 ± 1.81, kidneys = 13.48 ± 1.35 vs 10.49 ± 0.53, respectively). Interestingly, the T/N ratios in these organs were much lower than those in tumors, and as expected, no significant blocking effect was observed (Figure 4B). These results, along with those from *in vitro* and *in vivo* imaging studies, provide strong evidence on the binding specificity of NIR760-XLP6.
To determine the gross structure of tumors and examine local toxicity of NIR760-XLP6 in mice, tumors and selected organs (heart, lung, liver, spleen, pancreas, kidneys, brain, and muscle) were sectioned and stained with H&E. Tumor H&E staining images confirmed the presence of human PDAC cells evidenced by the irregularly shaped nucleus with hyperchromatin, polymorphism, and increased mitotic activity (Figure 5A). Figure 5C shows the representative H&E staining images of organs, and no evidence of inflammation or necrosis from the tissue sections was observed.

**In Vivo and Ex Vivo Optical Imaging Study in PDAC Lymph Node Metastasis Model**

To further explore the imaging potential of NIR760-XLP6 for PDAC, we performed *in vivo* optical imaging in lymph node metastasis mice. Three mice were treated with 100 nmol of NIR760-XLP6. One representative mouse was shown, and all images were displayed on the same scale (Figure 6A). Upon injection, visible signal contrast was observed in bilateral axilla and bladder region; the signal contrast reached maximum rapidly at 3 hours postinjection and then underwent a delayed clearance after 9 hours. To study the biodistribution of NIR760-XLP6 in lymph nodes, all mice were euthanized after the last imaging time point; selected lymph nodes (left proper axillary lymph node, right proper axillary lymph node, left superficial parotid lymph node, and right superficial parotid lymph node) were excised and imaged (Figure 6B). Lymph nodes exhibited significant signal contrast, but lower to bladder (3.34±0.53 vs 5.03±0.79 3.58±1.39 vs 5.03±0.79 3.24±0.53 vs 5.03±0.79 2.76±0.31 vs 5.03±0.79, respectively), this maybe because the probe is excreted...
through the bladder. Subsequently, we performed H&E to confirm lymph nodes and metastatic lymph nodes. Figure 5 shows a representative H&E staining image of metastatic lymph node.

**Discussion**

To our best knowledge, this is the first report on CB₂R-targeted NIR fluorescence imaging of PDAC. Much effort has been spent on exploring highly specific molecular target of PDAC to improve the diagnosis and treatment of this dismal disease in the past decade. However, long blood circulation times and suboptimal tumor accumulation constrained their potential clinical application [34,35]; effective therapy has only been shown in few examples in experimental pancreatic tumor models, including nanomedicines targeting the epidermal growth factor, integrin α₅β₃ receptor, and transferrin receptor [36–39]. In this study, we demonstrate that an NIR probe (NIR760-XLP6) specifically binds to CB₂R in PANC-1 cells and tumors and led to high imaging contrast (3.2-fold *ex vivo* and 50-fold *in vivo*) with minimal uptake in organs. These encouraging results suggest that NIR760-XLP6 may have great potential in imaging PDAC using low-cost fluorescence imaging systems.
High overexpression level is of critical importance to a target candidate for diagnostic and therapeutic purposes. We therefore performed real-time PCR to quantify the expression of CB2R in patient PDAC samples as compared to normal pancreas tissues. We found that CB2R was highly overexpressed in randomly selected patient samples. This is consistent with previous studies, which have demonstrated strong upregulation of CB2R in various cancers, including pancreatic cancer, whereas it is undetectable or expressed at rather low levels in the corresponding normal tissues \[21, 40\]. In addition to patient samples, we also examined CB2R expression in five PDAC cell lines and found CB2R expression in all tested cell lines, with high level in PANC-1, CAPAN-1, and BxPC3 PDAC cells and relatively low level in MIA PaCa-2 and CFPAC-1 cell lines. The overexpression of CB2R in PDAC patient samples and cell lines suggests that CB2R is a promising target for PDAC.

Cellular fluorescence imaging showed that NIR760-XLP6 primarily localized in the cytoplasm of PANC-1 cells, which is seemingly unexpected as CB2R belongs to transmembrane GPCR family. However, this observation is consistent with recent studies reported by us and others that CB2R is primarily located at intracellular sites in certain cell lines \[41–43\]. This may be contributed to ligand-induced internalization, which is part of membrane trafficking of GPCR in regulating complex signaling pathways \[44\]. Recent studies have shown that CB2R underwent internalization after sustained exposure to agonists \[42, 43\]. When blocked with 4Q3C, NIR760-XLP6 showed a much lower degree of cell uptake. Furthermore, cells treated with free dye did not show significantly fluorescence signal. These results are similar to our previously reported cellular imaging studies using DBT-CB2 cells, indicating that NIR760-XLP6 specifically binds to CB2R \[33\].

Building upon the findings from cellular imaging studies, we further evaluated the \textit{in vivo} imaging potential of NIR760-XLP6 in PANC-1 tumor-bearing mice. Free dye control mice showed rapid biodistribution and quick bioclearance throughout the whole bodies without tumor contrast. Conversely, significant fluorescence signal was observed in the mice injected with NIR760-XLP6, and the T/N ratio increased gradually over time. In addition, blocking agent 4Q3C partially decreased the uptake of NIR760-XLP6 in tumors. Although we collected similar results in our previous study using the same probe and the DBT-CB2 tumor mouse model, the imaging contrast shown in this study is far more remarkable. Specifically, T/N ratios of 2.0 (\textit{in vivo}) and 7.9 (ex vivo)
were recorded in our previous study, whereas T/N ratios as high as 3.2 (in vivo) and 49.6 (ex vivo) were observed here. This is likely due to the high expression level of CB2R in PANC-1 tumors as compared to DBT-CB2 cells that are delayed brain tumor cells transfected to express CB2R at a medium (endogenous) level. We also found that mice injected with NIR760-XLP6 showed no local toxicity in major organs based on the H&E staining. The safety profile and high imaging contrast provide strong support on the potential of NIR760-XLP6 as a new PDAC imaging probe.

To further explore the imaging potential of NIR760-XLP6 for PDAC, we performed i v in vivo imaging of NIR760-XLP6 in PDAC lymph node metastasis mice. After injection of the probe, significant signal contrast was observed in bilateral axilla at all time points, especially 3, 6, and 9 hours postinjection, which were confirmed to be proper axillary lymph node with PDAC metastasis by H&E staining. The safety profile and high imaging contrast provide strong support on the potential of NIR760-XLP6 as a new PDAC imaging probe.

Conclusion
In conclusion, we successfully imaged PDAC cells and tumors using our CB2R-targeted NIR fluorescent probe, NIR760-XLP6. Our results suggest that CB2R is a promising target for PDAC imaging. We plan to embark on CB2R-targeted PDAC imaging using more sophisticated tumor models, for example, comparing the imaging effect of NIR760-XLP6 in PDAC lymph node metastasis model to inflammation model, as well as further exploring the potential of cannabinoid therapy for PDAC in our future studies.

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References
[1] Siegel RL, Miller KD, and Jemal A (2017). Cancer statistics, 2017. CA Cancer J Clin 67, 7–30.
[2] Kamisawa T, Wood LD, Itoi T, and Takaori K (2016). Pancreatic cancer. Lancet 388, 73–85.
[3] Gillen S, Schuster T, Meyer Zum Buschenfelde C, Friess H, and Kleeff J (2010). Preoperative/neoadjuvant therapy in pancreatic cancer: a systematic review and meta-analysis of response and resection percentages. PLoS Med 7, e1000267.
[4] Konstantinidou IT, Warshaw AL, Allen JN, Blazkowsky LS, Castillo CF, Deshpande V, Hong TS, Kwak EL, Lauwers GY, and Ryan DP, et al (2013). Pancreatic ductal
adencarcinoma: is there a survival difference for R1 resections versus locally advanced unresectable tumors? What is a ‘true’ R0 resection? *Ann Surg 257*, 731–736.

[5] Frampton AE, Gall TM, Krell J, Ahmad R, and Jiao LR (2013). Is there a ‘margin’ for error in pancreatic cancer surgery? *Future Oncol* 9, 31–34.

[6] Provenzano PP and Hingorani SR (2013). Hyaluronidase, fluid pressure, and stromal resistance in pancreatic cancer. *Br J Cancer* 108, 1–8.

[7] Wolfgang CL, Herman JM, Laheri DA, Klein AP, Erdak MA, Fishman EK, and Hruban RH (2013). Recent progress in pancreatic cancer. *CA Cancer J Clin 63*, 318–348.

[8] Iovannia J and Dusetti N (2017). Speeding towards individualized treatment for pancreatic cancer by taking an alternative road. *Cancer Lett 410*, 63–67.

[9] Neesse A, Hahnenkamp A, Griesmann H, Buchholz M, Hahn SA, Mnhøjou A, Fendrich V, Ring J, Sips B, and Tuveson DA et al (2013). Claudin-4-targeted optical imaging detects pancreatic cancer and its precursor lesions. *Nat Med 19*, 1034–1043.

[10] Trajkovic-Arcis M, Mohajerani P, Sarantasopoulos A, Kalidiris E, Steiger K, Esposito I, Ma X, Themelis G, Burton N, and Michalski CW, et al (2014). Multimodal molecular imaging of integrin alphavbeta3 for in vivo detection of pancreatic cancer. *J Nucl Med 55*, 446–451.

[11] Luo H, England CG, Goel S, Graves SA, Ai F, Liu B, Theuer CP, Wong HC, Nidkies RJ, and Cai W (2017). ImmunoPET and near-infrared fluorescence imaging of pancreatic cancer with a dual-labeled bisacopeptide fragment. *Med Phys* 44, 1646–1655.

[12] Li H, Wang P, Gong W, Wang Q, Zhou J, Zhu WH, and Cheng Y (2017). Dendron-grafted polystyrene dual-modal nanoprobe for early-diagnosis of pancreatic precancerosis via targeting a urokinase-type plasminogen activator receptor. *Ado Health Mater*. doi: 10.1002/adhm.2017009121700912 (1 to 9).

[13] Lwin TM, Murakami T, Miyake K, Yazaki PJ, Shively JE, Hoffman RM, and Bouveret M (2018). Tumor-specific labeling of pancreatic cancer using a humanized anti-CEA antibody conjugated to a near-infrared florescence. *Ann Surg Oncol 25*, 1079–1085.

[14] Hudson SV, Huang JS, Yin W, Albeituni S, Rush J, Khanal A, Yan J, Ceresa BP, Bouvet M (2018). Tumor-specific labeling of pancreatic cancer using a humanized anti-CEA antibody conjugated to a near-infrared florescence. *Ann Surg Oncol 25*, 1079–1085.

[15] Aiuppa-Olaitoza O, Elagrayi I, Rico-Barrio I, Zasandona I, Itsexabarria N, and Usobiaga A (2017). Targering the endocannabinoid system: future therapeutic strategies. *Drug Discov Today* 22, 105–110.

[16] Matsuda LA, Loolit JS, Brownstone MJ, Young AC, and Bonner TJ (1990). Structure of a cannabinoid receptor and functional expression of the cloned cDNA. *Nature 346*, 561–564.

[17] Munro S, Thomas KL, and Abu-Shaar M (1993). Molecular characterization of a peripheral receptor for cannabinoids. *Nature 365*, 61–65.

[18] Volkow ND, Baler RD, Compton WM, and Weiss SR (2014). Adverse health effects of marijuana use. *N Engl J Med* 370, 2219–2227.

[19] Deng L, Guindon J, Cornett BL, Makriyannis A, Mackie K, and Hohmann AG (2014). Antibody targeting of integrin alphavbeta3 for in vivo detection of pancreatic cancer model. *J Clin Invest 111*, 43–50.

[20] Morales P, Blasco-Benito S, Andradas C, Gomez-Cantos M, Flores JM, Goya P, Fernandez-Ruiz J, Sanchez C, and Jagerovic N (2015). Selective, nontoxic CB2 cannabinoid α-quione with in vivo activity against triple-negative breast cancer. *J Med Chem 58*, 2256–2264.

[21] Evens N and Bormans GM (2010). Non-invasive imaging of the type 2 cannabinoid receptor, focus on positron emission tomography. *Curr Top Med Chem 10*, 1527–1543.

[22] Caille F, Cacheux F, Peyronneau MA, Jego B, Jaumain E, Portier G, Ullner C, Grether U, Winkler A, and Dolle F, et al (2017). From structure-activity relationships on thiazole derivatives to the in vivo evaluation of a new radiotracer for cannabinoid subtype 2 PET imaging. *Mol Pharm* 14, 4064–4078.

[23] Evens N, Vandeputte C, Muccioli GG, Lambert DM, Baekelandt V, Verbruggen AM, Debyser Z, Van Laere K, and Bormans GM (2011). Synthesis, in vitro and in vivo evaluation of fluoxetine-18 labelled FE-GW405833 as a PET tracer for type 2 cannabinoid receptor imaging. *Bioorg Med Chem* 19, 4499–4505.

[24] Frangioni J (2003). In vivo near-infrared fluorescence imaging. *Curr Opin Chem Biol 7*, 626–634.

[25] Wu Z, Shao P, Zhang S, Ling X, and Bai M (2014). Molecular imaging of human tumor cells that naturally overexpress type 2 cannabinoid receptors using a quinolone-based near-infrared fluorescent probe. *J Biomed Opt* 1976016.

[26] Zhang S, Shao P, and Bai M (2013). In vivo type 2 cannabinoid receptor-targeted tumor optical imaging using a near infrared fluorescent probe. *Biomol Cryst Eng* 14, 1907–1916.

[27] Wu Z, Shao P, Zhang S, and Bai M (2014). Targeted zwirneric near infrared fluorescent probe for improved imaging of type 2 cannabinoid receptors. *J Biomed Opt 19* 036006.

[28] Ling X, Zhang S, Shao P, Li W, Yang L, Ding Y, Xu C, Stella N, and Bai M (2015). A novel near-infrared fluorescence imaging probe that preferentially binds to cannabinoid CB2R over CB1R. *Biomaterials 57*, 169–178.

[29] Aung W, Tsuji AB, Sudo H, Sugyo A, Furukawa T, Ukiy Y, Kuroasa Y, and Saga T (2016). Immuno-targeting of integrin alphabeta3 for single photon emission computed tomography and near-infrared fluorescence imaging in a pancreatic cancer model. *Mol Imaging 15*, doi:10.1016/j.molimaging.2016.02.009.

[30] Huyhn AS, Chung WJ, Cho HI, Moberg VE, Cells E, Morse DL, and Vagner J (2015). Novel toll-like receptor 2 ligand for targeted pancreatic cancer imaging and immunotherapy. *J Med Chem 55*, 9751–9762.

[31] Camp ER, Wang C, Little EC, Watson PM, Pirillos KF, Rait A, Cole DJ, Chang EH, and Watson DK (2013). Transferrin receptor targeting nanomedicine delivering wild-type p53 gene sensitizes pancreatic cancer to gemcitabine therapy. *Cancer Gene Ther 20*, 222–228.

[32] Salvati A, Pitek AS, Monopoli MP, Prapatnaporn K, Bombelli FB, Hirstov DR, Kelly PM, Abeg C, Mahon E, and Dawson KA (2013). Transferrin-functionalized nanoparticles lose their targeting capabilities when a biomolecule corona adsorbs on the surface. *Nat Nanotechnol 8*, 137–143.

[33] Kudguus RA, Szabolcs A, Khan JA, Walden CA, Reid JM, Robertson JD, Bhattacharya R, and Mukherjee P (2013). Inhibiting the growth of pancreatic adenocarcinoma in vitro and in vivo through targeted treatment with designer gold nanotherapeutics. *PLoS One* 8,57522.

[34] Ji S, Xu J, Zhang B, Yao W, Xu W, Wu W, Xu Y, Wang H, Ni Q, and Hou H, et al (2012). RGD-conjugated albumin nanoparticles as a novel delivery vehicle in pancreatic cancer therapy. *Cancer Biol Ther* 13, 206–215.

[35] Michalski CW, Ott FE, Erkan M, Sauluinate D, Bergmann F, Pacher P, Barkai S, Muller MW, Giese NA, and Fries H, et al (2008). Cannabionoids in pancreatic cancer: correlation with survival and pain. *Int J Cancer 122*, 742–750.

[36] Zhang S, Jia N, Shao P, Tong Q, Xie QP, and Bai X (2014). Target-selective phototherapy using a ligand-based photosensitizer for type 2 cannabinoid receptor. *Chin Biol 21*, 338–344.

[37] Castaneda JT, Hariu A, Kiertscher SM, Roth JD, and Roth MD (2013). Differential expression of intracellular and extracellular CB2 cannabinoid receptor protein by human peripheral blood leucocytes. *J Neurimmun Pharmacol 8*, 323–332.

[38] Atwood BK, Wagner-Miller J, Haskins C, Straker A, and Mackie K (2011). Functional selectivity in CB2 cannabinoid receptor signaling and regulation: implications for the therapeutic potential of CB2 ligands. *Mol Pharmacol 81*, 250–263.

[39] Jean-Alphonse F and Hanyaloglu AC (2011). Regulation of GPCR signal networks via membrane trafficking. *Mol Cell Endocrinol 331*, 205–214.

[40] Zhang S, Shao P, Ling X, Yang L, Hou W, Thorne SJ, Beaino W, Anderson DJ, and Bai M (2015). In vivo inflammation imaging using a CB2R-targeted near infrared fluorescent probe. *Am J Nucl Med Mol Imaging 5*, 246–258.