PEOPLE: PatiEnt prOstate samPLes for rEsearch, a tissue collection pathway utilising MRI data to target tumour and benign tissue in fresh radical prostatectomy specimens.

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Keywords: Prostate, cancer, MRI, tissue, sampling, specimen, biobank

Abstract

Background

Over one million men are diagnosed with prostate cancer each year worldwide, with a wide range of research programs requiring access to patient tissue samples for development of improved diagnoses and treatments. Random sampling of prostate tissue is sufficient for certain research studies, however there is a growing research need to target areas of aggressive tumour as fresh tissue. Here we set out to develop a new pathway “PEOPLE: PatiEnt prOstate samPLes for rEsearch” to collect high quality fresh tissue for research use, using magnetic resonance imaging (MRI) to target areas of tumour and benign tissue.

Methods

Prostate tissue was sampled following robotic radical prostatectomy, using MRI data to target areas of benign and tumour tissue. Initially, 25 cases were sampled using MRI information from clinical notes. A further 59 cases were sampled using an optimised method that included specific MRI measurements of tumour location along with additional exclusion criteria. All cases were reviewed in batches with detailed clinical and histopathological data recorded. For one subset of samples DNA was extracted and underwent quality control. Ex vivo culture was carried out using the gelatin sponge method for an additional subset.

Results

Tumour was successfully fully or partially targeted in 64% of the initial cohort and 70% of the optimised cohort. DNA of high quality and concentration was isolated from 39 tumour samples, and ex vivo culture was successfully carried out in three cases with tissue morphology, proliferation and apoptosis remaining comparable before and after 72 hours culture.

Conclusion

Here we report initial data from the PEOPLE pathway; using a method for targeting areas of tumour within prostate samples using MRI. This method operates alongside the standard

clinical pathway and minimises additional input from surgical, radiological and pathological teams, while preserving surgical margins and diagnostic tissue.

Introduction

Prostate cancer is the second most common cancer in men worldwide, with an estimated 1.1 million men having been diagnosed with the disease in 2012 [1]. Current prostate cancer research programmes are often focused on better ways to diagnose and treat aggressive prostate cancer, while sparing men with more indolent cancers from undergoing unnecessary procedures and treatments. In order to best address these priorities, there is an increasing research need for high quality prostate tissue which represents the index lesion. With a wide range of emerging technologies being adopted in prostate cancer research, there is also a growing need for fresh tissue that can either be used immediately or banked for larger studies, for example in ex vivo culture, ex vivo MRI for assessment of new treatments and/or biomarkers or for large cohort genomic studies such as the International Cancer Genome Consortium (ICGC) and The Cancer Genome Atlas (TCGA). For studies requiring live cells researchers must be able to identify areas of tumour and benign tissue for experimental use immediately upon tissue collection, rather than fix or freeze tissue and wait for results from histological analysis.

Numerous methods have been published for sampling radical prostatectomy specimens for research without impeding on the diagnostic pathway. Key considerations across each published method include the minimisation of ischemia time, control of temperature during transport and processing, preservation of surgical margins for pathological examination and careful recording of positions of samples within the prostate. One early method involved slicing the prostate in half immediately in theatre, taking research biopsies for freezing and then suturing the prostate back together for fixation [2]. Another method involved slicing the whole prostate fresh in 4 mm slices, and storing one whole slice for research in RNAlater, while the rest were fixed and stored as formalin fixed paraffin embedded (FFPE) blocks for pathological assessment [3]. Sooriakumaran et al. published a method in 2011 which involved slicing and taking mapped punch biopsies for immediate storage in RNAlater in theatre, followed by fixation of remaining sections in histology cassettes [4]. The team later confirmed

consistently high RNA integrity independent of ischemia time [5]. In order to generate larger frozen specimens suitable for inclusion in the ICGC, another group quartered and flash froze alternate slices of the prostate for biobanking. This resulted in the generation of large amounts research specimens, but also large proportions of surgical margins not being available for routine histological processing, although the banked samples could be accessed later by pathology if required for further diagnostic tests [6].

Our previous prostate sampling publication [7] built upon these published methods to generate large quantities of high quality biopsy punches for research from radical prostatectomy specimens without affecting surgical margins. The method included pinning the prostate capsule to a cork board following sampling in order to ensure surgical margins did not become warped during fixation. Samples were taken randomly across a full transverse slice and stored without knowledge of tumour content. The method was highly successful for the ICGC study, where tumour content could be assessed at a later date. However, the ability to be reasonably confident of tumour content in real time is essential for many research applications requiring fresh tissue. The ability to use tissue immediately with high confidence of tumour content opens up a wide range of new technologies for prostate cancer research, in particular new imaging techniques and ex vivo culture.

With numerous Magnetic Resonance Imaging (MRI) clinical trials and studies reporting the efficacy of MRI in diagnosing clinically significant prostate cancer, there is an increasing trend towards using MRI prior to transperineal template biopsy to diagnose patients with suspected prostate cancer [8-11]. This represents an ideal opportunity to better target research samples, by incorporating a review of MRI data into the sampling procedure.

Here we set out to use MRI to build upon previously published prostate sampling methods, enabling researchers to target specific areas of tumour and benign tissue within radical prostatectomy specimens, and use tissue either immediately fresh, or frozen or fixed as applicable to the specific study requirements.

Materials and methods

Tissue samples

Patients were recruited and consented under Genomics England's 100,000 Genome Project ethics at University College Hospital between March 2016 and July 2017, with a subset of patients also consented under UCL/UCLH Biobank ethics (REC 15/YH/0311). A summary of the clinical characteristics of these patients is included in Table 1.

Tissue sampling from radical prostatectomy specimens

Prostates were collected from theatres immediately upon removal from the patient during robotic radical prostatectomy at UCLH and transported under UN3373 guidelines to the UCLH Pathology Department. If suitable staff were not available to sample the prostate immediately, the specimen was stored at 4°C for up to 24 hours in accordance with Genomics England guidelines. The prostate was weighed and inked, and a 5mm transverse slice was removed using the parallel blade device as previously published [7]. Tumour and benign areas were identified and 1-2 samples of each removed as per the requirements of each study, using 3mm or 6mm biopsy punches. Tumour regions typically felt slightly denser and occasionally looked paler and this was taken into account when selecting regions to sample.

Remaining tissue was pinned to cork, fixed in 10% neutral buffered formalin and processed as per local protocols. This process was initially carried out by pathologists, and later two trained postdoctoral researchers signed off as competent to carry out the procedure, under supervision of a pathologist.

Tumour targeting using MRI

All patients received a multiparametric (mp) prostate MRI prior to their robotic radical prostatectomy, either at UCLH or at their referring hospital (Table 2). Two methods were used to target tumour samples using MRI data (Table 3).

Initial method: All patients undergoing robotic radical prostatectomy at UCLH are discussed at a surgical planning meeting, where a surgical planning sheet was filled out with details of tumour location according to both biopsy and MRI data (Figure 1A). This sheet was uploaded centrally and accessed when planning the tissue sampling to take a transverse slice towards the apex, mid gland or base as indicated, and then take a 3mm or 6mm tissue punch from the region identified as containing a suspicious lesion.

Optimised method: Following review of the surgical planning sheet, MRI images were visualised using IMPAX software (version 6.5, AGFA-Gevaert, Mortsel, Belgium) and T2 weighted axial, T2 weighted coronal, diffusion weighted imaging (DWI) and apparent dynamic contrast (ADC) images used to identify the index lesion, defined as the largest tumour focus within the prostate. A measurement was then taken from the base of the prostate to the target lesion in mm within the software, using the image where the tumour was most visible – typically the T2 weighted axial image (Figure 1B). Once trained by a board certified radiologist (EJ), a postdoctoral researcher could carry out this procedure in less than five minutes per patient. During the sampling procedure, a transverse slice was removed at the position identified previously using the MRI image, measured from the base of the prostate by a ruler in mm (Figure 1C). After the first 6 cases were sampled, an additional step was carried out to identify any shrinkage that could affect the accuracy of this method. Here, length of the prostate was measured from base to apex on the coronal T2 MRI image, and compared with the length of the prostate measured by ruler immediately before slicing. Based upon the amount of shrinkage observed an ad hoc correction was applied before identifying the position to slice. Exclusion criteria were applied to omit patients who had undergone prior therapy, had no distinct lesion by MRI (i.e. only diffuse changes) and patients who had lesions smaller than 5mm visible on MRI.

Tissue storage

Tissue cores were stored depending on the individual study. At least one core from each patient was embedded in optimised cutting temperature compound (OCT), snap frozen in

liquid nitrogen immediately and then stored at -80°C. For subsets of cases, additional benign or tumour tissue was either snap frozen dry in cryovials in liquid nitrogen, fixed immediately in 10% neutral buffered formalin and stored as FFPE blocks, or transported in warm media for ex vivo culture (ex vivo culture was not carried out where prostates had been stored overnight before sampling).

Assessment of tumour content

All 84 samples assessed for tumour content had been stored frozen in OCT. Samples were sectioned at 5 µm thickness using sterile technique on the cryostat, then stained immediately with haematoxylin and eosin (H &E) as per 100,000 Genomes Project standard operating procedures. Slides were assessed by an experienced consultant uropathologist and tumour content was reported as 0, 5, 10, 15, 20, 30, 40, 50, 60, 70, 80, 90 or 100%.

Tissue data review

All specimens were reviewed in batches, by a multidisciplinary team including at least one consultant uropathologist along with a pathology registrar, postdoctoral researchers and technicians. All anonymised data is included in Supplementary Data File 1 and includes whether the specimen was refrigerated prior to sampling, number of samples taken, whether the initial or optimised tumour targeting method was used, which member of the pathology team supervised cut up, which area(s) were sampled, which level the sample was taken, distance to tumour on the same level if tumour was missed, whether the tumour could have been hit if samples were taken on the next/previous level in the same position, the total number of levels, the number of levels the tumour was present in, the level where tumour(s) reached maximum dimension, the maximum tumour dimension, the number of tumours in the specimen, the most affected area of the prostate by MRI versus biopsy, prostate volume and weight, tumour volume, Gleason grade, pathological tumour staging, MRI (Likert), the hospital the MRI was carried out in, tumour volume, patient age, PSA, base-apex length (MRI), base-apex length (ruler), base-apex shrinkage (%), cellularity (very low (<700 total cells), low (4,000 cells), medium (4,000-10,000 total cells), high (> 10,000 total cells), very

high (>50,000 total cells)), number of 10 µm sections submitted for DNA extraction, which sample used for frozen sections (if more than one was available), DNA concentration and 260:280 ratio.

DNA isolation

DNA was isolated from 20-40 frozen tissue sections per sample (10 µm) from 39 cases, taken adjacent to sections used for tumour content assessment, as per 100,000 Genomes Project standard operating procedures. The Maxwell 16 LEV Blood DNA Kit (AS1290; Promega) was used to extract DNA. Prior to extraction samples were homogenised by the addition of 300 µl lysis buffer and 30 µl Proteinase K (Promega) to each sample, followed by incubation at 56°C for at 30 minutes. Samples were then transferred to Maxwell 16 LEV Cartridges for extraction, according to the manufacturer's protocol. DNA was eluted in 70 µl Elution Buffer (Promega). Concentration was assessed using the Qubit dsDNA HS Assay Kit with the Qubit Fluorometer (ThermoFisher) and 260:280 ratio assessed using the Nanodrop Fluorometer (ThermoFisher) according to manufacturer's instructions.

Ex vivo culture

Ex vivo culture was carried out using the gelatin sponge method [12]. Sponges were placed in a 24 well plate with 200 µl / well Roswell Park Memorial Institute media (RPMI) supplemented with 10% fetal bovine serum 2-3 hr prior to tissue collection to allow the sponges to draw up the media in a 37 °C, 5% CO_2 incubator. Fresh 3 mm or 6 mm cores were divided as appropriate using a sterile scalpel and placed on the damp sponges, then incubated for 72 hr. Both uncultured control samples and samples that were cultured for 72 hr were fixed in 10% neutral buffered formalin and stored as FFPE blocks. 4 µm sections from the FFPE blocks were stained with H&E, or assessed for Ki67 by immunohistochemistry performed on the BondMax autostainer (Leica) (Dako m7240, 1:100 dilution, F. Dabe 1 min haematoxylin, ER2 30 min) and cleaved caspase 3 (Cell Signalling 9664) 1:200 dilution, F. Dabe 1 min haematoxylin, ER2 20 min).

Results

84 prostates were sliced and sampled for various research projects under the PEOPLE pathway, with tumours targeted using the initial method for 25 prostates and optimised method for 59 prostates (Figure 1). Patient characteristics are reported in Table 1. All surgery was carried out in UCLH and MRIs were carried out in 13 different hospitals across Greater London and Essex (Table 2).

After the first 25 cases a detailed case review was carried out and seven cases were identified where the tumour was missed. All could potentially have been accurately targeted had the transverse slice been taken more towards the base or apex of the prostate (Supplementary Data File 1). This resulted in a change to the optimised method where measurements from the MRI image were used to target the transverse slice and tumour.

In order to account for shrinkage that can occur following surgery, 46 prostates were measured using a ruler from base to apex, and compared with MRI measurements of the same distance. The majority of prostates did shrink, with a mean shrinkage of 5.71% ± 10.57% and a trend towards larger prostates shrinking more and smaller prostates shrinking less ($p = 0.0002$) (Figure 2A). Some prostates increased in size, possibly due to growth between the time the MRI image was taken and surgery.

All tumour samples were assessed for tumour content by a consultant uro-pathologist, using frozen H&E sections. 40% of samples taken using the initial method and 60% of samples taken using the optimised method had tumour content of at least 40%, and therefore could be submitted without macrodissection for sequencing under the 100,000 Genomes Project (Figure 2B). Samples that had between 5-30% tumour content were considered ‘partial hits’ and could potentially be used for sequencing following macrodissection. The sum of hits and partial hits for the initial method was 64% and for the optimised method was 70% (Figure 2C).

As well as the risk of missing the tumour when taking the transverse slice, there was also a risk of missing the tumour within the slice when taking punch biopsies. It appeared that accuracy in hitting tumour within the slice improved slightly over time, although this trend was not statistically significant ($p = 0.344$), based on measurements taken from the sampled area to the nearest tumour within that slice (Figure 2D).

The crucial aspect of the PEOPLE method is tumour targeting, and in order to maximise the efficacy of this we used both MRI and biopsy data to assess tumour location within the prostate for sampling. Where biopsy and MRI data did not agree, both indicated areas were targeted if possible, and if not, MRI data was used alone. During retrospective histopathological assessment, the consultant pathologist noted which area of the radical prostatectomy slides was most affected, e.g. left posterior. This was subsequently compared to the planning protocol. In cases where the surgical planning sheet estimated by both biopsy and MRI that the tumour was in this position (left posterior), it was considered that both biopsy and MRI estimated tumour position well. If only one or neither of these indicated accurately the most affected area of the prostate, this was noted (Supplementary Data File 1, tab 2). Following assessment of all samples it was found that both MRI and biopsy data only correctly estimated the same location of highest Gleason tumour in 35% of cases, with MRI outperforming biopsy in 46% of cases and biopsy outperforming MRI in 16% of cases (Figure 2E). There were three cases where neither the MRI nor the biopsy correctly estimated the location of the tumour. In all three cases the tumour was present in the transverse slice, but the tissue sample was not taken from the area of the slice where the tumour was present. Two of these cases were reported Likert 2 by MRI (low likelihood of tumour presence) and one was reported Likert 3 (equivocal likelihood of tumour presence). These three prostates were also low in weight (mean 36 g versus 48 g cohort mean) and tumour volume (mean 2.6 ml versus 4.4 ml cohort mean) (highlighted orange in Supplementary Data File 1).

Hit rate was found not to significantly differ by tumour volume (Supplementary Figure 1A), prostate volume (Supplementary Figure 1B) or ISUP grade (Supplementary Figure 1C). MRI (likert) did not correlate well with Gleason grade (Supplementary Figure 1D) or tumour volume (Supplementary Figure 1E), although this cohort is underpowered to consider the accuracy of MRI in this regard. The initial and optimised method did not differ by ISUP grade (Supplementary Figure 1F).

39 samples were submitted for sequencing under the 100,000 Genomes Project. DNA was eluted in a final volume of 70 µl, and was analysed using spectrophotometry. All had 260:280 ratios of 1.8-2.0, with an average concentration of 41.7 ng/µl ± 18.92 ng/µl. Cellularity of each sample was noted by a pathologist as very low (<700 total cells), low (4,000 cells), medium (4,000-10,000 total cells), high (>10,000 total cells), very high (>50,000 total cells), and there was no significant difference in DNA concentration between each cellularity grouping (Figure 2F).

Three samples were cultured ex vivo using the gelatin sponge method [12]. Control samples were stored as FFPE blocks at the time of sampling and after 72 hr culture at 37 °C, and 4 µm sections were stained with H&E, the proliferation marker Ki67 and apoptosis marker cleaved caspase 3. Minimal-no differences in morphology, proliferation or apoptosis were noted between cultured and uncultured samples in cases one and two, while some loss of tissue integrity was noted for patient three, which could be optimised in future using different media or shorter culture. (Figure 3).

Discussion

A number of methods have been described for the collection of prostate cancer tissue for research following radical prostatectomy. A 2017 review on these existing methods highlighted the importance of reducing numbers of samples taken from surgical specimens, and suggested the use of imaging and biopsy data to better target lesions for sampling [13].

Here, we address this by using MRI to target specific areas of tissue that can then be used immediately for research, vastly increasing the range of experimental techniques that can be exploited, while still integrating well within the clinical pathway.

Previous prostate tissue collection methods have sampled areas of the gland randomly, or by palpating or viewing the tumour as slightly more dense or pale. These methods are sufficient for certain downstream techniques, but are unlikely to be reliable for routine collection of high tumour content samples based upon our data that demonstrates that the average tumour only occupies 11% of the overall prostate volume, and is often not discernible by eye or touch from the surrounding benign tissue (Figure 1D). MRI has been heralded as a major step forward for prostate cancer diagnosis, with findings from multiple clinical trials advocating its routine use [8-11]. With this gain in popularity comes an opportunity for researchers to exploit MRI data to improve fresh tissue collection, here allowing us to successfully target tumour in 70% of samples (Figure 2C). We have incorporated MRI into the sampling method in order to improve tumour targeting and whilst UCLH is a world leader in MRI, most patients in our cohort had imaging at one of twelve other hospitals across the Greater London / Essex area before coming for surgery at UCLH. The varying quality of the MRI data did not impact on our ability to target tumours and we predict that this method can be successful wherever MRI is carried out prior to surgery (Table 2).

The major disadvantage of previous methods was the lack of confidence in the pathology of the sampled tissue that limited its utility and often required extensive sampling and/or pathologist time to identify samples with high tumour content [2, 3]. The key benefit of incorporating MRI guided tumour targeting into the sample collection pathway is the increased freedom this provides to carry out a much wider range of downstream experiments. As with previous methodologies, samples can be stored in RNAlater for expression analysis, fixed and stored as FFPE for immunohistochemistry, or frozen for genomics studies, all with greater confidence in the content of each sample prior to histological assessment [4, 5]. Here, tumour samples were frozen and DNA of consistently high

purity and quantity for sequencing was isolated for the 100,000 Genomes Project (Figure 2F). Crucially, this targeting method also allows for the development of new methodologies which require fresh matched tumour and benign tissue. For example, ex vivo MRI or other imaging techniques can be carried out on fresh tissue quickly following surgery, allowing researchers to more effectively study density and other physical properties than with fixed or frozen tissue[14]. The method we describe here allows techniques such as ex vivo culture, more widely used in breast cancer research, to be carried out to test new drugs or biomarkers [12, 15-17]. We cultured tumour and benign tissue for 72 hr using the gelatin sponge method and were able to show that untreated tissue post culture had minimal degradation in morphology, proliferation and apoptosis as uncultured tissue (Figure 3). As prostate cancer research progresses, we predict more methods will emerge which require high quality fresh tumour tissue. In order to do these experiments using previously described tissue collection methods, researchers would need to over-collect samples and use a substantial excess to ensure presence of tumour. This is unfeasible in terms of both cost, time and an unethical use of human tissue that could be better utilised in other studies.

Although this method contains additional steps compared with previously published methods, including our own, we succeeded in reducing the time burden on clinical staff using a protocol that dovetailed with the standard clinical pathway and could be performed by trained postdoctoral researchers and technicians [7]. This included measurement of MRI data, meaning the only radiology department input required was for training at the beginning of the study. This reduced the burden on the overall clinical system and allowed for flexible sample collection for research projects when required by the researchers. Additionally, surgical margins and diagnostic tissue are preserved as before[7].

Although this new method does allow for a wider range of downstream applications and minimises any additional burden on the pathologist, it does not significantly improve upon previously published tumour hit rates overall. This is due to the reduced tissue sampling carried out here, where 1-2 indicated tumour and matched benign punch biopsies are taken

for immediate experimentation, leading to a tumour hit rate of 70%, rather than the previous methods of taking many samples, often from several prostate slices and assessing tumour content of each sample later, which has led to tumour hit rates from 69%-100%[13]. It remains useful to discuss the 30% of cases where tumour was missed here, with a view to further refinement of the pathway for implementation in new centres. Interestingly, in the cases where tumour was missed, there was no discernible difference in ISUP grade, MRI (Likert), tumour volume, tumour location or prostate volume, and as such we have not added further restrictions to our recruitment criteria based on these parameters. There were three cases where neither the biopsy nor the MRI indicated the location of the tumour well. These cases had lower tumour volume, prostate weight and MRI Likert score, none of which were out of the range of this cohort, but perhaps these criteria together, and taken in the context of differing biopsy & MRI results could be used to identify potential patients to avoid recruiting in future iterations of this method.

High quality prostate cancer research relies on access to high quality human tissue for experimentation. Here we have built upon existing tissue sampling methodologies to introduce the PEOPLE pathway, providing high quality MRI targeted fresh tissue for a wide range of research.

Conclusions

We report our initial data from the PEOPLE pathway, an improvement to our previously published prostate slicing method, where we utilise MRI data to target and collect high quality tumour and benign prostate tissue that can be used for a wide variety of research applications such as next generation sequencing or ex vivo culture, with minimal impact on the clinical pathway.

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Legends

Table 1: Patient characteristics

Research specimens were collected from 84 prostate cancer patients who underwent robot assisted radical prostatectomy at UCLH between March 2016 and July 2017. Key clinical characteristics are noted here with additional information in Supplementary data file 1.

Table 2: MRI location

Although all patients underwent surgery at UCLH, MRI was carried out across 13 hospitals with the majority of patients referred to UCLH post MRI. The number of patients from each hospital is given with the percentage of the overall cohort in brackets. Hospitals where less

than three patients underwent MRI were grouped to avoid potential identification of patients, and include Homerton University Hospital, Whittington Hospital, Enfield Alliance Hospital, Queen's Hospital and St. Margaret's Hospital.

Table 3: Tumour Targeting Methods

The first 25 patients recruited under PEOPLE were part of an initial cohort, where a basic tumour targeting approach was used and outlined here. The following 59 specimens were sampled using an optimised approach with more specific MRI targeting, also outlined here.

Figure 1: Tumour Targeting Methods.

Following review of the surgical planning sheet, the index lesion is identified in MRI images. The position of the optimal transverse slice is identified and a measurement is taken from the base to this position and from the base to apex using a coronal T2 weighted image (A). The position of the tumour is noted, here in an axial T2 weighted image (B). Following radical prostatectomy, the full surgical margins are inked right side blue, left side black according to local protocol, then the prostate is measured from base to apex and a correction factor is applied if the prostate has shrunk in comparison with the MRI image. The position of the desired 5 mm transverse slice is identified (C). Following slicing, the area of expected tumour is confirmed if possible visually and/or palpably, with some tumours appearing paler and denser than benign tissue. 6 mm biopsy punches are used to remove samples from indicated tumour and benign areas (D). One tumour sample is submitted to Genomics England for DNA extraction, quality control, whole genome sequencing and data analysis as part of the 100,000 Genomes Project (E). Matched tumour and benign samples are submitted for use in ex vivo gelatin sponge culture, or other ethically approved research projects in a subset of cases (F).

Figure 2: Key data from case review.

Prostate length was measured in mm in MRI images and using a ruler post-surgery and an ad hoc correction for shrinkage applied each time prior to slicing (A). Tumour content was

assessed by a uropathologist based on H&E staining of frozen sections of tumour targeted samples. Mean tumour content was 29.6% for the initial targeting method and 44.8% for the optimised targeting method. All samples above the threshold of 40% tumour content were deemed eligible for next generation sequencing as per 100,000 Genome Project Guidelines. A two tailed Mann-Whitney was performed and the difference between the two cohorts was deemed non-significant (B). Samples where 5%-40% tumour was identified were noted as 'partial hits' and could be macrodissected in order to be submitted for next generation sequencing. (C). Distance from punch taken to nearest tumour on the same slice was measured and plotted sequentially in the order of patients sampled, with linear regression not identifying a significantly non-zero slope (D). The location of most significant tumour was recorded based on MRI, biopsy and radical prostatectomy data. It was recorded whether MRI, biopsy, both or neither best indicated the location of most significant disease post radical prostatectomy (E). DNA was isolated from 39 cases for next generation sequencing under the 100,000 Genomes Project. Almost all cases yielded concentrations over 20 ng/µl and those that did not were repeated with more sections and submitted for sequencing. Data is noted for the first DNA isolation of each case, with non-significant q values from a one way ANOVA and Tukey test (F).

Figure 3: Ex vivo culture

Tissue from three cases was cultured ex vivo for 72 hr using the gelatin sponge method. Samples were stored as FFPE blocks after 72 hr (untreated tissue), with matched uncultured samples also stored FFPE (uncultured tissue). Sections were then stained with H&E (morphology), Ki67 (proliferation) and cleaved caspase-3 (apoptosis) to assess whether tissue from PEOPLE is of sufficient quality for culture.

Supplementary Figure 1: Further data on tumour targeting

Hits (>40% tumour), partial hits (5-30% tumour) and misses (0% tumour) are indicated for the initial and optimised methods in comparison with tumour volume (A), prostate volume (B)

and ISUP grade (C). MRI (likert score) was compared with Gleason grade from radical prostatectomy specimens (D) and tumour volume from radical prostatectomy specimens (E). The cohorts from the initial and optimised method were compared by ISUP grade (F).

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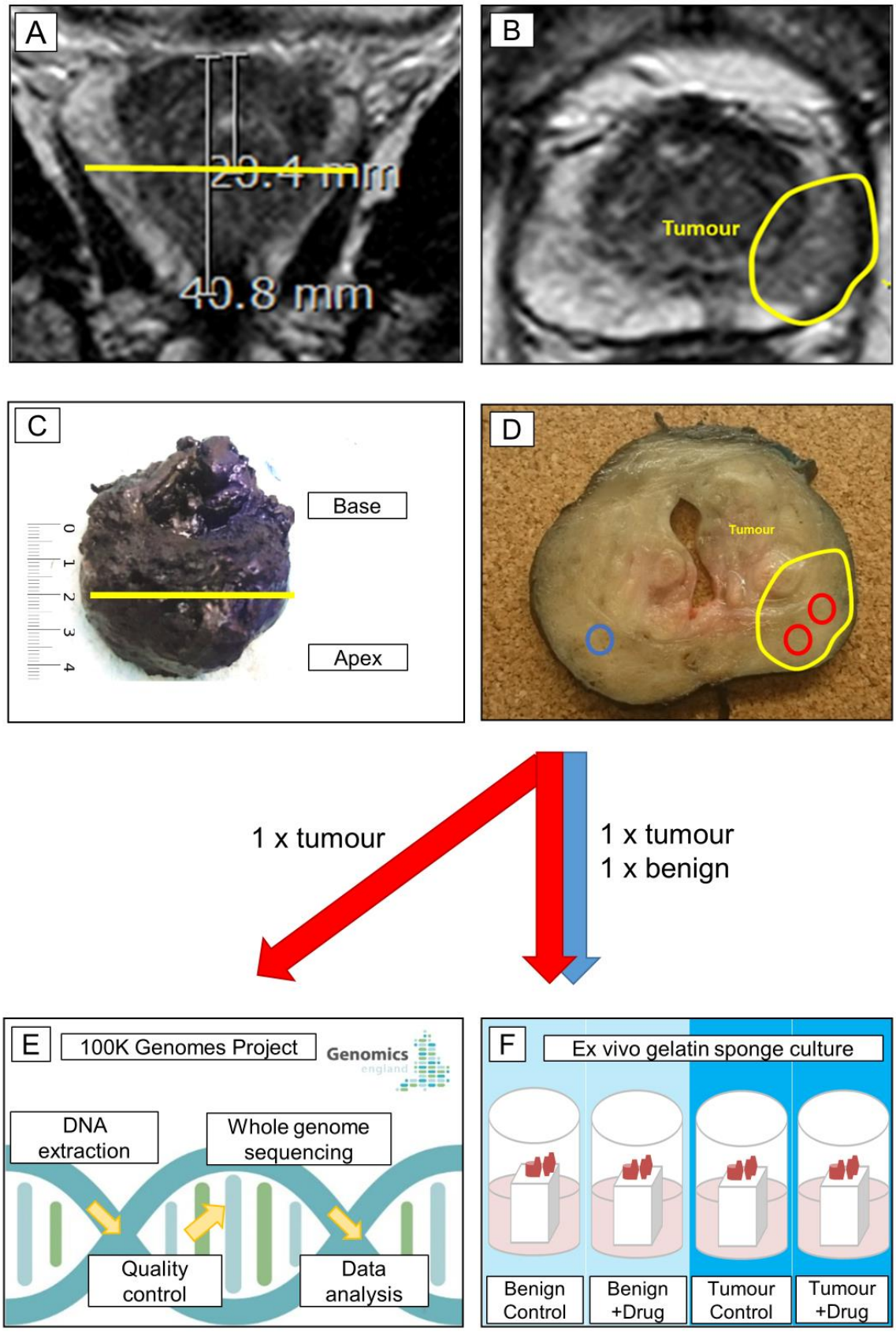
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E
100K Genomes Project
Genomics england
DNA extraction
Whole genome sequencing
Quality control
Data analysis
F
Ex vivo gelatin sponge culture
Benign Control
Benign +Drug
Tumour Control
Tumour +Drug

Figure 1

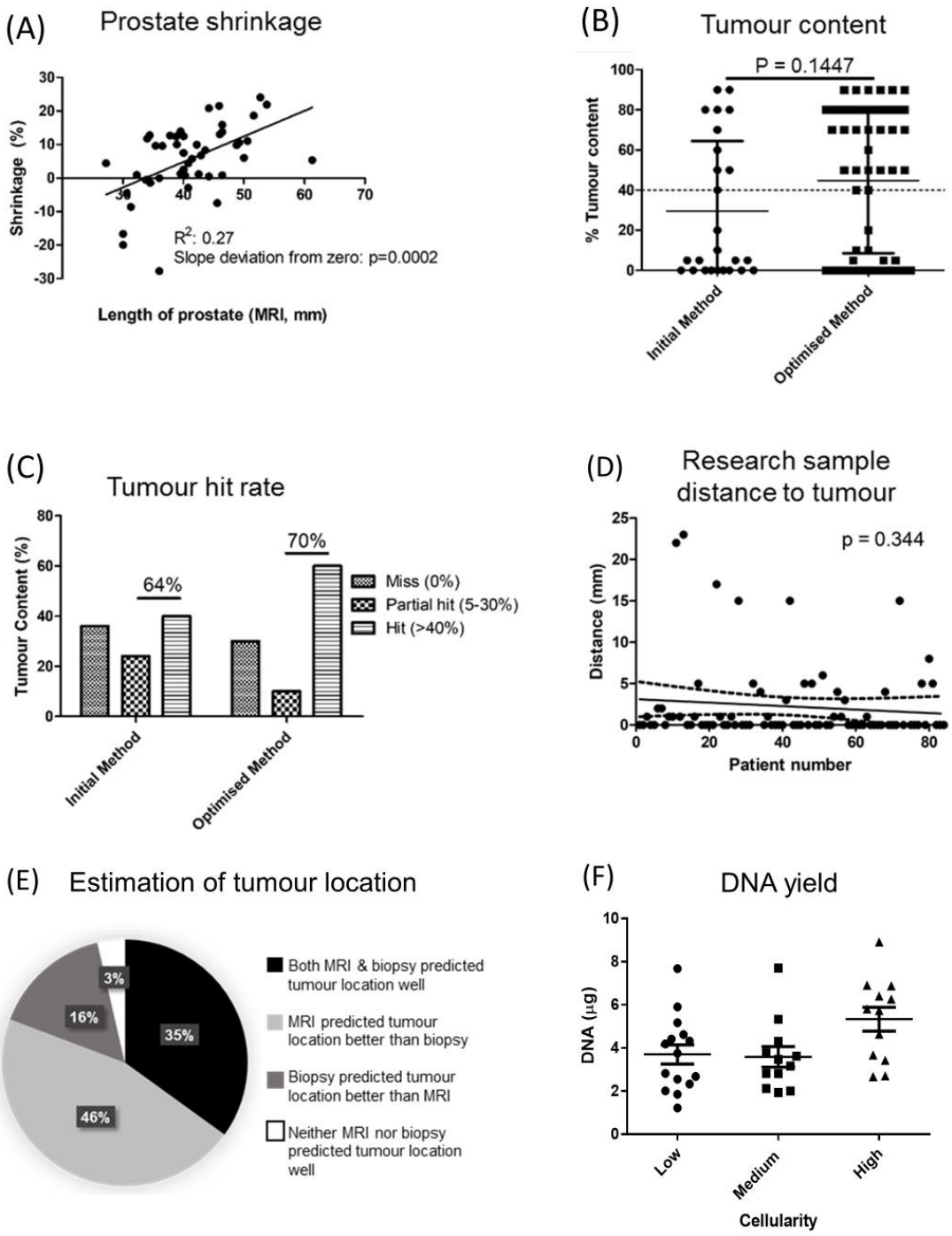

(A)
Prostate shrinkage
Shrinkage (%)
Length of prostate (MRI, mm)
R2: 0.27
Slope deviation from zero: p=0.0002
(B)
Tumour content
P = 0.1447
% Tumour content
Initial Method
Optimised Method
(C)
Tumour hit rate
Tumour Content (%)
64%
70%
Miss (0%)
Partial hit (5-30%)
Hit (>40%)
Initial Method
Optimised Method
(D)
Research sample distance to tumour
p = 0.344
Distance (mm)
Patient number
(E)
Estimation of tumour location
3%
16%
35%
46%
Both MRI & biopsy predicted tumour location well
MRI predicted tumour location better than biopsy
Biopsy predicted tumour location better than MRI
Neither MRI nor biopsy predicted tumour location well
(F)
DNA yield
DNA (µg)
Low
Medium
High
Cellularity

Figure 2

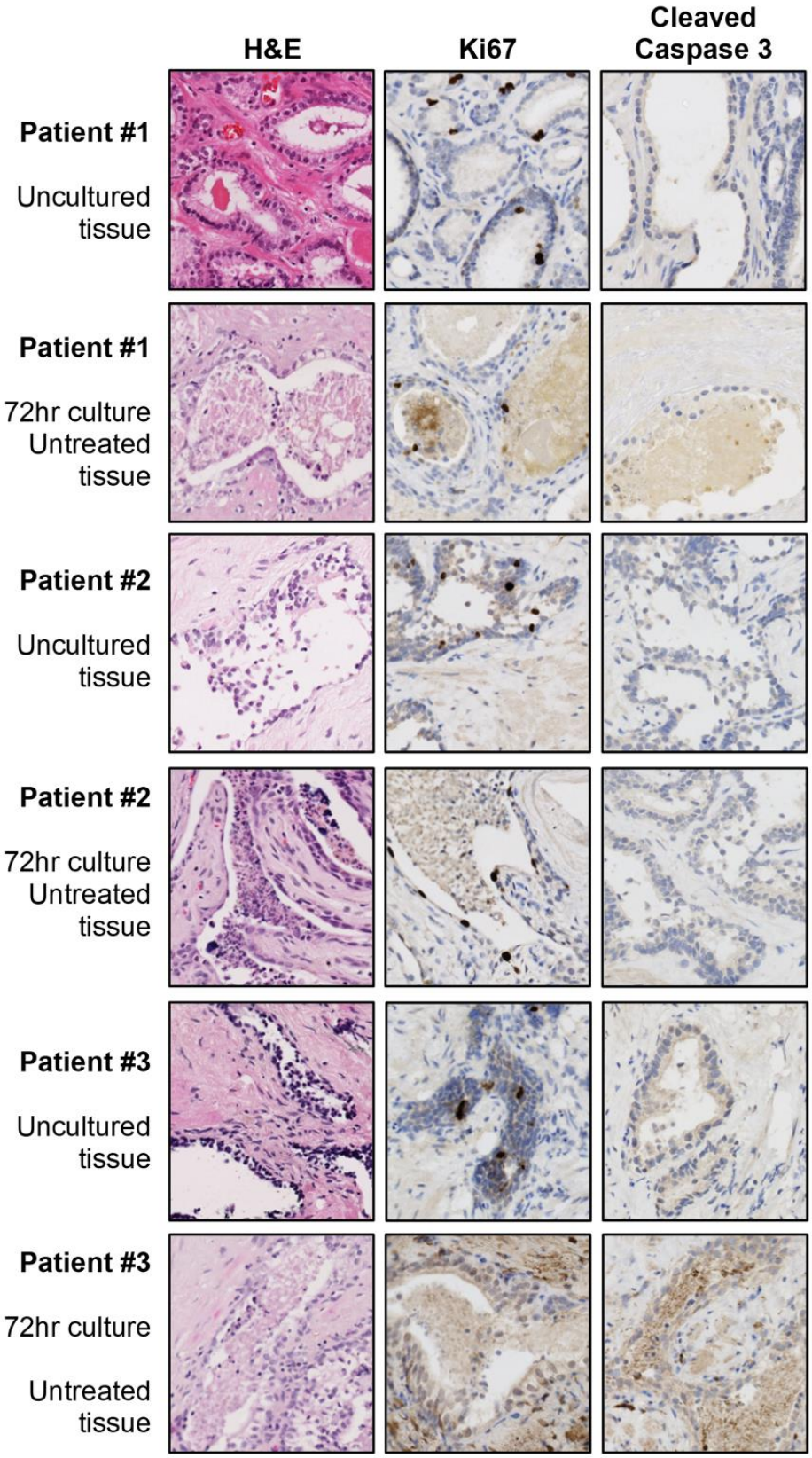
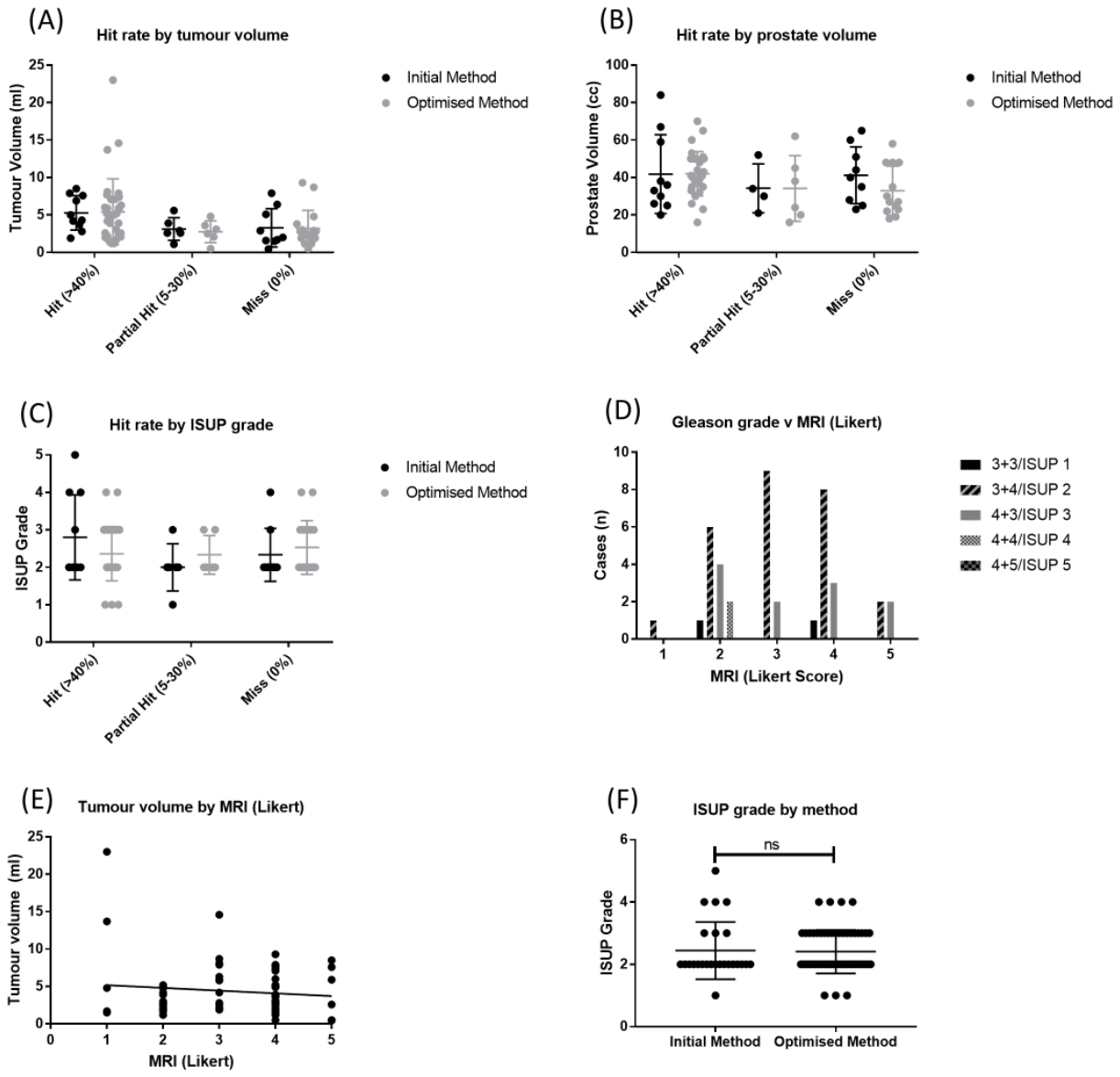

H&E
Ki67
Cleaved Caspase 3
Patient #1
Uncultured tissue
Patient #1
72hr culture Untreated tissue
Patient #2
Uncultured tissue
Patient #2
72hr culture Untreated tissue
Patient #3
Uncultured tissue
Patient #3
72hr culture
Untreated tissue

Figure 3



Supplementary Figure 1