Peer Review File

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Reviewer A

This retrospective study describes the outcomes of 26 men who underwent focal I-125 brachytherapy. Although this is a small study, there is relatively little published on this subject in the literature. Altogether, I think this paper is well written, clearly reports the pertinent details of their study, and of interest to the readership. I have noted some minor concerns below that should be considered prior to publication.

Comment 1: While the authors do report the volume of the PTV relative to the volume of the whole prostate, it would be helpful to report some dosimetric parameters related to the whole gland volume (i.e. V100%, D90 or D100%). These numbers would provide some perspective about the amount of prostate that was actually irradiated.

Reply 1: We agree that adding a dosimetric parameter would be informative for describing the proportion of the prostate irradiated. As requested by the reviewer, we have added the Prostate V100% (ie. volume of prostate receiving 145Gy).
Changes in the text:

- Prostate V100% summary statistics have been added to Table 3 (previously Table 2) and have also been stated in the results (Results, pp. 11, line 19-20).

Comment 2: In Figure 3c, the relationship between PSA nadir and % of prostate irradiated is plotted. Please clarify if this x-axis value is PTV/whole prostate volume ratio or based on some actual dosimetric parameter calculated in the post-plan. I suspect it is the former, but clarification would be appreciated.

Reply 2: In response to Reviewer B Comment 7 (see below), we have removed Figure 3C.

Comment 3: The authors report one patient experiencing biochemical failure and being treated with a salvage RP. If possible, it would be informative to report whether this recurrence was within or outside the region of radiation. It would also be interesting to note whether this was one of the patients with a "poor" implant.

Reply 3: We agree with this comment and it is a good point to expand on. The patient had an **in-field** recurrence detected at 30 months. As per the response to Reviewer B (Comments 4 and 5), we will no longer apply the BCAA post-implant criteria to the patients treated with focal LDR brachytherapy in this study. To determine the quality of the implant we will continue to report the focal-GTV (F-GTV) V100% and D90% V100% and discuss the use of these metrics in previously published focal LDR brachytherapy studies.
Changes in the text:

- We have clarified that the patient had an 'in-field recurrence' and have stated that the V100% for this patient was 97.0% and D90% was 163.6 Gy (pp. 13, line 16-17).

Comment 4: Figure 3A indicates n=28 at baseline, but the remainder of the text indicates only 26 men were included. Please clarify.
Comment 5: The text of the results notes 25 men (96.2%) were FFBF at a median of 18 months follow-up. However, Figure 3A only has PSA values for 20 men at three months out from RT and only 15 PSAs at 18 months. Please address this discrepancy.

Reply 4 + 5: Thank you for bringing this to our attention, comment 4 refers to a typographical error - the original Figure 3A should have stated N=26 at baseline. Comment 5 identifies a lack of clarity in the actual number of patients with a recorded PSA at each timepoint. This manuscript presents follow-up data for 26 consecutive patients; therefore, the follow-up time for the patients in this study is staggered. This means that the number of patients with follow-up data decreases at longer follow-up timepoints. The median follow-up time for PSA outcomes for the entire cohort was ~18 months; only 15 patients had reached 18 months or greater follow-up at the time of writing the original manuscript, which is why Figure 3A stated n=15 under the 18-month timepoint. In addition to this, as this is a retrospective study, patients did not have PSAs at every reported time-point, which adds further variation to the number of results at each time-point. We have made several changes to Figure 3 in order to improve the clarity of the results.

Changes in the text: In order to improve the clarity of the reported PSA outcomes we have:

- Changed Figure 3A to illustrate the time-course of PSA levels per patient and removed the original Figure 3C (refer to Reviewer B, Comment 6) and replaced this with a Kaplan-Meier curve for time to biochemical relapse. These new figures clearly illustrates the number of patients FFBF over time as well as the last available PSA reading per patient.
- Added further PSA follow-up data which has become available since writing the original manuscript – the median follow-up for PSA outcomes, for the entire cohort is now 24.2 months (Results pp. 12, line 7).
- The FFRF statement in the results (pp. 12, lines 10-13) has been changed to: "The median PSA follow-up time for the cohort was 24.2 (IQR: 17.9–30.0) months….Of the 18 patients who had reached 18 months follow-up, all were free from biochemical failure (FFBF) (PSA >2 ng/mL above post-radiotherapy nadir). At 24 months, 13 out of 13 patients were FFBF."

Comment 6: I think the discussion is very well-written. Consider adding a Table with results from prior focal brachytherapy studies (inclusive of the current findings) to help provide context for these results.

Reply 6: We thank the reviewer for their kind feedback. We agree that that brief summary table of prior focal LDR brachytherapy studies would be useful to place our study, and the importance of our findings, into context.

Changes in the text:

- A new table (Table 1) has been created and summarizes the scope and results of previously published focal LDR brachytherapy studies.

Comment 7: When reporting dosimetric measures within the text (e.g. V100, D90, etc) consider attaching % or Gy to the subscript to ensure clarity (i.e. V100%). Although it is typically clear which parameter is being reported based on the context, this may help clarify findings for some readers who are less familiar with these terms.

Reply 7: Thank you for your suggestion, we have appended '%' to the dose-volume parameters referred to throughout the text.
Changes in the text:

- All instances of V_{100} have been changed to V100%, (likewise for V_{150}, V_{200} and D_{90}).

Comment 8: Consider moving the second paragraph of Page 7 (lines 7-13) to the results section.

Reply 8: We agree that this paragraph is more appropriately placed in Results section.
Changes in the text:

- The second paragraph (original manuscript pp. 7, lines 7-13) has been moved to new section at the start of the Results section (pp. 10 line 21 – pp. 11 line 2) which has been entitled 'Patient characteristics'.

Reviewer B

The authors report preliminary results of focal low dose-rate brachytherapy for 26 patients. The patients have uni-focal disease defined by multiparametic MRI and template biopsies. 145 Gy was prescribed to the PTV which was on average about 25% of the prostate volume. Patients had a median follow up of 18 months. (IQR biochemical follow up 14-27 months). The authors conclude that the procedure has favorable toxicity and a high rate of control at 12- 18 months.

I have several concerns about this manuscript. The follow up is much too short to make any comment about efficacy. It is also too soon for the PSA nadir to occur as this generally takes 4-5 years for whole gland therapy. It is also too soon for a biopsy to be interpretable.

My specific comments are given below.

Methods:
Comment 1: Focal low-dose rate brachytherapy line 11: How is the radiologic extent of the disease defined? Was the abnormality defined on T2 images, ADC images, or diffusion enhanced images? These are not often in 100% agreement. Did the authors use a Boolean addition of any area of abnormality, or was it only the intersection where all the imaging modalities agreed? How was PSMA PET information incorporated?

Reply 1: This is a good point to clarify in our manuscript. The radiological extent of the disease was determined by a Boolean addition of the areas of abnormality visible on the different phases of mp-MRI; ADC, T1, T2 and Dynamic contrast enhanced (DCE) images where available. In the situation where a staging PSMA-PET was done, the PSMA-PET was also co-registered.

Changes to the text:

- We have clarified how a Boolean addition was used, based on the multiple mp-MRI sequences captured, and the PSMA-PET scan in the instance where it was performed, to define the focal gross tumour volume (pp. 8, lines 20-22)

Comment 2: Same page, line 25: Please define 'zulu' seeds. New terminology for me and probably for many readers.

Reply 2: Thank you for identifying this. 'Zulu' seeds are additional free seeds ordered extra to the plan that can be utilised in the event that intra-operative adjustments need to be made when analysing intra-operative real time dosimetry.

Changes to the text:

- We have added a clarification that 'zulu' seeds are extra 'free' seeds used in the intra-operative setting if any real-time differences between the planned and actual dosimetry is observed (pp. 9 line 12).

Comment 3: Line 27 Quality assurance was performed using a CT Scan at 30 days. Was this co- registered with the mp-MRI or the pre-treatment ultrasound?

Reply 3: The Day 30 CT scan was co-registered with a same-day mp-MRI scan to assist with the definition of anatomical features and target volumes. Specific regions of interest were utilised to assist with registration, including urethral markers, prostate apex and base, and seeds.

Changes to the text:

- We have clarified that the post-implant CT scan (at 30 days post-implant) was co-registered with a same day mp-MRI (pp. 9, line 14).

Outcome Measures

Comment 4: Why did the authors choose the British Columbia Cancer Agency criteria for implant quality? To my knowledge, these are the least stringent criteria in publication, and not widely accepted. This sets a very low bar for quality. On the contrary, there is a lot of published data on dose response related to implant quality. It is generally accepted that 180 to 200 Gy is required for optimal control. (references: R Stock and N Stone). Furthermore, the BCCA criteria are for whole prostate treatment. The actual target disease would be within the prostate, usually in the peripheral zone, and would thus receive a much higher dose than that prescribed for the entire prostate. These criteria are not easily transferrable to focal treatment where 100% coverage is critical. GTV V100 of < 100% would be unacceptable. The authors state that their GTV V100 ranged from 24 to 100% with a mean of 93%. They haven't reported the dosimetry for their GTV with the margins.

Comment 5: Dosimetry line 7: The postplan QA revealed a good implant for 22 patients; however, "good" by BCCA Criteria means V100 > 85% and this is not an appropriate quality measure for coverage of the gross tumour.

Reply to Comments 4 + 5: We would like to respond to comments 4 and 5 together as they both relate to the validity of applying the BCAA post-implant dosimetry criteria to focal LDR brachytherapy. One difficulty for LDR brachytherapy is that formal dosimetric assessment criteria specifically for focal LDR brachytherapy does not exist, and this was the reason for using the BCAA criteria. Our rationale was that it would be widely known in the brachytherapy fraternity. However, we acknowledge the validity of the reviewer's concerns.

Within the literature, the D90% and V100% are routinely reported by previous studies of Focal LDR brachytherapy (Cosset et al. 2013, Kunogi et al. 2020, Mahdavi et al. 2017). A clinical trial of hemi-gland LDR brachytherapy report dosimetry outcomes based on the V100% and D90% (Laing et al. 2016, *Radiother Oncol*). As such we intend to report these metrics for our study (see Figure 1A and 1B), however, we acknowledge your point and will remove reference to the BCAA criteria. To provide further clarity we have included a comparison between the outcomes of our study and the previously reported dosimetry for focal LDR brachytherapy.

Changes to the text: In recognition of the reviewer's comments, we have:

- Removed the BCAA criteria as an outcome measure (Figure 1A and B, methods)
- Presented the F-GTV V100% and D90% in the context of other focal LDR brachytherapy literature (Results pp. 11 lines 10-13, Table 1, Discussion pp. 14 lines 8-21)
- Discussed possible evaluation criteria for dosimetry implant quality for focal LDR brachytherapy, explicitly mentioning the limitations of the BCAA criteria and the need to develop specific criteria for focal LDR brachytherapy (Discussion pp. 14, lines 8-12), and
- Clarified the conclusion that focal LDR brachytherapy is technically feasible (Discussion pp. 14 line 5).

Comment 6: Oncologic outcomes line 12: 12 to 18 month oncologic outcomes were available for 18 patients via multiparametic MRI and transperineal biopsy. How were the lesions targeted? This is much too soon to be assessing the histological response.

Reply 6: Given that the follow-up MRIs were predominantly negative, the lesion defined by the pre-treatment mp-MRI was used to define the target region for the repeat transperineal biopsy.

We understand your concern regarding the time-point for assessing histological response and we are grateful to have an opportunity to explain our protocol. The basis for our biopsy regimen was the International Multidisciplinary Consensus paper (van den Bos et al. 2016, published in *European Urology*) on clinical trial design for the focal treatment of prostate cancer that stated

the "primary objective should be focal ablation of clinically significant disease with negative biopsies at 12 mo after treatment as the primary endpoint". We have tried in our discussion to acknowledge the limitations of a 12-18 months biopsy following brachytherapy and identify the need for future prospective studies to provide longer follow-up for oncological outcomes. By way of comparison to previously published focal LDR brachytherapy studies, most of these report repeat biopsy results at 12-24 months: Graff et al. 2018, n=17 patients all at 12 months; Cosset et al. 2013, n=6 patients with median of 18 months; Mahdavi et al., n=2 patients at 24 months. In addition, we have added further follow-up data to our study. We now have a total of 21 patients with a repeat mp-MRI and biopsy at a median time of 18.4 months (Range 12 – 37 months, IQR 13 – 19 months). This number of patients with a repeat biopsy, and the length of time post-treatment, compares very favorably to the previously published literature.

Changes to the text:

- We have clarified how lesions were targeted for the repeat biopsy (Results pp. 10, line 10-11).
- We have added specific median (IQR) for time to repeat biopsy to the results section (Result pp. 12 lines 21-23).
- We have added a discussion of the results and timing of repeat biopsy in our study compared to previously published studies (Discussion pp. 17, line 8-13, Table 3).

Comment 7: Oncologic outcomes line 23: What is a PSA remission when only 25% of the prostate is treated? And on line 27 they mention PSA nadir but it is much too soon for these patients to reach a nadir. Freedom from biochemical failure at 18 months with any treatment for prostate cancer is quite meaningless.

Reply 7: Our data suggests that some, but not all, of the men treated with focal LDR brachytherapy do reach a PSA nadir within the follow-up period reported in our study; we agree that not all patients will have reached the nadir. We define a PSA remission as a decline if PSA level following treatment, which is now clarified in the results section. We believe that the biochemical data we present is still of value especially compared with the relatively short follow-up of previously published focal LDR brachytherapy studies. With additional follow-up data which has become available since writing the original manuscript, the median follow-up for PSA outcomes is 24.2 months (IQR: 17.9-30.0), with several patients reaching more than 3 to 5 years follow-up. This compares to Graff et al. (2018, *Int J Radiat Oncol Biol Phys*) and Cosset et al. (2013, *Brachytherapy)* who present data only up to 12 months, and Mahdavi et al. (2017, *J Contemp Brachytherapy)* with only n=4 patients with PSA data (mean follow-up of ~20 months).

Changes to the text:

- For clarity, we have removed the term "PSA remission" and changed this to "reduction in PSA" (Results pp. 13, line 8)
- We have changed Figure 3A to present a more complete dataset, showing the PSA time-course per patient rather than group data, and showing the outcomes for patients who have reach longer follow-up (3 to 5 years).

- Given that the PSA of some patients in this study have not reached the nadir, we have removed the original Figure 3C (which had plotted PSA nadir versus proportion of prostate irradiated).

Comment 8: For the patient that had the radical prostatectomy, what were the pathologic findings?

Reply 8: The final histopathology demonstrated an in-field recurrence of PCa (ISUP grade group 3) that was staged as T2N0M0 disease with clear margins. The patient's post-operative PSA remained undetectable after 12 months of follow-up.')
Changes in the text:

- We have added the above histopathological details to the results section (pp. 13 lines 16-17)

Comment 9: Conclusions: line 17: "Focal LDR Brachytherapy is technically feasible". This statement is questionable since they have not demonstrated acceptable dosimetry. "

Reply 9: We acknowledge the reviewer's concern. Referring back to response 3 and 4 a formal assessment criterion for focal LDR brachytherapy dosimetry does not exist. We have now provided a discussion of this in our manuscript (see response to comment 3 and 4). It is our hope that this study shows that Focal LDR Brachytherapy is technically feasible in the context of the previously published literature.
Changes in the text:

- We have clarified the above statement to acknowledge the challenges of dosimetry for focal LDR brachytherapy (pp. 14, line 5).
- We have provided additional discussion of evaluation criteria for focal LDR brachytherapy dosimetry (see response to comments 4 and 5) and a comparison to previously published studies (pp. 14, line 8-21)

Comment 10: "Focal LDR Brachytherapy has a favorable toxicity profile" and this is a legitimate claim.

Reply 10: No response required.

Comment 11: "Focal LDR Brachytherapy" shows promising cancer control outcomes at 12-18 months" is a completely meaningless statement.

Reply 11: Referencing our responses to Comments 6 and 7, the median follow-up time for repeat biopsy and PSA is now 18 and 24 months, respectively. We have provided additional data to clarify the range of follow-up timepoints for both outcomes in order to provide readers with additional perspective on the validity (and limitations) of our findings and conclusions. Regarding our studies finding that LDR brachytherapy controls clinically significant cancer at 18–24 months, our post-treatment biopsy results found 7 men negative for malignancy with radiation effect, two men negative for malignancy no neoplastic change visible and 12 men

adenocarcinoma with radiation effect. It has been demonstrated in the whole-gland external beam radiotherapy literature (Zelefesky et al 2019 *J Urol*, Crook et al 2009 *Cancer)* that patients with adenocarcinoma showing radiation treatment effects at 2 to 3 years post treatment have long-term disease-free survival equivalent to patients with a negative biopsy. In the context of the criterion for a negative biopsy 12 months post focal therapy outlined by van den Bos et al. 2016, published in *European Urology,* we believe that this study demonstrates promising cancer control at this time point.

Changes in the text:

- We have changed the above statement to read, "…focal LDR Brachytherapy controls clinically significant cancer at 18–24 months" (pp. 14, lines 6-7).
- We have added a reference to an International Multidisciplinary Consensus paper (van den Bos et al. 2016, published in *European Urology*) which provides criteria for focal therapy to be considered effective and states that "primary objective should be focal ablation of clinically significant disease with negative biopsies at 12 mo after treatment as the primary endpoint" (pp. 16 line 23 to pp. 17 line 1)

Comment 12: The discussion of toxicity of whole gland brachytherapy quotes extremes of reported toxicity. In general grade 2 rectal toxicity is seen in about 2% of men following LDR brachytherapy and fistulas in less than 1 in 1000.

Reply 12: We thank the reviewer for their suggestion in regard to the rates of proctitis, we will now report the range of rates of grade 2 gastrointestinal toxicity, rather than only the maximum rate, as noted by the reviewer. In regard to significant complications, we referenced Stock and Stone (2002, European Urology) who found that significant injury (fistula) occurs in 1-2.4% of patients, across the reviewed studies. We believe that this rate is in line with the current literature with a more recent study reporting long term (10 year) rates of GI toxicity found that the cumulative rate of Gr 3-4 rectal toxicity was 1.65% (Cosset et al. 2016). Additionally, a large database of over 1000 whole-gland LDR brachytherapy patients, Keyes et al. (2012, *Brachytherapy)* report a rate of late RTOG grade 2 and 3 gastrointestinal toxicity of 7.2% and 0.9%, respectively.

Changes to the text:

- We now report the range of rates of grade 2 gastrointestinal toxicity from the literature (1-19%), rather than just reporting the maximum from this range (pp. 15, line 24-25)
- We have added references to Cosset et al. 2016 and Keyes et al. 2012 (pp. 16, line 1).

Comment 13: The authors do concede that the histologic resolution of tumors can take up to 3 years. They also point out that the applicability of standard definitions of biochemical failure following whole gland radiotherapy "may not be valid in a Focal setting". This is certainly true.

Reply 13: No response or changes to the text required and we thank the reviewer for their feedback.

Comment 14: In conclusion, I would say that at this time the manuscript does not contribute

significantly to the literature. The authors are to be commended for their work on Focal LDR brachytherapy and should continue to follow these patients and submit an update in another 3-4 years.

Reply 14: While duly acknowledging the limitations of this manuscript, we believe the data presented still makes an important contribution to the limited Focal LDR brachytherapy literature. We believe that the length of interim follow-up in our study for oncological outcomes is sufficient based on the consensus of a large international multidisciplinary panel (van den Bos et al. 2016, European Urology) and compares favorably to previously studies which have a shorter follow-up time (Cosset et al. 2013, Graff et al. 2014, Mahdavi et al. 2014) or a smaller study size (Kunogi et al. 2020, Mahdavi et al. 2017).

Furthermore, this study has served as the genesis for the prospective registry for Focal LDR brachytherapy (LIBERATE) to which we are currently recruiting patients. This registry will allow for the collection of 5-year oncological outcomes. Follow-up data for these patients is currently maturing and unfortunately will not be available for publication until 5 years following the recruitment of the last patient to the registry.

As such, we contend that the findings presented in the current manuscript (median follow up of 24 months for toxicity and PSA outcomes, 18 months for repeat biopsy outcomes), are still of relevance and publication is justified considering 1) the limited existing literature, and 2) the projected time until prospectively collected long term (5-year) outcomes will be available for publication.

Changes to the text:

- Given the time that has elapsed since writing the original manuscript, we have added extended follow-up data to the manuscript, which has increased the median follow-up for patients to 24 months for toxicity and PSA outcomes and 18 months for repeat biopsy outcomes.
- As per the response to Reviewer A, comment 6, we have added Table 3 to place the significance of our results - particularly the study size and length of follow up - into the context of the existing Focal LDR brachytherapy literature.