Molecular Profiling to Determine Clonality of Serial Magnetic Resonance Imaging/Ultrasound Fusion Biopsies from Men on Active Surveillance for Low-Risk Prostate Cancer


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Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

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doi: 10.1158/1078-0432.CCR-16-1454
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Abstract

Purpose: To determine whether MRI/ultrasound (MRI/US) fusion biopsy facilitates longitudinal resampling of the same clonal focus of prostate cancer and to determine whether high-grade cancers can evolve from low-grade clones.

Experimental Design: All men on active surveillance who underwent tracking MRI/US fusion biopsy of Gleason 6 prostate cancer, on at least two distinct occasions, between 2012 and 2014 were enrolled. MRI/US fusion was used to track and resample specific cancer foci. IHC for ERG and targeted RNA/DNA next-generation sequencing (NGS) were performed on formalin-fixed paraffin-embedded prostate biopsy specimens to assess clonality.

Results: Thirty-one men with median age and PSA of 65 years and 4.6 ng/mL, respectively, were analyzed. The median sampling interval was 12 months (range, 5–35). Of the 26 evaluable men, ERG IHC concordance was found between initial and repeat biopsies in 25 (96%), indicating resampling of the same clonal focus over time. Targeted NGS supported ERG IHC results and identified unique and shared driving mutations, such as IDH1 and SPOP, in paired specimens. Of the nine men (34.6%) who were found to have Gleason ≥7 on repeat biopsy, all displayed temporal ERG concordance. Prioritized genetic alterations were detected in 50% (13/26) of paired samples. Oncogenic mutations were detected in 22% (2/9) of Gleason 6 cancers prior to progression and 44% (4/9) of Gleason ≥7 cancers when progression occurred.

Conclusions: Precise tracking of prostate cancer foci via MRI/US fusion biopsy allowed subsequent resampling of the same clonal focus of cancer over time. Further research is needed to clarify the grade progression potential of Gleason 6 prostate cancer.

Introduction

Contemporary advances in our understanding of the biology and clinical trajectory of low-risk prostate cancer have led to the growing adoption of active surveillance (AS) strategies (1–3). The main objective of AS is to reduce prostate cancer overtreatment, while reserving curative therapy for when disease progression is detected (4, 5). Because of prostate cancer multifocality, precise sampling of prostate cancer foci to assess true disease status is paramount to optimizing AS strategies. Currently, this is typically done with either systematic, yet random sampling of the prostate or by cognitively directed prostate biopsy. Unfortunately, both of these techniques lack precision. Traditional transrectal ultrasound (TRUS)–guided biopsy platforms are confounded by prostate cancer multifocality and sampling bias (i.e., only ~0.04% of the prostate is normally assessed; ref. 6).

Recently, MRI/ultrasound (MRI/US) fusion–guided prostate biopsy platforms have been introduced to facilitate targeted sampling of regions of interest (i.e., areas considered at risk of harboring high-grade prostate cancer on imaging) as well as longitudinal assessment of specific sites (7–10). Tracking biopsy sites within the prostate, based on needle tracks recorded by the
Cohort description

Patients and Methods

Cohort description

Subjects were consecutive men with Gleason 6 prostate cancer foci who underwent an initial diagnostic and a subsequent confirmatory biopsy between January 2012 and December 2014. All were enrolled in an Institutional Review Board (IRB)–approved AS registry at the University of California, Los Angeles (UCLA; Los Angeles, CA). Inclusion criteria for this analysis were that two biopsies were performed at least 4 months apart and that evaluable tissue was available at both time points. Exclusion criteria included any previous form of prostate ablative treatment, androgen deprivation therapy, or 5α-reductase inhibitor use. Characteristics of the group are shown in Table 1.

Biopsy strategy

Biopsy methods and resampling technique using the Artemis device are shown in Figs. 1 and 2 and Supplementary Fig. S1. Prior to the initial biopsy, multiparametric MRI (mpMRI) of the prostate was obtained using a 3T (Siemens Medical Solutions) magnet and a transabdominal coil, as described previously (9, 11). Of 16 men, the prostate cancer focus was within an MRI target [region of interest (ROI)]; in 15 men, the focus was not in an ROI. Segmentation and initial MRI/US fusion biopsy were performed as described previously, using the Artemis device to obtain samples from any ROI seen on mpMRI and systematically via the 12-point template incorporated into the device (Fig. 1; refs. 9, 11). All biopsy sites were mapped, electronically tracked, and saved to enable resampling of the same site. Follow-up biopsy was targeted at the previously identified prostate cancer focus (obtaining 1 core every 3 mm along the longest axis of the lesion; 3–5 cores), which was recorded on the Artemis device (Fig. 2; refs. 9, 11). The ability of this system to resample the same site has been previously reported to be within 1.2 ± 1.1 mm margin of error (11, 17). MRI was not repeated prior to undergoing resampling of tracked biopsy sites.

Tissue preparation

Diagnostic formalin-fixed and paraffin-embedded (FFPE) prostate biopsy tissue obtained from the initial and repeat MRI/US fusion–guided prostate biopsy for each participant was procured. Pathology slides selected for next-generation sequencing (NGS) were re-reviewed independently by two board-certified anatomic pathologists with genitourinary pathology interest (J. Huang and S.A. Tomlins) to confirm Gleason score, volume of cancer, and to identify areas for NGS (discrepancies were resolved by a third pathologist, L.P. Kunju). IHC and NGS were performed with IRB approval on a single FFPE biopsy block with cancer (representing the highest Gleason score) per time point. FFPE sections (10–11 × 5 μm) were cut from each block, with H&E staining performed on the first and last sections to confirm tumor. The penultimate slide was used for ERG IHC. The remaining slides were used for manual tumor dissection with a scalpel for DNA/RNA isolation.

Table 1. Demographic and clinical characteristics of the study cohort (N = 31)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>65 (46–74)</td>
</tr>
<tr>
<td>Race/ethnicity</td>
<td></td>
</tr>
<tr>
<td>African American</td>
<td>1 (3.2)</td>
</tr>
<tr>
<td>Asian</td>
<td>3 (9.7)</td>
</tr>
<tr>
<td>Caucasian</td>
<td>26 (83.9)</td>
</tr>
<tr>
<td>Hispanic</td>
<td>1 (3.2)</td>
</tr>
<tr>
<td>Family history of prostate cancer</td>
<td>13 (41.9)</td>
</tr>
<tr>
<td>Abnormal DRE</td>
<td>5 (16.1)</td>
</tr>
<tr>
<td>Serum PSA (ng/mL)</td>
<td></td>
</tr>
<tr>
<td>Initial biopsy</td>
<td>4.56 (0.49–21.00)</td>
</tr>
<tr>
<td>Repeat biopsy</td>
<td>4.60 (0.47–10.90)</td>
</tr>
<tr>
<td>Prostate volume (cm³)</td>
<td>42.0 (17.0–81.1)</td>
</tr>
<tr>
<td>PSA density at initial biopsy (ng/mL/cm³)</td>
<td>0.097 (0.023–0.328)</td>
</tr>
<tr>
<td>Final Gleason score*</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>20 (64.5)</td>
</tr>
<tr>
<td>3 + 4</td>
<td>8 (25.8)</td>
</tr>
<tr>
<td>4 + 3</td>
<td>2 (6.5)</td>
</tr>
<tr>
<td>8–10</td>
<td>1 (3.2)</td>
</tr>
<tr>
<td>Cancer core length (mm)</td>
<td></td>
</tr>
<tr>
<td>Initial biopsy</td>
<td>2.5 (0.5–7)</td>
</tr>
<tr>
<td>Repeat biopsy</td>
<td>2.0 (0.5–13)</td>
</tr>
<tr>
<td>Interval between biopsies (months)</td>
<td>12 (5–35)</td>
</tr>
</tbody>
</table>

NOTE: Median (range) and frequency (percentages) are presented for continuous and categorical variables, respectively. Abbreviation: DRE, digital rectal exam.

*All patients had Gleason 6 disease at study entry.
ERG IHC

ERG rearrangement status is a clonal marker in prostate cancer (16, 18–20). Thus, to determine clonality of cancer specimens, we assessed ERG status on cancerous tissues obtained from the same focus at both time points (Supplementary Fig. S1). IHC for ERG was performed using the Ventana Benchmark System and rabbit monoclonal anti-ERG (clone 5B7, Ventana Medical Systems), as described previously (21, 22). ERG positivity was defined as diffuse, moderate, to strong nuclear immunoreactivity (21).

Targeted DNA/RNA NGS

DNA and RNA were coisolated from each specimen as described previously (23). DNA and RNA libraries were generated per sample using the Ion AmpliSeq Library Kit (Life Technologies), as described previously (23). We prepared templates for DNA and RNA libraries using the Ion PI Template OT2 200 Kit v3 on the Ion One Touch 2, and sequencing was performed on Ion Proton P1 chips using the Ion PI Sequencing 200 Kit v3 (200 base pair reads), essentially as described previously (24, 25). NGS data analysis was performed using Torrent Suite (4.2.0) and the Coverage Analysis Plug-ins (both v4.0.773765), along with the Ion Reporter (4.2.0) Targeted NGS, fusion analysis workflow and in-house–validated pipelines as described in the Supplementary Methods (24–27). A sample was classified as fusion positive if a fusion isoform was supported by ≥20 reads and ≥3.0% total mapped reads; otherwise, it was classified as fusion negative.

Statistical analysis

Demographic, relevant clinical and pathologic data of the cohort were abstracted from medical records and entered into a secure electronic HIPAA-compliant database. Biopsies were performed at UCLA and genetic studies were performed at University of Michigan (Ann Arbor, MI) with IRB approval. For each tissue sample, genetic alterations were classified as present or absent, and compared between paired “initial” and “repeat” tissue samples to assess clonality and reclassification from Gleason 6 to Gleason >6 prostate cancer. Statistical analyses were performed using R (R Foundation for Statistical Computing; http://www.R-project.org). Two-tailed statistical tests were used for all comparisons, and \( P < 0.05 \) was considered statistically significant.

Figure 1.
Targeted biopsy using MR/US fusion system. A, A lesion was identified on MRI and delineated on T2WI by radiologist (blue ellipse). The MRI was fused with real-time ultrasound images. B, Lesion was identified in sagittal and axial planes (blue enclosures), and biopsy targeting the lesion was established (parallel lines overlaying blue enclosures). C, Sites of targeted and 12-core biopsies were recorded in a 3D reconstruction, confirming that several targeted biopsies penetrated region of interest. Reprinted with permission from Natarajan et al. (11).

Figure 2.
Tracking technique for repeat sampling with Artemis device. A, The 3D model of the prostate from the second biopsy (brown) was superimposed on the model from the first biopsy (blue), showing a close match in size and shape. The models were created in real time at biopsy by the Artemis device. An MRI target (red) was displayed in the model. B, The location of prior positive sites (1 and 2) was mapped by the device (green dots). Site 1 was a systematic site; site 2 was from the MRI-targeted core. C, Four cores (black cylinders) were taken from each site. 3D, 3-dimensional. Reprinted with permission from Sonn et al. (9).
Results

Of the 275 men on AS for prostate cancer during the study period, 31 met eligibility criteria of whom 26 (84%) had cancer present on initial and repeat biopsy sections in quantities sufficient for ERG staining. Concordant ERG status in initial and repeat biopsies was observed in 25 of 26 (96%) patients, with 10 (38%), 15 (58%), and 1 (4%) patient demonstrating concordant ERG+/+ and discordant ERG+/ERG−/ERG− paired biopsies, respectively (Fig. 3 and Supplementary Fig. S1). Our observation of 96% concordance is highly significant compared with that expected by chance [expected: 50% (13/26) concordant (ERG+/+ and ERG−−/ERG−−); observed: 96% (25/26) concordant (ERG+/+ and ERG−−/ERG−−); P = 0.0003]. RNA sequencing (RNA-seq) using the Oncomine Comprehensive Panel (OCP), which targets recurrent cancer gene fusions (including all known 5' and 3' partners in prostate cancer ETS gene fusions), was evaluable in paired initial and repeat specimens from 13 patients. Concordant ETS fusion status in initial and repeat samples was present in 12 of 13 (92%) patients, and RNA-seq results were consistent with ERG IHC status in all samples with evaluable staining. Of interest, patient 29, who did not show grade progression on repeat biopsy, had ERG− and ERG+ cancer by IHC on initial and repeat biopsy, respectively. RNA-seq identified a TMPRSS2:ETV1 fusion in the ERG−/C0 initial biopsy sample and a TMPRSS2:ERG fusion in the ERG+ repeat biopsy sample (Table 2), supporting the sampling of two distinct clonal foci in this case.

In addition to ETS gene fusions, SPOP and IDH1 mutations are early driving molecular alterations in prostate cancer and define molecular subtypes (~10% and 1% of all prostate cancers, respectively) that are mutually exclusive with ERG gene fusions (24, 28, 29). Both alterations are targeted by the DNA component of the OCP; thus, we assessed for SPOP and IDH1 mutation status as clonal markers in serial samples. In patient 4, who showed grade progression on repeat biopsy and was ERG+ on initial (IHC/RNAseq−) and repeat biopsy (IHC+/RNAseq+), we identified a high-confidence prioritized SPOP F133L mutation by targeted DNA sequencing (DNA-seq) exclusively in the repeat biopsy specimen [3/670 reads (0.4%) in the initial sample vs. 111/864 reads (13%) in the repeat sample], consistent with serial sampling of two clonally distinct foci. In contrast, patient 22, who did not show grade progression and was ERG− on initial and repeat biopsy (both IHC+/RNAseq−), harbored high-confidence prioritized SPOP F125V mutations by targeted DNA-seq in both serial samples [7/124 reads (6%) in the initial sample vs. 20/264 reads (8%) in the repeat sample]. Similarly, patient 23, who also progressed to Gleason score 3+4=7 on repeat biopsy and was ERG− on initial and repeat biopsy (both IHC+/RNAseq−), harbored high-confidence prioritized IDH1 R132C mutations by targeted DNA-seq in both serial samples [128/400 reads (32%)]...
in the initial sample vs. 85/448 reads (19%) in the repeat sample. IDH1 R132H mutations were confirmed in both samples by Sanger sequencing (data not shown). Concordant IDH1 mutations in this case with grade progression support a clonal relationship between serially sampled low- and high-grade cancer components. Taken together, through IHC and targeted RNA-seq/DNA-seq, our data support MRI/US fusion as being able to sample the same prostate cancer focus over time.

Overall, of the 26 evaluable cases, nine (34.6%) progressed to high-grade disease. Repeat biopsies, however, showed only a small focus (≤10%) of a higher Gleason grade component in three of the nine cases. All nine cases (100%) that progressed to high-grade disease demonstrated ERG concordance between the initial and repeat biopsies, strongly suggesting that these high-grade cancers shared a clonal relationship with their low-grade counterparts.

### Discussion

In this study, we demonstrate, using electronic biopsy site tracking and ERG IHC status as a clonal marker, that a clonal focus of prostate cancer may be serially sampled over a median interval of 1 year. In men on AS, a 96% ERG status concordance between paired biopsies obtained from the same location over time was found using MRI/US fusion biopsy guidance. This finding strongly suggests that the tissues assayed at two different time points were of the same clonal origin (18). In addition, the results also provide support for the notion that high-grade prostate cancer (i.e., ≥Gleason 7) may arise clonally from Gleason 6 disease and further implies that some Gleason 6 prostate cancer may not be indolent.

Effective AS rests on accurate patient selection and the ability to precisely detect changes in disease status over time. Results from our study, and others, suggest that MRI/US fusion-guided biopsy facilitates both (14). It is not uncommon to obtain a negative TRUS-guided prostate needle biopsy during AS, even when biopsies are cognitively guided toward areas of presumed disease (14). One explanation is the lack of precision of TRUS in tracking and locating areas of previously diagnosed prostate cancer. In prior work, the precision of MRI/US fusion biopsy for resampling areas to within 3 mm was confirmed in phantom models and validated in a patient cohort (9, 11). Clinical validation in this study was assessed by targeting the same site as determined by repetitive 3D modeling during the same biopsy accession. Furthermore, a recent report by Ukimura and colleagues, employing a commercial cell cycle–based gene expression signature interrogating biopsy samples assayed 1 year apart, suggested a same site biopsy precision of 86% but could not determine clonality (30). In the current study, we present molecular data to clearly show that MRI/US-targeted biopsy can serially sample the same clonal focus of prostate cancer.

At present, it is unclear whether high-grade prostate cancers arise de novo or whether Gleason 6 cancers possess the biological potential for high-grade progression. In a recent epidemiologic study, Penney and colleagues concluded that Gleason grade progression of prostate cancer is uncommon (31). Similar to other reports, the evidence presented stems from the decline in advanced stage disease in the PSA era compared with the pre-PSA era, without a corresponding decrease in the proportion of high-grade disease across the same time period (31–33). Such analyses, however, are unable to evaluate the possibility of grade progression on an individual level. In a cross-sectional study evaluating the clonal origin of Gleason grades 3 and 4 cancer, Sowalsky and colleagues examined adjacent foci of disease (16). The authors reported 100% concordance for the TMPRSS2:ERG gene fusion and identical TMPRSS2:ERG fusion breakpoints in selected cases, suggesting a common clonal origin between contiguous areas of cancer. In another analysis of multifocal prostate cancer with metastasis, VanderWeele and colleagues concluded that (i) a single progenitor can give rise to both low- and high-grade disease; (ii) early divergence occurs between low- and high-grade foci; and (iii) late divergence occurs between high-grade foci and metastases. Although both studies suggest that high-grade disease...

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**Table 2. Pathologic and genetic profile of the biopsy samples exhibiting prioritized genetic alterations**

<table>
<thead>
<tr>
<th>ID</th>
<th>ERG status</th>
<th>Gleason score</th>
<th>Prioritized mutations</th>
<th>Variant allele frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>Repeat</td>
<td>Gene</td>
<td>Mutation</td>
</tr>
<tr>
<td>1</td>
<td>+</td>
<td>+</td>
<td>SPEN</td>
<td>P9284L</td>
</tr>
<tr>
<td>4</td>
<td>–</td>
<td>N/A</td>
<td>SPOP</td>
<td>F133L</td>
</tr>
<tr>
<td>7</td>
<td>–</td>
<td>–</td>
<td>BRCA2</td>
<td>K2524fs</td>
</tr>
<tr>
<td>8</td>
<td>+</td>
<td>+</td>
<td>NOTCH1</td>
<td>P743S</td>
</tr>
<tr>
<td>9</td>
<td>–</td>
<td>N/A</td>
<td>ZC3H5</td>
<td>N55H</td>
</tr>
<tr>
<td>18</td>
<td>–</td>
<td>6</td>
<td>TP53</td>
<td>R196P</td>
</tr>
<tr>
<td>25</td>
<td>+</td>
<td>+</td>
<td>PIK3CA</td>
<td>V344M</td>
</tr>
<tr>
<td>27</td>
<td>+</td>
<td>+</td>
<td>APC</td>
<td>A1718V</td>
</tr>
<tr>
<td>28</td>
<td>+</td>
<td>+</td>
<td>SPOP</td>
<td>F125V</td>
</tr>
<tr>
<td>29</td>
<td>–</td>
<td>+</td>
<td>NOTCH1</td>
<td>P168S</td>
</tr>
</tbody>
</table>

**Note:** ERG status (by IHC) and Gleason score of profiled initial and repeat biopsies is indicated. The variant allele frequencies (in %) of high-confidence somatic mutations identified in initial and/or repeat biopsies is given (bold indicates detected).

Abbreviations: ID, patient identification; N/A, insufficient sample for analysis.
may arise from low-grade lesions, neither study can ascertain the temporal progression of high-grade disease (34).

The ability to longitudinally assess the same focus of prostate cancer over time has only recently been made possible by MRI/US fusion biopsy platforms. In our cohort using MRI/US fusion biopsy with longitudinal sampling, we found that 100% of cases that progressed from Gleason 6 to $\geq 7$ cancer demonstrated concordance for ERG status. These data obtained via molecular profiling are the first to show that high-grade disease may arise clonally from Gleason 6 prostate cancer over time. A notable corollary to this is the notion that some Gleason 6 cancers may not be indolent and should be followed carefully. A case report of a lethal clone arising from Gleason pattern 3 (although in the presence of additional distinct large high-grade tumors) is in line with this and suggests that heterogeneity may also exist within low-grade lesions viz-a-viz aggressive potential (35). More work is needed to discern the molecular profile of Gleason 6 prostate cancer destined to progress.

Our study has several limitations. First, repeat biopsy was performed after 5 to 35 months based on routine clinical practice, with the ideal time to track grade progression unknown. Second, as fusion biopsy technology continues to accumulate, the reproducibility of our findings with more generalized use of MRI/US fusion biopsy technology needs to be evaluated. Third, our cohort is relatively small, and our findings should be tested in larger populations. And fourth, an element of sampling bias remains an important confounder of our study. We cannot say with certainty that high-grade lesions did not exist at the time of initial biopsy where low-grade lesions were observed. As only a small focus of high-grade component was found in some repeat biopsies, initially, nonsampled high-grade lesions that were present in close continuity with low-grade lesions might have given us the false impression of grade progression (Supplementary Fig. S1). Future studies that aim to additionally sample areas around targeted biopsy sites, at high density, may address this issue.

The strength of this study lies in its longitudinal nature and the performance of contemporary molecular techniques on minute FFPE biopsy samples. The current findings provide molecular data to support the clinical use of MRI/US fusion biopsy platforms in the management of men on AS. In addition, our results add to our understanding of the biology of low-grade prostate cancer and suggest that some Gleason 6 cancers may not be indolent. If these findings are confirmed, MRI/US fusion biopsy may become a new standard for monitoring the growing number of men on AS. Additional work is needed to confirm our findings and to develop genomic predictors of Gleason 6 prostate cancer progression.

**Conclusions**

In this study, we demonstrate that serial MRI/US-targeted prostate biopsy allows accurate assessment of the same clonal focus of cancer over time, even in the absence of an MRI target. Molecular profiling of tissue obtained in a longitudinal fashion suggests that Gleason $\geq 7$ prostate cancer may arise clonally from Gleason 6 disease. These findings may have significant impact on the clinical management of the growing number of men with low-grade prostate cancer being managed with AS. Larger studies are needed to validate our findings and to definitively determine the risk of low-grade prostate cancer progression.

**Disclosure of Potential Conflicts of Interest**

D.H. Hovelson reports receiving travel support from Thermo Fisher. T.M. Morgan reports receiving commercial research grants from and is a consultant/ advisory board member for Myriad Genetics. S.A. Tomlins is an employee of Strata Oncology; reports receiving commercial research grants from Astellas and Thermo Fisher; holds a patent on ETS gene fusion licensed to Hologic/Gen-Probe and Ventana Medical Systems, is a consultant/advisory board member for Abbvie, Astellas/Medivation, Janssen, and Ventana Medical Systems; and reports receiving travel support from Thermo Fisher. No potential conflicts of interest were disclosed by the other authors.

**Disclaimer**

The content is solely the responsibility of the authors and does not necessarily represent the official views of the NCI or the NIH.

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Study supervision: G.S. Palapattu, S.A. Tomlins, L.S. Marks

**Grant Support**

This work was supported in part by award number R01CA158627 (to L.S. Marks) and 5 P50 CA186786-05 (to G.S. Palapattu) from the NCI. Additional support was provided by the Beckman Coulter Foundation, the Jean Perkins Foundation, and the Steven C. Gordon Family Foundation. This work was also supported in part by the Department of Defense PC130652 (to S.A. Tomlins), W81XWH-14-1-0287 (to T.M. Morgan), S.S. Salami is supported by the Urology Care Foundation. S.A. Tomlins and T.M. Morgan are supported by the A. Alfred Taubman Medical Research Institute and the Prostate Cancer Foundation. Research reported in this publication was supported by the Pilot Training Grant in Translational Research (to K.R. Vandenberg) provided by the Department of Pathology, University of Michigan.

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Received June 8, 2016; revised August 21, 2016; accepted September 11, 2016; published OnlineFirst September 11, 2016.

**References**


