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In Treating Localized Prostate Cancer the Efficacy of Cryoablation is Independent of DNA Ploidy Type

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While the prognostic value of DNA ploidy has been well established for radical prostatectomy, external beam radiation, brachytherapy and androgen deprivation therapy its role as a survival outcome predictor for prostate cancer patients treated with cryoablation has not yet been examined. Anecdotal evidence suggesting that cryoablation may be independent of DNA ploidy type led to the implementation of the current study. Retrospective analysis of data including flow digital cytometry was performed on 447 archival specimens taken from patients who had undergone cryosurgical ablation of primary prostate cancer. Five-year biochemical disease free survivals (bDFS) (defined as PSA thresholds of 0.5 and 1.0 ng/ml) were determined with Kaplan-Meier analysis. Patients were grouped according to DNA ploidy types then stratified by Gleason grade, risk group, pre-surgical PSA level, and disease stage.

Mean and median age of the cohort was 65 and 64.6 years. Mean follow-up was 65.7 months. The DNA ploidy status of the population was found to be 59% diploid, 13% tetraploid, and 28% aneuploid. Using PSA < 1.0 ng/ml criterion, the bDFS rates for diploid, tetraploid, and aneuploid were 78%, 75%, and 79% respectively. The bDFS rates using a PSA < 0.5 ng/ml criterion were 67%, 59%, and 69% for diploid, tetraploid, and aneuploid groups. No significant outcome differences were found in stratified analysis. This investigation demonstrates that the efficacy of cryoablation is independent of DNA ploidy type.

Introduction

Pretreatment PSA, pathologic stage, and Gleason score are regularly employed as prognostic measures of prostate cancer progression and survival and are used in the treatment decision process. These outcome determinants, though, are not meant to forecast the efficacy of treatment in a given individual patient (1). Within any multivariate patient group, significant dissimilarities in treatment response, time to progression, and patient survival are inevitable. Determination of deoxyribonucleic acid (DNA) DNA ploidy is a laboratory test that can differentiate localized prostate cancer with a slow, indolent course from aggressive tumors indicative of a poor prognosis and has tremendous utility as an indicator of specific treatment response (2).

Differences in DNA ploidy were first identified as a predictor of treatment outcome by Tavares and colleagues in 1966, when they observed differences in clinical course following radical surgery that distinguished patients with tetraploid and hexaploid tumors from patients with diploid tumors (3). There is substantial evidence in the literature that indicates that differences in DNA ploidy are highly predictive of differential treatment response of individual patients to radical surgery and radiation therapy (4-20). These studies found that patients with aneuploid tumor suffer from substantially worse outcomes than patients with diploid tumor on such measures as extracapsular spread, systemic spread, time to pro-

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gression, metastases-free survival and biochemical disease free status (bDFS) among patients receiving salvage radical surgery after radiation failure. The World Health Organization echoed these findings by declaring that the outcome of radiotherapy in patients with aneuploid tumor is extremely poor (21). Song and colleagues at the Mayo Clinic made substantial contributions to the body of research that validated DNA ploidy as a predictor of radical surgery and radiation therapy outcome for localized prostate cancer during the late 1980's to the mid 90's, concluding that DNA ploidy is perhaps the most reliable prognostic indicator in prostate cancer for individual patients (15). Conversely, some research have found that DNA ploidy provides little additional prognostic information that Gleason grade (22).

Targeted cryoablation of the prostate (TCAP) is an established minimally invasive treatment option for localized prostate cancer (23-25). There are, to our knowledge, no reports of the link between outcomes following TCAP and DNA ploidy. We present a retrospective analysis of patients who underwent TCAP as the primary therapy for their localized prostate cancer and whose DNA ploidy was known prior to treatment. Five-year bDFS are presented and stratified according to DNA ploidy type.

Materials and Methods

Procedure

The methodology used in cryosurgical ablation of the prostate has evolved significantly since its reintroduction by Onik *et al*. in 1993 (26). The modern procedure incorporates the following advances: 2 freeze cycles, use of US Food and Drug Administration (FDA)-approved urethral catheter warming devices in conjunction with a circulating pump, use of 6 to 8 cryoprobes rather than 5, and the use of argon-based cryomachines. As one of the first centers performing TCAP, the patients we have treated, whom we report on in this article, have experienced the evolution of the procedure. It was not until 1996 that the procedure had matured to its current state of the art. Although all patients in our series were treated with 2 complete freeze-thaw cycles using a target temperature of _40º C, the first 350 patients were treated with a liquid nitrogen cryomachine, which we have shown to yield inferior results compared with the argon-based cryomachine we now use. As well, an FDA approved warming catheter was used during all treatments.

The TCAP procedure, previously described in detail by Lee *et al*., uses 3.4-mm diameter cryoprobes (Endocare, Inc., Irvine, CA) inserted transperineally into the prostate under guidance of transrectal ultrasound (27). Temperature probes are also guided transperineally to strategic locations within and around the prostate to evaluate the extent of the freezing injury. Temperature probes are placed in the right and left neurovascular bundles and the apex of the gland to ensure that the margins of the gland reach temperatures sufficient for efficacious treatment. Temperature probes are also placed in the Denonvilliers fascia and the external sphincter and are used to minimize complications by ensuring that the sensitive anatomy adjacent to the prostate is not frozen. A urethral warming catheter is used during the procedure to maintain the integrity of the urethra by not allowing it to be frozen. Androgen ablation therapy was given to 91.5% of the sample before treatment to downsize the gland and consisted of luteinizing hormone-releasing hormone, combined with an antiandrogen agent 3 months to 1 year before cryoablation. Hormonal therapy was not continued on any patient after cryoablation unless disease progression was observed.

Digital Flow Cytometry

Biopsy samples were sent for analysis to University Laboratories, Detroit Medical Center. Scrapes of biopsies of prostatic carcinomas received in saline were centrifuged and resuspended in ICC of 50% fetal calf serum/bpmi. The cells were then fixed by the slow, dropwise addition of 3cc of cold 70% ethanol while vortexing. Samples were tightly sealed and stored at refrigerator temperature until staining.

Fixed cells were pelleted and washed twice with wash media (PBS/1%FCS). The cell pellets were incubated (room temperature in the dark) 20 minutes with 20_l of monoclonal FITC conjugated CAM 5.2 (Becton-Dickenson). Cells were washed twice and treated with RNASE (Sigma – 1_g/mL saline) for 20 minutes at 37 degrees. 500_l of propidium iodide (Sigma – 100_g/mL saline) was added to each tube and held in the cold, a minimum of 15 minutes, until analysis. Samples were filtered through a 41_ nylon mush prior to running.

Samples were run on the Becton-Dickenson FacScan digital flow cytometer. Optimal performance was documented daily using B-D's Calibrite beads and DNA QC particles, and also fixed human thymocytes that were treated with PI and RNASE each run. For each CAM stained sample, 15,000 events were acquired using a low flow rate. 15,000 CAM-positive events from the same tube were then acquired using a live gate. Accrued list-file data was then analyzed using B-D's Cellfit software for cell cycle analysis. The DNA index, synthesis phase fraction, and CV were reported.

Statistical Analysis

Kaplan Meier analysis was used to determine the five year survival rates for the three different DNA ploidy types using both definitions bDFS namely, PSA thresholds of 0.5 and 1.0 ng/ml. The populations were then sub stratified according to Gleason (< 7, 7, >7), PSA (<4, 4-10, >10), stage (T1-

Table I
Clinical characteristics of the study population.

Demographic	Total Patients N (%)	Diploid (%)	Aneuploid (%)	Tetraploid (%)
Gleason (mean 6.59, median 7)				
< 7	155 (35)	72	20	8
= 7	273 (61)	54	31	15
> 7	19 (4)	28	44	28
Risk Group				
Low	50 (11)	76	18	6
Medium	136 (30)	68	23	9
High	261 (59)	51	33	16
PSA (mean 7.62, median 6.5)				
< 4	69 (15)	58	33	9
4 to 10	268 (60)	64	24	13
> 10	110 (25)	48	35	16
Stage				
T1-T2	350 (78)	62	26	13
T3-T4	96 (22)	49	36	15

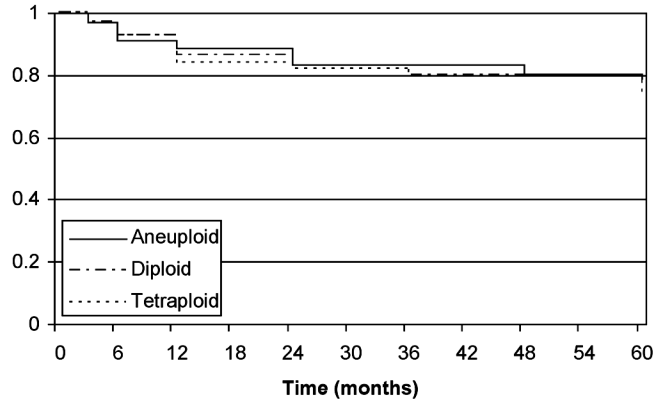

Figure 1: bDFS using a definition of PSA < 1.0 ng/ml for aneuploid, diploid and tetraploid tumor types.

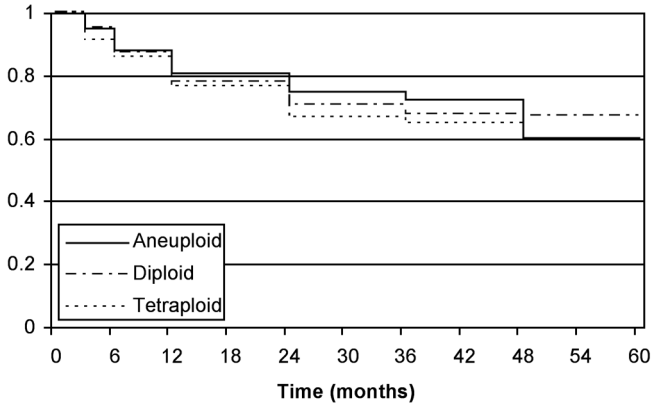

Figure 2: bDFS using a definition of PSA < 0.5 ng/ml for aneuploid, diploid and tetroploid tumor types.

2, T3-4) and risk group. Risk groups were defined using three risk factors: PSA > 10, Gleason > 6 and Stage T2b or higher. If a patient had no risk factors their disease was considered low risk, if one risk factor moderate risk and if two or three risk factors the were classified as having high risk disease. In each sub-stratification the survivals were compared for statistical significance.

Results

A consecutive series of 447 patients with known DNA ploidy type received TCAP with curative intent as a primary treatment for clinically localized prostate cancer. Patient accrual spanned from 3/1993 to 11/2001, and all analyses were performed retrospectively. The mean age of the sample was 65, with the median being 64.6. Mean follow-up time was 65.68 months, and the median follow-up time was 68.82 months. Clinical characteristics are summarized in Table I.

Digital cytometry revealed a patient population consisting of 28% aneuploids, 59% diploids, and 13% tetraploids. Kaplan-Meier analysis, using a PSA cutoff of 1.0 ng/mL, revealed rates of freedom from biochemical relapse for diploid, tetraploid, and aneuploid stratifications as 78%, 75%, and 79% respectively with a p-value of 0.9148 (Figure 1). A PSA cutoff of 0.5 ng/mL produced survival rates of 67%, 59%, and 69% for the diploid, tetraploid, and aneuploid groups with p-value 0.1492 (Figure 2).

Kaplan Meier analysis was also performed on the different DNA ploidy groups sub stratified according to Gleason, PSA, stage and risk group. These results are summarized in Table II. There were no significant differences ($p < 0.05$) between any of the sub-stratified groups.

Table II
Stratified 5-year disease-free rates by DNA ploidy group.

		bDFS	Diploid (%)	Aneuploid (%)	Tetraploid (%)
PSA	< 4	PSA < 0.5	91.5	44.4	73.0
		PSA < 1.0	97.4	66.7	82.2
	4 – 10	PSA < 0.5	66.0	59.6	76.6
		PSA < 1.0	77.7	76.2	85.0
	> 10	PSA < 0.5	54.7	63.4	55.9
		PSA < 1.0	67.1	75.1	66.7
Gleason	4 – 6	PSA < 0.5	70.1	83.3	40.0
		PSA < 1.0	83.2	91.7	90.1
	7	PSA < 0.5	66.0	62.1	66.9
		PSA < 1.0	75.7	67.2	75.5
	8 – 10	PSA < 0.5	50.0	80.0	41.7
		PSA < 1.0	50.0	80.0	75.0
Stage	T1 – 2	PSA < 0.5	68.6	62.9	77.3
		PSA < 1.0	80.1	81.1	85.1
	T3 – 4	PSA < 0.5	61.1	49.0	50.1
		PSA < 1.0	69.7	56.3	63.7
Risk group	Low	PSA < 0.5	60.7	0.0	100
		PSA < 1.0	83.7	100	100
	Moderate	PSA < 0.5	76.9	50.0	74.1
		PSA < 1.0	84.8	66.7	82.3
	High	PSA < 0.5	62.8	67.0	65.1
		PSA < 1.0	72.5	74.5	75.6

*PSA <0.5 and PSA < 1.0 definitions of bDFS.

Discussion

Tavares *et al*. (1966) was the first to report a relationship between DNA ploidy and prognosis in prostate carcinoma (3). Three important findings were noted; that survival of patients with diploid and tetraploid tumor was identical to survival of an age-matched control population of healthy adults, that these diploid and tetraploid tumors exhibited a favorable response to hormonal therapy, and that patients with aneuploid tumor exhibited poor prognosis and were resistant to hormonal therapy (21). Additionally, in cases where there is a discrepancy between tumor grade and DNA ploidy, DNA ploidy is considered to be the more valid prognostic indicator; a high grade tumor with a diploid DNA configuration will exhibit the behavior of a diploid tumor, whereas a well or moderately differentiated tumor with an aneuploid DNA configuration is likely to result is a worse prognosis than a similar diploid tumor. For tumors of identical staging and grading, the difference between diploid and aneuploid tumors varies approximately three-fold (21).

The majority of studies published on DNA ploidy analysis of prostate cancer treatment outcomes have involved radical surgery, because of the ease in ability to obtain sufficient material for analysis. Studies examining the relationship between DNA ploidy and radiation therapy outcome are fewer in number because the needle biopsy technique of assessment often yields insufficient archival tissue for analysis (15, 16).

Targeted cryoablation of the prostate appears to deliver the same efficacious treatment to those with tetraploid and aneuploid DNA ploidy type as patients with diploid tumor. The present series of 447 patients allowed a sufficient number of patients to be evaluated across most DNA ploidy by outcome parameter cells. Analysis revealed that there was no statistically significant relationship between DNA ploidy type and biochemical outcomes in patients stratified by Gleason score, PSA, stage and risk group.

There is no consensus on how to best treat localized prostate cancer. The clinical management of the disease is complicated by difficulty in predicting the malignant potential of the tumor in individual patients. Numerous studies indicate that patients with non-diploid tumor exhibit a poor response and prognosis to radical surgery, external beam radiation, interstitial I-125 brachytherapy, and hormonal therapy.

However, DNA ploidy is not usually one of the diagnostic factors considered during the treatment decision process. This is because patients with non-diploid tumors do not respond well, in comparison to those with diploid tumors, to either radiation therapy or radical prostatectomy which are the most common therapies used to treat localized prostate cancer.

This study demonstrates that the efficacy of prostate cryoablation is independent of tumor DNA ploidy. A possible explanation is the fundamental nature of the injury a tissue experiences when it is exposed to temperatures below the freezing point. The mechanisms of tissue damage during cryoablation result not only in the destruction of individual cells through membrane disruption but also the destruction of the microvasculature (29). Complete vascular stasis is well documented in tissues that have been subjected to a sufficient freezing injury. This damages the tissue as a whole and all cells within this volume will die of ischemia regardless of their individual characteristics including DNA ploidy type.

Our observation that TCAP treats non-diploid tumors as well as it does diploid tumors has led us to preferentially recommend TCAP to our patients with non-diploid tumors.

Conclusion

Targeted cryosurgical ablation treats all prostate cancer DNA ploidy types with equal efficacy, suggesting a broader indication for this therapy. Patients with non-diploid DNA tumor configurations undergoing cryoablation may expect the same favorable treatment response and prognosis as patients with diploid DNA configurations.

References and Footnotes

1. Brown, C., Sauvageot, J., Kahane, H. *et al*. Cell Proliferation and Apoptosis in Prostate Cancer – Correlation with Pathologic Stage? *Mod. Pathol. 9*, 205-209 (1996).
2. Montgomery, B. T., Nativ, O., Blute, M. L. *et al*. Stage B Prostate Andenocarcinoma. Flow Cytometric Nuclear DNA Ploidy Analysis. *Arch. Surg. 125*, 327-331 (1990).
3. Tavares, A. S., Costa, J., de Carvalho, *et al*. Tumour DNA Ploidy and Prognosis in Carcinomas of the Bladder and Prostate. *Br. J. Cancer 20*, 438-441 (1966).
4. Lerner, S. E., Blute, M. L., Zincke, H. Critical Evaluation of Salvage Surgery for Radio-recurrent/resistant Prostate Cancer. *J. Urol. 154*, 1103-1109 (1995).
5. Ross, J. S., Figge, H., Bui, H. X. *et al*. Prediction of Pathologic Stage and Post-prostatectomy Disease Recurrence by DNA Ploidy Analysis of Initial Needle Biopsy Specimens of Prostate Cancer. *Cancer 74*, 2811-2818 (1994).
6. Adolfsson, J. Prognostic Value of Deoxyribonucleic Acid Content in Prostate Cancer: A Review of Current Results. *Int. J. Cancer 58*, 211-216 (1994).
7. Winkler, H. Z., Rainwater, L. M., Myers, R. P. *et al*. Stage D1 Prostate Adenocarcinoma: Significance of Nuclear DNA Ploidy Patterns Studied by Flow Cytometry. *Mayo Clin. Proc. 63*, 103-112 (1988).
8. Stephenson, R. A., James, B. C., Gay, H. *et al*. Flow Cytometry of Prostate Cancer: Relationship of DNA Content to Survival. *Cancer Res. 47*, 2504-2507 (1987).
9. Lee, W. R., Hanks, G. E., Hanlon, A. Increasing Prostate-specific Antigen Profile Following Definitive Radiation Therapy for Localized Prostate Cancer: Clinical Observations. *J. Clin. Oncol. 15*, 230 (1997).
10. van den Ouden, D., Tribukait, B., Bloom, J. H. Deoxyribonucleic Acid DNA Ploidy of Core Biopsies and Metastatic Lymph Nodes of Prostate Cancer Patients: Impact on Time to Progression. The European Organization for Research and Treatement of Cancer Genitourinary Group. *J Urol 150* (2 Pt 1), 400-406 (1993).
11. Di Silverio, F., D'Eramo, G., Buscarini, M. *et al*. Is There Always a Role for Radical Prostatectomy in the Treatment of Localized Prostate Cancer? *Minerva Urol. Nefrol. 47*, 117-124 (1995).
12. Peters-Gee, J. M., Miles, B. J., Cerny, J. C. *et al*. Prognostic Significance of DNA Quantitation in Stage D1 Prostate Carcinoma with the Use of Image Analysis. *Cancer 70*, 1159-1165 (1992).
13. Pollack, A., Zagars, G. K., El-Naggar, A. K. *et al*. Near-diDNA Ploidy: A New Prognostic Factor for Clinically Localized Prostate Cancer Treated with External Beam Radiation Therapy. *Cancer 73*, 1895-1903 (1994).
14. O'Sullivan, D. C., Barrett, D. M., Colby, T. V. *et al*. Effect of External Beam Radiotherapy on Prostatic Carcinoma DNA Content as Measured by Static Image Cytometry. *Eur. Urol. 21*, 235-239 (1992).
15. Song, J., Cheng, W. S., Cupps, R. E. *et al*. Nuclear Deoxyribonucleic Acid Content Measured by Static Cytometry: Important Prognostic Association for Patients with Clinically Localized Prostate Carcinoma Treated by External Beam Radiotherapy. *J. Urol. 147* (3 Pt 2), 794-797 (1992).
16. Gauwitz, M. D., Pollack, A., El-Naggar, A. K. *et al*. The Prognostic Significance of DNA Ploidy in Clinically Localized Prostate Cancer Treated with Radiation Therapy. *Int. J. Radiat. Oncol. Biol. Phys. 28*, 821-828 (1994).
17. Khoo, V. S., Pollack, A., Cowen, D. *et al*. Relationship of Ki-67 Labeling Index to DNA-DNA Ploidy, S-phase Fraction, and Outcome in Prostate Cancer Treated with Radiotherapy. *Prostate 41*, 166-172 (1999).
18. Cheng, L., Sebo, T. J., Cheville, J. C. *et al*. p53 Protein Overexpression is Associated with Increased Cell Proliferation in Patients with Locally Recurrent Prostate Carcinoma after Radiation Therapy. *Cancer 85*, 1293-1299 (1999).
19. Amling, C. L., Lerner, S. E., Martin, S. K. *et al*. Deoxyribonucleic Acid DNA Ploidy and Serum Prostate Specific Antigen Predict Outcome Following Salvage Prostatectomy for Radiation Refractory Prostate Cancer. *J. Urol. 161*, 857-862 (1999).
20. Tribukait, B. Nuclear Deoxyribonucleic Acid Determination in Patients with Prostate Carcinomas: Clinical Research and Application. *Eur. Urol. 23,* 64-76 (1993).
21. Schroder, F., Tribukait, B., Bocking, A. *et al*. Clinical Utility of DNA Measurements in Prostate Carcinoma. Consensus Conference on Diagnosis and Prostatic Parameters in Localized Prostate Cancer. *Scand. J. Urol. Nephrol. 162*, 51-63 (1994).
22. Epstein, J. I., Pizov, G., Steinberg, G. D. *et al*. Correlation of Prostate Cancer Nuclear Deoxyribonucleic Acid, Size, Shape and Gleason Grade with Pathological Stage at Radical Prostatectomy. *J Urol. 148*, 87-91 (1992).
23. Donnelly, B. J., Saliken, J. C., Ernst, D. S. *et al*. Prospective Trial of Cryosurgical Ablation of the Prostate: Five-year Results. *Urology 60*, 645-649 (2002).
24. Bahn, D. K., Lee, F., Badalament, R. *et al*. Targeted Cryoablation of the Prostate: 7-year Outcomes in the Primary Treatment of Prostate Cancer. *Urology 60*, 3-11 (2002).
25. Katz, A. E., Rewcastle, J. C. The Current and Potential Role of Cryoablation as a Primary Therapy for Localized Prostate Cancer. *Curr. Oncol. Rep. 5*, 231-238 (2003).
26. Onik, G. M., Cohen, J. K., Reyes, G. D. *et al*. Transrectal Ultrasound-guided Percutaneous Radical Cryosurgical Ablation of the Prostate. *Cancer 72*, 1291-1299 (1993).
27. Lee, F., Bahn, D. K., Badalament, R. A. *et al*. Cryosurgery for Prostate Cancer: Improved Glandular Ablation by Use of 6 to 8 Cryoprobes. *Urology 54*, 135-140 (1999).
28. Borre, M., Hoyer, M., Nerstrom, B. *et al*. DNA Ploidy and Survival of Patients with Clinically Localized Prostate Cancer Treated Without Intent to Cure. *The Prostate 36*, 244-249 (1998).
29. Hoffman, N. E., Bischof, J. C. The Cryobiology of Cryosurgical Injury. *Urology 60*, 40-49 (2002).

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