Neuroendocrine prostate cancer (NEPC)

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INTRODUCTION

Neuroendocrine prostate cancer (NEPC) is an aggressive variant of prostate cancer that often occurs as a result of hormonal therapy for prostate adenocarcinoma (PCA). Histologically, NEPC can be identified by the observance of small round blue neuroendocrine cells. Previous studies have shown that alisertib could potentially benefit patients with NEPC: In a lab, alisertib was found to block the interaction between N-myc and Aurora-A and thereby suppressing tumor growth. Our study will further explore the effect of alisertib in the treatment of NEPC. We are primarily interested in exploring if there is a difference in three-month progression-free survival for NEPC and non-NEPC patients who are treated with alisertab. In addition, we will identify clinical and molecular characteristics that are associated with NEPC status and three-month progression-free survival status.

STUDY COHORT

Our study includes 60 men treated with alisertib 50 mg twice daily for 7 days every 21 days. The men eligible for the study must have metastatic prostate cancer. Patients obtained pretreatment biopsies to evaluate the entire exome and RNA-seq in order to confirm any gene abnormalities. Therefore, the available data includes for this study includes clinical and molecular profiles of patients. Patients were followed up for three months to confirm disease progression.

RESULTS

Description of study cohort

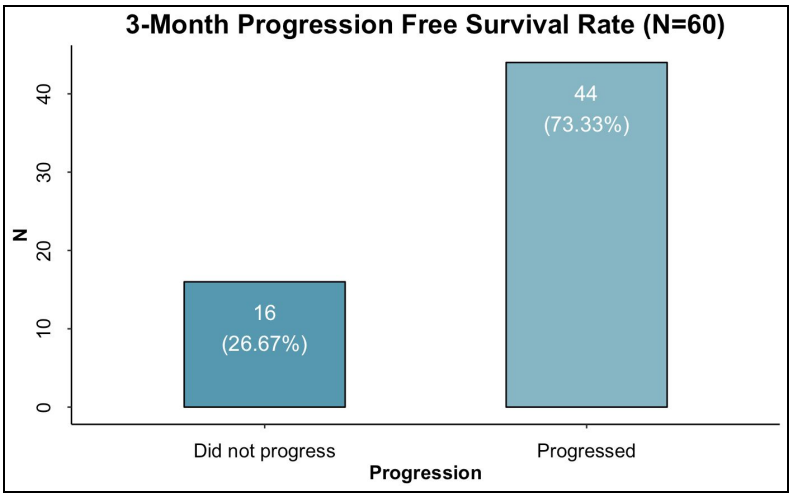
Our study cohort has 60 men who were previously treated with alisertib. Each observation has a unique subject identification number. Specific information that were collected include the Gleason score at baseline (ranges from 1-10), PSA at baseline (ng/mL), number of prior systemic therapies (integer), NEPC status (Yes or No), time from initial diagnosis to study start (months), visceral disease status (Yes or No), elevated LDH level at baseline (Yes or No), disease progression status at three months (Yes or No). Each patient was also tested for any genetic abnormalities (Yes or No) for the following mutations: RB1, TP53, PTEN, BRCA2, AR, AURKA and MYCN.

There are missing values in our data. We do not have a record of Gleason score for 15 of our observations and we do not have a record of time from initial diagnosis to study start and elevated LDH level at baseline for 3 of our observations. In addition, we are missing values for all gene abnormalities for 16 of our observations.

What is the observed 3-month progression free survival rate for men with metastatic prostate cancer treated with alisertib?

Our primary outcome of interest is three-month progression free survival rate. Of the 60 men in our study cohort, 16 men or 27% (95% CI 0.16 - 0.40) reported a status of progression at three months (Figure 1).

Figure 1. *Three-month progression free survival rate for men with metastatic prostate cancer treated with alisertib*

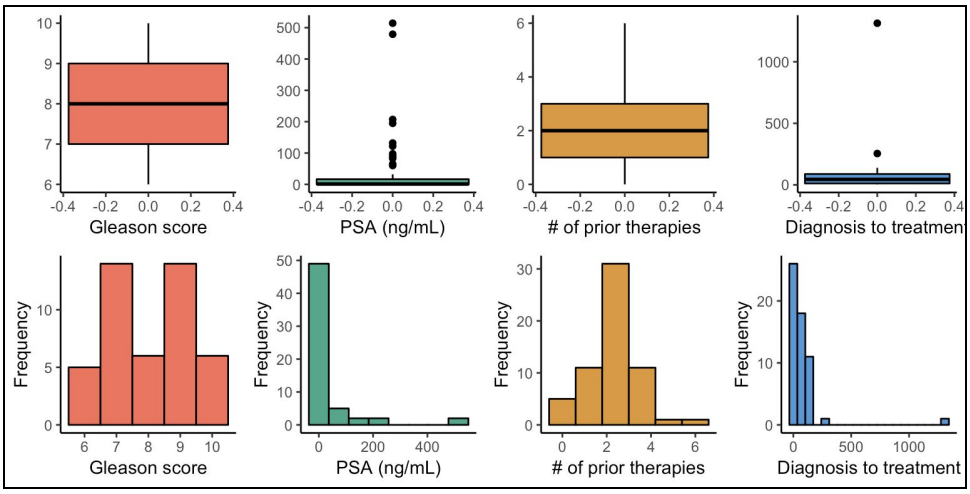


What variables are associated with three-month progression status?

We determined if there is association between three-month progression status for patients treated with alisertib and clinical variables in our dataset.

Before testing the association, we first plotted the distributions of PSA and month(s) from initial diagnosis to study start (Figure 2). We observe that the distributions are both right-skewed and have outliers. Because we believe this violates the assumptions of normality for a two-sample t-test, we instead employ the Wilcoxon rank-sum test to determine if there is association. In contrast, gleason score and number of prior therapies are relatively less skewed so we will use the two sample t-test to test the association with the three-month progression status (Figure 2). For NEPC status, visceral disease and abnormal LDH status, we use the chi-square test to determine if there is association with three-month progression status. We examined the expected value tables for the variables and chose to use the chi-square test because it satisfies the requirement that all cells have a value greater than five. Overall, the median PSA level is 1.075 ng/ml (Min-Max: 0.008-514.2 ng/ml), the median Gleason score is 8 (Min-Max: 6-10) and the median number of prior therapies is 2 (Min-Max:0-6) and the mean number of months from initial diagnosis to starting alisertib treatment is 44 months (4-1313 months).

Figure 2. *Boxplots and histograms of Gleason score, PSA level, number of prior cancer therapies, and number of months from initial diagnosis to treatment*



At a significance level of α=0.05, we do not find a significant difference between the variables explained above and three-month progression status (p-values > 0.05) (Appendix Table 1). This is additionally supported by the reported 95% confidence interval because all of the confidence intervals contain the number 0 (Appendix Table 3).

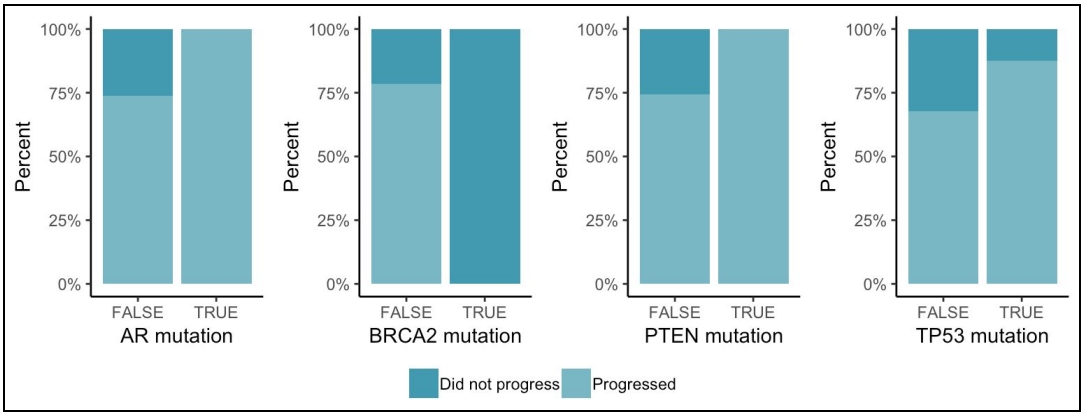
Is there an association between gene abnormalities and 3 month progression status?

We are additionally interested in defining any gene abnormalities that may be associated with three month progression status for patients treated with alisertib. There are six genetic mutations recorded in our data. As previously described, genetic mutation information was available for only 44 out of the 60 patients in our cohort. For our available data, no patients were profiled to have AURKA, MYCN or the RB1 mutation. Therefore, we were unable to test the association between AURKA, MYCN and RB1 mutations and three month progression status.

For the TP53 mutation, of those who had the mutation, 2 out of 16 (13%) of the patients did not progress and of those who did not have the mutation, 9 out of 28 (32%) did not progress. Only one patient had the PTEN mutation, and that patient did have disease progression at three months (0%). For the BRCA2 mutation, of those who had the mutation, 2 out of 2 (100%) of patients did not show disease progression at three months, and of those who did not have the mutation, 9 out of 42 (21%) did not have disease progression at three months. For the AR mutation, of those who had the mutation, 0 out of 2 (0%) of patients did not show disease progression at three months, and of those who did not have the mutation, 11 out of 42 (26%) did not show disease progression at three months. To visualize proportions of three-month progression by gene abnormalities, we created stacked bar plots (Figure 3).

To test the association for the TP53, PTEN, BRCA2, and AR mutations, we employ the Fisher's exact test to test the difference in two proportions of gene abnormality status and three-month disease progression. We chose the Fisher's exact test, instead of a chi-square test for contingency table because we have a small sample size and all the expected tables have at least one cell that has a value less than 5. For each of the included genetic mutations, we hypothesize that there is no association between gene mutation status and progression at of disease at three months. As seen in Table 1, we find that at a significance level of α=0.05, we find that there is not significant association between gene abnormalities and three-month disease progression (p-values > 0.05). This is additionally supported by the reported odds ratio 95% confidence interval because they all contain the 1 (Appendix Table 3).

Figure 3. *Stacked bar graphs of genetic abnormality status by three-month progression status*



Are there different gene abnormalities and other variables associated with NEPC?

We explore if there are any patient characteristics or gene abnormalities associated with NEPC status. Overall in our cohort, we had 38 patients (63%) with NEPC and 22 patients (37%) with non-NEPC metastatic prostate cancer.

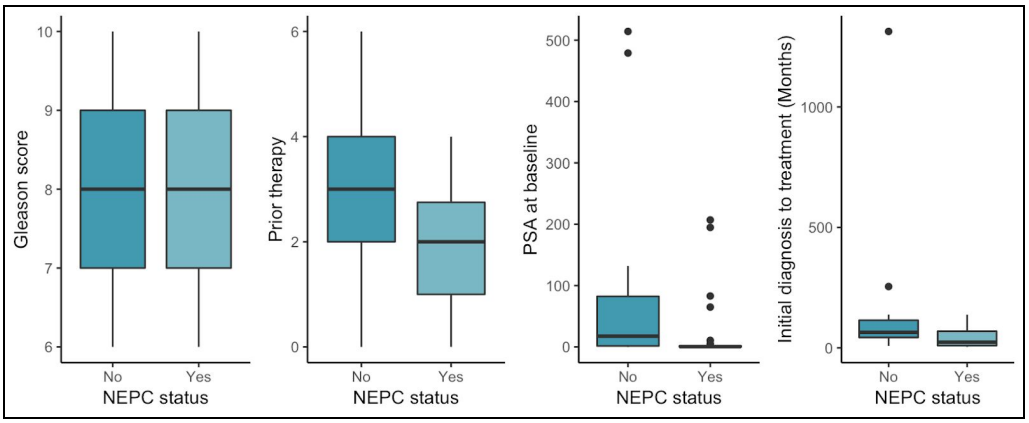
Clinical characteristics

To test the association between NEPC and PSA and month(s) from initial diagnosis to study start, we employ the Wilcoxon rank sum test for the same justifications as stated above (Figure 3). We use a two sample t-test to test the association between variable Gleason score and the number of prior therapies. For the variables indicating visceral disease, abnormal LDH and three month progression status, we use a chi-square test to determine if there is association between NEPC with each variable.

For each variable, we hypothesized that there is no association between NEPC and each variable. At a significance level of α=0.05, we find a statistically significant difference between groups in average PSA level and in average time from initial diagnosis to study start, patients in the non-NEPC group (74.2 ± 142.9) have a higher average PSA level than the patients in the NEPC group (15.5 ± 47.4) (Appendix Table 2). For the months from initial diagnosis to study start, patients in the non-NEPC group (144.9 ± 288.9) tend to have less time between initial diagnosis to study start than patients in the NEPC group (43.7 ± 41.9).

For variables including number of prior systemic therapies, gleason score, visceral disease status, abnormal LDH status, three-month progression status, the difference between NEPC and non-NEPC groups was not significant at a significance level of 0.05 (Appendix Table 2). Therefore, we have no evidence that the NEPC and non-NEPC groups differ in terms of gleason score, visceral disease status and abnormal LDH status. We also did not observe a difference in the three-month progression status between the NEPC and non-NEPC groups.

Figure 3. *Boxplots of Gleason score, PSA level, number of prior cancer therapies, and number of months from initial diagnosis to treatment by NEPC status*



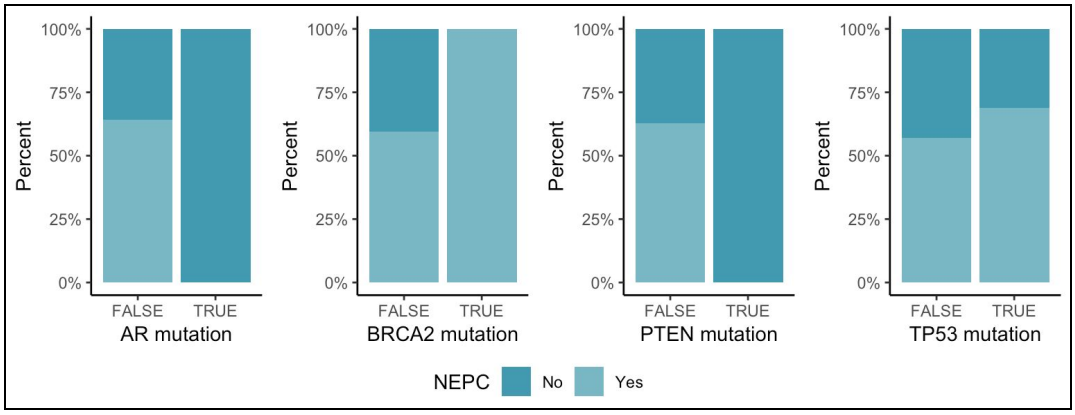
Gene abnormalities

For our available data, no patients were profiled to have RB1, AURKA, and MYCN mutation for both NEPC and non- NEPC groups. Thus we were unable to test the association between RB1, AURKA, and MYCN mutation and NEPC.

To test the association between NEPC and gene abnormalities, we employed a chi-squared test to test the association for TP53 mutation and NEPC status and we utilized the Fisher's exact test to test the association between NEPC with PTEN mutation, BRCA2 mutation and AR mutation because the expected tables have at least one cell that has a value less than five.

For each variable, we hypothesized that there is no association between NEPC and each mutation. At the 0.05 significance level, we find there is no significant association between NEPC and gene abnormalities (Table 2).
Of the 16 patients with the TP53 mutation, there are 11 patients in the NEPC group (68%) and of the 28 patients without the TP53 mutation, there are 16 patients in the NEPC group (57%). Of the 1 patient with the PTEN mutation, there are no patients (0%) in the NEPC group and among the 43 patients without the PTEN mutation, 27 patients (63%) are in the NEPC group. For the 2 patients with BRCA2 mutation, both are in the NEPC group (100%) and for the 42 patients without the BRCA2 mutation there are 25 patients in the NEPC group (60%). For the 2 patients with the AR mutation, none are in the NEPC group (0%) and for the 42 patients with the AR mutation, there are 25 in the NEPC group (60%). These proportions are depicted in Figure 4 below.

Figure 4. Stacked bar graphs of genetic abnormality status by NEPC status



CONCLUSION

Overall, our sample contains patients with severe and advanced prostate cancer. In summary, we find no difference in the proportions of three-month progression status between patients in the NEPC and non-NEPC groups treated with alisertib. Similarly, we found no association between three-month progression-free survival for all clinical characteristics and gene abnormalities. However, we did find an association between PSA level and prior systemic therapies between the NEPC and non-NEPC groups.

Overall, our study had many limitations including small sample size and many missing values for the gene abnormalities status; a full-scale efficacy trial might needed to further stimulate research on NEPC and treatment with alisertib. To ensure that there are cases of specific genetic abnormalities in the next sample, recruitment should require pre-screen for genetic abnormalities before including a patient on a trial.

APPENDIX TABLES AND FIGURES

Appendix Table 1. Molecular and clinical characteristics by three-month progression-free survival status

Table 1: 3-Month Progression Status and Patient Characteristics (N=60)

Characteristics	Not Progressed, N=16	Progressed, N=44	p-value
Gleason score at baseline (from 1-10)			0.69****
Mean ± SD	8.2 ± 1.2	8 ± 1.3	
Median (min, max)	8.5 (6, 10)	8 (6, 10)	
PSA at baseline (ng/mL)			0.58*****
Mean ± SD	8.9 ± 21.5	47.2 ± 111.7	
Median (min, max)	0.9 (0, 83)	1.3 (0, 514.2)	
Number of priror systemic therapies (integer)			0.06****
Mean ± SD	1.8 ± 1.1	2.5 ± 1.4	
Median (min, max)	2 (0, 4)	2 (0, 6)	
NEPC status			0.82***
No	5 (31.2%)	17 (38.6%)	
Yes	11 (68.8%)	27 (61.4%)	
Time from diagnosis to study start (months)			0.76*****
Mean ± SD	141.9 ± 319.8	52.3 ± 41.9	
Median (min, max)	48 (4, 1313.4)	44.2 (3.9, 138)	
Visceral disease status			0.13***
No	8 (50%)	11 (25%)	
Yes	8 (50%)	33 (75%)	
Elevated LDH level at baseline			0.5***
No	9 (60%)	19 (45.2%)	
Yes	6 (40%)	23 (54.8%)	
RB1			.*
No	11 (100%)	33 (100%)	
TP53			0.28**
No	9 (81.8%)	19 (57.6%)	
Yes	2 (18.2%)	14 (42.4%)	
PTEN			1**
No	11 (100%)	32 (97%)	
Yes	0 (0%)	1 (3%)	
BRCA2			0.06**
No	9 (81.8%)	33 (100%)	
Yes	2 (18.2%)	0 (0%)	
AR			1**
No	11 (100%)	31 (93.9%)	
Yes	0 (0%)	2 (6.1%)	
AURKA			.*
No	11 (100%)	33 (100%)	
MYCN			.*
No	11 (100%)	33 (100%)	

***** Wilcoxon rank sum test was used to generate p-value.
**** T-test was used to generate p-value.
*** Chi-square test was used to generate p-value.
** Fisher's exact test was used to generate p-value.
* Since there is no TRUE or YES values, any test was not employed.

Appendix Table 2. Molecular and clinical characteristics by NEPC status

Table 2: NEPC and Patient Characteristics (N=60)

Characteristics	No NEPC, N=22	NEPC, N=38	p-value
Gleason score at baseline (from 1-10)			0.66****
Mean ± SD	8 ± 1.2	8.1 ± 1.3	
Median (min, max)	8 (6, 10)	8 (6, 10)	
PSA at baseline (ng/mL)			<0.001*****
Mean ± SD	74.2 ± 142.9	15.5 ± 47.4	
Median (min, max)	17.5 (0, 514.2)	0.3 (0, 207)	
Number of prior systemic therapies (integer)			0.06****
Mean ± SD	2.8 ± 1.5	2.1 ± 1.1	
Median (min, max)	3 (0, 6)	2 (0, 4)	
Time from diagnosis to study start (months)			0.01*****
Mean ± SD	144.9 ± 288.9	43.7 ± 41.9	
Median (min, max)	64.5 (8, 1313.4)	23.6 (3.9, 138)	
Visceral disease status			0.38***
No	9 (40.9%)	10 (26.3%)	
Yes	13 (59.1%)	28 (73.7%)	
Elevated LDH level at baseline			0.65***
FALSE	9 (42.9%)	19 (52.8%)	
TRUE	12 (57.1%)	17 (47.2%)	
Disease progression status at three months			0.82***
No	5 (22.7%)	11 (28.9%)	
Yes	17 (77.3%)	27 (71.1%)	
RB1			.*
No	17 (100%)	27 (100%)	
TP53			0.66***
No	12 (70.6%)	16 (59.3%)	
Yes	5 (29.4%)	11 (40.7%)	
PTEN			0.39**
No	16 (94.1%)	27 (100%)	
Yes	1 (5.9%)	0 (0%)	
BRCA2			0.51**
No	17 (100%)	25 (92.6%)	
Yes	0 (0%)	2 (7.4%)	
AR			0.14**
No	15 (88.2%)	27 (100%)	
Yes	2 (11.8%)	0 (0%)	
AURKA			.*
No	17 (100%)	27 (100%)	
MYCN			.*
No	17 (100%)	27 (100%)	

***** Wilcoxon rank sum test was used to generate p-value.
**** T-test was used to generate p-value.
*** Chi-square test was used to generate p-value.
** Fisher's exact test was used to generate p-value.
* Since there is no TRUE or YES values, any test was not employed.

Appendix Table 3. Table of confidence intervals by three-month progression free survival status

Characteristics	Point estimate	Confidence Interval(0.95)
Gleason score	0.167	(-0.694, 1.027)***
PSA	-0.128	(-5.000, 0.330)****
Number of Previous Therapies	-0.688	(-1.391, 0.016)***
Since initial diagnosis(in months)	2.557	(-17.607, 39.836)****
Visceral disease	0.115	(-0.027, 0.479)**
LDH Abnormality	0.226	(-0.113, 0.342)**
NEPC	-0.062	(-0.289, 0.165)**
RB1 mutation	-	-
TP53 mutation	3.234	(0.542, 35.384)*
PTEN mutation	NA	(0.009, NA)*
BRCA2 mutation	0	(0.000, 1.701)*
AR mutation	NA	(0.0612, NA)*
AURKA mutation	-	-
MYCN mutation	-	-

* Fisher's exact test - the corresponding point estimate is the odds ratio
** Chi-squared test of proportions - the corresponding point estimate is difference in the proportions of the two groups
*** T-test of significance without continuity correction - the corresponding point estimate is the difference in the means of the two groups
**** Wilcoxon-rank sum test without continuity correction - the corresponding point estimate is the difference in location

Appendix Table 4. Table of confidence intervals by NEPC status

Characteristics	Point estimate	Confidence Interval(95%)
Gleason score	-0.17	(-0.944, 0.604)***
PSA	12.899	(1.250,25.290)****
Number of Previous Therapies	0.720	(-0.045,1.486)***
Since initial diagnosis(in months)	41	(6.885,65.803)****
Visceral disease	0.146	(-0.139, 0.430)**
LDH Abnormality	-0.099	(-0.404, 0.206)**
Progresssion status	-0.062	(-0.325, 0.201)**
RB1 mutation	-	-
TP53 mutation	0.113	(-0.220, 0.446)**
PTEN mutation	0	(0, 24.556)*
BRCA2 mutation	0	(0, 8.486)*
AR mutation	0	(0, 3.288)*
AURKA mutation	-	-
MYCN mutation	-	-

* Fisher's exact test - the corresponding point estimate is the odds ratio
** Chi-squared test of proportions - the corresponding point estimate is difference in the proportions of the two groups
*** T-test of significance without continuity correction - the corresponding point estimate is the difference in the means of the two groups
**** Wilcoxon-rank sum test without continuity correction - the corresponding point estimate is the difference in location

Bibliography

Beltran H, Rickman D, Park K, et al. Molecular characterization of neuroendocrine prostate cancer (NEPC) and identification of new drug targets. *Journal of Clinical Oncology*. 2011;29(7_suppl):19-19. doi:10.1200/jco.2011.29.7_suppl.19.