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Biomarker-driven Phase 2 study of Nivolumab in Advanced Metastatic NSCLC

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Abstract

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By Yilin Yang

Background: Non-small cell lung cancer (NSCLC) is the most lethal one among various human cancer. Nivolumab is a new effective anti-cancer treatment as a monoclonal antibody, which can bind PD-1 and prevent the combination of PD-L1 and PD-1. It's easy to obtain peripheral blood and to observe changes of immune response to nivolumab by sampling peripheral blood at different time points. After the treatment of PD-1 and PDL-1 inhibitors, there will be a proliferation of CD8 T cells, and we could treat the sustained proliferation of T cell as a predictive biomarker of response to nivolumab.

Methods and Materials: We enrolled a total of 48 patients (36 to 87 years) for the retrospective study, 25 in biomarker positive group and 23 in biomarker negative group. Both groups had been treated with nivolumab 240 mg per two weeks and the response had been assessed by the sample of peripheral blood. The primary endpoint was the response rates between two groups. T-test, Wilcoxon rank sum test, Chi-square test, Mantel-Haenszel test, Log-rank test, and Cox proportional-hazards model were used for statistical analysis.

Results: The number of patients with objective response is 7 out of 25 in biomarker positive group (expected rate = 0.280, p-value = 0.03). The hazard ratio of groups stratified by biomarker is 0.281 (0.088, 0.900) with the reference of negative group (p-value = 0.023). Two variables of biomarker and IRAE are included in the final model. The hazard rate of biomarker positive group is 0.232 (0.066, 0.819) (p-value = 0.023), IRAE group between grades 2-4 is 0.091 (0.017, 0.482) (p-value = 0.005), and patients in biomarker positive and IRAE group between grades 2-4 have the highest survival probability.

Conclusion: There are more patients with objective response in biomarker positive group and patients in biomarker positive group have significantly higher survival probability than those in biomarker negative group due to the greater immune response with the proliferation of CD8 T cells. Therefore, the sustained proliferation of CD8 T cells can be treated as a predictive biomarker of response to Nivolumab.

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CONTENTS

1. INTRODUCTION

In various forms of human cancer, non-small cell lung cancer (NSCLC) is the most lethal one and there are almost 221,200 new lung cancer cases with both sexes in 2015.[1] Most patients with this lung cancer also have other advanced stage disease due to the side effects of chemotherapy. Chemotherapy can help patient shrink the tumor size and slow the growth of cancer cells, but its toxicity will lead to many side effects, which will make great damage to human normal cells. Therefore, novel medical treatments are needed in cancer patients, especially the patients with NSCLC, to improve the clinical outcomes.

One feature of cancer is the ability to avoid the immune destruction.[2] Programmed cell death protein 1 (PD-1) is an immunosuppressive molecule, which will be expressed on immune cells, belonging to the CD28 family. With the situation of chronic viral infection, the T cells will firstly show the high expression of PD-1.[3] PD-L1 and PD-L2 are two ligands of PD-1. The combination of PD-1 and PD-L1 will result in the programmed cell death of T cells, and this will induce immune escape for tumor cells. The overexpression of PD-L1 on tumors will decrease the activation of T cell and then increase the immunity of tumor cells. Nivolumab, a kind of monoclonal antibodies which can block the PD-1 pathway, is a new effective anti-cancer treatment for various kinds of tumor type. The response of this new treatment is dramatic and durable for cancer patients. [4, 5]

As a monoclonal antibody, nivolumab will bind PD-1 and prevent the combination of PD-L1 and PD-1, which will break the inhibitory signaling pathway and therefore

improve the antitumor response.[6] For nivolumab, the binding of PD-1has great affinity and specificity in vitro, which can prevent the bond of other family members, such as ICOS, BTLA, CD28 and so on. For safety and efficacy of nivolumab, the study showed the dosage did not reach the maximum tolerated dose (MTD) in test activity of various kinds of tumor type.[7] Nivolumab monotherapy has been studied broadly in multiple kinds of tumor, which includes NSCLC, melanoma, renal cell carcinoma and others. Many patients could benefit from the treatments of PD-1 blockade, but we have not totally understood how to identify the patients who will have responses to this specific treatment. Although the expression of PD-1on tumor cells might be relative to clinical response, some patients without PD-1 expression are also responsive to PD-1 inhibitors. [7, 8] Moreover, there are some other restrictions of PD-1 expression. For example, getting adequate tissue for the PD-1 sample will be a big problem. Besides, it is not easy to estimate the probability of a response through observing the static markers of the tumor, due to the dynamics of immune response.

We supposed the analysis of peripheral blood might solve some limitations. It's easy to obtain peripheral blood and the changes of immune response can be observed by sampling peripheral blood at different time points. For patients with advanced non-small cell lung cancer, their peripheral blood has already been studied and they also have gotten monoclonal antibodies against PD-1 or PDL-1.[9] By the treatment of PD-1 and PDL-1 inhibitors, the number of CD8 T cells has increased mostly, and there is no increase of regulatory T cells.[10, 11] In this study, we aim to study the sustained proliferation of CD8 T cell as a predictive biomarker of response to nivolumab between the positive and

negative groups of patients stratified by the status of T cells. Biomarker is the indicator which can be measured to reflect the recurrence of severity of a disease. In this study, patients with a two-fold proliferation in this CD8 T cell population will be considered biomarker positive. In remaining of this thesis, we will describe the study design and methods including patients' parameters and statistical analysis in Section 2. In Section 3, the results of descriptive and statistical analysis will be presented to compare the objective response rate (ORR), which means the proportion of patients having the reduction in tumor burden with a predefined amount, between the two groups stratified by biomarker.

2. METHODS AND MATERIALS

The data for analysis is from a retrospective study on patients performed by Winship Cancer Institute of Emory University, and the use of the data for this paper has been approved by the Emory University Institutional Review Board. Demographic data and related clinical features are obtained from the electronic medical records.

2.1 Study Design

In this open label phase II study, we will study the development of biomarker based on the patients with non-small cell lung cancer. We have screened these patients firstly, and then provide them the treatment of 240 mg nivolumab per two weeks until they have any disease progression or unacceptable toxicity. In the study period, we will take the

peripheral blood samples at each treatment point for these patients and then evaluate patients' responses to biomarker based on the study of blood samples.

2.2 Population and Observation parameter

In this study, we have enrolled 48 patients with non-small cell lung cancer (NSCLC), and 25 patients in biomarker positive group and 23 patients in biomarker negative group. We will treat each patient with nivolumab, and then access their responses. The primary endpoint is the response rates in biomarker positive group and negative group. We will continue to enroll 2-3 patients every month until the completion of 48 patients in 12 months.

The key inclusion criteria for patients in this study includes the age of participants above 18 years old with the ability to offer consent, subjects with the tumor expression and measurable disease, participants willing to be treated with biopsy for analysis at the enrollment time and each disease progression time, patient's ECOG performance status no larger than 2, and so on.

Demographic and physical indicators (Age, Sex, Tumor burden, Tumor burden status and Tumor type), past medical history and past treatment history (Therapy type, Eastern Cooperative Oncology Group Performance Status (ECOG), Line of treatment, Immune-Related Adverse Events (IRAE) and Clinical response) has been recorded to allow for control of covariates and the impact of biomarker on survival in the analysis of outcomes.

2.3 Statistical Analysis

2.3.1 Descriptive analysis

The descriptive table for patients' characteristics and prognostics factors is constructed. We divide the whole dataset into two groups: the positive group and negative group, stratified by biomarker. For continuous variables, we will present them as mean and standard deviation based on the normal distribution, otherwise present them as median and range. For categorical variables, the frequencies and column percentage are summarized.

2.3.2 Statistical analysis

First, we want to discover the difference between biomarker positive and negative group for each variable. For continuous variables, two sample t-test between two groups is conducted based on the normal distribution, otherwise Wilcoxon rank sum test will be conducted. For categorical variables, Chi-square test is used to compare two groups.

After that, we want to estimate the proportion of objective response rate and the proportion of progressive disease response rate for each variable, as percentage with 95% confidence interval. Mantel-Haenszel test will be tested with the p-value, to compare the objective response rate and progressive response rate, respectively, between different levels of each variable, assuming a binomial distribution.

2.3.3 Univariate and Multivariate Survival analysis

Firstly, we conduct univariate survival analysis to test whether there is a significant

association between each variable and survival. We split continuous variables by median and separate these variables into 2 groups, less than median group and no less than median group. Overall survival for each group will be estimated by Kaplan Meier method and the comparison between different groups for each variable will be conducted by Log-rank test. The basic model for univariate survival analysis is shown here:

$$Model\,1\colon Survival = \beta_0 + \beta_1 * x_1 + \varepsilon$$

We choose one of the categories of each variable as reference group and present the hazard ratio with 95% confidence interval to see whether there is a significant difference between each category. Then, we conduct multivariate survival analysis with Cox proportional-hazards model to test the effect of biomarker on overall survival after the control of confounders. Also, we will calculate the point estimate of overall survival with 95% confidence interval at 1, 3, and 5 years in the multivariate survival analysis. The full model is shown here:

$$Model\,2\colon Survival = \beta_0 + \sum_{i=1}^{11} \beta_i * x_i + \varepsilon$$

Here the x_i's are Biomarker, Age, Tumor burden, Sex, ECOG, Therapy, Line of treatment, IRAE, Tumor burden status, Clinical response and Tumor type. We also choose one of the categories of each variable as reference group and present the hazard ratio with 95% confidence interval. The final model is selected by the backward variable selection method with an α = 0.05 removal criteria. The significant level is set at α = 0.05. The data in this study is analyzed using SAS 9.4.

3. RESULTS

3.1 Results of Descriptive Analysis

The 784 observations of 48 patients were consisted of two biomarker groups (negative/positive). The descriptive statistics of study variables was shown by the Table 1. From the table, we can know there are 23 patients in biomarker negative group and 25 patients in biomarker positive group. The mean age of patients is 65.91years old (standard deviation = 9.13) in negative group and 64.92 years old (10.85) in positive group, and the median of tumor burden is 8.45 cm (range = 26.50) in negative group and 10.00 cm (19.70) in positive group. There are about 47.8% vs. 76.0% male in biomarker negative group and positive group. For patients with ECOG performance status between 1-2 which means patients are capable of all selfcare but unable to carry out any work activities [12], there are 18.2% vs. 24.0% of patients in biomarker negative group and positive group. The therapy type of combination therapy is more likely among those in biomarker positive group than in the biomarker negative group with the percentage of 52.0% vs. 17.4%. For the variable of line of treatment, there are 55.0% vs. 39.1% patients with more than one line of therapy in biomarker negative group and positive group. Immune-related adverse events (IRAE) means the side effects which are caused by immune checkpoint inhibitors [13], and there are 30.4% vs. 52.2% patients with IRAE between grades 2-4 in biomarker negative group and positive group. The number of patients with low tumor burden status in biomarker negative group is the same as that in biomarker positive group, with the percentage of 50.0% vs. 47.8%. For the variable of clinical response, there are 4.3% vs. 28.0% patients with objective response, and 34.8% vs. 16.0% patients with progressive disease response in biomarker negative group and

positive group, respectively. There are 60.9% vs. 44.0% patients with the tumor type of lung cancer, and 4.3% vs. 16.0% patients with the tumor type of melanoma, in biomarker negative group and positive group, respectively. In various forms of cancer for human people, non-small cell lung cancer (NSCLC) is the most lethal one.[1]

3.2 Results of Statistical Analysis

As for **Table 1**, we test the difference of all variables in biomarker negative and positive groups. The null hypothesis is H_0: Negative $=$ Positive. By comparing corresponding p-value at the significance level $\alpha = 0.05$, we find that there are significantly more male patients in the biomarker positive group (p-value = 0.044), and patients with monotherapy treatment are significantly more likely among those in the biomarker negative group than in the biomarker positive group (p-value = 0.012). However, for other variables, there is no significant difference between these two groups.

The comparison of objective response rates between different level of each variable is shown in **Table 2**, including the number of patients with objective response and the estimate of objective response rate with 95% confidence interval, at the significance level $\alpha = 0.05$. There are 1 patient in biomarker negative group with the expected rate of 0.044 (0.001, 0.220), and there are 7 patients in biomarker positive group with the expected rate of 0.280 (0.121, 0.494). In the age less than median group, there are 5 patients with the expected rate of 0.208 (0.071, 0.422), and in the age no less than median group, there are 3 patients with the expected rate of 0.125 (0.027, 0.324). In the tumor burden less than median group, there are 5 patients with the expected rate of 0.200

(0.068, 0.407), and in the tumor burden no less than median group, there are 3 patients with the expected rate of 0.130 (0.028, 0.336). For sex, there are 1 patient with objective response in female group with the estimate rate of 0.056 (0.001, 0.273), and there are 7 patients with objective response in male group with the estimate rate of 0.233 (0.099, 0.423). For the two levels of ECOG, the group of performance status between 0-1 has 6 patients and the estimate rate is 0.162 (0.062, 0.320), and the group of performance status between 1-2 has 2 patients and the estimate rate is 0.200 (0.025, 0.556). In the monotherapy therapy group, there are 5 patients with objective response with the estimate rate of 0.161 (0.055, 0.337), and in the combination therapy group, there are 3 patients with objective response with the estimate rate of 0.177 (0.038, 0.434). For the line of treatment, there are 6 patients with objective response of first line therapy with the estimate rate of 0.261 (0.102, 0.484), and there are 1 patient with objective response of more than one line of therapy with the estimate rate of 0.050 (0.001, 0.249). There are 3 patients in the group of IRAE between none - grade 1 with the estimate rate of 0.111 (0.024, 0.292), and there are 5 patients in the group of IRAE between grades 2-4 with the estimate rate of 0.263 (0.092, 0.512). For tumor burden status, 4 patients in low group have objective response with the estimate rate of 0.182 (0.052, 0.403) and 3 patients in high group have objective response with the estimate rate of 0.130 (0.028, 0.336). The number of patients in lung cancer group, melanoma group and other group are 3, 2, 3, with the estimate rates of 0.120 (0.026, 0.312), 0.400 (0.053, 0.853), and 0.167 (0.036, 0.414), respectively. According to the p-values of all variables, there are more patients with objective response in the biomarker positive group with the significant difference in the objective response rate between positive and negative groups (p-value = 0.03).

The comparison of progressive disease response rates between different level of each variable is shown in **Table 3**, including the number of patients with progressive disease response and the estimate of progressive disease response rate with 95% confidence interval, at the significance level $\alpha = 0.05$. There are 8 patients in biomarker negative group with the expected rate of 0.348 (0.164, 0.573), and there are 4 patients in biomarker positive group with the expected rate of 0.160 (0.045, 0.361). In the age less than median group, there are 7 patients with the expected rate of 0.292 (0.126, 0.511), and in the age no less than median group, there are 5 patients with the expected rate of 0.208 (0.071, 0.422). In the tumor burden less than median group, there are 7 patients with the expected rate of 0.280 (0.121, 0.494), and in the tumor burden no less than median group, there are 5 patients with the expected rate of 0.217 (0.075, 0.437). For sex, there are 4 patients with progressive disease response in female group with the estimate rate of 0.222 (0.064, 0.476), and there are 8 patients with progressive disease response in male group with the estimate rate of 0.267 (0.123, 0.459). For the two levels of ECOG, the group of performance status between 0-1 has 10 patients and the estimate rate is 0.270 (0.138, 0.441), and the group of performance status between 1-2 has 2 patients and the estimate rate is 0.200 (0.025, 0.556). In the monotherapy therapy group, there are 11 patients with progressive disease response with the estimate rate of 0.355 (0.192, 0.546), and in the combination therapy group, there are 1 patient with progressive disease response with the estimate rate of 0.059 (0.002, 0.287). For the line of treatment, there are 4 patients with progressive disease response of first line therapy with the estimate rate of 0.174 (0.050, 0.388), and there are 8 patients with progressive disease response of more than one line of therapy with the estimate rate of 0.400 (0.191, 0.640). There are 10

patients in the group of IRAE between none - grade 1 with the estimate rate of 0.370 (0.194, 0.576), and there is no patient in the group of IRAE between grades 2-4 with progressive disease response. For tumor burden status, 5 patients in low group have progressive disease response with the estimate rate of 0.227 (0.078, 0.454) and 5 patients in high group have progressive disease response with the estimate rate of 0.217 (0.075, 0.437). The number of patients in lung cancer group, melanoma group and other group are 4, 1, 7, with the estimate rates of 0.160 (0.045, 0.361), 0.200 (0.005, 0.716), and 0.389 (0.173, 0.643), respectively. According to the p-values of all variables, the variable of therapy and the variable of IRAE have significant difference in the progressive disease response rate between different levels. Progressive disease response is significantly more likely among those in the monotherapy treatment group than in the combination treatment group (p-value = 0.025), and those in the group of IRAE between none - grade 1, than in the group of IRAE between grades 2-4 (p-value = 0.003).

3.3 Results of Univariate and Multivariate Regression Analysis

3.3.1 Results of univariate regression

As for **Table 4**, we present the outcome of univariate association between different variables and survival, including variables of biomarker, age, tumor burden, sex, ECOG, therapy, line of treatment, IRAE, tumor burden status, clinical response and tumor type. The hazard ratio of groups stratified by biomarker is 0.281 (0.088, 0.900) with the reference of negative group, which means the biomarker positive group has a 71.9% risk reduction of death compared to the negative group. We divide age and tumor burden into 2 groups by median, and the hazard ratio is 2.351 (0.737, 7.497) and 1.613 (0.559, 4.655)

and the reference group is whose values less than median. For sex, the hazard rate of male group is 0.690 (0.239, 1.994), and the male has a lower risk of death compared to the female. For ECOG, the hazard rate of the group with ECOG performance status between 0-1 is 0.432 (0.056, 3.334), which means the patients with ECOG performance status between 0-1has a 56.8% risk reduction of death compared to those with ECOG performance status between 1-2. There are two groups of therapy and the hazard ratio is 0.812 (0.265, 2.482) with the reference of monotherapy group, and this shows the combination therapy group has the lower risk of death. For line of treatment, the hazard rate of the group with more than one line of therapy is 1.525 (0.528, 4.403), with the higher risk of death compared to the group with first line therapy. The hazard rate of the IRAE group between grades 2-4 is 0.111 (0.024, 0.514), with a greatly risk reduction of death. For tumor burden status, the hazard ratio is 1.460 (0.506, 4.212) with the reference of low tumor burden group, which means the high tumor burden group has a higher risk of death. For clinical response, we treat the group of progressive disease response as reference group and the hazard rate is 0.000 of objective response group and 0.221 (0.071, 0.694) of stable disease response group, which means these two groups all have a lower risk of death compared to the progressive disease response group. There are three groups of tumor type and the group of other tumor type is reference group. The hazard rate of lung cancer group is 1.239(0.413, 3.715) and the hazard rate of melanoma group is 0.000, and this shows the lung cancer group has the highest risk of death among the three groups. From the results of comparisons for all of these variables, there are three variables significantly associated with survival. The biomarker positive group, the IRAE group between grades 2-4, and the groups of objective response and stable disease

response, are significantly associated with lower death rate compared with the biomarker negative group, the IRAE group between none - grade 1, and the group of progressive disease response, with the p-value of 0.023, 0.001 and 0.002, respectively.

3.3.2 Results of multivariate regression

We construct **Table 5**, for the outcome of final model and relative p-value of multivariate regression for overall survival. By using the basic model in method section, we want to examine which variable of patients will have the significant association with overall survival. We exclude the variables that are not statistically significant with the backward elimination method and we find there are only two variables, biomarker and IRAE, have significant impact on survival in final model. The patients in biomarker positive group with IRAE between grades 2-4 are more likely to survive than others. The hazard rate in biomarker positive group is 0.232 (0.066, 0.819) and p-value is 0.023. The hazard rate of the IRAE group between grades 2-4 is 0.091 (0.017, 0.482) and p-value is 0.005.

We constructed **Table 6**, for the point estimate of overall survival and 95% confidence interval at 1, 3, and 5 years for 4 groups stratified by biomarker and IRAE. In biomarker negative and IRAE between none - grade 1 group, the estimate of survival at 1, 3, and 5 years is 0.376 (0.105, 0.616), 0.2505 (0.000, 0.520), and 0.2505 (0.000, 0.520), respectively. In biomarker negative and IRAE between grade 2-4 group, the estimate of survival at 1, 3, and 5 years is 1.000 (1.000, 1.000), 0.6 (0.000, 1.000), and 0.6 (0.000, 1.000). In biomarker positive and IRAE between none - grade 1 group, the estimate of survival at 1, 3, and 5 years is 0.846 (0.569, 1.000), 0.604 (0.157, 1.000), and 0.604 (0.157, 1.000), respectively. In biomarker positive and IRAE between grade 2-4 group,

the estimate of survival at 1, 3, and 5 years is 0.917 (0.760, 1.000), 0.825 (0.604, 1.000), and 0.825 (0.604, 1.000), respectively.

The Cox proportional-hazards model is presented as **Figure 1.** This outcome confirms the stability and reliability of our study design of using sustained proliferation of T cells as a predictive biomarker of response to Nivolumab.

4. DISCUSSION

The purpose of developing biomarker is to find available biomarker and then improve clinical treatments outcomes for patients.[14] There are various biomarkers have been developed and we find the immunotherapy of T-cell has great value in clinical experiments for cancer study and it has become an effective way.[15] According to other researches, we know the overexpression of PD-L1 on tumors will decrease the activation of T cell and then affects the inhibition of immune checkpoint.[16] Nivolumab can block the PD-1 pathway, as an effective anti-cancer treatment. The results in our study shows the proliferation of CD8 T cells is a good response to nivolumab and we can treat the sustained proliferation of CD8 T cell as a predictive biomarker of response to nivolumab.

However, there are also some limitations. Firstly, the sample size is small. We just have 48 patients in this study. The study can be more precise and reliable if we can hire more participants. The outcome is also affecting by some outlier and missing values, but these effects are all in an acceptable range. Secondly, it will be better for this study to record

related data and then conduct progression free survival, due to the advantages of shorter study time and fewer participants. Finally, we also need to conduct additional tests to access the treatment response and practical value of treating the sustained proliferation of CD8 T cell the predictive biomarker.[17]

5. CONCLUSION

According to our study, the type of combination therapy and male sex will influence the proliferation of CD8 T cells, with more patients in the biomarker positive group. Also, there are more patients with objective response in biomarker positive group, which means the sustained proliferation of T cells can be treated as a predictive biomarker. Because the proliferation of CD8 T cells, as the good response to nivolumab, can bring better immune response with the objective response. According to the results of multivariate survival analysis, we also find that patients in biomarker positive group have significantly higher survival probability than those in biomarker negative group, which also verifies the greater immune response with the proliferation of T cells. Therefore, we conclude that the sustained proliferation of CD8 T cells can be treated as a predictive biomarker of response to Nivolumab.

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7. FIGURES AND TABLES

Table 1. Descriptive statistics of study variables

Characteristic	Level	Biomarker		P-value
		Negative (N=23)	Positive (N=25)	
Age (mean ± SD)		65.91 (9.13)	64.92 (10.85)	0.734
Tumor burden (cm)		8.45 (26.50)	10.00 (19.70)	1.000
Sex	Female	12 (52.2%)	6 (24.0%)	0.044*
	Male	11 (47.8%)	19 (76.0%)	
ECOG	0-1	18 (81.8%)	19 (76.0%)	0.627
	1-2	4 (18.2%)	6 (24.0%)	
Therapy	Monotherapy	19 (82.6%)	12 (48.0%)	0.012*
	Combination	4 (17.4%)	13 (52.0%)	
Line of treatment	First line therapy	9 (45.0%)	14 (60.9%)	0.298
	More than one line of therapy	11 (55.0%)	9 (39.1%)	
IRAE	None - Grade 1	16 (69.6%)	11 (47.8%)	0.134
	Grades 2-4	7 (30.4%)	12 (52.2%)	
Tumor burden status	low	11 (50.0%)	11 (47.8%)	0.884
	high	11 (50.0%)	12 (52.2%)	
Clinical response	Objective response	1 (4.3%)	7 (28.0%)	0.056
	Stable disease	14 (60.9%)	14 (56.0%)	
	Progressive disease	8 (34.8%)	4 (16.0%)	
Tumor type	lung cancer	14 (60.9%)	11 (44.0%)	0.316
	Melanoma	1 (4.3%)	4 (16.0%)	
	Other	8 (34.8%)	10 (40.0%)	

** Statistical significant, $\alpha = 0.05$*

Table 2. The comparison of objective response rate

Characteristic	Level	Objective Response Rate			P-value
		N	Estimate	95% CI	
Biomarker	Negative	1	0.044	(0.001, 0.220)	0.030*
	Positive	7	0.280	(0.121, 0.494)	
Age	< Median	5	0.208	(0.071, 0.422)	0.443
	≥ Median	3	0.125	(0.027, 0.324)	
Tumor burden	< Median	5	0.200	(0.068, 0.407)	0.523
	≥ Median	3	0.130	(0.028, 0.336)	
Sex	Female	1	0.056	(0.001, 0.273)	0.113
	Male	7	0.233	(0.099, 0.423)	
ECOG	0-1	6	0.162	(0.062, 0.320)	0.780
	1-2	2	0.200	(0.025, 0.556)	
Therapy	Monotherapy	5	0.161	(0.055, 0.337)	0.894
	Combination	3	0.177	(0.038, 0.434)	
Line of treatment	First line therapy	6	0.261	(0.102, 0.484)	0.065
	More than one line of therapy	1	0.050	(0.001, 0.249)	
IRAE	None - grade 1	3	0.111	(0.024, 0.292)	0.185
	Grades 2-4	5	0.263	(0.092, 0.512)	
Tumor burden status	Low	4	0.182	(0.052, 0.403)	0.638
	High	3	0.130	(0.028, 0.336)	
Tumor type	Lung cancer	3	0.120	(0.026, 0.312)	0.633
	Melanoma	2	0.400	(0.053, 0.853)	
	Other	3	0.167	(0.036, 0.414)	

* *Statistical significant, $\alpha = 0.05$*

Table 3. The comparison of progressive disease response rate

Characteristic	Level	Progressive Disease Response Rate			P-value
		N	Estimate	95% CI	
Biomarker	Negative	8	0.348	(0.164, 0.573)	0.137
	Positive	4	0.160	(0.045, 0.361)	
Age	< Median	7	0.292	(0.126, 0.511)	0.510
	≥ Median	5	0.208	(0.071, 0.422)	
Tumor burden	< Median	7	0.280	(0.121, 0.494)	0.621
	≥ Median	5	0.217	(0.075, 0.437)	
Sex	Female	4	0.222	(0.064, 0.476)	0.733
	Male	8	0.267	(0.123, 0.459)	
ECOG	0-1	10	0.270	(0.138, 0.441)	0.655
	1-2	2	0.200	(0.025, 0.556)	
Therapy	Monotherapy	11	0.355	(0.192, 0.546)	0.025*
	Combination	1	0.059	(0.002, 0.287)	
Line of treatment	First line therapy	4	0.174	(0.050, 0.388)	0.103
	More than one line of therapy	8	0.400	(0.191, 0.640)	
IRAE	None - grade 1	10	0.370	(0.194, 0.576)	0.003*
	Grades 2-4	0	0.000	(0.000, 0.000)	
Tumor burden status	Low	5	0.227	(0.078, 0.454)	0.937
	High	5	0.217	(0.075, 0.437)	
Tumor type	Lung cancer	4	0.160	(0.045, 0.361)	0.094
	Melanoma	1	0.200	(0.005, 0.716)	
	Other	7	0.389	(0.173, 0.643)	

** Statistical significant, $\alpha = 0.05$*

Table 4. Univariate overall survival analysis

Characteristic	Level	Overall Survival		
		N	Hazard Ratio (95% CI)	Log-rank P-value
Biomarker	Negative	23	Ref	0.023*
	Positive	25	0.281 (0.088, 0.900)	
Age	< Median	24	Ref	0.137
	≥ Median	24	2.351 (0.737, 7.497)	
Tumor burden	< Median	22	Ref	0.372
	≥ Median	23	1.613 (0.559, 4.655)	
Sex	Female	18	Ref	0.491
	Male	30	0.690 (0.239, 1.994)	
ECOG	0-1	37	Ref	0.408
	1-2	10	0.432 (0.056, 3.334)	
Therapy	Monotherapy	31	Ref	0.714
	Combination	17	0.812 (0.265, 2.482)	
Line of treatment	First line therapy	23	Ref	0.432
	More than one line of therapy	20	1.525 (0.528, 4.403)	
IRAE	None - grade 1	27	Ref	0.001***
	Grades 2-4	19	0.111 (0.024, 0.514)	
Tumor burden status	Low	22	Ref	0.482
	High	23	1.460 (0.506, 4.212)	
Clinical response	Objective response	8	0.000 (0.000, 0.000)	0.002***
	Stable disease	28	0.221 (0.071, 0.694)	
	Progressive disease	12	Ref	
Tumor type	Lung cancer	25	1.239(0.413, 3.715)	0.249
	Melanoma	5	0.000 (0.000, 0.000)	
	Other	18	Ref	

** Statistically significant, $\alpha = 0.05$*

**** Extremely Statistical significant, $\alpha = 0.005$*

Table 5. Multivariate overall survival analysis

Covariate	Level	Overall Survival	
		Hazard Ratio (95% CI)	Log-rank P-value
Biomarker	Negative	Ref	0.023*
	Positive	0.232 (0.066, 0.819)	
IRAE	None - Grade 1	Ref	0.005***
	Grades 2 - 4	0.091 (0.017, 0.482)	

** Statistically significant, $\alpha = 0.05$*
**** Extremely Statistical significant, $\alpha = 0.005$*

Table 6. Point estimate of overall survival at 1, 3, and 5 years

Covariate		Overall Survival		
Biomarker	IRAE	Survival at 1 year (95% CI)	Survival at 3 years (95% CI)	Survival at 5 years (95% CI)
Negative	None - Grade 1	0.376 (0.105, 0.616)	0.251 (0.000, 0.520)	0.251 (0.000, 0.520)
Negative	Grades 2-4	1.000 (1.000, 1.000)	0.600 (0.000, 1.000)	0.600 (0.000, 1.000)
Positive	None - Grade 1	0.846 (0.569, 1.000)	0.604 (0.157, 1.000)	0.604 (0.157, 1.000)
Positive	Grades 2-4	0.917 (0.760, 1.000)	0.825 (0.604, 1.000)	0.825 (0.604, 1.000)

** Statistically significant, $\alpha = 0.05$*

Figure 1. Cox proportional-hazards model for multivariate overall survival analysis