

The relationship between the androgen receptor CAG repeat polymorphism length and the response to intermittent androgen suppression therapy for advanced prostate cancer

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Objective: To determine whether the duration of the off-treatment interval in patients being treated with intermittent androgen deprivation therapy can be predicted by the length of the CAG trinucleotide repeat polymorphism in the androgen receptor.

Methods: This is a companion study to a prospective randomized trial, NCIC CTG PR-7, comparing intermittent to continuous androgen deprivation therapy in men with PSA progression after radiation therapy. The duration of the first off-treatment interval was established for 76 participants randomized to the intermittent therapy arm of the trial. Androgen receptor CAG repeat polymorphism lengths were determined for these 76 participants. Statistical analysis was completed to determine a relationship between CAG repeat length and the duration of the off-treatment interval.

Results: A significant correlation was not established ($P = 0.424$) between androgen receptor CAG repeat length and the duration of the first off-treatment interval. Categorical analysis of CAG repeat lengths using 18 and 22 as cutoff points did not find any significant difference in the mean duration of the off-treatment interval between categories ($P = 0.672$ and 0.774 , respectively).

Conclusion: No relationship was established between the duration of the first off-treatment interval and the CAG repeat length.

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Background and introduction

Management of advanced or metastatic prostate cancer is based on androgen deprivation therapy. While most patients initially respond to this treatment, after a variable period of time, an androgen-independent tumour state usually develops. Intermittent androgen deprivation therapy (ADT) reduces the side effects and

costs of therapy, and may increase the time to androgen independency.

Intermittent androgen deprivation for patients with biochemical failure is currently being evaluated in a prospective international randomized phase III clinical trial, NCIC CTG (PR-7). Patients with biochemical failure following radiotherapy have been randomized to receive hormone therapy either through a continuous or intermittent schedule. The phase II experience with intermittent therapy has demonstrated marked heterogeneity in the duration of the off-treatment interval, from 3 to 60 months (median 8 months).¹

Molecular prognostic factors such as the CAG repeat polymorphisms of the androgen receptor may contribute to a more rational and individualized use of intermittent

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androgen suppression (IAS) in the treatment of advanced prostate cancer. In this translational study, we have examined the CAG trinucleotide repeat polymorphism lengths in 76 patients randomized to the intermittent arm of this study, in whom the duration of the 1st off-treatment interval is established. The hypothesis is that the duration of the off-treatment interval is correlated with the CAG repeat length.

Alterations in the androgen receptor in the prostate cancer cells of men treated with androgen deprivation therapy (ADT) are thought to be responsible, at least in part, for the development of androgen independent progression.² Of particular interest are two polymorphic trinucleotide repeats that exist within the transcription activation domain of the N-terminal region of exon 1 in the AR gene, which explain part of the variability in the molecular size of the AR. The CAG repeat has an average length of 21 repeat units in the general population, with a range from 11 to 31.³ Given that the length of the CAG repeat is inversely related to the transactivation function of the AR gene,^{4,5} it has been suggested that a shorter CAG repeat length is associated with a greater risk of developing prostate cancer, as the increased response to the proliferative signalling by androgens may facilitate conditions for malignant growth.^{6,7} The clinical evidence to support this relationship, however, is inconclusive.^{8–17}

Suzuki *et al*¹⁸ found that a *shorter* CAG repeat length predicted for a better response to endocrine therapy. In contrast, Bratt *et al*¹⁰ reported that a *long* CAG repeat length predicted for a better response to hormone therapy. No previous studies have attempted to identify a relationship between CAG repeat length and the response to intermittent androgen deprivation. This study seeks to identify if such a relationship exists using the duration of the off-treatment interval as an end point. Proof of a relationship between CAG repeat length and the duration of the off-treatment interval would help clinicians to identify patients suitable for IAS, and further clarify the general relationship between AR CAG length and the response to ADT.

Materials and methods

Study subjects

Study subjects were drawn from participants who were randomized to the IAS arm of the NCIC NTG (PR-7) trial. At the time of this study, 187 participants were randomized to the IAS arm; however, only 85 participants had completed the first cycle. Of these 85 participants, CAG repeat lengths were available on 76.

Eligibility for the NCIC NTG (PR-7) trial included having previous pelvic radiation therapy for prostate cancer either post-radical prostatectomy or as a primary management. Radiation therapy had to be completed at least 12 months prior to randomization. Patients treated with brachytherapy were eligible for the trial if the implant date was greater than 30 months prior to randomization. Men randomized to the trial had a PSA value greater than 3 ng/ml, and higher than the lowest level recorded since the end of their radiation therapy. Serum testosterone levels were required to be greater than 5 nmol/l within 28 days prior to randomization.

Any hormonal therapy participants had received must have been for a maximum of 12 months and completed at least 12 months prior to randomization. Evidence of metastatic disease was an exclusion criterion for trial entry.

Patients randomized to the IAS arm of the trial received an LHRH analog (Buserelin) and a nonsteroidal antiandrogen (Nilutamide) for 8 months. The PR-7 trial was structured according to the following treatment algorithm. After the 8 months of treatment, if a patient's PSA value was less than 4 ng/ml and less than their baseline PSA, patients were observed until their PSA rose to greater than 10 ng/ml at which time they would restart treatment for another 8 months. If a patient's PSA failed to fall below 4 ng/ml following the 8 months of treatment, or if their PSA rose above 10 ng/ml within the first 2-month interval following the 8 months of treatment, patients were converted to a continuous treatment regimen until hormone resistance developed. The duration of the off-treatment interval was calculated as the date of the first antiandrogen restarted or date of the first LHRH analog restarted (whichever was the earliest) minus the stop date of the last antiandrogen or expiry date of the last LHRH analog (whichever was the latest).

Prior to randomization, a complete history and physical examination was completed, as was a haematology and biochemical workup including serum PSA and testosterone measurements. Chest X-rays and bone scans were completed prior to randomization to identify and exclude any patients with metastatic activity. Serum PSA and testosterone measurements were collected every 2 months for follow-up. Blood samples were also taken from all participants prior to the start of treatment for RT-PCR testing.

Molecular analysis

DNA was extracted from peripheral blood leukocytes using standard protocols. The AR exon 1 CAG trinucleotide repeat was amplified by a nested PCR protocol using outside primers (forward primer 5'-GTGGAAGATT CAGCCAAGCT-3' and reverse primer 5'-TTGCTGT TCCTCATCCAGGA-3') and inside primers (forward primer 5'-CCCGGCCAGTTTGCTGCTG-3' labelled with cyanine-5; reverse primer is the same as the first PCR). The final products were analysed by electrophoresis on 6% denaturing polyacrylamide gels. Following direct sequencing PCR products, the CAG repeats were calculated from the size of the predominant PCR product in relation to a series of previously determined CAG size standards.

Data analysis

Univariate descriptive analyses were conducted to determine means, ranges, and frequency distributions for all variables in the data set. CAG repeat length and time to retreatment were first considered as continuous variables, and Pearson, Spearman, and Kendall's tau-b correlation coefficients were calculated to determine if a significant relationship exists between these two variables. The same correlation coefficients were also calculated for other variables and their relation to time

to retreatment, including baseline PSA and total testosterone levels, age at randomization, the time passed in months from the subject's last radiation treatment to randomization in the trial, and PSA and total testosterone levels at the start of their second cycle of therapy. Univariate and multivariate regression analysis was also conducted to examine the relationship between CAG repeat length and time to retreatment. Multivariate regression was performed in a stepwise forward method, considering baseline PSA and total testosterone levels, age at randomization, and time from last radiation to randomization as covariables.

The relationship between CAG repeat length and time to retreatment was then analysed categorically. Subjects were divided into two groups, the first with CAG repeat lengths ≤ 18 repeat units, and the second with CAG repeat lengths ≥ 19 repeat units. One-way analysis of variance of the mean time to retreatment for each group was then conducted. A second one-way variance analysis was completed, but with subjects instead being categorized according to a CAG repeat length ≤ 22 or ≥ 23 repeat units. Among published literature, both 18 and 22 repeat units have been used as cutoff definitions for short CAG repeat length with which significant relationships have been found with risk of developing prostate cancer, earlier age of diagnosis, and cancer aggressiveness.^{5,9,17} Thus, similar CAG repeat lengths cutoff points were used in this study.

All significance tests were two-tailed, using a *P*-value less than or equal to 0.05 as significant. All statistical analyses were conducted with SPSS software version 10.0 (Chicago, IL, USA).

Results

The mean and median length of the CAG repeats in this study was 21.78 and 21 units, with a range from 14 to 27, and a standard deviation of 2.8. A frequency distribution of CAG repeat lengths can be seen in Figure 1. The mean and median duration of the off-treatment interval was 18.1 and 17.84 months, with a large range from 0.03 to 41.27 months. The mean and median age of study subjects at the time of randomization was 73.2 and 74.3 y with a range from 54 to 83 years, and a standard deviation of 6.2. The mean and median time passed from the last radiation treatment to randomization in the study and first hormone therapy treatment was 49.76 months and 49.28 ± 24.3 , with a large range from 12.7 to 134.5 months (Table 1).

CAG repeat length and duration of the off-treatment interval

Pearson, Kendall's tau-b, and Spearman correlation tests did not identify a statistically significant relationship between these two variables ($P = 0.424, 0.208, 0.226$, respectively; Table 2). A weak but nonsignificant positive correlation was seen. The nonsignificance may reflect lack of power of the study, or absence of a true correlation. Other variables tested for association with the duration of the off-treatment interval (Table 2) were also all found to be nonsignificant. Univariate and multivariate regression analysis did not identify a significant relationship between CAG repeat length and the duration of the off-treatment interval, nor were baseline PSA, baseline total testosterone, age at randomization, or time from last radiation to randomization found to have significant associations with time to retreatment.

Categorical analysis of CAG repeat length and the association with the duration of the off-treatment interval showed no difference. The duration of the off-treatment interval for patients with a CAG repeat length ≤ 18 and ≥ 19 was 17.17 and 18.25 months, respectively. One-way analysis of variance did not find this difference to be significant. Using the median CAG repeat length of 22 as the cut point, the off-treatment interval was 17.89 vs 18.39 months for CAG lengths less than or equal to 22, and greater than 22, respectively. One-way analysis of variance did not find this difference to be significant (Table 3).

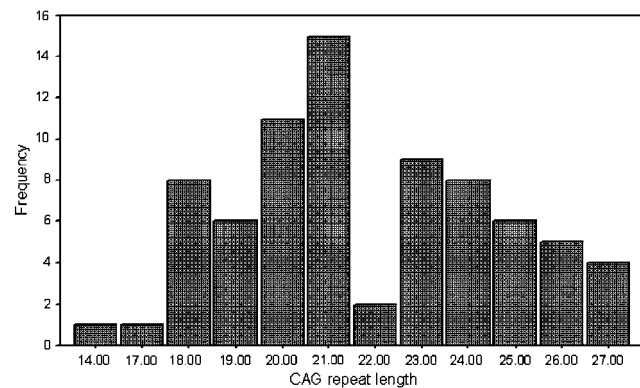


Figure 1 Frequency distribution of CAG repeat lengths, n = 76.

Table 1 Minimum, maximum, mean and standard deviations for data variables

	N	Minimum	Maximum	Median	Mean	s.d.
CAG repeat length	76	14.00	27.00	21.00	21.78	2.83
Duration of off-treatment interval (months)	76	0.03	41.27	17.84	18.10	7.41
Time from radiation to randomization (months)	76	12.65	134.54	49.28	49.76	24.31
Baseline PSA	76	6.90	118.00	13.66	17.85	15.71
Baseline total testosterone	74	7.30	36.40	12.45	14.66	5.65
PSA at restart of treatment	75	1.00	29.96	11.70	11.83	4.42
Total testosterone at restart of treatment	71	0.30	38.20	8.20	10.67	7.56
Age (y)	76	54.00	83.00	74.30	73.23	6.22

Table 2 Correlation coefficients and significance for time to retreatment, CAG repeat length, and other variables

Variable		Time to retreatment		
		Pearson	Kendall's Tau-b	Spearman
CAG repeat length	Correlation coefficient	0.093	0.103	0.140
	Sig. (two-tailed)	0.424	0.208	0.226
Time from radiation to randomization (months)	Correlation coefficient	0.092	0.077	0.119
	Sig. (two-tailed)	0.428	0.328	0.307
Baseline PSA	Correlation coefficient	-0.056	-0.088	-0.143
	Sig. (two-tailed)	0.630	0.262	0.219
PSA at retreatment	Correlation coefficient	0.121	-0.066	-0.060
	Sig. (two-tailed)	0.302	0.402	0.612
Baseline total testosterone	Correlation coefficient	-0.015	-0.110	-0.159
	Sig. (two-tailed)	0.902	0.168	0.176
Total testosterone at retreatment	Correlation coefficient	0.123	0.079	0.118
	Sig. (two-tailed)	0.306	0.328	0.326
Age at randomization	Correlation coefficient	-0.092	-0.056	-0.075
	Sig. (two-tailed)	0.429	0.479	0.517

Table 3 Average time to retreatment and significance of one-way variance analysis for subjects categorized by CAG repeat length, first using 18 repeat units as a cutoff, and then again using 22 repeat units as a cutoff

CAG repeat length	N	Mean	P-value
<i>Categorized as</i>			
≤ 18 repeat units	10	17.17	0.672
≥ 19 repeat units	66	18.25	
<i>Categorized as</i>			
≤ 22 repeat units	44	17.89	0.774
≥ 23 repeat units	32	18.39	

Discussion

The proliferative response of prostate cancer cells to androgens is related to CAG repeat length. An inverse relationship exists between CAG repeat length and the activity of the androgen receptor, in that a short CAG repeat length translates to higher transactivation of the androgen receptor and proliferation of prostate cancer cells. Clinically, evidence to support this inverse relationship is inconclusive. African-American men have been found to have on average, shorter CAG repeat lengths and a higher risk of prostate cancer. Caucasian and Asian men tend on average, to have longer CAG repeat lengths and a lower risk of developing prostate cancer.^{8,9} Short CAG repeat length has also been associated with younger age at diagnosis,^{10,11} and increased risk of developing prostate cancer.^{9,12,13} Giovannucci *et al*,⁹ and Stanford *et al*,¹⁴ have also described an association between CAG repeat length and prostate cancer aggressiveness. In contrast, a number of studies have not found an association between CAG repeat length and overall risk of prostate cancer, age of diagnosis, or aggressiveness.^{8,10,13,15-17} This discrepancy in the significance of the CAG repeat length as a predictor for prostate cancer behaviour may reflect the limited number of studies and

small patient numbers, differences in patient selection between studies, technical differences in evaluating the CAG length, or a combination of factors.

Standard treatment in advanced prostate cancer involves ADT to halt tumour progression. However, over time the tumour becomes androgen-independent and progresses further. It has been suggested that cells with a short CAG repeat length divide more rapidly and therefore are more likely to accumulate genetic alterations that lead to androgen-independence.^{7,19}

In prostate cancer patients treated with IAS, the duration of the off-treatment interval is highly variable. In most patients, the testosterone recovery occurs fairly quickly, while the PSA recovery lags behind. The rate of PSA recovery clearly is a function of the interaction between the androgen response elements in the genome and the AR-mediated transactivation. It seems likely that this interval would also be related, in part, to cellular responsiveness to androgens, and therefore be a function of the CAG length. The ability to predict the off-treatment duration would be clinically useful. In particular, patients who could be predicted to have a short off-treatment interval would not be offered this approach.

Suzuki *et al*¹⁸ and Bratt *et al*¹⁰ both established relationships between CAG repeat length and a positive marker response (i.e. slower PSA progression) during endocrine therapy. In contrast, Hardy *et al*¹¹ found no association between CAG repeat length and response to continuous hormonal therapy. In the PR-7 trial of IAS, biochemical progression served as the indication for receiving the next cycle of treatment. Therefore, examining the relationship between CAG repeat length and duration of the off-treatment interval in IAS is a means to test the relationship between CAG length and the response to ADT.

In this study, no relationship existed between the duration of the off-treatment interval and the CAG repeat length.

The lack of correlation between CAG repeat length and the duration of the off-treatment interval may reflect the modest size of this study (76 participants) resulting in a lack of statistical power. Furthermore, the duration of the off-treatment interval may also be a function of other factors not examined in this study, such as the rate of testosterone recovery during the off-treatment interval. We are currently examining the value of clinical and pathologic parameters in predicting the off-treatment duration, including base line PSA, pre-treatment PSA doubling time, and Gleason grade.

IAS therapy remains investigational. Current ongoing randomized prospective trials, including the PR-7 trial that this study is a companion to, will provide definitive information as to the benefits of IAS over the next 5–7 years. A means to predict the duration of prolonged PSA suppression after a short course of ADT would be useful, but the CAG repeat length does not appear to do this.

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