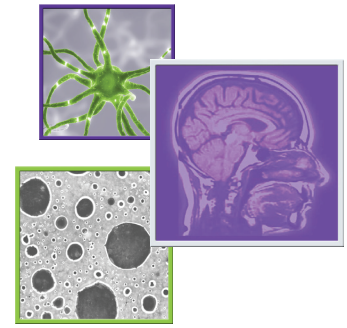
SPECIAL REPORT

CNS Oncology

Diapeutic cancer-targeting alkylphosphocholine analogs may advance management of brain malignancies



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Practice points

- Glioblastoma is the most prevalent primary brain cancer with more than 10,000 cases a year in the USA.
- Ten to thirty percent of cancer patients with systemic malignancies develop metastatic brain cancers, which comprise the majority of all brain cancers.
- Advanced imaging of brain malignancies, improving surgical resections, more effective therapies and post-treatment distinctions between progression and pseudo-progression are clinical challenges in the management of brain malignancies.
- Alkylphosphocholine (APC) analogs are a special class of 'diapeutic' (diagnostic imaging and therapy) agents that display impressive broad-spectrum avidity for malignancies and low background in normal brain.
- The multimodal imaging and therapy capabilities, and identical mechanism of uptake and retention of our APC analogs may address unmet clinical needs in brain malignancies by providing synergism between diagnostic imaging, therapy and post-therapy follow-up.
- Positron emission tomography APC analog can be used for localizing and imaging cancer throughout the body in surgical planning and clinical follow-up.
- Fluorescent APC analog can be used for intraoperative cancer visualization and to improve resections.
- Cancer-targeted radiotherapeutic APC analogs can be used to increase radiotherapy efficacy and decrease off-target toxicities.
- Together, this suite of diapeutic analogs can have transformative implications on the management of brain malignancies, addressing limitations in diagnostic imaging, treatment and follow-up.

The following is a special report on alkylphosphocholine analogs as targeted imaging and therapy agents for cancer, and their potential role in diagnosis and treatment in glioblastoma and brain metastases. These novel cancer-targeting agents display impressive tumor avidity with low background in the normal brain, and multimodal diagnostic imaging and therapy capabilities. The use of these agents may significantly improve diagnosis, treatment and post-treatment follow-up in patients with brain malignancies

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'Theranostics' are defined as diagnostic tests that inform treatment choice and clinical outcome. To improve patient outcomes, theranostics would ideally be used in therapy decision-making to predict treatment efficacy and response, and change clinical management by avoiding unnecessary risks and associated costs of ineffective care [1,2]. Optimizing therapeutic efficacy and safety is especially warranted in treating primary brain tumors or brain metastases, where tumor cells are infiltrating or adjacent to vital functional brain tissue, and deleterious side effects carry significant morbidity and mortality. Therefore, theranostics can have transformative implications in managing brain tumor patients. In this report, we highlight alkylphosphocholine (APC) analogs as a special theranostics type: the 'diapeutic' (diagnostic imaging and therapy) agent. APC analogs have a cancer-selective retention mechanism mediated by an identical cancer-targeting chemical backbone, exhibit broad specificity for tumor cells in multimodal imaging and therapy, and could predict, inform and monitor therapy outcomes. We discuss how this versatility may ultimately transform clinical management of primary and metastatic brain tumors.

^{124}I-CLR1404, ^{131}I-CLR1404 and the near-infrared fluorescent analog CLR1502 are related novel small molecule, cancer-targeting APC analogs. These APC analogs share a common targeting moiety that is selectively retained by tumor cells of many different histologies linked to a functional moiety fashioned for diagnostic imaging or therapy (Figure 1) [3,4]. The lead compound CLR1404 was selected from over 30 radioiodinated phospholipid ether analogs from four different structural classes including an APC class [5]. CLR1404 exhibited the highest tumor signal to background ratio in rodent flank xenograft models and excellent *in vivo* stability against deiodination due to iodine's position on the aromatic ring [5]. APC derivatives localize to cellular and intracellular membranes via uptake into cholesterol-rich plasma membrane lipid raft domains overexpressed in cancer cells [3,6]. The relative deficiency of phospholipid catabolizing enzymes in tumor versus normal cells results in prolonged tumor retention, in contrast to APC clearance by normal tissues. Remarkably, substitution of iodine with a large optically active functional group (e.g., bodipy, IR-775) does not alter APC's tumor selectivity. APCs are being further evaluated in multiple early phase human clinical imaging and radiotherapy trials, after extensive testing and demonstration of prolonged selective retention in over 60 *in vivo* rodent and human cancers and cancer stem cell models [3,7,8]. Two radioinodinated CLR1404 analogs have recently undergone five multi-institutional human imaging and therapy clinical trials,^{124}I-CLR1404 for positron emission tomography (PET) imaging and ^{131}I-CLR1404 for therapy and single photon emission computed tomography imaging. Preliminary data from a multi-institutional Phase II trial for glioblastoma (GBM) imaging with ^{124}I-CLR1404 is currently being evaluated [7,8]. The near-infrared fluorescent analog CLR1502 is also nearing clinical trial testing.

While theranostic strategies often rely on technologies and techniques that are radically different to inform clinical decisions, the identical tumor-targeting backbone of all three APC analogs confers a shared mechanism of uptake and retention. Due to their identical uptake and retention mechanisms, CLR1404 derivatives have similar pharmacological half-life, tissue biodistribution and cancer-selective retention characteristics. This commonality ensures that the synergistic use of these agents reliably and specifically targets the same cancer cells in diagnosis and staging, treatment planning and dosimetry and potentially subsequent clinical follow-up and monitoring of treatment response. To illustrate the potential advantage of diapeutic agents, consider a patient with a brain malignancy: ^{124}I-CLR1404 is administered initially for whole body or global assessment of cancer lesion(s) and staging. Fluorescent

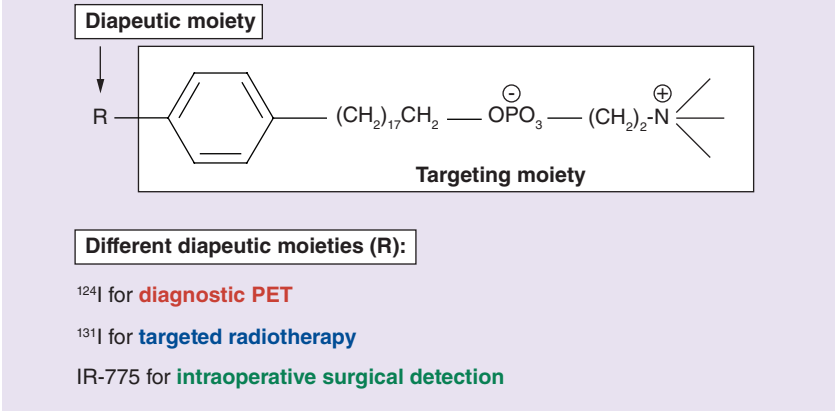

Figure 1. Alkylphosphocholine analogs are composed of a targeting moiety and a diapeutic moiety. The diapeutic moiety can be substituted with ^{124}I for diagnostic PET imaging, ^{131}I for targeted radiotherapy, and a near-infrared dye (IR-775) for intraoperative surgical detection.
PET: Positron emission tomography.

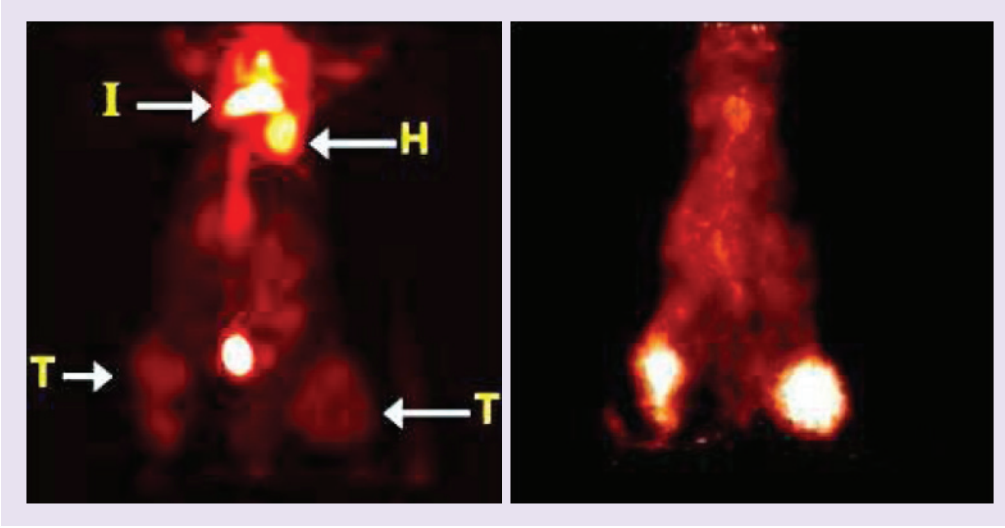

Figure 2. MicroPET projection view images of a mouse with human PC3 tumors (T) in the right flank and pseudo metastasis in the left tibia (T), a carrageenan-induced inflammatory lesion (I) located between the scapulae, and heart (H). Left image was acquired 1 h after administration of ^{18}F-FDG. Right image was obtained 24 h after administering 124I-CLR1404 to the same mouse, 24 h after the FDG scan.
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analog CLR1502 would then be used locally for optical cancer visualization to optimize surgical resection, and illuminate residual unresectable tumor. Postoperative uptake of APC PET imaging tracer can be used to calculate personalized dosimetry for the targeted radiotherapy agent ^{131}I-CLR1404. Follow-up APC PET imaging may further inform patient management through possible detection of tumor recurrence. As this example demonstrates, due to the identical cancer-targeting mechanism of diapeutic APCs, this suite of APC analogs can be used at multiple clinical phases, potentially maximizing effectiveness and simultaneously minimizing complications during surgery and radiotherapy on a patient-to-patient basis.

Diapeutic APCs have four primary advantages over current standard of care for brain cancer diagnostic imaging and therapy, which include: targeting of metastatic malignancies in addition to primary brain malignancies; impressive PET signal to background in the brain; long retention profiles that permit evaluation of postsurgical outcomes; and patient-specific staging and dosimetry information for the next phases of clinical care [9]. Currently, GBM is the most prevalent primary brain cancer with approximately 20,000 diagnosed per year in the USA, and 10–30% of patients with systemic malignancies develop brain metastases, with 200,000 cases per year in the USA [10–12]. Primary and metastatic tumors in the brain can be detected and treated with APC analogs due to their observed broad-spectrum selective uptake by many kinds of cancer histologies, with potential benefit for a significant number of cancer patients [3,13].

PET with fludeoxyglucose (^{18}F-FDG), a positron-emitting glucose analog, is used widely in solid tumor imaging for the purpose of diagnosing, staging, restaging and monitoring therapy response in most cancers with the notable exception of primary and metastatic lesions to the brain [14–16]. In the context of brain lesions, ^{18}F-FDG currently suffers from low sensitivity and specificity due to its high physiologic uptake in normal brain tissue, low uptake in necrotic lesions and propensity for false-positive inflammation [17,18]. Importantly, ^{124}I-CLR1404 has shown impressive resolution on brain cancer PET-imaging in rodents and humans due to its selectivity and retention in multiple tumor histologies including GBMs and metastatic brain malignancies. APC analogs show high sensitivity and specificity for tumor detection and low uptake in background tissues and inflammatory lesions **(Figure 2)** [3]. ^{124}I-CLR1404 PET imaging of a malignant glioma patient demonstrated

probable recurrence with very high signal to background that was surgically verified **(Figure 3)** [3]. Lastly, the diapeutic paradigm and long retention in cancers permit ^{124}I-CLR1404 imaging to provide critical information for biopsy or surgery, personalized, tailored dosimetry for the targeted radiotherapy compound ^{131}I-CLR1404 and follow-up monitoring.

Diapeutic APCs may also improve resection of brain tumors. The goal of maximal safe resection of brain tumors is often difficult due to tumor proximity to normal brain tissue, or infiltration into normal brain parenchyma [19]. Patients with gross total resection for GBMs and low-grade gliomas have longer median survival than patients with subtotal resection outcomes [20–22]. Recently, the use of optical agents for intraoperative imaging has shown promise in surgical oncology to improve resection outcomes, and thus patient survival. A randomized Phase III clinical trial in Europe demonstrated that patients who received fluorescence-guided surgery with 5-aminolevulinic acid followed by radiotherapy had improved resection outcomes, and progression-free survival by 6 months without a decline in performance status compared

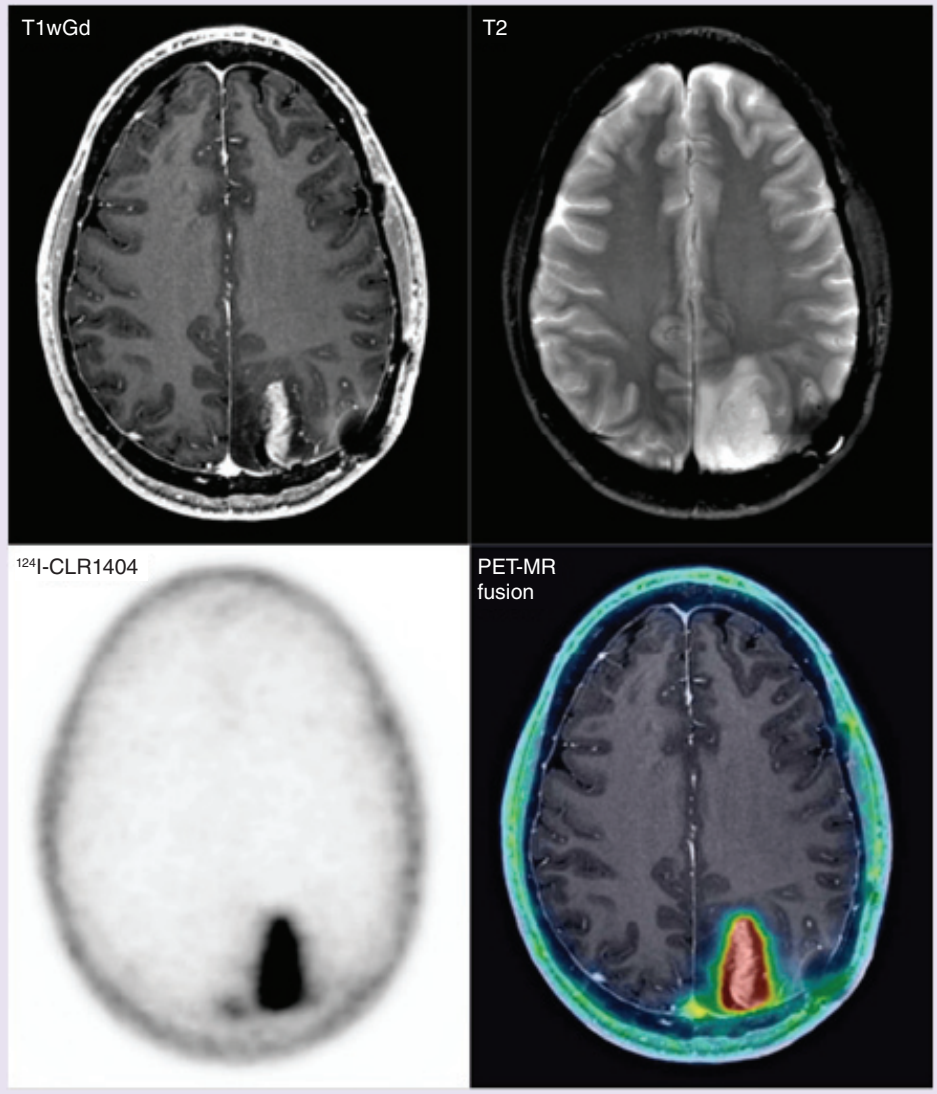

Figure 3. Malignant glioma was resected 2 years before this axial T1-contrast MR and positron emission tomography imaging that shows likely recurrence in a patient.
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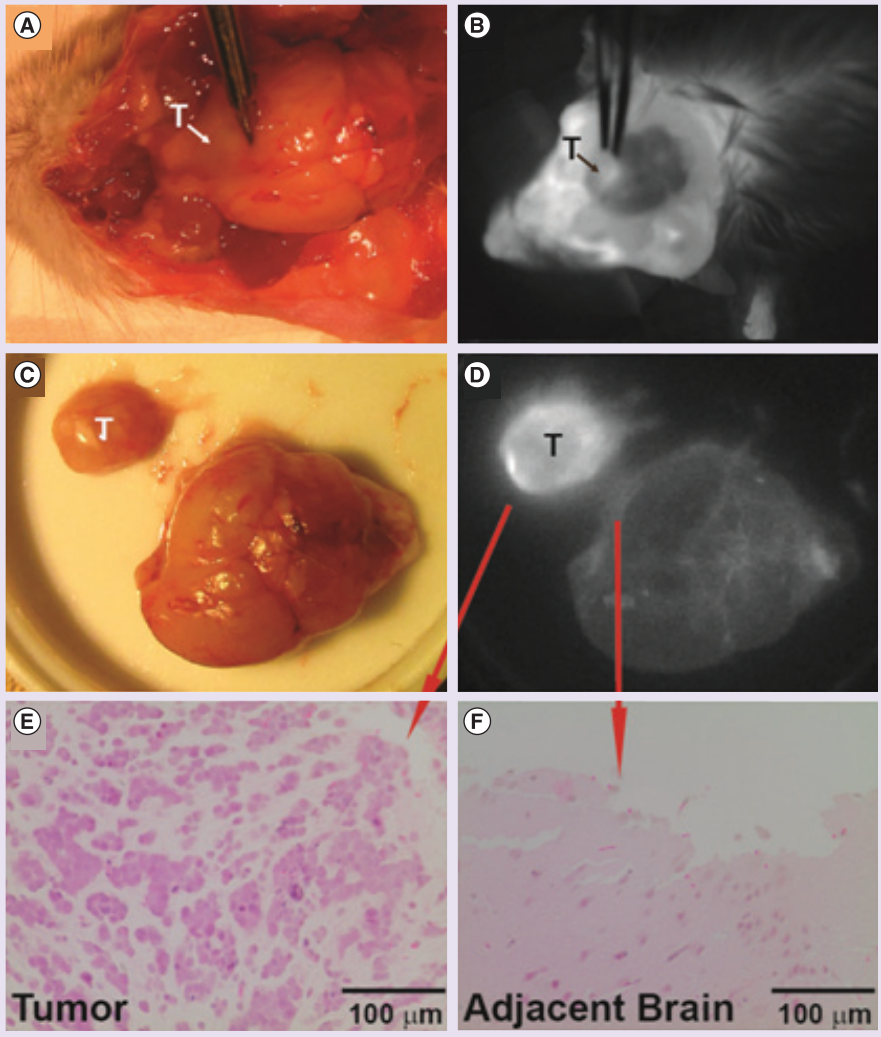

Figure 4. CLR1502 fluorescence imaging of a patient-derived glioblastoma stem cell orthotropic xenograft. (A & B) White light and fluorescence imaging of glioblastoma stem cell xenograft and **(C & D)** after resection. **(E & F)** Verification of tumor and normal brain by histology.
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with those that received conventional surgery under white light followed by radiotherapy [23,24]. Real-time tumor visualization may achieve additional clinical benefits including more efficient surgeries, decreased operative times, decreased anesthesia exposure and reduced complications due to avoidance of normal structures. The near-infrared fluorescent APC analog CLR1502 showed robust tumor fluorescence in orthotopic GBM xenografts derived from human cancer stem cells [13] **(Figure 4 & Supplementary Video 1)**. The high tumor fluorescence of CLR1502 may result from active targeting and penetration and lower near-infrared range background characteristics. This agent has also been used as a fluorescent tumor label in preclinical colon cancer, breast cancer and prostate cancer models in addition to GBM models, and shows promise as an intraoperative surgical adjunct [25–27]. Furthermore, postresection fluorescence signal from CLR1502 and APC PET uptake signal can provide information about the extent of resection.

In addition, the targeted radiotherapeutic analog ^{131}I-CLR1404 may be a safe and more effective alternative to current radiotherapy

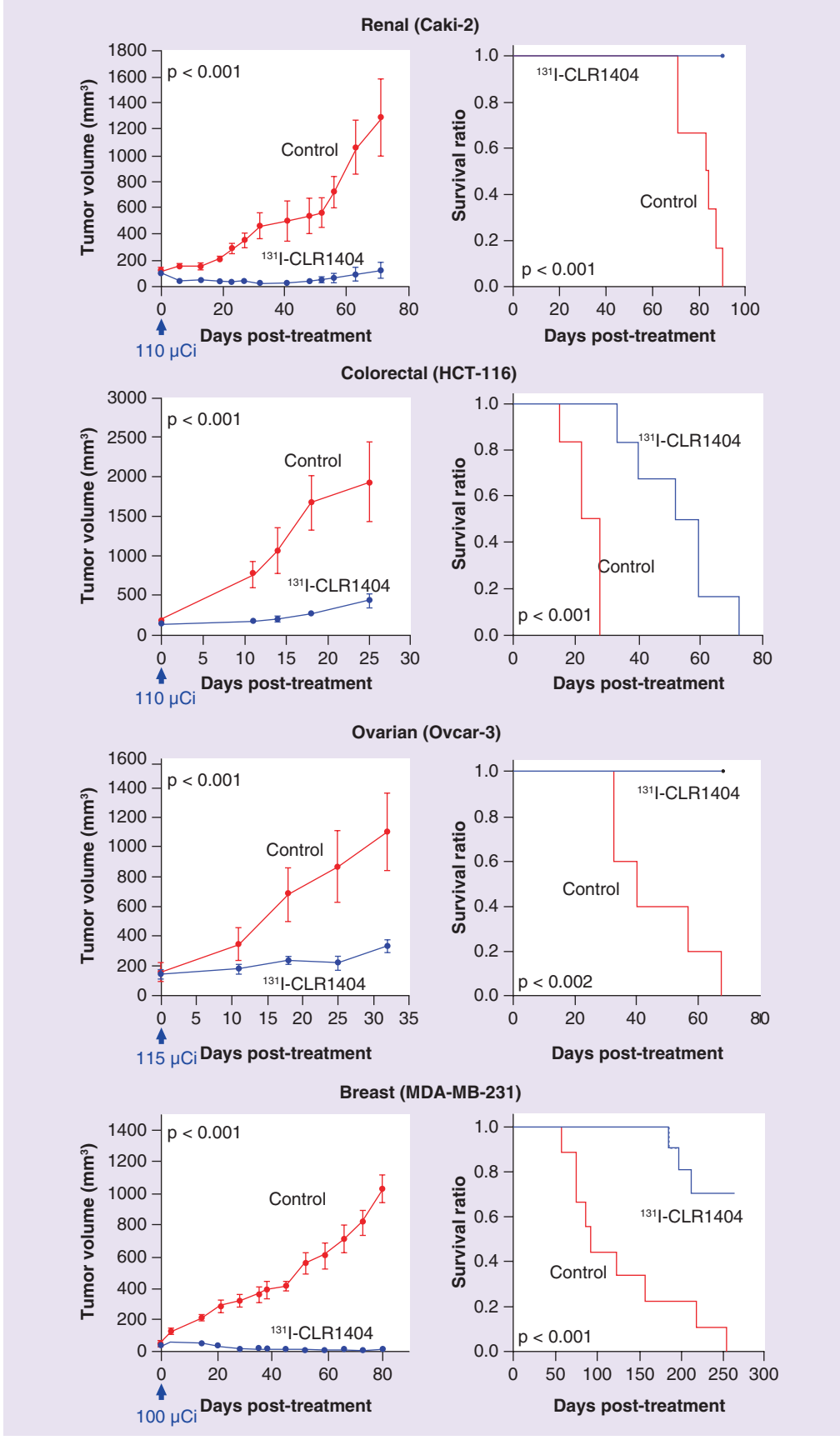
Renal (Caki-2)
Tumor volume (mm³)
p < 0.001
Control
131I-CLR1404
110 µCi
Days post-treatment
Survival ratio
131I-CLR1404
Control
p < 0.001
Days post-treatment
Colorectal (HCT-116)
Tumor volume (mm³)
p < 0.001
Control
131I-CLR1404
110 µCi
Days post-treatment
Survival ratio
131I-CLR1404
Control
p < 0.001
Days post-treatment
Ovarian (Ovcar-3)
Tumor volume (mm³)
p < 0.001
Control
131I-CLR1404
115 µCi
Days post-treatment
Survival ratio
131I-CLR1404
Control
p < 0.002
Days post-treatment
Breast (MDA-MB-231)
Tumor volume (mm³)
p < 0.001
Control
131I-CLR1404
100 µCi
Days post-treatment
Survival ratio
131I-CLR1404
Control
p < 0.001
Days post-treatment

Figure 5. Tumor growth and animal survival after ^{131}I-CLR1404 therapy (see facing page). Nude mice harboring human tumor xenografts (n = 6 renal, 6 colorectal, 5 ovarian and 9 breast) were administered a single, nonoptimized ^{131}I-CLR1404 dose (arrow). Control animals were administered a mass-equivalent CLR1404 dose (n = 6).
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regimens in targeting tumor cells that are not resectable during initial treatment. Preclinical work with human cancer cell lines that frequently metastasize to the brain demonstrated that a single, nonoptimized dose of 100–115 μCi of ^{131}I-CLR1404 significantly improved survival in mice with subcutaneously implanted flank xenografts **(Figure 5)**. Furthermore, glioblastoma stem cells are resistant to radiation therapy and chemotherapy with temozolomide, and may account for tumor recurrence despite current therapies [28]. Importantly, ^{131}I-CLR1404 targets brain cancer progenitor cells in addition to bulk tumor cells, while avoiding normal brain tissues, which may improve radiotherapy outcomes [3]. Optimizing ^{131}I-CLR1404 dosing on patient-specific basis, minimizing off-target toxicities and monitoring radiotherapy or chemotherapy outcomes can be achieved using ^{124}I-CLR1404 PET imaging in the proposed diapeutic paradigm.

At last, diapeutic APCs are proposed for use in clinical follow-up imaging to assess treatment efficacy or disease progression. Approximately 20% of patients treated with chemoradiotherapy show post-treatment-related enhancement on MRI also known as pseudoprogression, frequently difficult to distinguish from tumor progression or recurrence [29,30]. Much research in tumor imaging technologies and strategies is being pursued to address this clinical challenge to distinguish between pseudoprogression and true tumor progression [31]. Several amino-acid tracers such as ^{18}F-fluorothymidine and ^{18}F-fluoro-glutamine undergoing clinical assessment have variable signal sensitivity and specificity based on tumor metabolism [16,29,32]. In contrast, ^{124}I-CLR1404 is being evaluated as a possible agent that may detect tumor progression due to its favorable selectivity and retention profile, and lack of uptake in areas of inflammation or necrosis [3].

Conclusion

In summary, the diapeutic utility of APC analogs, which rely on the identical APC cancer specific mechanism of uptake and retention, synergizes each step in patient management from diagnostic imaging to post-treatment monitoring to a degree that is not seen with other theranostic strategies using different distinct modalities or mechanisms. Diapeutic APC analogs are a class of cancer-targeted agents with broad-spectrum cancer targeting and multimodal diagnostic imaging and therapy capabilities that may significantly advance care of patients with brain malignancies.

Synthesis and testing of APC analogs with other capabilities may increase the diagnostic and therapeutic arsenal for this targeting platform. The use of APC agents as diapeutics will be advanced by studies on dosimetry with the PET agent, microdosimetry studies of targeted radiotherapeutics and studies on therapeutic effects. Notably, regulatory approval bodies (e.g., US FDA) do not have a current framework for evaluating the planned clinical combination trials with multiple APC agents that are needed to evaluate the diapeutic synergism of these agents or for evaluating optical detection technologies. While we are able to visualize high-grade gliomas and metastatic lesions that have disrupted barriers, visualization of lower grade gliomas has not been adequately studied. Studies are underway to determine and characterize the blood–brain barrier permeability of APC analogs [33]. Despite the need for further work, the promise of this diapeutic paradigm has already started to materialize with ^{124}I-CLR1404 as a diagnostic agent for cancer diagnosis, staging and dosimetry of therapeutic analogs, its companion ^{131}I-CLR1404 as a therapeutic agent for targeted radiation therapy and CLR1502 as intraoperative fluorescent agent to aid surgical resection. Introduction of next-generation APC analogs will increase the complement of diapeutic APC analogs in imaging and therapy to potentially advance the clinical management of brain cancer patients.

Supplementary data

Supplemental Video 1: Real-time CLR1502 fluorescence visualization of orthotopic brain cancer in mouse. Data taken from [13]. *To view the supplementary data that accompany this paper please visit the journal website at: www.futuremedicine.com/doi/full/10.2217/cns-2016-0017*

Financial & competing interests disclosure

JP Weichert is founder of Cellectar Biosciences, Inc., which holds the licensing rights to the described APC analogs. Cellectar Biosciences, Inc. provided the APC analogs used for this research. The authors grant permission to publish this information or an appropriate summary thereof with the abstract. This work was supported in part by NIH grants R01CA158800 (JP Weichert, LT Hall, JS Kuo), R01NS75995 (JS Kuo), T32 GM008692 (RR Zhang), UL1RR025011 and P30CA014520 (UW Carbone Cancer Center), Headrush Brain Tumor Research Professorship (JS Kuo) and Roger Loff Memorial Fund (JS Kuo). The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations.

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