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Clinical Investigation

50-Gy Stereotactic Body Radiation Therapy to the Dominant Intraprostatic Nodule: Results From a Phase 1a/b Trial

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Summary

After prostate radiation therapy, local recurrences are observed in the dominant intraprostatic nodule. This phase 1a/b study safely escalated the radiation therapy dose to the dominant intraprostatic nodule up to

Purpose: Although localized prostate cancer (PCa) is multifocal, the dominant intraprostatic nodule (DIN) is responsible for disease progression after radiation therapy. PCa expresses antigens that could be recognized by the immune system. We therefore hypothesized that stereotactic dose escalation to the DIN is safe, may increase local control, and may initiate tumor-specific immune responses.

Patients and Methods: Patients with localized PCa were treated with stereotactic extreme hypofractionated doses of 36.25 Gy in 5 fractions to the whole prostate while simultaneously escalating doses to the magnetic resonance image–visible DIN (45 Gy, 47.5 Gy, and 50 Gy in 5 fractions). The phase 1a part was designed to

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50 Gy by using stereotactic body radiation therapy without grade 3 acute toxicity. Stereotactic body radiation therapy increased antigen-specific immune responses in a subset of patients.

determine the recommended phase 1b dose in a "3 + 3" cohort-based, dose-escalation design. The primary endpoint was dose-limiting toxicities defined as ≥grade 3 gastrointestinal (GI) or genitourinary (GU) toxicity (or both) by National Cancer Institute Common Terminology Criteria for Adverse Events (version 4) up to 90 days after the first radiation fraction. The secondary endpoints were prostate-specific antigen kinetics, quality of life (QoL), and blood immunologic responses.

Results: Nine patients were treated in phase 1a. No dose-limiting toxicities were observed at either level, and therefore the maximum tolerated dose was not reached. Further characterization of tolerability, efficacy, and immunologic outcomes was conducted in the subsequent 11 patients irradiated at the highest dose level (50 Gy) in the phase 1b expansion cohort. Toxicity was 45% and 25% for grades 1 and 2 GU, and 20% and 5% for grades 1 and 2 GI, respectively. No grade 3 or worse toxicity was reported. The average (±standard error of the mean) of the QoL assessments at baseline and at 3-month posttreatment were 0.8 (±0.8) and 3.5 (±1.5) for the bowel (mean difference, 2.7; 95% confidence interval, 0.1-5), and 6.4 (±0.8) and 7.27 (±0.9) for the International Prostate Symptom Score (mean difference, 0.87; 95% confidence interval, 0.3-1.9), respectively. A subset of patients developed antigen-specific immune responses against prostate-specific membrane antigen (n = 2), prostatic acid phosphatase (n = 1), prostate stem cell antigen (n = 4), and prostate-specific antigen (n = 2).

Conclusions: Irradiation of the whole prostate with 36.25 Gy in 5 fractions and dose escalation to 50 Gy to the DIN was tolerable and determined as the recommended phase 1b dose. This treatment has promising antitumor activity, which will be confirmed by the ongoing phase 2 part. Preliminary QoL analysis showed minimal impact in GU, GI, and sexual domains. Stereotactic irradiation induced antigen-specific immune responses in a subset of patients.

Introduction

In 2016, more than 241,000 US men received a diagnosis of prostate cancer (PCa), approximately 90% of whom had localized disease.[1]

Standard treatment options include surgery, radiation (with or without androgen deprivation therapy [ADT]), and active surveillance.[2] For external beam radiation therapy (EBRT), dose escalation is known to improve tumor control,[3,4] albeit not without increased toxicity because of the proximity of organs at risk (OARs), particularly the bladder and rectum.[5]

Several studies have suggested that PCa has a low alpha-to-beta ratio (1.4-2 Gy) that is similar to normal tissue late effects, suggesting that larger doses per fraction (hypofractionation) would be beneficial to increasing the therapeutic ratio.[6] Stereotactic body radiation therapy (SBRT) is a promising technique that allows the use of extreme hypofractionation to treat localized PCa by using 36.25 Gy administered in 5 fractions (7.25 Gy per fraction). Results from both retrospective and prospective series have shown excellent biochemical outcomes and acceptable toxicity profiles in patients with low- and intermediate-risk disease, which serves to reaffirm the concept of a low alpha-to-beta ratio for PCa.[7]

Studies of patterns of failure after conventional EBRT have shown that the main site of tumor recurrence—in more than 90% of cases—was the dominant intraprostatic nodule (DIN).[8,9]

The DIN is defined as the largest nodule that harbors the most aggressive biological behavior and therefore dictates the overall clinical prognosis of mutifocal PCa in a multifocal disease.[10] State-of-the-art imaging allows reliable identification of DINs.[11] Indeed, multiparametric magnetic resonance imaging (mpMRI), using T1- and T2-weighted sequences, dynamic contrast enhancement to assess perfusion, and diffusion-weighted imaging to calculate the different diffusion capability of PCa versus normal tissues, has markedly improved the sensitivity and specificity of magnetic resonance imaging (MRI) to detect and characterize prostatic carcinomas.[12] This approach enables high-precision targeting of DINs and escalation of the dose of radiation therapy (RT) to the MRI-visible dominant nodule, which should increase biochemical control while avoiding the increase in side effects seen with whole-gland SBRT dose escalation.

Previous landmark trials of dose escalation to the whole prostate using 45 Gy and 47.5 Gy have shown no evidence of severe toxicity.[13,14] However, further whole-prostate dose escalation up to 50 Gy has led to an increase in

severe late toxicity.[14,15] Although those studies seem to support the use of dose escalation to the whole prostate at least for the first 2 dose levels studied, questions remain regarding the feasibility and safety of heterogeneous dose-escalated SBRT delivered to the DIN, particularly considering the difficulties in manipulating hot spots located in proximity to healthy organs (urethra, bladder, and rectum). We therefore performed this SBRT regimen in the context of a dose-escalated phase 1 study.

We proposed irradiating the whole prostate gland, which may contain multifocal cancers, with tumoricidal doses of 36.25 Gy in 5 fractions while simultaneously escalating the dose specifically to the DINs to up to 50 Gy by using heterogeneous dose distribution (prescription isodose line 80%). We postulated that this approach would increase local tumor control while reducing genitourinary (GU) and rectal side effects in patients with intermediate- to high-risk PCa. Here, we report the results of a phase 1a/b study investigating boosting DINs by using SBRT while protecting the rectum, the bladder, and the urethra from high doses of radiation. To maximize protection of the rectum, we also used a rectal spacer.

Although the proposed extreme hypofractionated RT approach was expected to provide excellent local control of the primary PCa lesion(s), a proportion of patients with intermediate- or high-risk disease may have recurrences with distant metastases in the future, requiring strategies to minimize systemic disease. Hypofractionated EBRT is known to trigger occasional abscopal effects, meaning the eradication of a nonirradiated distant metastasis, which is thought to be mediated largely by adaptive immunity, specifically T cells.[16] Indeed, the triggering of antitumor immune response by RT has been reported before, but the optimal dose and schedule of fractionation to maximize immune effects are not entirely understood and are presently being debated.[16] To better understand the immune effects of the extreme hypofractionated RT applied, we also investigated the T-cell responses to PCa-specific antigens in these patients at different time points before, during, and after SBRT.

Methods and Materials

This phase 1 dose-escalation study was approved by the Ethics Committee of the Canton Vaud (ClinicalTrials.gov ID: NCT02254746). Informed consent was obtained from all patients. The primary endpoint of the study was to assess acute (up to 90 days after the first RT fraction) urinary and rectal toxicity. Secondary endpoints were prostate-specific antigen (PSA) kinetics, quality of life (QoL), and immunologic response to SBRT. Patients were enrolled from October 2014 to April 2017.

Eligible patients were those with newly diagnosed and previously untreated PCa, intermediate- and high-risk disease according to the D'Amico risk classification,[17] and stage T2 to T3 adenocarcinoma of the prostate, N0, M0. All patients had to have at least 1 visible nodule at the endorectal coil (ERC) mpMRI. The serum PSA level was required to be < 50 μg/L, and the International Prostate Symptom Score (IPSS) ≤ 15 (alpha blockers allowed). Patients were excluded if they had a pre-SBRT prostate volume on MRI greater than 70 cm^3 or a tumor located at less than 3 mm from the urethra when measured at the ERC MRI. They were also excluded if they had evidence of inflammatory colitis or previous RT in the pelvis. Concomitant or adjuvant ADT was allowed, but neoadjuvant ADT was an exclusion criterion.

The Ethics Committee of the Canton Vaud approved the translational research part of the study, and an independent informed consent was obtained from patients. Blood was collected at the following time points: SBRT-0 (day 0 of SBRT, before the introduction of a rectal spacer), SBRT-5 (the day the patient received the fifth fraction of SBRT), SBRT-15 (15 days after the last fraction of SBRT), and SBRT-40 (40 days after the last fraction of SBRT). Patients with human leukocyte antigen (HLA) allele A2+ were included in the translational analyses. Exclusion criteria from the translational analyses were anemia (hemoglobin <100 g/dL at baseline), human immunodeficiency virus seropositivity with or without hepatitis B and C active infection, or any other condition, such as autoimmune diseases or medication that could have affected the results of the immune monitoring.

Radiation therapy planning and delivery

Approximately 8 to 10 days before the RT scanner simulation, a biodegradable spacer device (BioProtect Balloon, Ltd., Kfar Saba, Israel) was transperineally inserted between the prostate and the rectum under transrectal ultrasound guidance after the patient received sedative anesthetics. During the same procedure, 4 fiducial markers consisting of gold anchors (Gold Anchor, Naslund AB, Sweden) were placed in the prostate with at least 2 cm of separation among them to accommodate the fiducial spacing threshold of at least 1 cm of separation on orthogonal imaging to ensure accurate rotational corrections. All angles formed by at least 3 fiducials had to be greater than 15° to comply with the colinearity threshold.[18] MRI and computed tomography (CT) scans for treatment planning were performed 7 days after fiducial implantation to ensure adequate fiducial marker positioning and to allow for resolution of edema/inflammation.[18] The planning MRI was immediately followed (after <2 hours) by a planning CT scan to minimize anatomic changes in the rectum that may interfere with image fusion. Both planning MRI and planning CT were performed with the same slide thickness (1 mm) to favor correct fusion.

Precautions were taken to minimize prostate motion during the planning scans and treatment. Specifically, starting 5 days before acquisition of the planning scans until the end of treatment, patients maintained a low-fiber

diet to reduce intestinal gas. They were instructed to use a mild laxative 48 hours before the planning CT scan. When necessary, enemas were performed 1 hour before acquisition of the planning scans and before each treatment session to minimize rectal volume. Patients were also instructed to drink 200 mL of water 1 hour before the scans, after voiding completely. All patients had images taken and were treated in the supine treatment position with a knee cushion to maximize patient comfort and limit prostate motion in response to respiration.[19]

For accuracy on contouring and appropriate visualization of the DIN and OARs, the planning T2-weighted MRI image sets after rectal spacer/fiducial markers insertion were rigidly fused to the planning-CT images using Velocity Advanced Imaging Software (Varian Medical Systems, Palo Alto, CA). Intraprostatic fiducials were used to guide image coregistration and limit fusion errors.[20] Axial and sagittal planes of both planning MRI and planning CT scans were used to maximize the visualization of the fiducials and guide coregistration.

The diagnostic ERC mpMRI performed before rectal spacer and fiducial marker insertion was deformably coregistered to the planning MRI and the planning CT scans to aid in accurate localization of the target volume and OARs.[21] This process was performed with Velocity Advanced Image Software (Varian Medical Systems, Palo Alto, CA) using the fade correction algorithm.

Anatomical contours of the prostate, DIN, seminal vesicles, and OARs were performed on the planning-CT scan by the principal investigators of the trial (FH and JB) before being scrutinized by a panel of board-certified radiation oncologists. A radiologist (J-YM) delineated the DIN as the region of interest in the MRI. The prostate was expanded uniformly by 3 mm to create the planning target volume (PTVp).

The DIN was contoured as the gross tumor volume and expanded by 3 mm to create a PTV_{DIN} (no clinical target volume was used around the gross tumor volume). The prescribed dose to the PTVp was 36.25 Gy in 5 fractions (7.25 Gy per fraction). The prescribed dose to the PTV_{DIN} was 45, 47.5, and 50 Gy in 5 fractions, corresponding to the 80% isodose line; therefore the maximum dose point corresponded to 56.25, 59.38, and 62.5 Gy, respectively. To allow gradients for DIN boosting and to maximize PTV_{DIN} doses, there were no limits on dose heterogeneity. At least 95% of the volume of interest (PTV_{DIN} and PTVp) had to be covered by >95% of the prescription dose. A minimum of 2 days and a maximum of 6 days had to separate each treatment fraction. No more than 2 fractions would be delivered per week. The overall treatment time had to be no more than 20 days.

We recognize that this treatment is a more conservative treatment schema than the ones tested in previous trials, which usually delivered 5 fractions in consecutive days or every other day. This fractionation schema was based on that of Fowler et al,[22] who advised to prolong the total treatment time to at least 5 weeks to avoid side effects. From the disease-control perspective, the prostate carcinoma cell repopulation time was assumed to be 40 days (range, 15-60 days), and thus the proliferation between fractions was theoretically negligible.[22]

Dose-volume histogram goals for the rectum were maximum dose to 1 cm^3 < 38 Gy, maximum dose to 0.1 cm^3 < 41 Gy, and V25 < 20 (ie, the volume receiving 25 Gy < 20 cm^3). The bladder dose-volume histogram was limited to no more than 1 cm^3 receiving 41 Gy or greater, 0.1 cm^3 to receive less than 45 Gy, and the bladder median dose was not to exceed 20 Gy. The urethra dose was limited to no more than 1 cm^3 of urethra receiving more than 39 Gy and 0.1 cm^3 not to exceed 41 Gy (Fig. 1).

SBRT was delivered via CyberKnife (Accuray Inc, Sunnyvale, CA) with energies of 6 MV. The CyberKnife unit is equipped with 2 orthogonal x-ray imaging devices for automated image guidance based on fiducial markers. Before treatment delivery, the system determined the absolute position of the fiducial markers via CT image-to-digitally reconstructed radiograph registration. This method allowed for the assessment of potential marker migration or misalignment between fractions (interfraction motion).[23,24]

During treatment delivery, orthogonal x-ray images were used to locate the center of mass of implanted gold fiducial markers within the prostate and allowed for targeting and correction of the therapeutic beam during an individual treatment (intrafraction motion). A minimum of 3 properly placed fiducials was required to accommodate 6-dimensional tracking. The goals of fiducial tracking were to keep the translational shifts (X, Y, Z) to less than 2 mm and the rotational shifts to less than 5° (roll maximum 2°, pitch maximum 5°, yaw maximum 3°) throughout treatment. Imaging was obtained every 30 to 60 seconds to assure submillimeter tracking accuracy.

The shift of x-ray images from the planning CT scan was monitored in real time during each fraction. If the calculated shift was more than the given threshold, the treatment would be paused and manual couch movement would be required until the shift was below the limit. The implementation of this technique for daily setup and intrafraction corrections was shown by others to help decrease the required PTV margins to less than 3 mm.[25]

Treatment schema, definitions, and statistical analysis

The radiation dose to the whole prostate was 36.25 Gy in 5 fractions of 7.25 Gy. For dose escalation to the DIN, we used the traditional 3 + 3 design, which remains the prevailing method for conducting phase 1 cancer clinical trials; one of its theoretical main advantages is that it is recognized as being safe.[26] The dose-expansion cohort was used to confirm the safety of the treatment at the recommended phase 2 dose.[27]

Patients in the phase 1a part (in cohorts of 3 per dose level) were treated with a starting dose to the DIN of 9 Gy per fraction up to 45 Gy. If no DLT was observed in the first

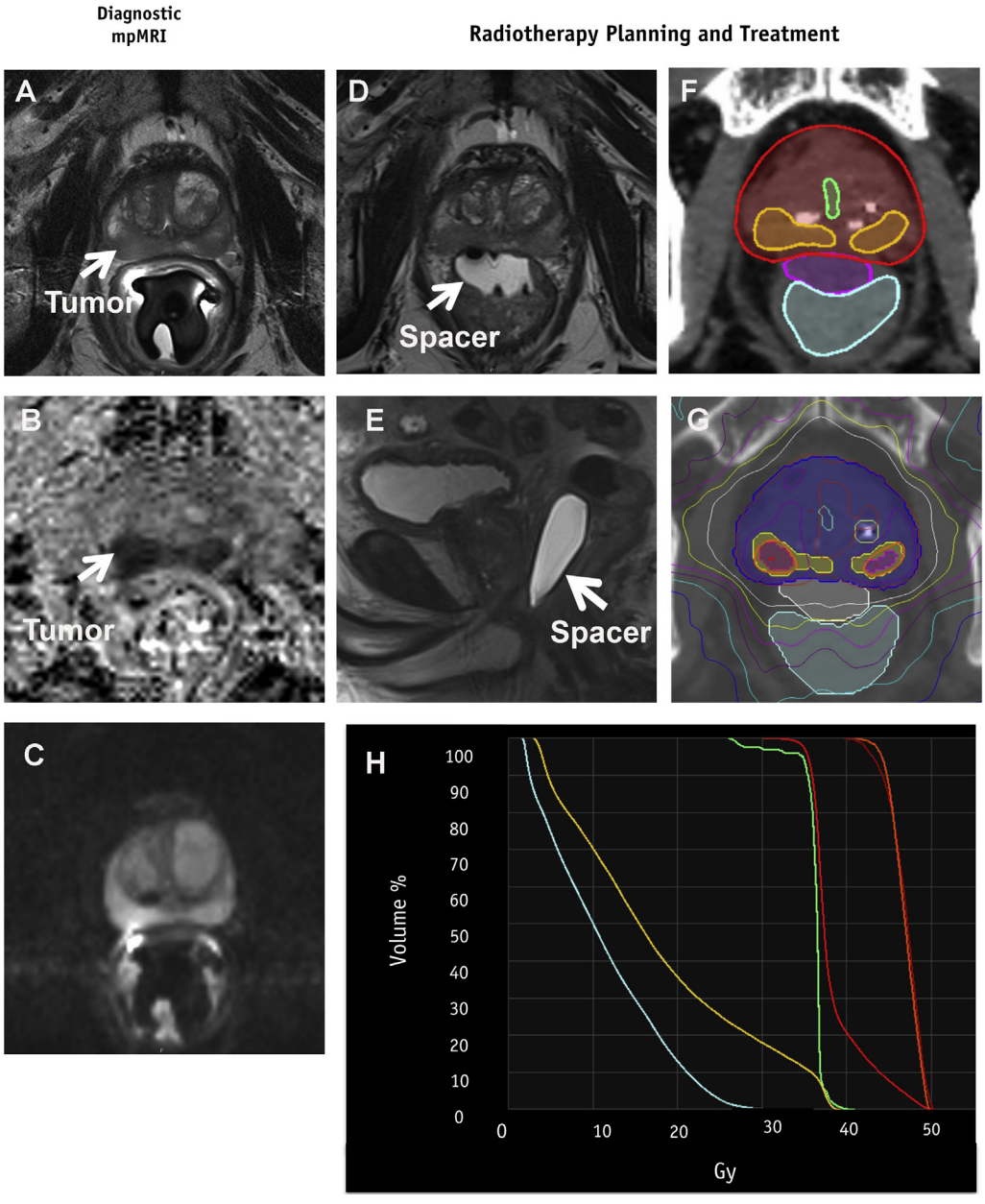

Fig. 1. Patient with a prostate cancer (T2, N0, M0; Gleason Score, 3 + 4 = 7; prostate-specific antigen, 20 μg/L). Magnetic resonance imaging shows 2 lesions in the medial region, in the right and left posterior peripheral zones, with hypointensity on axial T2-weighted image (A), restriction of diffusion on apparent diffusion coefficient map (B) and on diffusion-weighted images (C), and Prostate Imaging Reporting and Data System (4 on the left and 5 on the right). RT planning magnetic resonance imaging scan shows a hyperintense rectal spacer on the axial (D) and sagittal (E) T2-weighted images. (F) RT planning was performed on a CT scan, and an axial image shows the RT volumes: prostate in red, tumor in yellow, rectal spacer in pink, rectum in light blue, and urethra in green. Fiducial markers were placed in the prostate for robotic-assisted tracking purposes. (G) Stereotactic body radiation therapy plan using CyberKnife. The whole prostate was treated with 36.25 Gy in 5 fractions of 7.25 Gy (dark-blue isodose) with a boost of 47.5 Gy to the dominant intraprostatic nodule (orange isodose). (H) Dose-volume histogram shows the maximal dose delivered to the urethra: 0.1 cm^3 = 38 Gy (light-green curve); the anterior rectal wall, 1 cm^3 = 25.9 Gy (light-blue curve); and the bladder: 1 cm^3 = 38 Gy (yellow curve). The prostate (red curve) and the tumors (orange curve) received the prescribed dose. *Abbreviations:* CI = computed tomography; RT = radiation therapy.

cohort, an additional 3 patients were entered at the next dose level (47.5 Gy in 5 fractions of 9.5 Gy), with dose escalation continuing until DLT was observed or if the maximum dose level (50 Gy in 5 fractions of 10 Gy) was reached in the absence of a DLT. If 1 of the 3 patients experienced a DLT at a particular dose level, an additional 3 patients were planned to be included at that level. If 2 or more patients experienced a DLT at a given dose level, a lower dose level would have been explored to define the maximum tolerated dose. The 3 patients included in a cohort could be enrolled simultaneously or sequentially without any waiting period among them. However, dose escalation in the phase 1a was not allowed until the last patient included in a specific cohort completed a minimum follow-up period of 90 days without experiencing DLT.

DLTs were defined as grade 3 or higher gastrointestinal (GI) or GU toxicity that appeared from the first fraction of RT and up to 90 days after completing treatment. Once the maximal dose level was reached, an interim analysis was to be conducted per protocol and evaluated by the Independent Data and Safety Monitoring Board, with a cutoff date of April 4, 2015. If this evaluation did not identify any safety issues, additional patients were to be treated at the maximal dose level to confirm the safety and tolerability of the combination in a phase 1b expansion cohort.

All adverse events were graded by the National Cancer Institute Common Toxicity Criteria, version 4. Secondary endpoints were late toxicity (occurring >90 days from first fraction), PSA kinetics, and patient-reported outcome. European Organisation for Research and Treatment of Cancer QoL Form PR25 (prostate module) together with the IPSS score were collected at baseline and 1, 3, and 6, months after treatment. Health-related QoL outcomes were scored using the European Organisation for Research and Treatment of Cancer guidelines[28] into values ranging from zero to 100. A difference of 10 points or more was considered clinically relevant.[29] Patients were followed up by having PSA measurements, a history, and a physical examination performed every 3 months for the first 2 years, and every 6 months thereafter. The nadir +2 μg/mL failure definition was used for biochemical control.[30] Analyses were done in Prism 7 (GraphPad Software, Inc, La Jolla, CA).

Immune monitoring

All analyses were performed after Minimal Information About T cell Assays (MIATA) guidelines.[31] In squared brackets, the reader can follow the Minimal Information About T cell Assays modules and submodules submitted as Appendix E1 (available online at https://doi.org/10.1016/j.ijrobp.2018.09.023).

Patient material

Peripheral blood mononuclear cells (PBMCs) were isolated from Li-heparin whole blood with drawls (1.2, 1.3, 1.4) by density gradient using Ficoll-Paque Plus (GE Health care), according to the laboratory standard of practice (1.6, 5.1, 5.4), and immediately cryopreserved in 90% FCS (Fetal Calf Serum; Gibco) and 10% dimethyl sulfoxide in CoolCells devices (BioCision) at −80 °C and transferred after 24 hours in liquid nitrogen until analysis (1.10, 1.11, 1.12). Cryopreserved PBMCs were thawed in Roswell Park Memorial Institute (RPMI) medium (Invitrogen), 20% FCS, and counted with the Trypan Blue method in Neubauer counting chambers (1.20). Viability after thawing was >85% (1.15). Cells were then split between flow cytometry analyses (3 × 10E6) or rested for 5 hours in RPMI-8% human AB serum (HS; Biowest) at 37°C before the in vitro stimulation.

Analysis of T-cell responses against Human leukocyte antigen (HLA) serotype A2 (HLA-A2) restricted epitopes of the PCa-specific antigens prostate stem cell antigen (PSCA), prostate-specific membrane antigen (PSMA), PSA, and prostatic acid phosphatase (PAP) were quantified by IFNγ Enzyme-Linked ImmunoSpot (ELISpot). We also performed enumeration of circulating leukocytes and phenotypic and functional characterization of T cells.

All assays were performed using general research investigative assays (5.5).

In vitro stimulation of antigen-specific T cells

Given the precursor frequencies of antigen-specific T cells in peripheral blood samples, a conventional highly sensitive assay was used to quantify antigen-specific T-cell responses.[32-34] Thawed PBMCs rested for 5 hours in RPMI (Invitrogen), and 8% human AB serum (HS; Biowest) supplemented with penicillin or streptomycin and beta-mercaptoethanol at 37°C (2.1) were plated in 96-well plates at 10E5 cells per well in RPMI-8% human serum. Peptide pools (see the following list) were added in replicates (2-6 wells per condition). Peptide pools were composed of the following:

- Prostate stem cell antigen ($PSCA_{7-15}$ ALLMAGLAL, $PSCA_{14-22}$ ALQPGTALL, $PSCA_{21-30}$ LLCYSCKAQV)
- Prostate-specific membrane antigen ($PSMA_{4-12}$ LLHETDSAV, $PSMA_{27-38}$ VLAGGFFLL, $PSMA_{441-450}$ LLQERGVAYI, $PSMA_{663-671}$ MMNDQLMFL)
- Prostate-specific antigen (PSA_{53-61} VLVHPQWVL, $PSA_{146-154}$ KLQCVDLHV, $PSA_{165-174}$ FLTPKKLQCV, $PSA_{178-187}$ VISNDVCAQV)
- Prostatic acid phosphatase (PAP_{18-26} FLFLLFFWL, $PAP_{112-120}$ TLMSAMTNL, $PAP_{299-307}$ ALDVYNGLL)

Recombinant human interleukin-2 was added to the culture after 48 hours at a final concentration of 100 U/mL (2.4). At day 12, IFN-γ ELISpot assays were performed using precoated 96-well ELISpot plates ($ELISpot^{PRO}$ kit for Human IFN-γ from Mabtech, ref 3420). Analyses were performed with an established laboratory protocol and under Good Laboratory Practice conditions (5.1, 5.4). Each

individual well was split into 2 identical fractions, and only 1 fraction was rechallenged with 1 μg/mL of the corresponding peptide pool for 16 to 18 hours at 37°C and 5% CO_2.

As a positive control, 5 × 10E4 cells stimulated with staphylococcal enterotoxin B at a concentration of 0.25 ng/mL or respective cells without rechallenge (medium only) were used as negative control (2.5). Plates were washed according to the manufacturer's instructions and counted with the iSpot Robot ELISpot reader (AutoImmun Diagnostika GmbH).

For each condition, background was subtracted from each replicate (unstimulated fraction was considered as a negative control), and the relative magnitude of tumor-specific T-cell responses was determined for each antigen, time point, and patient as the proportion of positive replicates (ie, see the background described in the preceding paragraphs [3.4, 4.4]) of the total number of replicates tested (4.6). The relative magnitude of prostate cancer antigen-specific T-cell responses ranged from 0 to 1, corresponding to the proportion of positive replicates. The cumulative prostate antigen T-cell response was measured for each patient as the sum of the individual responses against the 4 distinct prostate cancer antigen peptide pools and ranged from 0 to 4. Row data of ELISpot assay can be provided to the reader on request.

Flow cytometry phenotypic analyses

Fresh thawed patient PBMCs were washed and stained in phosphate-buffered saline with the following distinct panels (2.1, 2.2):

- PBMC basic phenotype panel: CD3 APC (IM2467, BC), CD4 PE-Cy7 (737660, BC), CD8 Pacific Blue (558207, BD), CD14 APC-H7 (641394, BD), CD16 FITC (555406, BD), CD56 PE (A07788, BC), CD11c Alexa Fluor 700 (561352, BD), CD19 Brilliant Violet 711 (563036, BD), CD123 PerCP-Cy5.5 (45-1239-42, eBioscience), HLA-DR ECD (IM3636, BC), Zombie UV (77474 BioLegend)
- CD8 T-cell panel: CD3 PerCP-Cy5.5 (300430, BioLegend), CD8 Brilliant Violet 650 (301042, BioLegend), CD45RA Alexa Fluor7 00 (560673, BD), CCR7 (CD197) Brilliant Violet 711 (353228, BioLegend), CD127 Brilliant Violet 605 (351334, BioLegend), PD1 (CD279) Brilliant Violet 421 (329920, BioLegend), ICOS (CD278) APC-eFluor 780 eBioscience (47-9948-42), CCR4 (CD194) PE-Cy7 (557864, BD), Zombie UV (77474 BioLegend), Granzyme B PE-Texas Red (GRB17, Life Technologies), Perforin-PE (AB47226 Abcam), BAX Alexa Fluor 488 (633604, BioLegend), BCL-2 (658706, BioLegend)
- CD4 Treg panel: CD3 Brilliant UV496 (564809, BB), CD4 Brilliant Violet 510 (317444, BioLegend), CD45RA Alexa Fluor 700 (560673, BD), CCR7 (CD197) Brilliant Violet 711 (353228, BioLegend), CD127 Brilliant Violet 605 (351334, BioLegend), CD25 PE (A07774, BC), ICOS (CD278) APC-eFluor 780 eBioscience (47-9948-42), CCR4 (CD194) PE-Cy7 (557864, BD), CD73 PE-Dazzle 594 (344020, BioLegend), CD14 PerCP-Cy 5.5 550787, BD), Zombie UV (77474 BioLegend), FoxP3 eFluor 450 (48-4776-02, eBioscience), BAX Alexa Fluor 488 (633604, BioLegend), BCL-2 (658706, BioLegend)

Staining was performed at 4°C in the dark, and for intracellular staining, the FoxP3 Fix/Perm kit (eBioscience) was used (2.4). Analyses were performed with an established laboratory protocol and under Good Laboratory Practice conditions [5.1, 5.4].

Samples were acquired on a 5-laser BD LSRFortessa cytometer equipped with FACSDiva software version 8.0.1 (3.1). Cytometer Setup and Tracking (CS&T) settings were performed daily and before each experiment (3.2). Analyses were performed with FlowJo (FLOWJO.LLC) software version 10 (3.3, 3.4), and all data were processed in GraphPad PRISM version 7. FCS files can be provided to the reader on request (4.3).

Analyses of the T-cell proliferation capacity

Cryopreserved PBMCs were thawed in RPMI (Invitrogen), 20% FBS. After an overnight rest in RPMI (Invitrogen) and 8% human AB-serum (HS; Biowest) supplemented with penicillin, streptomycin, and beta-mercaptoethanol at 37°C and 5% CO_2 (2.1), cells were labeled with 1.5-μM final concentration Carboxyfluorescein succinimidyl ester (CFSE) (CellTrace CFSE Cell Proliferation Kit, Life Technologies) according to the manufacturer's instructions and laboratory robust standard operating procedures (5.4) and stimulated with 50 ng/mL of an anti-CD3 antibody (Mabtech). After 6 days of in vitro expansion, cells were washed and stained for dead cells (Zombie UV Fixable viability kit, BioLegend) and with CD3 APC-Fire 750 (344840, BioLegend), CD4 Pacific Blue (558116, BD), and CD8 Brilliant Violet 650 (301042, BioLegend). The percentage of proliferated $CFSE^{low}$ cells and $CD4^+$ and $CD8^+$ T cells was determined among viable T cells (4.4). A representative data set is shown in Figure E3D-E; available online at https://doi.org/10.1016/j.ijrobp.2018.09.023 (3.4]). As negative control, nonstimulated cells were used (2.5). FCS files for all samples can be provided on request (4.3).

Results

Early PSA kinetics, side effects, and patient-reported outcomes

A total of 20 patients were treated and are evaluable for study endpoints (9 patients in phase 1a and 11 patients in phase 1b). Patients' pathologic and treatment characteristics are detailed in Table 1. The median age was 73.5 years (range, 58-85 years). The PSA was 12.3 (range, 2.7-40 μg/mL), the IPSS was 7 (range, 0-12), and the prostate volume

Table 1 Patient characteristics

Characteristics	Number of patients (N = 20)	Patients (%)
Tumor stage		
T2a	6	30
T2b	6	30
T2c	5	25
T3a	3	15
Gleason Score		
3 + 3	2	10
3 + 4	8	40
4 + 3	5	25
4 + 4	2	10
3 + 5	1	5
4 + 5	2	10
Intermediate-risk*		
(Gleason Score = 7 or PSA 10-20, T2b)	7	35
High-risk*		
(Gleason Score ≥8 or PSA >20 or ≥T2c)	13	65
Hormone therapy		
Yes	1	5
No	19	95

Abbreviations: PSA = prostate-specific antigen.
* Risk stratification according to D'Amico classification.[17]

Table 2 Genitourinary and gastrointestinal toxicity according to grade and time

	Genitourinary toxicity				Gastrointestinal toxicity			
	≤90 d		>90 d		≤90 d		>90 d	
Grade	N	%	N	%	N	%	N	%
1	9	45	6	30	4	20	1	5
2	5	25	2	10	1	5	0	0

Abbreviations: N = number of patients having developed grade 1 or 2 toxicity.
Toxicity graded according to Common Terminology Criteria of Adverse Events, version 4.
No grade 3 or higher toxicity was reported. Period: from day 1 of stereotactic radiation therapy and up to 90 days after the first fraction or more than 90 days after the first radiation fraction.

at baseline was 36 cm^3 (range, 12-60 cm^3). The median number of DINs treated was 2 (range, 1-3), and the median DIN volume was 2 cm^3 (range, 0.6-4). Sixty-five percent of the patients were high risk, and 35% were intermediate risk, according to the D'Amico classification.[17] The median RT treatment duration was 19 days (range, 14-19 days). All patients were able to complete their treatments. None of the patients died or were unavailable for follow-up. Despite ADT being offered to the entire patient population, only 1 intermediate-risk patient accepted to undergo 6 months of ADT.

No DLT was observed within 90 days from the start of treatment, and thus dose escalation proceeded through all planned dose levels. Consequently, 3 patients were entered in each dose level (N = 9), and 11 additional patients were enrolled at the 50-Gy dose level (N total = 20).

The number of patients experiencing GI and GU toxicity by grade and time is shown in Table 2. Table E1 (available online at https://doi.org/10.1016/j.ijrobp.2018.09.023) shows the most common reported acute adverse events. Only grade 1 and 2 GI and GU toxicity was observed within 90 days. The most common early GU toxicity was urinary frequency and urgency. No grade 3 toxicity occurred over the complete course of follow-up in any of the dose levels. No complications or side effects were observed because of the placement of the rectal spacer.

With a median follow-up of 24 months (range, 6-39 months), 19 of 20 men showed a marked reduction in early PSA measurements. Figure E1A (available online at https://doi.org/10.1016/j.ijrobp.2018.09.023) shows the decline in PSA as a function of patients' initial PSA. One patient experienced a biochemical failure 1 year after treatment; he had a baseline of cT3a, N0, M0 and a Gleason Score of 9 disease; he was ultimately found to have distant metastases without evidence of recurrence in the prostate.

All of the patients completed the patient-reported outcome questionnaires at 4 time points. Figure E1 B-E (available online at https://doi.org/10.1016/j.ijrobp.2018.09.023) shows trends in (B) PR-25 change in urinary function, (C) IPSS scores, (D) PR-25 bowel symptoms scores, and (E) PR-25 sexual activity scores.

The average (±standard error of the mean) of the QoL assessments at baseline and at 3 months posttreatment for all patients was 0.8 (±0.8) and 3.5 (±1.5), respectively, for PR25 bowel module (mean difference, 2.7; 95% confidence interval [CI], 0.1-5); 11 (±2.8) and 18 (±3.3) for the PR25 GU module (mean difference, 7; 95% CI, 3.3-10); 6.4 (±0.8) and 7.27 (±0.9) for IPSS (mean difference, 0.87; 95% CI, 0.3-1.9); and 42.4 (±7.25) and 36.3 (±7.41) for PR25 sexual activity module (mean difference, 6; 95% CI, 0.09-12).

Tables 3 and 4 show the planned versus the delivered doses to the targets and OARs.

Immunologic monitoring

We measured peripheral blood leukocyte subsets longitudinally in 8 of 20 HLA-A2+ patients treated in this phase 1 study. We found no radiation-induced decline in peripheral blood leukocyte populations including natural killer cells; $CD3^+$, $CD4^+$, and $CD8^+$ T cells; $CD4^+$-to-$CD8^+$ ratio; $CD19^+$ B cells; and dendritic cells and monocytes up to 40 days after treatment (Fig. 2A-D). Analysis of absolute cell counts confirmed this observation (Fig. E2 A-B; available online at https://doi.org/10.1016/j.ijrobp.2018.09.023). We did not observe variations in lymphocyte phenotype during or after treatment (Fig. E2 C-D; available online at https://doi.org/10.1016/j.ijrobp.2018.09.023). The overall

Table 3 Dosimetry analysis for the organs at risk

Organ at risk	Dose planned per protocol	Median delivered (range)
Rectum	0.1 cm^3 <41 Gy	0.1 cm^3 <33 Gy (26-41 Gy)
	1 cm^3 <38 Gy	1 cm^3 <27 Gy (24-36 Gy)
	V25 Gy <20 cm^3	V25 Gy 3 cm^3 (0.6-20 cm^3)
Bladder	0.1 cm^3 <45 Gy	38 Gy (37.5-41 Gy)
	1 cm^3 <41 Gy	37 Gy (36-39.5 Gy)
	Median dose <20 Gy	14.16 (4-20 Gy)
Urethra	0.1 cm^3 <41 Gy	38 Gy (35-41 Gy)
	1 cm^3 <39 Gy	36.33 Gy (33.4-39 Gy)

ability of CD4 and CD8 T cells to expand and proliferate upon T cell receptor (TCR) stimulation also remained unaffected by the treatment (Fig. E3A-C; available online at https://doi.org/10.1016/j.ijrobp.2018.09.023), and the viability of T cells expanded ex vivo and in vitro remained stable before and after treatment (Fig. E3D; available online at https://doi.org/10.1016/j.ijrobp.2018.09.023).

Next, we assessed whether radiation induced T-cell responses against PCa antigens, including PSMA, PSA, PSCA, and PAP. To this end, we stimulated peripheral CD8 purified T cells with pools of peptides derived from prostate cancer antigens for 12 days in vitro and then rechallenged expanded T cells with the same cognate antigens to determine the relative magnitude of tumor antigen-specific CD8 T-cell responses, as recently described.[34] We detected a prostate cancer antigen−specific immune response after treatment in 5 of 8 patients. We detected radiation-induced T-cell responses directed against PSCA in 4 patients, PSA in 2 patients, PSMA in 2 patients, and PAP in 1 patient. Responses were observed as early as 15 days after the end of SBRT and persisted on day 40 after the end of treatment (for PSCA, n = 4 patients, and for PSMA, n = 1 patient; Fig. 3A). A significant increase in the overall magnitude of tumor antigen−specific T-cell responses was observed 40 days after the end of treatment relative to the pretreatment baseline ($P = .014$; Fig. 3B). The responses against PAP and PSA were generally transient and substantially lower in magnitude compared with the PSCA-specific responses. We did not observe any correlation between peptide-specific immunoreactivity and clinical outcome because the 8 patients participating in the translational part had a decline in PSA measurements.

Discussion

The essential goal of PCa RT is to administer a risk-adjusted and patient-tailored treatment offering maximal cancer control and minimal side effects. We demonstrate the feasibility of dose escalation to the DINs to up to 50 Gy in 5 fractions, without grade 3 or higher acute urinary and rectal toxicity. However, in our study, 70% of the patients experienced acute grade 1 or 2 urinary toxicity that persisted at more than 90 days in 40% of them. It is difficult to ascertain whether these grade 1 or 2 symptoms will persist or worsen with longer follow-up.

Reported rates of acute grade 1 or 2 GU toxicity in previous SBRT trials were between 28% and 78%.[35-40] Most of these SBRT series limited PTV doses to 35 to 36.25 Gy in 5 fractions.[35-40] Series using heterogeneous dose distribution to 50 Gy using CyberKnife have reported grade 1 to 2 acute GU toxicities of 50% to 60%.[41,42]

A recent study using high-dose-rate brachytherapy to deliver a boost (18.75 Gy in 1 fraction) to the DIN after SBRT (37.5 Gy in 15 fractions) reported 20% acute grade 2 GU toxicity that persisted in 6.7% of the patients.[43] In a multicenter dose-escalation phase 1-2 SBRT study in patients treated to 50 Gy in 5 fractions, Boike et al[13] reported a grade 1 to 2 acute GU toxicity of 60%. Similarly, Quon et al reported an acute grade 1 to 2 GU toxicity of 64% when irradiating the whole prostate gland to 40 Gy in 5 fractions every other day.[44] Therefore, the grade 1 to 2 acute GU toxicity profile reported in our study is in line with the ones previously reported. We have not observed acute grade 3 or higher GU toxicity or GI toxicity in our study. Reported rates of acute grade 3 urinary or rectal toxicity were between 0% and 7% in previous trials.[13,35-40,44]

Previous SBRT studies irradiating the whole prostate gland with a dose between 35 and 37 Gy have reported acute grade 1 or 2 GI toxicity rates of 16% to 80%.[35-40] Quon et al reported 82% acute grade 1 to 2 GI toxicity using 40 Gy in 5 fractions to the whole prostate.[44] Boike et al reported 54% acute grade 1 to 2 GI toxicity in the whole-prostate, 50-Gy dose cohort.[13] Gomez-Iturriaga et al reported 13% acute grade 1 to 2 GI toxicity using high-dose-rate brachytherapy to the DIN.[43] We have observed a 25% incidence of acute grade 1 to 2 GI toxicity that persisted at more than 90 days in 5% of the patients. This

Table 4 Dosimetry analysis for targeted volumes

Target volume	Median dose Gy (ranges)	Minimum dose Gy (ranges)	Maximum dose Gy (ranges)	Median D95 Gy (ranges)	V36.25 cm^3 (ranges)	V50 cm^3 (ranges)
PTV_{DIN} phase 1a	50 (47-55)	41 (34-44)	56.5 (50-62)	45 (40-50.4)	NA	4 (2-6)
PTV_{DIN} phase 1b	53 (50-57)	43 (32-48)	60 (54-63)	48 (40-51.3)	NA	3.2 (0.5-6.7)
PTV_p	40 (37-42)	31 (27-35)	57 (45-62.5)	36 (35-37.6)	76.7 (47-101)	NA

Abbreviations: NA = not applicable; PTVDIN = planning target volume of the dominant intraprostatic nodule; PTVp = planning target volume of the prostate.

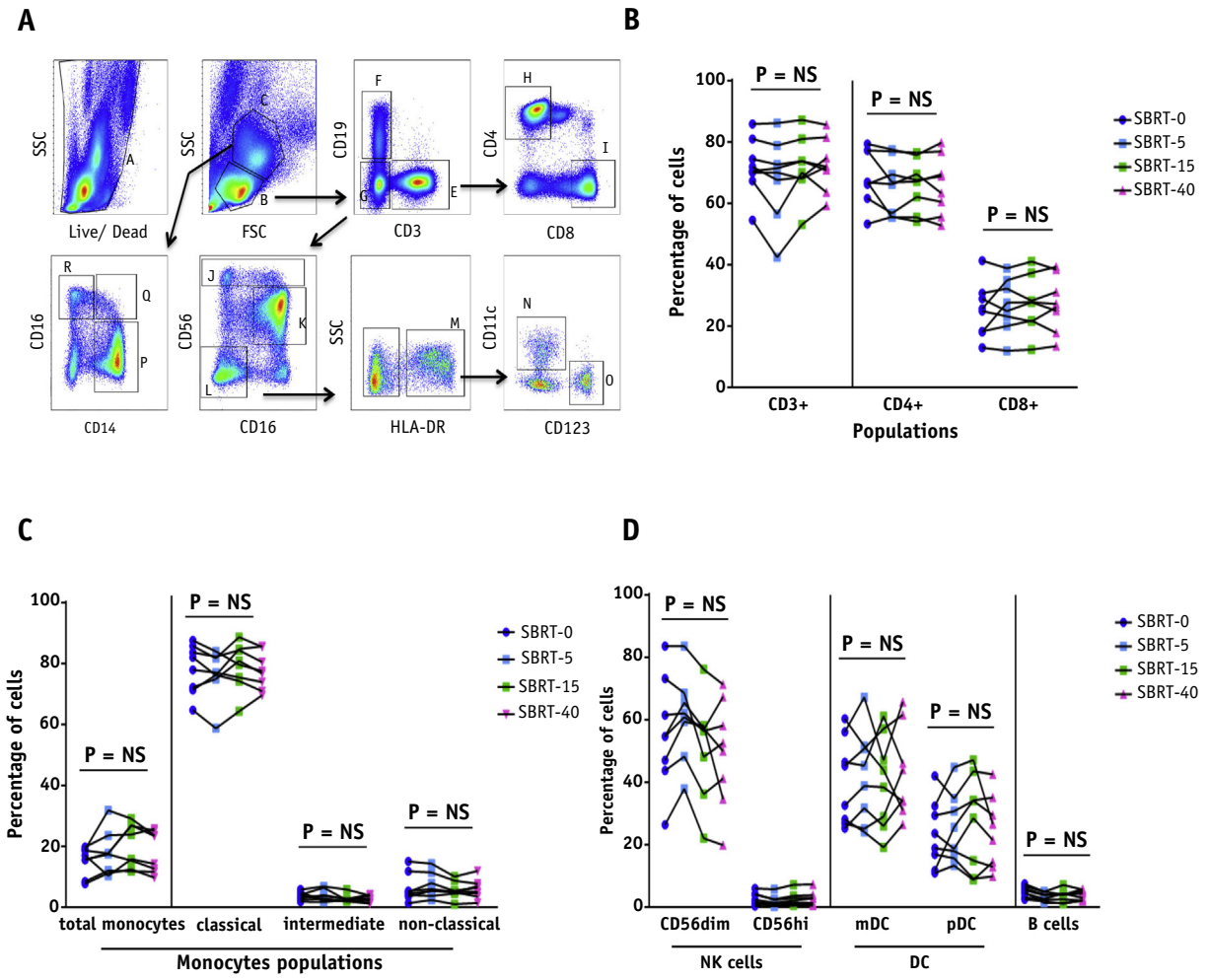

Fig. 2. Longitudinal analyses of peripheral lymphocyte subpopulations: (A) Flow cytometry gating strategy of a patient's peripheral blood mononuclear cells. The gating strategy involved first identifying the lymphocyte population by forward scatter and side scatter. Nonviable cells were gated out (A and B), and then B cells ($CD19^+$) were separated (F) from all other cell populations. Viable T cells ($CD3^+$) were selected (E), and then the 2 main types of T cells were defined by $CD4^+$ (T-helper cells; H) and $CD8^+$ (cytotoxic T cells; I). Classical (P), intermediate (Q), and nonclassical monocyte (R) populations were gated based on their forward scatter and side scatter characteristics (C) and CD14 and CD16 expression. By using CD16 and CD56, natural killer cells were identified in the CD2-negative population (G) and subdivided to give the 2 main types: $CD56^{high}CD16^-$ (J) and $CD56^{dim}CD16^+$ (K) cells. mDC - N and pDC - O were gated based on the positive expression of HLA-DR (M) and were stained for expression of CD11c and CD123. $HLA\text{-}DR^+$ populations that are $CD11c^+$ and $CD123^{lo}$ are considered to be mDCs; those that are $CD11c^-$ and $CD123^{hi}$ are considered to be pDCs. (B) Percentages of $CD3^+$, $CD4^+$, and $CD8^+$ T cells over the study period. (C) Percentages of total monocytes ($CD14^+$), composed of classical ($CD14^+$), intermediate ($CD14^{dim}CD16^+$), and nonclassical ($CD14^-CD16^+$) monocytes over the study period. (D) Percentages of natural killer cells ($CD56^{dim}$, left, and $CD56^{high}$, right), dendritic cells (mDC [$HLA\text{-}DR^+$ $CD11c^+$ $CD123^-$] and pDC [$HLA\text{-}DR^+CD11c^-CD123^+$]) gated on $CD3^-$ cells and B cells ($CD19^+$) over the study period. *Abbreviations:* HLA = human leukocyte antigen; mDC = myeloid dendritic cells; NS = not significant; pDC = plasmacytoid dendritic cells; SBRT = stereotactic body radiation therapy; SBRT-0 = day 0 of SBRT; SBRT-5 = day 5 of SBRT; SBRT-15 = 15 days after completing SBRT; SBRT-40 = 40 days after completing SBRT.

rate is lower than the acute rates previously reported when using SBRT 40 to 50 Gy to the whole gland in 5 fractions. It may be due in part to the incorporation of a mandatory rectal spacer to create a distance of several millimeters between the rectal wall and the DIN. We thus were able to keep our dose limits below the rectal dose limits previously reported by Kim et al as being predictors of rectal toxicity (>3 cm^3 of the anterior rectal wall exposed to 50 Gy or >35% of the rectal wall circumference exposed to 39 Gy).[14]

Longer follow-up is required in our study to evaluate late radiation-induced toxicity, which can occur even

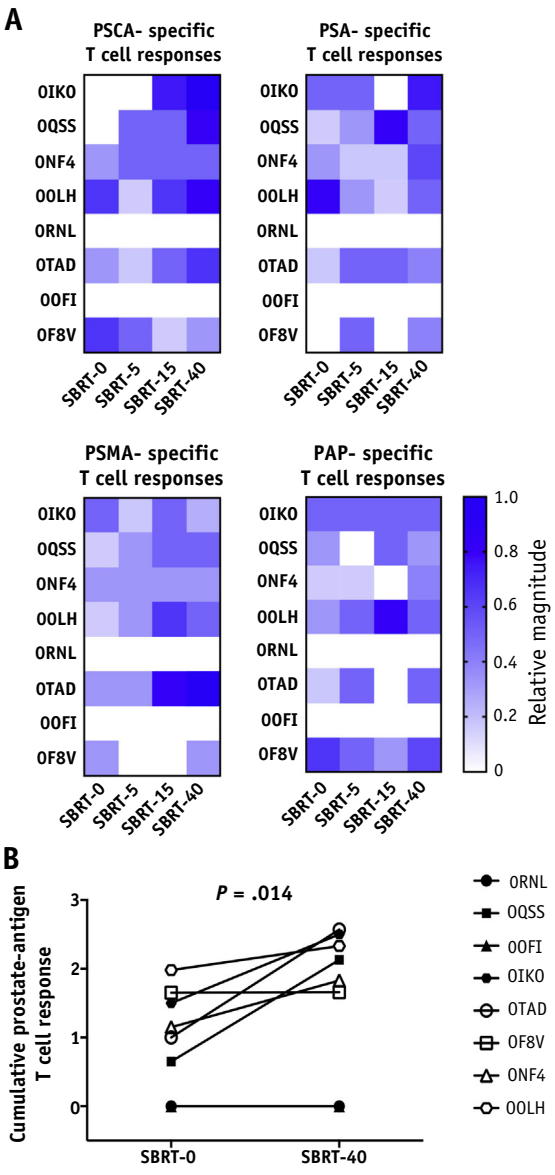

Fig. 3. (A) Longitudinal analyses of the relative magnitude of prostate antigen−specific T-cell responses. Heatmaps represent relative magnitude of antigen-specific T-cell responses measured by IFN-γ ELISpot against the 4 distinct peptide pools derived from PSCA, PSMA, PSA, and PAP. The relative magnitude of prostate antigen−specific T-cell responses was determined, for each antigen/time point/patient, as the relative proportion of positive replicates (ie, above internal background) of the total number of replicates tested. Patients are represented with alphanumeric codes. (B) Comparison of detection of prostate antigen−specific T cells in peripheral blood by the ELISpot assay at baseline (SBRT-0) and at day 40 after completing SBRT. The cumulative magnitude, ranging from 0 to 4, corresponds to the sum of relative magnitudes of tumor antigen−specific T-cell responses shown in (A) for each of the 4 antigens. A nonparametric Mann-Whitney *U* (Wilcoxon) test was used to compare the matched results, with a *P* value of $< .05$ considered statistically significant. A significant increase in the overall magnitude of tumor antigen−specific T-cell responses was observed 40 days after the end of treatment relative to pretreatment baseline ($P = .014$). *Abbreviations:* IFN-γ = interferon gamma; PAP = prostatic acid phosphatase; PSA = prostate-specific antigen; PSCA = prostate stem cell antigen; PSMA = prostate-specific membrane antigen; SBRT = stereotactic body radiation therapy; SBRT-0 = day 0 of SBRT; SBRT-5 = day 5 of SBRT; SBRT-15 = 15 days after completing SBRT; SBRT-40 = 40 days after completing SBRT.

decades after treatment. For instance, in a multicenter study of 309 patients treated with 40 Gy in 5 fractions with a median follow-up of 61 months, there were 2% grade 3 or higher late GU toxicities and no grade 3 or higher GI toxicities.[45] Along the same lines, Hannan et al[15] reported the long-term toxicity of 50 Gy in 5 fractions with a median follow-up of 54 months: late grade 3 urinary and rectal toxicities was 5.5% and 7%, respectively. This toxicity appeared with doses greater than 47.5 Gy in 5 fractions delivered every other day to the whole prostate. When Quon et al reported the results of 40 Gy in 5 fractions with a median follow-up of 48 months, late grade 3 toxicity was 1.33% for GI and 6.7% for GU.[44]

Diminishing GU and GI acute and long-term toxicity while increasing the therapeutic outcome should be the aim of SBRT dose-escalation techniques. This approach firmly relies on the accurate delivery of radiation to the target volume. Interfraction and intrafraction motion control guaranteeing that the fiducial position matches the original position on the planning CT scan is a prerequisite that can avoid hot spots to coincidently enter in contact with healthy organs, such as the urethra.

We have made use of fiducial markers in our patients and have delivered the treatment with CyberKnife. CyberKnife precisely delivers highly conformal radiation to the prostate with 6-dimensional correction of intrafraction prostate motion.[18,46] We have also excluded patients with tumors located close to the urethra (within 3 mm). In addition, we have incorporated a mandatory rectal spacer to create a distance of several millimeters between the rectal wall and the prostate to reduce rectal toxicity. Longer follow-up is required in our study to monitor late effects and eventually observe the benefits of those interventions.

In our study, patient-reported outcomes and clinician-reported assessments were performed with the aim of being complementary and to more fully document the burden of toxicities and subjective symptoms, such as sexual dysfunction. Nevertheless, we recognize the obvious limitation of our study in performing QoL analysis in a relatively small number of patients, and therefore our results should be interpreted with caution. Several key studies have prospectively examined the importance of health-related

QoL after definitive treatment of PCa.[5,15,47] Sanda et al[5] reported Expanded Prostate Cancer Index Composite (EPIC)-based QoL after prostate intensity modulated RT, brachytherapy, or surgery. That study, which followed patients for up to 2 years after treatment, showed patterns of urinary and bowel decline in scores after EBRT of a magnitude range of 10 to 15 EPIC score points, occurring at approximately 2 months posttreatment and returning to baseline scores at approximately 6 months. Our analysis after extreme hypofractionated treatment showed increases of 2.7 and 7 PR-25 points in GI and GU symptoms at 3 months posttherapy, respectively.

Regarding the sexual QoL domain, the study by Sanda et al showed a decline of approximately 10 EPIC score points, peaking at around 2 months.[5] This outcome is in striking contrast to our study, where we observed a decrease of only 4 score points at 3 months. Although we acknowledge that sexual dysfunction may increase with longer follow-up, the differences observed between our study and the one reported by Sanda et al may exist, in part, because most of our patients refused to undergo ADT, which generally causes immediate and long-term sexual dysfunction.

Although ADT was recommended to all, most of our patients declined to undergo this treatment. This outcome could be the result of a cognitive bias because most patients declined standard ADT in the hopes that experimental, higher-dose radiation to the prostate could provide equal disease control with better QoL, and especially sexual QoL, compared with the addition of ADT. King et al did not reveal any benefit from ADT, either in local or systemic control, when given during SBRT in intermediate-risk and high-risk groups.[7] Despite the strong level-1 scientific evidence supporting the use of ADT with conventional EBRT in intermediate- and high-risk patients, a recent systemic review reported that only 27.8% of the patients treated in the context of prostate dose escalation received ADT.[48] According to the current evidence, it is difficult to rule out that dose escalation to the DIN could provide a benefit for local tumor control in the same magnitude as the addition of ADT. Certainly the lack of use of ADT in our study limits generalizability of our results because according to the standard approach, these patients should have all received ADT.

Because of the small number of patients included and the short patient follow-up (median, 24 months), we were not able to reliably evaluate the biochemical outcome. Therefore, the early PSA kinetics provided in Figure E1 (available online at https://doi.org/10.1016/j.ijrobp.2018.09.023) should be interpreted with caution. Mature prostate SBRT trials have been summarized in a recent review.[48] Most SBRT trials have focused on patients with low- to intermediate-risk disease, with limited experience in higher-risk patients. King et al performed a combined analysis of 1100 patients treated in SBRT trials to 35 to 40 Gy in 4 to 5 fractions. Thirty percent had intermediate-risk disease (median follow-up, 30.5 months), and 11% had high-risk disease (median follow-up, 23 months). Biochemical control for intermediate-risk and high-risk patients was 84% and 81%, respectively.[7] Katz reported 10-year biochemical control of 93% for low-risk patients who received 35 to 36.25 Gy in 5 daily fractions.[49]

A Canadian trial recently reported 5-year biochemical control rate of 97% in patients at low-to intermediate risk who were treated with 40 Gy in 5 fractions delivered over 29 days (once-a-week treatment).[50] This biochemical control rate was comparable to that of studies using 40 to 50 Gy in 5 fractions delivered every other day[14,45,51] and showed better tolerability.[44]

Longer follow-up will be needed in our study to see if the early PSA kinetics obtained in this population of intermediate- and high-risk patients translates into lower biochemical failure rates. Therefore, biochemical outcome and long-term toxicity will be reported after all patients in the ongoing phase 2 study have been followed for a minimum of 5 years.

Phase 3 randomized trials are now underway to directly compare prostate SBRT versus conventional and moderately hypofractionated RT. They include HYPO-RT-PC (78 Gy/39 fractions vs 42.7 Gy/7 fractions, ISRCTN4590532), PACE-A (radical prostatectomy vs 36.25 Gy/5 fractions, NCT01584258), PACE-B (78 Gy/39 fractions or 62 Gy/20 fractions vs 36.25 Gy/5 fractions, NCT01584258), HEAT (36.25 Gy/5 fractions vs 70.2 Gy/26 fractions, NCT01794403), and NRG GU005 (36.25 Gy/5 fractions vs 70 Gy/28 fractions, NCT03367702). We believe our study provides further evidence to develop clinical trials aimed at optimizing SBRT for patients with high-risk disease who may require doses higher than those currently reported in the literature.

The abscopal effect of local RT has been shown to be T-cell mediated and antigen specific and indicates that tumor-associated antigens specific to T-cell activation can take place during an ongoing radiation treatment.[16] However, EBRT also has negative effects on lymphoid cells. Lymphopenia often occurs after local RT, as observed in prostate, testicular, and gynecologic cancers.[52-55] Pioneering work by Tabi et al showed decreased circulating lymphocyte numbers in the blood after pelvis RT (55 Gy in 20 fractions to the prostate, 2.75 Gy per fraction; and 44 Gy in 20 fractions to pelvic nodes, 2.2 Gy per fraction).[56] The reduction in lymphocyte numbers was observed 4 weeks after completing pelvis RT and affected mainly the T-cell population, which also showed decreased IFN-γ production.[56] We were unable to observe lymphopenia in our patients. Our hypofractionated treatment plans incorporated a significantly smaller PTV than the one reported by Tabi et al,[56] who also included elective nodal irradiation. Thus, the smaller PTV could explain the absence of lymphopenia.[56]

Previous comprehensive translational research by Nesslinger et al showed antibody responses against shared PCa antigens in 14% of patients undergoing standard pelvis and prostate RT and in 20% of patients undergoing prostate

brachytherapy.[57] Another study determined the frequency of HLA-A2−restricted, surviving peptide (LMLGEFLKL)−specific, tetramer-positive T cells in patients with PCa who were undergoing standard RT. Increased tetramer-positive $CD8^+$ T-cell frequencies were observed in 1 of 10 patients (10%) during RT and in 2 of 7 patients (28%) after RT.[58] We found evidence of immune recognition of prostate cancer tumor-associated antigens at baseline in many patients, which may explain why immunotherapy approaches have shown some promise in the management of recurrent forms of PCa, especially when administered in conjunction with interventions that can mitigate immunologic tolerance or with vaccines that increase antigen availability.[53,59,60] We show for the first time that SBRT overall enhanced such antigen-specific T-cell responses in patients where such responses were detected also at baseline (ie, 5 of 8 HLA-A2+ patients evaluated).

Given the small number of patients, our study does not allow us to ascertain whether radiation-induced antibody response has an important antitumor effect. Interestingly, the in situ vaccination effect of RT persisted also at the 40-day follow-up period, suggesting that RT-induced immune activation may be persistent for a while, thus creating a window of opportunity for combinations with immunotherapy drugs. In future trials, the ideal partners for SBRT should be monoclonal antibodies that boost the in situ vaccination effect through antigen-presenting cell stimulation (OX40 agonists, CD40 agonists, toll-like receptor agonists, CD137 agonists, CTLA-4 inhibitors, or vaccines).[16] In addition to direct antigen-presenting cell activation, additional immunotherapy interventions that simultaneously sustain the effector capacity of T cells could be very useful in combination with SBRT (PD1 and PDL1 inhibitors).[16]

Several limitations of our study are acknowledged. The sample size is too small to derive any definitive conclusions. The follow-up time is too short to assess biochemical outcome, QoL, and long-term toxicity, but these outcomes will be reported after the ongoing phase 2 study has reached maturity. An additional limitation is that the number of patients included in the translational study was limited by the selection of patients with HLA-A2. Because HLA alleles are highly polymorphic, we focused our efforts on the HLA-A2 population, which is the most frequent HLA allele in the white population, and most presently known PCa specific T-cells epitopes are HLA-A2 restricted. Therefore, only 40% of our patients were eligible to participate in the translational research.

In the future, we plan to approach this problem by incorporating cancer genome sequencing in tumor biopsies to enable rapid identification of private nonsynonymous mutations. This can be done through a single nucleotide mutation that results in changes of the amino acid sequence of a protein, expressed at different frequencies in a patient's tumor, that can be used as personalized immunogens. Predictive algorithms for major histocompatibility class I binding, which choose epitopes on the basis of predicted affinity, will provide a rapid and unbiased approach to epitope prioritization. Neoantigens induced upon tumor cell death provoked by SBRT may be included in a personalized tumor vaccine that could be combined with immunotherapy, ADT, or both in this category of patients. Furthermore, because of the limited number of patients and because we applied a gold standard (highly sensitive antigen-screening assays), we believe that our data need to be reproduced in a larger cohort of patients using the same assay to confirm our observations. We thus acknowledge that the work presented in this article is preliminary, but it opens the door for more translational research in PCa, a disease that is poorly responsive to immunotherapy.

Conclusions

Dose escalation to the DIN in this phase 1 trial was possible up to 50 Gy delivered in 5 fractions. The early PSA kinetics were considered promising, whereas the preliminary QoL analysis showed a favorable profile. This outcome was likely a result of the significant efforts made to spare healthy tissues from high doses of radiation. Because no DLT was observed and the MTD was not reached, we are currently enrolling patients into the phase 2 part of this study that uses the 50-Gy dose level. This extreme hypofractionated regimen enhanced antigen-specific immune recognition in a subset of patients. Therefore, immunologic mechanisms may contribute to clinical outcomes after SBRT, an effect that could potentially be exploited with immune-activation approaches, including checkpoint blockade and vaccination.

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