Radioimmunotherapy for solid tumors: spotlight on Glypican-1 as a radioimmunotherapy target

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Abstract: Radioimmunotherapy [i.e., the use of radiolabeled tumor targeting antibodies] is an emerging approach for the diagnosis, therapy, and monitoring of solid tumors. Often using paired agents, each targeting the same tumor molecule, but labelled with an imaging or therapeutic isotope, radioimmunotherapy has achieved promising clinical results in relatively radio-resistant solid tumors such as prostate. Several approaches to optimize therapeutic efficacy, such as dose fractionation and personalized dosimetry, have seen clinical success. The clinical use and optimization of a radioimmunotherapy approach is, in part, influenced by the targeted tumor antigen, several of which have been proposed for different solid tumors. Glypican-1 (GPC-1) is a heparan sulfate proteoglycan that is expressed in a variety of solid tumors, but whose expression is restricted in normal adult tissue. Here, we discuss the preclinical and clinical evidence for the potential of GPC-1 as a radioimmunotherapy target. We describe the current treatment paradigm for several solid tumors expressing GPC-1 and suggest the potential clinical utility of a GPC-1 directed radioimmunotherapy for these tumors.

Keywords: glypican-1, personalized dosimetry, radioimmunotherapy, solid tumors, theranostic

Introduction

Despite significant advances in the development of anti-cancer therapies, cancer remains a leading cause of death in Australia and globally, with one in two Australian men and women being diagnosed with cancer by the age of 85. Rare and less common cancers account for one in three new cancer cases diagnosed and one in two cancer deaths. Cancer is the leading cause of disease burden in Australia and, although improvements have been seen in survival rates over the past three decades in tumors such as breast, bowel, and lung cancer, there has been minimal improvement in overall survival rates for pancreatic, esophageal, and brain cancers.1

The clinical landscape in solid tumors has changed dramatically over the past decades. The advent of chemotherapy and radiotherapy provided non-targeted, “carpet bomb” therapy, damaging tumor and normal tissue alike. The next generation of therapeutics used antibodies with some success, exploiting their native effector functions and/or blocking ability to destroy tumor cells via recruitment of the immune system or inhibition of critical signaling pathways.2 Immunotherapy in the form of immune checkpoint inhibitors (ICIs) represented a major breakthrough in the treatment and survival of patients with immunogenic cancers including melanoma, non-small cell lung cancer, renal cell and urothelial carcinoma, and Merkel cell carcinoma, where durable clinical responses were observed.3–5 However, the majority of patients do not respond to these treatments, and a reliable and consistent predictive biomarker is yet to be validated to identify those that will. In those patients who do demonstrate an initial response, a proportion will ultimately progress to refractory disease when the cancer develops resistance to the treatment.
Furthermore, there are certain cancers that do not respond at all to checkpoint inhibitors. This may be expected given that several solid tumors, such as pancreatic and prostate cancer, are considered “non-immunogenic.” For those immunogenic tumors, recent clinical practice has seen the combination of immune modulating substances with other agents in order to increase the proportion of patients who respond and result in a prolonged response. For example, in advanced melanoma and renal cell carcinoma, combination therapy with two checkpoint inhibitors or the combination of a VEGF inhibitor or tyrosine kinase inhibitor (TKI)-targeted therapy and a checkpoint inhibitor has become the new standard of care (SOC), resulting in increased response rates and prolonged survival times when compared with single agent therapy.

Pioneered in the 1940s with the use of radioiodine to image and manage thyroid cancers, a “theranostic” approach to the treatment and monitoring of cancer has become an area of increasing interest. The coupling of a therapeutic agent with a diagnostic allows for patient selection, dose optimization, and monitoring of treatment outcomes. “Molecular targeting” refers to the use of a tumor targeting molecule (usually a monoclonal antibody or small molecule, specific for tumor cells and with limited binding to normal tissue), to target tumors either with an imaging or therapeutic agent. From the therapeutic perspective, molecular-targeted radiotherapy allows selective delivery of therapeutic radiation to the tumor, while minimizing the dose delivered to normal tissue, a potential advantage over traditional radiotherapy. Further, molecular-targeted radiotherapy, unlike conventional radiotherapy, can achieve systemic exposure following intravenous infusion and so has the potential to target micro-metastatic disease after resection. The targeting molecule may be conjugated to an imaging isotope alongside a therapeutic isotope, the former enabling visualization/detection of tumor lesions thus allowing tailoring of therapeutic drug dosage to achieve optimal therapeutic radiation dose to tumor (personalized dosimetry), as well as monitoring of disease post treatment.

Radioimmunotherapy (RIT) utilizes antibodies as targeting molecules, harnessing their exquisite specificity for their target antigen, along with their relatively long circulating half-life, to optimally deliver radiation to tumor, sparing healthy tissue. Historically, despite the very promising clinical responses achieved using RIT in hematological malignancies, RITs have been broadly less effective in solid tumors. However, a number of approaches hold promise for unleashing the potential of RIT in solid tumors, including the combination of RIT with other therapies. Ultimately, the success of an RIT approach requires targeting of an appropriate tumor antigen, that is, an antigen that is expressed at high level and preferably homogenously on tumor tissue and is not expressed on normal tissue. Several antigens have been proposed for the molecular targeting of various solid tumors, with varying characteristics and expression profiles, for example, carcinoembryonic antigen (CEA) in colorectal cancers and epidermal growth factor receptor (EGFR) in non-small cell lung cancer.

GPC-1 is a heparan sulfate (HS) proteoglycan that is critically involved in tumor cell signaling, and cancer growth, invasion, and metastasis. GPC-1 is over-expressed in a variety of solid tumors, including prostate, breast, pancreatic, bladder, mesothelioma, esophageal, cervical, and ovarian cancers, as well as brain cancers glioma and glioblastoma. Importantly, the expression of GPC-1 is restricted in normal adult tissue. Moreover, the safety of targeting GPC-1, both in mice and in humans, is well established. This review explores the clinical utility of RIT in solid tumors, including promising approaches for the optimization of therapeutic efficacy of RIT in these relatively radio-insensitive tumors. We discuss evidence for the potential role of GPC-1 as a novel molecular target in these tumors, particularly as an RIT, and, finally, propose potential clinical utility for a GPC-1 directed RIT. GPC-1 may have advantages over current RIT approaches due to the wide range of tumors in which it is overexpressed, and its lack of expression in normal tissues.

Radioimmunotherapy: a solid tumor perspective

There is preclinical and clinical evidence for the utility of antibodies as targeting agents for RIT. Antibodies as a class have several characteristics
that make them appropriate delivery agents for radiation. Assuming an appropriate tumor target antigen has been selected (an antigen that is expressed on the tumor but is lacking in normal healthy tissue), antibodies are able to deliver radiation specifically to the tumor, sparing healthy tissue. The long circulating half-life (1–3 weeks) of antibodies, owing to their engagement of neonatal Fc receptor (FcRn), improves the likelihood of tumor exposure.\textsuperscript{32} Tumor accumulation, penetration, and retention are, in part, a function of antibody affinity, which can be engineered. Binding of antibody to antigen often triggers cellular internalization – a characteristic critical to the cytotoxicity of radiolabels with short path lengths. For example, the alpha emitter, \textsuperscript{225}Actinium, requires immediate proximity to the nucleus for cell killing, relying upon internalization into the cell for efficacy.\textsuperscript{33} Finally, some antibodies may serve the dual purpose of mediating effector function alongside delivery of the therapeutic payload. For example, in pre-clinical studies, anti-human epidermal growth factor receptor 2 (anti-HER2) antibodies block signaling while mediating antibody-dependent cellular cytotoxicity (ADCC) alongside delivery of the therapeutic radioisotope, and it is thought that the combination of these mechanisms contribute to tumor cell killing.\textsuperscript{34} Manufacturability and radiolabeling of antibodies are well characterized processes. Immunogenicity is generally not of concern for fully human or humanized antibodies, allowing safe multi-dose administration.\textsuperscript{35}

Clinically, most molecular-targeted radiotherapies, including RITs, have been developed for therapy of the most radiosensitive tumors including leukemias and lymphomas. Indeed, the radio-sensitivity of the tumor is defined by the radio-sensitivity of the parent cell. Thus, hematological malignancies tend to be more radio-sensitive than solid tumors, with complete responses possible with radiation doses in the range of 1500–2000 cGy, as compared with the 3500–10,000 cGy required to demonstrate a clinical response in solid tumors.\textsuperscript{36,37} Regardless of this lower radio-sensitivity, there is substantial preclinical and clinical evidence for the potential utility of molecular targeted therapies (including RIT) in the treatment of solid tumors.\textsuperscript{38–49} Solid tumor RIT trials have been reviewed in depth by Larson,\textsuperscript{50} Bartholomä,\textsuperscript{13} and Krčéisz.\textsuperscript{51} A selection of recent trials completed or in progress is presented in Table 1.

Radiolabelled full-length antibodies have long half lives in patients, with reported values of 24–111 h.\textsuperscript{52,53} While the long half life is beneficial in maintaining high serum concentrations of the radiolabelled antibody to allow maximum tumor targeting, it also exposes the bone marrow to prolonged exposure to the radioisotope. As a result, bone marrow suppression is almost exclusively the dose-limiting toxicity for full-length antibodies in the treatment of solid tumors.\textsuperscript{50} A number of strategies have been investigated to reduce bone marrow toxicity while maintaining the anti-tumor response. Dose fractionation has been employed successfully,\textsuperscript{54} while other approaches such as smaller antibody fragments with shorter half lives are also being tested in the clinic.\textsuperscript{55} Pre-targeting approaches are also being investigated preclinically and clinically\textsuperscript{56–58}; however, the benefits of a shorter half life need to be weighed against reduced uptake to the tumor.\textsuperscript{59}

The efficacy of \textsuperscript{177}Lutetium (\textsuperscript{177}Lu) using a humanized antibody against prostate specific membrane antigen (PSMA), J591, has been demonstrated in patients with metastatic castration-resistant prostate cancer (mCRPC). In the phase I trial of \textsuperscript{177}Lu-J591, 35 patients with mCRPC were treated with doses of \textsuperscript{177}Lu ranging from 10 to 75 mCi/m². A total of 20 patients had either a stable or partial biochemical [prostate specific antigen (PSA)] response to treatment. The main dose limiting toxicity was myelosuppression and the maximum tolerated dose was determined to be 70 mCi/m².\textsuperscript{60} This dose was then used for the phase II study in which 47 patients were enrolled. Approximately 60% of patients experienced a decline after receiving a single dose. The overall response rate in patients with measurable disease was 75%. All patients experienced hematological adverse events caused by radiation dose to the bone marrow, which was reversible.\textsuperscript{61} Of note, in both phase I and II studies, tumor targeting by \textsuperscript{177}Lu-J591 was sensitive and specific for known tumor metastases, with 100% and 94% of lesions identified on planar imaging, respectively. A phase I/II trial of \textsuperscript{177}Lu-J591 tested fractionated dosing in which fractionated doses of between 20 and 45 mCi/m² were administered 2 weeks apart. The study concluded that higher doses of radiation could be delivered safely using dose fractionation than could be achieved with a single dose regimen, with an acceptable safety profile.\textsuperscript{54}

These clinical data complement the studies using a small molecule binder of PSMA (PSMA-617) to deliver the same therapeutic radionuclide (\textsuperscript{177}Lu) and have demonstrated further clinical
| Indication | Target | Antibody | Isotope | Phase | Trial status | Key results | ClinicalTrials.gov identifier | Reference |
|------------|--------|----------|---------|-------|--------------|-------------|-------------------------------|-----------|
| High-risk and recurrent medulloblastoma | GD2 | 3F8 | $^{131}$I | II | Active not recruiting, has results | Intracerebroventricular injection. Mean OS 24.9 months. Toxicities included acute bradycardia with somnolence, headache, fatigue, and CSF pleocytosis consistent with chemical meningitis and dystonic reaction | NCT00445965 | Kramer et al. 62 |
| Refractory, recurrent, or advanced CNS or leptomeningeal cancer | B7H3 | Omburtamab | $^{131}$I | I | Active not recruiting | | NCT0089245 |
| Leptomeningeal metastasis from solid tumors | B7H3 | Omburtamab | $^{177}$Lu | I | Not yet recruiting | | NCT04315246 |
| Recurrent medulloblastoma and ependymoma | B7H3 | Omburtamab | $^{131}$I | II | Not yet recruiting | | NCT04743661 |
| Recurrent or refractory medulloblastoma | B7H3 | Omburtamab | $^{177}$Lu | I/II | Not yet recruiting | | NCT04167618 |
| Non-progressive diffuse pontine gliomas previously treated with external beam radiation therapy | B7H3 | Omburtamab | $^{124}$I | I | Recruiting | | NCT01502917 |
| Neuroblastoma central nervous system/leptomeningeal metastases | B7H3 | Omburtamab | $^{131}$I | II/III | Recruiting | | NCT03275402 |
| Breast cancer | HER2 | Trastuzumab | $^{177}$Lu | I | Has results | One patient suffered from mild fever post administration of $^{177}$Lu-trastuzumab and was managed by antipyretics. No adverse reactions were observed in other nine patients. Localization of $^{177}$Lu-trastuzumab observed at primary as well as metastatic sites (HER2 positive) in the planar and SPECT/CT images | Histo/15/IMEC/211/30/3/15 | Bhusari et al. 63 |
| Unresectable hepatocellular carcinoma | HAb18G/CD147 | Metuximab | $^{131}$I | IV | Has results | Hepatic intra-arterial injection. 1-year survival rate was significantly higher (79.47% versus 65.59%; $p = 0.041$) and TTP significantly improved ($6.82 \pm 1.28$ versus $4.7 \pm 1.14$ months, $p = 0.037$) compared with the control group. Significantly different toxicities in leukocytopenia, thrombocytopenia and increased total bilirubin level ($p < 0.001$, $p = 0.013$, $p < 0.01$), but no differences in severe AEs of upper GI hemorrhage and severe liver dysfunction between the groups (5.39% versus 2.3%, $p = 0.136$) | SFDA and Ethics Committee of Affiliated Zhongshan Hospital, Fudan University | Ma and Wang 64 |

(continued)
| Indication                                                                 | Target                | Antibody | Isotope | Phase | Trial status | Key results                                                                                     | ClinicalTrials.gov identifier | Reference                        |
|---------------------------------------------------------------------------|-----------------------|----------|---------|-------|--------------|-----------------------------------------------------------------------------------------------|-------------------------------|---------------------------------|
| Liver metastases in patients with metastatic colorectal cancer previously treated with surgery | CEA                   | cT84.66  | ^90^Y   | I/II  | Has results  | ^90^Y-labeled cT84.66 radioimmunotherapy, gemcitabine, and HAI of FUdR; 10 patients had no evidence of visible disease and remained without evidence of disease after completion of protocol therapy. The remaining six patients demonstrated radiological visible disease after surgery and after protocol therapy two patients had a CR, one patient had PR, two had stable disease, and one had progression. With a median follow up of 41.8 months (18.7–114.6), median PFS was 9.6 months. Two patients demonstrated long-term disease control out to 45+ and 113+ months | NCT00645710                  | Cahan et al.⁵⁵                   |
| Ovarian                                                                   | NaPi2b                | MX35 F(ab)² | ^21^At | I     | Has results  | A total of 12 patients with relapsed epithelial ovarian cancer, achieving a second complete or nearly complete response with chemotherapy received intraperitoneal administration, dose escalation from 20 to 215 MBq/L without any dose-limiting toxicities. At progression, chemotherapy was given without signs of reduced tolerability. Overall median survival was 35 months, with a 1-, 2-, 5-, and 10-year survival of 100%, 83%, 50%, and 25%, respectively | NCT04461457                  | Hallqvist et al.⁶⁶              |
| Pancreatic                                                                | CA19-9                | MVT1075  | ^17^Lu  | Active not recruiting |                                                                                               | NCT03118349                  |                                 |
| Desmoplastic small round cell tumor                                        | B7H3                  | Omburtamab | ^131^I  | I     | Active not recruiting, has results | Intraperitoneal injection 1.11–3.33 GBq/m². Maximum tolerated dose was not reached; there were no dose-limiting toxicities. Recommended phase II activity was established at 2.96 GBq/m² | NCT01099644                  | Modak et al.⁶⁷                   |
| Desmoplastic small round cell tumors and other solid tumors in the peritoneum | B7H3                  | Omburtamab | ^131^I  | II    | Recruiting   |                                                                                               | NCT04022213                  |                                 |
| Prostate                                                                  | PSMA                  | J591     | ^17^Lu  | I     | Has results  | Men with progressive mCRPC received docetaxel 75 mg/m² every 3 weeks with escalating two fractionated doses of ^17^Lu-J591; 15 men with progressive mCRPC received dose-escalated targeted radionuclide therapy in four cohorts up to the highest planned dose [2.96 GBq/m²]. No DLT was seen at any dose level. All patients had targeting of known sites of disease by planar ^17^Lu-J591 imaging | NCT00916123                  | Batra et al.⁶⁸                   |
| Indication                     | Target | Antibody | Isotope | Phase | Trial status | Key results                                                                                                                                                                                                 | ClinicalTrials.gov identifier | Reference |
|-------------------------------|--------|----------|---------|-------|--------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------|-----------|
| Prostate                      | PSMA   | J591     | $^{177}$Lu | I/II  | Has results  | A total of 49 men received fractionated doses of $^{177}$Lu-J591 ranging from 20 to 45 mCi/m$^2$ about 2 weeks apart. The DLT in phase I was neutropenia. The recommended phase II doses (RP2Ds) were 40 and 45 mCi/m$^2$. At the highest RP2D (45 mCi/m$^2$), 35.3% of patients had reversible grade 4 neutropenia, and 58.8% of patients had thrombocytopenia. This dose showed a greater decrease in PSA levels and longer survival (87.5% with any PSA decrease, 58.8% with >30% decrease, 29.4% with >50% decrease; median survival, 42.3). Fractionated administration of $^{177}$Lu-J591 allowed higher cumulative radiation dosing. The frequency and depth of PSA decrease, OS, and toxicity (dose-limiting myelosuppression) increased with higher doses. | NCT00538668 | Tagawa et al. |
| Prostate                      | PSMA   | TLX591   | $^{225}$Ac | I     | Active not recruiting |                                                                                                                                                                                                 | NCT03276572 |           |
| Prostate                      | PSMA   | JNJ-69086420 | $^{225}$Ac | I     | Recruiting | Patients were imaged with $^{111}$In OTSA-101. Patients with significant tumor uptake were randomized between 370 MBq (Arm A) and 1110 MBq (Arm B) of $^{90}$Y-OTSA-101. Best response was stable disease in 3/8 patients lasting up to 21 weeks for one patient. The most common Grade $\geq$ 3 AEs were reversible haematological disorders, which were more frequent in Arm B. | NCT02002312 | Giraudet et al. |
| Advanced renal cancer         | Carbonic anhydrase | cG250 | $^{177}$Lu | II    | Has results  | 2405 MBq/m$^2$ of $^{177}$Lu-girentuximab administered intravenously. RIT with $^{177}$Lu-girentuximab resulted in disease stabilization in 9 of 14 patients with progressive metastatic ccRCC, but myelotoxicity prevented retreatment in some patients. | NCT02002312 | Muselaers et al. |
| Indication                                                                 | Target                      | Antibody          | Isotope | Phase | Trial status       | Key results                                                                                                                                                                                                 | ClinicalTrials.gov identifier: | Reference |
|---------------------------------------------------------------------------|-----------------------------|-------------------|---------|-------|-------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------|-----------|
| Relapsed or refractory synovial sarcoma                                   | DFZ10                       | OTSA-101          | $^{90}$Y | I     | Recruiting        |                                                                                                                                                                                                             | NCT04176016                    |           |
| Mesothelin positive cancers                                              | Mesothelin                  | BAY2287411        | $^{227}$Th | I     | Active not recruiting |                                                                                                                                                                                                             | NCT03507452                    |           |
| Non-prostate metastatic solid tumors                                     | PSMA                        | J571              | $^{177}$Lu | I     | Active not recruiting |                                                                                                                                                                                                             | NCT00967577                    |           |
| Advanced/metastatic HER2-positive breast, gastric, and GEJ cancer         | HER2                        | Anti-HER2 fragment | $^{131}$I | I     | Has results       | No drug related AEs were observed, drug is primarily eliminated via the kidneys, focal uptake of $^{131}$I-OMIB-Anti-HER2-VHH1 observed in metastatic lesions via the kidneys                                                                 | NCT04467515                    | D’Huyvetter et al.⁵⁵            |
| Advanced/metastatic HER2-positive breast, gastric, and GEJ cancer         | HER2                        | CAM-H2            | $^{131}$I | I     | Not yet recruiting |                                                                                                                                                                                                             | NCT04467515                    |           |
| Advanced cancer expressing the HER2 protein                              | HER2                        | BAY2701439        | $^{227}$Th | I     | Recruiting        |                                                                                                                                                                                                             | NCT04147819                    |           |
| Advanced solid cancers                                                   | IGFR1                       | FPI-1434          | $^{211}$Ac | I     | Recruiting        |                                                                                                                                                                                                             | NCT03746431                    |           |
| P-cadherin positive cancers                                              | P cadherin                 | FF21101           | $^{90}$Y | I     | Recruiting, has results | DLT was not observed in any patient on study and dose reductions were not required over the course of the dose escalation. A complete clinical response was observed in a patient with clear cell ovarian carcinoma, correlating with a high tumor P-cadherin expression. Stable disease was observed in a variety of other tumor types, without dose limiting toxicity | NCT02454010                    | Subbiah et al.⁷¹               |

AE, adverse events; CNS, central nervous system; CR, complete remission; DLT, dose-limiting toxicity; FUdR, fluorodeoxyuridine; GEJ, gastro-esophageal junction; GI, gastrointestinal; HAI, hepatic arterial infusion; mCRPC, metastatic castration-resistant prostate cancer; OS, overall survival; PFS, progression free survival; PR, partial remission; PSA, prostate-specific antigen; RIT, radioimmunotherapy; SPECT, single photon emission computed tomography.
success. The published literature, which is limited to retrospective single arm studies, describes PSA responses (defined as a serum PSA fall of >50%) in 30–70% of patients with mCRPC. Given the nature of the studies with varying treatment regimens and doses of $^{177}$Lu, interpretation of this data is difficult. In one of the larger studies of 56 patients, overall survival at 28-month follow up was 78.6%. The first prospective phase II study, which was conducted in Australia, saw a PSA response of >80% achieved in 44% of patients. The main side effects observed, which are attributed to targeting of PSMA expressed in normal tissue, included dry mouth, nausea, and thrombocytopenia. In view of these results, trials are currently underway investigating the effect of combination therapy of $^{177}$Lu-PSMA with immunotherapy (PRINCE) and with a poly ADP ribose polymerase (PARP) inhibitor (LuPARP) as well as the effect of using LuPSMA in hormone naïve patients (UpFrontPSMA). It is important to note that use of $^{177}$Lu labelled full-length antibody J591, as opposed to the PSMA-617, have differences in side-effect profile, with more hematological toxicity observed for J591 and more non-hematological adverse events observed for the small molecule.

The size of the antibody limits exposure of certain normal tissue that the small molecule accesses, for example, the lacrimal glands.

**Dosimetry and personalized medicine: a new paradigm**

Achieving an optimal therapeutic window, that is, delivering sufficient radiation dose to the tumor with minimal dose to healthy tissue, in part defines the clinical success of an RIT. The use of paired diagnostic and therapeutic agents (the same targeting molecule conjugated to an imaging or therapeutic isotope) allows assessment of the in-body distribution of the tumor targeting molecule with an imaging radionuclide prior to delivery of cytotoxic radiation. The biodistribution of the imaging radionuclide allows calculation of the expected therapeutic radionuclide dose to tumor and healthy tissue. Commonly, monoclonal antibodies are distributed to the liver, spleen, and, in some cases, kidney, as a result of normal clearance mechanisms. This results in an inevitable dose of radiation to these organs, each of which vary in radio-sensitivity. For example, bone marrow and skin are more radio-sensitive, tolerating just 150 cGy, while lung and kidney may tolerate 1500–2000 cGy, and liver up to 3500 cGy. Dosimetry calculations can inform the optimal therapeutic dose to be delivered based on an understanding of the dose to tumor required for efficacy and safe levels of exposure to normal tissue and is performed on a patient-to-patient basis – an approach known as “personalized dosimetry”.

Clinical studies using J591 labelled with $^{111}$Indium ($^{111}$In), $^{90}$Yttrium ($^{90}$Y), and $^{177}$Lu demonstrated the predictive value of $^{111}$In biodistribution for radiation dose received during $^{90}$Y therapeutic studies. However, there was some variation, with an overestimation of liver dose by 25%. This study proposed the use of $^{177}$Lu distribution as a potential surrogate for $^{90}$Y dose estimation. Optimally, the same element would be used as the diagnostic and therapeutic agent, as different elements may exhibit different *in vivo* behaviors. The use of paired copper radionuclides [copper-64 ($^{64}$Cu) for imaging, and copper-67 ($^{67}$Cu) for therapy] is an approach in development. Currently, $^{64}$Cu conjugated to SARTATE, which targets somatostatin receptor type 2 (SSTR 2), is in clinical trials as an imaging agent for neuroendocrine tumors and phase IIa efficacy studies, pairing SARTATE $^{64}$Cu (imaging) and $^{67}$Cu (therapy) for meningioma (ACTRN12618000309280). Preclinically in animal models, $^{64}$Cu conjugated to a bivalent PSMA binder has shown improved targeting as compared with monovalent PSMA binder.

The small molecule PSMA-617 has been used theranostically, with $^{68}$Ga-PSMA-11 imaging to guide clinical decisions as well as a treatment with therapeutic $^{177}$Lu-PSMA-11. In a study using a different ligand-binding small molecules, PSMA-TUM1, in patients with mCRPC, all lesions identified on $^{68}$Ga labelled PSMA-TUM1 screening demonstrated high $^{177}$Lu uptake on post treatment planar imaging. A phase II study of $^{177}$Lu-PSMA-617 screened patients with mCRPC with a $^{68}$Ga-PSMA 617 scan, defining tumor uptake greater than 1.5 times the level of liver uptake as a positive scan. Patients with a positive scan went on to $^{177}$Lu-PSMA-617 treatment, which amounted to more than 70% of screened patients.

**Optimising therapeutic window**

A number of approaches have been proposed to improve the efficacy of RITs by minimizing antibody accumulation in non-tumor tissue resulting from on-target off-tumor binding or normal
clearance mechanisms. This approach aims to deliver minimal radiation dose to normal tissue whilst maximizing the dose delivered to the tumor, optimizing the so called “therapeutic window”. A dose of non-radiolaabeled tumor targeting antibody (“cold antibody”) may be delivered prior to (or simultaneously with) the radiolabeled version of the antibody, with the aim of saturating normal clearance mechanisms with the cold antibody, which reduces radiolabeled antibody uptake and radiation dose in normal tissues such as liver and spleen. The use of cold antibody also serves to deplete any circulating soluble antigen that may limit tumor exposure to the drug. Indeed, this approach has seen success clinically. Liver clearance of J591 was reduced significantly by pre-infusion with 25–100 mg unlabeled antibody prior to infusion of the radiolabeled antibody, resulting in improved tumor lesion uptake.84 Similarly, initial clinical studies showed that Lilotomab (anti-CD37 mAb) was localized predominantly to the liver, presumably due to clearance mechanisms, but tumor targeting could be improved by pre-dosing with both 40mg flat dose and 100 mg/m² dose of cold antibody as compared with no pre-dose.85 Interestingly, the amount of unlabeled antibody required to optimize tumor targeting is empirical, depending on the unique antibody itself, possibly a function of several factors including affinity, tumor antigen expression, and levels of circulating soluble antigen.

Dose-fractionation is the frequent dosing at lower radiation dose levels and is another means to increase cumulative tumor dose whilst minimizing off target exposure to healthy tissue. Fractionated dosing can also overcome antigen heterogeneity in the tumor by allowing more stable and uniform distribution of dose over time.86,87 Different parts of the tumor are accessed with each subsequent dose of radiation and, as the tumor responds, improved vascular permeability and access to the tumor permits the delivery of further radiation. This approach has been tested clinically for 177Lu-J591, where fractionated dosing achieved a dose dependent increase in overall survival in men with mCRPC, suggesting that dose fractionation is more efficacious than less frequent, higher dosing.54 Using a similar approach, 90Y-labelled humanized antibody clivatuzumab tetraxetan (hPAM4) allowed delivery of higher doses of radiation without dose limiting toxicity in patients with advanced pancreatic ductal carcinoma.88

Radioimmunotherapy: the target antigen

Ideally, a targeting antigen for RIT would be exclusively and highly expressed on tumor but not on normal tissue, theoretically eliminating any risk of “on-target, off-tumor” antibody binding. However, in practice, there are few antigens that exhibit such absolute specificity. Largely, “tumor antigens” are expressed at low levels on normal tissue or moderately expressed in a limited number of normal tissues. An example is PSMA, which has high expression in prostate cancer, moderate expression in salivary glands, and minimal expression in other normal tissues.72,89 Targeting of mutated tumor antigens is one way to achieve targeting that is highly tumor specific, since the mutations are expressed only by the tumor cell. The caveat to this approach is that tumor mutations are often not ubiquitous in patient populations, limiting the utility of targeting such antigens during drug development. Identification of a relatively specific tumor-associated antigen with limited normal tissue expression, is critical to the successful design of an RIT. Indeed, the clinical implication of low-level normal tissue expression may be mitigated by the above described approaches, such as dose fractionation and dosimetry.

GPC-1: a new targeting antigen with potential for therapy of solid tumors

GPC-1 is one of six members of the vertebrate family of glypican – a family of cell surface proteoglycans consisting of a core protein covalently linked to HS side chains, all encoded by separate genes.90,91 GPC-1 exists on the surface of cells attached by a glycosylphosphatidylinositol (GPI) anchor. The role of GPC-1 is thought to be developmental,92 with expression restricted in normal adult tissue,22,93 and its expression is not required for normal homeostasis.28 GPC-1 is expressed on tumor cells, controlling signaling of Wnts, Hedgehog, fibroblast growth factors (FGF), and transforming growth factor-beta (TGF-β). Acting on these pathways, GPC-1 promotes tumor and cancer stromal cell growth, tumor cell invasion, and metastasis.94 GPC-1 is known to be overexpressed in a variety of solid tumors and is associated with poor clinical prognosis and an aggressive phenotype, which is perhaps not surprising, given its known role in tumor biology, as discussed below. The biology of GPC-1 in several of these solid tumors has been investigated, with a wealth of evidence in pancreatic cancer.95
GPC-1 in solid tumors: expression and biology

Pancreatic cancer
The first study establishing the overexpression of GPC-1 in pancreatic cancer was published in 1998, demonstrating overexpression of GPC-1 in pancreatic cancer cells as well as adjacent stromal cells, but not in normal pancreatic tissue or chronic pancreatitis specimens.96 These findings were confirmed in a study by Kayed et al. comparing GPC-1 mRNA expression in normal pancreatic tissue with pancreatic ductal adenocarcinoma (PDAC) tissue samples.90 Although there was no statistically significant difference in the quantitative analysis of GPC-1 expression between node-positive and node-negative PDAC, there was a statistically significant difference in the expression of GPC-1 mRNA levels between all PDAC and normal pancreatic tissue, whereby PDAC tissue exhibited higher levels.91 Immunohistochemical (IHC) assessment of GPC-1 on surgically resected pancreatic cancer specimens and normal pancreatic tissue show that GPC-1 expression was significantly higher in cancer specimens compared with normal tissue.97 Importantly, GPC-1 expression was linked to prognosis in PDAC, where high GPC-1 expression as measured by IHC was associated with shorter overall survival time.24 There is a growing body of evidence demonstrating the potential of GPC-1 as a biomarker for pancreatic cancer, with several reports demonstrating correlation between serum levels of presumably tumor derived GPC-1+ exosomes with PDAC.98,99 However, there is no consensus, as some reports have shown no correlation between the two – an area yet to be clarified by future work in the field.91,98,100,101

Functional experiments explain a biological role for increased expression of GPC-1 in PDAC. In vitro, knockdown of GPC-1 in PDAC cell lines COLO-357 and PANC-1, reduced anchorage-dependent and independent growth, and inhibited mitogenic responses to FGF2 and HB-EGF.96,102,103 Knockdown of GPC-1 in athymic mice resulted in decreased tumor angiogenesis and tumor dissemination when mice were implanted with human pancreatic cancer cell lines (PANC-1 and T3M4).104 Moreover, knockdown of GPC-1 in a KRASG12D model of PDAC inhibited tumor growth through influence on angiogenesis and tumor metastasis.104 GPC-1 appears to play a role in PDAC progression both in tumor cells themselves, and in the tumor microenvironment.93

Breast cancer
GPC-1 staining was shown to be expressed differentially in human breast cancer, as compared with normal breast tissue, by both IHC and in situ hybridization.23 Higher levels of GPC-1 expression were seen in Stage 2 and 3 breast cancer tissue as compared with Stage 1, suggesting that GPC-1 expression correlates not only with the presence of tumor but is increased with disease progression, in line with its known biology.23 In vitro data using an GPC-1 antisense construct transfected breast cancer cells, demonstrated that knockdown of GPC-1 protein levels led to a decreased mitogenic response to heparin binding growth factors, supporting its role in disease progression in this tumor type.23

Brain tumors: glioblastoma, gliomas
IHC analysis of brain cancers, including astrocytoma and oligodendroglioma, demonstrated significant overexpression of GPC-1 when compared with benign gliosis.27 In studies evaluating the role of HS proteoglycans (HSPG) in human glioma cell lines, the glioma HSPGs were found to promote FGF-dependent tumor growth signaling via FGFR1c, with the level of GPC-1 expression reflecting FGF-2 signalling.27 More recently, higher expression of GPC-1 has been associated with increased metastatic potential and significantly decreased overall survival (OS) in patients with glioblastoma.105

Prostate cancer
The expression of GPC-1 in prostate cancer biopsy tissue was identified by IHC staining with BLCA-38, murine parent antibody to Miltuximab® – an anti-GPC-1 antibody in clinical phase development (GlyTherix Ltd). Expression was observed in 80% of malignant prostate biopsy tissue samples but not benign prostatic tissue and antibody reactivity increased with increasing Gleason score, indicating an association of GPC-1 with aggressive phenotype.22 Moreover, expression of GPC-1 in metastatic prostate cancer lesions has been observed by IHC (Bradley J Walsh, personal communication). Expression of GPC-1 in prostate cancer cell lines has also been reported.106 Miltuximab® conjugated to 67Ga for imaging has progressed through a first-in-human phase I clinical trial,
demonstrating targeting to prostate cancer lesions.107

**Bladder cancer**

BLCA-38 (also known as MIL-38) is an IgG1 murine monoclonal antibody (mAb) raised against the human bladder cancer cell line, UCRU-BL-17CL, and is the murine parent antibody to clinical stage antibody Miltuximab® (GlyTherix Ltd).22,108 BLCA-38 antibody detected cells in the voided urine from patients with transitional cell carcinoma of the bladder but showed no reactivity to cell sediments from normal control patients.108 The antibody also bound human bladder cancer cell lines. Binding of intraperitoneally injected radiolabeled (131Iodine or 153Samarium) BLCA-38 to orthotopic bladder tumor xenograft tissue was later demonstrated in a rat model.109 Subsequent work by GlyTherix Ltd. showed that the BLCA-38 antibody recognized GPC-1 as its target antigen.106

**Colorectal cancer**

Colorectal cancer (CRC) is a very heterogenous disease, with evidence supporting a different disease course for left- and right-sided primary cancers. In a study looking specifically at right-sided CRCs, strong staining for GPC-1 protein was seen in well differentiated neuroendocrine tumors by IHC, while there was less staining seen in poorly differentiated cancers. This is in line with observations in prostate cancer where higher Gleason grades are associated with higher expression levels of GPC-1.110 Transcription levels of genes encoding core proteins carrying HS chains for GPC-1 demonstrated a statistically significant difference between healthy tissue and tumors in both non-metastatic and metastatic colorectal cancers originating in the right side of the colon.110

**Cervical cancer**

Matsuzaki et al.111 investigated the expression of GPC-1 via IHC in cervical cancer. High levels were detected in approximately half of uterine cervical cancer tissues as well as in a relapsed tumor post radiotherapy.

**Gastro-esophageal cancer**

IHC analysis in esophageal squamous cell (ESCC) cancer tissues revealed GPC-1 expression in gastric cancer tissue as well as lymph node metastases, with none observed in normal adjacent tissue.112 IHC analysis of close to 200 esophageal cancer patients revealed 98.8% presence of GPC-1. High GPC-1 expression was associated with worse clinical prognosis, as compared with low GPC-1 expression.113 Furthermore, GPC-1 expression correlated with chemoresistance to platinum-based chemotherapy, pointing to the expression of GPC-1 in aggressive cancer, but also the potential for GPC-1 directed therapies in resistant tumours.26,112,114

Additionally, knockdown of GPC-1 controlled cell proliferation in ESCC tumor cells in vitro.112,115 Knockdown of GPC-1 resulted in a decrease in anti-apoptotic proteins as well as inhibition of EGFR, AKT, and p44/42-MAPK signaling pathways in these cancer cell lines in vitro.112 In vivo, blockade of GPC-1 with an antibody inhibited tumor growth in xenografts and patient-derived xenografts, in a manner partly dependent upon signaling blockade, influencing angiogenesis.112

**GPC-1 directed therapies in solid tumors**

The critical role of GPC-1 in modulating tumor biology and the microenvironment, its overexpression in a variety of solid tumors and the association with poor clinical prognosis and a more aggressive and resistant tumor phenotype, suggests its potential as a therapeutic target. Indeed, several pre-clinical studies have demonstrated the therapeutic efficacy of targeting GPC-1 in different cancers using different mechanisms.23,96,102 In a mouse xenograft of esophageal cancer, targeting of GPC-1 with an antibody was shown to inhibit tumor growth. This effect was seen independently of complement dependent cytotoxicity (CDC) or antibody dependent cellular cytotoxicity (ADCC) but was reliant on direct interaction of the antibody with GPC-1, likely by a signaling blockade. Interestingly, this study demonstrated a reduction in angiogenesis using staining for CD31 within the tumor as a proxy, suggesting a role for GPC-1 in establishment/maintenance of angiogenesis.112 In an animal model of uterine cervical cancer, a cytoxic antibody drug conjugate targeting GPC-1 demonstrated significant and potent tumor growth control.111

Importantly, both preclinical and clinical data demonstrate the safety of targeting GPC-1. Protein expression of GPC-1 in normal adult tissue is absent,22,28 and knockdown of GPC-1 in mice does not alter homestasis.93 Moreover, safety studies in mice delivering an anti-GPC-1 antibody...
(50 mg/kg) that recognizes mouse GPC-1, showed no adverse effects. A phase I first-in-human clinical trial of Miltuximab® targeting GPC-1, at a total antibody dose of 25 mg, proved safe.

**GPC-1 as a radioimmunotherapy target**

Given the clinical potential for RIT in the treatment of solid tumors, novel antigens for the delivery of radiolabels are required. Overexpression of GPC-1 in a variety of solid tumors, its role in tumor biology, its restricted expression in normal tissue and the demonstrated safety of targeting GPC-1, suggest its potential as a delivery molecule for RIT.

In a phase I study of Miltuximab® conjugated to $^{67}$Ga for imaging, targeting of GPC-1 was assessed primarily for an endpoint of safety, which was met. The secondary endpoint was tumor lesion targeting, which was seen in two patients with active prostate cancer lesions. The observation of targeting in this study suggests the potential of targeting GPC-1 clinically for imaging and therapy of prostate cancer lesions.

Preclinical studies have demonstrated the utility of GPC-1 as an RIT target for the imaging and therapy of prostate tumors. Miltuximab® radiolabeled with $^{89}$Zirconium via a DFO chelate was used for the PET visualization of DU-145 tumor xenografts in mice. The study demonstrated specific accumulation and retention of the antibody in the tumor out to 7 days, which was the experimental endpoint, as well as expected clearance in normal organs including the liver. Therapeutic studies using Miltuximab® conjugated to beta emitting $^{177}$Lutetium via DOTA in mice, demonstrated specific accumulation and retention in the tumor (imaged by Cerenkov luminescence at 3 and 5 days post injection, and $ex$ $vivo$ organ biodistribution by measure of radioactivity). Mice that received 3, 6, or 10 MBq $^{177}$Lu-Miltuximab® showed significant inhibition of tumor growth compared with control mice (Figure 1). In survival studies using 3 and 10 MBq doses, improved survival was achieved with a 3 MBq dose, and significantly improved survival was observed with 10 MBq. Importantly, the treatment was well tolerated with no adverse safety signals, which included assessment of organ histopathology.

Rationale for the targeting of GPC-1 for the delivery of therapeutic radiolabel is supported by several studies using BLCA-38 (parent antibody to MIL-38/Miltuximab®). BLCA-38 conjugated to $^{153}$Samarium was used to target UCRU-BL-17 human bladder cancer orthotropic xenografts in mice. Intrapertitoneal injection of BLCA-38, radiolabeled with $^{131}$I or $^{153}$Sm allowed visualization of the tumor. BLCA-38 tumor targeting and biodistribution in animals was compared with that of the J591 mAb that recognizes PSMA. Both were labelled with $^{125}$I to treat human prostate tumor xenografts of LNCaP-LN3 and DU-145 cell lines in mice. The study showed comparable targeting and no unusual localization in non-tumor tissue with both antibodies. BLCA-38 was also used as part of a cocktail of antibodies labelled with Bismuth-213 ($^{213}$Bi) to inhibit PC3 tumor growth and metastases in a mouse xenograft model.

The use of alpha-emitters such as $^{213}$Bi and Actinium-225 ($^{225}$Ac) as a component of targeted therapy has shown great promise both preclinically and clinically. In prostate cancer (with metastatic bone disease), for example, the United States Food and Drug Administration (FDA) in 2018 approved the use of the first alpha-emitter, Radium 223 ($^{223}$Ra). The real interest in alpha-emitters is their potency and short path length, minimizing the risk of damage to surrounding tissue. Internalization of the antibody–antigen complex allows proximity of the alpha particle to the nucleus and potentially better efficacy, thus internalizing antibodies are optimal for targeting of alpha particles. Interestingly, there may be some advantage to antibodies that internalize over those non-internalizing, for example, Boudousq et al. compared efficacy of internalizing anti-HER2 mAbs and non-internalizing anti-CEA mAbs using $^{212}$Pb in peritoneal carcinomatosis, finding better efficacy with the internalizing mAb, though the different target antigens should be noted. The anti-GPC-1 antibody BLCA-38 demonstrated efficacy against PC3 primary tumor and metastases, and, although internalization of the antibody was not studied in the report, findings in our laboratory have demonstrated rapid internalization of Miltuximab® antibody in GPC-1 expressing cell lines (unpublished findings), suggestive of its suitability for alpha targeting.
Molecular targeting therapy, as a theranostic or solely therapeutic approach, using antibodies, is an emerging field in cancer treatment. Exciting developments, particularly in the treatment of prostate cancer, have brought this approach to the forefront, with the success of agents such as 177Lu-PSMA-617. However, novel targets are required to achieve optimal tumor targeting with minimal normal tissue exposure and to allow targeting of different solid tumors refractory to available treatment.

GPC-1 is a target antigen with great potential, exhibiting overexpression in a variety of solid tumors, but without normal adult tissue expression. Indeed, its utility as an RIT target for prostate cancer has been demonstrated in preclinical models, and a first-in-human clinical trial using 67Ga-labelled Miltuximab® has been completed in prostate, pancreatic and bladder cancer patients. A phase I trial for GPC-1 positive solid tumor patients using 89Zr Miltuximab® for imaging and 177Lu Miltuximab® for therapy is planned. Anti-GPC-1 RITs have potential for use in a number of diseases where novel imaging agents and treatments are needed, including lethal tumors like glioblastoma, gastroesophageal cancer, and pancreatic cancer. The use of different radioisotopes (alpha versus beta emitters) may have specific advantages in some tumors. GlyTherix has ongoing preclinical programs examining targeting and therapy of Miltuximab® and Miltuximab® antibody fragments in combination with 89Zr, 177Lu, and 225Ac in prostate, bladder, pancreatic cancer, and glioblastoma.

**Figure 1.** (a) Safety of GPC-1-directed RIT 177Lu-Miltuximab® (3–10 MBq) in mice. (b–d) Efficacy measured by growth inhibition of 177Lu-Miltuximab® (3–10 MBq) in mice bearing DU-145 prostate cancer xenografts, showing tumor volumes [b], tumor control index [c], and survival [d]. Reproduced from Yeh et al.116.

**A GPC-1 RIT: potential clinical utility**

GPC-1 is a target antigen with great potential, exhibiting overexpression in a variety of solid tumors, but without normal adult tissue expression. Indeed, its utility as an RIT target for prostate cancer has been demonstrated in preclinical models, and a first-in-human clinical trial using 67Ga-labelled Miltuximab® has been completed in prostate, pancreatic and bladder cancer patients. A phase I trial for GPC-1 positive solid tumor patients using 89Zr Miltuximab® for imaging and 177Lu Miltuximab® for therapy is planned. Anti-GPC-1 RITs have potential for use in a number of diseases where novel imaging agents and treatments are needed, including lethal tumors like glioblastoma, gastroesophageal cancer, and pancreatic cancer. The use of different radioisotopes (alpha versus beta emitters) may have specific advantages in some tumors. GlyTherix has ongoing preclinical programs examining targeting and therapy of Miltuximab® and Miltuximab® antibody fragments in combination with 89Zr, 177Lu, and 225Ac in prostate, bladder, pancreatic cancer, and glioblastoma.

**Glioblastoma**

The advent of stereotactic radiosurgery (SRS) has changed the way we treat glioblastoma and forms...
a component of standard of care together with debulking surgery and chemotherapy. Despite this, the prognosis of glioblastoma is poor, due to recurrent disease, with a 5-year survival rate of less than 5%,\textsuperscript{125} with ultimate disease progression causing neurological deficit and eventual death. The utility of high-dose SRS is limited to smaller lesions away from critical neural structures; however, these lesions need to be seen radiologically to be treated – a barrier to effective use – particularly given the infiltrative nature of the tumor. Moreover, one of limitations of radiation treatment of glioblastoma is the associated neurological defect caused by radiation dose to surrounding brain tissue. Targeting of radiation \textit{via} the use of an RIT to treat unresectable tumor while minimizing dose to normal brain tissue, may have clinical utility, and this approach has reviewed recently been.\textsuperscript{126} Drug delivery to brain tumors has its own considerations, given the generally poor accessibility of systemically delivered drugs; however, there is precedence for antibody access to the brain when delivered systemically, and other delivery routes are available.\textsuperscript{126}

Given the expression of GPC-1 in glioblastoma, targeting by radiation \textit{via} a GPC-1-directed RIT may have utility as an adjuvant treatment postsurgery, specifically targeting minimal residual disease or micro-metastatic disease in order to prevent recurrence, whilst sparing healthy brain tissue. Indeed, calculation of dosimetry using an accompanying GPC-1 targeting imaging agent would allow determination and minimization of radiation dose to normal brain tissue, whilst ensuring sufficient radiation dose to tumor. Fractionation of RIT can potentially be used in older, more fragile patients, whose tolerance to radiation is dose limiting for SRS, in order to achieve therapeutic levels of radiation to the tumor whilst minimizing toxicity to surrounding normal tissue.\textsuperscript{127} Alpha particle RIT has previously been investigated in the treatment of glioblastoma, conjugated to an antibody against vascular endothelium cadherin (E4G10).\textsuperscript{128} In mouse models of high-grade glioblastoma \textsuperscript{225}Ac-labelled E4G10 showed high accumulation in tumor tissues expressing the antigen, with improved survival when compared with controls. Furthermore, the combination of temozolomide with this RIT resulted in further survival benefit,\textsuperscript{128} supporting the hypothesis that RIT may be useful as a monotherapy and/or in combination with SOC treatments to increase clinical benefit.

**Gastroesophageal cancer**

Gastroesophageal cancer is aggressive, increasing in global prevalence, and treatment options are limited. Definitive chemoradiotherapy is accepted as standard of care for these cancers; however, the outcomes for these patients remain poor, with only one in five patients surviving at 5 years. Studies investigating increasing the dose of radiotherapy to the primary tumor have suggested that this may increase local control and survival; however, this has not been proven in phase III studies.\textsuperscript{129} In addition, more intense radiation doses confer higher incidence of toxicity, which can limit treatment. Targeting of radiation using a GPC-1 directed RIT may have utility for this class of cancer. Targeting of esophageal tumors with an anti-GPC-1 antibody has been demonstrated in animal models.\textsuperscript{112} The use of RIT with an alpha emitter in this group of patients may be particularly useful. Alpha emitters have short path length and intense energy expenditure so permit targeted tumor cell killing with less damage to surrounding normal tissue.\textsuperscript{33} The increased expression of GPC-1 seen in chemotherapy resistant esophageal tumors would suggest the utility of a GPC-1 RIT in this resistant patient population for whom treatment options are limited.

**Prostate cancer**

Whilst targeting of PSMA with \textsuperscript{68}Ga-PSMA-PET and \textsuperscript{177}Lu-PSMA-617 or \textsuperscript{177}Lu-J591 has shown promise for imaging and therapy of prostate cancer, these therapies are not applicable to all patients since not all patients’ tumors overexpress PSMA.\textsuperscript{73} Furthermore, PSMA expression can be heterogeneous within a patient. PSMA is not expressed exclusively in prostate tumor tissue; rather, it is overexpressed, with evident expression in normal prostate, small intestine, proximal renal tubules and salivary glands, which results in a radiation dose to these tissues during therapy and related side effects. IHC studies using BLCA-38 indicate that GPC-1 is not expressed in normal adult tissue,\textsuperscript{22} and other studies show it is not required for normal homeostasis,\textsuperscript{28} thus GPC-1 may represent an attractive target antigen for those patients not suitable for, or who have failed, PSMA-directed therapy. Given the increase in GPC-1 expression observed with increasing Gleason grade, and the role for GPC-1 in tumor progression, invasion, and metastasis, a GPC-1 targeted RIT may have special utility for monitoring of disease and/or treatment response. Indeed,
in a phase I study of $^{67}$Ga-Miltuximab®, targeting was observed to prostate cancer lesions in two men with particularly aggressive disease.\textsuperscript{118}

**Pancreatic cancer**

Pancreatic cancer treatment has seen minimal improvement over recent decades.\textsuperscript{8} Disappointing responses have been seen despite the advent of immunotherapy.\textsuperscript{8} Novel strategies for targeting this cancer are required, and the well described expression of GPC-1, and its role in the biology of this tumor, suggest its potential clinical utility as a targeting agent for RIT. The utility of RIT in the management of pancreatic cancer is being explored. In a study of $^{90}$Y labelled anti-$\alpha$$\beta$4 integrin antibody, single and double doses of the radiolabeled mAb showed significant reduction in cell proliferation and tumor growth when compared with treatment with unlabeled mAb.\textsuperscript{130} Of note, the double dose of radioimmunotherapy resulted in increased myelosuppression compared with the single dose.\textsuperscript{130} This suggests that further studies of dose fractionation are warranted to minimize toxicity whilst maintaining radiation dose delivery and improving efficacy in this tumor type.

**Conclusion**

The potential for RIT in the treatment of solid tumors has been demonstrated clinically, and methods to optimize the therapeutic efficacy of an RIT approach in these less radio-sensitive tumors are well established. Novel tumor targets with ideal expression profiles (tumor expression with limited expression in normal tissue) are required to expand RIT treatment options for solid tumors. GPC-1 plays a critical role in the growth factor signaling pathways, controlling and promoting multiple aspects of tumor growth and metastasis. Its expression has been identified in a number of solid tumors, many of which have limited therapeutic options despite the evolution of novel anti-cancer therapies. Pre-clinical studies have demonstrated that RIT using GPC-1 targeting antibody is well-tolerated and effective way of treating prostate cancer. Clinically, targeting of GPC-1 is expected to be safe based on both clinical and pre-clinical data. Further studies investigating the therapeutic potential of a GPC-1 RIT in a range of solid tumors where novel therapies are needed is warranted.

**Conflict of interest statement**

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