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Radiation-Activated Cobalamin-Kinase Inhibitors for Treatment of Pancreatic Ductal Adenocarcinoma

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Abstract

Pancreatic ductal adenocarcinoma (PDAC) remains one of the most dismal diagnoses that a patient can receive. PDAC is extremely difficult to treat, as drug delivery is challenging in part due to the lack of vascularization, high stromal content, and high collagen content of these tumors. We have previously demonstrated that attaching drugs to the cobalamin scaffold provides selectivity for tumors over benign cells due to a high vitamin demand in these rapidly growing cells and an overexpression of transcobalamin receptors in a variety of cancer types. Importantly, we have

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Author Contributions

The manuscript was written through contributions of all authors.

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ASSOCIATED CONTENT

Supporting Information

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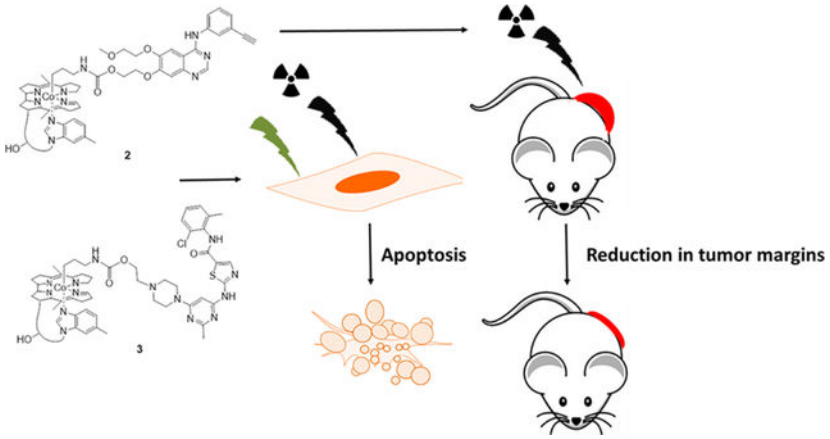
Full vitamin B_{12} scaffold structure, dark controls for visible light and X-ray cell viability studies and representative H & E for mouse studies (PDF)

shown the ability to deliver cobalamin derivatives to orthotopic pancreas tumors. Tyrosine kinase inhibitors have shown promise in treating PDAC as well as other cancer types. However, some of these inhibitors suffer from drug resistance, and as such, their success has been diminished. With this in mind, we synthesized the tyrosine kinase inhibitors erlotinib (EGFR) and dasatinib (Src) that are attached to this cobalamin platform. Both of these cobalamin-drug conjugates cause visible light-induced apoptosis, and the cobalamin-erlotinib conjugate (**2**) causes X-ray-induced apoptosis in MIA PaCa-2 cells. Both visible light and X-rays provide spatial control of drug release; however, utilizing X-ray irradiation offers the advantage of deeper tissue penetration. Therefore, we explored the utilization of **2** as a synergistic therapy with radiation in athymic nude mice implanted with MIA PaCa-2 tumors. We discovered that the addition of **2** caused an enhanced reduction in tumor margins in comparison with radiation therapy alone. In addition, treatment with **2** in the absence of radiation caused no significant reduction in tumor size in comparison with the controls. The cobalamin technology presented here allows for the spatial release of drugs in conjunction with external beam radiation therapy, potentially allowing for more effective treatment of deep-seated tumors with less systemic side effects.

Graphical Abstract



Keywords

cobalamin; X-ray; radiation therapy; kinase inhibitors; pancreatic adenocarcinoma

INTRODUCTION

Patients diagnosed with pancreatic ductal adenocarcinoma (PDAC) have a very low long-term survival rate of approximately 3%.[1] The recurrence rate is high among patients who do respond to initial treatment due to the development of resistance. Delivery of chemotherapeutics to pancreatic tumors remains challenging due to the lack of vascularization, as well as the high stromal and collagen content in these tumor types.[2,3] PDAC clinical outcomes are poor, and there is a critical need to develop new strategies for treatment of this terrible disease.

Gemcitabine is widely used as a general treatment for PDAC. Approximately one-half of PDAC patients exhibit aberrant EGFR pathway signaling.[4] The combination of gemcitabine with the small-molecule EGFR inhibitor erlotinib provides patients with a statistically significant enhancement in survival.5 In addition, significant research has been conducted investigating the addition of other kinase inhibitors as part of a treatment cocktail and have been utilized for PDAC that has been demonstrated to overexpress protein kinases.[6–8] While these combination therapies have shown promise, significant improvements are needed to enhance the survival of PDAC patients. These chemotherapies have extensive systemic side effects due to a lack of selectivity for malignancies over healthy tissue. Kinase inhibitor therapy can cause widespread disruption of a large number of facets of the endocrine system, including thyroid function, bone and glucose metabolism, and adrenal function.[9] A more targeted therapy is necessary to improve patient outcomes.

Light-activated chemotherapy affords spatial control of drug release in a region of interest, thereby mitigating systemic side effects. Photodynamic therapy (PDT) has proven to be invaluable for treating a variety of cancers, including skin, lung, brain, bladder, esophagus, and head and neck cancer. This type of treatment involves the use of photosensitizers to generate singlet oxygen that is activated in a specific area, typically with laser light. Traditional PDT is effective only up to several millimeters depth, which limits this therapy to relatively shallow tumors.[10,7] External beam radiation therapy (EBRT) allows the penetrating, precise delivery of high-dose radiation, limiting damage to surrounding tissue. X-ray-activated chemotherapy provides promise for the ability to treat deep-seated tumors, such as those in PDAC, in conjunction with EBRT.[2,11–15] Valuable studies have focused on the excitation of photosensitizers via nanoscintillators, which release luminescence when exposed to X-rays.[5,16–18] This technology allows the X-ray-induced production of singlet oxygen, provided that there is efficient energy transfer between the nanoscintillator and the photosensitizer.

We developed a drug delivery technology derived from alkylcobalamins. Alkylcobalamins are vitamin B12 derivatives that are known to be photosensitive.[19–21] We have demonstrated that these compounds undergo a tunable Co–C bond cleavage when exposed to light spanning the electromagnetic spectrum from X-ray to near-infrared (NIR).[22–29] We have characterized this precedent in numerous model systems via fluorescence quenching and mass spectrometry assays, as well as translational applications that have included light-induced cell viability assays, cytoskeleton rearrangements, DNA cleavage, and hydrogel formation.[22–29] We have previously demonstrated that these alkylcobalamin-drug conjugates are tumor-selective in vivo. This is likely due to an enhanced demand for vitamin B12 and overexpression of transcobalamin receptors (TCblR) in a range of cancer types, including PDAC. Importantly, we found that fluorescently labeled cobalamins accumulated not only in PDAC flank tumors but also in orthotopic pancreas tumors.[22] This is an exciting discovery, as it indicates that the cobalamin platform can deliver drugs directly to the pancreas tumor, which is something that is difficult to accomplish with traditional chemotherapy.[2,3]

We have discovered that cargo can be released from the cobalamin platform at radiation doses as low as 0.2 Gy, with maximal release of cargo at 2 Gy, which is the equivalent of a single fraction dose in radiation therapy.[22]

We wanted to expand on the X-ray chemotherapy that the cobalamin platform can provide. Therefore, in this article, we discuss the synthesis of the conjugation of two tyrosine kinase inhibitors, erlotinib and dasatinib, to the cobalamin scaffold to provide two new cobalamin-drug conjugates, cobalamin-erlotinib (Cbl-Erl, **2**) and cobalamin-dasatinib (Cbl-Das, **3**). We show that these cobalamin-drug conjugates cause light-induced apoptosis in the K-Ras mutant, drug-resistant PDAC cell line MIA PaCa-2. In addition, we found that **2** causes X-ray-dependent cell killing at clinically relevant radiation doses. Finally, we demonstrate the in vivo application of this technology in athymic nude mice bearing MIA PaCa-2 tumors, whereby we see enhanced reduction in tumor margins with the combination of the cobalamin technology with radiation therapy. This work represents X-ray-activated chemotherapy, whereby a known cancer drug is released synergistically with radiation therapy in a finite area, providing the potential to reduce systemic side effects of traditional chemotherapy (Figure 1).

EXPERIMENTAL PROCEDURES

General.

3-Chloropropylamine was purchased from Sigma-Aldrich. Desmethylerlotinib hydrochloride (OSI-420) was obtained from SelleckChem, and dasatinib was purchased from LC Laboratories. All were used without further purification. Isolation of cobalamin compounds was accomplished by utilizing a Biotage SP1 chromatography system. The MIA PaCa-2 (CRL-1420) cell line was purchased from ATCC. Dubecco's Modified Eagle's Medium (DMEM), fetal bovine serum (FBS), phosphate buffered saline (PBS), and Cell Titer 96 Aqueous One Cell Proliferation Assay were purchased from Thermo Fisher. Athymic nude mice were purchased from Charles River Laboratories. Illumination with visible light was accomplished with a Zeiss Colibri II LED system, and X-ray activation was accomplished with a linear accelerator (Varian LINAC 2100CD).

Synthesis and Characterization of Cobalamin-Erlotinib Conjugate (Cbl-Erl, 2).

Desmethylerlotinib hydrochloride (3.6 mg, 0.0087 mmol), triethylamine (NEt_3, 16 μL, 0.116 mmol), and anhydrous Na_2SO_4 (excess) were combined in 500 μL of DMSO, and allowed to incubate at room temperature with agitation for 30 min. 1,1′-Carbonyl-di(1,2,4-triazole) (CDT, 18.9 mg, 0.116 mmol) was added to this mixture and allowed to react for 1 h at room temperature with agitation. Finally, β-aminopropylcobalamin **1** (8.0 mg, 0.0058 mmol) was added, and the mixture was agitated for 18 h at room temperature in the dark. The desired compound was purified by reverse phase column chromatography (C-18 column) using a linear gradient binary solvent system (solvent A: 0.1% TFA/H_2O; solvent B: 0.1% TFA/CH_3CN) with a ratio of A/B that varied from 97:3 (0 min) to 10:90 (40 min). Solvent was removed by lyophilization to afford an orange solid (7.8 mg, 76%). ESI-MS calcd for $C_{87}H_{115}CoN_{17}O_{19}P$: ($M^+ + 2H$) m/z = 1794.0, found 1793.8; ($M^{2+} + 3H$): m/z = 897.4, found 897.2; calcd for ($M^{3+} + 4H$) m/z = 598.6, found 598.5.

Synthesis and Characterization of Cobalamin-Dasatinib Conjugate (Cbl-Das, 3).

Dasatinib (4.2 mg, 0.0087 mmol), triethylamine (16 μL, 0.116 mmol), and anhydrous Na_2SO_4 (excess) were combined in 500 μL of DMSO, and allowed to incubate at room

temperature with agitation for 30 min. CDT (18.9 mg, 0.116 mmol) was added to this mixture and allowed to react for 1 h at room temperature with agitation. Subsequently, β-aminopropylcobalamin **1** (8.0 mg, 0.0058 mmol) was added, and the mixture was agitated for 18 h at room temperature in the dark. The desired compound was purified by reverse phase column chromatography (C-18 column) using a linear gradient binary solvent system (solvent A: 0.1% TFA/H_2O; solvent B: 0.1% TFA/CH_3CN) with a ratio of A:B that varied from 97:3 (0 min) to 10:90 (40 min). Solvent was removed by lyophilization to afford an orange solid (8.1 mg, 76%). ESI-MS calcd for $C_{88}H_{120}ClCoN_{21}O_{17}PS$ (M^{2+} + H_2O + 2H): m/z = 959.9, found 960.1.

Cell Culture.

MIA PaCa-2 cells were grown in DMEM supplemented with 10% FBS and 1% penicillin/streptomycin. Cells were placed in an incubator at 37 °C with 5% CO_2 and 100% humidity and allowed to grow until the desired confluency was reached.

Cell Viability Assays.

MIA PaCa-2 cells were seeded in black 96-well plates with clear bottoms (8000 cells/well), with a recovery period of 24 h. Media was removed, and cells were then treated with the following: 1 μM erlotinib, 1 μM dasatinib, 1 μM 2, and 1 μM 3 in phenol red-free media supplemented with 0.5% BSA and 0.1% DMSO (cobalamindrug conjugates, negative and radiation controls), and allowed to incubate at 37 °C for 24 h. The cells were irradiated with a Zeiss Colibri II LED system at 530 nm for 10 min or treated with a 2 Gy dose of radiation from a linear accelerator, followed by incubation at 37 °C for 72 h. Cell viability was assessed utilizing the Cell Titer 96 Aqueous One Cell Proliferation Assay (Promega, absorbance read at 492 nm).

In Vivo Studies with 2.

Athymic nude mice were injected in the flank with 1×10^5 MIA PaCa-2 cells and mice with subcutaneous PDAC tumors in the range of 125–175 mm^3 were selected for these studies. Mice were anesthetized by inhaling isofluorane to maintain blood flow and tumor oxygenation. Compound **2** (10, 50, 100 μM) was injected intravenously (IV), and mice were allowed to rest in the dark for 24 h post-injection. The tumors were irradiated with a 6 MeV electron beam using a 5 mm cone and 1 cm bolus over the tumor area to ensure that Dmax was reached at the tumor site. A single dose of 8 Gy was used for radiation therapy. The tumor volume was then measured at regular intervals for 30 days. Controls included sham (PBS) injection, **2** without radiation, radiation only, and erlotinib (100 μM).

RESULTS AND DISCUSSION

Synthesis of Cbl-Erl (2) and Cbl-Das (3).

We have extensive experience with the synthesis of cobalamin-drug conjugates.[22,23,27] Our previous reports were limited to conjugating drugs with a modifiable amine or a modifiable carboxylic acid to form amide bonds between the alkylcobalamin and the drug. The synthesis of these new conjugates invokes a different methodology that allows the modification of drugs that contain an alcohol functionality to the cobalamin platform

through a carbamate linkage (Scheme 1). β-aminopropylcobalamin **1** was synthesized from hydroxocobalamin acetate and 3-chloropropylamine hydrochloride according to the literature procedure.[27] Erlotinib, an EGFR kinase inhibitor, was attached to the cobalamin scaffold **1** utilizing a functionalizable desmethylerlotinib via a carbamate bond between the amine handle on the cobalamin and the primary alcohol of the erlotinib derivative. This bond formation was facilitated by the coupling agent CDT to form Cbl-Erl **2**. Dasatinib, a Src kinase inhibitor, was attached to this cobalamin platform in the same fashion to form the cobalamin-drug conjugate Cbl-Das **3** (Scheme 1). These reactions are facile to complete and require no special equipment or inert atmosphere. The successful synthesis of these cobalamin-drug conjugates was confirmed via highresolution electrospray mass spectrometric analysis, which is the standard method of characterization for cobalamin derivatives.[22,24–29]

Radiation-Induced Apoptosis.

The MIA PaCa-2 PDAC cell line, shown to overexpress TCblR[30,31] and to be drug resistant,[7,32–34] was treated with 1 μM **2** or 1 μM **3**, respectively, for 24 h. Following incubation with cobalamindrug conjugate, the cells were illuminated with 530 nm as a positive control since this is the natural absorbance of the cobalamin scaffold. We have previously demonstrated that the release of cargo from the cobalamin platform is tunable.[27] The incubated cells were also given a 2 Gy radiation dose with a clinical linear accelerator (Figure 2). Cell death was assessed with an 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxy-phenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium, inner salt (MTS) assay. Cells exposed to 530 nm light in combination with the cobalamin-drug conjugate showed significant apoptosis that was more effective than just erlotinib or dasatinib alone (Figure 2a). Remarkably, a similar trend was observed when the cells were exposed to radiation in the presence of **2**. The synergism of the cobalamin-drug conjugate with radiation was more efficient at killing PDAC cells than erlotinib alone (Figure 2b). This is a promising result since MIA PaCa-2 cells have been shown to be resistant to erlotinib treatment.[7,32–34]

However, the combination of 3 with radiation in MIA PaCa-2 cells did not seem to result in significant cell death, most likely due to lower photon flux with radiation in comparison to 530 nm light as well as lower basal expression of Src in MIA PaCa-2 cells.[35] Incubation of the cells with the cobalamin-drug conjugates stored in the dark (Figure S2), illumination with 530 nm, or radiation alone showed no significant cell killing, as expected.

Synergistic Treatment of Mice Implanted with MIA PaCa-2 Xenografts.

Due to the promising results obtained in vitro showing successful apoptosis with the combination of **2** and radiation in MIA PaCa-2 cells, we embarked on a study to explore this combination in vivo. A series of cohorts of athymic nude mice implanted with flank MIA PaCa-2 tumors were treated as follows: 1. sham injection of PBS; 2. erlotinib (100 μM); 3. radiation only; 4. **2** (50, 10 μM) as a dark control; and 5. **2** (50, 10 μM) with radiation therapy. The cohorts undergoing radiation therapy were exposed to a single dose of 8 Gy, as recommended by our medical physicist consultant, and tumor volumes were measured at regular intervals for 30 days post-treatment. We observed no significant reduction in tumor growth in the sham or dark controls that were injected with multiple concentrations

of **2** without radiation therapy. However, we assessed a marked reduction in tumor volumes when **2** (50 and 10 μM) was utilized in conjunction with radiation therapy (28 and 29%, respectively, relative to no treatment control) in comparison to radiation therapy (53%), or the parent erlotinib (89%) alone (Figure 3). These results were recapitulated by seeing a marked reduction in tumor cellularity with the combination of **2** with radiation therapy by H&E staining (Figure S3).

CONCLUSIONS

We synthesized two new cobalamin-drug conjugates where tyrosine kinase inhibitors, desmethylerlotinib and dasatinib, with alcohol functionality are linked to the cobalamin scaffold with a synthetic methodology different from that previously reported. We have shown visible light-induced apoptosis with both drug conjugates in the K-Ras-mutated, multidrug-resistant PDAC cell line MIA PaCa-2. In addition, erlotinib conjugate **2** displayed enhanced cell killing in combination with clinical radiation doses (2 Gy) in comparison to radiation or the parent drug erlotinib alone. Excitingly, it was demonstrated that the combination of the cobalamin platform with radiation therapy in vivo elicited an enhanced reduction in tumor margins in mice implanted with MIA PaCa-2 tumors in comparison with radiation alone or erlotinib treatment. This is an interesting result because MIA PaCa-2 cells have been shown to be insensitive to erlotinib treatment.[7,32–34]

We developed a tumor-targeted radiochemotherapy that can be directly activated by X-rays from a linear accelerator. This technology could allow tumor-selective stereotactic release of a drug in accordance with a physician's designed plan for radiation treatment. We are currently studying the application of the cobalamin platform in other pancreatic tumors as well as expanding this versatile technology to other cancer types.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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ABBREVIATIONS

PDAC	pancreatic ductal adenocarcinoma
EBRT	external beam radiation therapy
NIR	near-infrared

TC	transcobalamin
TCblR	transcobalamin receptor
Gy	gray
TFA	trifluoroacetic acid
CDT	1,1′-carbonyl-di(1,2,4-triazole)
NEt_3	trimethylamine
DMSO	dimethyl sulfoxide
MTS	3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt
H & E	hematoxylin and eosin

REFERENCES

(1). Warshaw AL; Fernandez-del Castillo C Pancreatic carcinoma. N. Engl. J. Med. 1992, 326 (7), 455–465. [PubMed: 1732772]

(2). Dimou A; Syrigos KN; Saif MW Overcoming the stromal barrier: technologies to optimize drug delivery in pancreatic cancer. Ther Adv. Med. Oncol 2012, 4 (5), 271–279. [PubMed: 22942909]

(3). Nieskoski MD; Marra K; Gunn JR; Hoopes PJ; Doyley MM; Hasan T; Trembly BS; Pogue BW Collagen Complexity Spatially Defines Microregions of Total Tissue Pressure in Pancreatic Cancer. Sci. Rep. 2017, 7 (1), 10093. [PubMed: 28855644]

(4). Dancer J; Takei H; Ro JY; Lowery-Nordberg M Coexpression of EGFR and HER-2 in pancreatic ductal adenocarcinoma: a comparative study using immunohistochemistry correlated with gene amplification by fluorescencent in situ hybridization. Oncol. Rep. 2007, 18 (1), 151–155. [PubMed: 17549361]

(5). Moore MJ; Goldstein D; Hamm J; Figer A; Hecht JR; Gallinger S; Au HJ; Murawa P; Walde D; Wolff RA; Campos D; Lim R; Ding K; Clark G; Voskoglou-Nomikos T; Ptasynski M; Parulekar W Erlotinib plus gemcitabine compared with gemcitabine alone in patients with advanced pancreatic cancer: a phase III trial of the National Cancer Institute of Canada Clinical Trials Group. J. Clin Oncol 2007, 25 (15), 1960–6. [PubMed: 17452677]

(6). Alcalá S; Mayoral-Varo V; Ruiz-Cañas L.; López-Gil JC.; Heeschen C.; Martín-Pérez J.; Sainz B Jr Targeting SRC Kinase Signaling in Pancreatic Cancer Stem Cells. Int. J. Mol. Sci. 2020, 21 (20), 7437–7459. [PubMed: 33050159]

(7). Diep CH; Munoz RM; Choudhary A; Von Hoff DD; Han H Synergistic Effect between Erlotinib and MEK Inhibitors in KRAS Wild-Type Human Pancreatic Cancer Cells. Clin. Cancer Res. 2011, 17 (9), 2744–2756. [PubMed: 21385921]

(8). Poh AR; Ernst M Functional roles of SRC signaling in pancreatic cancer: Recent insights provide novel therapeutic opportunities. Oncogene 2023, 42 (22), 1786–1801. [PubMed: 37120696]

(9). Lodish MB; Stratakis CA Endocrine side effects of broadacting kinase inhibitors. Endocr. Relat. Cancer 2010, 17 (3), R233–R244. DOI: 10.1677/ERC-10-0082. [PubMed: 20603395]

(10). Mallidi S; Anbil S; Bulin A-L; Obaid G; Ichikawa M; Hasan T Beyond the Barriers of Light Penetration: Strategies, Perspectives and Possibilities for Photodynamic Therapy. Theranostics 2016, 6 (13), 2458–2487. [PubMed: 27877247]

(11). Han G; Xie J Light-Mediated Deep-Tissue Theranostics. Theranostics 2016, 6 (13), 2292–2294. [PubMed: 27877234]

(12). Kotagiri N; Sudlow GP; Akers WJ; Achilefu S Breaking the depth dependency of phototherapy with Cerenkov radiation and low-radiance-responsive nanophotosensitizers. Nat. Nano 2015, 10 (4), 370–379.

(13). Oldham M; Yoon P; Fathi Z; Beyer WF; Adamson J; Liu L; Alcorta D; Xia W; Osada T; Liu C; Yang XY; Dodd RD; Herndon JE 2nd, ; Meng B; Kirsch DG; Lyerly HK; Dewhirst MW; Fecci P; Walder H; Spector NL X-Ray Psoralen Activated Cancer Therapy (X-PACT). PloS one 2016, 11 (9), No. e0162078. [PubMed: 27583569]

(14). Wang GD; Nguyen HT; Chen H; Cox PB; Wang L; Nagata K; Hao Z; Wang A; Li Z; Xie J X-Ray Induced Photodynamic Therapy: A Combination of Radiotherapy and Photodynamic Therapy. Theranostics 2016, 6 (13), 2295–2305. [PubMed: 27877235]

(15). Larue L; Ben Mihoub A; Youssef Z; Colombeau L; Acherar S; André JC; Arnoux P; Baros F; Vermandel M; Frochot C Using X-rays in photodynamic therapy: an overview. Photochemical & Photobiological Sciences 2018, 17 (11), 1612–1650. [PubMed: 29938265]

(16). Clement S; Deng W; Camilleri E; Wilson BC; Goldys EM X-ray induced singlet oxygen generation by nanoparticle-photosensitizer conjugates for photodynamic therapy: determination of singlet oxygen quantum yield. Sci. Rep. 2016, 6, 19954. [PubMed: 26818819]

(17). Cline B; Delahunty I; Xie J Nanoparticles to mediate X-rayinduced photodynamic therapy and Cherenkov radiation photodynamic therapy. Wiley Interdiscip. Rev. Nanomed. Nanobiotechnol. 2019, 11 (2), No. e1541. [PubMed: 30063116]

(18). Kamkaew A; Cheng L; Goel S; Valdovinos HF; Barnhart TE; Liu Z; Cai W Cerenkov Radiation Induced Photodynamic Therapy Using Chlorin e6-Loaded Hollow Mesoporous Silica Nanoparticles. ACS Appl. Mater. Interfaces 2016, 8 (40), 26630–26637. [PubMed: 27657487]

(19). Hogenkamp HP The photolysis of methylcobalamin. Biochemistry 1966, 5 (2), 417–22. [PubMed: 5940929]

(20). Hogenkamp HPC; Ladd JN; Barker HA Identification of a nucleoside derived from coenzyme B12. J. Biol. Chem. 1962, 237, 1950–2. [PubMed: 13908222]

(21). Pratt JM; Whitear BRD Photolysis of methylcobalamin. J. Chem. Soc. A 1971, 2, 252–5.

(22). Gendron LN; Zites DC; LaRochelle EPM; Gunn JR; Pogue BW; Shell TA; Shell JR Tumor targeting vitamin B12 derivatives for X-ray induced treatment of pancreatic adenocarcinoma. Photodiagnosis Photodyn Ther 2020, 30, No. 101637. [PubMed: 31899377]

(23). Shell TA; Lawrence DS Vitamin B12: a tunable, long wavelength, light-responsive platform for launching therapeutic agents. Acc. Chem. Res. 2015, 48 (11), 2866–74. [PubMed: 26479305]

(24). Priestman MA; Shell TA; Sun L; Lee HM; Lawrence DS Merging of confocal and caging technologies: selective three-color communication with profluorescent reporters. Angewandte Chemie (International ed. in English) 2012, 51 (31), 7684–7. [PubMed: 22740297]

(25). Rodgers ZL; Hughes RM; Doherty LM; Shell JR; Molesky BP; Brugh AM; Forbes MD; Moran AM; Lawrence DSB(12)-mediated, long wavelength photopolymerization of hydrogels. J. Am. Chem. Soc. 2015, 137 (9), 3372–8. [PubMed: 25697508]

(26). Shell TA; Lawrence DS A new trick (hydroxyl radical generation) for an old vitamin (B12). J. Am. Chem. Soc. 2011, 133 (7), 2148–50. [PubMed: 21275391]

(27). Shell TA; Shell JR; Rodgers ZL; Lawrence DS Tunable visible and near-IR photoactivation of light-responsive compounds by using fluorophores as light-capturing antennas. Angewandte Chemie (International ed. in English) 2014, 53 (3), 875–8. [PubMed: 24285381]

(28). Gendron LN; Shell JR; Shell TA Light-mediated double-strand DNA cleavage by an alkyldicobalamin. J. Photochem. Photobiol. 2023, 18, No. 100209.

(29). McCue AC; Moreau WM; Shell TA Visible Light-Induced Radical Mediated DNA Damage. Photochem. Photobiol. 2018, 94 (3), 545–551. [PubMed: 29377197]

(30). Sysel AM; Valli VE; Bauer JA Immunohistochemical quantification of the cobalamin transport protein, cell surface receptor and Ki-67 in naturally occurring canine and feline malignant tumors and in adjacent normal tissues. Oncotarget 2015, 6 (4), 2331–2348. [PubMed: 25633912]

(31). Sysel AM; Valli VE; Nagle RB; Bauer JA Immunohistochemical quantification of the vitamin B12 transport protein (TCII), cell surface receptor (TCII-R) and Ki-67 in human tumor xenografts. Anticancer Res. 2013, 33 (10), 4203–4212. [PubMed: 24122983]

(32). Cao J; Yang J; Ramachandran V; Arumugam T; Deng D; Li Z; Xu L; Logsdon CD TM4SF1 Promotes Gemcitabine Resistance of Pancreatic Cancer In Vitro and In Vivo. PloS one 2015, 10 (12), No. e0144969. [PubMed: 26709920]

(33). Hao J; Yang X; Ding X-L; Guo L-M; Zhu C-H; Ji W; Zhou T; Wu X-Z Paeoniflorin Potentiates the Inhibitory Effects of Erlotinib in Pancreatic Cancer Cell Lines by Reducing ErbB3 Phosphorylation. Sci. Rep. 2016, 6 (1), 32809. [PubMed: 27609096]

(34). Quiñonero F.; Mesas C; Doello K; Cabeza L; Perazzoli G; Jimenez-Luna C; Rama AR; Melguizo C; Prados J The challenge of drug resistance in pancreatic ductal adenocarcinoma: a current overview. Cancer Biol. Med. 2019, 16 (4), 688–699. [PubMed: 31908888]

(35). Li Y; Xi Z; Chen X; Cai S; Liang C; Wang Z; Li Y; Tan H; Lao Y; Xu H Natural compound Oblongifolin C confers gemcitabine resistance in pancreatic cancer by downregulating Src/MAPK/ERK pathways. Cell Death Dis. 2018, 9 (5), 538. [PubMed: 29749405]

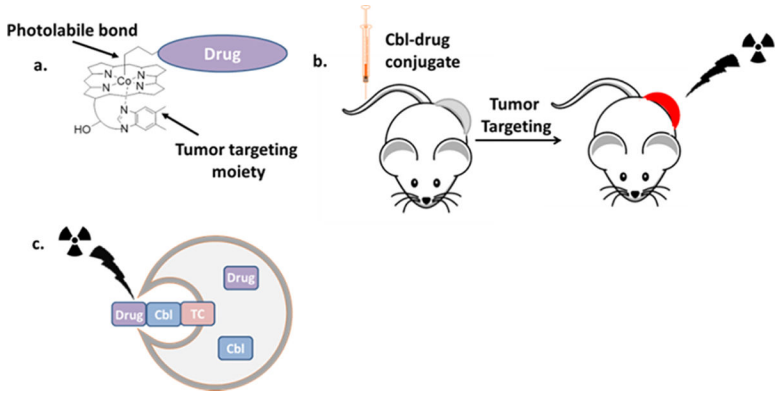


Figure 1.

Radiation-activated cobalamin technology. (a) The general cobalamin-drug conjugate structure (see Supporting Information Figure S1 for the full structure of the vitamin B_{12} scaffold); (b) tumor targeting with IV injection of the cobalamin-drug conjugate; and (c) selective drug uptake at the cellular level of drug conjugates via the transcobalamin (TC) pathway and release of free drug upon radiation into the cell.

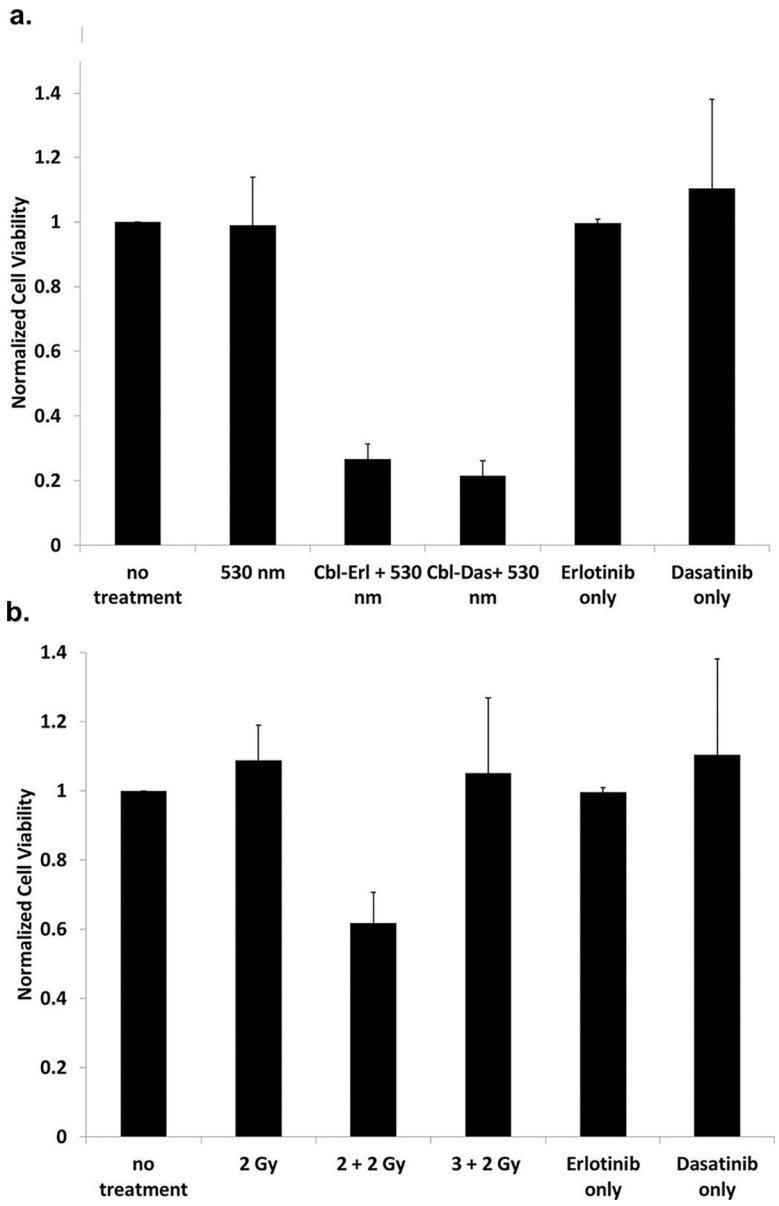


Figure 2.

Light-triggered release of kinase inhibitors from cobalamin scaffold induces apoptosis of MIA PaCa-2 cells. (a) Cells were loaded with 1 μM 2 or 3 and illuminated at 530 nm for 10 min. (b) Cells were loaded with 1 μM 2 or 3 and exposed to a 2 Gy radiation dose. Cell viability was assessed after 72 h via an MTS assay (absorbance measured at 492 nm and normalized to the untreated control). The data shown are the average of six experiments ± SEM.

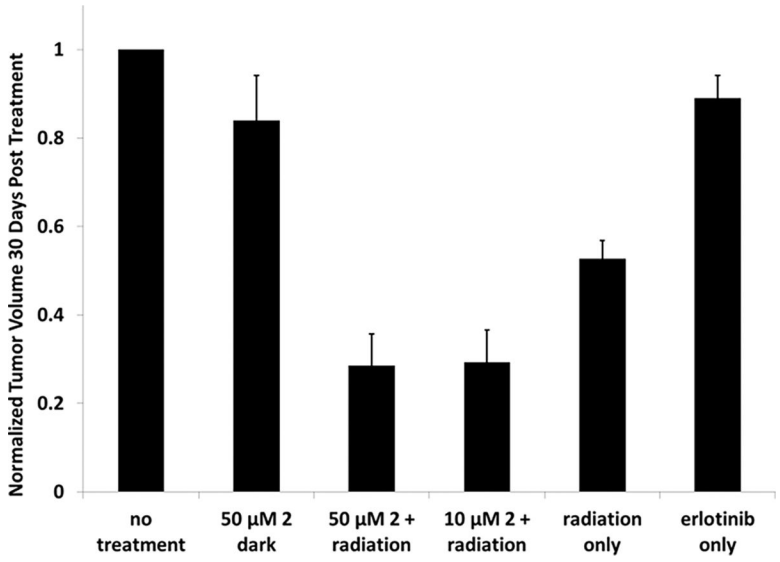
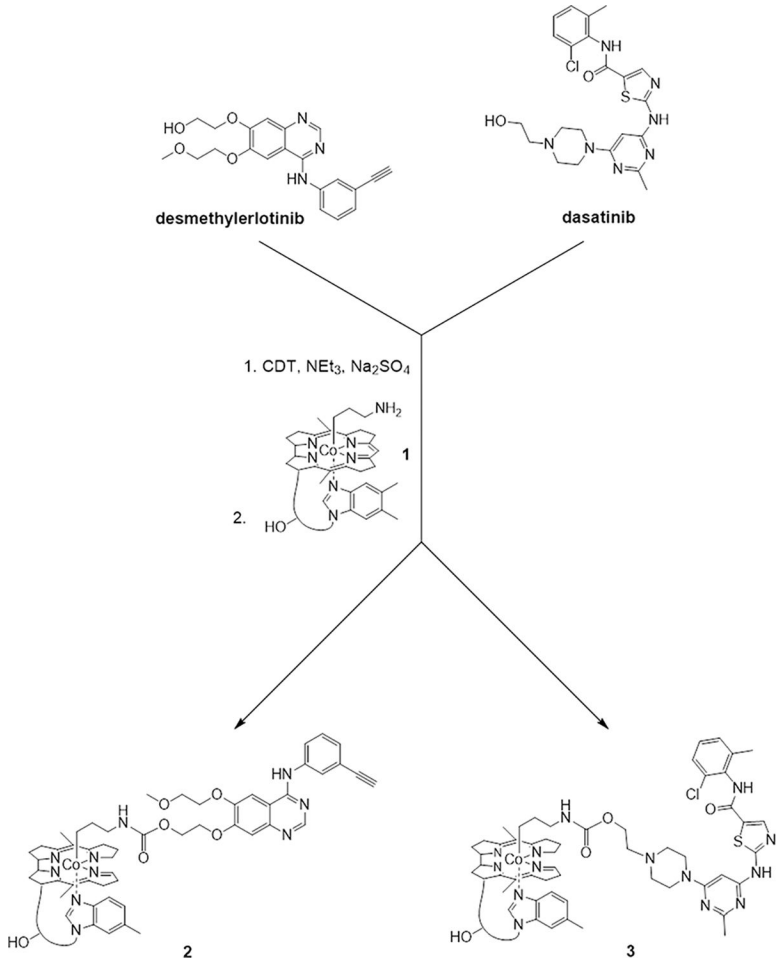


Figure 3.

Synergistic pancreatic tumor reduction with **2**. Mice were IV-injected with 2 (50 and 10 μM) and exposed to a single dose of targeted 8 Gy radiation. Tumor volumes for all cohorts were monitored at regular intervals for a period of 30 days. The data shown is the relative end-point tumor volume normalized to no treatment and is the average of n = 3–6 mice ± SEM.



Scheme 1. Synthesis of Cobalamin-Drug Conjugates[a]

[a]Desmethylerlotinib and Dasatinib were conjugated to the cobalamin scaffold via a carbamate bond linkage.