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Meeting Report from the Prostate Cancer Foundation PSMA Theranostics State of the Science Meeting

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Conflicts of Interest:

JWB is an inventor on the some of the constructs described in this manuscript and holds equity in Noria Therapeutics, Inc.

NHB is an inventor on patents that are assigned to Cornell University for anti-PSMA antibody technology, is a consultant (compensated) and equity holder in BZL Biologics, LLC, the company to which the patents were licensed by Cornell University for further research and development, and is a SAB member (uncompensated) and equity holder in Telix Pharmaceuticals Ltd.

JC reports consulting activities for Blue Earth Diagnostics Inc., Janssen Pharmaceuticals Inc., Curium Pharma, Advanced Accelerator Applications, GE Healthcare, Telix Pharmaceuticals, Progenics Pharmaceuticals, Inc. and Radiomedix, Inc. outside of the submitted work.

MSH receives research support from Endocyte Inc. (a Novartis company). Unrelated to this work he has received honoraria and travel support for educational talks from Janssen, Ipsen and Sanofi Genzyme.

SML was named as one of the inventors in the following patent applications relating to GPA33: SK2014-074, SK2015-091, SK2017-079, SK2018-045, SK2014-116, SK2016-052, and SK2018-068 filed by MSK. SML reports receiving commercial research grants from YMABS Therapeutics Inc., Genentech, Inc., WILEX AG, Telix Pharmaceuticals Limited, and Regeneron Pharmaceuticals, Inc.; holding ownership interest/equity in Elucida Oncology, Inc., and holding stock in YMABS Therapeutics Inc., ImaginAb, Inc. SML is the inventor and owner of issued patents both currently unlicensed and licensed by MSK to Samus Therapeutics, Inc., Elucida Oncology, Inc., and Y-mAbs Therapeutics, Inc. SML serves or has served as a consultant to Cynvec LLC, Eli Lilly & Co., Prescient Therapeutics Limited, Advanced Innovative Partners, LLC, Gerson Lehrman Group, Progenics Pharmaceuticals, Inc., and Janssen Pharmaceuticals, Inc.

MJM is an unpaid advisor to Bayer, Advanced Accelerator Applications, Endocyte, Progenics, and Johnson and Johnson, is a compensated advisor to ORIC and Curium, and has institutional contracts for clinical trials conduct with Bayer, Sanofi, Endocyte, Progenics, Corcept, Roche/Genentech, and Janssen.

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MGP is a co-inventor on a U.S. patent covering [^{18}F]DCFPyL and as such is entitled to a portion of any licensing fees and royalties generated by this technology. This arrangement has been reviewed and approved by the Johns Hopkins University in accordance with its conflict-of-interest policies.

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Abstract

Introduction. The Prostate Cancer Foundation (PCF) convened a PCF PSMA Theranostics State of the Science Meeting on November 18, 2019, at Weill Cornell Medicine, New York, NY.

Methods. The meeting was attended by 22 basic, translational, and clinical researchers from around the globe, with expertise in prostate-specific membrane antigen (PSMA) biology, development and use of PSMA theranostics agents, and clinical trials. The goal of this meeting was to discuss the current state of knowledge, the most important biological and clinical questions, and critical next steps for the clinical development of PSMA positron emission tomography (PET) imaging agents and PSMA-targeted radionuclide agents for patients with prostate cancer.

Results. Several major topic areas were discussed including the biology of PSMA, the role of PSMA-targeted PET imaging in prostate cancer, the physics and performance of different PSMA-targeted PET imaging agents, the current state of clinical development of PSMA-targeted radionuclide therapy (RNT) agents, the role of dosimetry in PSMA RNT treatment planning, barriers and challenges in PSMA RNT clinical development, optimization of patient selection for PSMA RNT trials, and promising combination treatment approaches with PSMA RNT.

Discussion. This article summarizes the presentations from the meeting for the purpose of globally disseminating this knowledge to advance the use of PSMA-targeted theranostic agents for imaging and treatment of patients with prostate cancer.

Key Words

PET imaging, radiopharmaceuticals, radiation therapy, radionuclides, prostate-specific membrane antigen (PSMA), clinical trials, nuclear medicine, radiology, urology, medical oncology

Introduction

Prostate specific membrane antigen (PSMA) has emerged as one of the most promising theranostic targets for prostate cancer. Several PSMA-targeted small molecules and antibodies have been developed and are being tested as positron emission tomography (PET) imaging and radionuclide therapy (RNT) agents. PSMA-PET imaging agents, including ^{68}Ga-PSMA-11 and ^{18}F-DCFPyL, have been demonstrated in numerous clinical trials to outperform current standard imaging for both specificity and sensitivity for detecting sites of prostate cancer. ^{68}Ga-PSMA-11 is currently under FDA review for biochemical recurrence localization and initial staging of prostate cancer and ^{18}F-DCFPyL will shortly be considered by the FDA likely for a similar indication with a response expected later in 2020-2021. PSMA-RNT agents have also demonstrated significant promise in phase 2 trials and case reports of individual patients treated outside the US. The beta-emitting therapeutic agent ^{177}Lu-PSMA-617 is now being tested in the international phase 3 VISION trial and the Australian randomized phase 2 TheraP trial. However, despite promising results and high interest from academic and pharmaceutical drug developers, many questions yet remain about how best to deploy these agents to maximize patient benefit along with the implications of PSMA-targeted theranostics.

In recognition of this highly promising class of theranostic agents, the Prostate Cancer Foundation (PCF) convened the PCF PSMA Theranostics State of the Science Meeting to

discuss and outline the most urgent questions surrounding the biology and optimal clinical use of these agents. This meeting is a follow-up to a previous PCF PSMA-directed radionuclide scientific working group held in 2017 [1].

The meeting was held at Weill Cornell Medicine, New York, New York on November 18, 2019. The meeting was attended by 22 basic, translational, and clinical researchers with expertise in PSMA biology and theranostics, from several academic institutions in the U.S. and Australia, the NIH/NCI and Bayer Pharmaceuticals. There were 13 speakers who discussed different aspects of PSMA biology in prostate cancer, PSMA-targeted PET imaging, and PSMA-targeted RNT.

This review provides a summary of the presentations from the meeting for the purposes of disseminating this knowledge and the critical next steps identified to the global community, in order to rapidly optimize the use of PSMA-targeted agents for the imaging and treatment of prostate cancer.

The Biology of PSMA

PSMA, also known as glutamate carboxypeptidase II (GCP-II) and folate hydrolase 1 (FOLH1), is a cell surface transmembrane glycoprotein enzyme with several features that qualify it as an excellent prostate cancer theranostic target. The extracellular domain makes up 95% of the PSMA protein providing an easily accessible target for both small molecule and antibody-based agents. PSMA does not appear to function as a cellular receptor, though it is hypothesized to function in signaling and it may have a role in cleaving glutamate from folate to activate molecular pathways [2]. PSMA is highly overexpressed on most prostate cancer cells, being consistently found on over 94% of prostate cancer samples across many independent immunohistochemistry studies. While there is PSMA expression on non-prostate tissues, mainly in kidney, duodenum, salivary and lacrimal glands and non-myelinated ganglia nerves [3], the high levels of PSMA overexpression in prostate cancer (up to 100-1000 fold) makes PSMA an excellent prostate cancer theranostic target.

PSMA-Targeted Agents

A number of PSMA-targeting small molecule and antibody agents have been developed and tested for imaging by single-photon emission computerized tomography (SPECT) and PET, and for RNT applications. Development of PSMA-targeted small molecules was accelerated by the discovery of urea-based ligands with the binding motif (glutamate-urea-lysine [Glu-urea-Lys]) that bind to the extracellular domain of PSMA [4-6]. Most PSMA-targeting ligands currently under development are derivatives of these early urea-based compounds. PSMA-targeted small molecule ligands may be preferable to antibodies as PET imaging agents due to their rapid clearance kinetics resulting in a higher tumor to background ratio, ability to read images within 1-2 hours (as opposed to days with antibodies), as well as the ease and cost of manufacture of small molecule ligands relative to antibody-based approaches.

PSMA-targeted agents are also being investigated for use in MR imaging [7], chemical exchange saturation transfer (CEST) imaging [8], photoacoustic imaging [9], and optical imaging for surgical guidance [9].

PSMA PET imaging in prostate cancer

^{111}In-capromab pendetide (ProstaScint™) was an early strategy using a PSMA-directed radiolabeled antibody as a prostate cancer imaging agent [10]. While ^{111}In-capromab pendetide could detect sites of disease, the images produced were substantially inferior to the current largely small molecule-based PSMA PET imaging technologies because capromab binds to an intracellular region of PSMA that was difficult to reach for circulating antibody (except where there were dead or dying cells) and planar/SPECT imaging lacks the resolution of current PET technology. Subsequent studies utilized anti-PSMA antibodies and minibodies radiolabeled with the PET emitter ^{89}Zr with substantially improved results. However, because of the long circulating times of antibody-based agents, injection and imaging cannot be performed on the same day, limiting practical use in clinical development as an imaging agent [11, 12]. Nonetheless, these studies, which included biopsy confirmation, provided strong rationale for developing PSMA-directed molecules targeting the external domain of PSMA, with a short half-life, and that can be conjugated with a positron-emitting radionuclide.

Since this time, ^{18}F- and ^{68}Ga-based PSMA-targeted PET imaging agents have made significant progress in clinical development. These tracers enable the detection of metastatic lesions as small as 2mm (a volume-based estimation of ~10-14 million cells) [13-15]. As a comparison, the circulating tumor DNA based cancer screening tests that are currently under development have a limit of detection estimated at 50 million tumor cells [16].

PSMA-directed PET imaging agents under development for prostate cancer imaging include unpatented free-for-use agents which are typically labeled with ^{68}Ga (^{68}Ga-PSMA-11 and ^{68}Ga-PSMA-I&T), and those under development by biopharma: ^{18}F-based (^{18}F-DCFPyL, ^{18}F-rhPSMA and ^{18}F-PSMA-1007) and ^{68}Ga-based (^{68}Ga-THP-PSMA and TLX591-CDx). Of these, ^{68}Ga-PSMA-11 and ^{18}F-DCFPyL are furthest along, and will be the focus of discussion in this review.

PSMA PET imaging in prostate cancer has been widely investigated for many contexts of use including initial staging in high-risk patients and the detection and localization of disease in the setting of a biochemical recurrence (BCR), and has demonstrated superiority in multiple studies against other standard agents and modalities [17-19]. A team led by academic investigators from UCLA and UCSF have recently submitted data [17, 18] for an New Drug Application (NDA) to the U.S. Food and Drug Administration (FDA) for ^{68}Ga-PSMA-11 as a PET imaging agent for initial and subsequent management decisions in patients with prostate cancer. The phase 3 CONDOR trial (NCT03739684) evaluating ^{18}F-DCFPyL PET/CT in patients with suspected biochemical recurrence (BCR) of prostate cancer completed enrollment in August 2019 [20, 21]. Topline results and submission of an NDA to the FDA for ^{18}F-DCFPyL are anticipated in 2020 and FDA approval is anticipated in 2021 [20]. In these settings, the improved sensitivity afforded by PSMA PET imaging for early detection of metastatic lesions may change treatment planning and could improve patient outcomes. PSMA PET imaging may also be useful for selecting candidates for active surveillance [22].

PSMA PET imaging is also being studied as a theranostic tool to guide treatment planning for patients with both oligometastatic non-castrate disease and metastatic CRPC. For example, in a recent study by Tran et al., the use of ^{18}F-DCFPyL PET imaging to guide treatment with stereotactic ablative radiotherapy (SABR) to sites of spread in the oligometastatic setting significantly improved distant metastasis-free survival times compared to conventional imaging [23]. PSMA PET is also being used for the selection and therapeutic monitoring of patients for

PSMA-targeted radionuclide therapy (discussed extensively in following sections) and other PSMA-targeted approaches. Other roles of PSMA PET in patients with advanced / metastatic castration-resistant disease remain to be defined.

Separately, and because PSMA is expressed on the tumor neovasculature of many non-prostate solid tumors, applications outside of prostate cancer are also being explored [24-29].

Gallium vs Fluorine PSMA-PET: Differences in Physics and Production

The major differences between ^{18}F- and ^{68}Ga-based agents stem from the physical properties of the isotopes, including their half-lives, positron energies and the methods necessary for their production [17].

^{68}Ga-PSMA-11 can be produced by any center with the capability and a facility able to perform Gallium-68 labelling. The short half-life (68 minutes) of Gallium-68 is beneficial for onsite facility production but reduces ability to distribute to satellite sites. ^{68}Ga is obtained from germanium-68/gallium-68 radionuclide generators. Radiolabeling of the PSMA-ligand necessitates qualified staff and can be performed only in small batches, usually 2 doses at a time, up to 6 per day per generator. But due to the unpatented chemical structure of PSMA-11 (HBED-CC) [30], ^{68}Ga-PSMA-11 has been widely used at academic centers internationally and data from over 10,000 patients has been published, despite a lack of industry support. Among other unpatented ^{68}Ga-based agents that have been investigated (^{68}Ga-PSMA-11, ^{68}Ga-PSMA-I&T), PSMA-11 is the most widely used [30].

In contrast, ^{18}F-based agents are more amenable to commercial development. ^{18}F production requires a cyclotron and can be obtained in high quantity. Its longer half-life (110 minutes) enables central production and distribution to satellite sites. As an example, ^{18}F-FDG is widely available. ^{18}F-based agents being developed by biopharma include ^{18}F-DCFPyL, ^{18}F-rhPSMA and ^{18}F-PSMA-1007. An ^{18}F-based agent may be more practical and more commonly used in some parts of the world for patients and health care providers due to the high production yields and because deliveries can parallel those for ^{18}F-FDG PET, with ready-to-inject syringes provided for single patient use.

Ultimately, however, it is thought that the choice of PSMA-PET agents used in practice will be highly influenced by the IP and financial considerations surrounding the different PSMA-targeted ligands, as the performance of currently used ligands may be similar.

Gallium vs Fluorine PSMA-PET: Detection Performance

Diagnostic performance parameters for PSMA PET imaging agents have been reported in several clinical settings including the detection of pelvic lymph node metastases in patients with high risk prostate cancer and detection of recurrent prostate cancer sites in patients with BCR.

A prospective multicenter trial evaluated the performance of ^{68}Ga-PSMA-11 in 635 patients with biochemically recurrent prostate cancer [18]. The sensitivity of ^{68}Ga-PSMA-11 for detecting sites of recurrent prostate cancer increased with PSA levels: 38% in patients with PSA <0.5 ng/mL (N = 136), 57% in patients with PSA 0.5 -1.0 ng/ML (N = 79), 84% in patients with PSA 1.0 - 2.0 ng/mL (N = 89), 86% in patients with PSA 2.0 - 5.0 ng/mL (N = 158), and 97% in patients with

PSA ≥5.0 ng/mL (N = 173) [18]. Overall, in this study, the positive predictive value for ^{68}Ga-PSMA-11 in detecting sites of recurrent prostate cancer was 92% [18].

Similar detection rates have been reported for ^{18}F-DCFPyL in the BCR setting. A single site study at Johns Hopkins University in patients with biochemically recurrent prostate cancer reported sensitivity for ^{18}F-DCFPyL ranging from 59.1% at PSA levels between 0.20 – 1.00 ng/mL (N = 22) to 88.9% in patients with PSA > 1.00 ng/mL (N = 9). In a study at the NCI, the sensitivity of ^{18}F-DCFPyL in 90 patients with BCR was 47.6% in patients with PSA 0.20 – 0.5 ng/mL, 50.0% in patients with PSA 0.5 – 1.0 ng/mL, 88.9% in patients with PSA 1.0 – 2.0 ng/mL, and 94.0% in patients with PSA >2.0 ng/mL [31]. On a per-patient basis, the positive predictive value in this study was 93.3% by histopathologic validation and 96.2% by histology, imaging and/or clinical follow-up [31].

In patients with high risk prostate cancer, in a single site study at Johns Hopkins, ^{18}F-DCFPyL had a sensitivity of 71.4% and specificity of 88.9% for detecting sites of pelvic lymph node metastases (N = 25) [32]. In the OSPREY study, the sensitivity of ^{18}F-DCFPyL in patients with high risk prostate cancer (cohort A, N = 268) was 40.3%. The lower sensitivity in OSPREY may be due to the inclusion of community practices in the trial and scans being read by clinicians who have less experience with PSMA PET.

Meta-analyses have also been conducted to evaluate detection rates of ^{68}Ga-PSMA-11 (37 articles including 4,790 patients, [33]) and pooled analysis of ^{18}F-based agents (6 articles including 645 patients, [34]) in prostate cancer patients with BCR. At PSA levels > 2.0 ng/mL, ^{18}F-PSMA and ^{68}Ga-PSMA-11 PET agents had a similar detection rates (92%, 95%, respectively) [33, 34]. However, at lower PSA levels, ^{18}F-PSMA agents appear slightly but increasingly superior to ^{68}Ga-PSMA-11 (detection rates of 73% vs. 59% for PSA levels between 0.5 – 1.0 ng/mL, respectively) [33, 34].

Several studies have compared ^{18}F-PSMA and ^{68}Ga-PSMA-11 in consecutive cases and/or head-to-head [35, 36]. A study in biochemically recurrent prostate cancer compared detection rates per-patient and per-lesion for 62 patients imaged with ^{18}F-DCFPyL vs 129 patients imaged with ^{68}Ga-PSMA-11 [35]. At PSA levels between 0.5 - 3.5 ng/mL, ^{18}F-DCFPyL detected ~22% more lesions than ^{68}Ga-PSMA-11 (88% vs 66%). However, outside of this range, detection rates for ^{18}F-DCFPyL vs ^{68}Ga-PSMA-11 were comparable (13% vs 11% at PSA levels < 0.5 ng/mL; 84% vs 91% at PSA levels >3.5 ng/mL) [35]. In this study, in 25 patients imaged with both scans, lesions were detected by both scans in 11 of 25 (44%) of patients [35]. ^{18}F-DCFPyL detected additional lesions in 4 of 25 patients (16%), but without resulting in patient upstaging [35].

Overall, the diagnostic performances of ^{68}Ga-PSMA-11 and ^{18}F-DCFPyL appear to be either similar, or slightly superior with ^{18}F-DCFPyL [33-36]. The lower energy and positron range of ^{18}F (E_{max} = 633 keV) compared with ^{68}Ga (E_{max} = 1,899 keV) may result in improved spatial resolution and a cleaner image [17, 36, 37]. However, a meaningful difference will be hard to prove in a prospective head-to-head comparative trial and will require a large study.

^{18}F-PSMA-1007 is a newer agent in development that differs by having predominantly hepatobiliary instead of urinary excretion. This enables improved imaging of recurrent lesions in the prostate bed compared with other PSMA-PET agents [38]. Urinary excretion poses limits on PSMA PET imaging for detecting prostate bed recurrences, although this can be partially overcome with approaches including adjusting imaging scales, delayed imaging after forced

diuresis, hyperhydration, or voiding of the bladder before imaging [39]. Studies have suggested that ^{18}F-PSMA-1007 may perform equally or slightly better than ^{68}Ga-PSMA-11 at detecting lesions in patients with biochemically recurrent prostate cancer [40]. However, some suspected bone lesions found on ^{18}F-PSMA-1007 were not corroborated on contrast enhanced MRI, suggesting false positive findings in bone may occur with this agent [41]. Additionally, in a matched-pair comparison study, ^{18}F-PSMA-1007 detected an equal number of malignant lesions compared with ^{68}Ga-PSMA-11, but five-times more lesions that were found to be benign [42].

PSMA PET vs Metabolic PET Imaging Agents for Prostate Cancer

Currently, there are two PET imaging agents approved for detection and localization of biochemically recurrent prostate cancer: the metabolic tracers ^{11}C-Choline and ^{18}F-fluciclovine (^{18}F-FACBC, Axumin). ^{18}F-sodium fluoride (^{18}F-NaF), which measures bone production, is also FDA approved, but its clinical use has been limited because it is generally not reimbursed. Despite significant impact on clinical management data from the National Oncologic PET Registry (NOPR) [43], the Centers for Medicare & Medicaid Services (CMS) has denied its coverage since 2018.

^{18}F-fluciclovine is a metabolic PET imaging agent which measures amino acid uptake. It is an amino acid analog (l-leucine) that is taken up into cells via the LAT1 and ASCT2 amino acid transporters, both of which have been shown to be upregulated in prostate cancer. Choline is an essential cell membrane phospholipid precursor, and ^{11}C-choline is rapidly taken up in proliferating cells. ^{18}F-fluciclovine was shown to have slightly better detection rates than ^{11}C-Choline (37.1% vs. 33.7%) in biochemically recurrent prostate cancer patients (N=89, mean PSA 6.99 ± 17.5 ng/ml) [44]. ^{18}F-fluciclovine became FDA approved as the new standard of care molecular imaging agent for patients with prostate cancer recurrence in 2016.

A number of studies have focused on comparing the sensitivity and specificity of PSMA PET imaging agents with these metabolic tracers. A study which directly compared ^{68}Ga-PSMA-11 vs. ^{18}F-Fluromethylcholine PET/CT in 38 prostate cancer patients with BCR (mean PSA 1.74 ± 2.54 ng/mL) after curative treatment demonstrated a detection rate of recurrent lesions of 66% for ^{68}Ga-PSMA-11 vs. 32% for ^{18}F-Fluromethylcholine [45]. A study which compared ^{18}F-PSMA-1007 vs. ^{18}F-Flurocholine PET/CT in 40 prostate cancer patients with a biochemical recurrence (PSA < 0.2 ng/mL), demonstrated a detection rate of recurrent lesions of 60% for ^{18}F-PSMA-1007 vs. 5% for ^{18}F-Flurocholine [46]. Lesions detected by both agents in this study had a significantly higher standardized uptake value (SUV) for ^{18}F-PSMA-1007 than ^{18}F-Flurocholine [46]. These studies support PSMA-PET imaging as superior to ^{18}F-labelled choline derivatives in the biochemically recurrent prostate cancer setting.

A head-to-head comparison of ^{18}F-fluciclovine vs. ^{68}Ga-PSMA-11 PET/CT (NCT03515577) was performed in 50 patients with early biochemically recurrent prostate cancer (PSA <2.0 ng/mL, median PSA 0.5 ng/mL) [17]. In this study, each scan was independently read by three blinded independent central readers (BICR). ^{68}Ga-PSMA-11 was found to be the superior imaging modality, with a detection rate of 56% vs. 26% for ^{18}F-fluciclovine [17] and in particular detected more lesions than ^{18}F-fluciclovine in pelvic lymph nodes, extra-pelvic nodes, bone, other organs, and extra-pelvic lesions, while ^{18}F-fluciclovine detected more lesions than ^{68}Ga-PSMA-11 only in the prostatic bed. SUV_{max} was also higher in the same lesions detected by ^{68}Ga-PSMA-11 than ^{18}F-fluciclovine in this study. These differences are likely mediated in part by the pharmacokinetics of the agents. ^{18}F-fluciclovine has a high background due to its uptake by

metabolically active tissue such as muscle, but a low bladder activity at early imaging because renal excretion is delayed. Inter-reader agreement was higher for ^{68}Ga-PSMA-11 than ^{18}F-fluciclovine in this study, due to the higher target to background ratio of ^{68}Ga-PSMA-11.

False negatives and false positives with PSMA PET imaging

Despite the substantially improved sensitivity of PSMA PET, false negatives can still arise due to absent or insufficient expression levels or heterogeneity of expression of PSMA in the tumor, or tumors < 4mm that are below the level of resolution of PET technology. PSMA expression levels are lower in lower Gleason grade tumors. And with lower grade, more of the expression is on the luminal side of the glandular structures making it less accessible [47-52]. In addition, PSMA has been shown to be lost in very advanced, de-differentiated prostate cancer, such as neuroendocrine prostate cancer (NEPC) [47]. Thus, not all prostate cancers can be detected using PSMA PET.

PSMA PET imaging can also produce false positive findings in some patients. False positive findings on PSMA PET have been reported in patients with various disorders, including fibrous dysplasia, fractures, and schwannoma [53]. A common false positive is uptake in the cervical, celiac, and sacral ganglia [53, 54]. PSMA expression has also been observed in a variety of non-prostate solid tumors by PSMA PET imaging [55-60].

Imaging Discordance between PSMA PET and other PET Imaging Modalities

At the National Cancer Institute (NCI), patients with metastatic disease are routinely imaged with both ^{18}F-PSMA PET and ^{18}F-NaF PET. Imaging discordance, where one type of scan is positive and the other is not, or where the two scans have minimal overlap in the same lesion, have been observed [61, 62]. Biopsy of some of the lesions that were ^{18}F-PSMA-negative/^{18}F-NaF-positive confirmed the presence of cancer in some cases [62]. This suggests that PSMA PET may underestimate bone disease in some patients, for example those under ADT with PSA levels <0.2 ng/mL [61]. This may be due to an insufficient “mass” of PSMA-positive cells in some lesions, or “burned out” lesions in other cases. However, PSMA-PET findings may better reflect actual prostate cancer whereas ^{18}F-NaF PET depicts bone reaction to the tumor. ^{18}F-NaF PET false-positivity is also observed in patients with benign bone degenerative conditions. Accordingly, it is not unexpected that some discordance is seen. For the majority of lesions, PSMA PET is equivalent or better than ^{18}F-NaF at detecting bone lesions in patients with castration resistant prostate cancer, particularly when the disease extends beyond sites of osteoblast activity [61].

Discordance has also been observed between PSMA PET and ^{18}F-Fluorodeoxyglucose (FDG) PET imaging, particularly in very advanced mCRPC, when the cancer has become more heterogeneous and de-differentiated [63, 64]. Co-registered PSMA and FDG PET imaging for selecting mCRPC patients to receive PSMA-targeted radionuclide therapy was used in the Australian TheraP trial, allowing exclusion of patients with PSMA-negative/FDG-positive lesions [63]. While FDG PET imaging is generally considered to be suboptimal in prostate cancer and thus not a standard imaging method, it can provide biological information such as detecting more aggressive and rapidly growing, de-differentiated disease that is poorly responsive to treatment including PSMA-RNT (if PSMA PET-negative) and associated with poorer outcomes [65-67].

PSMA RADS for PSMA PET Imaging Standardization

A structured and inclusive reporting schema, PSMA RADS, has been developed for ^{18}F and ^{68}Ga-based PSMA PET imaging [68, 69]. A study which evaluated inter-observer reliability of four readers with different experience levels found a relatively high concordance rate for determining PSMA RADS scores from ^{18}F-DCFPyL PET images [70]. This rate was considered to be better than what has been seen for PI-RADS inter-reader concordance rates [71]. Artificial intelligence algorithms are now being developed to analyze PSMA PET images and determine PSMA RADS scores.

Recent Developments in PSMA-PET Imaging

Recently, shortly after this meeting, high level evidence from the 300 patient multi-center phase 3 proPSMA trial directly comparing ^{68}Ga-PSMA-11 to conventional imaging (CT and bone scanning) were published [19]. ^{68}Ga-PSMA-11 had 27% greater accuracy than conventional imaging (92% vs 65%) for identifying regional nodal or distant metastases. ^{68}Ga-PSMA-11 had a higher patient management impact defined by change in treatment modality or treatment technique of 28% of patients compared to 15% of patients for conventional imaging. Average radiation exposure was 8 mSv ^{68}Ga-PSMA-11 compared to 19 mSv for conventional imaging. Furthermore, ^{68}Ga-PSMA-11 had less equivocal findings (7% vs. 23%) and high reporter agreement (kappa 0.87 for nodal and 0.88 for distant metastases). The trial included a cross-over component to second-line imaging and this demonstrated similar high utility for ^{68}Ga-PSMA-11 and little benefit from conventional imaging. The combined findings provide compelling data that ^{68}Ga-PSMA-11 is a suitable replacement for conventional imaging.

Whether the use of new molecular imaging agents actually improve patient outcomes is a critical question. The STOMP trial, which tested metastasis-directed therapy vs. surveillance in patients with recurrent oligometastatic prostate cancer on choline PET has suggested an improvement in ADT-free survival with metastasis-directed therapy (p=0.11), but no clear overall survival or quality of life benefit [72]. It remains unknown whether change in management improves oncologic outcomes. PSMA PET is still limited by the spatial resolution of PET and shows only the visible emerging part of the “iceberg” in many cases and thus still underestimates the disease burden [13, 15]. Randomized prospective trials powered for outcome are needed to formally address this question and some are ongoing (NCT03582774, NCT03525288, NCT01666808, NCT03762759), [73]. Yet, unlike any therapy, PET/CT has few if any side effects, minimal risks, and enables better patient selection and disease state identification. Its integration into routine clinical care would represent a major step towards individualized medicine: selecting the right treatment for the right patient.

Ultimately, PSMA PET imaging has been proven to be superior to all other current standard imaging modalities for prostate cancer, and wide-spread use will become possible following the anticipated FDA approval of ^{68}Ga-PSMA-11 later in 2020 and ^{18}F-DCFPyL shortly thereafter. However, multiple factors are likely to determine the use of PSMA PET vs metabolic PET imaging agents in community practice, including availability of the agent, the impact on patient outcomes, and reimbursement.

PSMA-Targeted Radionuclide Therapy

Prostate cancer is a radiosensitive cancer, and various forms of radiation treatment, including external beam radiotherapy and brachytherapy, are standard options for localized or locally recurrent disease. Stereotactic body radiation therapy (SBRT) is currently being tested as an option for delivering high dose radiation to tumor sites in patients with oligometastatic prostate cancer. Radium-223, an alpha particle-emitting calcium mimetic is an established treatment for metastatic prostate cancer that is localized to bone. Targeted radionuclide therapy (RNT) is now being explored as a new class of treatment agents in metastatic prostate cancer that enables delivery of radiation to bone, soft tissue and visceral tumor deposits.

Several PSMA-targeted RNT agents have been developed for the treatment of metastatic prostate cancer and are being tested in clinical trials or used to treat patients outside of clinical trials where permitted under compassionate use. These include PSMA-targeted small molecule ligands such as MIP-1095 [74], PSMA I&T, PSMA-617 [75], and anti-PSMA antibodies such as J591, labeled with alpha or beta particle emitting isotopes. The beta-emitter ^{177}Lu-PSMA-617 [63, 76-81] is furthest on in clinical testing with the randomized phase 3 (VISION, NCT03511664) and randomized phase 2 (TheraP, NCT03392428) trials now completed recruitment. Other PSMA-targeted RNT agents in clinical development include the alpha emitter ^{225}Ac-PSMA-617, the J591 antibody labelled with Lutetium (^{177}Lu-J591) or with Actinium (^{225}Ac-J591), PSMA I&T labelled with Lutetium (^{177}Lu-PSMA I&T) or with Actinium (^{225}Ac-PSMA I&T), MIP-1095 labelled with Iodine-131, ^{177}Lu-PSMA-R2, and a PSMA-targeting monoclonal antibody linked to the alpha-emitter thorium-227 (BAY 2315497; ^{227}Th-PSMA-TTC). Results from trials testing several of these agents in mCRPC patients have been highly promising and are discussed in more detail in the following sections.

While highly promising, the studies discussed below collectively find that 50-75% of patients respond to single agent PSMA-targeted RNT, in either unselected mCRPC populations, or in trials using PSMA PET alone or in combination with FDG PET to select patients. Other therapeutic strategies to improve response rates and duration of response may include the addition of other biomarkers for patient selection or rational therapeutic combinations. Trials are also seeking to define the optimal clinical states for use of PSMA RNT. These issues are discussed in more detail below.

Clinical Development of ^{177}Lu-PSMA-617

Promising results for ^{177}Lu-PSMA-617 have been reported from multiple early stage investigations, many of which were based on retrospective data derived from patients treated in national compassionate use programs that furnish radionuclide therapy outside of formal clinical trials, with no formal data recording or reporting obligations.

In a multicenter retrospective aggregation of German data from patients treated on a compassionate use program, the clinical outcomes of 145 patients were analyzed. In this series, ≥50% PSA declines were observed in 40% of the 99 patients with available repeat PSA values after one cycle and 45% after all cycles [77]. Although there was not a formal plan for enforcing follow-up toxicity assessments or a data management plan, at least preliminarily the hematologic toxicity appeared to be quite modest. Based on physician-reported toxicity from 145 patients and laboratory-based toxicity from 121 patients, 8% of patients experienced grade 3-4 leukopenia, 2% experienced high grade thrombocytopenia, and 4% experienced some combination of these [77].

These data suggested that further prospective examination of this agent was warranted using robust clinical trials methodology.

An investigator-initiated bi-centric prospective single-arm phase 2 trial of ^{177}Lu-PSMA-617 RNT (RESIST-PC, NCT03042312) randomized patients with progressive mCRPC into 2 treatment activities groups (6.0 or 7.4 GBq). Patients received up to 4 cycles of ^{177}Lu-PSMA-617 every 8±1 weeks. Overall, of 64 patients treated, 59% experienced any PSA decline, 38% experienced a >50% PSA decline, and 16% experienced a >90% PSA decline. There was no difference between the 6.0 GBq and 7.4 GBq treatment arms [82]. In the UCLA cohort of 43 patients after a median follow-up of 19.5 months, the median OS was 14.8, 15.7 and 13.5 months in the whole cohort, the 6.0 GBq and 7.4 GBq treatment arms, respectively (p=0.68). Patients showing a PSA decline of ≥50% after 2 cycles and at any time had a longer OS: median 20.1 months vs. 13.6 (p=0.091) and 20.1 vs. 11.6 (p=0.002), respectively [83].

A prospective single-center phase 2 trial in Australia evaluated up to four cycles of ^{177}Lu-PSMA-617 in mCRPC patients who had failed conventional therapies and were selected to have high PSMA avidity on ^{68}Ga-PSMA-11 PET scans and no PSMA negative metastases that were detectable on FDG PET imaging. In 50 patients treated on this trial, 22 (44%) had PSA responses ≥ 80% (8 of which are depicted in Figure 1), 32 (64%) had PSA responses ≥ 50%, 37 (74%) had PSA responses ≥ 30%, and only two had no PSA response [63, 64]. Fifteen patients (30%) who had attained a response initially and subsequently developed disease progression were permitted to received further cycles of ^{177}Lu-PSMA-617 therapy through a compassionate use program and 73% of these patients had PSA responses ≥ 50% [64]. However disease eventually recurred in all patients on this trial [63, 64]. Notable treatment-emergent adverse events (TEAEs) observed in this trial included 66% with grade 1-2 xerostomia and 10% with grade 1-2 renal injury. Grade 3 TEAEs included lymphocytopenia (32%), thrombocytopenia (8%), anemia (10%), neutropenia (6%), and fatigue (2%). The only grade 4 TEAEs observed were thrombocytopenia (2%). Treatment with ^{177}Lu-PSMA-617 improved quality of life measures including pain severity and pain interference [64].

The higher response rates observed in the Australian trial compared with other trials/reports may reflect the stringent imaging selection criteria applied in this study that required patients to have highly PSMA avid disease without any FDG-positive/PSMA-negative lesions. Biopsy studies are being initiated at Peter MacCallum Cancer Center to investigate the biology and clinical impact of tumor heterogeneity based on PSMA and FDG PET imaging. The median OS of patients with PSMA-positive/FDG-positive (concordant) or PSMA-positive/FDG-negative lesions who were included on the trial (N = 50) was significantly better than patients who were excluded from the trial due to being either PSMA-low/negative or having any FDG-positive/PSMA-negative (discordant) lesions (N = 16) (13.3 months vs. 2.5 months) [65]. Imaging and blood biomarkers from patients on this trial were evaluated to identify any with potential prognostic value. FDG volume and PSMA intensity were identified as most prognostic of overall survival, followed by LDH, ALP, and bone scan index [84].

Despite already progressing to testing in a phase 3 trial, the optimal activity dose and maximum tolerated dose (MTD) for ^{177}Lu-PSMA-617 remains undefined, and a conventional phase 1 trial was not previously performed. To address this, a phase 1/2 trial was initiated at Weill Cornell Medicine to determine the MTD for ^{177}Lu-PSMA-617 in mCRPC (NCT03042468), and to evaluate the possible benefits of a fractionated activity dose schedule [2 doses, 2 weeks apart]. This regimen, previously utilized with ^{177}Lu-J591 [85], is designed to avoid resistance due to

repopulation by delivering a shorter but more intense dose, relative to dosing every 6-8 weeks. The dose escalation phase of the trial tested two activity doses given 2 weeks apart ranging from 7.4 GBq (200 mCi) to 22.2 GBq (600 mCi) per fractionated cycle (5 cohorts). No dose limiting toxicities were observed and MTD was not achieved [86]. The recommended phase 2 activity dose (RP2D) for the trial was chosen to be 22.2 GBq (600mCi) per single fractionated cycle and preliminary data from the partially completed combined phase 1/2 trial have been presented [87]. The most common adverse events observed were temporary and low grade pain flares (82%; 43.2% Grade 1, 38.6% grade 2) and xerostomia (61%; 56.8% Grade 1, 4.5% grade 2). No grade 3 xerostomia events were observed. Rare grade 3 events observed were thrombocytopenia (2.3%) and anemia (6.8%). Other Grade 1-2 AEs observed included fatigue, AST elevation, and neutropenia. Overall the treatment was considered well tolerated. Of note, this trial was not restricted to patients with positive/high PSMA PET scans. The rationale for this was to determine whether some PSMA-negative/low patients may benefit. However, all patients treated had PSMA uptake in at least one lesion and 80% of the patients had a mean tumor PSMA SUV_{max} of >5x liver. In preliminary analyses of the first 44 patients treated, 82% of the patients had any PSA decline and 59% had a >50% PSA decline. Of 21 patients treated with the RP2D (600mCi), 67% had a >50% PSA decline.

The Current Landscape of ^{177}Lu-PSMA-617 Clinical Trials

Several ongoing randomized prospective trials were noted as important for delivering critical insights into the efficacy and optimal clinical space for PSMA RNT.

The randomized phase 2 TheraP trial (ClinicalTrials.gov Identifier: NCT03392428) conducted by the Australian and New Zealand Urogenital and Prostate Cancer Trials Group (ANZUP) is evaluating ^{177}Lu-PSMA-617 vs. cabazitaxel in 200 mCRPC patients who had prior docetaxel. 91% of patients on the trial had prior abiraterone or enzalutamide. The trial completed enrollment in Q3 2019 and results were recently reported [88]. Patients on this trial were required to have positive ^{68}Ga-PSMA-11 PET scans defined by SUVmax levels > 20 at a site of tumor and SUVmax > 10 at sites of measurable disease to be eligible. All patients also underwent ^{18}F-FDG PET/CT and exhibited no PSMA PET-negative/FDG PET-positive lesions. 28% of patients were excluded following PSMA/FDG PET evaluation. The primary endpoint, the percentage of patients experiencing a PSA decline of 50% or more, occurred in 37% of patients randomized to cabazitaxel compared to 66% randomized to ^{177}Lu-PSMA-617, representing a 29% absolute improvement.

Secondary endpoints in the TheraP trial include pain, PFS, objective tumor response rate, radiographic PFS (rPFS), OS, and safety. Preliminary analyses demonstrate a delay in PSA progression with ^{177}Lu-PSMA-617 with a hazard ratio of 0.69. Further patient follow-up is ongoing to evaluate these secondary endpoints. Grade III-IV adverse events (AEs) occurred in 35% of ^{177}Lu-PSMA-617-treated patients vs 54% of cabazitaxel-treated patients. Toxicities related to ^{177}Lu-PSMA-617 were similar compared to phase II data. Although this investigator initiated Australian trial was not designed in liaison with the U.S. FDA, the results will provide important data that may contribute to regulatory approval. Overall, these early results suggest that in patients with progressive disease following docetaxel, ^{177}Lu-PSMA-617 was more active than cabazitaxel, with relatively fewer grade III-IV adverse events and PSA responses favoring ^{177}Lu-PSMA-617. These results are particularly relevant given the recent publication of data from the randomized phase 3 CARD trial comparing cabazitaxel to an androgen-signaling-targeted inhibitor (abiraterone or enzalutamide) in patients who had previously been treated with docetaxel

and the alternative androgen-signaling-targeted inhibitor [89]. The CARD trial demonstrated that cabazitaxel improved a number of clinical outcomes including OS and imaging-based PFS [89]. Thus, the TheraP trial will provide data comparing ^{177}Lu-PSMA-617 to a relevant current standard-of-care.

The VISION study (ClinicalTrials.gov Identifier: NCT03511664) is an international phase 3, FDA registration trial that is testing ^{177}Lu-PSMA-617 in patients with progressive mCRPC who have previously progressed on docetaxel and an anti-androgen therapy with PSMA PET-positive lesions. Patients are randomized (2:1) to receive ^{177}Lu-PSMA-617 plus best supportive/best standard-of care vs. best supportive/best standard-of-care alone. Patients randomized to the investigational arm received the best standard of care plus 7.4 GBq (±10%) ^{177}Lu-PSMA-617 administered once every 6 weeks (±1 week) for a maximum of 6 cycles. There are two co-primary endpoints, OS and rPFS, only one of which needs to reach statistical significance for the trial to be considered positive. One interim analysis will be performed for OS and rPFS when 457 rPFS events are reached. In order to have an OS endpoint, no crossover was allowed in this study. A key issue to the integrity of this study, is the selection and maintenance of proper best standard of care management. Whilst alternative androgen-signaling-targeted inhibitor therapy (e.g. enzalutamide in a patient who previously received abiraterone) was allowed, other established standards-of-care such as cabazitaxel were not allowable in this study. Another key issue in the design of this study was the lack of blinding, and thus patients knew whether or not they were receiving ^{177}Lu-PSMA-617, a potential source of patient and investigator bias. This is, however, the largest trial of ^{177}Lu-PSMA-617 to date and it is anticipated will be adequately powered to address these limitations. The first results are expected in 2020.

If these trials are positive, the next challenge for the development of PSMA-RNT will be to develop evidence for its use as an earlier line of mCRPC treatment. The standard-of-care for first-line mCRPC is currently unclear, but will likely be docetaxel if patients have already progressed on ADT plus an AR-targeted therapy. Randomization against docetaxel may be challenging, as numerous phase 3 trials testing new agents vs. docetaxel in mCRPC have failed, while adding treatment to docetaxel would first require a demonstration of safety, delaying development. However, the radiolabeled antibody ^{177}Lu-J591 has been combined with docetaxel in a pilot dose-escalation study, demonstrating safety of the combination and setting the stage for future combinations [90, 91]. As PSMA RNT is moved earlier in the disease history and patients have longer to live, trajectory issues of delayed toxicity such as renal toxicity and myelosuppression may become the most concerning TEAEs

Development of Anti-PSMA J591 Antibody-based Radionuclide Therapy

The anti-PSMA antibody, J591, predates the development of small molecules and was developed at Weill Cornell by Bander and colleagues. Various J591-based theranostic agents, conjugated to different isotopes, have been tested in prostate cancer clinical trials. Compared with small molecule PSMA-targeting agents, the far larger size of the J591 antibody results in a much longer circulation time (days vs. hours) and reaches target mostly via vasculature as opposed to rapid diffusion to all tissues. These differences result in different imaging properties (optimal tumor imaging at 3-8 days vs. hours) [11, 12]. The dose-limiting toxicity for J591-RNT agents is off-tumor exposure to bone marrow [85, 92-95]. In contrast, PSMA ligand-based RNT results in include on-target, off-tumor radiation exposure to salivary glands and the digestive tract and possible late renal toxicity [63-65, 82, 84-87].

Beta particle emitting ^{90}Y- and ^{177}Lu-labeled J591 agents have demonstrated promise as theranostic agents in clinical studies [85, 92-97]. In SPECT imaging, ^{90}Y- (using ^{111}Indium imaging) and ^{177}Lu-labeled J591 agents demonstrated accurate targeting in 89% of patients across unselected populations. Dose-responses in PSA declines and overall survival have been observed in patients treated with ^{177}Lu-J591. In a phase 2 study testing single doses of ^{177}Lu-J591, median OS was 21.8 months in patients who received a single dose of 70 mCi/m^2 compared to 11.9 months in patients who received a dose of 65 mCi/m^2 [95]. However higher activity doses also had a higher incidence of toxicity, including predictable, reversible myelosuppression. Dose fractionation enabled administration of higher cumulative activity doses with less myelosuppression and also allowed for concurrent administration of docetaxel. In a phase 2 study that tested fractionated activity doses of ^{177}Lu-J591, improved PSA declines and overall survival were seen at higher fractionated activity doses, along with increased toxicity [85]. Median overall survival was 42.3 months in patients who received a cumulative fractionated dose of 90 mCi/m^2, 19.6 months in patients who received a cumulative fractionated dose of 80 mCi/m^2, and 14.6 months in patients who received a cumulative fractionated dose of 40-70 mCi/m^2 [85].

The survival data from ^{177}Lu-J591 studies should not be compared to the ^{177}Lu-PSMA-617 literature absent a head-to-head, randomized trial. There may be a role for J591-RNT agents in combination with small molecule PSMA-targeted agents due to non-overlapping toxicities. A trial testing ^{177}Lu-PSMA-617 combined with ^{177}Lu-J591 is ongoing (NCT03545165). Development of alpha-labeled J591 RNT is discussed in further detail below.

Optimizing Alpha-Emitting PSMA-Targeted Radionuclide Therapy

Early anecdotal reports of treatment with the alpha-emitting PSMA-targeted small molecule ^{225}Ac-PSMA-617 suggest significant efficacy but also severe xerostomia [80]. Alpha-emitters have been shown to have anti-tumor effects in patients who have not responded to, or have progressed on treatment with ^{177}Lu-PSMA-617 [80, 98, 99]. This suggests that one of the mechanisms of failure to beta-emitting PSMA-targeting RNT might be under-treatment. Indeed alpha-emitters have a higher linear energy transfer (LET) (~x 100) than beta-emitters and thus induce more DNA damage, DNA damage incidence being proportional to the absorbed radiation dose (40 double-strand breaks /cell/Gy, 1000 DNA base lesions /cell/Gy). Alpha particles emit a much higher energy (4-8 MeV for alpha vs 0.1-3 MeV for beta) over a shorter range energy (14-42uM in tissue for alpha vs 0.6-10mm in tissue for beta), effectively ~100 keV/um. This translates to a significantly greater capacity for tumor damage Strategies to develop targeting agents that can deliver potent alpha radiation to tumors, but with reduced toxicity include the use of antibody-based and albumin-binding PSMA-targeting agents, both which alter tissue distribution properties with the goal of reducing xerostomia and dry eye effects.

Antibody-based agents are larger and have different pharmacokinetics and biodistribution than small molecules, resulting in a different side effect profile. PET imaging with J591 has not demonstrated uptake in the salivary and lacrimal glands or kidneys, further suggesting xerostomia may be avoided with J591-based RNT [11, 12]. The PSMA-targeted J591 antibody has also been shown to bind a different site of PSMA compared with the small molecule PSMA ligands (Bander et al, unpublished), suggesting co-targeting with the two agents could result in an additive radiation dose to tumor without added side effects. Based on these features, it is hypothesized that treatment with an alpha-labeled anti-PSMA antibody will have a differing toxicity profile than ^{225}Ac-PSMA-617, including decreased or absent xerostomia and renal exposure.

A phase 1 trial testing ^{225}Ac-J591 has been initiated at Weill Cornell Medicine [98, 100]. In the phase 1 dose escalation trial, 22 patients were treated at 7 dose levels. The treatment was found to be well tolerated and the MTD was not reached. A single subject treated with 80 KBq/Kg had grade 4 anemia and thrombocytopenia, but 0 of 6 patients treated at the highest planned dose (93.3 KBq/Kg) had dose-limiting toxicities, so the RP2D for a single dose was declared to be that dose level [98, 100]. Promising anti-tumor activity was observed, including exceptional responses in patients who had previously progressed on ^{177}Lu-PSMA-617 [98]. Of 22 patients treated, 14 (64%) experienced any PSA decline and 9 (41%) experienced > 50% PSA decline [100]. The trial recently began the multicenter expansion phase. This study will also lay the foundation for additional planned studies testing ^{225}Ac-J591 + pembrolizumab and ^{225}Ac-J591 + PSMA ligand-based RNT.

A phase I dose escalation study is also ongoing for a novel anti-PSMA antibody labeled with the alpha emitter ^{227}Th (BAY 2315497; ^{227}Th-PSMA-TTC). This international study is enrolling patients with progressive mCRPC following at least one potent AR pathway inhibitor and 1-2 lines of taxane chemotherapy; no prior PSMA-TRT or radium-223 is allowed [NCT03724747].

A novel small molecule PSMA inhibitor that includes in its structure an albumin-binding moiety to modulate pharmacokinetics is under development as an alpha-emitting PSMA RNT agent. The albumin-binding motif is hypothesized to increase the effective agent size and thus reduce the renal excretion and increase blood circulation time, with the goal of enabling a greater number of "passes" though tumor tissue while reducing uptake to normal tissues that express lower PSMA levels (salivary glands and kidneys). Albumin-binding motifs can be chemically modified [101], in order to select a motif with a significantly weaker (1,000-fold) affinity for albumin compared with affinity of the PSMA-ligand for PSMA. A series of albumin-binding PSMA-targeted RNT lead compounds, conjugated to either ^{177}Lu or ^{225}Ac, have been developed that have improved pharmacokinetics and efficacy in prostate xenograft models [102-104]. Clinical studies testing the most promising of these compounds (^{225}Ac-RPS-074, [103]) in prostate cancer patients are planned in 2020.

Other PSMA-targeted RNT Agents in development

While the agents above were most highly discussed at this meeting, it is worth noting that promising efficacy has been observed with other beta emitting PSMA-targeted RNT agents under clinical development. For instance, among 100 patients treated with ^{177}Lu-PSMA-I&T, ≥50% PSA declines were observed in 38 patients (38%) [105]. A study testing a single dose of ^{131}I-MIP-1095 (2.0-7.2 GBq) in 28 consecutive patients reported a 60.7% response rate (decline in PSA ≥ 50%) [74]. A phase 1/2 dose escalation study of ^{177}Lu-PSMA-R2 in patients with PSMA+ mCRPC is also being completed [NCT03490838].

Other Strategies to Optimize PSMA-Targeted Radionuclide Therapy

Overall, these studies find that ~50-75% of patients with mCRPC exhibit responses to PSMA-targeted RNT agents. Response rates have been higher in mCRPC trials with more stringent selection, such as in the Australian ^{177}Lu-PSMA-617 study which required that any FDG PET-positive lesions were also apparent on PSMA PET scans. Whether PSMA PET imaging is necessary for patient selection at all stages of prostate cancer remains unclear, and may vary in clinical contexts. For instance, PSMA PET imaging may be more important in settings where PSMA expression loss has been seen, such as in NEPC. Studies have found that responses to

^{177}Lu-PSMA-617 are more likely and deeper in patients with higher PSMA PET uptake [84] or when higher delivered radiation doses to tumor are evident on post-therapy SPECT/CT imaging [64], but even those with no PSMA uptake on imaging may occasionally respond [Tagawa et al., manuscript in review, [106]]. Studies in animal models in support of this have found that PSMA expression levels and the fraction of PSMA-positive cells (PSMA heterogeneity) in prostate tumors correlate with uptake and efficacy of PSMA RNT [107].

These studies also demonstrate that a subset of patients do not exhibit significant responses to ^{177}Lu-PSMA-617, despite high uptake on PSMA PET scans. Furthermore, even in patients who initially exhibit deep PSA and radiographic responses to ^{177}Lu-PSMA-617, disease eventually recurs in nearly all patients and in some patients responses may not be durable. Long term disease control vs. recurrence rates for patients who exhibit deep responses to treatment with ^{225}Ac-PSMA-617 have yet to be reported. Mechanisms of pre-existing and acquired tumor resistance likely include insufficient radiation dose reaching tumors, heterogeneity in target expression, and biologic pathways leading to lack of radiation responsiveness (radioresistance). It is critical that future studies identify mechanisms of resistance to PSMA-RNT and develop strategies to overcome them.

Thus far, PSMA-targeted RNT have primarily been tested as single agents in either unselected mCRPC patients or have used PSMA PET alone or in combination with FDG PET to select patients. However, it is possible that combination treatments or the use of other biomarkers, such as homologous recombination deficiency (HRD), may improve selection of populations who may be more sensitive to RNT. Trials testing PSMA-RNT in combination with AR-targeted agents, PARP-inhibitors or checkpoint immunotherapy in patients with mCRPC are ongoing (See section on PSMA RNT combinations below).

Whether certain genomic alterations can be used as biomarkers to identify patients who are more or less likely to respond to PSMA-RNT is a major question under investigation [108]. An analysis of available germline (targeted) or/and somatic (targeted or whole exome) DNA testing results from patients treated with various PSMA-RNT agents at Weill Cornell Medicine (N = 53; 58% with ^{177}Lu-PSMA-617, 31% with ^{177}Lu-J591, 7% with ^{177}Lu-PSMA-617 + ^{177}Lu-J591, 4% with ^{225}Ac-J591) found that *BRCA2* inactivating mutations, losses or deletions were associated with improvements in PSA response (HR = 0.26) and overall survival (HR = 0.09) [109]. *AR* amplifications or mutations and *MYC* amplifications were associated with shorter OS (HR = 7.26 for *AR* amp/mut; HR = 2.61 for *MYC* amp) [109].

Trials are also testing the role of PSMA RNT earlier in prostate cancer disease history, including as first-line therapy in newly diagnosed patients. For instance, the UpFrontPSMA trial (NCT04343885), led by the Peter MacCallum Cancer Centre, is testing ^{177}Lu-PSMA-617 (2 cycles) + ADT followed by docetaxel vs. ADT + docetaxel in patients with newly diagnosed high volume metastatic prostate cancer. The LuTectomy trial (NCT04430192), also led by the Peter MacCallum Cancer Centre, is testing ^{177}Lu-PSMA-617 (1-2 cycles) followed by prostatectomy + pelvic lymph node (LN) dissection in patients with high risk localized prostate cancer with positive lymph nodes (N1) and PSMA-positive scans.

Challenges facing PSMA RNT Clinical Trials

A number of critical factors are necessary to fulfill demonstration of clinical benefit of a new treatment agent. These include active agents, willingness to perform clinical trials, equipoise,

uniform eligibility criteria, and informative endpoints. For PSMA RNT, treatment effects of several agents have been shown (PSA changes), as discussed above, but important clinical endpoints – such as progression free survival, overall survival, improved quality of life, improved pain, or other measures of how patients feel, function, or survive, have not been prospectively demonstrated compared to other therapies.

In the U.S., the regulatory requirements for developing new drugs and the methods for demonstrating clinical benefit (or biomarkers of such) have been clearly outlined by the FDA. These requirements include substantial evidence of effectiveness and specifies that this evidence must be derived from adequate and well-controlled clinical investigations [110]. Clinical benefits that have supported drug approval state that the treatment must improve how patients feel, function, or survive, and that surrogate endpoints must be known to have significant associations with clinical benefit [111, 112]. The FDA has not allowed PSA changes to be considered as an indicator of clinical benefit in prostate cancer trials. On the other hand, standard imaging using cross-sectional imaging and bone scintigraphy has been associated with clinical benefit, and has received at least qualified regulatory recognition when using the Prostate Cancer Working Group 2 and 3 definitions of radiographic progression [113, 114]. These criteria correlate with overall survival in the range of 0.5-0.7, depending on the study and the statistical test of correlation being applied [110, 115]. Recent new treatments for prostate cancer have also been FDA-approved based on endpoints beyond overall survival and rPFS, including symptomatic skeletal event (SSE) prevention [116, 117], and metastasis free survival (MFS) [118, 119].

In the development of PSMA-RNT, data to date have revealed significant treatment effects using PSA and PSMA imaging, but the VISION trial (with two co-primary endpoints, OS and rPFS, only one of which needs to reach statistical significance for the trial to be considered positive) is the first clinical study to be adequately powered to demonstrate a clinical benefit, as described above.

While PSMA imaging has been a mainstay of demonstrating treatment effects of PSMA-RNT trials, the use of PSMA imaging as a response indicator remains understudied, and to date unqualified as a biomarker of clinical benefit. Prospective studies to do so are planned or underway. These images however, have been widely promulgated as evidence of clinical benefit, which has the potential to prematurely assume clinical benefit, or conversely, prematurely terminate treatment. Until associations between PSMA imaging and clinical benefit are determined, these scans should be treated as exploratory.

Overall, current challenges for PSMA-targeted RNT trials include incentivization of provisioning of drug off-study by some countries, availability of drug for patients randomized to arms not containing up-front drug; a loss of equipoise amongst patients fueled by investigators, sponsors, and institutions publicizing inaccurate portrayals of purported efficacy; no standardization on eligibility criteria across trials, and a poor understanding of how to use imaging in this context, as standard imaging is no longer used in many countries and novel imaging available in other countries is acted upon but has not been validated.

To enable success in the development of this new class of agents, it will be necessary to develop consensus between regulators, investigators, and sponsors on trial design, and to include uniform criteria for eligibility and informative endpoints. Validation of PSMA PET as an informative biomarker for patient selection and/or measurement of meaningful clinical responses including correlation with OS is critical.

Development of PSMA RNT using radiotherapy vs. drug dosing paradigms

^{177}Lu-PSMA-617 has been conventionally delivered through multiple cycles given every 6-8 weeks, with follow-up assessments at 4 week intervals [120]. PSMA PET/CT images are obtained at baseline and post-therapy imaging is typically performed after each cycle. Patients may progress between treatment intervals, and many do not receive the full number of cycles. The treatment schedule used in the VISION trial is 7.5 GBq ^{177}Lu-PSMA-617 administered every 6 weeks for up to 6 cycles. If this trial is successful this will likely became the de-facto standard. The TheraP trial [88] uses an identical schedule, but with a declining amount of administered radioactivity, commencing at 8.5 GBq and decreasing by 0.5 GBq per cycle to 6 GBq for cycle 6. The total administered radioactivity across 6 cycles is very similar with these two regimens.

The dose of a drug given is typically determined by factors including body weight, excretion, pharmacodynamics, pharmacogenomics, normal organ tolerance, tumor sensitivity, and goals of the therapy. When used in combinations, maximum tolerated doses of each agent and overlapping toxicities, as well as target manipulation and tumor sensitization must be considered.

In contrast, radiation therapy doses are prescribed and determined using dosimetry, which is a calculated assessment of the dose of radiation absorbed by a particular tissue (discussed in detail below).

PSMA RNT is a novel treatment that in some contexts has been developed using radiotherapy paradigms, but in others has been treated as a drug. Both approaches have merits as well as disadvantages.

If RNT is treated as a drug (Table 1), dosimetry is not required, and instead a phase 1 dose escalation study would be used to determine a maximum tolerated dose (MTD) and a recommended activity dose which all patients would receive (such as in the VISION trial, in which no dosimetry or post-treatment PSMA PET imaging was done). This approach would enable acute toxicities to be observable and definable. However, some predictable delayed cumulative toxicities such as myelodysplasia and renal toxicities may be missed.

In the radiotherapy approach (Table 1), dosimetry would be used to define and deliver doses which have biologic efficacy but don't exceed known maximum tolerated limits to critical organs. The advantage of this approach is to avoid delayed toxicities, such as renal toxicity. Use of dosimetry also defines tumor absorbed dose which enables dose-response relationships to be defined and optimization of treatment including defining thresholds that may define high likelihood of response of treatment failure. However, this model is theoretical for RNT agents as the approach is extrapolated from external-beam radiotherapy. The current lack of robust standardized and reproducible dosimetry methods for RNT precludes widespread clinical use. Further work and improvement are critically needed to see personalized RNT dosimetry in routine practice.

Individualized Dosimetry for PSMA RNT

Dosimetry is the measure of radiation doses that reach tumors vs. normal tissue (unit: Gray, Gy). Dosimetry is necessary for establishing safety and validating efficacy of systemically administered radionuclide therapies, can be used to optimize individualized activity, and may serve as a

prognostic biomarker for treatment response or futility. Molecular imaging such as PET, planar gamma camera, or SPECT/CT is used to measure dosimetry following RNT administration.

In many institutions, patients treated with PSMA-RNT are imaged several (~5) times over a week-long period with 2-dimensional planar scanners and dosimetry is estimated using a schema developed by the Medical Internal Radiation Dose (MIRD) Committee [121]. This dosimetry method is time-consuming and reading of a single set of scans can take a trained medical physicist several hours.

An improved approach is voxel-based dosimetry, in which multi-time point (such as 4hr, 24hr, 96hr) 3D quantitative SPECT/CT is performed (Figure 2). The multi-time point images are co-registered, and an algorithm is used to determine dose (in Gy) per voxel for selected tumor or normal regions according to the kinetics of dose washout over time [122]. Voxel-based methods are now emerging as a standard, owing to highly accurate quantitative SPECT/CT technologies becoming commercially available, that have cross-calibration factors within 1%. Artificial intelligence algorithms are being developed to further automate dosimetry calculations, for instance auto-determination of the location of normal organs [123]. However, multi-time point dosimetry studies are not feasible for all patients. Dosimetry studies with ^{177}Lu-PSMA-617 have found that a single image taken at 72 hours post-therapy administration can be used to estimate dosimetry with 5-10% accuracy, although anytime within 24-96 hours may be sufficient [124].

Importantly, dosimetry evaluation of the Australian phase 2 ^{177}Lu-PSMA-617 study demonstrated that the dose to tumor is strongly predictive of response to ^{177}Lu-PSMA-617 [125]. Although there were some patients who received high doses to tumor and did not have responses, there were very few patients who received low doses to tumor and responded. 10 of 11 patients who received on average less than 10 Gy to tumor did not have a PSA response, defined by decline in PSA of more than 50%. This suggests that dosimetry may be used as a futility measure, as patients who do not receive a sufficient dose to tumor after the first dose are unlikely to respond and could opt to discontinue treatment in favor of other options. In the same research, the SUV_{mean} on ^{68}Ga-PSMA PET/CT scans has also been shown to correlate with ^{177}Lu-PSMA-617 dosimetry, though this may not be sufficient for dose planning.

Dosimetry may also provide useful information about likely toxicity and aid with dose optimization. The tumor has been found to act as a sink for PSMA RNT, as the dose to the parotid glands and other non-tumor organs diminishes with larger tumor uptake [125-127]. This indicates that it may be possible to give larger activity doses of PSMA-RNT to patients with greater tumor burden without increasing toxicity. Injected activity may also be adjusted for individual patients based on tumor volume and body weight. Whether the presence of a sensitizing genomic alteration may also be used to adjust dose activity is of interest and deserves further study. This type of individual optimization of administered amount of radioactivity may be logistically challenging, especially in the context of a phase 3 study which may be necessary to prove superiority. The added costs and complexity of delivering customized doses to each patient also need to be considered.

Lessons may be learned from other PET imaging agents being developed as theranostic tools to individualize administration of treatments. For instance, doses of traztuzumab may be determined based on ^{89}Zr-traztuzumab PET scans [128] and doses of rituximab may be determined based on tumor volume on FDG PET [129]. Strategies are being tested to optimize ^{177}Lu-DOTATATE based on serial imaging, though this may not be practical. Unfortunately, toxicities such as

myelodysplasia, which occurs in ~5% of patients with neuroendocrine tumors treated with ^{177}Lu-DOTATATE may not be predicted by dosimetry [130].

Assessing the Biological Effect of Therapeutic Radiation

In conventional radiation oncology approaches using external beam radiation therapy (EBRT), the biological effect of the radiation is determined by several factors aside from the total radiation dose to tumor (Gy). Radiation biologists have long been able to incorporate such factors into tumor control probability (TCP) models to enable accurate predictions of cell kill in vitro and useful tumor control prediction in vivo. Normal tissue calculations are typically more complicated and must take into context the structure of the tissue. Ideally such models could be extended to use in the clinic to calculate equivalent doses (usually in terms of equivalent dose in 2Gy per fraction EBRT) when confronted with tumor dosimetry from varied methods of therapeutic radiation administration and would be clinically useful in understanding dosimetry seen with agents such as ^{177}Lu-PSMA. The term “isoeffect” distills variations of radiation treatment parameters such as total dose, fractionation regimen, and relative biological effectiveness (RBE) into a theoretical estimate of damage in tissue, thus enabling regimen comparison [131, 132].

A key physical characteristic of isoeffect calculation is the linear energy transfer (LET) of the radiation. Alpha particles have a high LET, as they carry moderate mass and charge, can densely ionize and damage DNA, and are highly effective at producing double-stranded DNA breaks. Conversely, particles such as beta particles (electrons) which have a tiny mass and charge, and gamma rays (photons / X-rays) which have no mass and no charge, have low LET. Low LET radiation is sparsely ionizing, and is less effective at inducing dsDNA breaks, producing 20-50-fold more ssDNA than dsDNA breaks.

The relative proportions of ssDNA and dsDNA breaks are also central in predicting the interaction of total radiation dose and the individual radiation fraction size with EBRT. Smaller fractional doses create a higher proportion of ssDNA damage which is associated with high rates of DNA repair usually, while high fractional doses result in higher levels of the more lethal dsDNA damage. The ratio of ssDNA vs dsDNA damage is traditionally thought to be the basis of the α/β ratio (ratio of total dose to dose-per-fraction). In primary prostate cancer, the α/β ratio for a lethal dose is estimated at ~ 1.5 Gy which is considerably lower than most cancers (with α/β ratios of 6-10 Gy) [133, 134]. A lower α/β ratio implies that larger doses are needed to overwhelm sub-lethal damage repair and maximize the effectiveness of radiation.

A third parameter that impacts the isoeffect is dose homogeneity. Modern EBRT technologies can enable high levels of dose modulation, such as high doses to the prostate target, lower doses to the surrounding pelvic lymph node regions, while also respecting the tolerance doses of organs at risk. Radiation plans must take these issues into consideration to assure that isoeffective doses are being delivered to the various targeted sites. In brachytherapy, dose delivery is complicated and extremely heterogeneous, with different regions of the prostate gland receiving 90 to >200% of the target dose. For unsealed source therapy such as PSMA RNT, the extent and effect of homogeneity is difficult to quantify.

A further physical parameter that impacts the isoeffect is dose-rate. Dose rate is a function of radioactive decay of the given isotope. Dose rates need to be optimized such that rates at which dose is delivered overwhelms the rates at which the cancer cells can sufficiently repair DNA

damage. Other biological features determining radiation response are described (such as hypoxia or *BRCA1/2* mutation), but are yet to impact clinical practice.

Overall, RBE models incorporating LET, dose and fraction size to predict dose isoeffect for EBRT have been robust enough to accurately predict clinical outcomes of alternative fractionation schedules [135]. For brachytherapy, adding decay kinetics and homogeneity to the models has been used to develop clinical protocols with low-dose-rate brachytherapy. For unsealed sources such as PSMA RNT, more remains to be known about dose homogeneity in tissue, path length, and determining predictors of dose vs. administered activity, in order to develop an accurate RBE model. Developing an accurate model for predicting dose isoeffect with RNT will require either improved RBE models based on in vitro models, or alternatively may be determined using data from patients in RNT clinical trials.

PSMA RNT Rational Combinations

Despite the promising response rates seen in patients treated with ^{177}Lu-PSMA-617, nearly all patients eventually experience disease progression, highlighting the need to improve this treatment strategy. Several combination treatment strategies to improve the efficacy of PSMA RNT are currently under investigation. Rational combinations should aim to improve efficacy by leveraging synergistic mechanisms of actions between the therapeutic agents while mitigating any possible overlapping toxicities. In the phase 2 Australian ^{177}Lu-PSMA-617 trial [63, 64], while most patients experienced only grade 1-2 AEs, a subset experienced Grade 3 events, particularly myelosuppression, which should be taken into account when considering possible combinations. Other sites of physiologic PSMA expression and consequent toxicity including kidney, small bowel and salivary gland are relevant when considering combinations. The timing of concomitant drug administration may also be important. Commencing a drug 24-48 hours after ^{177}Lu-PSMA-617, when plasma clearance of radiation has occurred but tumor uptake remains high, may be a mechanism to maximize the therapeutic index of combination therapeutics. Identifying patient populations most likely to respond to certain combinations based on molecular and clinical characteristics should also be a critical component of any combination treatment strategy.

Several rational treatment combinations with ^{177}Lu-PSMA-617 include AR-targeted agents, targeting DNA repair, and immune checkpoint inhibition [136]. Clinical studies evaluating these combinations are underway and discussed in more detail below.

Combining ^{177}Lu-PSMA-617 with AR-targeted agents

Acute AR blockade has been shown to upregulate PSMA mRNA production and PSMA receptor density on the cell surface [137, 138]. In addition, AR pathway inhibitors may also lead to radiosensitization [139, 140]. These observations serve as rationale for combining ^{177}Lu-PSMA-617 with AR-targeted agents such as enzalutamide, apalutamide, abiraterone acetate, or darolutamide. In animal models, improved tumor control was observed with ^{177}Lu-PSMA-617 and enzalutamide combination treatment compared with either agent alone [137]. In the phase III VISION study in patients with late stage, heavily pre-treated mCRPC, ^{177}Lu-PSMA-617 is being added to best standard of care. In most cases, the best standard of care utilized in the study is likely to be AR-targeted drugs. However, the effect of AR targeted agents appear to have dichotomous effects on PSMA expression in patients at different disease states [141]. In hormone-sensitive prostate cancer patients, treatment with AR-targeted agents has been observed to cause a decline in PSMA expression, while in patients with CRPC, AR-targeted agents caused an increase in PSMA expression [141]. These data suggest that careful

consideration needs to be given to disease state when combining PSMA-targeted therapy with AR-targeted therapy.

ENZA-P (ANZUP 1901; NCT04419402) is a multicenter, 1:1 randomized, phase 2 trial that is testing enzalutamide + ^{177}Lu-PSMA-617 vs. enzalutamide monotherapy in 160 patients with mCRPC. Patients on this trial must have a rising serum PSA (PSA ≥ 10ng/mL), no prior novel hormonal agents or chemotherapy (except for abiraterone acetate or docetaxel in the hormone sensitive setting), sufficient PSMA expression on PSMA PET/CT (SUVmax > 15 of disease ≥ 10mm in size), and at least 3 risk factors for early treatment failure on enzalutamide alone based on data from the PREVAIL and PROPHECY trials [142, 143]. Patients randomized to the treatment arm will receive up to four doses of 7.5GBq ^{177}Lu-PSMA-617 in combination with 160mg enzalutamide daily. The primary endpoint is PSA PFS. Secondary endpoints include rPFS, PSA reduction of ≥50% from baseline, pain response, overall survival, health related quality of life, and frequency and severity of adverse events. The study includes multi-time point PSA PET and evaluation of biomarkers including circulating tumor cells (CTCs). Altogether, this study will annotate 960 PET/CT scans across 160 patients providing useful insights into the longitudinal impact of enzalutamide on PSMA expression in the control arm in addition to evaluating response across both treatment arms.

While there is strong rationale supporting the combination of ^{177}Lu-PSMA-617 with AR-targeted agents, it will be crucial to understand long term safety and the impact of early use of ^{177}Lu-PSMA-617 on the ability to administer subsequent lines of therapy in particular chemotherapy, define the optimal dose and schedule, optimal patient selection, patterns of relapse, and mechanisms of disease resistance. Given that AR-targeted agents can be highly effective and result in a rapid reduction in tumor volume and PSMA expression, questions remain on whether sequential treatment using ^{177}Lu-PSMA-617 to first debulk tumors followed by ADT + AR-targeted therapy would be a more rational approach.

Combining ^{177}Lu-PSMA-617 with inhibitors of DNA damage repair

Targeting DNA repair is widely considered to be a synergistic approach with radiation therapy [144]. Radiation induces ssDNA breaks and dsDNA breaks through the generation of oxidative free radicals. Poly (ADP-ribose) polymerase (PARP) plays a central role in repairing radiotherapy-induced ssDNA breaks, minimizing potentially lethal radiation-induced damage and conferring resistance [145]. Therefore, PARP inhibition is a rational therapeutic approach for radiosensitization with RNT. Treatment with single agent PARP-inhibitors has been shown to be effective against mCRPC with *BRCA1/2* alterations as well as in some other DNA repair genes in phase 2 and phase 3 trials [146-149]. Somatic alterations in DNA repair genes are present in 20-25% of mCRPC, and germline alterations are present in ~12% of patients with mCRPC [146, 150, 151]. These patients, particularly those with *BRCA1/2* alterations, represent a subset who may benefit from treatment with single agent PARP inhibitors [147, 148].

Several preclinical studies have shown enhanced anti-tumor activity from the combination of PARP inhibitor and radiotherapy including RNT [Cullinane and Sandhu et al., unpublished, [144, 152]]. Combination treatment with PSMA-directed RNT and PARP-1 inhibitors has also been studied in LNCaP cells cultured as multicellular tumor spheroids [153]. It was observed that the PARP-1 inhibitor olaparib synergized with ^{131}IMIP-1095 in delaying growth of LNCaP spheroids. Significantly, it has been shown that PARP inhibitors are especially effective in the enhancement of radiation kill at low doses such as observed in RNT [154]. These data suggest that PARP

inhibitors may be appropriate for combination with targeted radiopharmaceuticals characterized by a low dose-rate radiation.

It will be important to delineate the biological mechanisms underlying synergy between ^{177}Lu-PSMA-617 and PARP-inhibitors. Increasing evidence has suggested an interaction between tumor DNA damage and the immune system during the treatment of cancers, through pathways including cGAS/STING. The c-GAS/STING pathway is an innate immune signaling pathway that senses cytosolic pathogenic or self-DNA via the DNA sensor cGAS, which produces the second messenger cGAMP, which in turn activates STING signaling and subsequent production of type I interferons (IFNs) and pro-inflammatory cytokines. Recent studies suggest that a STING-dependent cytosolic DNA sensing pathway mediates the efficacy of PARP inhibitors, radiation therapy and chemotherapy [155-157].

Sandhu, Hofman and team have opened the phase 1 LuPARP trial (ClinicalTrials.gov Identifier: NCT03874884). The primary end point is to establish the maximum tolerated dose (MTD) and dose-limiting toxicities (DLTs) of ^{177}Lu-PSMA-617 in combination with the PARP inhibitor olaparib in patients with mCRPC. Patients on this trial must have PSMA-avid disease, and have progressed on a second generation AR-targeted agent. Secondary objectives include evaluating the safety and preliminary anti-tumor activity of ^{177}Lu-PSMA-617 in combination with olaparib. Paired biopsies and liquid biopsies (CTCs and circulating tumor DNA) will be collected from these patients to identify mechanisms and predictive biomarkers of response and resistance. In this trial, the administered radioactivity is 7.4 GBq of ^{177}Lu-PSMA-617, and the dose of olaparib will be escalated from 50mg to 300mg, in 6 increments. Patients will receive up to 6 cycles of ^{177}Lu-PSMA-617, 6 weeks part, with olaparib administered for 14 days commencing 1 day after each administration of ^{177}Lu-PSMA-617.

Other agents known to alter DNA-damage responses that may have synergy with ^{177}Lu-PSMA-617 include inhibitors of DNA-PK, ATM, ATR, and RNA polymerase I inhibitors. Preclinical studies in animal tumor models have demonstrated synergy between the potent and selective DNA-PK inhibitor AZD7648 with radiation therapy or olaparib [158]. The ATM inhibitor AZD0156 has also been shown to potentiate both radiation and olaparib responses in preclinical xenograft tumor models [159]. The ATR inhibitor BAY 1895344 has demonstrated some anti-tumor activity in a single agent phase 1 study in solid tumors [160]; this agent could be considered for combination with ^{177}Lu-PSMA-617. The RNA polymerase I inhibitor CX-5461 has also demonstrated synergy with talazoparib in preclinical models of ovarian cancer [161] and prostate cancer [Sandhu et al., unpublished, [162]]. In vitro evaluation of CX-5461 and ^{177}Lu-PSMA-617 is under way.

Combining ^{177}Lu-PSMA-617 with immune checkpoint inhibitors

Immune checkpoint inhibitors that enhance T cell effector function such as the anti-PD-1 monoclonal antibodies (mAbs) (e.g., nivolumab, pembrolizumab) and anti-CTLA-4 mAbs have been designated breakthrough treatments for many cancers including melanoma, lung cancer and others because of the marked and durable responses and unprecedented survival benefit [163, 164]. However these agents have had limited activity in prostate cancer [[165-170], Sweeney et al., 2020 AACR Virtual Annual Meeting I], which has diminished enthusiasm for testing immunotherapy in this space.

The low level of responses in prostate cancer patients are attributed to the relatively low level of neo-antigens and other immunogenic mutations seen in the majority of prostate tumors [171],

resulting in lower levels of T cell recognition and tumor infiltration by immune cells, aka a “cold tumor”. Research has more recently focused on strategies and biomarkers to identify subsets of prostate cancer patients who are more likely to benefit from immune checkpoint therapy, namely those associated with a higher likelihood of greater neoantigen loads. Promising biomarkers include mismatch repair (MMR) gene defects or microsatellite instability-high (MSI-high) tumors, bi-allelic loss of *CDK12*, and alterations in other DNA damage repair gene defects such as *BRCA1*, *BRCA2*, or *ATM* [172-178]. Prior treatment, such as chemotherapy [179] may also impact the efficacy of immune checkpoint therapy in prostate cancer patients, possibly due to impacts on the tumor microenvironment. Studies are underway to define the biology of the tumor microenvironment during treatment with various prostate cancer treatments, and to identify strategies to turn “cold” prostate tumors “hot.”

Radiation therapy is one of the most promising treatment combinations with immunotherapy, as it can have a variety of immunomodulatory effects, based on dose and type of radiation delivered [180]. The abscopal effect, in which non-irradiated tumors have been observed to shrink in some patients following radiation therapy targeted to other tumor sites, is hypothesized to be mediated by the generation of systemic anti-tumor immune responses following radiation-induced immunogenic tumor cell death. Ongoing studies are seeking to determine the immunomodulatory effects of PSMA-RNT and optimal dose and scheduling for achieving the greatest synergy with checkpoint immunotherapy.

Immunotherapeutic agents that could potentially be combined with ^{177}Lu-PSMA-617 include anti-PD1/anti-PDL-1, anti-CTLA-4 + anti-PD-1, inhibitors of TGF-beta signaling, inhibitors of myeloid derived suppressor cells (MDSCs), amongst others. Treatment efficacy may also be enhanced by rational combinations of these different modalities.

The phase Ib/II PRINCE trial (NCT03658447) is testing the combination of pembrolizumab with ^{177}Lu-PSMA-617 in mCRPC patients who have progressed on second generation anti-androgen treatment. Patients enrolled on this trial are required to have PSMA-PET and FDG-PET scan concordant disease using similar criteria to the TheraP trial. Patients will receive continual dosing with pembrolizumab for up to two years (35 cycles given every 3 weeks) and up to 6 cycles of ^{177}Lu-PSMA-617 (6 weeks apart). The primary objectives of this study are PSA response rates and to evaluate safety and tolerability of the treatment combination. Secondary objectives include OS, rPFS, PSA-PFS, objective response rates, duration of response, duration of disease control, time to treatment response, and changes in pain and health related quality of life measures. This study plans to enroll 37 patients across four sites overall.

Overall, immunotherapy may offer a rational combination approach with ^{177}Lu-PSMA-617 in prostate cancer, but remains unproven and responses may be affected by clinical characteristics, prior treatments, and somatic genomic alterations. Research is needed to better understand the prostate tumor microenvironment and develop novel combinations and biomarkers to select patients for monotherapy versus combination treatments.

Lessons from other radiopharmaceuticals

Radium-223 is an alpha-particle emitting radionuclide treatment approved for CRPC patients with bone-only metastatic disease. Radium-223 was approved based on results from the phase III ALSYMPCA trial, which demonstrated a median overall survival benefit of 3.6 months for radium-223 (14.9 months) compared with placebo (11.3 months) [181]. Radium-223 also delayed the median time to the first symptomatic skeletal event by 5.8 months [181].

A number of clinical trials have been conducted to test the efficacy of Radium-223 with other standard and experimental prostate cancer treatments, including AR-targeted therapy, immunotherapy, and chemotherapy.

Based on rationale that both radium-223 and AR-targeted agents (abiraterone acetate and enzalutamide) delay skeletal-related events and extend overall survival in mCRPC [181-183], phase 3 trials were conducted to combine radium-223 with either of these agents. Of note, the ERA-223 trial (NCT02043678) found an increased number of skeletal fractures with the combination of abiraterone/prednisone + radium-223 vs abiraterone/prednisone + placebo (23% vs. 10%), even in patients who used bone health agents (BHAs) [184], leading to regulatory warnings that this combination cannot be used. In contrast, the EORTC 1333/PEACE III trial (NCT02194842), which tested radium-223 in combination with enzalutamide, found that when BHA were mandated, risk of increased bone fractures with these agents alone or in combination were nearly abolished [185]. The risk of fracture may have been attributed to the physiologic distribution of radium-223 to bone cortex. In patients with high volume disease such as the ALSYMPCA trial, high uptake of radium-223 to sites of osseous metastatic disease may limit uptake into normal bone cortex. This so-called “sink effect” has been described with other radiopharmaceuticals [126]. This suggests extrapolating results of theranostic agents from patients with large tumor burdens to patients with small tumor burdens should be cautioned.

A phase I/II randomized trial which tested radium-223 + docetaxel versus docetaxel alone in bone metastatic CRPC patients demonstrated an improvement in median time to PSA progression (7 months vs. 5 months) [186]. This combination is now being tested in an open-labeled, randomized, phase III study in patients with mCRPC (NCT03574571). There may be similar opportunities to assess PSMA RNT in combination with docetaxel, owing to its properties as a radiosensitizer and single agent efficacy.

As discussed above, DNA damage caused by RNTs, particularly by targeted alpha therapies, suggest there is rationale to combine these agents with DNA damage repair inhibitors and immunotherapeutic agents. The combination of radium-223 plus olaparib vs. radium-223 alone is currently being tested in a randomized phase II trial (NCT03317392).

Several trials are testing radium-223 in combination with immunotherapy agents, including sipuleucel-T and checkpoint inhibitors. A phase II trial testing the combination of radium-223 with the cellular immunotherapy vaccine sipuleucel-T (NCT02463799), found an improvement in clinical outcomes (radiographic/clinical PFS, PSA response (≥50% decline), and AlkPhos response (≥30% decline)) with the combination, with no safety concerns noted [187]. Thus further study of this combination is warranted. Ongoing trials are also testing radium-223 in combination with pembrolizumab (NCT03093428) and nivolumab (NCT04109729). Results were recently reported from a phase 1b trial which tested the safety and tolerability of radium-223 plus atezolizumab in participants with metastatic CRPC and multiple bone metastases, visceral metastases and/or lymphadenopathy who have progressed after treatment with an androgen pathway inhibitor (NCT02814669). In 44 evaluable patients (out of 45 treated), the ratio of toxicity to anti-tumor effects were such that the investigators concluded that this regimen should not be pursued further [188]. As detailed above, there is interest in studying whether immunotherapy has synergistic effects with PSMA RNT.

The Need for Multidisciplinary Patient Management Teams

As the use of PET/CT imaging and radionuclide therapy in oncology becomes more widespread, it will become critical to consolidate multidisciplinary patient management teams that incorporate nuclear medicine specialists alongside medical oncologists, radiation oncologists, urologists, radiologists, pathologists, nursing, allied health, and others including researchers (Table 2, adapted from [189]). This expertise is required to interpret the increasing number of PSMA PET scans used in the evaluation of patients with metastatic prostate cancer. Furthermore, optimal selection of patients and application of radionuclide therapy requires specialists in nuclear medicine [190]. Multi-disciplinary teams enable cross-fertilization of ideas between team members which is vital for optimal management of patients. Other notable benefits include the development of successful concepts for clinical trials, and optimization of service quality and performance.

Conclusion

Overall, the PCF PSMA Theranostics State of the Science Meeting addressed the current state of understanding and most critical next steps surrounding the clinical development of PSMA theranostics for prostate cancer. These included the role and best use of PSMA PET imaging agents in patient management and differences in PSMA imaging agents, ongoing and planned clinical trials to optimize and position PSMA RNT therapy as single or combination agents, and the critical unknowns and barriers to successful use of these agents by the global clinical community. It remains critical to appropriately design trials that demonstrate efficacy based on survival or proven survival-associated endpoints, to maintain equipoise of clinical trials, and to develop standardized imaging and other biomarkers for patient selection for treatment. PSMA-targeted theranostics agents are a highly promising class of new agents that have significant potential to impact survival, but more research is needed to validate efficacy, extend disease control and improve outcomes. We hope that the knowledge shared at this meeting will help to focus studies on those most critical for advancing these agents and ultimately improve the lives of patients with prostate cancer.

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Figure and Table Legends

Figure 1. PSMA PET images taken before and after treatment with ^{177}Lu-PSMA-617 in 8 patients with mCRPC who exhibited exceptional PSA responses. This image was selected as the 2018 Society of Nuclear Medicine and Molecular Imaging (SNMMI) Image of the Year. Reprinted with author permission from [64].

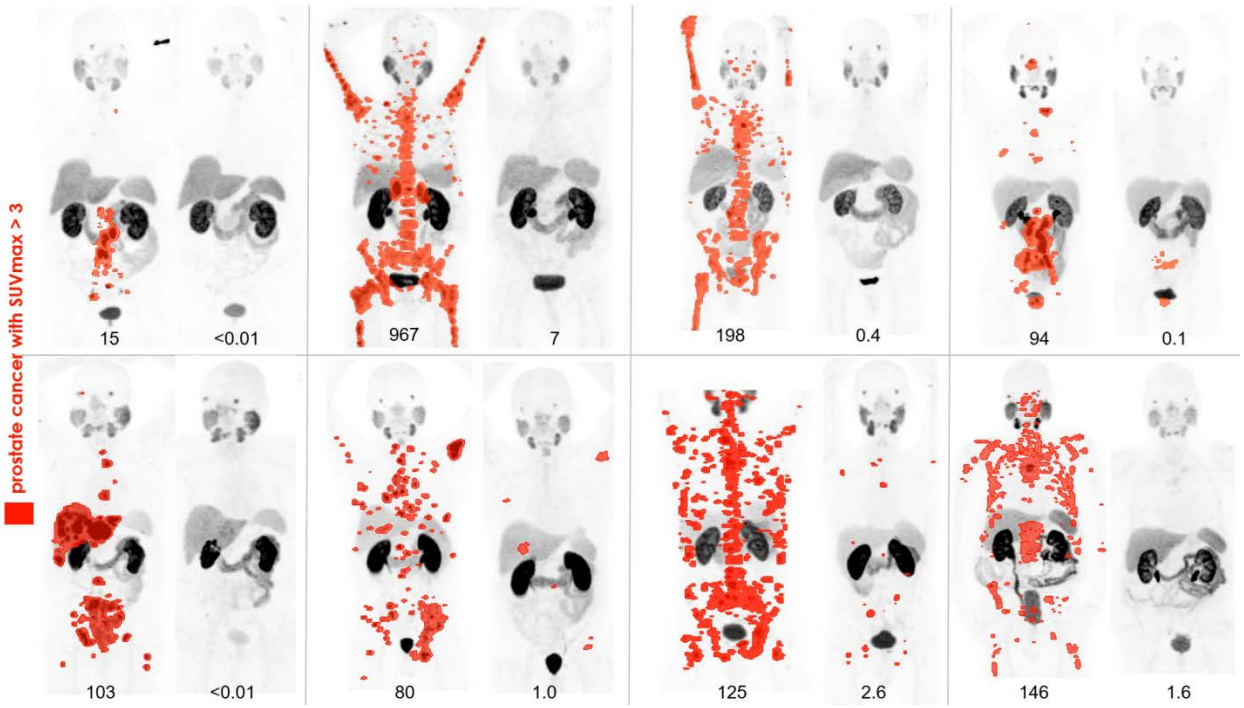

Figure 2. Voxel-based dosimetry Imaging. Voxel-based Monte-Carlo dosimetry was determined using 3 time-point qSPECT/CT following 8GBq ^{177}Lu-PSMA617.

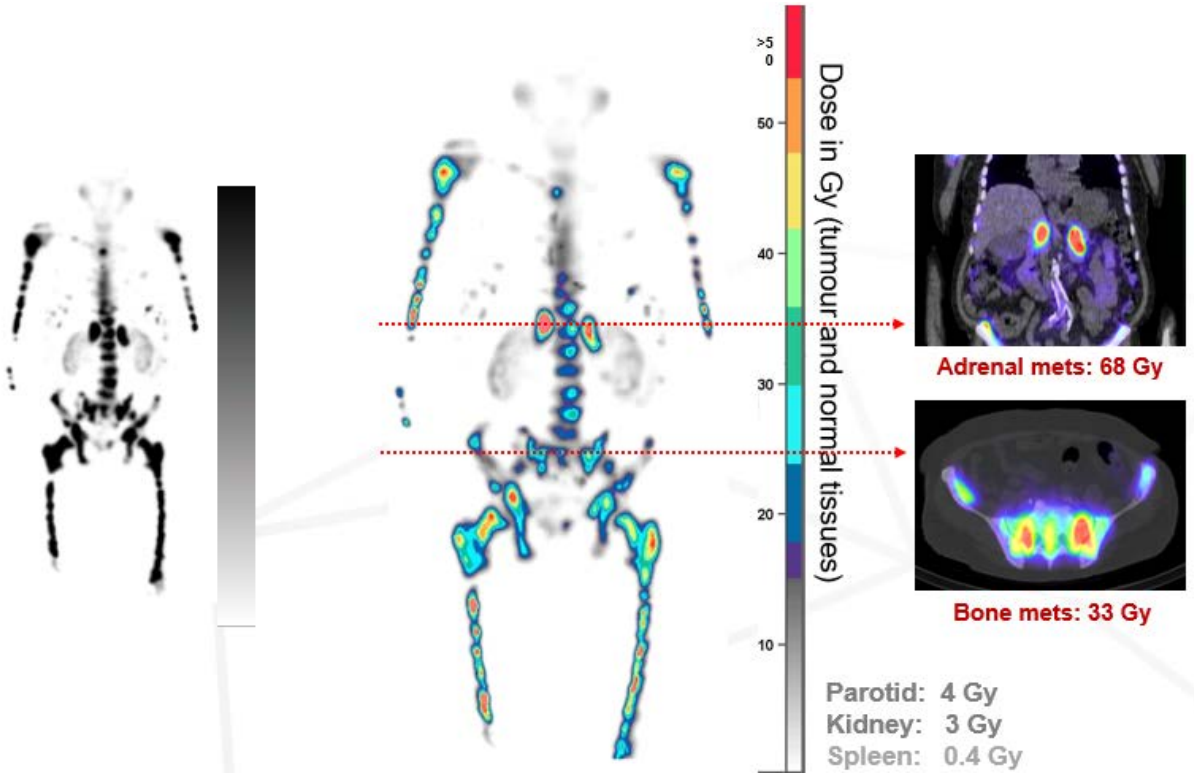

Table 1. Models for development of PSMA RNT as a "drug" vs. as a "radiopharmaceutical."

Radiotherapy Model	Drug Model
• Use dosimetry to define dose to critical organs • Don't exceed known maximum tolerated limits to critical organs • Disadvantage: limits theoretical, extrapolated from external-beam radiotherapy • Advantages: predict and avoid delayed toxicities, personalise and optimise administered activity	• Don't need dosimetry • Use phase 1 dose escalation study to determine maximum tolerated dose (MTD) • Disadvantage: predictable delayed cumulative toxicities (MDS / renal) will be missed • Advantage: acute toxicities observable and definable

Table 2. The multidisciplinary prostate cancer patient management team. Adapted from [189].

Medical Specialists	Nursing and Allied Health	Others
Urologist	Specialist nurse	Researchers
Medical oncologist	Psychologist	Administrative support
Radiation oncologist	Dietician	Clinical trial coordinators
Nuclear medicine physician	Exercise physiologist	Genetic counsellor
Radiologist	Physiotherapist	General practitioner
Pathologist	Intimacy specialist	Patient
Endocrinologist		

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