



# Anti-PD-1 Based Immunotherapy in Melanoma: Application of Machine Learning to Predict Survival and Elucidate Complex Relationships

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**ANTI-PD-1 BASED IMMUNOTHERAPY IN MELANOMA: APPLICATION OF  
MACHINE LEARNING TO PREDICT SURVIVAL AND ELUCIDATE COMPLEX  
RELATIONSHIPS**

**“AIM2SURVIVE”**

by

Girish S. Naik

A Dissertation Submitted to the Faculty of Harvard Medical School

in Partial Fulfillment of

the Requirements for the Degree of Master of Medical Sciences in Clinical  
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I have reviewed this thesis. It represents work done by the author under my guidance/supervision.

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## **OVERVIEW OF THE TWO THESIS PROJECTS**

### *Project 1*

Presence of brain metastases from melanoma usually portends a poor prognosis with a one-year survival rate reported to be as low as 9 - 19 % (1,2) before the era of immunotherapy. Anti-Programmed Death-1 (PD-1) based immunotherapy has emerged as the standard of care for unresectable or metastatic melanoma due to superior response rates and improved survival compared to chemotherapy and ipilimumab (3–8). However, patients with melanoma brain metastases were mostly excluded during clinical development due to poor prognosis and trials which included such patients were asymptomatic, stable and treated with standard of care treatment which include radiation and/or surgery. Recent studies have reported clinical activity of anti-PD-1 based immunotherapies in melanoma brain metastases (9–11). However, there is limited data on overall survival estimates and predictive factors in the real-world setting. Given the relatively limited number of treatment options available in this setting, accurate prediction of survival is critical to prioritize available systemic treatment options.

Brain metastases cohorts in general have fewer patients and even more so in the context of newer immunotherapy agents that have become available in the past three years (11); this scenario has introduced the problem of high dimensionality when using conventional statistical methods to study several important clinical covariates in a model. Therefore, we used data adaptive machine learners (for survival) which have unique properties suited to address these challenges given improved performance, ability to handle high dimensionality, incorporate complex relationships and ability to allow elucidating complex inter-relationships of predictors under a non-parametric framework (12–15). Because no

single learner is known to have a good performance in all settings, we evaluated the predictive performance of multiple tree based and ensemble learners compared to the traditional Cox-Proportional Hazards (Cox-PH) model which is commonly used. We also assessed the performance of individual features deemed clinically relevant and incorporated performance of brain metastases prognostic indices either alone or in combination with individual features to identify the feature subset that maximizes the predictive performance.

### *Project 2*

Five year survival rate in metastatic melanoma has improved substantially due to anti-PD-1 based immunotherapies (3,4,6,8). However, many responders eventually develop resistance (16). While translational biomarkers such as PD-L1 expression, mutational load, interferon gamma signature have some value in predicting response, clinical predictors may have a role for identification of patient subgroups likely to develop long term benefit and/or survival to PD1 blockade (17–19). In this context, obesity paradox has been studied in several solid cancers where, overweight/obese patients have shown improved survival than normal weight patients (referred to as the “obesity paradox”) (20). This phenomenon is not well understood in melanoma and there is currently no published data which characterizes the relationship of BMI with survival and clinical benefit outcomes in the context of anti-PD-1 based immunotherapy. It would be important to characterize the relationship and assess whether BMI is important in the prediction of survival and clinical benefit outcomes in the context of anti-PD-1 based immunotherapy as nearly 70% patients in the US fall in this category (21) and could represent an important subgroup that may have survival benefit. We therefore characterized the relationship of BMI with survival and

clinical benefit outcomes in a real world setting where patients are expected to have a more representative obese patient population with regards to comorbidities that may qualify as exclusion criteria in clinical trials. We collected extensive baseline data pertaining to demographics, disease attributes, disease severity, prior treatments, clinical chemistry parameters, previous weight loss impact to study complex inter-relationships that may help explain the observed relationship of BMI with survival. We used machine learners, Random Survival Forests/RSF (for survival outcomes) and Random Forests/RF (for clinical benefit outcome) as our primary method of analyses as the adaptive nature of the forests allow uncovering complex patterns, inter-relationships among predictors and assess predictive importance in the context of other predictors of survival (12,14,15). We also confirmed the findings using traditional methods (frequentist approach).

## **PROJECT 1**

**Title: Machine Learning to Predict Overall Survival in Patients with Melanoma Brain Metastases Treated with Anti-PD-1 based Immunotherapy**

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## **Abstract**

**Background:** We assessed if machine learners outperform Cox-PH models in a high dimensional setting in the context of anti-PD-1 treatment for melanoma brain metastases as accurate prediction of overall survival/OS is critical to prioritize limited treatment options.

**Methods:** In this retrospective cohort study, metastatic melanoma patients had radiological evidence of brain metastases (treated/untreated) at the time of anti-PD-1 (monotherapy/combination) immunotherapy (June-2014–September-2016). The primary outcome was OS. Five tasks were evaluated (Task-1: 22-features; Task-2: diagnosis specific-graded prognostic assessment/ds-GPA; Task-3: Recursive Partitioning Analysis/RPA; Task-4: ds-GPA plus 20-features; Task-5: RPA plus 21-features) using machine learners (Random Survival Forests/RSF, Boosting, Cox-Boost, Survival-Tree) and Cox-PH models based on test-set performance (5-fold cross-validated C-index) in a benchmark experiment followed by feature filtering and sequential forward selection (best performing learner).

**Results:** The median OS was 59 days (IQR:13-97) for ds-GPA 0-1, 473 days (IQR:150-Not Reached/NR) for ds-GPA 2 and was NR for ds-GPA 3-4 (N=56 patients/33 deaths). RSF had the lowest mean rank and performed significantly better than Cox-PH (mean-rank 1.3vs.4.3; Critical-Difference=2.73;  $p$ -value=0.023). Average C-index for Task-2/ds-GPA (0.867;95%CI:0.777-0.947) and Task-4 (0.878; 95%CI:0.760-0.961) feature-subset was comparable. Task-3/RPA performed poorly (C-index:0.664; 95%CI:0.560-0.779). Task-4 subset included ds-GPA,  $\geq 4$  extra-cranial metastases, prior surgical resection and

stereotactic radiotherapy/SRT, neurological symptoms and spine metastases. Prior surgical resection and SRT predicted improved survival for ds-GPA>1.

**Conclusions and Impact:** RSF had the best relative performance compared to Cox-PH models in a high dimensional setting. OS was prolonged for ds-GPA>1 with anti-PD-1 immunotherapy compared to historical estimates (surgery and/or radiation). Baseline ds-GPA was the strongest predictor.

## **Introduction**

Metastases of melanoma to the brain account for approximately 10% of all brain metastases, and are associated with a dismal prognosis (1,2); Historically the one-year survival has been reported to be as low as 9-19%, with a median survival of 3-4 months (2,3). The revised tumor, lymph node, and metastasis (TNM) staging criteria for melanoma, by the American Joint Committee on Cancer in 2017, now include a separate category for brain metastases (M1d), to reflect the poor prognosis (4).

Anti-Programmed Death-1 (PD-1) based immunotherapy, either anti-PD-1 monotherapy or a combination of anti-PD-1 and Cytotoxic T Lymphocyte Antigen-4 (anti-CTLA-4), has substantially improved the treatment of metastatic melanoma; given the documented improvement in overall survival compared to ipilimumab and chemotherapy and the observation of durable objective responses in 40- 60% of patients, immunotherapy is now a standard of care first line treatment option for patients with advanced melanoma (5–10).

The treatment of patients with brain metastases from melanoma has included surgical resection, stereotactic radiotherapy (SRT), and whole brain radiation therapy (WBRT); however, with the availability of more active systemic therapies for melanoma, this multi-disciplinary treatment approach has increasingly incorporated systemic therapy as an additional treatment tool (11,12).

Prior to the availability of oncogene (BRAF<sup>V600</sup>) directed therapy and immunotherapy, systemic treatment options for melanoma brain metastases were largely restricted to temozolomide or other chemotherapies, which have modest clinical activity in this setting (13,14). Targeted therapy using BRAF inhibition (dabrafenib or vemurafenib) or combined BRAF/MEK inhibition (dabrafenib plus trametinib) show activity in patients with

melanoma brain metastases that harbor BRAF<sup>V600</sup> mutations, and lead to rapid onset responses of relatively short duration (11,15–17). Among checkpoint inhibitors, ipilimumab (anti-CTLA-4) has documented anti-tumor activity for brain metastases; when used in combination with radiation and/or surgery, it has been shown to improve survival outcomes(18,19). Recent studies also reveal that anti-PD-1 monotherapy (pembrolizumab/nivolumab) and combination ipilimumab and nivolumab, show clinical activity in patients with melanoma brain metastases (20–23). In general, however, data on survival outcomes and clinical prognostic factors of survival in melanoma brain metastases for anti-PD-1 based immunotherapy is sparse, and is largely limited to interventional clinical trial protocols with narrow inclusion criteria (20,22,23). As such, we have studied survival, and prognostic factors of survival, in a real world clinical setting.

Given the poor prognosis for melanoma brain metastases, and the relatively limited therapeutic options available for these patients, being able to accurately predict overall survival is critical for prioritizing treatments. Existing prognostic indices for brain metastases, such as ds-GPA (diagnosis specific-GPA) and RPA (Recursive Partitioning Analysis), do not incorporate prior treatments. Others, including the Basic Score-Brain Metastases (BS-BM) and Score Index for Radiosurgery in Brain Metastases (SIR) only incorporate stereotactic radiosurgery, and do not fully reflect the landscape of prior treatments that patients may have received prior to consideration for anti-PD-1 immunotherapy (24–26).

Several machine learning algorithms (learners) have been developed for survival analysis, including tree-based and ensemble learners (27–31). Random Survival Forests (RSF) are ensemble learners and a non-parametric alternative to the semi-parametric Cox-PH model

that offers several advantages, such as data adaptive incorporation of non-linear relationships of covariates, improved performance in high dimensional settings (large number of predictors relative to sample size), and allow exploration of complex interactions using powerful visualization techniques (29,32,33). However, limited data are available on comparisons of these learners with the traditional Cox-PH model in a clinical prediction modeling context for high dimensional problems. Hence, we have evaluated the predictive performance of tree-based, ensemble learners and Cox-PH models in a benchmark experiment, to allow direct comparison with a goal to identify the best performing learner. Subsequently, the best performing learner was used to identify a parsimonious feature sub-set that yielded the best performance. We incorporated the assessment of performance of existing prognostic brain metastases indices, such as ds-GPA or RPA, both univariably and in combination with other predictors of survival.

## **Methods**

### *Design, setting and eligibility criteria*

A retrospective observational study was performed. The eligibility criteria were broad, given the goal to study prognostic factors of survival in the “real world” clinical setting. Patients had received at least one dose of commercially available or expanded access program-supplied anti-PD-1 based immunotherapy between June-2014 and September-2016 at the Dana-Farber Cancer Institute. Patients also had MRI evidence of brain metastases (treated/untreated) at the time of treatment. Treatments included monotherapy (pembrolizumab every 3 weeks at 2mg/kg, intravenous infusion or nivolumab every 2 weeks at 3mg/kg, intravenous infusion) or combination therapy (Ipilimumab 3 mg/kg intravenous infusion plus nivolumab 1mg/kg intravenous infusion every 3 weeks for 4

cycles, followed by nivolumab 3mg/kg maintenance therapy every two weeks until unequivocal disease progression as determined by the clinician, unacceptable toxicity, or death). Patients who had prior treatment with stereotactic radiotherapy (SRT), Whole Brain Radiotherapy (WBRT), surgical resection, or a combination of radiation and surgery, were included. Prior treatment with investigational immunotherapies/checkpoint-based blockers other than ipilimumab or interferon for metastatic melanoma was permitted, provided the patient did not have brain metastases at earlier exposure.

#### *Ethics statement*

This study was approved by Dana-Farber/Harvard Cancer Center's Institutional Review Committee.

#### *Data collection and outcome*

All baseline, clinical and neuro-radiological data were collected from electronic health records. No adjudication was required for the primary outcome, namely, overall survival (OS). Neuro-radiological variables were retrieved from MRI/CT/PET scan reports at baseline. OS was calculated from the date of administration of mono- or combination immunotherapy, until the date of death or last follow-up. The outcome was censored at the last follow-up, in the absence of death. Patients who received other forms of treatment (radiation/investigational treatments) due to clinical and/or radiological disease progression were not censored, and actual survival duration was recorded. Neurological outcomes were not analyzed.

#### *Model features*

Twenty-two individual features were included in the model based on subject matter knowledge, literature review of known predictors and review of components of existing

validated prognostic indices for brain metastases, including ds-GPA, RPA, BS-BM and SIR (3,24,26,34–38). Baseline features assessed were Karnofsky Performance Score/KPS (<70, 70-80 and 90-100), lactate dehydrogenase (LDH) (>ULN/upper limit of normal or  $\leq$ ULN), presence of  $\geq 4$  brain metastases, neurologic symptoms (focal/non-focal), infratentorial lesion, hemorrhagic brain metastases, spine metastases, mass effect, hydrocephalus, leptomeningeal metastases, presence of  $\geq 4$  extra-cranial metastases, BRAF mutation and treatments received prior to anti-PD-1 based immunotherapy that included SRT alone, WBRT alone, prior immunotherapy, surgical resection/craniotomy alone, surgical resection and SRT, surgical resection and WBRT and prior targeted therapy (BRAF/BRAF plus MEK inhibitors). In addition, age ( $\leq 45$ ,  $>45$ - $<65$  and  $\geq 65$  years), gender, and anti-PD-1 immunotherapy type (monotherapy/combination) were included. The prognostic indices ds-GPA and RPA were included separately (see Tasks).

### **Statistics**

Descriptive statistics were used to summarize baseline demographic and clinical characteristics for the overall group and type of immunotherapy (monotherapy/combination). A complete case analysis was performed for the modeling exercise. The Kaplan-Meier (KM) method was used for survival analysis.

### *Tasks, learners and benchmark experiment*

Five tasks (feature combinations) were assessed. Task-1 had the 22 individual features listed above under model features. Task-2 had ds-GPA as the only feature. Task-3 had the RPA Class alone. Task-4 had 20 features from Task-1 plus ds-GPA (KPS and  $\geq 4$  brain metastases were excluded as these features are components of ds-GPA). Task-5 had 21 features from Task 1, plus RPA Class (but excluded KPS). The learners evaluated were

random survival forests (RSF), survival tree (decision tree), model-based boosting, likelihood-based Cox-Boost (tuned for optimal number of boosting steps) and Cox-PH (see Supplementary Text).

In a benchmark experiment, five learners were compared across five tasks, such that the same training and test sets were used for all learners for direct comparison of the five-fold cross-validated performance metric, C-index (concordance probability between predicted and observed survival; Supplementary Text). Relative performance was assessed based on ranking of the algorithms (calculated from five-fold cross-validated C-index across tasks) and were compared using the non-parametric Friedman test (alpha of 0.05) and post-hoc pairwise comparisons using Nemenyi multiple comparison tests with q approximation;  $p$ -values  $< 0.05$  for pairwise comparisons were considered statistically significant (39). A learner was considered the best if it had the lowest mean rank and was considered significantly better if not connected by a bar to the reference learner (Cox-PH) in the critical differences (CD) plot.

#### *Feature importance and selection, partial dependences and complex interactions*

Predictive features were identified from learners by measures of feature importance. The learner with the best relative performance was used for feature sub-selection. Feature filtering was based on variable importance (VIMP); only filtered features were subject to selection performed by sequential forward selection (Supplementary Text) (40). Nested resampling was applied, wherein features were sub-selected via inner resampling loop and performance assessed through outer resampling loop in a benchmark setup (5-fold cross-validation for each). The feature-subset that yielded the best performance was identified. Prediction error curves were additionally assessed for each of the feature subsets selected

and the Integrated Brier Score (IBS) was computed. The partial dependence of features (adjusted risk) was explored, to identify the relationship of individual features (adjusted for remaining features in the model) on OS. Conditioned partial dependences of top predictive features were studied for anti-PD-1 monotherapy and combination groups separately, to assess whether the trend for the RSF predicted risk was similar (Supplementary Text). We used co-plots to assess complex interactions for features identified in the subset. Because the analysis was performed on a relatively small dataset, learning curves were used to assess the impact of increasing sub-sample size on the predictive performance of learners (sensitivity analysis).

All statistical analyses were performed using R v3.3.3 with “RandomForestSRC”, “ggRandomForest”, “Survival”, “Survminer”, “mboost”, “CoxBoost”, “rpart”, “mlr” and “pec” packages (41–43).

## **Results**

A total of 263 patient charts were reviewed of which 56 eligible patients were included in the final analysis. The maximum follow-up period was 1006 days and the median follow up was 674 days (IQR: 520-829 days). Patients were approximately equally distributed in ds-GPA risk groups 0-1, 2 and 3-4 in the overall cohort; most had received prior radiation for brain metastases (most commonly SRT), more than half had  $\geq 4$  brain metastases with brain metastases predominantly located supratentorially, whereas less than half had neurological symptoms and  $\geq 4$  extra-cranial metastases. Fewer patients who received combination therapy had previously received immunotherapy, most commonly ipilimumab (Table-1). One patient had missing data for BRAF mutation and another had baseline LDH value missing. These patients were only used for KM analysis while for prediction, 54

patients were used for whom complete data was available. In addition, 7/56 (12.5%) patients received radiation and 4/56 (7.14%) underwent surgical resection for brain metastases after baseline.

Survival curves and estimates for the overall cohort and treatment groups are shown in Figure-1 and Table-2.

Absolute performance comparisons from the benchmark experiment (5-fold cross-validated aggregate C-index and 95%CI) for five learners across five tasks are shown in Figure-2A and Supplementary Table-S1. Cox-PH based models had the widest distribution and worse performance which was more apparent in a high dimensional setting (Task-1/Task-4/Task-5) compared to other learners (Figure-2A). Rank based comparisons showed that RSF had the lowest mean rank (best) of 1.3 and Cox-PH the highest mean rank of 4.3; The mean rank was 2.7 for model-based boosting, 2.9 for Cox-Boost and 3.8 for Survival Tree (Figure-2B). The global Friedman rank sum test  $p$ -value was statistically significant (chi-squared=12.37, degrees of freedom=4,  $p$ -value: 0.015). The CD was 2.73 for the Nemenyi test. The CD plot showed a significantly better relative performance for RSF compared to Cox-PH models (RSF vs. Cox-PH rank comparison: 1.3 vs. 4.3, difference:  $3 > CD$ , Nemenyi  $p$ -value=0.023) (Figure-2C and Supplementary Table-S2).

VIMP for tasks which had more than one feature (1/4/5) showed that KPS and  $\geq 4$  brain metastases (Task-1), ds-GPA and  $\geq 4$  ECM (Task-4) and  $\geq 4$  brain metastases and RPA Class (Task-5) were the top two features respectively (Figure-3A). Other baseline predictive features included prior surgical resection and SRT, LDH, neurological symptoms, spine metastases, leptomeningeal metastases, infratentorial lesion and age (Figure-3A and Supplementary Figure-S1). Partial dependence and conditioned partial

dependence plots (mono/combination immunotherapy) for top predictors are shown in Figure-3B/3C and Supplementary Figure-S1. The mean C-index was 0.878 (95%CI:0.822-0.934) for Task-1, 0.867 (95%CI:0.777-0.947) for Task-2, 0.664 (95%CI: 0.560-0.779) for Task-3, 0.878 (95%CI: 0.760-0.961) for Task-4 and 0.741 (95%CI: 0.668-0.838) for Task-5 feature subsets selected by sequential forward selection respectively (Figure-3D). The prediction error curves for the feature subset selected are shown in Figure-3 E. The IBS (Integrated Brier Score) for Task-1 subset was 0.104, Task-2 subset was 0.108, Task 3 subset was 0.146, Task-4 subset was 0.098 and Task-5 subset was 0.122 (Reference IBS was 0.189). Co-plots for ds-GPA, neurological symptoms, prior treatment with surgical resection and SRT and ds-GPA,  $\geq 4$  ECM and LDH levels are shown in Figure-3F. RSF achieved the highest C-index with increasing sample size (Supplementary Figure-S2).

## **Discussion**

### *Overall survival*

Brain metastases prognostic indices, ds-GPA (0-4) and RPA (Class I-III) were studied due to their wide spread use and ease of assessment. Historically the OS has been the lowest for ds-GPA 0-1 and Class III RPA (24,37,38). In our cohort, patients with ds-GPA 0-1 or RPA Class III showed poor OS with anti-PD-1 based immunotherapy. However, median OS in patients with ds-GPA $>1$  or RPA Class I-II was more than three times the historical estimates (Figure-1 and Table 2). Importantly, ds-GPA was developed based on data from a patient population that had newly diagnosed brain metastases who went on to receive radiation and/or surgery (24). In our cohort, despite patients being largely pretreated, survival estimates were much longer than historical estimates (Table-2). Median survival

even in the best non-systemic treatment setting has been reported to be about 8-10 months for this patient population (44).

A retrospective analysis of twenty-six melanoma patients who received nivolumab monotherapy after prior or concurrent treatment with stereotactic radiation for brain metastases showed a median OS of 12 months for non-resected disease and was not reached for resected disease (21). In our cohort, median survival for patients with prior surgery and SRT was about 24 months (Table-2) and this difference was likely due to inclusion of patients with ds-GPA $\leq$ 1 (poor prognosis). Survival estimates in melanoma patients with predominantly asymptomatic brain metastases (mostly RPA Class II) treated with ipilimumab in combination with SRT appeared favorable (median OS:18.5 months) in a retrospective study (18). In comparison, our cohort had about 43% patients who were symptomatic and the median OS for RPA Class II was about 19.5 months. Overall survival among patients with brain metastases from BRAF<sup>V600</sup> mutant melanomas treated with BRAF inhibitors such as vemurafenib was about 9 months, with added risk of radiosensitization in the context of radiation; Immunotherapy is well tolerated with radiation on the contrary (17),(18,21,45–47).

*Comparison of relative performance of machine learning clinical prediction models to Cox-PH models*

An accurate clinical prediction model can help prioritize treatments according to the risk in this setting. We compared predictive performance of machine learning based clinical prediction models with Cox-PH models. RSF had the best relative performance and Cox-PH the worst performance. Survival tree had the lowest performance amongst machine learners evaluated and is consistent with previous reports. Boosting did not show an

improved performance compared to RSF in this study (Figure-2). Our findings confirm that RSF models perform better than Cox-PH models in a high dimensional setting in a clinical prediction modeling context.

*Predictive features and feature subset performance*

A higher predicted risk was apparent for ds-GPA 0–1 and RPA Class 3 based on inflection points in partial dependence analysis (Figure-3B). Individual features which predicted worse survival in RSF(Task-1) were low KPS ( $<70$ ),  $\geq 4$  brain metastases,  $\geq 4$  ECM, spine metastases, high LDH, neurologic symptoms, leptomeningeal disease, infratentorial lesion, and young age ( $\leq 45$  years) while prior surgical resection and SRT predicted improved survival (Figure-3A/3B). Monotherapy or combination had negligible importance relative to other features (Figure-3A). Predictive features identified were consistent with known predictors and apply in the setting of anti-PD-1 treatment for melanoma brain metastases as well(36). Task-1 and 4 feature subsets each had a C-index of 0.878 and Task-2 had a comparable performance (C-index: 0.867) indicating that the single strongest prognostic feature was ds-GPA. RPA alone or RPA based feature subset performed poorly; Task-1 feature subset outperformed Task-3 as indicated by non-overlapping confidence intervals (Figure-3D). These findings are consistent given that RPA is not disease specific. Although ds-GPA alone had good performance, performance maximized, and prediction error minimized with inclusion of prior surgical resection and SRT, neurological symptoms,  $\geq 4$ ECM and spine metastases (Task-4 subset); Comparable results were achieved by KPS,  $\geq 4$  brain metastases, neurological symptoms, LDH and prior surgical resection and SRT subset (Task-2 vs. Task-4 vs. Task-1 subsets; Figure-3D and 3E).

### *Impact of predictive features within treatment groups and exploration of complex interactions using RSF*

A similar trend for RSF estimated mortality was noted for top predictive features for mono and combination therapy. Although the magnitude of effect of the features on mortality were not identical, they were similar with no reversal in trend (Figure-3C). Exploration of complex interactions for features identified on sub-set selection revealed that patients who received treatment with surgical resection and SRT prior to anti-PD-1 had better two-year predicted survival among patients with ds-GPA>1 (low to moderate risk). This was seen in patients with or without neurological symptoms, although patients without neurological symptoms had the best survival. RSF predicted two-year survival was much lower for patients who had  $\geq 4$  ECM irrespective of high LDH (even for ds-GPA>1); the risk was accentuated for ds-GPA 0-1 (Figure-3F).

### *Strengths and Limitations*

We extracted a wealth of information despite analyzing a small data set with many covariates, which is commonly encountered in brain metastases cohorts, by using data adaptive methods. In addition to improved performance, complex relationships and interactions were easily elucidated due to the adaptive nature of the forests.

The main limitation was the absence of external validation given the lack of an external dataset given the relative rarity of this brain metastases cohort which included patients treated with ipilimumab and nivolumab combination. Therefore, we used cross-validation to obtain efficient estimates of generalization performance on a held-out test set (validation set). We used cross-validation at every step of the modeling exercise. Additionally, nested resampling was used to reduce overfitting and provide fair comparisons. The study design

has inherent limitations of retrospective studies where unmeasured covariates cannot be accounted for. Missing data was minimal due to robust documentation and follow-up information. Neurological outcome was not assessed in this study.

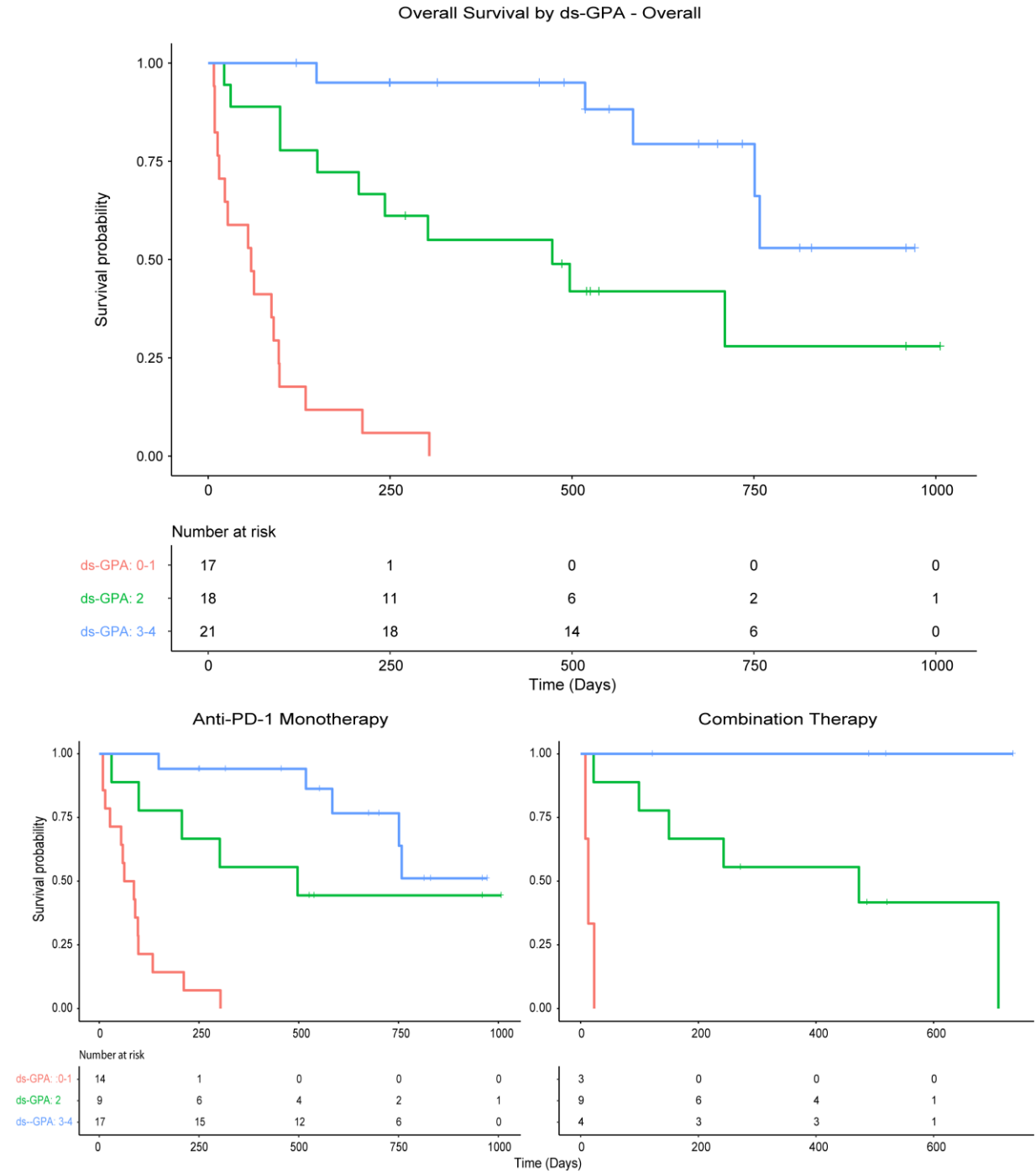
### **Conclusion**

RSF had the best relative predictive performance compared to Cox-PH models in a high dimensional setting. The OS of patients with brain metastases from melanoma treated with anti-PD-1 based immunotherapy who had baseline ds-GPA > 1 was substantially longer than historical estimates in the treated setting (radiation and/or surgery). This study provides key insights into baseline intra-cranial, extra-cranial and prior treatment prognostic factors of survival for anti-PD-1 based immunotherapy in the real world. Baseline ds-GPA had the strongest predictive value among all features in this treatment setting. Prior surgery and SRT predicted improved survival for moderate to low risk patients (ds-GPA > 1). These findings should be externally validated.

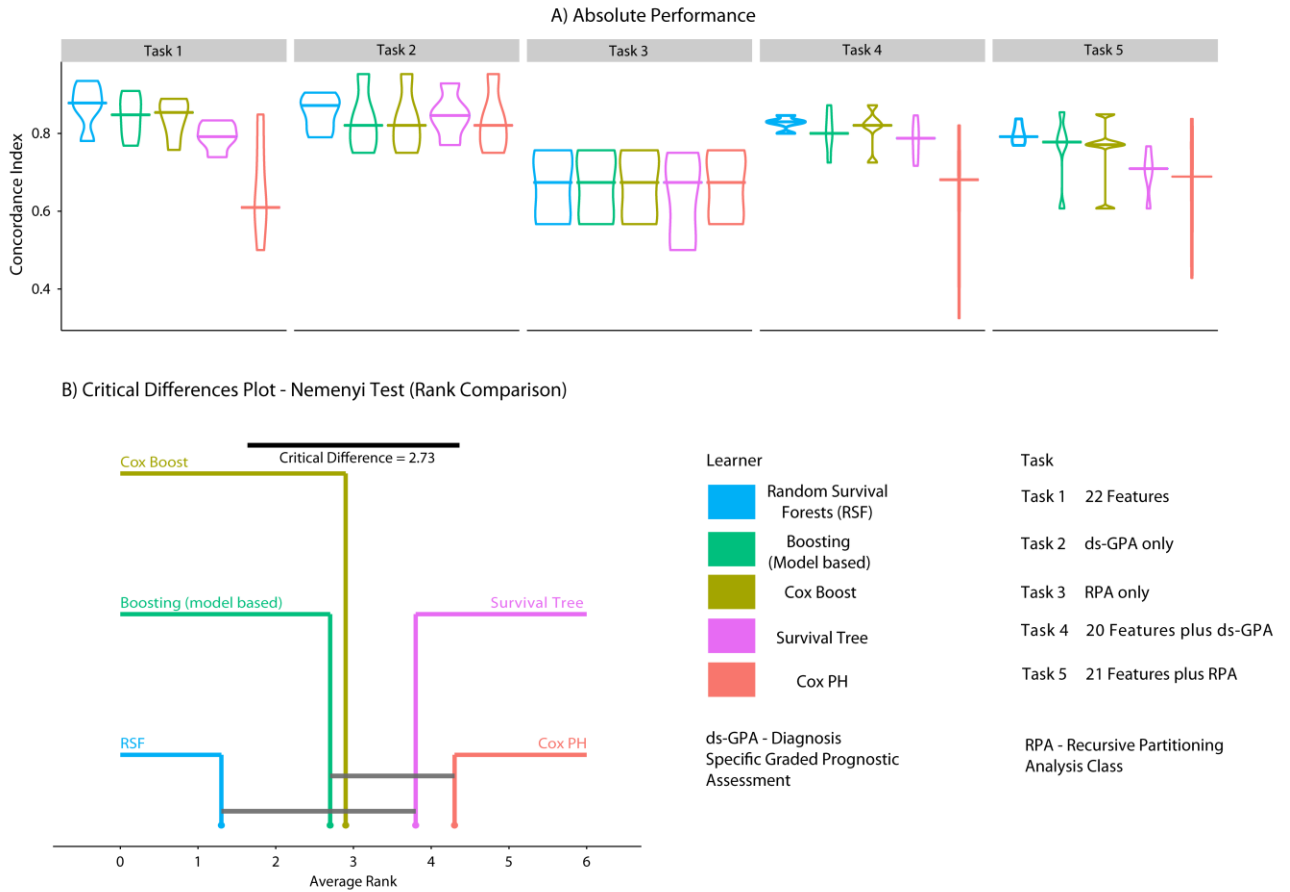
### **Acknowledgements**

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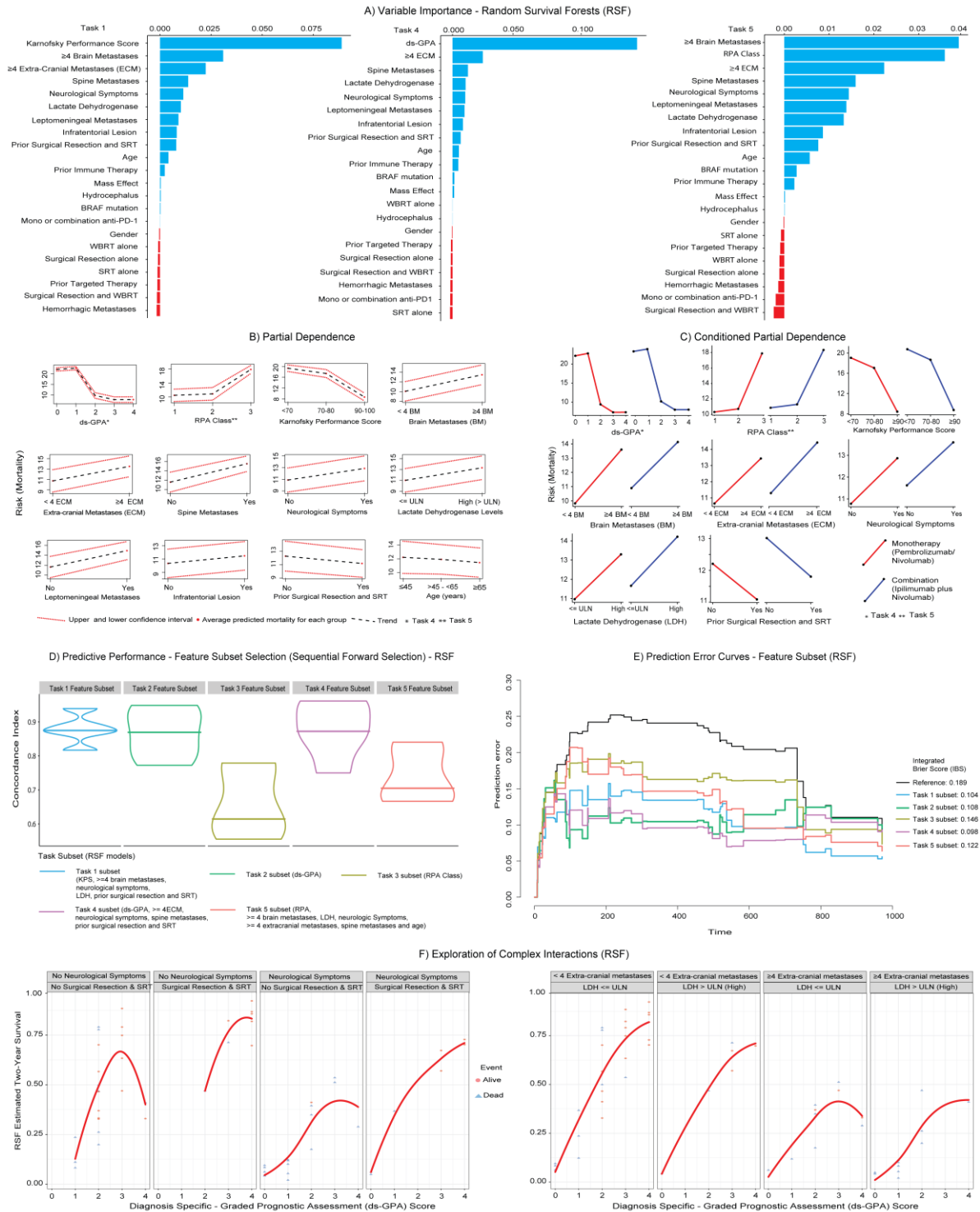
**FIGURES (PROJECT 1)**



**Figure-1. Overall Survival for ds-GPA (Diagnosis Specific Graded Prognostic Assessment) risk groups for the overall cohort and anti-PD-1 based treatment groups (mono and combination therapy).**



**Figure-2. Benchmark experiment – Comparison of performance of machine learning and Cox-PH based prediction models. Absolute performance comparison is shown in A and rank based relative performance comparison is shown in B.**



**Figure-3. Feature importance (A), partial dependences of top features (B), conditioned partial dependence of top features for mono and combination anti-PD-1 immunotherapy (C), predictive performance of feature subset (D), prediction error curves for selected feature-subsets (E) and exploration of complex interactions using Random Survival Forests (F).**

**TABLES (PROJECT 1). Table-1. Baseline characteristics for the overall cohort and anti-PD-1 based treatment groups.**

Baseline characteristics	Overall N=56 (%)	Anti-PD-1 monotherapy N=40 (%)	Combination (Ipilimumab and Nivolumab) N=16 (%)
Age in years (Mean and SD)	59 (15.3)	60.4 (15.6)	55.6 (14.4)
Gender	Male: 35 (62.5) Female: 21 (37.5)	Male: 28 (70) Female: 12 (30)	Male: 7 (43.75) Female: 9(56.25)
Race	Caucasian: 54 (96.4) Asian: 1 (1.8) Unknown: 1 (1.8)	Caucasian: 38 (95) Asian: 1 (2.5) Unknown: 1 (2.5)	Caucasian: 16 (100) Asian: 0 (0)
Type of melanoma	Cutaneous: 38 (67.8) Occult: 16 (28.6) Mucosal: 2 (3.6)	Cutaneous: 27(67.5) Occult: 13 (32.5) Mucosal: 0 (0)	Cutaneous: 11(68.75) Occult: 3 (18.75) Mucosal: 2 (12.5)
LDH (Lactate Dehydrogenase) (N=55)	High (>ULN): 17 (30.9) ≤ULN: 38 (69.1)	High (>ULN): 11/40 (27.5) ≤ULN: 29/40 (72.5)	High: 6/15 (40) ≤ULN: 9/15 (60)
Diagnosis specific -Graded Prognostic Assessment (ds-GPA)	0 -1: 17 (30.4) 2: 18 (32.1) 3-4: 21 (37.5)	0 -1: 14 (35) 2: 9 (22.5) 3-4: 17 (42.5)	0 -1: 3 (18.75) 2: 9 (56.25) 3-4: 4 (25)
RPA (Recursive Partitioning Analysis) Class	I: 4 (7.14) II: 41 (73.21) III:11 (19.64)	I: 1 (2.5) II: 30 (75) III: 9 (22.5)	I: 3 (18.75) II: 11 (68.75) III: 2 (12.5)
Karnofsky Performance Score (KPS)	90 – 100: 35 (62.5) 70-80: 10 (17.86) <70: 11 (19.64)	90 – 100: 23 (57.5) 70-80: 8 (20) <70: 9 (22.5)	90 – 100: 12(75) 70-80: 2 (12.5) <70: 2 (12.5)
BRAF mutation (n=55)	21 (38.2)	13/39 (33.3)	8/16 (50)
NRAS mutation (n=39)	9 (23.1)	7/30 (23.3)	2/9(22.2)
<b>Prior treatments</b>			
Prior immunotherapy	33 (58.9)	29 (72.5)	4 (25)
• Prior anti-CTLA4 based therapy	28 (50)	26 (65)	2 (12.5)
Prior investigational agent	9 (16.1)	8 (20)	1 (6.25)
Prior chemotherapy	4 (7.1)	4 (10)	0 (0)
Prior targeted therapy	15 (26.8)	10 (25)	5 (31.3)
Prior treatment with SRT alone	14 (25)	11 (27.5)	3 (18.75)
Prior treatment with WBRT alone	12 (24.43)	5 (12.5)	7 (43.75)
Prior surgical resection alone	3 (5.4)	3 (7.5)	0 (0)
Prior surgical resection and SRT	15 (26.8)	11 (27.5)	4 (25)
Prior surgical resection and WBRT	7 (12.5)	6 (15)	1 (6.3)
<b>CNS and extra-cranial metastases</b>			
Brain metastases (BM)	≥ 4 BM: 31 (55.4)	≥ 4 BM: 21 (52.5)	≥ 4 BM: 10 (62.5)
Neurologic symptoms	24 (42.9)	20 (50)	4 (25)
Infratentorial lesion	26 (46.4)	19 (47.5)	7 (43.75)
Leptomeningeal metastases	6 (10.7)	6 (15)	0 (0)
Spine metastases	9 (16.1)	7 (17.5)	2 (12.5)
Hemorrhagic metastases	25 (44.6)	18 (45)	7 (43.75)
Hydrocephalus	3 (5.4)	2 (5)	1 (6.25)
Mass effect	13 (23.2)	7 (17.5)	6 (37.5)
Extra cranial metastases (ECM)	≥ 4 ECM: 23 (41.1)	≥ 4 ECM: 16 (40)	≥ 4 ECM: 7 (43.75)

**Table-2. Overall Survival by ds-GPA, RPA, mono and combination anti-PD-1 treatments and prior radiation and surgery.**

Brain metastases prognostic score and immunotherapy type	No. of patients	Median (days)	Inter Quartile Range in days; NR: Not Reached)		Median in months	Historical median survival reported by brain metastases prognostic indices for melanoma treated with radiation and/or surgery (24,38)	
			25 <sup>th</sup> percentile	75 <sup>th</sup> percentile			
<b>ds-GPA for melanoma (scored based on KPS and cranial metastases)</b>							
0-1	17	59	13	97	<b>1.9 months</b>	3.4 months	
2	18	473	150	NR	<b>15.5 months</b>	4.7 months	
3-4	21	NR	584	NR	<b>&gt; 32 months (NR)</b>	~11 months (8.77 to 13.33 months)	
Overall	56	497	150	751	<b>16.3 months</b>	6.7 months	
<b>RPA Class</b>							
Class I	4	NR	NR	NR	<b>&gt;26 months</b>	10.5	
Class II	41	584	243	NR	<b>19.2 months</b>	5.9	
Class III	11	63	15	135	<b>2.1 months</b>	1.8	
Overall	56	497	150	751	<b>16.3 months</b>	5.5 months	
Immunotherapy type	No. of patients	Median (days)	IQR in days; NR: Not Reached)		Median in months	Number of deaths	One-year event rate (Overall - 59.1%)
			25 <sup>th</sup> percentile	75 <sup>th</sup> percentile			
Monotherapy	40	497	134	758	16.43 months	24/40	56.56%
Combination	16	473	23	NR	15.55 months	9/16	67.36%
Prior treatment for brain metastases	No. of patients	Median (days)	IQR in days; NR: Not Reached)		Median in months	-	
			25 <sup>th</sup> percentile	75 <sup>th</sup> percentile			
Prior surgical resection and SRT	15	758	99	NR	24.92 months	-	

## **SUPPLEMENTARY MATERIAL (PROJECT 1)**

Supplementary Text – 1

Supplementary Tables – S1 and S2

Supplementary Figures – S1 and S2

### **Supplementary Text**

*Machine learning modeling approach*

*Random Survival Forests (RSF) modeling approach*

The Random Survival Forests is an extension of random forests to model time to event outcomes with right censoring(1,2). The advantage of using an RSF method is in one pass, both model building, and internal validation can be achieved because RSF builds models in the training set and tests it on the out of bag “test” set. Randomization is introduced in two steps in the RSF algorithm. The first step involves random selection of “B” bootstrap samples and the second step involve random selection of “p” number of covariates at each node in each tree. In each boot strap sample drawn randomly from the main data, it is split into two parts. The first part constitutes 63% and is the training set in which the RSF model is built and the remaining 37% represents the out of bag “test” set on which the model is tested, and error rate computed and averaged across all trees. This method has been shown to reduce variance and bias with improved performance (1). Log rank splitting rule was used for the RSF models and 2000 trees (B) were grown in the forest. The optimal value for “mtry” tuning parameter was assessed through grid search.

*Other machine learning and regularized modeling approaches*

Boosting for survival outcome (right censored data) is an extension of the gradient and model based boosting methods which are machine learning algorithms relevant in the

analysis of high dimensional data as they prevent overfitting and are also suitable in situations where conformation to the proportional hazards assumption is unlikely (3). Two boosting methods were used to build clinical prediction models in this study. These included boosting through the componentwise version, an ensemble learner which improves performance by aggregating weak learners through an iterative process by minimizing pre-specified loss function and Cox-boost (based on partial log likelihood) tuned for optimal boosting steps. Survival tree is an extension for the decision tree model to handle right censored data (4).

#### *Model performance metric(s)*

The predictive performance of learners was assessed using 5-fold cross validated C-index. The C-index is the fraction of all pairs of subjects whose predicted survival times are correctly ordered among all subjects that can be ordered. In short, it is the probability of concordance between predicted and observed survival. Additionally, the cross-validated prediction error curves were used to assess the prediction error for the feature subset selected.

#### *Extraction of features of importance for RSF*

The feature importance measure used to identify top predictors for RSF model was variable importance (VIMP). VIMP represents the prediction error of the ensemble constructed originally minus a newly constructed ensemble obtained by randomizing “x” assignments (1,5).

### *Feature sub-selection strategy*

The best performing learner (based on rank comparison) was used for performing feature sub-selection. Sequential forward selection was performed by using an alpha of 0.001 (increase in performance for adding a feature in forward selection starting with an empty model)(6). Feature filtering was performed based on optimal number of features identified through 5-fold cross-validation based on VIMP. Filtered tasks with optimal number of filtered features were used for final sequential forward selection (At each step, a feature that increased the learner performance ( $\alpha=0.001$ ) was included, starting from a model with no features) performed through an internal resample loop and performance cross-validated through outer resample loops using 5-fold cross-validation for both resampling loops (nested resampling). A benchmark setup was used for this step to allow direct comparison of performances across all feature subsets (Task 1 to Task 5 feature subsets). Further, prediction error curves were used and the Integrated Brier Score (IBS) was computed for the feature subsets selected.

### *Exploration of RSF for studying relationship of variables with survival outcome*

RSF analysis was explored further for studying the relationship of predictors on survival. Variable dependence plots and the partial dependence plots (risk adjusted) were examined to assess the partial dependence of each covariate on predicted mortality (partial dependence is calculated for a covariate of interest after integrating out the effect of all other covariates). In addition, conditioned partial dependence plots in RSF were plotted for mono and combination anti-PD1 immunotherapy (stratified partial dependence) for top predictors to assess if the trend for RSF predicted risk (mortality) was similar across the two treatment groups.

### *Exploration of complex inter-relationships or interactions*

Complex inter-relationships or interactions including multi-dimensional interactions were assessed using co-plots for predictive features identified by feature selection methods. These provide additional information of how the predicted survival is dependent on more than two features and were assessed to identify inter-relationships (5).

### **Supplementary References**

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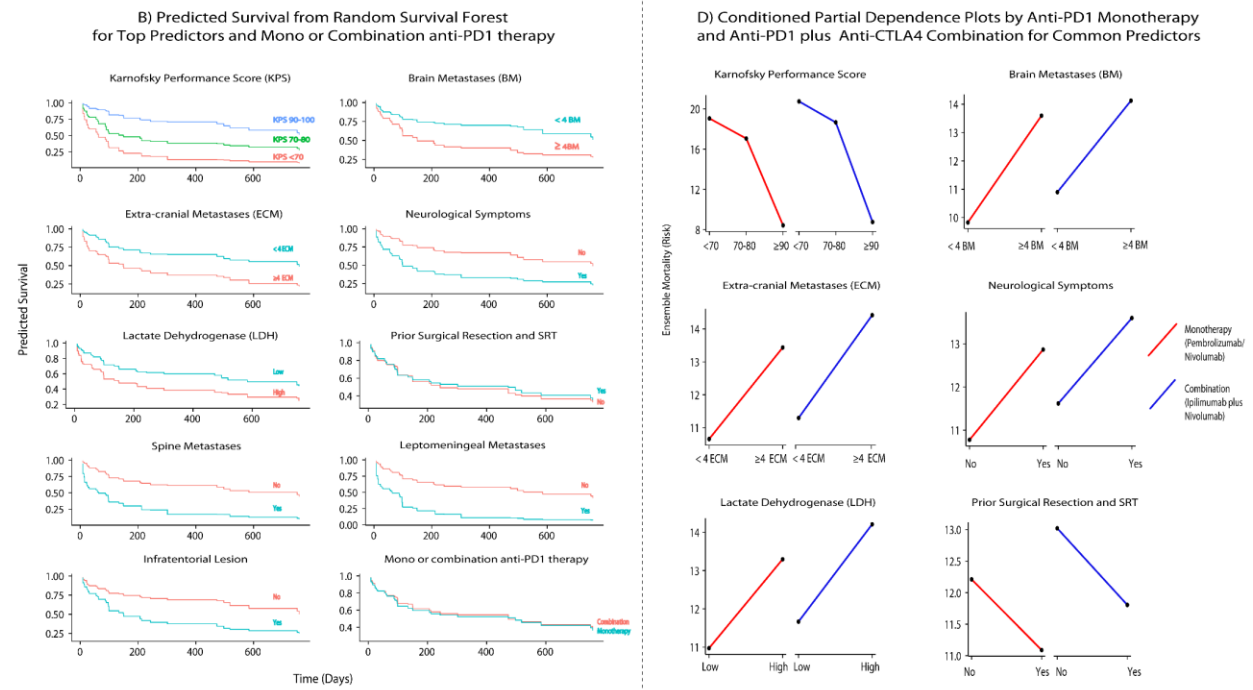
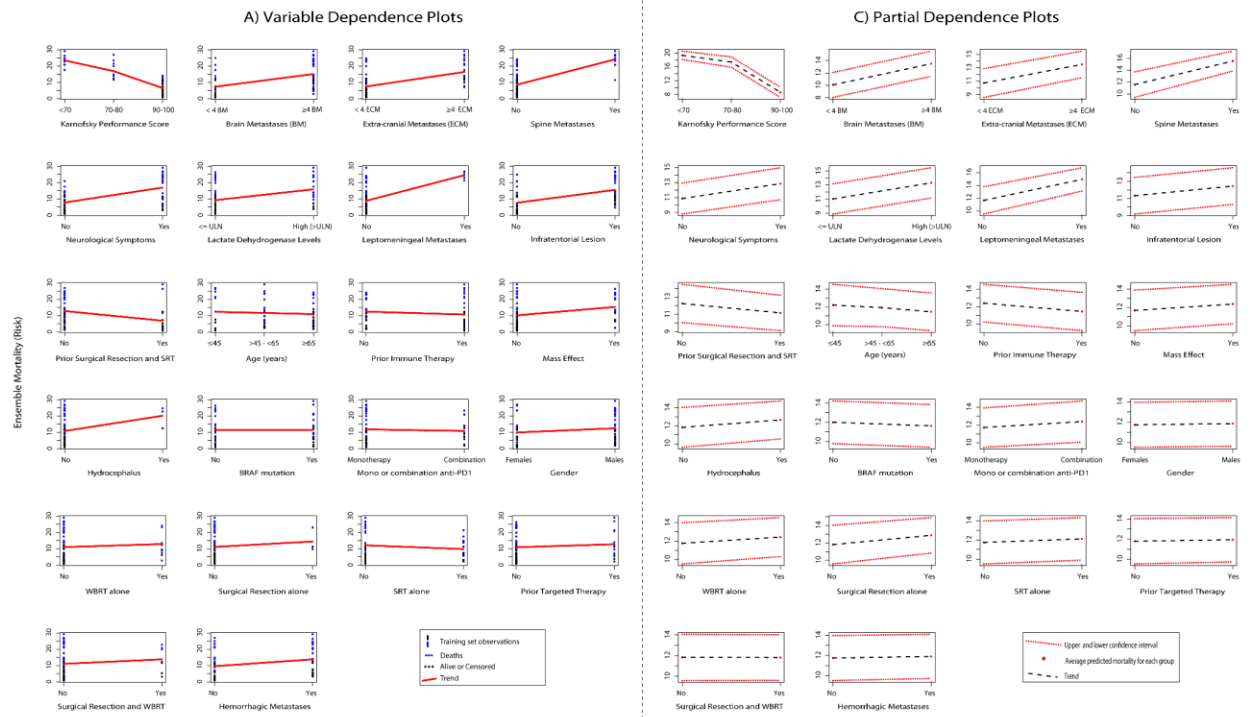
## Supplementary Tables

**Supplementary Table-S1: Performance (test) of learners across 5 Tasks from the benchmark experiment.**

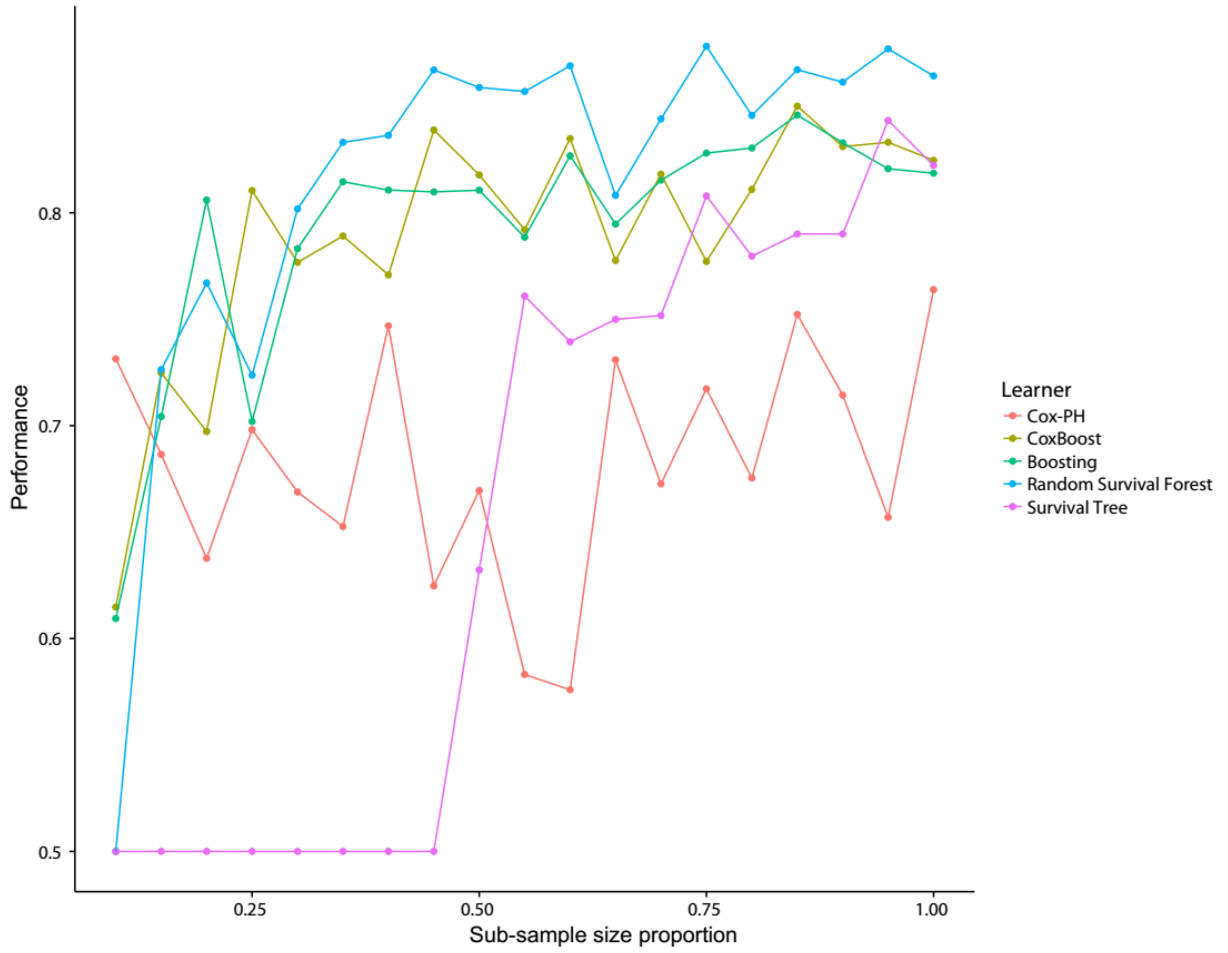
Task	Learner	Aggregate C-index (Mean)	95% Confidence Intervals (CI)	
			LCI (Lower Confidence Interval)	UCI (Upper Confidence Interval)
Task 1 (22 Features)	Random Survival Forest (RSF)	0.873	0.789	0.932
	Boosting (model)	0.843	0.772	0.907
	Cox Boost	0.840	0.764	0.888
	Survival Tree	0.792	0.743	0.832
	Cox-PH	0.660	0.511	0.837
Task 2 (1 Feature/ds-GPA)	RSF	0.853	0.793	0.902
	Boosting (model)	0.831	0.753	0.943
	Cox Boost	0.831	0.753	0.943
	Survival Tree	0.846	0.775	0.922
	Cox-PH	0.831	0.753	0.943
Task 3 (1 Feature/RPA)	RSF	0.664	0.568	0.756
	Boosting (model)	0.664	0.568	0.756
	Cox Boost	0.664	0.568	0.756
	Survival Tree	0.633	0.503	0.747
	Cox-PH	0.664	0.568	0.756
Task 4 (20 Features and ds-GPA)	RSF	0.823	0.803	0.845
	Boosting (model)	0.803	0.731	0.868
	Cox Boost	0.814	0.734	0.868
	Survival Tree	0.782	0.72	0.842
	Cox-PH	0.604	0.339	0.811
Task 5 (21 Features and RPA)	RSF	0.797	0.770	0.834
	Boosting (model)	0.762	0.623	0.849
	Cox Boost	0.755	0.623	0.842
	Survival Tree	0.700	0.615	0.763
	Cox-PH	0.661	0.440	0.835

**Supplementary Table-S2: Pairwise comparisons using Nemenyi multiple comparison test with q approximation for unreplicated blocked data.**

	<b>Random Survival Forest</b>	Boosting (Model)	Cox Boost	Survival Tree
Boosting (Model)	0.628	-	-	-
Cox Boost	0.497	1.000	-	-
Survival Tree	0.091	0.807	0.897	-
<b>Cox-PH</b>	<b>0.023</b>	0.497	0.628	0.987



**Supplementary Figure-S1. Variable dependence (A), predicted survival (B), partial dependence (C) and conditioned partial dependence plots (D) by mono or combination immunotherapy based on exploration of RSF analysis (Task 1).**



**Supplementary Figure-S2. Learning curve analysis for test set performance (5-fold cross validated C-index) of learners for Task 4 (ds-GPA plus 20 individual features).**

## **PROJECT 2**

### **Title: Complex Inter-relationship of Body Mass Index, Gender and Serum Creatinine on Survival: Exploring the Obesity Paradox in Melanoma Patients Treated with PD-1 Blockade**

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**Short Title:** Obesity Paradox, Metastatic Melanoma and PD-1 Blockade

**Key Words:** Anti-PD-1, Melanoma, Body Mass Index, Creatinine, Obesity Paradox

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**Number of Figures: 4; Number of Tables: 2; Number of Supplementary Material: 1**

(Figures: 12; Tables: 1; Text)

**Conflict of Interest**

GSN: Former employee of Biocon.

AEW: None to declare

EIB: Research funding paid to the institution – BMS (Bristol-Myers Squibb) and Merck.

RH: Grant support from BMS.

FSH: Consultant to Merck, BMS, Novartis and Genentech.

JDS: Research funding paid to the institution – BMS, Merck; Scientific Advisory Board - BMS, AstraZeneca, Debiopharm, Nanobiotix; Consulting - Tilos.

PAO: Research funding paid to the institution - BMS, Merck, AstraZeneca, Celldex, CytomX, Neon Therapeutics, ARMO Biosciences; Scientific Advisory Board: BMS, Merck, Genentech, Pfizer, Novartis, CytomX, Celldex, Neon Therapeutics.

## **Abstract**

**Background:** Obesity paradox (improved survival in overweight/obese patients compared to normal weight) is well documented in cancer but not in metastatic melanoma. We characterized the relationship of Body Mass Index (BMI) with survival outcomes and explored complex interactions in the context of PD-1 blockade.

**Methods:** Unresectable or metastatic melanoma patients who received at least one dose of pembrolizumab, nivolumab, or nivolumab plus ipilimumab (combination) from June-2014-September-2016 were included in this retrospective cohort study. Overall Survival and Progression Free Survival were the main outcomes. Analysis was performed using Random Survival Forests (RSF) and multi-variable Cox Proportional Hazards models.

**Results:** A “U” shaped relationship was noted for pre-treatment BMI where overweight/Class-I ( $25 < \text{BMI} < 35 \text{ kg/m}^2$ ) obese patients had a lower risk of mortality (HR:0.26; 95%CI:0.1-0.71;  $p$ -value=0.008) and progressive disease (HR:0.43; 95%CI:0.19-0.95;  $p$ -value:0.038) compared to normal-weight ( $18.5 < \text{BMI} < 25 \text{ kg/m}^2$ ) (Total N=139). Exploration of interactions (RSF) showed that the association was predominantly driven by males; it was attenuated in patients with serum creatinine  $< 0.9 \text{ mg/dL}$ , who were predominantly females. Findings were similar for anti-PD-1 monotherapy( $n=79$ )/combination( $n=60$ ).

**Conclusions:** The findings support the presence of “obesity paradox” where overweight/Class-I obesity was associated with substantially lower risk of mortality and progressive disease compared to normal weight; this association was driven predominantly by males who largely had higher serum creatinine levels (surrogate for skeletal muscle mass in the setting of metastatic disease). These findings suggest that instead of BMI alone,

sarcopenia (low skeletal muscle mass) or direct measures of body mass composition may be more suitable predictors of survival in melanoma patients treated with PD-1 blockade.

## **Introduction**

Anti-Programmed Death-1 (PD-1) based immunotherapies have led to a drastically improved 5-year survival rate in patients with advanced melanoma (1–4). Combination therapy with ipilimumab and nivolumab result in higher response rates albeit with significant toxicity (5). Despite this improved outlook, many patients have either primary resistance or develop acquired resistance (6). While tumor PD-L1 expression, mutational/neoantigen load, and IFN-gamma signatures have some utility as predictors of outcome with checkpoint inhibitors (7–9), clinical predictors also may have value for the identification of patient subgroups who have higher survival and/or response rates to these therapies.

Nearly 70% of the adult population in the United States are either overweight or obese according to the CDC/National Center for Health Statistics and has reached epidemic proportions (10). While obesity has generally been shown to be a risk factor for many chronic diseases (11–13) and solid cancers such as colon cancer, esophageal carcinoma and renal cell carcinoma (14), it has been associated with improved survival in cancers such as renal cell carcinoma (15), colorectal cancer (16) and non-small cell lung cancer (17). Observation of improved survival outcomes among overweight/obese patients compared to normal weight patients has been referred to as the “obesity paradox” (18).

Several hypotheses have been posed to explain the obesity paradox observed in chronic diseases and cancer. Reverse causality has been commonly proposed, a phenomenon where previously overweight/obese patients are classified as normal weight at study baseline due

to rapid cancer related weight loss (18). Methodological reasons cited for explaining the paradox include detection bias, selection bias, less aggressive disease among obese patients (15), confounding and inability of BMI to distinguish well between adiposity and lean body mass (18,19). Many of the studies exploring obesity paradox (non-melanoma indications) have limitations as the extent or differences in co-morbidities, medications for co-morbidities, differences in body mass composition (or surrogate markers), lifestyle habits or the influence of preceding disease related weight loss on survival were not adequately accounted for (18).

The relationship of BMI with survival outcomes is not characterized in metastatic melanoma and is unknown in the setting of anti-PD-1 based immunotherapy, which has emerged as the standard of care; as such we studied the relationship of pre-treatment BMI on overall survival, progression free survival and clinical benefit outcomes in the context of anti-PD-1 based treatment either as monotherapy (pembrolizumab or nivolumab) or nivolumab combined with ipilimumab (anti-CTLA-4) treatment for advanced melanoma in a real-world setting.

We collected extensive data on baseline characteristics and performed time fixed analysis using Random Survival Forests (RSF) and Random Forests (RF) given distinct advantages of these methods in incorporating non-linear relationships, handling multi-collinearity, inclusion of covariate interactions and ability to handle high dimensional data under a non-parametric framework with better or comparable performance to Cox-PH models (20–24). The adaptive nature of the forests and powerful visualization techniques which uncover complex patterns and inter-relationships/higher order interactions (20) with ease were of

interest to elucidate the potential factors driving the obesity paradox finding, if any, in this treatment setting. We confirmed the findings with conventional analysis.

## **Methods**

### *Patient population and study design*

Unresectable or metastatic melanoma patients who received at least one dose anti-PD-1 monotherapy (pembrolizumab or nivolumab) or combination (nivolumab plus ipilimumab) treatment from June 2014- September 2016 outside of a clinical trial were included in this retrospective cohort study. Patients with brain metastases prior to the first dose of anti-PD-1 monotherapy or combination (with ipilimumab) treatment were excluded. Prior exposure to investigational or approved immune checkpoint inhibitors (ICI) were not allowed except in the case of anti-CTLA-4 based therapies.

### *Ethics Statement*

Approval for the study was obtained from the Dana-Farber/Harvard Cancer Center Institutional Review Committee.

### *Outcomes*

The primary outcome was Overall Survival (OS). OS was calculated from the date of first dose of anti-PD-1 treatment (monotherapy/combination) until the date of death. The outcomes of patients who did not have an event at the last follow up were censored. Secondary outcomes included progression free survival (PFS) and durable clinical benefit as defined previously (8).

### *Model covariates*

We retrospectively collected data from the electronic health records. Baseline covariates included disease related characteristics, disease severity measures, co-morbidities, co-

medications, lifestyle habits, clinical chemistry and blood count profiles. A total of 42 features were included in RSF/RF models: age, gender, race, melanoma type, BRAF mutation, NRAS mutation, stage, Karnofsky Performance Score (KPS), Lactate Dehydrogenase (LDH), ever smokers, current drinkers, prior treatments (anti-CTLA-4/Immunotherapy, chemotherapy, radiation, targeted therapy), Charlson's Comorbidity Index (25,26), diabetes, hypertension, hyperlipidemia, chronic kidney disease (CKD)(27), cardiovascular disease (CAD/CHF/MI/AF), autoimmune/immune mediated disorders, co-medications (Anti-platelet agents such as aspirin/clopidogrel, anti-hypertensive medications (any), ACE inhibitors (Angiotensin Converting Enzyme) or ARBs (Angiotensin Receptor Blocker), metformin, statins and oral steroids), albumin, ANC (absolute neutrophil count), ALC (absolute lymphocyte count), hemoglobin, serum creatinine, eGFR (estimated glomerular filtration rate based on Cockcroft-Gault formula (27,28)), fasting glucose, alkaline phosphatase, ALT (Alanine transaminase), AST (Aspartate transaminase), type of anti-PD-1 based immunotherapy (monotherapy/combination).

### *Statistical Analyses*

Time fixed analysis was performed by RSF for survival outcomes and RF for the binary outcome of durable clinical benefit (See Supplementary Text). The minimal depth criteria and variable importance (VIMP) filter methods were used to assess the importance of BMI among other features studied. The threshold for minimal depth filtering was minimal depth below the mean value. Variables with positive VIMP values were considered to have predictive value (Refer Supplementary Text). A total of 42 features deemed clinically important by expert knowledge (listed above) were assessed in the RSF and RF models;

the number of features included for each individual tree was set at 7 and 2000 trees were grown in the forest. No feature selection was performed.

Partial dependence (adjusted risk estimate) plots were used to assess the relationship of BMI as a continuous variable on survival and clinical benefit outcomes. Subsequently, interactions were assessed by co-plots plotted as heat maps for the adjusted risk estimate (risk for survival outcomes and probability of achieving DCB for binary outcome). Two-way, three-way and four-way interactions were explored for features of importance and clinically relevant features as deemed by expert clinical knowledge, by examining partial dependence plots (for individual features) and baseline characteristics to find potential explanations or mechanisms for the observed relationship of BMI with outcomes. Missing covariate data was handled by forest imputation for analyses in RSF and RF. Discrimination of the model was evaluated using the average concordance index (C-index) within a 10-fold cross-validation scheme. RSF has previously been used to help explain paradoxical findings of association of high BMI and improved survival in cardiovascular disease (20). The packages used were “RandomForestsSRC”, “ggRandomForestsSRC”, “mlr”, “survminer”, “nephro” and “ggplot2” in R version 3.3.3 (29).

Results were confirmed by univariate and multi-variable Cox-PH model for survival outcomes and logistic regression for durable clinical benefit outcome (baseline characteristics with imbalance across BMI groups or covariates deemed clinically important in the context of studying BMI on survival outcomes were adjusted for; see Table 2 footnote). No imputation was performed for missing covariate data for multi-variable Cox-PH/logistic regression (complete case analysis).

### *Sensitivity Analyses*

BMI measured within the past three to six months (the earliest measure available within this window period) was analyzed in place of BMI measured at baseline (pretreatment BMI just prior to receiving anti-PD-1 based immunotherapy) by RSF/RF to assess if the results and/or conclusions obtained were similar to primary analysis with pre-treatment BMI, to account for patients crossing over to the lower weight category due to disease related or other causes of weight loss. The CKD-EPI equation (30) based eGFR as a continuous covariate was included instead of Cockcroft-Gault formula to assess if the findings were similar.

### **Results**

A total of 139 patients (79 patients treated with anti-PD-1 monotherapy and 60 patients treated with ipilimumab plus nivolumab) were included in the analysis after reviewing 263 patient records. Overall, the patient population mostly consisted of Caucasians with cutaneous metastatic melanomas (excluding brain metastases) with about 72% patients being overweight or obese ( $BMI \geq 25 \text{ kg/m}^2$ ) and was comparable to the US population distribution of BMI (10). The overweight/Class I obese group had a higher proportion of males, diabetes, hypertension, hyperlipidemia and more commonly received anti-hypertensives, ACE/ARB inhibitors, statins compared to patients with  $BMI < 25 \text{ kg/m}^2$  (Table-1). The overweight/Class I obese group had higher serum creatinine concentrations at baseline compared to patients with  $BMI < 25 \text{ kg/m}^2$  and Class II/III obese patients (Table-1).

After a median follow up: 760 days (IQR: 522 - 910), 25/38 patients with  $BMI < 25$  had died (1-year event-rate: 51.6% (95%CI: 34.9% - 76.4%)), 27/86 patients who were

overweight/Class I obese had died (1-year event-rate: 20.9% (95%CI: 14.3% - 30.5%)) and 9/15 patients who were Class II/Class III obese had died (1-year event-rate: 43.3% (95%CI: 22.5% - 83.2%)). The median OS was not reached (NR) for overweight/Class I obese patients. Median OS was 530 days (IQR: 157 days – 985 days) for patients with BMI<25 kg/m<sup>2</sup> and was 458 days (IQR: 152 days – NR) for Class II/III obese patients. The median PFS was 673 days (IQR: 109 - 1126) for overweight/Class I obese patients, was 135 days (IQR: 75 days – 463 days) for patients with BMI<25 kg/m<sup>2</sup> and was 168 days (IQR: 87 days – 377 days) for Class II/III obese patients. The KM survival curves (OS and PFS) are shown in Fig 1-C/1-F. The KM survival graphs stratified by treatment and gender are shown in Supplementary Fig-1

#### *RSF/RF findings*

Minimal depth statistic in RSF ranked BMI the top 8<sup>th</sup> predictor among fifteen predictors (of 42 features) of OS (KPS was ranked 1) and the VIMP for BMI was positive (Supplementary Fig-2). The mean C-index was 0.8003 for OS. Partial dependence plots in RSF analysis showed a “U” shaped relationship for pretreatment BMI with risk of mortality and progressive disease and an “inverted U” shaped relationship for probability of achieving DCB (Fig-1A/1D/1G). These findings were similar in both mono and combination therapies (Fig-1B/1E/1F). Partial dependence derived cutoffs/inflection points corresponded to WHO based BMI categories wherein, overweight or Class I (25-<35 kg/m<sup>2</sup>) obese patients had a lower predicted risk of mortality and progressive disease and higher probability of achieving DCB compared to normal BMI (18.5-<25 kg/m<sup>2</sup>) and Class II/III obese patients ( $\geq$ 35 kg/m<sup>2</sup>). Underweight patients had the highest risk of mortality and progressive disease (Fig-1A/1D/1G).

### *Exploration of complex interactions*

A gender driven difference in survival and clinical benefit outcomes was apparent in coplots, where overweight/Class I obese males had a lower predicted risk of worse outcomes than overweight/Class I obese females (Fig-2A/3A/4A). Further, an interaction was noted between BMI and serum creatinine where the obesity paradox was attenuated for serum creatinine levels  $< 0.9$  mg/dL and more strongly for levels  $< 0.7$  mg/dL (Fig-2B/3B/4B). A three-way interaction of BMI, serum creatinine and gender revealed that the obesity paradox was attenuated for both genders if serum creatinine  $< 0.9$  mg/dL (Fig-2C/3C/4C). These findings were similar for both mono and combination treatments (Fig-2D/3D/4D). Serum creatinine (VIMP rank of 6 and minimal depth rank of 14) was predictive in both filter methods while gender was predictive based on positive VIMP but was above the threshold for minimal depth criterion (Supplementary Fig-2). Examination of the partial dependence of serum creatinine on OS revealed a “L” shaped relationship with survival outcome where patients having levels  $< 0.9$ mg/dL had a high risk of mortality and levels lower than  $0.7$  mg/dL had the highest risk of mortality (Fig-2E/2F). Gender based density distribution of serum creatinine within the three BMI risk groups showed that most females had serum creatinine  $< 0.9$  mg/dL (Fig-2G). Baseline characteristics (Table-1 and Supplementary Table 1) grouped by serum creatinine ( $<$  and  $\geq 0.9$  mg/dL) showed only 13.3% of patients with serum creatinine  $\geq 0.9$  mg/dL were females and 87.7% males.

Exploration of other gender-based interactions of BMI and serum creatinine with co-morbidities, lifestyle habits, co-medications for co-morbidities, BRAF, NRAS mutations, prior therapies for melanoma (targeted, immunotherapy, chemotherapy and radiation) and

interaction of BMI with clinical chemistry parameters, disease severity and other demographics did not provide strong alternative explanations for gender-based difference (Supplementary Fig-3-8 and Text). Gender based density distribution of hemoglobin and albumin for BMI and creatinine risk groups are shown in Supplementary Fig-9.

#### *Cox-PH/logistic regression and sensitivity analyses findings*

The findings from Cox-PH and logistic regression (univariate and multi-variable) largely supported the results from RSF/RF and were statistically significant (Table-2). BMI measured within 3 to 6 months showed a similar relationship for survival outcomes as for pre-treatment BMI measured just prior to first dose of anti-PD-1 immunotherapy (Supplementary Fig-10). Primary findings were similar when CKD-EPI based eGFR was used as a continuous covariate in RSF analysis in place of Cockcroft-Gault based eGFR (Supplementary Fig-11).

### **Discussion**

#### *Nonlinear relationship of BMI with survival and inter-relationship of BMI and serum creatinine levels*

The relationship of BMI with survival was not monotonic and showed a “U” shaped relationship where underweight or normal weight patients were at high risk of poor outcome and less likely to achieve durable clinical benefit. Overweight/Class I obese patients had the lowest risk. The protective association noted with overweight/Class I obese patients compared to normal weight group was not observed for Class II/III obesity (Fig-1/Table-2). These findings support the presence of “obesity paradox” albeit for overweight/Class I obesity indicating two themes. First, having low lean body mass is a risk factor for poor outcome and second, high adiposity is also associated with adverse

outcome; both observations have been documented in other cancers such as colorectal cancer before (16,31,32). However, concerns were raised that BMI does not accurately indicate either lean mass or adiposity (33). Recent studies have shown the importance of assessing skeletal muscle mass/body mass composition to predict survival in cancers as even obese patients can have underlying sarcopenia (low skeletal muscle mass) and patients with sarcopenic obesity have been shown to have high risk of mortality (16,34). Low serum creatinine (<0.7 mg/dL) has been regarded as a surrogate marker for frailty and sarcopenia (low skeletal muscle mass) especially among the elderly and is a strong predictor of mortality among patients with normal BMI in the setting of chronic diseases such as coronary artery disease (35). Serum creatinine was shown to modify the association of BMI (paradox) with mortality, where patients with low creatinine had the worst outcomes among hemodialysis patients (36). In the setting of cancer, body mass composition was shown to modify the association of BMI with survival, where sarcopenic obesity was associated with the highest risk of mortality and the paradox was seen only when obesity was defined by BMI (34). Serum creatinine has been previously shown to be correlated with urinary creatine excretion/DEXA (Dual Energy X-ray Absorptiometry) based skeletal muscle mass measurements in cancers such as colorectal cancer and healthy volunteers although to a varying extent (37–41). Nevertheless, serum creatinine represents a simple alternative to indirectly assess skeletal muscle mass when renal function is accounted for (40–42) and is especially relevant given the setting of advanced cancer where the growing metabolic demands of the tumor mobilize nutrients from skeletal muscle (43); as such we included eGFR in the primary analysis to account for renal function. Less than 10% of the cohort had prior history of CKD at baseline. Causes of low serum creatinine

levels other than older age, female gender and chronic illness (cancer/other co-morbidities) such as rapid renal clearance, pregnancy, fever/acute illness and advanced liver disease were unlikely to explain low serum creatinine levels in this cohort (44,45).

*Gender-based difference in serum creatinine (surrogate for skeletal muscle mass), and the impact on outcomes*

A predominant male gender driven association of overweight/Class I obesity with lower risk of worse outcomes was evident (Fig-2A/3A/4A/Table-2) and was largely attenuated if serum creatinine was  $< 0.9$  mg/dL and the paradox was further attenuated for levels  $< 0.7$  mg/dL. Gender based density distribution of serum creatinine showed that most females had serum creatinine levels of  $< 0.9$  mg/dL and 87.7% of males had serum creatinine  $\geq 0.9$  mg/dL which was the threshold identified for serum creatinine in RSF for predicting outcomes and confirmed by Cox-PH analysis (Fig-2F/Table 1/Supplementary Table 1/Table-2). Overweight/Class I obese patients more commonly had serum creatinine  $\geq 0.9$  mg/dL and were males. Class II/III obese patients had lower serum creatinine and were more commonly females (Table-2/Fig-2F/Supplementary Fig-6). Comparable gender-based differences in distribution of serum creatinine has been shown in the setting of solid cancers before (46). Gender based differences in body mass composition and/or muscle mass (47) is well documented and provides a potential basis for explaining the finding as low skeletal muscle mass has been shown previously to increase risk of mortality in several solid cancers (16,48,49) given that skeletal muscle is a large reservoir of proteins and other minerals/metabolites to meet the high requirements in a catabolic state such as in advanced cancer; low muscle mass is associated with poor immune function as skeletal muscle provides key nutrients (glutamine) to the immune system (critical for lymphocyte and

monocyte function and cell surface expression (50,51)) and tissues which undergo rapid repair (52) and is likely relevant in the setting of checkpoint based immunotherapy as the mechanism of action is T-cell/ immune mediated via reversal of T cell exhaustion (53). It is well documented that in advanced cancers, sarcopenia results from mobilization of key nutrients in the form of amino acids by muscle catabolism and indirect mechanisms such as transport of glucose through liver gluconeogenesis to growing tumors in addition to other factors such as immobility/reduced activity, cancer treatments such as surgery and toxic chemotherapies; as such underlying cachexia has been shown to directly contribute to cancer specific mortality (43).

In our study, serum creatinine levels above 0.7 mg/dL up to 0.9 mg/dL were also associated with worse outcomes although the highest risk was for patients with levels deemed to indicate sarcopenia clinically (<0.7 gm/dL)(35). These findings suggest that extra muscle reserves could provide a survival advantage and an ability to cope in a stressful environment and the higher skeletal muscle mass in men may have offered a survival advantage in an advanced cancer setting. On the contrary, several studies have shown that males have a higher risk of worse outcomes in mostly early stage melanomas although the treatment setting was during the chemotherapy era and the relationship with BMI was not studied (54–56). Our study cohort received immunotherapy and >50% of patients had Stage IV M1c disease at baseline (Table-1); additionally, these observations suggest that our study findings may be predictive specifically in the setting of anti-PD-1 based immunotherapy. Preceding weight loss was unlikely to explain the findings as the relationship of BMI measured 3-6 months prior to treatment were similar to pre-treatment BMI based findings indicating that the crossover of patients to lower weight categories had

minimal impact on outcomes (Supplementary Fig-7). Predictors of survival were in agreement with known clinical prognostic markers in melanoma (57). To summarize, the hypothesized link between BMI, obesity, gender, serum creatinine, sarcopenia and outcomes in the context of PD-1 inhibition is illustrated in Supplementary Figure 12.

### *Strengths and Limitations*

We used a data adaptive approach to derive cutoffs for defining BMI risk groups to analyze the data in an unbiased way. Given the underlying non-linear relationship of BMI and complex interplay with other predictors of survival and progressive disease, adaptive tree-based methods (RSF/RF) were able to identify complex patterns and uncover higher order interactions with ease. Because the underlying philosophy of machine learning is prediction and not hypothesis testing, we confirmed the findings in the frequentist framework and such an integrated analytical approach is increasingly being adopted in clinical studies (58).

Information on diet and physical activity would have allowed a better exploration of potential mechanisms as it is known that serum creatinine levels can be affected by varying meat intake/diet (44) but is unlikely to affect the overall conclusions of the study given the setting of advanced metastatic cancer. Other unmeasured covariates could have contributed to the findings given the retrospective nature of the study. As serum creatinine is not the gold standard measure of skeletal muscle mass due to its relationship with renal and non-renal factors, the results are hypothesis generating and should be confirmed with DEXA or CT scan based skeletal muscle mass measurements in larger cohorts.

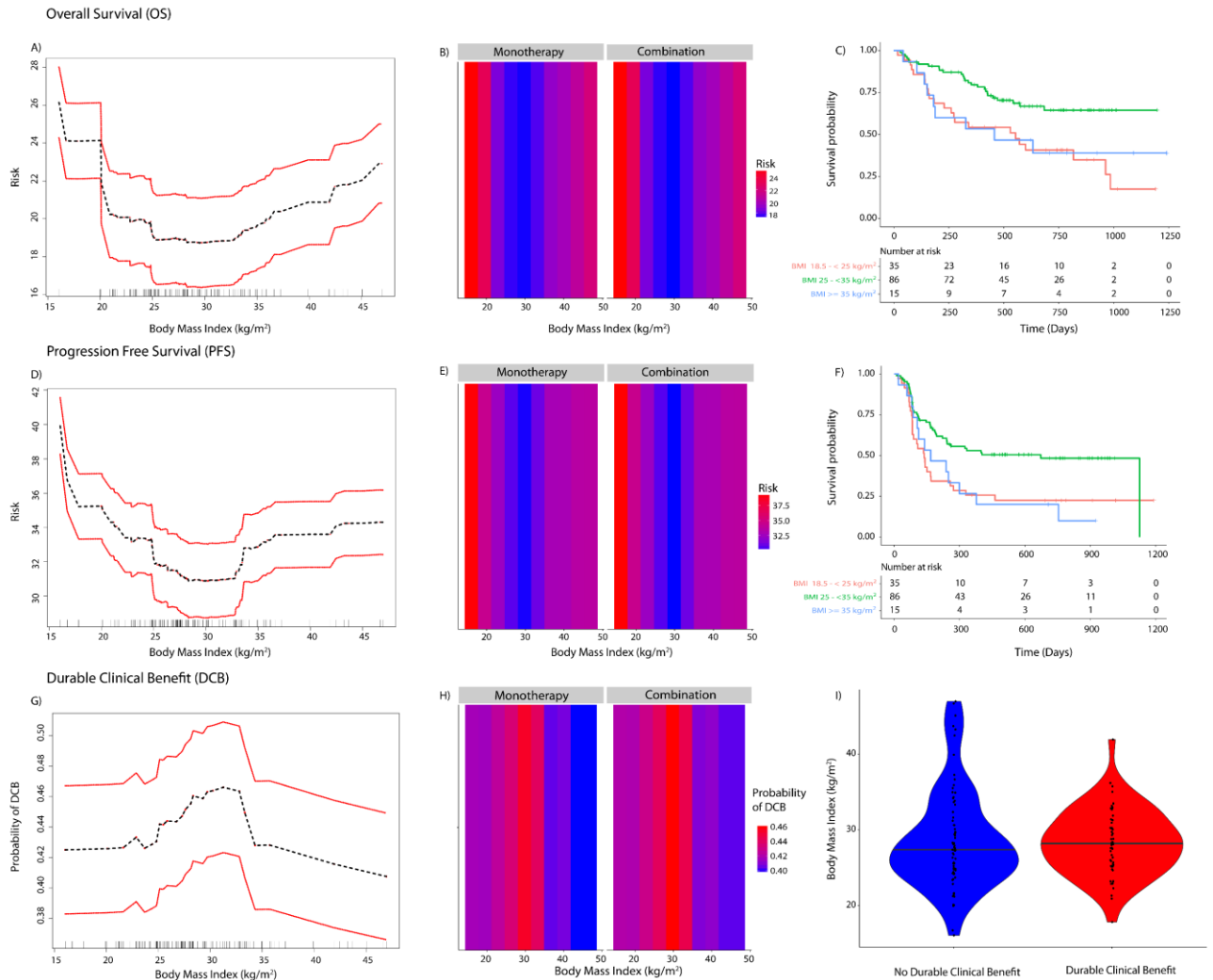
## **Conclusion**

Overweight or Class I obesity was associated with a significantly lower risk of mortality and progressive disease compared to normal weight; the findings support the presence of the “obesity paradox” restricted to overweight/Class I obesity in advanced melanoma patients treated with PD-1 blockade. Overweight/Class I obese male patients predominantly drove the association. Lower serum creatinine levels (predominant among females) was associated with worse survival outcomes and attenuated the obesity paradox. These findings suggest that sarcopenia (low skeletal muscle mass) or direct measures of body mass composition may be more suitable predictors of survival outcomes rather than BMI alone in the setting of anti-PD-1 based immunotherapy in patients with melanoma and other cancers.

## **Acknowledgements**

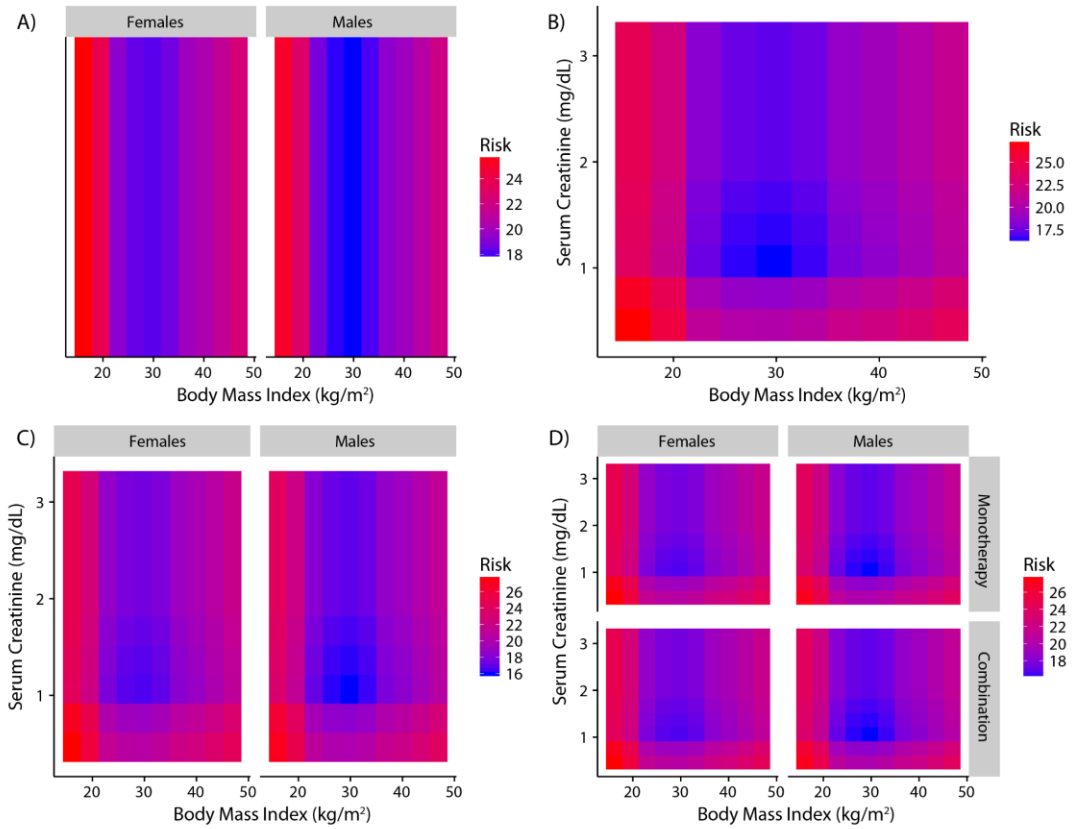
The authors would like to thank Dr. Brian Healy, Dr. Miguel Hernan, Dr. Ajay K. Singh, Dr. Finnian McCausland and Dr. Kate Madden (Harvard Medical School) for their support.

## FIGURES (PROJECT 2)

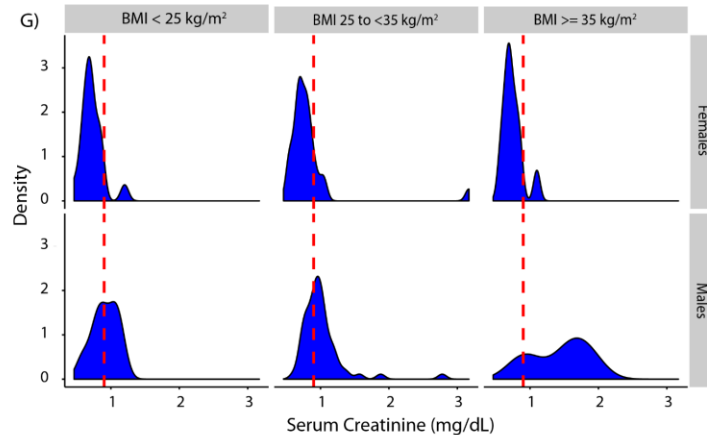
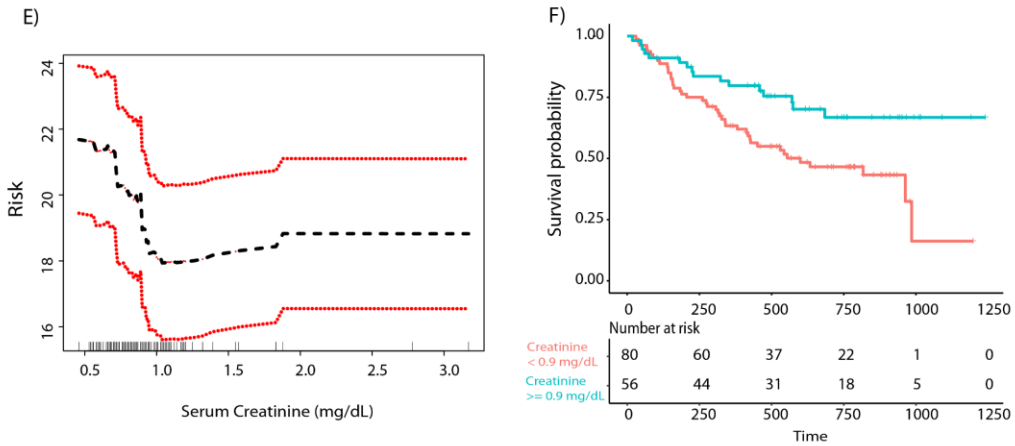


**Figure-1 (A-I).** Panel A shows a “U” shaped relationship between BMI and risk (mortality), with the risk being the lowest for overweight/Class I obese patients and the highest for underweight patients. Panel B shows the relationship is similar for both anti-PD-1 monotherapy/combination. Panel D, E and F shows similar findings for PFS. Panel G shows an “inverted U” shaped relationship for durable clinical benefit outcome. Panel H shows that overweight/Class I obese patients had a higher probability of achieving DCB for both mono/combination therapy. Panel I shows the distribution of BMI in patients with and without durable clinical benefit. Note: Figures C and F excluded 3 underweight patients.

Overall Survival (OS): Exploration of interactions (A-D)

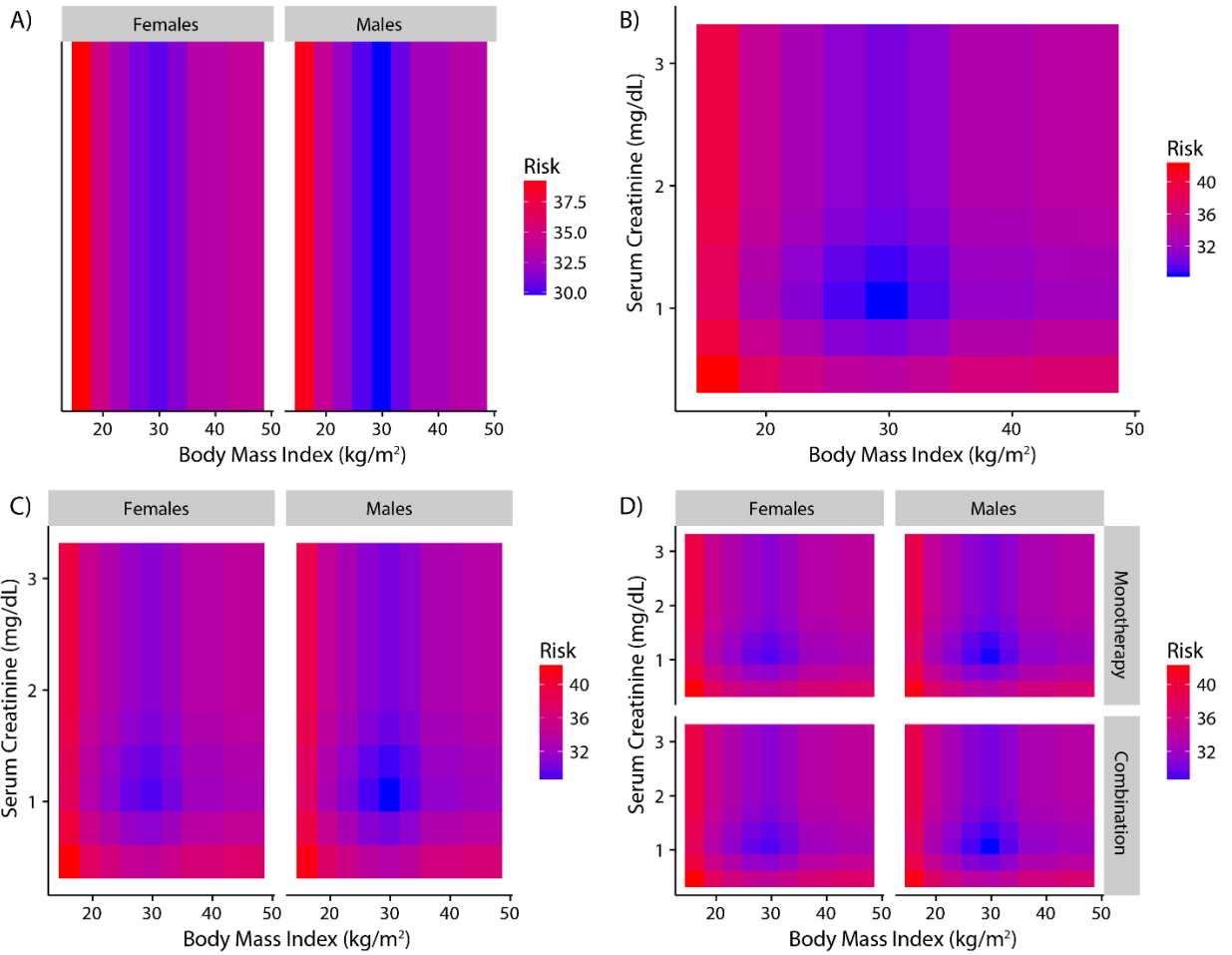


Impact of serum creatinine on OS and gender based differences in distribution within BMI groups (E-G)

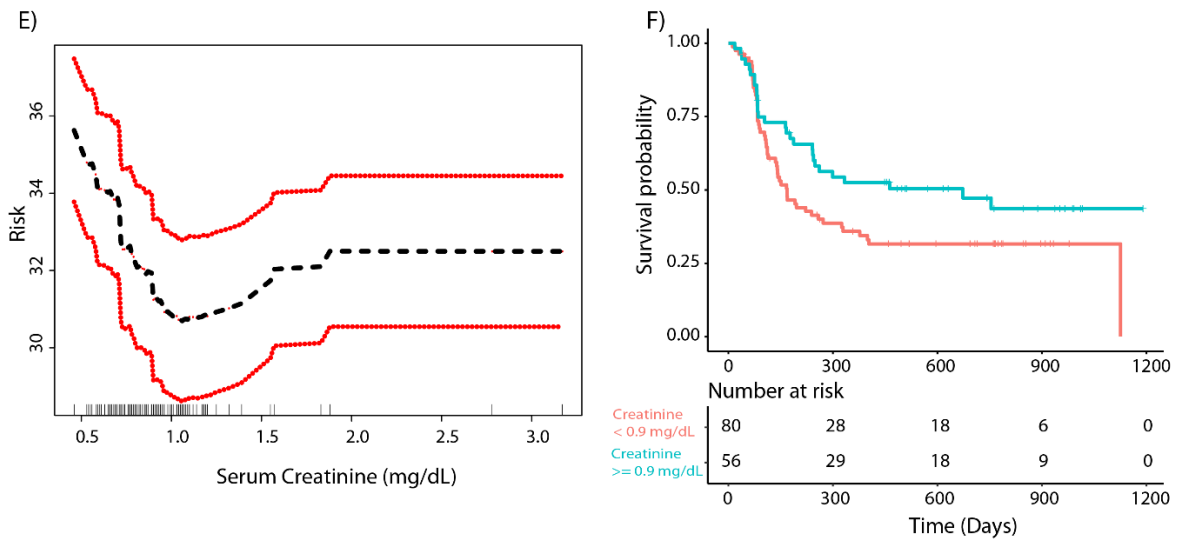


**Figure 2 (A-G).** Overall Survival (A-D): Panel A shows the predominant male gender driven association of overweight/Class I obesity with lower risk of mortality (dark blue) compared to normal weight/underweight patients and Class-II/III obese patients who had higher risk of mortality (red). Panel B shows that patients who had serum creatinine  $<0.9$  mg/dL had high risk of mortality and the obesity paradox pattern (blue) was largely attenuated. Panel C shows that the obesity paradox finding was attenuated for both genders if serum creatinine concentrations  $<0.9$  mg/dL. Panel D shows that findings from Panel C were not different between the two treatment arms. Panel F shows KM survival curves for the two creatinine risk groups per RSF thresholds (excluding 3 underweight patients). Panel G shows gender-based differences in distribution of serum creatinine within BMI groups where most females had serum creatinine  $< 0.9$  mg/dL (risk threshold identified by RSF and is indicated as a red dashed line).

Progression Free Survival (PFS): Exploration of interactions (A-D)

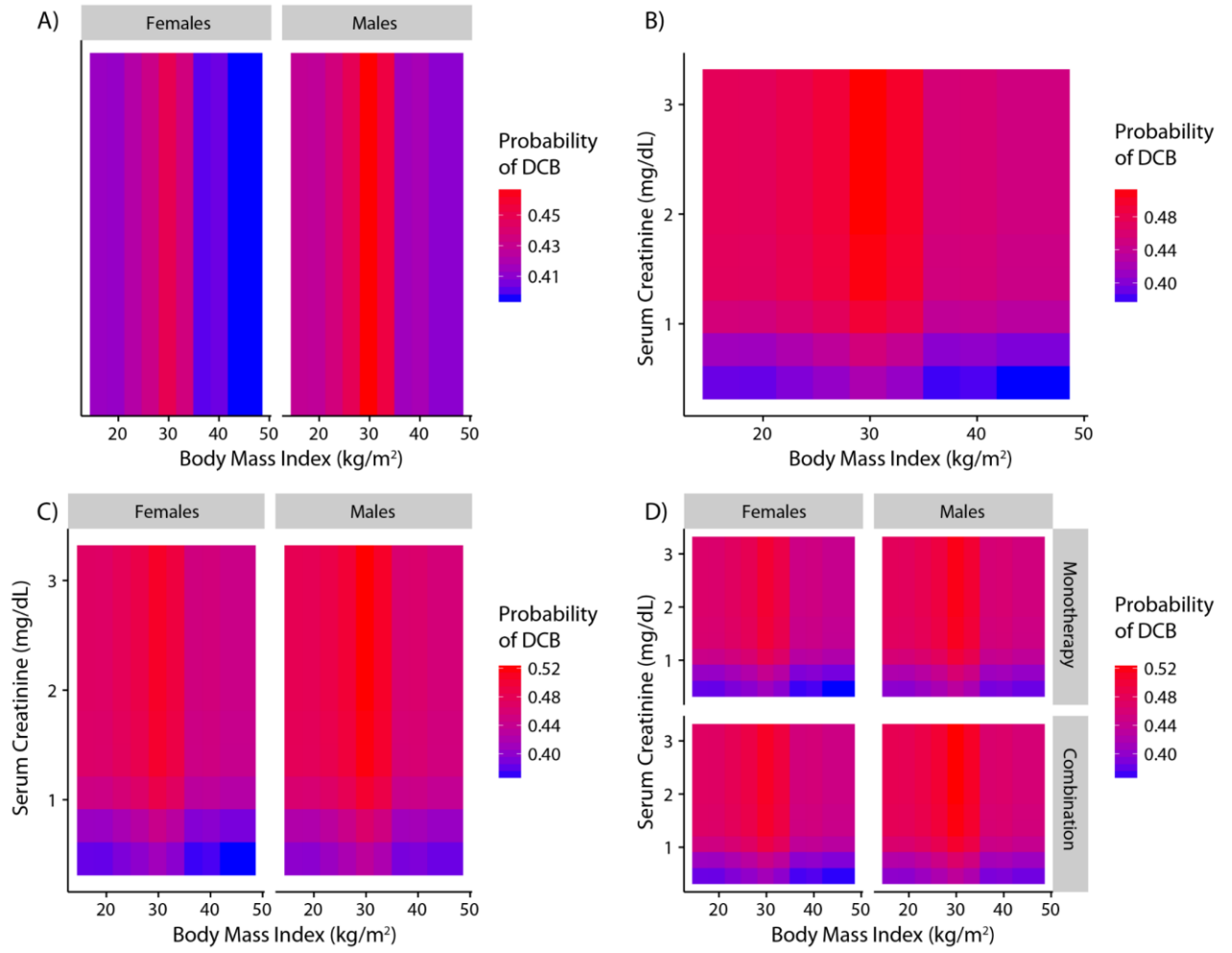


Impact of serum creatinine on PFS (E-F)

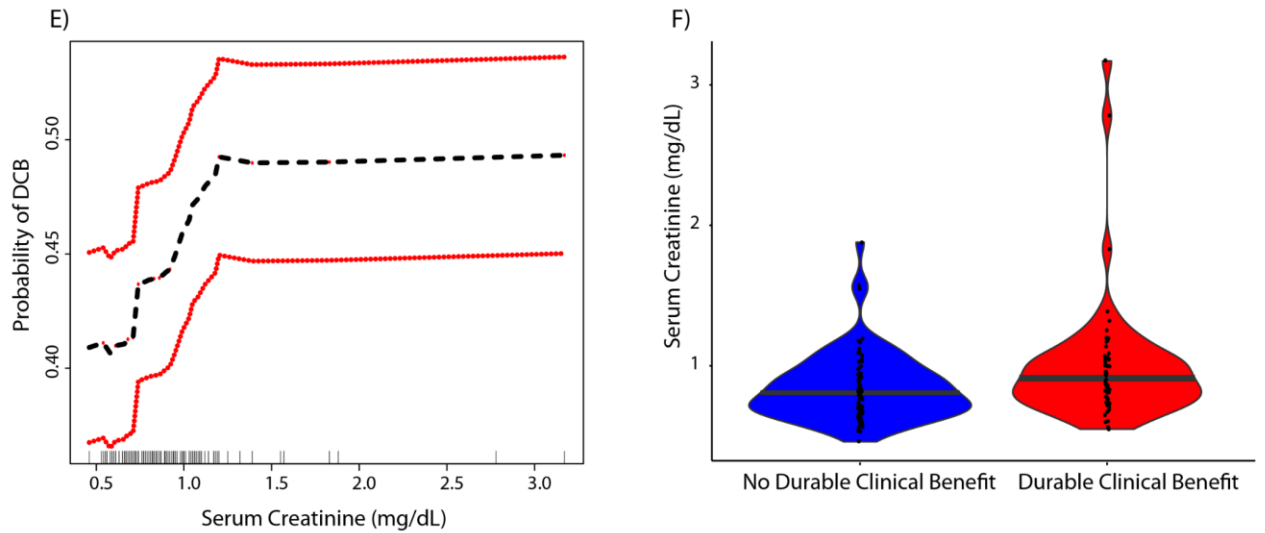


**Figure 3 (A-D).** Progression Free Survival (A-D): Panel A shows the predominant male gender driven association of overweight/Class-I obesity with lower risk of progressive disease (dark blue) compared to normal weight/underweight patients and Class-II/III obese patients who had higher risk of disease progression (red). Panel B shows that patients who had serum creatinine  $<0.9$  mg/dL had high risk of progressive disease and the obesity paradox pattern (blue) was largely attenuated. Panel C shows that for both genders the paradox was attenuated if serum creatinine was  $<0.9$  mg/dL. Panel D shows that the findings from Panel C were similar for anti-PD-1 based monotherapy and combination therapy. Panel E shows the relationship of serum creatinine with PFS and Panel F shows improved survival for patients with serum creatinine  $\geq 0.9$  mg/dL compared to patients with levels  $<0.9$  mg/dL.

Durable Clinical Benefit (DCB): Exploration of interactions (A-D)



Impact of serum creatinine on probability of achieving DCB (E-F)



**Figure 4 (A-F).** Durable Clinical Benefit (E-H): Panel A shows the predominant male gender driven association of overweight/Class-I obesity with a higher probability of achieving DCB (red) compared to normal weight/underweight patients and Class-II/III obese patients who had lower probability of DCB (blue). Panel B shows that patients who had serum creatinine  $<0.9$  mg/dL had lower probability of DCB and the obesity paradox pattern (red) was largely attenuated. Panel C shows that the paradox was attenuated for both genders for lower serum creatinine levels ( $<0.9$  mg/dL). Panel D shows that findings from Panel C were similar for both anti-PD-1 based monotherapy and combination therapy. Panel E shows the relationship of serum creatinine with probability of achieving DCB. Panel F shows the distribution of serum creatinine among patients with and without DCB.

## TABLES (PROJECT 2)

**Table 1. Baseline characteristics by BMI categories**

N = 139	Body Mass Index (kg/m <sup>2</sup> )		
	BMI < 25 kg/m <sup>2</sup> N = 38* (%)	Overweight and Class I Obesity (BMI 25-<35 kg/m <sup>2</sup> ) N = 86 (%)	Class II/III Obesity (BMI ≥ 35 kg/m <sup>2</sup> ) N = 15 (%)
<b>Demographics</b>			
Age	64.6 (15.2)	61.2 (13.8)	57.3 (13.7)
Male gender	19 (50)	57 (66.3)	3 (20)
White race	37 (97.4)	85 (98.8)	14 (93.3)
Cutaneous Melanoma	31 (81.6)	78 (90.7)	12 (80)
BRAF mutation	11 (28.9)	22 (25.6)	2 (13.3)
NRAS mutation	4 (10.5)	6 (7)	4 (26.7)
<b>Disease severity</b>			
Stage at baseline	IV M1c: 24 (63.2)	IV M1c: 49 (57)	IV M1c: 7 (46.7)
Karnofsky Performance Score (KPS)	<70: 2 (5.3) >=70: 36 (94.7)	<70: 2 (2.4) >=70: 83 (97.6) N=85/86	<70: 0 (0) >=70: 15 (100)
LDH in U/L (Median and IQR; N=131)	211.5 (158- 336) N=34/38	176.5 (137- 230) (N=82/86)	187 (158- 264)
<b>Lifestyle habits</b>			
Ever smokers	Former: 16 (42.1) Current: 3 (7.9)	Former: 34 (39.5) Current: 8 (9.3)	Former: 7 (46.7) Current: 0 (0)
Current drinkers	24 (63.2)	54 (62.8)	6 (40)
<b>Prior treatments</b>			
Immunotherapy	9 (23.7)	33 (38.4)	8 (53.3)
Chemotherapy	1 (2.6)	12 (14)	2 (13.3)
Radiation	10 (26.3)	22 (25.6)	6 (40)
Targeted therapy	5 (13.2)	5 (5.8)	0 (0)
<b>Co-morbidities</b>			
Charlson's Comorbidity Index (Mean and SD)	8.6 (1.7)	8.4 (2.1)	8.1 (1.7)
Diabetes	3 (7.9)	11 (12.8)	4 (26.7)
Hypertension	14 (36.8)	51 (59.3)	9 (60)
Hyperlipidemia	11 (28.9)	27 (31.4)	8 (53.3)
Chronic Kidney Disease (CKD)	3 (7.9)	7 (8.1)	1 (6.7)
Cardiovascular disease (CAD/CHF/MI/AF) #	6 (15.8)	11 (12.8)	4 (26.7)
Autoimmune/Immune mediated disorders	7 (18.4)	9 (10.5)	1 (6.7)
<b>Co-medications for co-morbidities</b>			
Anti-platelet agents (Aspirin/Clopidogrel)	10 (26.3)	22 (25.6)	4 (26.7)
Anti-hypertensive medications (any)	10 (26.3)	49 (57)	9 (60)
ACE or ARB inhibitors	7 (18.4)	28 (32.6)	4 (2.7)

Metformin	3 (7.9)	6 (7)	3 (20)
Statins	11 (2.9)	20 (23.3)	4 (26.7)
Oral Steroids	1 (2.6)	10 (11.6)	1 (6.7)
<b>Clinical chemistry and Vitals</b>			
Albumin in g/dL (Mean and SD)	3.8 (0.59)	4.1 (0.41)	4 (0.45)
ANC K/uL (Mean and SD)	5.5 (3.3)	4.8 (1.6)	5.5 (2.7)
ALC K/uL (Mean and SD)	1.3 (0.6)	1.6 (1.4)	1.8 (1.1)
Hemoglobin g/dL (Mean and SD)	12.4 (2)	13.1 (1.6)	12.2 (1.5)
Serum Creatinine mg/dL (Mean and SD)	0.81 (0.21)	0.95 (0.38)	0.88 (0.36)
eGFR <sup>^</sup> (ml/min/1.73m <sup>2</sup> )	>=60: 36; <60: 2	>=60: 77; <60: 9	>=60: 13; <60: 2
eGFR by CKD-EPI equation in ml/min/1.73m <sup>2</sup> (Median and IQR)	88.51 (71.09 – 98.91)	85.87 (74.83 – 94.73)	91.03 (70.99 – 100.6)
Fasting Glucose in mg/dL (Median and IQR)	106 (100-122)	101 (93 - 119)	102 (98-114)
BMI at baseline (kg/m <sup>2</sup> )	22.6 (2.3)	29.1 (2.7)	40.1 (4.4)
Alkaline Phosphatase in U/L (Median and IQR)	74 (63-100)	74 (62-90)	69 (60-129)
ALT in U/L (Median and IQR)	15 (11-27)	17 (12-21)	18 (14-27)
AST in U/L (Median and IQR)	19 (15-29)	18 (13-23)	21 (13-27)
Systolic blood pressure in mm Hg (Mean/SD)	124.7 (19.9)	133.8 (18.2)	135.3 (14.4)
Diastolic blood pressure in mm Hg (Mean/SD)	74.4 (11.9)	78.3 (13.2)	75 (10.6)
<b>Disease related weight loss</b>			
BMI measured up to 6 months before baseline (Mean and SD)	23 (2.6) (N=35/38)	29.3 (2.7) (N=81/86)	40.4 (4.8)
<b>Treatment</b>			
Anti-PD-1 immunotherapy type	Mono: 19 (50) Combination: 19 (50)	Mono: 49 (57) Combination: 37 (43)	Mono: 11 (73.3) Combination: 4 (26.7)

\*Includes three patients with BMI < 18.5 for descriptive purposes. Analyses by Cox-PH/logistic regression was performed by excluding underweight patients (n=3) but were included for RSF analysis where BMI was included as a continuous variable.  
<sup>^</sup>eGFR – Estimated Glomerular Filtration Rate (Cockcroft-Gault).  
# CAD: Coronary Artery Disease; CHF: Congestive Heart Failure; MI: Myocardial Infarction and AF: Atrial Fibrillation  
IQR: Inter Quartile Range

**Table 2. Hazard ratios for the association of BMI with OS and PFS (along with interactions) and odds ratios for the association of BMI with durable clinical benefit**

<b>Outcome (N=136) **</b>	<b>Effect Estimate</b>			
	<b>Unadjusted Hazard Ratio (95% CI)</b>	<b>p-value</b>	<b>Adjusted Hazard Ratio* (95% CI)</b>	<b>p-value</b>
<b>Overall Survival (OS)</b>				
<b>Overweight/Class-I Obese vs. Normal Weight (reference)</b>	<b>0.41 (0.24 – 0.72)</b>	<b>0.002</b>	<b>0.26 (0.1-0.71)</b>	<b>0.008</b>
Class-II/III Obese vs. Normal Weight	0.88 (0.41 – 1.91)	0.756	0.42 (0.1 – 1.77)	0.238
<i>Interaction Model<sup>+</sup> 1 (OS)</i> Interaction of BMI (Overweight/Class-I vs. normal weight) with gender (males vs. females)	-	-	0.19 (0.04-0.95)	0.044
<i>Interaction Model 1 (OS)</i> Overweight/Class-I Obese (vs. normal weight) HR in females	-	-	0.56 (0.16-1.89)	0.346
<b><i>Interaction Model 1 (OS)</i></b> <b>Overweight/Class-I Obese (vs. normal weight) HR in males</b>	-	-	<b>0.11 (0.03 – 0.4)</b>	<b>0.001</b>
<i>Interaction Model 2 (OS)</i> Interaction of BMI (Overweight/Class-I vs. normal weight) with serum creatinine ( $\geq 0.9$ mg/dL vs. $< 0.9$ mg/dL) <sup>+</sup>	-	-	0.11 (0.02 - 0.7)	0.020
<i>Interaction Model 2 (OS)</i> Overweight/Class-I Obese (vs. normal weight) HR in patients with serum creatinine $< 0.9$ mg/dL	-	-	0.43 (0.15 – 1.24)	0.119
<b><i>Interaction Model 2 (OS)</i></b> <b>Overweight/Class-I Obese (vs. normal weight) HR in patients with serum creatinine <math>\geq 0.9</math> mg/dL</b>	-	-	<b>0.045 (0.08 – 0.262)</b>	<b>0.001</b>
<b><i>Progression Free Survival (PFS)</i></b>				
<b>Overweight/Class-I Obese vs. Normal Weight (reference)</b>	<b>0.5 (0.31 – 0.81)</b>	<b>0.005</b>	<b>0.43 (0.19-0.95)</b>	<b>0.038</b>
Class-II/III Obesity vs. Normal Weight	1.03 (0.53 - 2)	0.932	1 (0.34 – 2.94)	0.991
<i>Interaction Model 1 (PFS)</i> Interaction of BMI (overweight/Class-I vs. normal weight) with gender (males vs. females)	-	-	0.29 (0.07 – 1.18)	0.084

<i>Interaction Model 1 (PFS)</i> Overweight/Class-I Obese (vs. normal weight) HR in females	-	-	0.80 (0.27 – 2.41)	0.695
<b><i>Interaction Model 1 (PFS)</i></b> <b>Overweight/Class-I Obese (vs. normal weight) HR in males</b>	-	-	<b>0.23 (0.08 – 0.66)</b>	<b>0.006</b>
<i>Interaction Model 2 (PFS)</i> Interaction of BMI (overweight/Class-I vs. normal weight) with serum creatinine ( $\geq 0.9$ mg/dL vs. $< 0.9$ mg/dL)	-	-	0.17 (0.04 – 0.77)	0.021
<i>Interaction Model 2 (PFS)</i> Overweight/Class-I Obese (vs. normal weight) HR in patients with serum creatinine $< 0.9$ mg/dL	-	-	0.71 (0.29 – 1.77)	0.464
<b><i>Interaction Model 2 (PFS)</i></b> <b>Overweight/Class-I Obese (vs. normal weight) HR in patients with serum creatinine <math>\geq 0.9</math> mg/dL</b>	-	-	<b>0.12 (0.03 – 0.45)</b>	<b>0.002</b>
<b>Durable Clinical Benefit (N=132) **</b>	<b>Unadjusted Odds Ratio (95% CI)</b>	<b>p-value</b>	<b>Adjusted Odds Ratio<sup>s</sup> (95% CI)</b>	<b>p-value</b>
<b>Overweight/Class-I Obese vs. Normal Weight (reference)</b>	<b>2.76 (1.18 – 6.46)</b>	<b>0.020</b>	<b>11.4 (1.65 – 78.6)</b>	<b>0.013</b>
Class-II/III Obesity vs. Normal Weight	0.91 (0.23 – 3.54)	0.891	3.3 (0.22 – 50.5)	0.391
<i>Interaction Model 1 (DCB)</i> Interaction of BMI (overweight/Class-I vs. normal weight) with gender (males vs. females)	-	-	10.39 (0.17 – 634.68)	0.265
<i>Interaction Model 1 (DCB)</i> Overweight/Class-I Obese (vs. normal weight) in females	-	-	2.91 (0.16 – 54.05)	0.473
<b><i>Interaction Model 1 (DCB)</i></b> <b>Overweight/Class-I Obese (vs. normal weight) in males</b>	-	-	<b>30.27 (2.01 – 455.72)</b>	<b>0.014</b>
<i>Interaction Model 2 (DCB)</i> Interaction of BMI (overweight/Class-I vs. normal weight) with creatinine ( $\geq 0.9$ mg/dL vs. $< 0.9$ mg/dL)	-	-	2.82 (0.03 – 229.67)	0.644
<i>Interaction Model 2 (DCB)</i> Overweight/Class-I Obese (vs. normal weight) HR in patients with serum creatinine $< 0.9$ mg/dL	-	-	8.03 (0.85 – 76.27)	0.070
<b><i>Interaction Model 2 (DCB)</i></b> <b>Main effect (Serum Creatinine <math>\geq 0.9</math> mg/dL): Overweight/Class-I Obese (vs. normal weight) HR in patients with serum creatinine <math>\geq 0.9</math> mg/dL</b>	-	-	<b>22.66 (0.51 – 1013.5)</b>	<b>0.108</b>

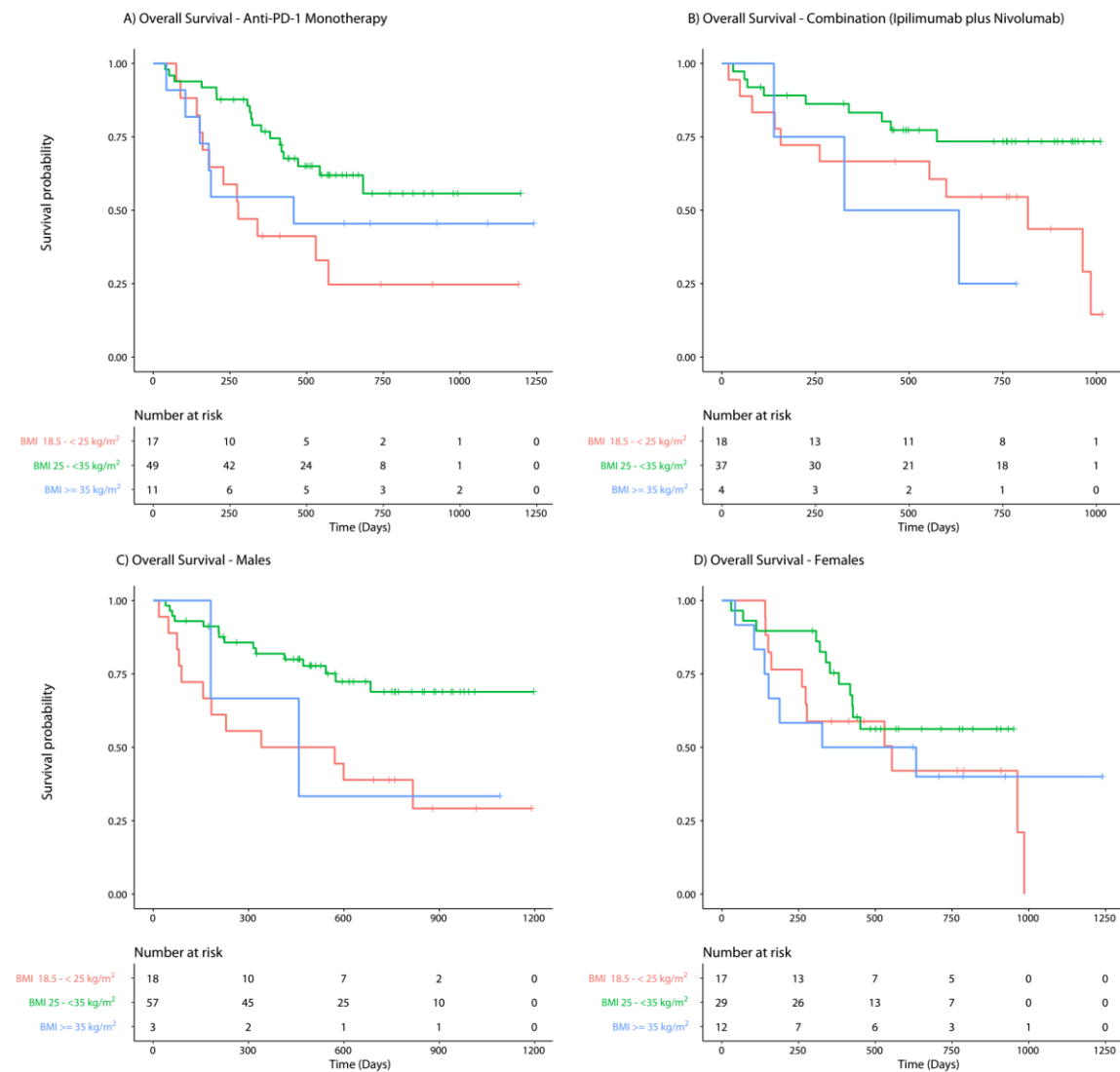
\* Adjusted for the following covariates: age ( $\leq 45$ ,  $>45-75$  and  $>75$  years), gender, serum creatinine ( $< 0.9$  and  $\geq 0.9$  mg/dL), treatment (monotherapy/combination), current drinker (vs. non-current/never-drinker), smoking history (ever vs. never), KPS ( $\leq 70$  and  $>70$ ), LDH ( $\leq 231$  vs.  $>231$  U/L), stage at baseline, Charlson's score ( $<10$  vs.  $\geq 10$ ), hemoglobin ( $<11.5$  vs.  $\geq 11.5$  g/dL), ANC ( $\leq 8$  and  $>8$  K/uL), ALC ( $<3$  and  $\geq 3$  K/uL), albumin ( $<3.5$  g/dL), autoimmune disease, diabetes, CV disease, CKD, BRAF mutation, NRAS mutation, hypertension, hyperlipidemia, comedications (anti-platelet agents, statins, metformin, ACE/ARB inhibitors), prior treatments (immunotherapy/CTLA-4, radiation, chemotherapy and targeted therapy), fasting glucose ( $\leq 110$  vs.  $>110$  mg/dL), type of melanoma, ; N=127 after excluding three patients who were underweight, 8 patients with missing LDH values and 1 patient whose KPS could not be assessed (missing).

\*\* Three underweight patients were excluded

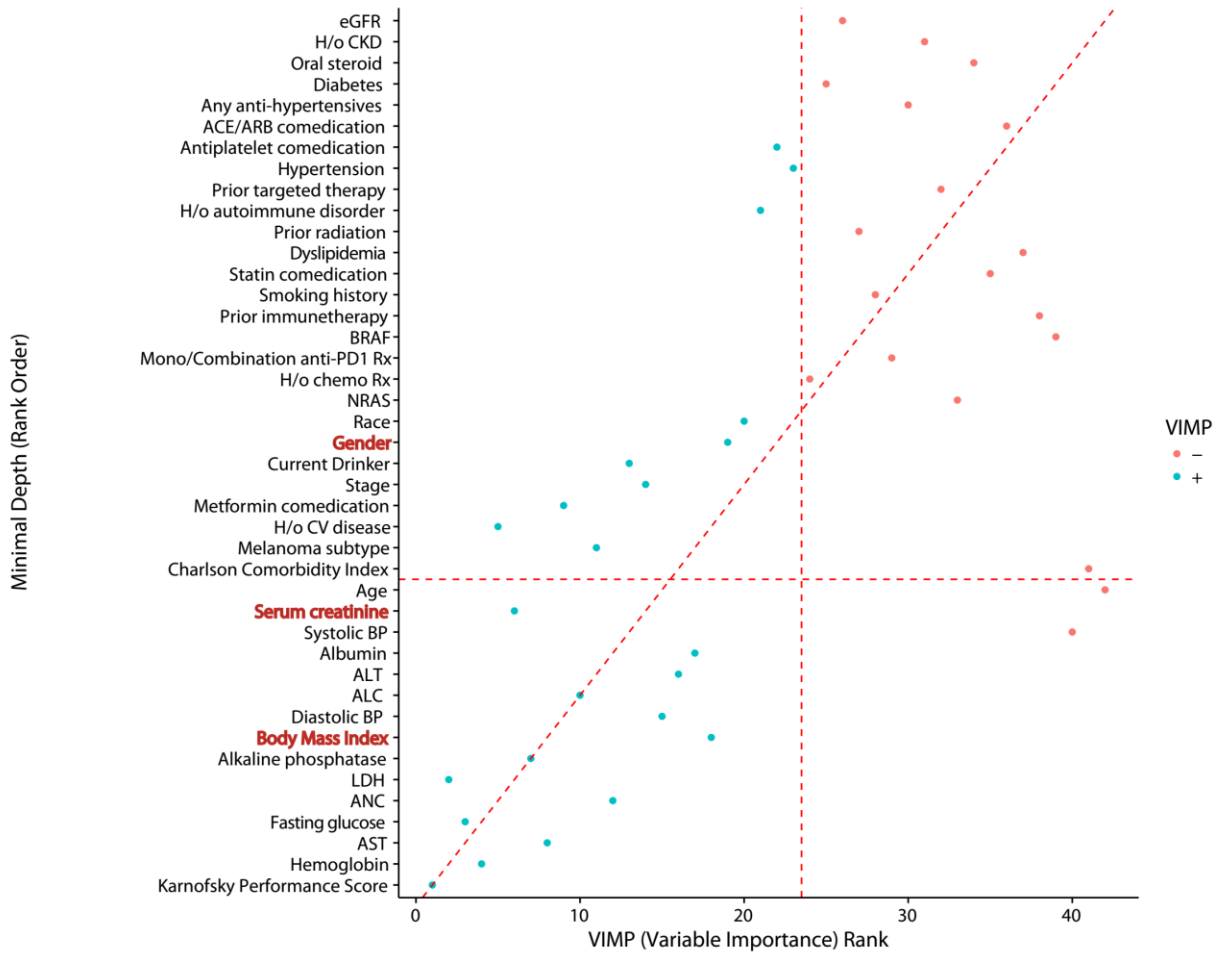
+ Interactions were studied in separate models along with main effects and adjusted for the same covariates as the main models.

§ Adjusted for the same covariates listed for OS and PFS (except KPS which was included as a continuous variable); N=123 (excluding 3 underweight patients; DCB was not assessable/available for 4 patients; patients with missing data for LDH, KPS were not included for the adjusted analysis).

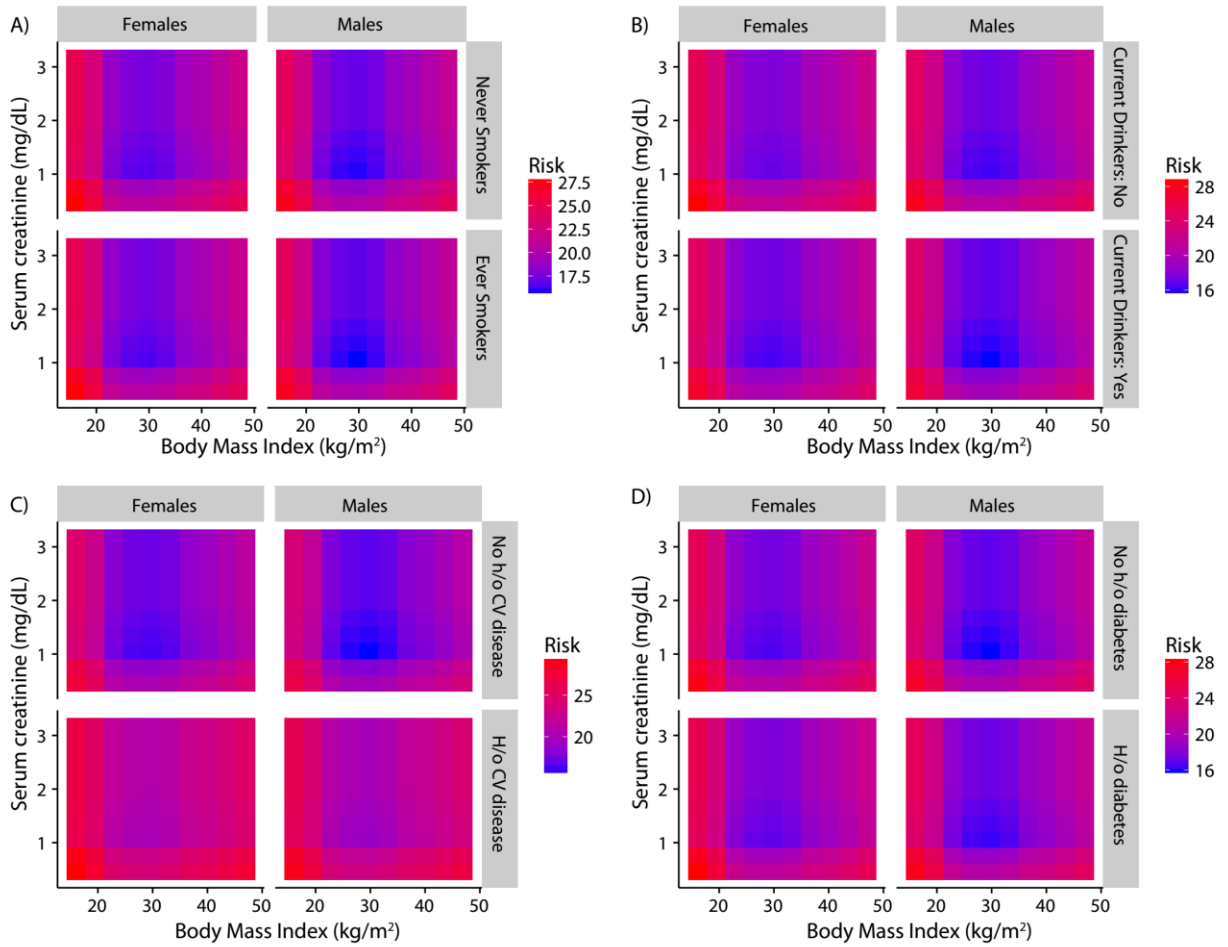
## SUPPLEMENTARY MATERIAL (PROJECT 2)



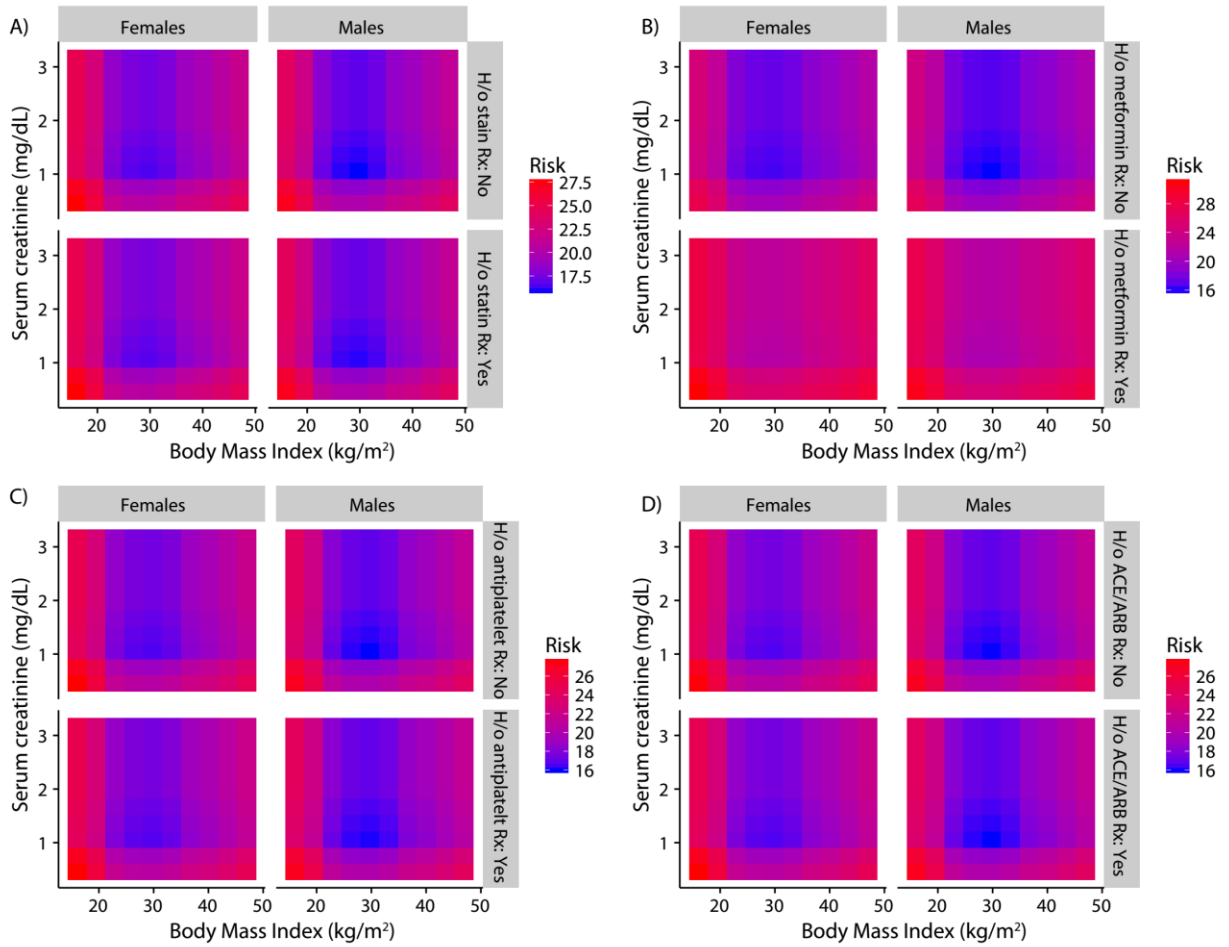
**Supplementary Figure 1. Kaplan-Meier survival curves for BMI and stratified by treatment and gender.**



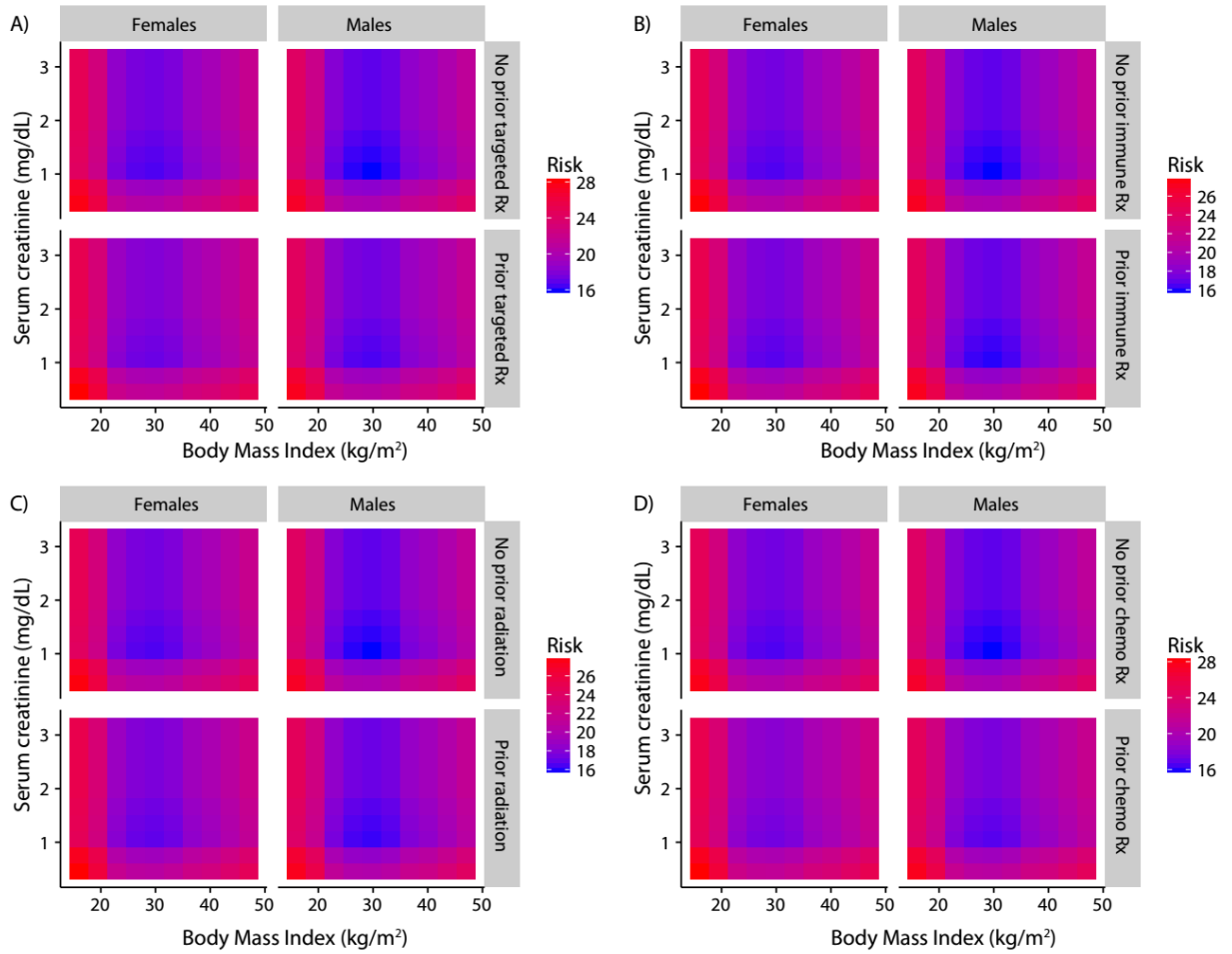
**Supplementary Figure 2. Variable Importance (VIMP) ranking and comparison of VIMP with MD ranking for predictors in RSF (Overall Survival). BMI and creatinine were predictive by both methods while gender was predictive only on VIMP but was not filtered by MD threshold criteria.**



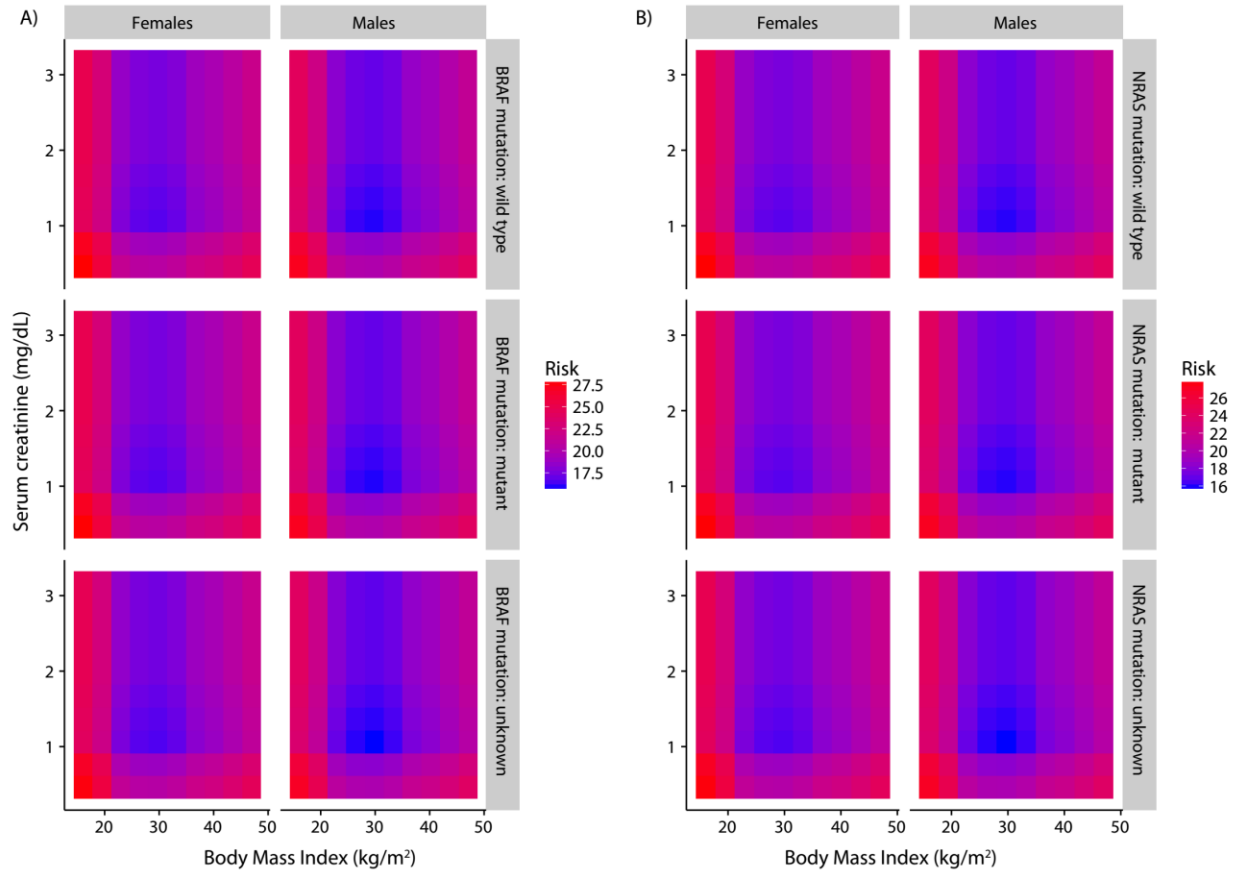
**Supplementary Figure 3. Exploration of gender based complex interactions with BMI and serum creatinine: Lifestyle habits and co-morbidities (CV disease and diabetes).**



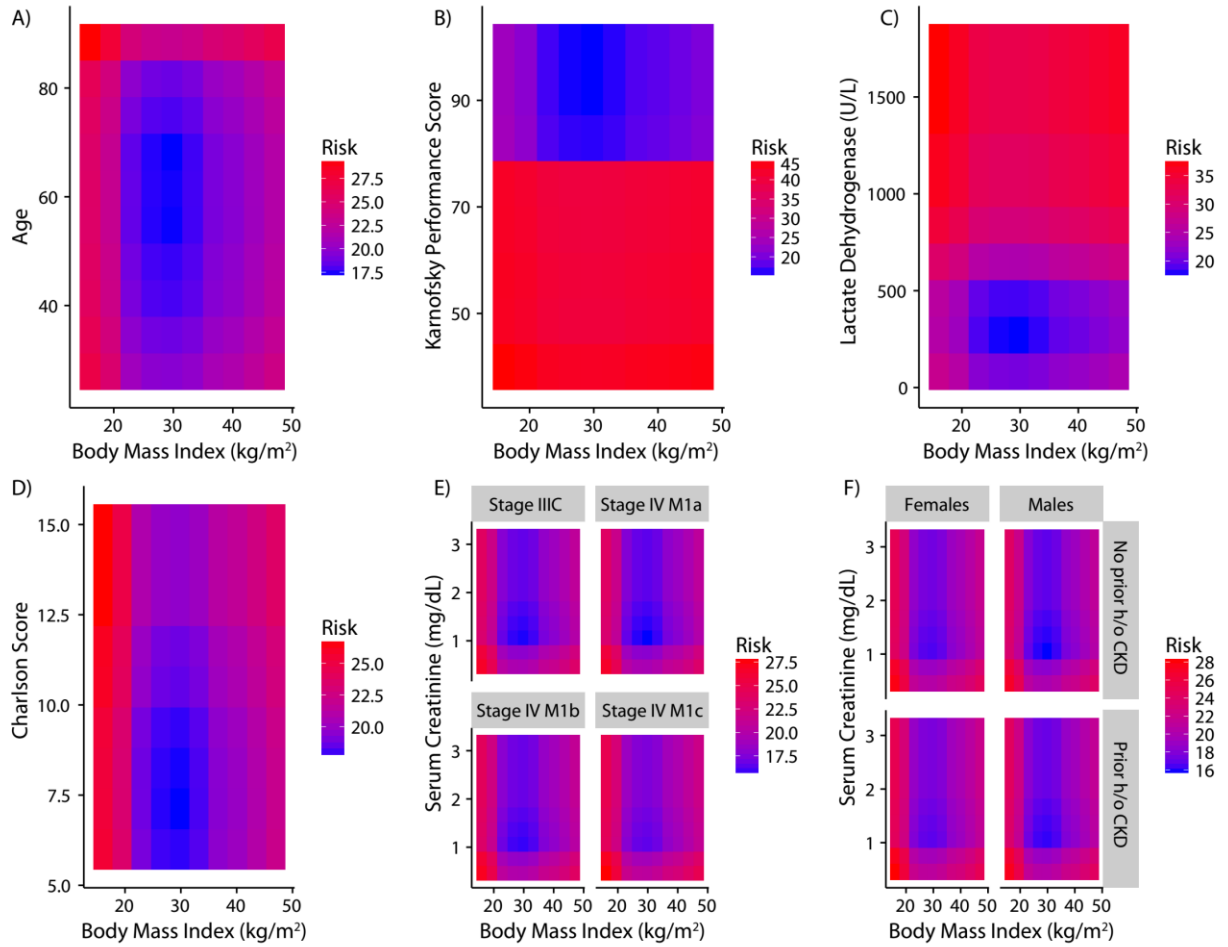
**Supplementary Figure 4. Exploration of gender based complex interactions with BMI and serum creatinine: Co-medications for co-morbidities.**



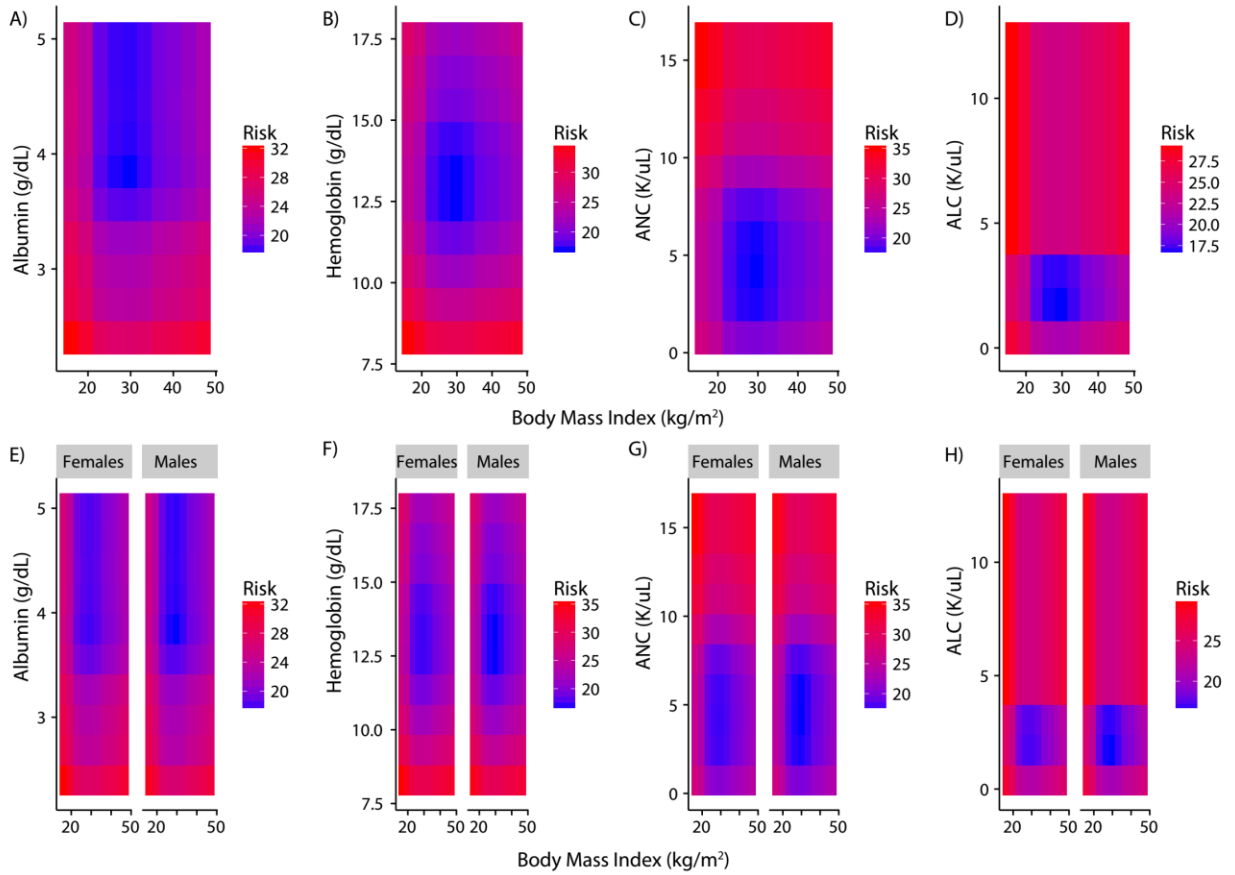
**Supplementary Figure 5. Exploration of gender based complex interactions with BMI and serum creatinine: Prior treatments.**



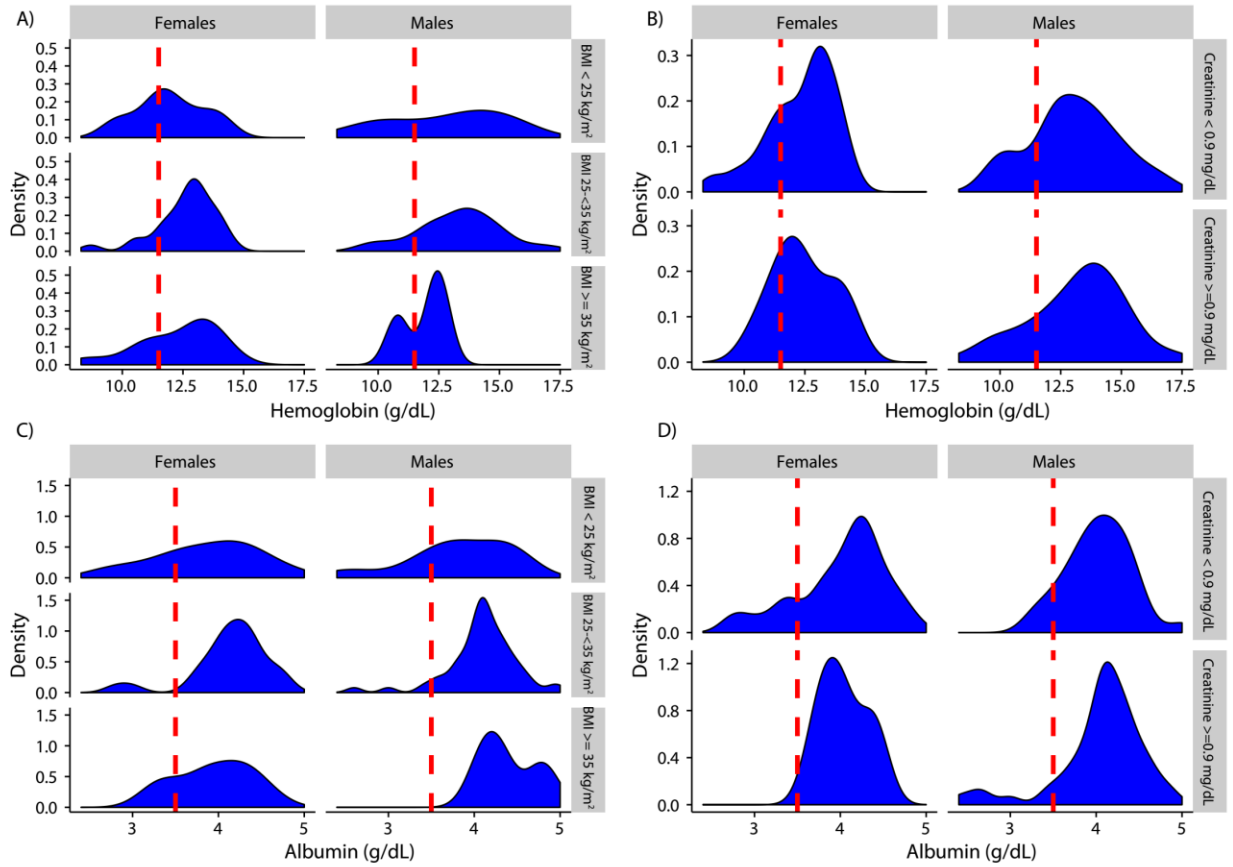
**Supplementary Figure 6. Exploration of gender based complex interactions with BMI and serum creatinine: BRAF (A) and NRAS (B) mutations.**



**Supplementary Figure 7. Exploration of interactions with BMI: Age, KPS, LDH, Charlson’s index, baseline, interplay of BMI, creatinine and stage at baseline and interplay of BMI, creatinine, gender and prior history of CKD.**

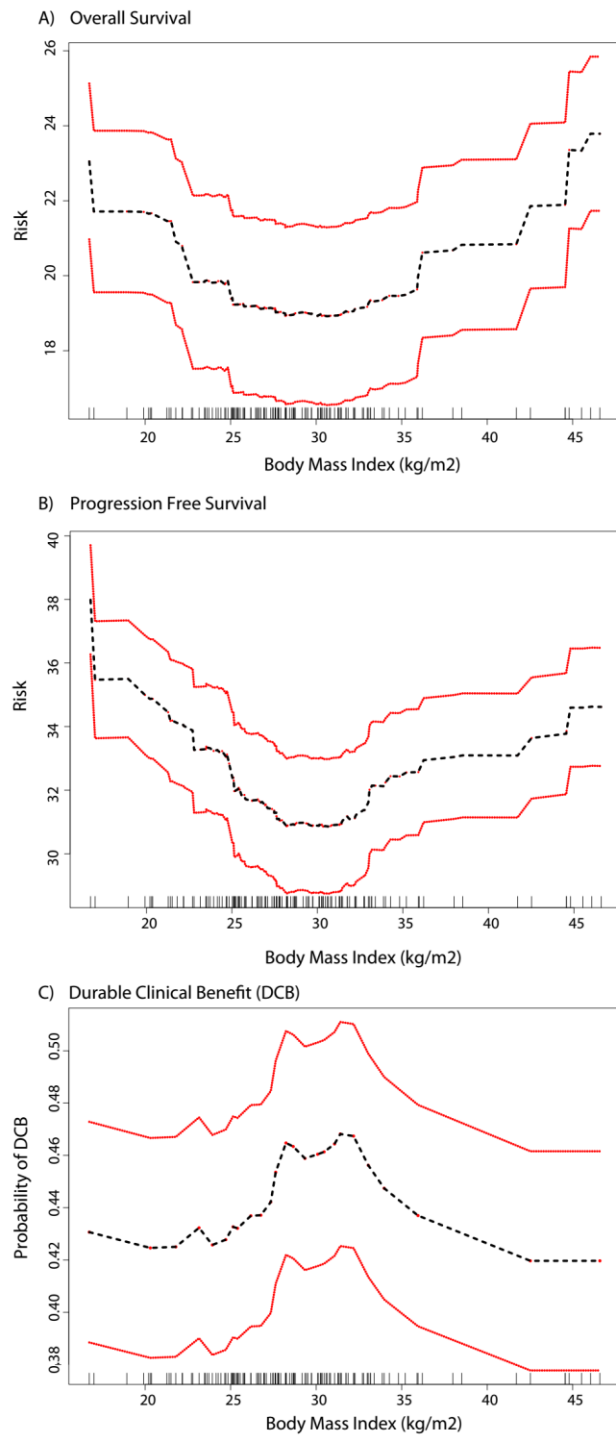


**Supplementary Figure 8. Exploration of interactions with BMI: vs. albumin, hemoglobin, ANC and ALC and their respective gender-based interactions.**

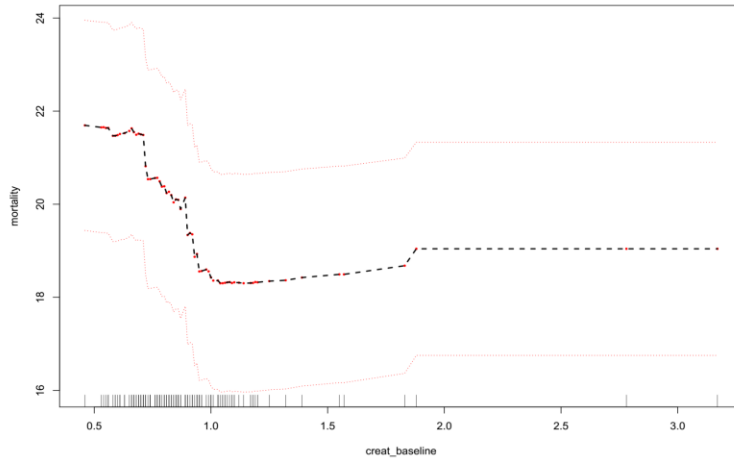


**Supplementary Fig 9. Gender based density distributions of hemoglobin and serum albumin in BMI risk groups and creatinine risk groups.**

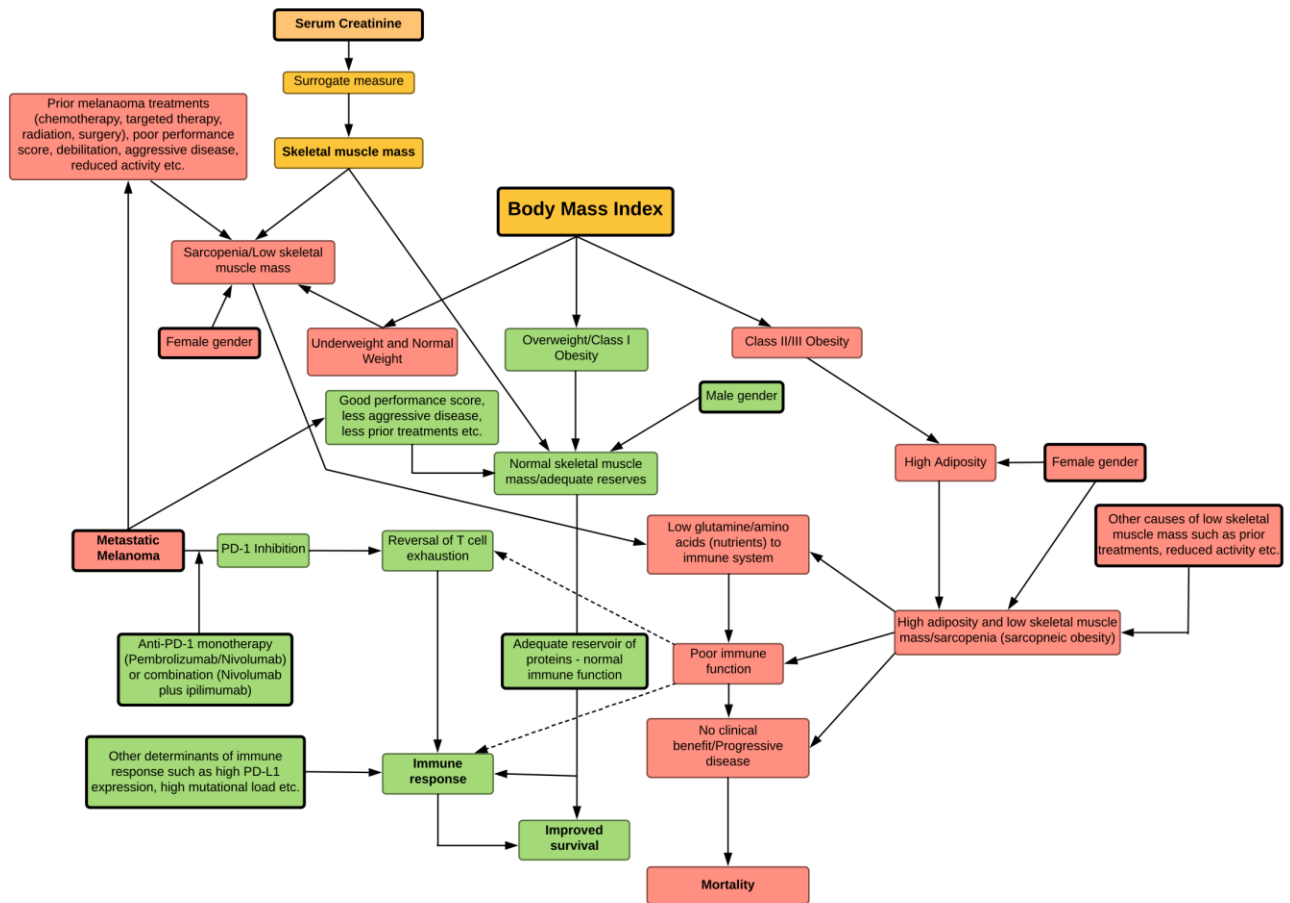
Sensitivity Analyses - BMI measured in the past 3-6 months



**Supplementary Figure 10. Sensitivity Analyses. BMI measured within the last 3-6 months and relationship with OS, PFS and DCB (RSF and RF analysis). Findings were similar to the results from primary analyses for pre-treatment BMI (refer Figure 1 for comparison).**



**Supplementary Figure 11. Relationship of serum creatinine on OS was similar when eGFR based on CKD-EPI was included (adjusted) as a continuous covariate.**



**Supplementary Figure 12. Illustration showing interplay of BMI, serum creatinine, sarcopenia and gender on survival outcomes in the context of PD-1 blockade.**

**Supplementary Table 1. Baseline characteristics by RSF estimated serum creatinine cutoffs and gender for anti-PD-1 based immunotherapy treated patients.**

N = 139	Serum Creatinine (mg/dL)		Gender	
	S. Cr. < 0.9 mg/dL N = 82 (%)	S. Cr. ≥0.9 mg/dL N = 57 (%)	Females N=60 (%)	Males N=79 (%)
<b>Demographics</b>				
Age	59.8 (13.1)	64.5 (15.4)	61.1 (13.2)	64.4 (14.8)
Male gender	29 (35.4)	50 (87.7)	-	-
White race	80 (97.6)	56 (98.2)	58 (96.7)	78 (98.7)
Cutaneous Melanoma	67 (81.7)	54 (94.7)	48 (80)	73 (92.4)
BRAF mutation	19 (23.2)	16 (28.1)	14 (23.3)	21 (26.6)
NRAS mutation	12 (14.6)	2 (3.5)	10 (16.7)	4 (5.1)
<b>Disease severity</b>				
Stage at baseline	IV M1c: 47 (57.3)	IV M1c: 33 (57.9)	IV M1c: 35 (58.3)	IV M1c: 45 (57)
Karnofsky Performance Score (KPS)	<70: 0 (0) ≥=70: 81 (100) N=81	<70: 4 (7) ≥=70: 53 (93)	<70: 0 (0) ≥=70: 60 (100)	<70: 4 (5.1) ≥=70: 74 (9.5) N=78
LDH in U/L (Median and IQR; N=131)	187 (147 – 277) N=78/82	177 (138 – 230) N=53/57	180.5 (145- 287.5) N=56/60	183 (146- 261) N=75/79
<b>Lifestyle habits</b>				
Ever smokers	Former: 27 (32.3) Current: 8 (9.8)	Former: 30 (52.6) Current: 3 (5.3)	Former: 19 (31.7) Current:6 (10)	Former: 38 (48.1) Current: 5 (6.3)
Current drinkers	44 (53.7)	40 (70.2)	30 (50)	54 (68.4)
<b>Prior treatments</b>				
Immunotherapy	35 (42.7)	15 (26.3)	26 (43.3)	24 (30.4)
Chemotherapy	10 (12.2)	5 (8.8)	6 (10)	9 (11.4)
Radiation	29 (35.4)	9 (15.8)	20 (33.3)	18 (22.8)
Targeted therapy	8 (9.8)	2 (3.5)	4 (6.7)	6 (7.6)
<b>Co-morbidities</b>				
Charlson's Comorbidity Index (Mean and SD)	8.1 (1.6)	8.9 (2.2)	7.9 (1.6)	8.8 (2.1)
Diabetes	11 (13.4)	7 (12.3)	7 (11.7)	11 (13.9)
Hypertension	39 (47.6)	35 (61.4)	23 (38.3)	51 (64.6)
Hyperlipidemia	25 (30.5)	21 (36.8)	16 (26.7)	30 (38)
Chronic Kidney Disease (CKD)	3 (3.7)	8 (14)	5 (8.3)	6 (7.6)
Cardiovascular disease (CAD/CHF/MI/AF) #	8 (9.8)	13 (22.8)	6 (10)	15 (19)
Autoimmune/Immune mediated disorders	10 (12.2)	7 (12.3)	8 (13.3)	9 (11.4)
<b>Co-medications for co-morbidities</b>				
Anti-platelet agents (Aspirin/Clopidogrel)	16 (19.5)	20 (35.1)	11 (18.3)	25 (31.6)
Anti-hypertensive medications (any)	33 (40.2)	35 (61.4)	24 (40)	44 (56)
ACE or ARB inhibitors	15 (18.3)	24 (42.1)	11 (18.3)	28 (35.4)

Metformin	8 (9.8)	4 (7)	4 (6.7)	8 (10.1)
Statins	18 (22)	17 (29.8)	11 (18.3)	24 (30.4)
Oral Steroids	8 (9.8)	4 (7)	3 (5)	9 (11.4)
<b>Clinical chemistry and Vitals</b>				
Albumin in g/dL (Mean and SD)	4 (0.47)	4 (0.49)	4 (0.5)	4 (0.47)
ANC K/uL (Mean and SD)	4.9 (2.3)	5.3 (2.3)	4.7 (2.1)	5.3 (2.4)
ALC K/uL (Mean and SD)	1.6 (1.4)	1.4 (0.7)	1.6 (0.8)	1.5 (1.4)
Hemoglobin g/dL (Mean and SD)	12.6 (1.6)	13.1 (1.9)	12.4 (1.4)	13.1 (1.9)
Serum Creatinine mg/dL (Mean and SD)	<b>0.73 (0.1)</b>	<b>1.16 (0.4)</b>	<b>0.78 (0.3)</b>	<b>1 (0.3)</b>
eGFR <sup>^</sup> (ml/min/1.73m <sup>2</sup> )	>=60: 82; <60: 0	>=60:44; <60: 13	>=60: 56 <60: 4	>=60: 70 <60: 9
eGFR by CKD-EPI equation in ml/min/1.73m <sup>2</sup> (Median and IQR)	93.16 (86.91-101.3)	72.12 (58.14 – 83.26)	90.38 (77.9 – 100.3)	83.62 (69.62 – 92.65)
Fasting Glucose in mg/dL (Median and IQR)	102 (92-115)	106 (95-122)	101.5 (93.5-113)	105 (94-126)
BMI at baseline (kg/m <sup>2</sup> )	28.5 (6.4)	28.6 (4.6)	29.3 (7.1)	28 (4.3)
Alkaline Phosphatase in U/L (Median and IQR)	75.5 (61-95)	71 (62-98)	70.5 (59 - 97)	75 (63-95)
ALT in U/L (Median and IQR)	17 (11-24)	16 (12-23)	15 (11-22.5)	18 (13-25)
AST in U/L (Median and IQR)	18 (13-25)	19 (13-25)	16 (12.5-21)	20 (14-28)
Systolic blood pressure in mm Hg (Mean/SD)	131.4 (19.3)	131.7 (17.9)	129 (20.3)	133.4 (17.3)
Diastolic blood pressure in mm Hg (Mean/SD)	77 (12)	76.7 (13.6)	74.3 (12.7)	78.8 (12.3)
<b>Disease related weight loss</b>				
BMI measured up to 6 months before baseline (Mean and SD)	28.8 (6.6) (N=79/82)	29 (4.3) (N=52/57)	29.5 (7.4) N=58/60	28.3 (4.1) N=73/79
<b>Treatment</b>				
Anti-PD-1 immunotherapy type	Mono: 47 (57.3) Combination: 35 (42.7)	Mono: 32 (56.1) Combination: 25 (43.4)	Mono: 34 (56.7) Combination: 26 (43.3)	Mono: 45 (57) Combination: 34 (43)

\*Includes three patients with BMI < 18.5 for descriptive purposes. Analyses by Cox-PH/logistic regression was performed by excluding underweight patients (n=3) but were included for RSF analysis where BMI was included as a continuous variable.

<sup>^</sup>eGFR – Estimated Glomerular Filtration Rate (Cockcroft-Gault).

# CAD: Coronary Artery Disease; CHF: Congestive Heart Failure; MI: Myocardial Infarction and AF: Atrial Fibrillation  
IQR: Inter Quartile Range

## Supplementary Text

### Methods

Random Survival Forests – an ensemble machine learning algorithm which is an extension of random forests suitable for handling right censored outcomes (20,24).

VIMP – is the prediction error of the original constructed ensemble minus the ensemble constructed newly obtained by randomizing “x” assignments (20,24).

Minimal depth – is the distance of the splitting variable from the root of the trunk (20,24).

Concordance index – fraction of all pairs of subjects whose predicted survival times can be correctly ordered that can be ordered among all subjects (20).

### Results

*Exploration of other complex interactions for explaining gender-based association of overweight/Class I obesity with improved outcomes*

Cardiovascular comorbidities abolished the obesity paradox pattern, presence of diabetes or hyperlipidemia largely attenuated the paradox but did not provide a strong basis for explaining gender-based difference in outcomes (Supplementary Fig-3). For lifestyle habits, there was no gender-based interaction with smoking (Supplementary Fig-3). There were no obvious interactions with different anti-hypertensive drugs, statins or anti-platelet agents but patients receiving metformin had worse survival (Supplementary Fig-4) although there were few patients who received metformin and did not provide an explanation for the gender-based association.

Among prior treatments, patients with prior history of targeted therapy and chemotherapy had worse outcomes in general but relatively few patients had these prior treatments and

did not explain the gender-based difference in outcomes (Supplementary Fig-5 and Table-1). There was no gender-based interaction noted however for BRAF and NRAS mutation for the association of BMI with outcomes (Fig-6). Exploration of other interactions of BMI with covariates related to demographics, disease severity showed that overweight/Class I obese patients were more likely to have improved survival outcomes if they were aged 45 -75 years, had Charlson's Comorbidity Index of <10, KPS  $\geq$ 80, normal LDH levels and Stage < IVM1c (Supplementary Fig-7) but these findings do not provide an explanation for a gender-based difference in outcomes. The obesity paradox was attenuated in the presence of low albumin (<3.5 g/dL), high ANC (>8 K/uL), low and high ALC (>3 K/uL), low hemoglobin (<11.5 g/dL) (Supplementary Fig-8). These results concur with findings from studies on clinical prognostic markers in melanoma (57). Patients with lower serum creatinine were more likely to have lower hemoglobin and serum albumin (Table-1) and although these factors likely have contributed to the findings, gender based density distributions of albumin and hemoglobin within BMI risk groups and creatinine groups show that albumin and hemoglobin on their own do not explain the gender based differences in survival outcomes as strongly as serum creatinine (Supplementary Fig-9) and the absolute numbers of patients who had hemoglobin and serum albumin below the risk threshold were relatively low (Supplementary Fig-9 and Table 1).

## **SUMMARY OF PROJECT 1 & 2 CONCLUSIONS**

The first project demonstrated that in a high dimensional setting (small “n” and large “p”), RSF machine learner had the best relative performance compared to Cox-PH model. Further, patients with ds-GPA  $> 1$  had prolonged survival, nearly 2-3 times longer than historical estimates in the treated setting (radiation and/or surgery). Baseline ds-GPA had the strongest predictive performance among all individual features and ds-GPA based risk groups were identified. Additionally, patients who had prior surgical resection and stereotactic radiation had improved survival for ds-GPA  $> 1$ . RPA based feature subsets or RPA alone performed poorly in the setting of anti-PD-1 based immunotherapy among patients with melanoma brain metastases.

The second project used RSF as the primary analytical tool and elucidated a complex “U” shaped relationship between BMI and survival outcomes, where overweight/Class-I obese patients had a lower risk of mortality, progressive disease and higher probability of achieving durable clinical benefit compared to normal weight. This study showed the presence of “obesity paradox” albeit for overweight/Class I obese patients. This association was predominantly driven by male gender and was attenuated among patients with low serum creatinine (predominant among females), a surrogate for skeletal muscle mass in the setting of advanced metastatic disease. These findings suggest that direct measures of skeletal muscle mass or sarcopenia (low skeletal muscle mass) may be more suitable predictors of survival/clinical benefit in the setting of anti-PD-1 based immunotherapy treatment for metastatic melanoma than BMI alone.

## **DISCUSSION AND PERSPECTIVES**

Performance of machine learning algorithms in a high dimensional setting (small “n” and large “p”) in a clinical prediction modeling context is not well documented. This is a common scenario for relatively rare cohorts like melanoma brain metastases and more so in the context of availability of newer cancer immunotherapies. The underlying philosophy of machine learning is prediction and is well suited for identification of complex patterns and inter-relationships and is increasingly being used in clinical studies (1); as such we used these methods in a high dimensional setting in a clinical context and demonstrated improved predictive performance in Project 1 and identified ds-GPA based risk groups likely to have survival benefit to anti-PD-1 based treatment after accounting for several covariates; In Project 2 we elucidated a U shaped relationship of BMI with survival and identified a complex inter-relationship of BMI, gender and serum creatinine, generating an important hypothesis that sarcopenia may be a more relevant predictor of survival in the setting of PD-1 blockade. Findings from Project I/II would need external validation from external datasets. Future work should focus on developing predictive models for intracranial response and molecular mechanisms underlying sarcopenia and survival outcomes in this setting. In conclusion, machine learning algorithms improve predictive performance, identify complex relationships and inter-relationships of clinical relevance and provides a feasible alternative along with standard methods to analyze relatively smaller data sets common in clinical cancer immunotherapy research.

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