Pre-implementation assessment of the acceptability of using circulating microRNAs for follow-up of malignant germ-cell-tumors.

Lorna A. Fern[1,2], Michelle Greenwood[3], Shievon Smith[3], Susan Brand[4], Nicholas Coleman[5], Daniel P. Stark[2,6] and Matthew J. Murray[2,5,7]

[1] Oncology Division, University College London Hospitals NHS Foundation Trust, London, United Kingdom.

[2] National Cancer Research Institute Teenage, Young Adult and Germ Cell Research Group, United Kingdom.

[3] Department of Medical Oncology, St Bartholomew's Hospital, London, United Kingdom.

[4] It's in the Bag Charity, Floor 3 Offices, Bristol Haematology & Oncology Centre, Horfield Road, Bristol, United Kingdom.

[5] Department of Pathology, University of Cambridge, Tennis Court Road, Cambridge, United Kingdom.

[6] Leeds Institute of Molecular Medicine, University of Leeds, Leeds, United Kingdom.

[7] Department of Paediatric Haematology and Oncology, Cambridge University Hospitals NHS Foundation Trust, Cambridge, United Kingdom.

Corresponding author: Dr Lorna Fern, Oncology Division, University College London Hospitals NHS Foundation Trust, London, UK, NW1 2BU; Telephone 0044 (0)203 456 7199; Fax: not applicable; email: lorna.fern@nhs.net

Running title: Patient acceptability of testis cancer miRNA test

Disclaimers: none

Conflicts of interest: none

Keywords. Germ cell tumor; user involvement; microRNA; miR-371a-3p; patient and public involvement; testicular cancer

Word count: 4,033 words

Abstract

Background. MicroRNAs from the miR-371~373 and miR-302/367 clusters, particularly miR-371a-3p, are promising biomarkers for blood-based diagnosis/disease-monitoring of malignant germ-cell-tumors (GCTs) and nearing clinical implementation. These biomarkers have superior sensitivity/specificity compared with current markers alpha-fetoprotein (AFP) and human-chorionic-gonadotropin (HCG). We explored patient acceptability of using circulating microRNAs to replace multiple serial CT-scans in malignant-GCT follow-up.

Methods. Two workshops, involving an interactive presentation and focus-group. Discussions were digitally recorded and transcribed verbatim. Qualitative thematic analysis of transcripts identified key themes.

Results. Prior to the workshops, potential participants expressed concern about adoption of new blood tests due to personal experiences of the limitations of existing (AFP/HCG) markers. Twelve males with malignant-GCT diagnosis aged 22-57y, currently 26-59y, participated; all in follow-up. Three had experienced recurrence. Participants had cumulative exposure to between 1-15 CT-scans. Data saturation was reached at the second workshop; five themes emerged underpinning preference for microRNA testing *versus* CT-scans: 1) *increased sensitivity/safety*; 2) *reduced financial costs*; 3) *reduced time for test/results*; 4) *practicalities*; and 5) *reduced anxiety*. However, some participants perceived an increased diagnostic capacity of CT-scans *versus* blood-testing.

Conclusion. This first user consultation of circulating microRNA testing for future malignant-GCT follow-up suggests high acceptability with potential patient and healthcare system benefits.

Introduction

Testicular germ-cell-tumors (GCTs) are the most common malignancy in young men, with increasing incidence[1]. Malignant testicular GCTs (TGCTs) are classified as seminomas or nonseminomatous tumors, the latter comprising the histological subtypes yolk sac tumor, embryonal carcinoma, and choriocarcinoma[2]. Clinical issues managing patients with TGCTs remain, despite generally highly successful treatment[3], which can include surveillance alone following orchidectomy for clinical stage I (CS-I) cases and chemotherapy, surgery and/or radiotherapy for more advanced stage (metastatic) cases[4]. The high success rate of treatment for TGCTs and the relatively young age at diagnosis means that many patients will be in follow-up for a long period of time, thus long-term follow-up schedules which detect recurrence without patient harm are of critical importance.

A current issue is the limited sensitivity and specificity of the conventional serum protein markers alpha-fetoprotein (AFP), human-chorionic-gonadotrophin (HCG) and lactate dehydrogenase (LDH)[3], which are positive at diagnosis in only approximately half of TGCT cases [5]. AFP is typically raised in yolk sac tumor and HCG in choriocarcinoma[3], but these markers are usually negative in seminoma, the commonest subtype, and consequently follow-up schedules to identify recurrence are heavily CT-imaging based[6]. For example, the current National Comprehensive Cancer Network (NCCN) Guidelines advise three CT scans in the first year, and up to nine across the first five years, for follow-up of such patients[6]. Concern exists regarding the associated cumulative radiation burden and potential second cancer risk from such serial CT imaging[3]. Moreover, the substantial anxiety associated with surveillance after TGCT has been quantified from national registers[7], subjected to recent systematic review[8] and its etiology systematically explored[9]. How to effectively screen and diagnose patients in

follow-up for development of either relapse and/or late-effects is the top research priority for adults living with and beyond cancer[10]. Specifically, circulating nucleic acid markers of disease/relapse are highlighted as a priority. A similar exercise undertaken in teenagers and young adults, where TGCT represent one of the commonest cancer types, identified effective screening and follow-up, which causes the least physical and psychological harm, as another 'top-10' research priority[11].

As a result, a universal biomarker of all malignant TGCT subtypes is urgently needed. Such a biomarker could be used in follow-up and would, for example, allow avoidance of multiple serial CT scans[3]. Quantification of circulating microRNAs may provide the solution[3]. MicroRNAs are short, non-protein-coding RNAs regulating protein-coding gene expression and cellular pathways[12]. We previously showed that all eight microRNA members of the miR-371~373 and miR-302/367 clusters were highly expressed in all malignant GCTs, regardless of patient age, tumor site or histologic subtype and this co-ordinate over-expression does not occur in any other tumor type or disease-state[13]. We developed a highly sensitive, pre-amplified PCR methodology and demonstrated that miR-371~373 and miR-302/367 microRNAs could act as useful blood-based markers of malignant GCTs[14]. Numerous subsequent studies have utilized this pre-amplified PCR approach to demonstrate that these microRNAs, particularly miR-371a-3p, are highly sensitive and specific for malignant GCT diagnosis, disease-monitoring and detection of recurrence[2]. Studies in TGCT cohorts are impressive, with diagnostic sensitivity and specificity both >90%, far outperforming the conventional serum markers AFP/HCG[5,15-18]. Data show that circulating miR-371a-3p levels accurately reflect malignant GCT burden and treatment response[5,19].

Accordingly, quantification of circulating microRNAs could be used for malignant TGCT follow-up, particularly for seminoma patients, thereby avoiding multiple serial CT scans, with imaging reserved for patients with elevated/increasing circulating microRNA levels[3]. A cost-analysis has shown that this approach could save the US healthcare system $69million per year[20]. As microRNA testing is rapidly being translated towards routine clinical practice[21], early user consultation exercises are warranted. Patient acceptability for new clinical tests is essential to their successful integration into practice, and a number of frameworks may be utilized to assess this. Implementation science is 'the scientific study of methods to promote the systematic uptake of research findings and other evidence-based practices into routine practice, and, hence, to improve the quality and effectiveness of health services'[22]. Within this implementation context, 'acceptability is the perception that the given innovation is agreeable, palatable, or satisfactory' and this can be determined using quantitative or qualitative methods[23]. Early engagement can flag potential issues or concerns which can be addressed[24], and through this early (pre-implementation) patient engagement, can improve disease, health service uptake and satisfaction outcomes during the later implementation process[23]. Here, we sought to determine such early (pre-implementation) patient acceptability of replacing multiple serial CT scans with a circulating microRNA blood test for future malignant TGCT follow-up.

Methods

Participants and setting

Data collection took place during two workshops held in March and June 2019. Participants were eligible if they had a previous malignant TGCT. Participants were recruited through social media, relevant charities and National Health Service (NHS) TGCT clinics. An advert (Supplementary Figure S1) was circulated during February and March 2019 for the first workshop and participants for the second workshop were recruited through an established testicular cancer support group with approximately 60 members during April and May 2019. Participants completed basic anonymised demographic information, which was optional. It was not possible to determine response rate due to recruitment via social media/cascading techniques.

Workshop format

Both workshops were conducted and reported consistent with the 'GRIPP2' reporting guidelines for patient and public involvement work[25]. Both workshops were similar in design, with the second workshop building upon the first. Workshops consisted of an interactive presentation (MJM) describing currently used serum tumor markers (AFP/HCG/LDH) for TGCTs, the new circulating microRNA test, literature overview, representative case studies and a cost-analysis evaluation; participants commented throughout. Participants were informed, based on current technical data, about the known practicalities, including costs and benefits, of the new and existing tests. This included that the microRNA test showed ~90-95% sensitivity/specificity for the presence of malignant/viable GCT disease[5], and that further prospective studies with associated outcomes were required prior to clinical implementation. It was explained that at diagnosis, a staging CT scan would still be performed, in addition to

blood tests, but that in follow-up, the aim was for the circulating microRNA test to replace routine CT scanning, with CT reserved for cases where circulating microRNA levels were persistently raised or increasing. Participants were also counselled on the small possibility of false negative testing and that occasional, infrequent CT scans would still be required to detect any development of pure teratoma, a GCT subtype typically managed with surgery alone, and not associated with raised levels of the circulating microRNA test[5,19].

The presentation in both workshops was followed by a focus group led by an independent researcher to the microRNA study group (LF); the second workshop was also observed by SS/MG. During the focus groups, the role of MJM was only to answer technical questions about microRNA test performance. The focus group discussion guide is shown in Box 1. Following the presentation, participants were asked if they had any further questions regarding the test. Participants were prompted to share their initial thoughts of the microRNA test; this section was allowed to flow organically to capture as much diversity in views about the test as possible. Participants were then asked to share their thoughts about the feasibility of the new test and of their existing CT scan experiences. We then asked about acceptability of the microRNA test based on currently available data, and sought to identify potential concerns of replacing CT scans with this new testing (patients may think a test can feasibly be implemented in practice, but it may still not be acceptable to them i.e. they would still prefer current follow-up methods). Finally, the participants were specifically asked about any additional concerns they had about the new blood test which had not yet been covered. Workshops were audio recorded and transcribed verbatim.

Data analysis

Thematic analysis of transcripts involved familiarization with the data, use of an iterative coding strategy, and then generating, reviewing and agreeing themes[26]. We considered a range of theoretical approaches to analysis, including theory of planned behaviour[27], but selected thematic analysis and framework structure. This was because we could identify no convincing existing hypothetical basis to propose a causal relationship between the service users' perspectives of a proposed change, when that change was the potential withdrawal of routine CT scanning[22]. The initial analysis phase was undertaken by one researcher (LF) with checking and further refinement by two additional researchers (MG/SS) in the second phase.

Ethical considerations

As a user consultation study, formal ethical approvals were not required, but ethical standards adhered to. Participants received information in advance, and gave written consent prior to workshop participation for audio recording and use of anonymized quotes. Following the workshop, participants were contacted (LF) to ensure that the workshop had not elicited any distress.

Results

In total, 12 males with a TGCT diagnosis aged 22-57 (mean 35.3) years, and currently aged 26-59 (mean 42.8) years participated; most had been in follow-up for over two years (n=8). The participants were representative of the spectrum of disease observed in clinical practice, with some having undergone surveillance following diagnosis of a CS-I malignant TGCT and others having received chemotherapy for metastatic disease. In total, three of the 12 participants had experienced recurrence. All had experience of blood testing and CT scan-based monitoring and surveillance, and of receiving adverse test/scan results in terms of a GCT diagnosis. As this was a user consultation exercise specifically regarding follow-up for patients with malignant TGCTs, and not primary diagnosis and treatment, it was not necessary or appropriate to collect further clinical details or information. The total number of CT scans experienced by the group as a whole was 65 (range 1-15, median 5). Table 1 shows patient demographics.

Of note, the wording within the text of the original workshop advert specifically stated that this was referring to a 'new' blood test (Supplementary Figure S1). Despite this, some potential participants had responded to the original advert on social media with comments expressing caution about the performance of blood testing due to their own negative personal experiences of existing (AFP/HCG/LDH) blood tests not detecting their disease, and reliance on imaging for diagnosis and/or relapse. Examples are shown below:

- *"I won't be able to make it there but I wouldn't trust a blood test over a CT scan….. I don't know how different this blood test would be. I won't be able to go (to the workshop).. I had markers before my surgery. After surgery, one year later no markers, blood all normal but CT showed spread into my lungs. Without the CT I wonder how much more spread there would have been."*

- *"I have had testicular cancer twice - once when I discovered it myself by physical examination and a year later a re-occurrence by an annual CT scan - my blood markers provided no evidence of anything abnormal - my cancer was seminoma. I feel more reassured by scans as a result. Sounds to me that in the excitement to save money, mens' wellbeing could be compromised."*

Although others were more positive about a new blood test pre-workshop, for example:

- *"Hi, sounds like a great idea. A blood test is going to be a lot less hassle and expense than a CT scan."*

As a result of these comments, the advert for the second workshop was altered to add the word 'new' to the title, becoming 'Can a new blood test replace CT scans in testicular cancer?', in addition to changes to the text, where the word 'new' was highlighted.

The presentation and focus group of the first workshop lasted two hours and fifty-eight minutes, excluding breaks. In the second workshop, the focus group was one hour and seventeen minutes, excluding the presentation. The second focus group was shorter as data saturation was reached, and no new data was being generated by the discussion.

Four initial themes arose in the first phase of analysis (LF). In the second phase (MG/MS), there was concordance with the identification of these four themes, which were supplemented by the emergence of an additional fifth theme (reduced anxiety). Participants of the first workshop received a copy of these five identified themes to confirm that we had understood the service users' views and perspectives correctly, prior to the second workshop. Data saturation was reached at the second workshop and no further themes subsequently identified;

all participants have received a copy of the themes and study outputs, in accordance with good patient and public involvement practice. Overall, five key themes were identified, which favored the circulating microRNA test:

1. Increased sensitivity and safety
2. Reduced financial costs
3. Reduced time
4. Practicalities
5. Reduced anxiety

Increased sensitivity and safety

All participants were reassured by the sensitivity of the microRNA test compared with current AFP/HCG markers. In particular, participants knew patients where current AFP/HCG markers had not detected relapse:

- *"…they've all got experience of blood tests not picking anything up and it's a CT scan that's picked something up… so, that's really reassuring to see that it's totally new, it's not HCG or anything like that…"*

Safety benefits were conferred by reducing exposure to radiation in patients previously exposed to cytotoxic chemotherapy:

- "*If it's as accurate as we hope it is, and we can reduce the number of CT scans, then, yes, we're not exposing patients to radiation that they may not need to have."*

Further safety gains were identified as some participants had experienced allergies/reactions to intravenous contrast agents administered prior to CT scanning and contrast would not be

required for a circulating microRNA test. In addition to follow-up, participants were keen to know if microRNA testing had diagnostic capability:

- *"…being misdiagnosed (at original diagnosis, with negative conventional serum markers AFP/HCG/LDH), that'll be very interesting to me, that if that blood test was available then, perhaps I wouldn't have been misdiagnosed…"*

Researcher MJM re-confirmed that circulating microRNA testing had high sensitivity/specificity for malignant TGCT diagnosis[5], but more data would be needed prior to implementing microRNAs as a diagnostic tool in routine clinical practice.

Reduced financial costs

There was complete concordance amongst participants of the cost advantages for patients and healthcare systems (e.g. NHS in the United Kingdom). Patient saving costs were attributed to time off work and paying for lengthy parking charges for the CT scan:

- *"There's the cost to the patient, you know, going in, I know I could go to the hospital and get a blood test done in twenty minutes, I'd only have to pay for my parking. If I'm going in for a CT scan, you know, it's time off work, it's all that other cost as well."*

Participants identified potential NHS savings in addition to the CT scan appointment, recognising the administrative preparation and radiology reporting costs associated with scans:

- *"it's not just the actual scan, is it? It's all the nurses and all the rest of it, and you'll sit and drink your thing (oral contrast) and all that. So, that is all a hidden cost"*

Reduced time

The majority of participants supported circulating microRNA testing as the return of results to patients would generally be quicker compared with CT scans. Participants recognised time spent waiting in hospitals and at home for results would be reduced.

- *"…not asking them to go into hospital for two or three hours…You can just nip in and get a blood test."*

Participants also acknowledged the multiple processes required for CT, including multidisciplinary team (MDT) review, and how these impact on professional time:

- *"…not just the costs, but the time of everybody involved in doing it."*

The possibility of 'dropping' into the hospital for blood testing was preferred, however variation in service configuration between hospitals meant a minority of participants felt it could take the same amount of time as a CT scan.

The period between tests and results was described as anxiety-provoking for participants; their experience of blood test result turnaround was superior compared with CT scans:

- *"My blood test I normally have done on a Monday, and then it's normally in the system by Thursday when I come in for my follow-up. It's a decent turnaround."*

Practicalities

The practical processes of investigation, particularly the ease-of-access of taking an additional blood sample alongside routine markers (AFP/HCG), compared with the physical and psychological impact of being in a CT scanning machine, was discussed and agreed by all participants:

- *"Well, the machine is claustrophobic, and I think a lot of people find the process where you have to sit and have the drink (oral contrast)…that is not very pleasant in itself. The machine is big and frightening to some people."*

Additional practical advantages of the circulating microRNA test included no preparation procedures, as the blood test doesn't require any prior fasting and drinking of oral contrast agents described as '*disgusting'*.

Reduced anxiety

All participants shared openly their anxiety regarding scans and fear of recurrence, not just within themselves but also for their families. Participants felt the circulating microRNA test would reduce *'scan anxiety'* while preparing for the scan and the *'fear factor'* when waiting for results. However, some participants felt parents/partners may prefer the 'what we know works' approach and favor CT scanning, perceiving some risk with adopting new technologies:

- *"I think with my parents in particular, I think they would be very 'Old Testament' in terms of it. This is what we know, this is what we stick to."*

Interestingly, some participants perceived the CT scan to have increased diagnostic capacity compared with blood tests and felt reassured by the capacity of the scan to detect other anomalies:

- *"The only concern I've got is that if you remove the CT scan, you will not be able to detect anything else. So, if there is any other cancer coming up, or anything else, you're relying on just the blood test. You won't be able to pick it up."*

Some anxiety was expressed around the possibility of not having a CT scan at all in follow-up. MJM clarified that if circulating microRNA levels were raised/increased in follow-up, a re-

staging CT scan would routinely be performed, prior to any management decisions being made, which offered appropriate reassurance.

Discussion

We report on the first patient acceptability consultation of replacing multiple serial CT scans with sequential circulating microRNA testing for future follow-up for malignant TGCT, using methodology consistent with recent guidelines developed for patient and public involvement work[25]. We identified positive elements of the proposed service change in each of the domains that are relevant early in an implementation process, notably in acceptability, appropriateness and feasibility[23]. This work provides the first evidence that this proposed change in surveillance testing satisfies many of the domains of health care quality put forward by the Institute of Medicine (IOM), in particular being patient-centred, timely and efficient (https://www.ahrq.gov/talkingquality/measures/six-domains.html). For any new test planned for clinical use, determining early patient acceptability is critical for successful implementation[24]. Poor acceptability may reduce follow-up adherence in patients, mitigating any theoretical advantages of the new test. The circulating microRNA test was favored by participants for multiple reasons, including its increased sensitivity/specificity compared with existing AFP/HCG serum markers, safety in reducing cumulative radiation exposure from serial CT scans, reduced time and cost-saving benefits, improved practicalities compared with CT scans and reducing anxiety. Participants described an associated fear of recurrence in preparation for, whilst undergoing, and waiting for the results of CT scans, a fear somewhat mitigated by the reduced timelines associated with blood tests. Interestingly, a minority of participants reported a perceived increased diagnostic capacity of the CT scan to detect other anomalies. Of note, such anomalies often lead to additional anxiety and investigations, which are frequently of no clinical significance[28]. This is particularly relevant in a young and otherwise well population, without associated co-morbidity, and in whom the additional radiation is not justified if no longer required for malignant TGCT surveillance.

As outcomes for patients with malignant TGCTs are generally excellent, there is a requirement for safe, effective and economically viable follow-up testing, which reliably detects early relapse without incurring additional psychological or physical harm to patients, such as the cumulative radiation risk from serial surveillance CT scans. Importantly, such follow-up testing has recently been identified as a research priority for young people with cancer [11] and older adults living with and beyond cancer[10]. In this regard, a major current issue is the limited sensitivity and specificity of the serum markers AFP/HCG in clinical follow-up for patients with malignant TGCTs[3]. Follow-up schedules are therefore heavily CT imaging-based[6], particularly for CS-I seminoma tumors, where only 3% of patients who relapse are identified by raised markers compared with 87% by CT[29]. Even for CS-I nonseminoma cohorts, >50% of all relapses are AFP/HCG negative, reinforcing the reliance on CT in follow-up[29]. Although recent advances mean that current CT scanners use low-dose radiation, concerns regarding cumulative radiation burden from TGCT follow-up schedules exist, along with second malignant neoplasm risk[3]. Consequently, replacing multiple serial CT scans with circulating microRNA testing in follow-up for malignant TGCT patients would overcome the issues with existing AFP/HCG markers and imaging, and importantly address patients' research priorities[11].

In the workshops, we provided technical information first, then elicited what was important to service users afterwards. Assessment of service user acceptability (such as relative advantage), appropriateness and feasibility of the new microRNA test using an implementation science framework[23] could only be judged once technical knowledge had been provided and these advantages and disadvantages understood. The importance of suitably equipping service users with the knowledge they need to make an informed assessment was evidenced by the comments posted on social media prior to the workshop. Here, potential participants based their

assumption on their own experience of the current AFP/HCG markers which had missed relapses, particularly in those with seminoma, which the new microRNA test would be expected to detect.

The participants in this study were representative of the observed age-range and clinical spectrum of patients with TGCTs. All were in follow-up and some had experienced recurrence. They were recruited nationally with potential subtle regional differences in standards-of-care for follow-up and represented a range of ethnicities (not formally recorded). Despite positive participant review of circulating microRNA testing, we acknowledge study limitations. The participants were a small, self-selecting cohort; therefore, their views may not necessarily reflect the views of a wider group. Having said this, recruiting males to research and user involvement is a well-recognized challenge[11,30,31], and therefore the workshops were relatively well-attended. Furthermore, there was considerable interest in the first national workshop, but the daytime schedule required to allow the necessary travel made it difficult to attend for those who were working, a common difficulty encountered in organising user involvement events in patients who are in follow-up. Of note, the second, local support group workshop was held in the evening and accordingly had greater attendance. An additional limitation was that participants experienced their care in a 'free' healthcare system (NHS); further work may be required to determine the acceptability of the test in a larger group and in different systems. Indeed, a small minority of participants offered contrasting views based on their experience of service configuration for blood taking (appointment times versus walk-in clinics). Further, some participants expressed the view that their families/partners may need additional information in order to deviate from the traditional CT scan follow-up; the acceptability of the new circulating microRNA test in such groups warrants further exploration. The role of researcher MJM during the focus groups in both workshops was only to answer technical

questions about microRNA test performance; all conversations were led and facilitated by LF, preventing bias. Participants were also made aware that occasional, infrequent CT scans would still be required to detect pure teratoma, a GCT subtype typically managed with surgery alone, and not associated with raised circulating microRNA levels[5,19]. Finally, we also acknowledge that the outcome of current prospective trials/studies with associated clinical outcomes will be required prior to routine clinical implementation of the microRNA test. However, early evidence of acceptability to patients is important[24].

In summary, our early pre-implementation data suggest that replacing CT scan-based follow-up with a circulating microRNA blood text for malignant TGCT follow-up in the future will be acceptable to patients. Implementation science approaches may now be applied in future studies to promote behaviour change by clinicians, organisations and policy-makers[22,23]. Upon clinical implementation, further long-term prospective studies are required to confirm the diagnostic and follow-up accuracy of microRNA testing for malignant TGCT disease and assess cost-effectiveness.

Additional information

Acknowledgements: We wish to thank the participants for taking part in the user consultation exercise. We would also like to thank the charities who circulated the advert for the first workshop.

Authors' contributions: MJM and NC were responsible for study concept. LF was responsible for study design. MG, SS, SB were involved in recruitment to the workshops. LF undertook the first phase of qualitative data analysis. MG and SS attended the second workshop and undertook the second phase of qualitative data analysis. All other authors (MJM/SB/DS/NC) contributed to the interpretation of the results but were not involved in the direct analysis of the qualitative data. LF wrote the manuscript with clinical and technical input from DS, NC and MJM. All authors (LF/MG/SS/SB/NC/DS/MJM) were involved in drafting and approving the final manuscript.

Ethical approval and consent to participate/publish: As a user consultation study, formal ethical approvals were not required, but ethical standards adhered to. Participants received information in advance, and gave written informed consent for participation and subsequent publication and use of anonymized quotes.

Data availability: Data will be made available upon reasonable request.

Conflicts of interest: None.

Funding: The authors acknowledge grant funding from the St. Baldrick's Foundation (reference 358099) and the Isaac Newton Trust (reference 15.40f). Lorna Fern is funded by Teenage Cancer Trust. We are grateful for support from the Max Williamson Fund and from

Christiane and Alan Hodson, in memory of their daughter Olivia. The funders were not involved in study design, data collection or interpretation.

References

1. Huyghe E, Matsuda T, Thonneau P. Increasing incidence of testicular cancer worldwide: a review. *J Urol* 2003;170(1):5-11.

2. Murray MJ, Coleman N. MicroRNA Dysregulation in Malignant Germ Cell Tumors: More Than a Biomarker? *J Clin Oncol* 2019;37(16):1432-35.

3. Murray MJ, Coleman N. Can circulating microRNAs solve clinical dilemmas in testicular germ cell malignancy? *Nat Rev Urol* 2019;16(9):505-06.

4. Murray MJ, Huddart RA, Coleman N. The present and future of serum diagnostic tests for testicular germ cell tumours. *Nat Rev Urol* 2016;13(12):715-25.

5. Dieckmann KP, Radtke A, Geczi L, et al. Serum Levels of MicroRNA-371a-3p (M371 Test) as a New Biomarker of Testicular Germ Cell Tumors: Results of a Prospective Multicentric Study. *J Clin Oncol* 2019;37(16):1412-23.

6. Gilligan T, Lin DW, Aggarwal R, et al. Testicular Cancer, Version 2.2020, NCCN Clinical Practice Guidelines in Oncology. *J Natl Compr Canc Netw* 2019;17(12):1529-54.

7. Kreiberg M, Bandak M, Lauritsen J, et al. Psychological stress in long-term testicular cancer survivors: a Danish nationwide cohort study. *J Cancer Surviv* 2020;14(1):72-79.

8. Smith AB, Rutherford C, Butow P, et al. A systematic review of quantitative observational studies investigating psychological distress in testicular cancer survivors. *Psychooncology* 2018;27(4):1129-37.

9. Stark D, Kiely M, Smith A, et al. Reassurance and the anxious cancer patient. *Br J Cancer* 2004;91(5):893-9.

10. National Cancer Research Institute (NCRI). Living With and Beyond Cancer Top 10 Research Priorities 2020 [Available from: www..ncri.org.uk/lwbc/ accessed 08/12/2020 2020.

11. Aldiss S, Fern LA, Phillips RS, et al. Research priorities for young people with cancer: a UK priority setting partnership with the James Lind Alliance. *BMJ Open* 2019;9(8):e028119.

12. Lu J, Getz G, Miska EA, et al. MicroRNA expression profiles classify human cancers. *Nature* 2005;435(7043):834-8.

13. Palmer RD, Murray MJ, Saini HK, et al. Malignant germ cell tumors display common microRNA profiles resulting in global changes in expression of messenger RNA targets. *Cancer Res* 2010;70(7):2911-23.

14. Murray MJ, Halsall DJ, Hook CE, et al. Identification of MicroRNAs From the miR-371~373 and miR-302 Clusters as Potential Serum Biomarkers of Malignant Germ Cell Tumors. *Am J Clin Pathol* 2011;135(1):119-25.

15. Dieckmann KP, Radtke A, Spiekermann M, et al. Serum Levels of MicroRNA miR-371a-3p: A Sensitive and Specific New Biomarker for Germ Cell Tumours. *Eur Urol* 2017;71(2):213-20.

16. Nappi L, Thi M, Lum A, et al. Developing a Highly Specific Biomarker for Germ Cell Malignancies: Plasma miR371 Expression Across the Germ Cell Malignancy Spectrum. *J Clin Oncol* 2019:JCO1802057.

17. Syring I, Bartels J, Holdenrieder S, et al. Circulating Serum miRNA (miR-367-3p, miR-371a-3p, miR-372-3p and miR-373-3p) as Biomarkers in Patients with Testicular Germ Cell Cancer. *J Urol* 2015;193(1):331-7.

18. van Agthoven T, Looijenga LHJ. Accurate primary germ cell cancer diagnosis using serum based microRNA detection (ampTSmiR test). *Oncotarget* 2017;8(35):58037-49.

19. Murray MJ, Bell E, Raby KL, et al. A pipeline to quantify serum and cerebrospinal fluid microRNAs for diagnosis and detection of relapse in paediatric malignant germ-cell tumours. *Br J Cancer* 2016;114(2):151-62.

20. Charytonowicz D, Aubrey H, Bell C, et al. Cost Analysis of Noninvasive Blood-Based MicroRNA Testing Versus CT Scans for Follow-up in Patients With Testicular Germ-Cell Tumors. *Clin Genitourin Cancer* 2019;17(4):e733-e44.

21. Fendler A, Stephan C, Yousef GM, et al. The translational potential of microRNAs as biofluid markers of urological tumours. *Nat Rev Urol* 2016;13(12):734-52.

22. Bauer MS, Damschroder L, Hagedorn H, et al. An introduction to implementation science for the non-specialist. *BMC Psychol* 2015;3:32.

23. Proctor E, Silmere H, Raghavan R, et al. Outcomes for implementation research: conceptual distinctions, measurement challenges, and research agenda. *Adm Policy Ment Health* 2011;38(2):65-76.

24. Gradinger F, Britten N, Wyatt K, et al. Values associated with public involvement in health and social care research: a narrative review. *Health Expect* 2015;18(5):661-75.

25. Staniszewska S, Brett J, Simera I, et al. GRIPP2 reporting checklists: tools to improve reporting of patient and public involvement in research. *BMJ* 2017;358:j3453.

26. Chapman AL, Hadfield M, Chapman CJ. Qualitative research in healthcare: an introduction to grounded theory using thematic analysis. *J R Coll Physicians Edinb* 2015;45(3):201-5.

27. West R, Godinho CA, Bohlen LC, et al. Development of a formal system for representing behaviour-change theories. *Nat Hum Behav* 2019;3(5):526-36.

28. Berland LL, Silverman SG, Gore RM, et al. Managing incidental findings on abdominal CT: white paper of the ACR incidental findings committee. *J Am Coll Radiol* 2010;7(10):754-73.

29. Kollmannsberger C, Tandstad T, Bedard PL, et al. Patterns of relapse in patients with clinical stage I testicular cancer managed with active surveillance. *J Clin Oncol* 2015;33(1):51-7.

30. Gadsby R, Snow R, Daly AC, et al. Setting research priorities for Type 1 diabetes. *Diabet Med* 2012;29(10):1321-6.

31. Layton A, Eady EA, Peat M, et al. Identifying acne treatment uncertainties via a James Lind Alliance Priority Setting Partnership. *BMJ Open* 2015;5(7):e008085.

Box 1: Focus group topic discussion guide

FOCUS GROUP – TOPIC DISCUSSION GUIDE

1. **Thinking back to the presentation, does anyone have any questions that didn't get answers or something that needs to be explained more clearly?**
    a. Ask individually
2. **OK, overall what are your thoughts about the feasibility of the blood test in clinical practice for replacing CT scans.**
    a. Expand on positive comments
    b. Expand on concerns
3. **Ok, I want to back track a little and I'd like to talk a little bit more about CT scans and your experience of going for CT scans?**
    a. Expand what it's like in the run up to an appointment
    b. Expand what it's like during the CT scan itself
    c. Expand what it's like waiting for results
4. **So, thinking back to the blood test can you see any advantages over what you now experience at the moment with CT scans?**
    a. Expand on advantages mentioned, potential responses – anxiety, time, radiation exposure, travel to hospital
5. **Thinking back to the blood test and the current results we looked at do any of you have any concerns about replacing CT scans with a blood test?**