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CLINICAL AND THERAPEUTIC SIGNIFICANCE OF OBESITY IN MELANOMA

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CLINICAL AND THERAPEUTIC SIGNIFICANCE OF OBESITY IN MELANOMA

A

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Acknowledgements

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CLINICAL AND THERAPEUTIC SIGNIFICANCE OF OBESITY IN MELANOMA

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While the FDA approval of targeted and immune therapies in metastatic melanoma (MM) have dramatically improved outcomes in this disease, *de novo* and/or acquired resistance can limit the clinical benefit of these agents. The IGF-1/PI3K/AKT pathway has been implicated in resistance to both targeted and immune therapy. The IGF-1/PI3K/AKT pathway has also been shown to play a key role in the pathogenesis of obesity in other malignancies. To date, the impact of energy balance on clinical outcomes and therapeutic response in MM has not been studied. I hypothesized that energy balance would impact the molecular biology, behavior, and drug sensitivity of melanoma.

The association of body mass index (BMI) with overall survival (OS) and progression-free survival (PFS) was studied in independent cohorts of >1900 MM patients treated with targeted therapy [dabrafenib and trametinib (D+T) and vemurafenib and cobimetinib], immunotherapy [ipilimumab and anti-PD-1/PDL-1], and chemotherapy. The functional significance of obesity was tested using a mouse model of diet-induced obesity (DIO) injected subcutaneously with murine melanoma cells. Tumors were followed for growth and assessed by proteomics and flow cytometry. The effect of DIO on therapeutic sensitivity was tested in tumor-bearing mice treated with a) D+T and b) anti-PD1.

Obesity was associated with significantly improved PFS and OS in MM patients treated with both targeted therapy and immunotherapy but not chemotherapy. Improved outcomes were not attributable to differences in clinical prognostic factors or treatment-related adverse events. The association of BMI with improved outcomes was driven by markedly improved survival in obese compared to normal BMI males, whereas no significant associations were observed in females. In a subcutaneous model of mouse melanoma, DIO led to increased tumor growth, increased PI3K pathway activation, and decreased immune infiltrates. There were no differences in sensitivity to D+T or anti-PD1 between diets in this model.

Obesity is associated with markedly improved outcomes in MM patients treated with targeted and immune therapies. In a subcutaneous model of murine melanoma, DIO increased tumor growth, recapitulating clinical associations in early stage melanoma. The biological basis for the paradoxical association of obesity with improved outcomes in MM should be explored further.

Abbreviations

AE= adverse event

BMI= body mass index

CI=confidence interval

CTLA-4= Cytotoxic T-lymphocyte associated protein 4

D+T= dabrafenib + trametinib

DIO= diet-induced obesity

DTIC= dacarbazine

ECOG PS=Eastern Cooperative Oncology Group performance status

FDA= Food and Drug Administration

HR=hazard ratio

IGF1R= insulin-like growth factor receptor

IPI= ipilimumab

IR= insulin receptor

irAE= immune related adverse

LDH=lactate dehydrogenase

MAPK= mitogen-activated protein kinase

MEK= mitogen-activated protein kinase kinase

MM= metastatic melanoma

OR= odds ratio

OS= overall survival

PD-1= Programmed cell death protein 1

PD-L1= Programmed death-ligand 1

PFS= progression-free survival

PI3K=phosphoinositide 3-kinase

PTEN= Phosphatase and tensin homolog

RECIST= Response Evaluation Criteria in Solid Tumors

ULN= upper limit of normal

V+C= vemurafenib + cobimetinib

WT= wild type

Metastatic melanoma is an aggressive disease with poor outcomes historically. However, the outcomes of patients with metastatic melanoma have improved dramatically with the FDA approval of MAPK pathway-directed targeted therapies and checkpoint inhibitor immunotherapies.(1-7) Despite these many new options, metastatic melanoma patient outcomes remain heterogeneous and many patients still succumb to this disease. An improved understanding of factors associated with clinical benefit from these treatments may improve their personalized use and provide new insights into mechanisms of resistance. In other malignancies, clinical metabolic phenotypes (obesity and the metabolic syndrome) have been shown to correlate with clinical outcomes.(8-13) In preclinical models, dietary manipulation impacts tumor growth and sensitivity to anti-cancer therapies.(14-18) However, the impact of energy balance on melanoma molecular signaling, immunology, and response to therapy is currently unknown.

The treatment of metastatic melanoma

Melanoma is the most deadly of the common skin cancers. While clinically localized melanoma is curable by surgical resection, melanoma that has metastasized is an aggressive disease. Outcomes in patients with metastatic melanoma have historically been poor as chemotherapy has only limited activity in this disease. However, the treatment landscape for patients with metastatic melanoma has dramatically improved with the FDA approval of 10 new drugs and combination regimens since 2011. These new therapies are the result of advances in the understanding of the molecular biology and immunology of this disease.

Nearly 50% of cutaneous melanomas have an activating V600E point mutation in BRAF (BRAF^{V600})(19) resulting in constitutive activation of the RAF/MEK/ERK MAPK pathway that promotes cell proliferation and survival. Randomized phase III trials demonstrated that

vemurafenib and dabrafenib (both BRAF inhibitors) significantly improved ORR and PFS in BRAF^{V600} metastatic melanoma patients, leading to their approval.(2, 20) However, ~50% of patients failed to respond, and 10% of patients had disease progression as their best response, indicating the presence of *de novo* resistance. In addition, the median duration of response was only ~6 months due to rapid development of acquired resistance. Concurrent inhibition of BRAF and MEK can help overcome MAPK pathway reactivation, as demonstrated by the improvement in ORR (75%) and PFS (~10 months) with combined BRAF and MEK inhibition (dabrafenib + trametinib and vemurafenib + cobimetinib). (4, 5) However, responses remain variable, and most patients still go on to develop resistance.(21)

The other major category of systemic therapies now approved in metastatic melanoma is immunotherapy. Melanoma has long been known to be a highly immunogenic tumor. High-dose interleukin-2 (HD-IL2), a cytokine therapy, was FDA approved in 1998 in metastatic melanoma based on rare but durable responses.(22) The newer immunotherapies are the checkpoint inhibitors which work by blocking the inhibitory checkpoints that limit T cell activation, effectively "taking the brakes off" the immune system to allow it to eradicate tumors. Anti-CTLA4 (Ipilimumab) was the first checkpoint inhibitor FDA approved in 2011.(1, 23) Though the response rate with ipilimumab is only 10-15%, these responses are extraordinarily durable. Subsequently, the anti-PD1 antibodies nivolumab and pembrolizumab have been FDA approved in metastatic melanoma (and other diseases). These agents have response rates ~40% in metastatic melanoma are much better tolerated than ipilimumab.(6, 24). However, while responses to checkpoint inhibitors are extremely durable, the majority of patients fail to respond, representing *de novo* resistance, and biomarkers to accurately predict response are lacking.

IGF1/PI3K/AKT signaling and therapeutic resistance

Oncogenic activation of the PI3K/AKT pathway is one of the most frequent events in cancer. The PI3K pathway can be activated by binding of ligands to receptor tyrosine kinases, including insulin-like growth factor receptor (IGF1R) and the insulin receptor (IR). The Davies lab previously demonstrated that BRAF[V600] melanoma cell lines with *de novo* resistance to BRAF and MEK inhibitors are characterized by increased expression of IGF1R and compensatory activation of the PI3K/AKT pathway following MAPK pathway inhibition (Figure 1). (25, 26) Both PI3K/AKT pathway activation and therapeutic resistance could be overcome by inhibition of IGF1, IGF1R, or AKT. Increased activation of IGF1R was also identified in both cell lines and patient samples with acquired resistance to BRAF inhibitors.(27) While combined inhibition of BRAF and MEK was able to slow growth of these cell lines, inhibition of IGR1R or PI3K was required to achieve cell death.(27)

We have also recently shown that the PI3K/AKT pathway can mediate resistance to immunotherapy. (28) Loss of the tumor suppressor PTEN is one of the most common ways that the PI3K/AKT pathway is activated and occurs in ~30% of melanoma.(29) In patients, loss of PTEN is correlated with decreased tumor infiltrating lymphocytes and worse outcomes with anti-PD1 immunotherapy. In preclinical models, PTEN loss decreases T cell trafficking into tumors and inhibits T cell-mediated tumor killing. (28)

These and other studies support the rationale for combining PI3K pathway inhibition with MAPK pathway inhibition and/or checkpoint inhibitors. However, clinical development of IGF-1R/PI3K pathway inhibitors has been slow due to the challenge of achieving significant target inhibition at clinically tolerated doses.(30) However, other less toxic strategies to inhibit this pathway could have benefit.

Energy balance and cancer

Obesity and the metabolic syndrome are well-established risk factors for many malignancies, including breast, endometrial, colon, and pancreatic cancers.(31) In fact, obesity is now poised to overtake smoking as the leading preventable cause of cancer. Obesity has also been associated with increased recurrence risk and mortality in some malignancies.(11, 12) Based on strong epidemiological evidence, preclinical studies demonstrating increased tumor growth in obese models, and biological plausibility, energy balance has become a major target for cancer prevention efforts and identified as a priority area of research by the American Society of Clinical Oncology and the National Cancer Institute.(32)

However, higher BMI has also been associated with improved survival in some cancers,(33-36) a phenomenon dubbed the "obesity paradox." Whether this unexpected association is due to disease biology, ability to tolerate cytotoxic treatments, or other factors such as concurrent medication use for obesity-related comorbidities that may impact cancer biology (i.e. statins, metformin, beta blockers) remains unclear.

Molecular and immunological effects of energy balance

The biology underlying the impact of energy balance on cancer risk and progression is complex, and includes the stimulation of signaling pathways by obesity-related cytokines and hormones such as insulin-like growth factor 1 (IGF1) and leptin, chronic inflammation, increased oxidative stress, and adipocyte cross-talk.(37) The insulin/IGF axis has been implicated as one of the key mediators in the relationship between obesity and cancer.(37, 38) Insulin and IGFs regulate growth and metabolism at both the organism and tissue level. Obesity results in high circulating levels of these hormones, which are in turn linked to increased incidence and worse outcomes in several cancers.(39, 40) Diet-induced obesity (DIO) has been shown in preclinical models of many tumor types to promote cancer

development and progression as well as induce resistance to therapy, often in association with increased IGF1 and activation of the PI3K-AKT pathway.(14-18) Conversely, calorie restriction (CR) has been shown to suppress tumor growth and prolong survival.(18, 41-44) IGF1 appears to be a key mediator of many of these effects as knock-down of IGF1 attenuates the growth stimulatory effects of DIO and restoration of IGF1 abolishes the inhibitory effects of CR on tumor growth.(45, 46)

Links between obesity, chronic inflammation, and cancer initiation have been described in many malignancies. Obesity has been demonstrated to negatively impair the adaptive immune response, with increased rate of vaccine failure in patients, and decreased cell-mediated immunity and immunological memory in DIO animal models.(47) However, the impact of energy balance on anti-tumor immunology remains largely unexplored and the impact of obesity on response to checkpoint inhibition has not been examined in any disease.

Energy balance in melanoma

The impact of energy balance in melanoma has not been well-studied to date. Limited data suggests that obesity is associated with a slightly increased risk of melanoma in men (48) and increased primary tumor Breslow thickness.(49) However, the association of obesity with clinical outcomes in patients with melanoma had not been previously investigated. (31) The Lee lab at MD Anderson recently demonstrated that obesity is associated with worse outcomes in a large cohort of patients with surgically resected melanoma.(50) In this study, we examined the association of body mass index (BMI) with overall survival and disease free survival in 1186 patients with surgically resected melanoma (75% Stage I/II, 24% Stage III, and 1% Stage IV). Obesity (BMI≥30) was associated with worse overall survival and disease free survival in this cohort, associations that remained significant after adjusting for sex, age, and disease stage. However, when serum C-reactive protein (CRP), a key marker of inflammation that has previously been associated with worse

prognosis in this population,(51) was added into the model, obesity was no longer significantly associated with survival but CRP was. This indicates that the association of obesity with poor outcomes in surgically resected melanoma may be mediated by chronic inflammation. While this study included patients of all stage of melanoma, the vast majority had clinically localized disease as this was a surgical cohort. Only 15 patients had Stage IV melanoma; therefore, this study was underpowered to examine the association of BMI with outcomes in Stage IV melanoma. Moreover, this cohort was accrued between 1998 and 2008, which was before the FDA approval of contemporary targeted and immune therapy in melanoma and could therefore not be used to examine outcomes with these therapies.

In preclinical mouse models of B16 melanoma, diet-induced obesity has been shown to increase melanoma tumor growth and progression, however, the mechanisms are poorly understood.(52-54) Thus far, energy balance has not been studied in genetically relevant melanoma models (such as BRAF mutant), nor has the impact of energy balance on sensitivity to clinically relevant targeted or immune therapies.

In summary, there is strong parallel evidence that (1) energy balance modulates the activity of the PI3K signaling pathway, and (2) activation of the PI3K signaling pathway can cause resistance to both targeted and immune therapy in melanoma (Figure 1). I thus hypothesized that energy balance could impact the biology of melanoma and the efficacy of targeted therapy and immune therapy. To test this hypothesis, I assessed the association of BMI with overall survival (OS), progression-free survival (PFS), and overall-response rate (ORR) in multiple large independent cohorts of metastatic melanoma patients treated with targeted and immune therapies. In addition, I conducted functional testing of the impact of obesity on melanoma tumor growth, molecular signaling, and therapeutic response in a genetically relevant, immunocompetent murine melanoma tumor model.

Figure 1: Molecular signaling in obesity and melanoma.

The PI3K pathway plays a key role in both melanoma and energy balance signaling. Obesity results in increased circulating insulin and IGF-1 which bind to their cell surface receptors leading to downstream activation of the PI3K pathway. The PI3K pathway is also commonly activated in melanoma and can lead to resistance to both targeted and immune therapies.

Chapter 2: Methods

Clinical cohorts

Analysis was conducted on independent cohorts of patients with metastatic melanoma, including 2 trial cohorts of patients treated with BRAFi + MEKi targeted therapy combinations, a multi-institutional cohort of patients treated with PD-1 immunotherapy, a trial cohort of patients treated with ipilimumab immunotherapy + DTIC chemotherapy vs. DTIC alone, and a control arm of DTIC chemotherapy (Table 1).

BMI at treatment initiation was calculated as weight (kilograms) divided by the square of height (in meters) and categorized according to standard World Health Organization definitions of underweight (BMI<18.5), normal weight (18.5-24.9), overweight (25-29.9), and obese (≥30).(55) Underweight patients were excluded from analyses due to low prevalence (<2%) across the cohorts.

Patients were followed from the date of treatment initiation or baseline randomization until disease progression [progression-free survival (PFS)] or death (OS). Disease progression and response rate were defined by Response Evaluation Criteria in Solid Tumors (RECIST) 1.1 criteria.(56) Survival curves for OS and PFS across BMI category and by sex were generated using the Kaplan-Meier method. The association of BMI with prospective survival outcomes was evaluated in Cox proportional hazards regression models adjusted for prognostic factors. Logistic regression was used to assess associations of BMI with treatment response and pharmacokinetics. In all analyses, normal BMI was used as the reference category.(57) Statistical analyses were performed utilizing SAS 9.4, JMP (SAS), R studio, and S+ 8.0.

Table 1: Metastatic melanoma patient cohorts

Therapy	Cohort	Participants	Men	Women
Targeted Therapy	D+T	599	347	252
	V+C	240	143	97
Immune Therapy	IPI + DTIC	207	138	69
	PD1/PDL1	331	214	117
Chemotherapy	DTIC	320	174	146
	DTIC	221	140	81

Cell lines

BRAF-mutant murine melanoma cell lines were provided by Marcus Bosenberg from the Yale University Mouse Melanoma (YUMM) lines, Yale University.(58) YUMM 3.1 cells (BrafV600E::Cdkn2a-/-) and YUMMM 5.2 (BrafV600E/wt p53−/−) cells were maintained in DMEM-F12 media with 10% FBS, 1% NEAA and 1% PS.

Mice and diets

Male C57/Bl6 18 week old diet-induced obesity (DIO) (#380050) and control mice (#380056) were obtained from Jackson laboratories. These mice have been fed either a 60% high-fat diet (Research labs D12492, composition- 60% fat, 20% protein, 20% carbohydrates) or a low fat matched purified ingredient isocaloric diet (Research labs D12451, composition- 20% protein, 70% carbs, 10% fat) since weaning at 6 weeks. Mice were acclimatized for 3 weeks and continued on the same diets. All animal experiments were approved by The University of Texas MD Anderson Cancer Center Animal Care and Use Committee

Mouse phenotypic analyses

Mice were weighed weekly. Following overnight fasting, serum IGF-1 (R&D System) and IGFBP-1 (EMD Millipore) were measured by ELISA and blood glucose was measured by glucometer at baseline.

Treatments

$1x10^6$ cells YUMM 3.1 or YUMM 5.2 cells were injected subcutaneously into the left flank of 21 week DIO or control mice. Tumor-bearing mice were treated with twice weekly IP injections of 200mg/kg of anti-PD1 antibody (Bioxcell Clone RMP1-14) or isotype control

(Bioxcell Clone 2A3) or daily oral gavage treatment of dabrafenib (30mg/kg) and trametinib (1 mg/kg) or vehicle. Tumors were measured every 3 days with calipers and tumor volume was calculated as the product of shortest length squared x longest length.

Cell line protein isolation and western blotting

Cells were plated in 6-well plates overnight. Cells were collected by scraping and washing 3x with cold PBS. The pellets were then lysed in buffer to isolate protein. Protein was quantified using the BCA reaction. Protein concentration was adjusted to 1.5mg/ml. Western blots of cell lines were performed for insulin receptor and IGF-1R (Cell Signal) with actin run to confirm equivalent protein loading between samples. For reverse phase protein array, cell lysate was mixed with 4 x SDS sample buffer and boiled for 5 minutes and then stored at -80.

Tissue handling

Day 14 tumor tissue was divided with 1/2 OCT-embedded, 1/4 FFPE, and 1/4 snap frozen. For each tumor sample used for protein extraction, a hematoxylin and eosin (H&E)-stained slide was prepared and reviewed by a pathologist (M Tetzlaff). Regions containing 80% or more viable tumor cells were identified. To isolate tumor tissue, the marked H&E slide was used to guide macrodissection of the matched tissue block.

Reverse-phase protein array

Extraction of protein from the dissected tumor samples was performed by the MD Anderson Functional Proteomics Core facility as previously described. (59) Reverse phase protein array (RPPA) analysis, which quantitatively measures >200 total- and phospho-proteins in oncogenic signaling pathways, was performed as previously described.(60) A

detailed description of the RPPA method and data normalization is available at the core facility's web page. [1]

Immunohistochemistry

FFPE tissue blocks were cut into 5-µm sections. Immunohistochemistry for pS6 (Clone D57.2.2E Cell Signaling, catalog #4858) was performed. Stained slides were reviewed by a pathologist (M.T. Tetzlaff) and an H-score was derived by multiplying the staining intensity (0, 1+, 2+, or 3+) by the percentage of positive cells.

Flow cytometry

Tumor tissues and spleens were weighed and dissociated into single cell suspensions. Erythrocytes in all samples were depleted using ammonium-chloride-potassium lysing buffer (Invitrogen). Cells were treated with Fc blocking antibody and then stained with mAbs against CD8, CD4, Gr-1, CD11b, F4/80 and CD25. Samples were analyzed using FACS cantoII (BD Biosciences).

Statistical analysis

GraphPad Prism was used to perform statistical tests and graphing. A t-test was used to test for significance and data was plotted with standard error of the mean (SEM) shown. *P* values less than 0.05 were considered statistically significant. Heatmaps were generated using Java Cluster and Tree View.

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[1] https://www.mdanderson.org/research/research-resources/core-facilities/functional-proteomics-rppa-core.html

Introduction

Nearly 50% of cutaneous melanomas have an activating BRAF^{V600} mutation (19) resulting in constitutive activation of the RAF/MEK/ERK MAPK pathway that promotes cell proliferation and survival. Treatments targeting the MAPK pathway have impressive activity in BRAF^{V600} mutant metastatic melanoma with response rates of >70% for both FDA-approved BRAF + MEK combinations. However, not all patients respond and most go on to develop resistance.(21) Understanding the clinical factors associated with benefit from these therapies is critical to risk-stratifying patients and making informed treatment decisions.

A key mechanism of resistance to MAPK pathway targeted therapy is PI3K pathway activation. This can occur at multiple nodes in this pathway, including via binding of receptor tyrosine kinases on the cell surface such as insulin and IGFR. Obesity results in higher circulating insulin and IGF-1, even in the absence of diabetes, and binding of these growth factors leads to downstream activation of the PI3K pathway. This interaction has been demonstrated in preclinical models in other malignancies to be key in the pathogenesis of obesity in promoting tumor growth and resistance to targeted therapy. (14-18, 61, 62) Given this, I hypothesized that obesity would be associated with worse outcomes in patients with BRAF^{V600} melanoma treated with targeted therapy.

Results

Initial analysis was conducted on a cohort of treatment-naive patients with *BRAF^{V600}*-mutant metastatic melanoma treated with the BRAF inhibitor + MEK inhibitor combination dabrafenib and trametinib (D+T; FDA approval, 2014) in the randomized clinical trials BRF113220 (part C), COMBI-d, and COMBI-v with available BMI at treatment initiation (n=610).(4, 63-65)

Eleven patients (1.8%) were underweight (BMI<18.5), 222 (37.1%) were normal weight (BMI 18.5-24.9), 231 (38.6%) were overweight (BMI 25-29.9), and 146 (24.4%) were

obese (BMI ≥30) (Figure 2). Clinical characteristics, including tumor burden, LDH, and ECOG PS were similar across BMI groups (Table 2). However, patients with higher BMI tended to be older, male, and less likely to have Stage M1C disease. As expected, obese patients were more likely to use metabolic syndrome-associated medications (metformin, statins, beta blockers, and aspirin).

With a median follow-up of 20.2 months, the median PFS and OS were 9.6 and 19.8 months, respectively, for normal BMI, 11.0 and 25.6 months for overweight, and 15.7 and 33.0 months for obese patients treated with D+T (Figure 3). Obese patients had significantly improved PFS (HR 0.73, 95% CI 0.56-0.95) and OS (HR 0.63, 95% CI 0.46-0.86) compared to normal BMI patients (Table 3). Analysis of BMI as a continuous variable demonstrated a dose-dependent inverse relationship between BMI and HR for PFS that extended through morbid obesity (Figure 4).

On multivariate analysis incorporating clinicopathological factors previously associated with outcomes with D+T(65) (age, sex, stage, LDH, $BRAF^{V600}$ mutation type, ECOG PS, sum of target lesion diameters, number of disease sites, and prior adjuvant therapies), obesity remained associated with improved PFS (multivariate HR 0.75, 95% CI 0.57-0.99) and OS (HR 0.59, 95% CI 0.43-0.82) (Table 3). I also examined the possible contribution of metabolic syndrome related medication use, including metformin, beta blockers, aspirin, and statins as these medications may have potential anti-cancer activity. Following adjustment for concomitant medication use, obesity remained strongly associated with improved OS (multivariate HR 0.63, 95% CI 0.44-0.88), while the association with PFS was slightly attenuated (HR 0.77, 95% CI 0.58-1.03). Exclusion of patients taking metformin (n=50) did not substantively change the association observed for obese BMI and OS (HR 0.63, 95% CI 0.44 – 0.91).

Clinical response rates with D+T were also modestly increased in obese patients

(OR 1.6, 95% CI 1.0-2.6) (Table 4) .Rates of adverse events were similar by BMI category

(Table 4).
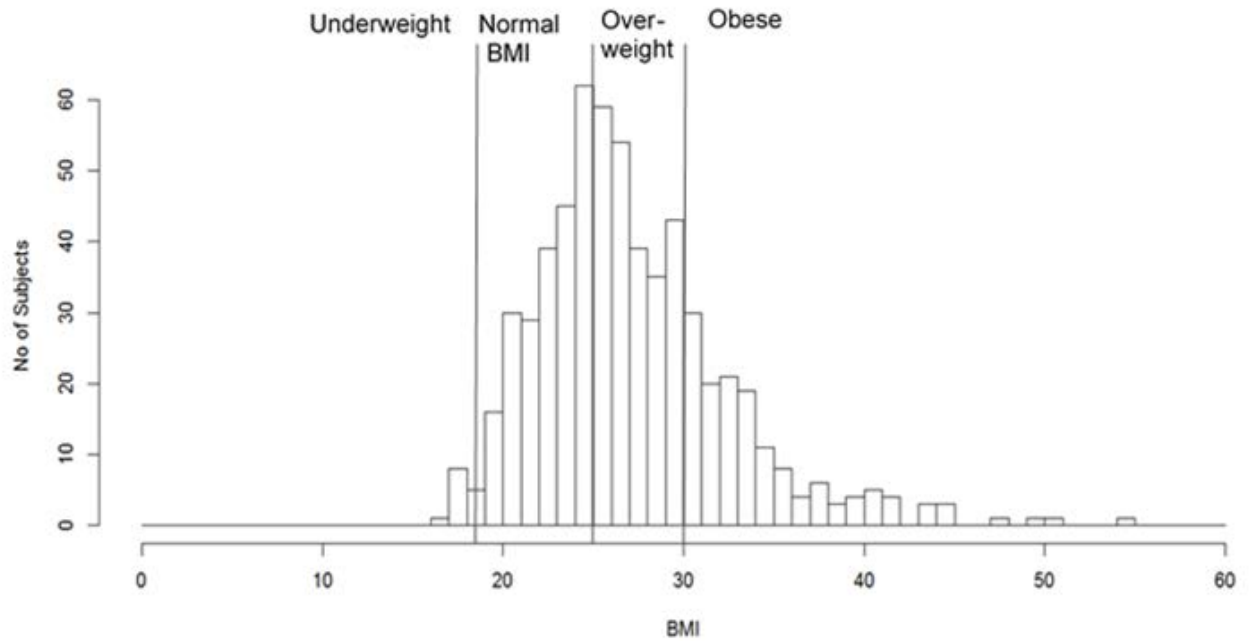
Figure 2: BMI distribution of D+T cohort.

Histogram of BMI distribution shows a similar distribution to the general US population. <2% of patients were underweight and >60% were overweight or obese.

Table 2: Baseline characteristics of patients treated with dabrafenib and trametinib

(D+T) by BMI category

Characteristic	BMI at treatment initiation		
	Normal BMI (18.5 to <25)	**Overweight (25 to <30)**	**Obese (≥30)**
Patients, No. (%)	222 (37.1)	231 (38.6)	146 (24.4)
Age, Mean, y (range)	52 (18-91)	56 (22-82)	56 (30-82)
Male, No. (%)	109 (49.1)	156 (67.5)	82 (56.2)
Stage, No. (%)			
III/M1a/M1b	71 (32.0)	81 (35.1)	59 (40.4)
M1c	151 (68.0)	150 (64.9)	87 (59.6)
LDH, No. (%)[a]			
>ULN	51 (23.2)	54 (23.4)	36 (24.7)
>2xULN	28 (12.7)	25 (10.8)	12 (8.2)
BRAF mutation, No. (%)			
V600E	201 (90.5)	192 (83.1)	129 (88.4)
V600K/V600E + V600K	21 (9.5)	39 (16.9)	17 (11.6)
ECOG PS, No. (%)[b]			
0	159 (72.3)	168 (73.0)	103 (70.5)
≥1	61 (27.7)	62 (27.0)	43 (29.5)
Sum of lesion diameter, No. (%)[c]			
<median (57mm)	101 (47.0)	117 (51.1)	72 (49.3)
≥median (57mm)	114 (53.0)	112 (48.9)	74 (50.7)
Number of organ sites with metastases, No. (%)			
<3	112 (50.5)	114 (49.4)	82 (56.2)
≥3	110 (49.5)	117 (50.6)	64 (43.8)
Prior adjuvant ipilimumab, No. (%)	2 (0.9)	3 (1.3)	4 (2.7)
Prior non- ipilimumab adjuvant therapy, No. (%)	26 (11.7)	25 (10.8)	14 (9.6)
Concomitant medications, No. (%)			
Metformin	8 (3.6)	13 (5.6)	29 (19.9)
Statin	24 (10.8)	27 (11.7)	39 (26.7)
Beta blocker	27 (12.2)	47 (20.3)	43 (29.5)
Aspirin	23 (10.4)	26 (11.3)	37 (25.3)

[a]Data missing for 2 patients. [b]Data missing for 3 patients. [c]Data missing for 9 patients
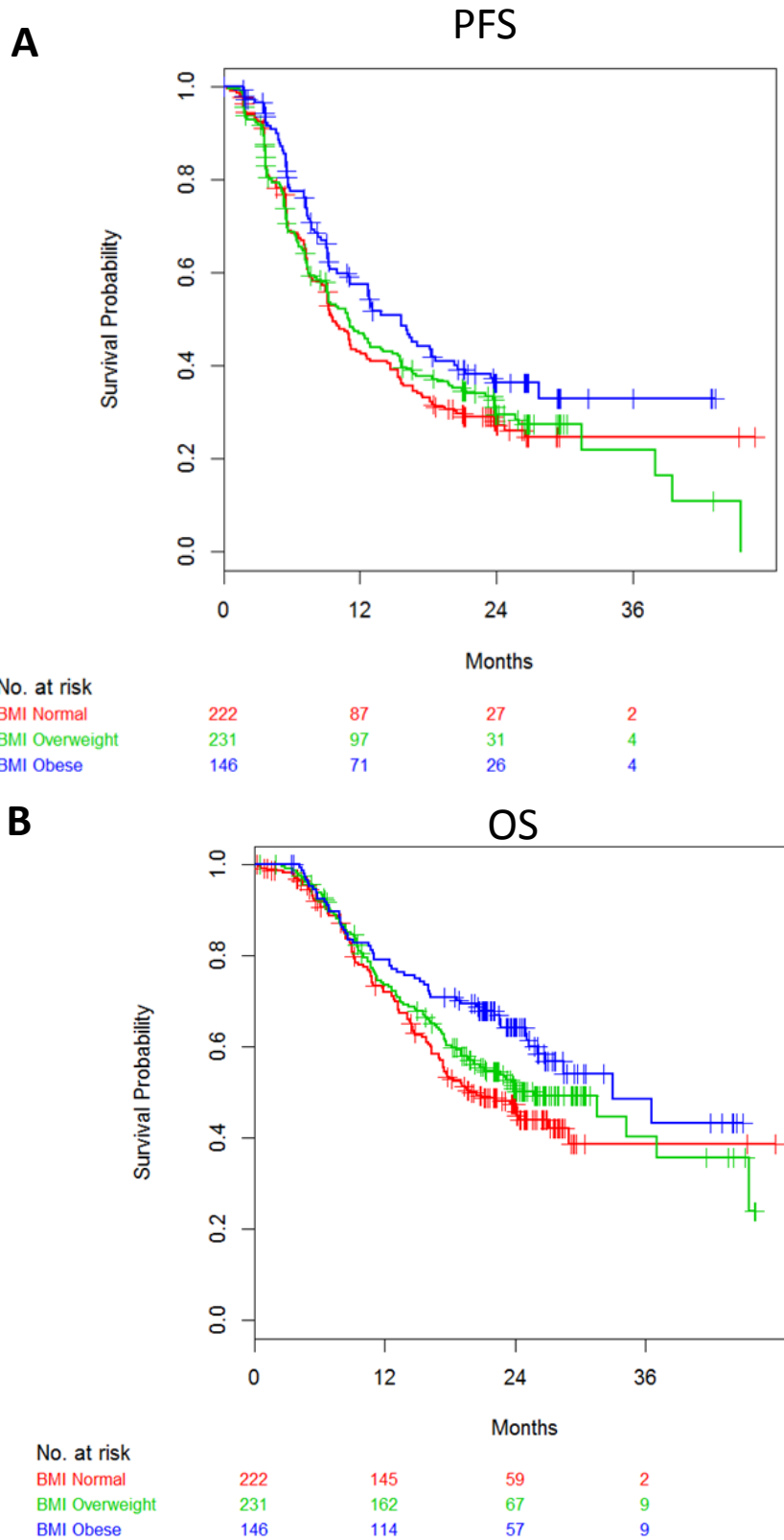
Figure 3: Outcomes by BMI category in patients treated with D+T. A. Progression-free survival. B. Overall survival. Blue=obese. Green=overweight. Red=Normal BMI

Table 3: Association between BMI and outcomes for patients treated with D+T

BMI	Patient No. (%)	PFS			OS		
		Median (mo)	Univariate Adjusted HR (95% CI)	Multivariate Adjusted HR (95% CI)[a]	Median (mo)	Univariate Adjusted HR (95% CI)	Multivariate Adjusted HR (95% CI) [a]
18.5 to <25	222 (37)	9.6	1.00	1.00	19.8	1.00	1.00
25 to <30	231 (39)	11.0	0.90 (0.76-1.19)	0.95 (0.75-1.21)	25.6	0.84 (0.65-1.10)	0.78 (0.59-1.02)
≥30	146 (24)	15.7	0.73 (0.56-0.95)	0.75 (0.57-0.99)	33.0	0.63 (0.46-0.86)	0.59 (0.43-0.83)

[a]Adjusted for age, gender, stage, LDH, BRAF mutation, ECOG performance status, sum of

target lesion diameters, number of disease sites, and prior adjuvant therapies
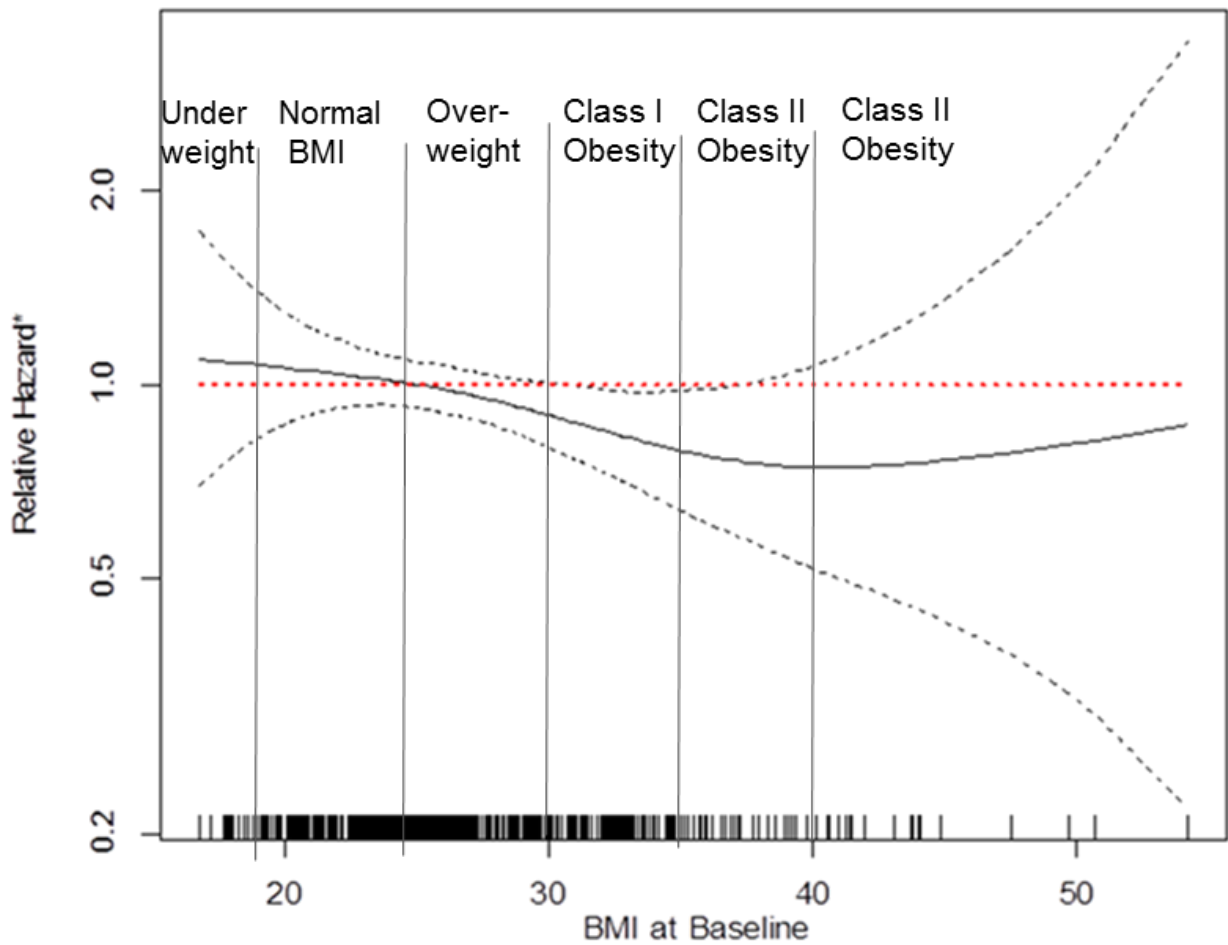
Figure 4: HR for PFS by BMI in patients treated with D+T. Spline analysis showing HR for

PFS (y-axis) by BMI (x-axis). Solid line indicates HR with curve centered at reference BMI 24.9.

Dotted lines indicate 95% CI.

Table 4: Clinical response rates and adverse events by BMI category in patients treated with D+T

Outcome	Normal BMI (18.5 to <25)	Overweight (25 to <30)	Obese (≥30)
Response rate	64%	65%	77%
OR vs Normal BMI (95%CI)		OR 0.90 (0.61-1.34)	OR 1.63 (1.0-2.63)
Adverse events			
Any AE	216 (98%)	226 (97%)	145 (>99%)
Grade III/IV AE	110 (50%)	125 (54%)	95 (65%)
AE leading to treatment discontinuation	28 (13%)	27 (12%)	30 (21%)

A cohort of patients treated with the only other FDA-approved BRAF inhibitor + MEK inhibitor combination for $BRAF^{V600}$-mutant metastatic melanoma, vemurafenib and cobimetinib (V+C; FDA approval, 2016), in the phase III coBRIM randomized controlled trial (n=241), was analyzed as a validation cohort.(5, 66) Clinical characteristics are presented in Table 5.

Obese patients treated with V+C again had improved PFS (HR 0.62, 95% CI 0.42-0.91) and OS (HR 0.64, 95% CI 0.41-0.98) compared to normal BMI patients, with hazard ratios very similar to those observed in the D+T cohort (Table 6). Following adjustment for clinical prognostic factors in this smaller cohort (age, gender, stage, LDH, BRAF mutation, ECOG performance status), the hazard ratios were minimally changed but statistical significance was lost (PFS HR 0.66, 95% CI 0.42-1.02; OS HR 0.62, 95% CI 0.37-1.02).

Pharmacokinetic data available for this cohort demonstrated no significant differences in serum Cobimetinib concentrations between BMI groups (Figure 5).

Table 5: Baseline characteristics of patients treated with V+C by BMI category

Characteristic	BMI at treatment initiation		
	Normal BMI (18.5 to <25)	Overweight (25 to <30)	Obese (≥30)
Patients, No. (%)	85 (35.4)	88 (36.7)	67 (27.9)
Age, Median, y (25-75%)	50 (39-61)	59 (50-67)	57 (44-65)
Male, No. (%)	40 (47.1)	59 (65.9)	44 (65.7)
Stage, No. (%)			
III/M1a/M1b	34 (40.0)	34 (38.6)	32 (47.8)
M1c	61 (60.0)	54 (61.4)	35 (52.2)
LDH >ULN, No. (%)[a]	45 (46.4)	41 (47.7)	26 (40.0)
BRAF mutation, No. (%)[b]			
V600E	61 (91.0)	62 (86.1)	44 (84.6)
V600K	6 (9.0)	10 (13.9)	8 (15.4)
ECOG PS, No. (%)[c]			
0	65 (77.4)	72 (82.8)	43 (64.2)
≥1	19 (22.6)	15 (17.2)	24 (35.8)

[a] Data missing for 3 patients. [b] Data missing for 49 patients. [c] Data missing for 2 patients.

Table 6: Association between BMI and outcomes for patients treated with V+C

	BMI	Patient No. (%)	PFS		OS	
			Univariate Adjusted HR (95% CI)	Multivariate Adjusted HR (95% CI)	Univariate Adjusted HR (95% CI)	Multivariate Adjusted HR (95% CI)
All patients (n=240)	18.5-24.9	85 (35)	1	1	1	1
	25-29.9	88 (37)	0.73 (0.51-1.04)	0.65 (0.43-1.00)	0.86 (0.58-1.28)	0.67 (0.43-1.06)
	≥30	67 (28)	0.62 (0.42-0.91)	0.66 (0.42-1.02)	0.64 (0.41-0.98)	0.62 (0.37-1.02)

Adjusted for age, sex, stage, and LDH
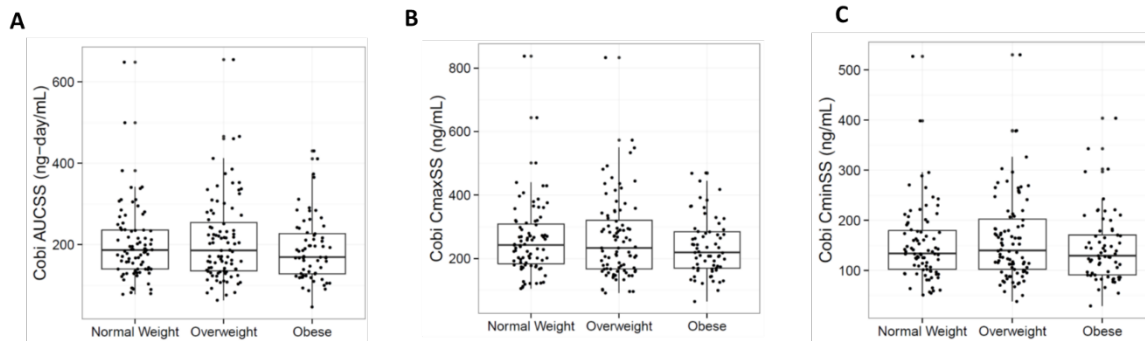
Figure 5: Pharmacokinetics by BMI category for Cobimetinib. No significant differences are seen in Cobimetinib serum pharmacokinetics by BMI category A. steady-state area under the curve (AUC) (p=0.39)   B. maximum concentration (Cmax) (p=0.34), and C. minimum concentration (Cmin) (p=0.37)

Sex differences in BMI and outcome associations with targeted therapy

As female sex was previously shown to be independently associated with improved survival in the D+T cohort,(65) and as there were sex differences in BMI distribution (Table 1), associations in men and women were next assessed separately. These analyses showed that obesity was associated with markedly improved outcomes in male patients but that BMI was not associated with outcomes in females (Table 7 and Figure 6). Median PFS and OS were 7.4 and 16.0 months respectively for normal BMI males, 10.1 and 21.3 months for overweight males, and 12.8 and 36.5 months for obese males

Obese males had significantly improved PFS (0.69, 95% CI 0.49-0.99) and OS (HR 0.46, 95% CI 0.30-0.70) versus normal weight males on univariate analysis, with differences in OS remaining significant after adjustment for other prognostic features (multivariate HR 0.44, 95% CI 0.29-0.69) (Table 7). These differences were marked, as the 2-year OS rate for obese males was 64% compared to 35% for normal BMI males (Figure 6 and Table 8). Obesity in males was also associated with improved response rates (ORR 76% vs. 58%, OR 2.26, 95% CI 1.20-4.26) (Table 8). In contrast, there were no significant differences in OS, PFS, or response rates by BMI in female patients treated with D+T (Tables 7 and 8).

Similar differences by sex were observed in patients treated with V+C. Obesity was associated with markedly improved PFS (HR 0.44, 95% CI 0.26-0.74) and OS (HR 0.53, 95% CI 0.29-0.93) in male patients. In contrast, no significant associations of BMI with outcomes were detected in female patients (Table 7).

Table 7: Outcomes by BMI stratified by sex for patients treated with targeted therapy

Population	BMI	Patient No. (%)	PFS		OS	
			Univariate Adjusted HR (95% CI)	Multivariate Adjusted HR (95% CI)	Univariate Adjusted HR (95% CI)	Multivariate Adjusted HR (95% CI)
D+T						
Male (n=347)	18.5-24.9	109 (31)	1	1	1	1
	25-29.9	156 (45)	0.85 (0.63-1.13)	0.93 (0.69-1.25)	0.73 (0.53-1.00)	0.80 (0.57-1.11)
	≥30	82 (24)	0.69 (0.49-0.99)	0.75 (0.52-1.08)	0.46 (0.30-0.70)	0.44 (0.29-0.69)
Female (n=252)	18.5-24.9	113 (45)	1	1	1	1
	25-29.9	75 (30)	0.95 (0.65-1.39)	1.05 (0.69-1.59	0.84 (0.52-1.35)	0.65 (0.37-1.13)
	≥30	64 (25)	0.74 (0.48-1.12)	0.83 (0.54-1.29)	0.89 (0.55-1.45)	0.93 (0.56-1.55)
V+C						
Male (n=143)	18.5-24.9	40 (28)	1	1	1	1
	25-29.9	59 (41)	0.69 (0.44-1.07)	0.62 (0.38-1.03)	0.82 (0.51-1.35)	0.67 (0.39-1.15)
	≥30	44 (31)	0.44 (0.26-0.73)	0.59 (0.31-1.08)	0.53 (0.29-0.93)	0.68 (0.35-1.29)
Female (n=98)	18.5-24.9	45 (46)	1	1	1	1
	25-29.9	30 (31)	0.64 (0.35-1.16)	0.66 (0.27-1.58)	0.71 (0.34-1.39)	0.72 (0.27-1.83)
	≥30	23 (23)	0.92 (0.50-1.64)	0.75 (0.37-1.51)	0.75 (0.35-1.50)	0.59 (0.25-1.29)

Table 8: ORR and 2 year survival by BMI stratified by sex for patients treated with D+T

Cohort	BMI	ORR	OR (95% CI)	2 year PFS	2 year OS
All patients	18.5-24.9	65%	1	27%	27%
	25-29.9	65%	0.9 (0.6-1.3)	29%	51%
	≥30	77%	1.6 (1.0-2.6)	36%	64%
Male	18.5-24.9	58%	1	20%	35%
	25-29.9	65%	1.4 (0.8-2.3)	27%	46%
	≥30	76%	2.3 (1.2-4.3)	33%	64%
Female	18.5-24.9	72%	1	34%	60%
	25-29.9	63%	0.7 (0.4-1.2)	36%	61%
	≥30	78%	1.4 (0.7-2.9)	41%	65%
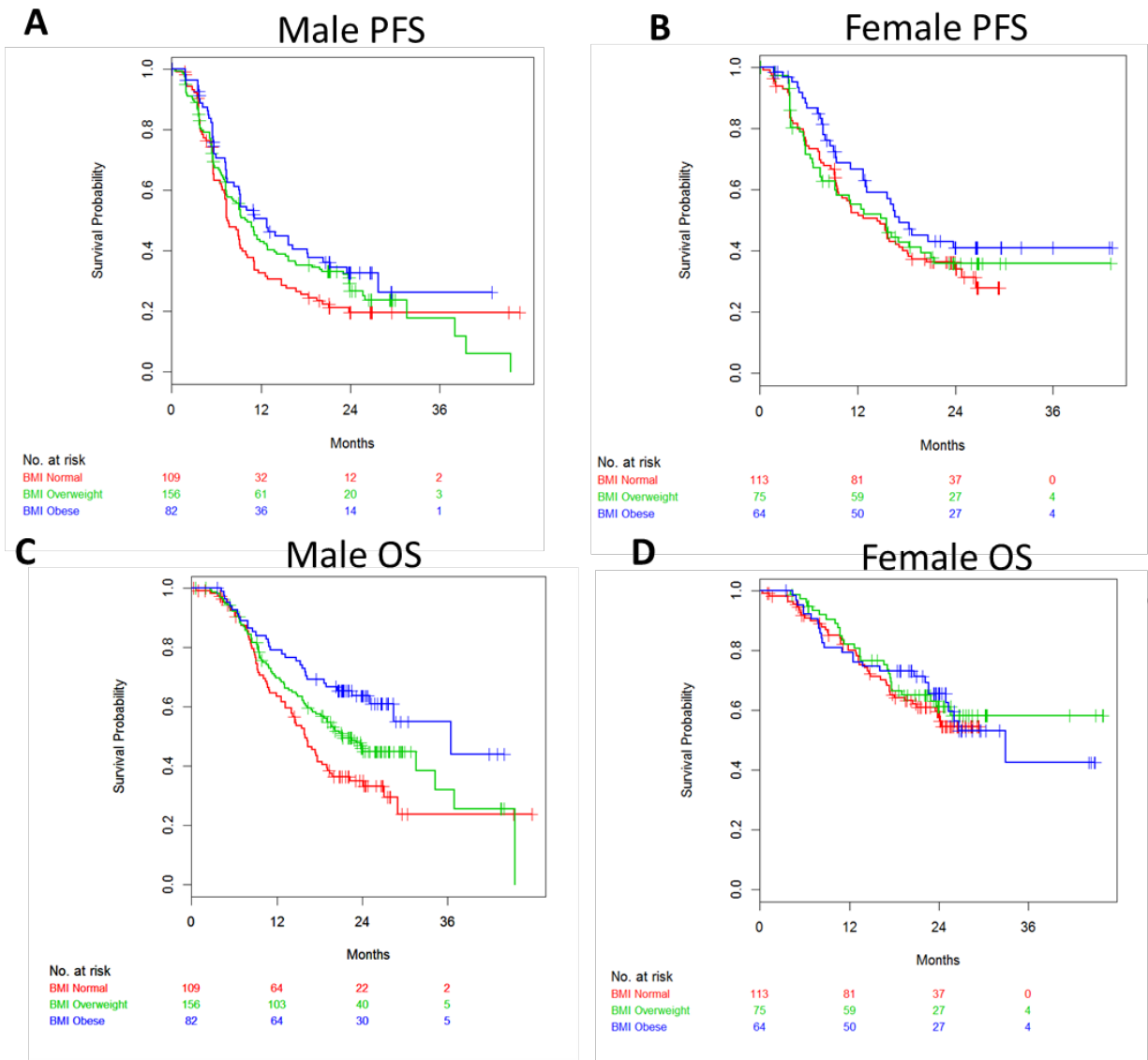
Figure 6: Outcomes by BMI stratified by sex for patients treated with D+T. A. Male progression-free survival. B. Female progression-free survival C. Male overall survival. D. Female overall survival. Red lines, normal BMI; Green lines, overweight; Blue lines, obese

Discussion

Obesity was unexpectedly associated with improved outcomes BRAF-mutant metastatic melanoma patients treated with the two BRAF and MEK inhibitor combinations approved in metastatic melanoma. In the larger cohort of patients treated with dabrafenib and trametinib, these associations were independent of multiple clinical prognostic factors previously found to be predictive of benefit from this therapy.(65) In this cohort, I also examined concomitant medications as a possible confounder as obese patients were more likely to be taking multiple medications associated with the metabolic syndrome which may have anti-cancer activity. However, BMI effects were independent of use of aspirin, metformin, beta blockers, and statins. Treatment tolerance was also examined as a possible explanation, but the Grade III/IV adverse events were actually slightly higher in obese patients, though this was not time adjusted and as obese patients had better response they stayed on therapy longer. The vemurafenib + cobimetinib validation dataset, which was significantly smaller, showed similar associations of higher BMI and better outcomes on univariate analysis but significance was lost on multivariate analysis. Cobimetinib pharmacokinetic data was available for this cohort and there was no difference by BMI category by pharmacokinetics.

As female sex was previously found to be associated with improved PFS in the dabrafenib + trametinib cohort(65), and as there are differences in BMI distribution and body composition by sex, I next looked at male and female patients separately. This analysis showed that in both cohorts, obesity was strongly associated with improved outcomes in males, but no BMI associations were observed in females.

Interestingly, there has only been one prior study of the association of BMI with outcomes in patients treated with targeted therapy.(36) In this study of patients with renal cell carcinoma (RCC), higher BMI was also found to be associated with improved survival. However, the association of BMI with outcomes has not been evaluated in metastatic RCC patients treated with other therapies. Thus, it is unclear if this paradoxical association is specific

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to targeted therapies, or whether higher BMI is generally prognostic in these diseases. Therefore, I next examined the association of BMI with outcomes in metastatic melanoma patients treated with checkpoint inhibitor immunotherapy.

Introduction

The association of BMI with outcomes in patients treated with immunotherapy has never been examined in any malignancy, as this form of therapy is very new with FDA approvals first occurring in melanoma (ipilimumab 2011; pembrolizumab and nivolumab both in 2014). Non-small cell lung cancer was the first non-melanoma malignancy in which anti-PD1 was approved in 2015, and they have now been approved in RCC, bladder cancer and head and neck cancer, with more approvals expected.

While targeted therapy has a robust predictive biomarker for response (BRAF V600 mutation), immunotherapy lacks a biomarker adequate to inform treatment decisions.(67)The most well-studied biomarker is tumor PD-L1 expression. However, PDL-1 expression is dynamic and thresholds vary by assay. More problematic is that PD-L1 expression does not adequately discriminate between patients who will or will not benefit. Though higher PD-L1 expression enriches for responders, patients with no tumor PD-L1 expression may still respond and vice-versa. Thus, identifying other clinical and/or molecular predictors of response in critical.

Interestingly, there is considerable cross-talk between molecular signaling pathways and the anti-tumor immune response.(68, 69) Our lab has recently shown that PI3K-AKT pathway can cause resistance to checkpoint inhibitor immunotherapy.(70) The success of targeted therapy may also in part depend on the immune response.(71) Thus, though immunotherapy and targeted therapy are fundamentally different modalities, there may be common factors underlying response and resistance. However, if opposing associations of BMI and outcomes were seen between targeted and immune therapy, this factor could be used to help decide which form of therapy is used first-line in BRAF-mutant patients.

The impact of obesity on the anti-tumor immune response has not been well-studied. Obesity leads to chronic inflammation and this is another key mechanism linking obesity to cancer initiation and progression.(72, 73) Obesity has been demonstrated to negatively impair the adaptive immune response, with increased rate of vaccine failure in patients, and decreased cell-mediated immunity and immunological memory in animal models.(47) However, the impact of obesity on tumor immunology has not been explored.

Therefore, I next examined the association of BMI with outcomes in patients treated with both anti-CTLA4 and anti-PD1 immunotherapy.

Results

Metastatic melanoma patients treated on the Phase III RCT CA 184-024 of ipilimumab (IPI, anti-CTLA-4, FDA approval 2011) + dacarbazine (DTIC) with available BMI (n=207) were analyzed.(23) In addition, a cohort of 335 metastatic melanoma patients treated with anti-PD-1 (pembrolizumab, FDA approval 2014, n=250; nivolumab, FDA approval 2014, n=73) or anti-PDL-1 (atezolizumab, n=8) antibodies at 4 centers in the USA and Australia with BMI at treatment initiation, clinical response assessment, and survival data available was analyzed. Patients initiated therapy between October 2009 and January 2016. Additional clinical characteristics extracted included age, sex, stage, tumor mutation, prior treatments, and immune-related adverse events (irAEs).

BMI distributions of both immunotherapy cohorts were similar to the targeted therapy cohorts. Patients with higher BMI were again older and more likely to be male, but there were otherwise no consistent differences between BMI categories (Table 9).

With a median follow-up of 38.8 months, obesity was associated with improved PFS (HR 0.67, 95% CI 0.45-0.99) and OS (0.64, 95% CI 0.42-0.97) compared to normal BMI in patients treated with IPI + DTIC (Table 10 and Figure 7). These associations remained significant after adjustment for age, sex, stage, and LDH. Similar to targeted therapy, obesity

was associated with large improvements in outcomes in men, with 2 year OS of 40.6% in obese versus 17.8% in normal BMI males. Multivariate analysis confirmed significantly improved PFS (HR 0.55, 95% CI 0.32-0.93) and OS (HR 0.40, 95% CI 0.22-0.72) in obese males. In contrast, BMI was not associated with either PFS or OS in females treated with IPI + DTIC (Table 10 and Figure 7).

Table 9: Patient characteristics of patients treated with immunotherapy

	IPI + DTIC			PD1		
	BMI 18.5-24.9 No. (%)	BMI 25-29.9 No. (%)	BMI ≥30 No. (%)	BMI 18.5-24.9 No. (%)	BMI 25-29.9 No. (%)	BMI ≥30 No. (%)
Patients, No. (%)	68 (33)	88 (43)	51 (25)	102 (31)	109 (33)	120 (36)
Age, Mean, y (range)	53 (24-83)	60 (31-87)	60 (34-80)	57 (18-86)	63 (34-86)	63 (22-86)
Male, No. (%)	41 (60)	64 (73)	33 (65)	58 (57)	70 (64)	83 (69)
Stage						
III/M1a/M1b	17 (25)	38 (43)	26 (51)	19 (19)	32 (29)	40 (33)
M1c	51 (75)	50 (57)	25 (49)	81 (79)	76 (70)	80 (67)
LDH >ULN, No. (%)	26 (38)	31 (35)	18 (25)	40 (39)	38 (35)	39 (32)
ECOG PS						
0	46 (68)	65 (74)	35 (69)	60 (59)	64 (59)	72 (60)
≥1	22 (32)	23 (26)	16 (31)	41 (40)	45 (41)	48 (40)
Mutation status						
BRAF mutant	-	-	-	34 (33)	32 (29)	34 (28)
V600E	-	-	-	-	-	-
Other V600	-	-	-	-	-	-
NRAS mutant	-	-	-	24 (24)	21 (19)	18 (15)
WT	-	-	-	37 (36)	50 (46)	67 (57)

Table 10: Association between BMI and outcomes for patients treated with IPI + DTIC

Population	BMI	Patient No. (%)	PFS		OS	
			Univariate Adjusted HR (95% CI)	Multivariate Adjusted HR (95% CI)	Univariate Adjusted HR (95% CI)	Multivariate Adjusted HR (95% CI)
All patients (n=207)	18.5-24.9	68 (28)	1	1	1	1
	25-29.9	88 (37)	0.87 (0.62-1.22)	0.88 (0.61-1.26)	0.76 (0.53-1.08)	0.70 (0.48-1.03)
	≥30	51 (21)	0.67 (0.45-0.99)	0.63 (0.41-0.95)	0.64 (0.42-0.97)	0.54 (0.34-0.86)
Male (n=138)	18.5-24.9	41 (29)	1	1	1	1
	25-29.9	64 (45)	0.76 (0.50-1.16)	0.77 (0.49-1.22)	0.69 (0.45-1.07)	0.63 (0.39-1.01)
	≥30	33 (23)	0.53 (0.32-0.88)	0.55 (0.32-0.93)	0.46 (0.27-0.80)	0.40 (0.22-0.72)
Female (n=69)	18.5-24.9	27 (28)	1	1	1	1
	25-29.9	24 (24)	1.02 (0.56-1.88)	1.29 (0.66-2.51)	0.79 (0.42-1.50)	0.84 (0.43-1.64)
	≥30	18 (18)	1.02 (0.55-1.92)	0.92 (0.45-1.86)	1.13 (0.58-2.18)	1.16 (0.55-2.46)

Adjusted for age, gender, stage, LDH, and ECOG performance status
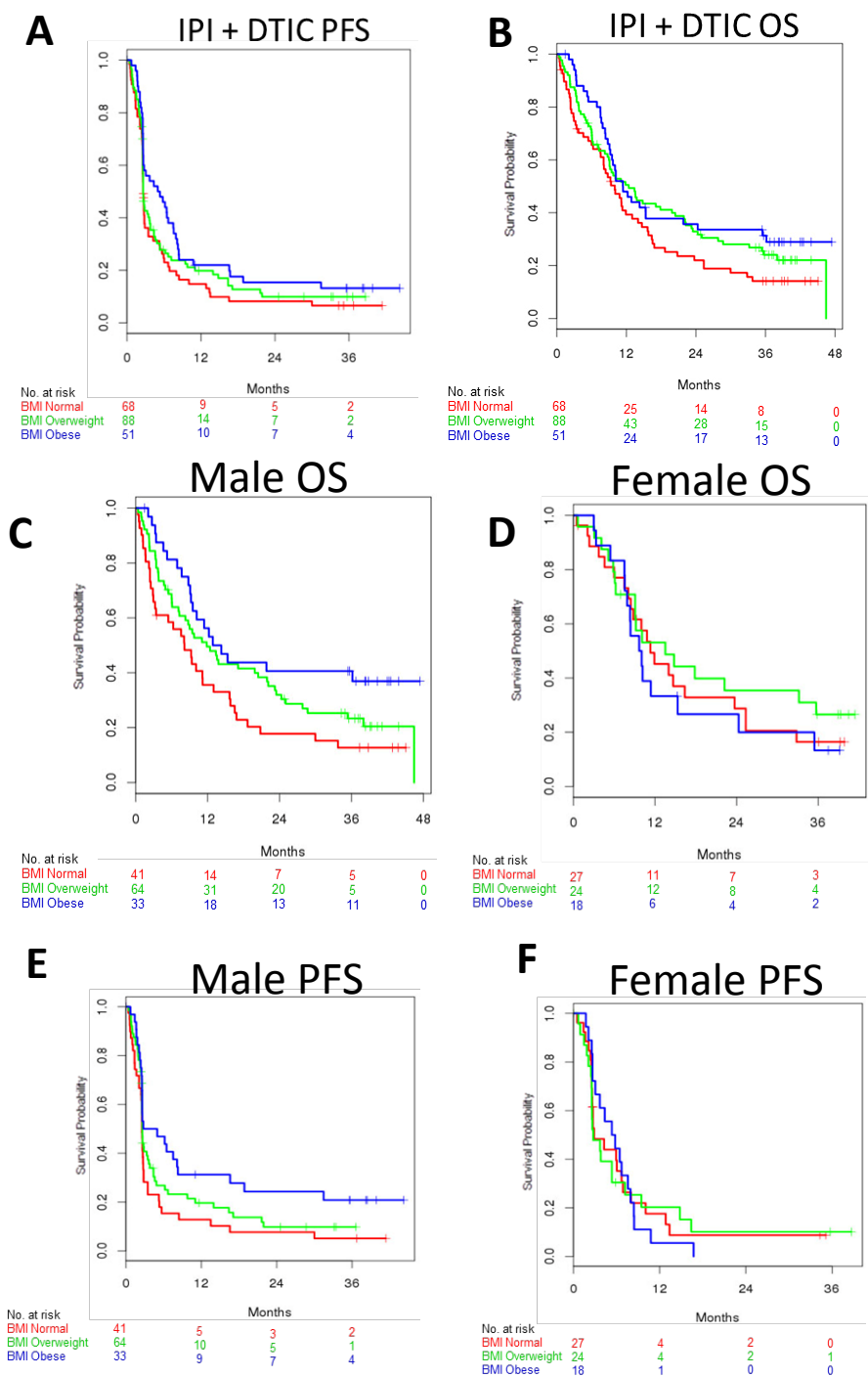
Figure 7. Outcomes by BMI in metastatic melanoma patients treated with Ipilimumab (IPI) + dacarbazine (DTIC). A. All patients' progression-free survival. B. All patients overall survival C. Male overall survival. D. Female overall survival. E. Male progression-free survival. F. Female progression-free survival. Red lines, normal BMI; Green lines, overweight; Blue lines, obese

Anti-PD1

Increased BMI was again associated with significantly improved outcomes in males but not females treated with anti-PD-1/PD-L1. For male patients, the 2 year PFS rate was 36% for overweight/obese versus 18% for normal BMI (HR 0.62, 95% CI 0.43-0.89) (Table 11 and Figure 8). Overweight/obese male patients also had improved OS (HR 0.60, 95% CI 0.41-0.90) (Table 11) and modestly improved response rates (45% vs 32%, OR 1.82, 95% CI 0.94-3.50) (Tables 12) In contrast, women treated with anti-PD-1/PD-L1 had identical response rates by BMI category (41%), and there were no significant BMI associations with PFS or OS (Table 12 and Figure 8).

Table 11: PFS and OS by BMI for patients treated with PD-1/PD-L1

Population	BMI	Patient No. (%)	PFS		OS	
			Univariate Adjusted HR (95% CI)	Multivariate Adjusted HR (95% CI)	Univariate Adjusted HR (95% CI)	Multivariate Adjusted HR (95% CI)
All patients (n=331)	18.5-24.9	102 (31)	1	1	1	1
	≥25	229 (69)	0.77 (0.58-1.02)	0.86 (0.63-1.16)	0.72 (0.52-1.00)	0.75 (0.53-1.07)
Male (n=214)	18.5-24.9	57 (27)	1	1	1	1
	≥25	157 (73)	0.62 (0.43-0.89)	0.66 (0.45-0.96)	0.60 (0.41-0.90)	0.68 (0.44-1.04)
Female (n=117)	18.5-24.9	45 (38)	1	1	1	1
	≥25	72 (62)	1.07 (0.67-1.68)	1.20 (0.73-1.97)	0.90 (0.52-1.56)	0.85 (0.46-1.56)

Table 12: Clinical response rates by BMI for patients treated with PD-1/PD-L1

	BMI<25	BMI≥25
All patients		
Response rate	34%	44%
OR vs BMI<25 (95%CI)		OR 1.5 (0.9-2.4)
Males		
Response rate	32%	45%
OR vs BMI<25 (95%CI)		OR 1.8 (1.0-3.5)
Females		
Response rate	41%	41%
OR vs BMI<25 (95%CI)		OR 1.0 (0.5-2.1)
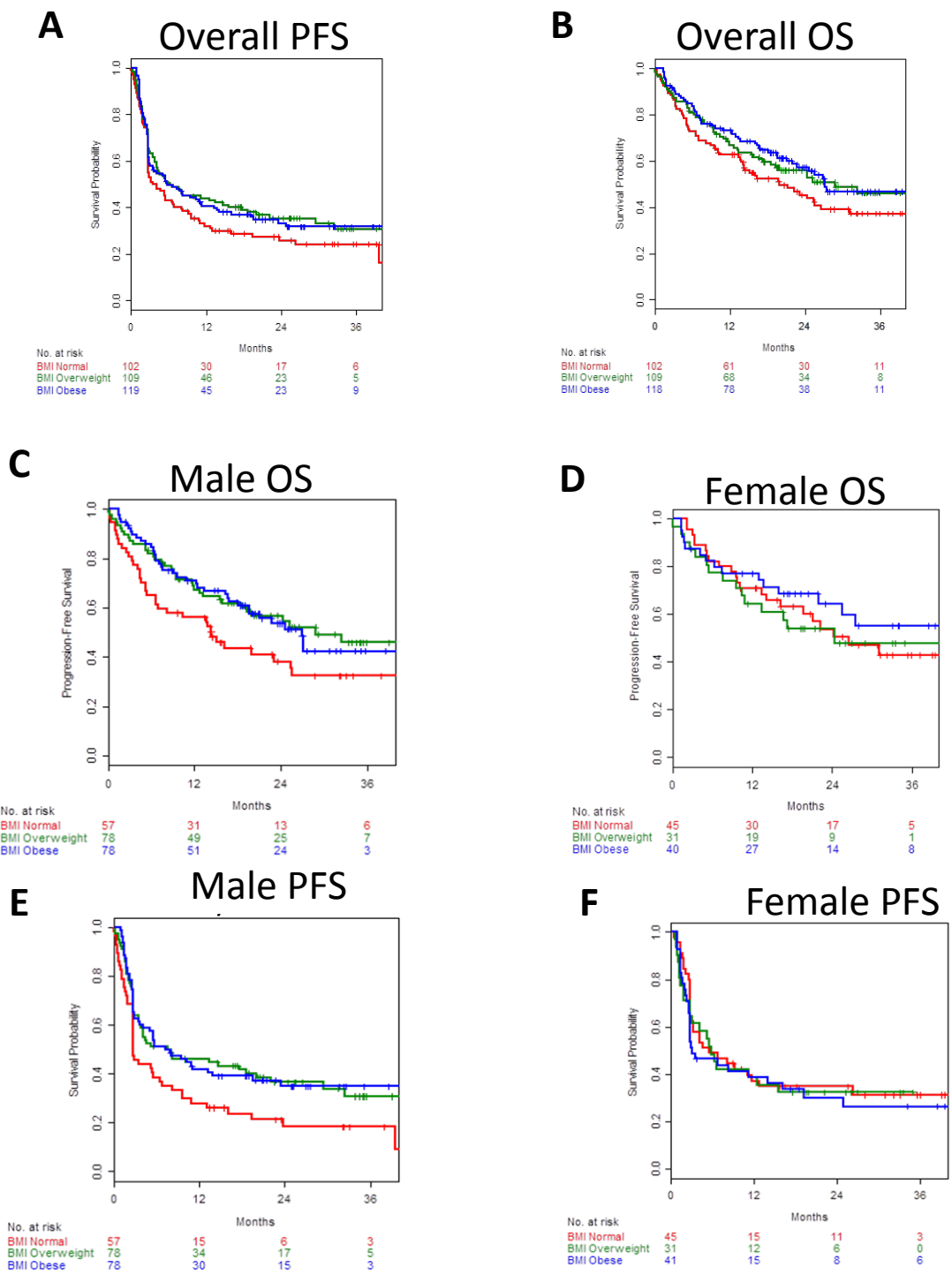
Figure 8. Outcomes by BMI in metastatic melanoma patients treated with PD1/PDL1. A. All patients progression-free survival. B. All patients overall survival C. Male overall survival. D. Female overall survival. E. Male progression-free survival. F. Female progression-free survival. Red lines, normal BMI; Green lines, overweight; Blue lines, obese

Discussion

Consistent with the association of obesity with improved outcomes seen with targeted therapy, higher BMI was also associated with improved outcomes in patients treated with both anit-CTLA4 and anti-PD1/PDL1 immunotherapy. Moreover, there was again a large sex difference in BMI associations with very strong associations of higher BMI with improved outcomes seen in males and no associations observed in females. Future work should focus on validating the findings of the PD1 cohort which was an off-protocol cohort and examining combination CTLA4 and PD1 therapy. These findings also support the rationale for examining the association of BMI with outcomes in other diseases in which immunotherapy has been approved.

As noted in the introduction, the impact of obesity on anti-tumor immunity has not been previously studied. The work from the Lee lab showing that the association of higher BMI with worse outcomes in early stage melanoma was lost when serum C-reactive protein was added into the model suggests that inflammation may mediate the deleterious relationship between obesity and outcomes seen in clinically localized melanoma.(50) However, this chronic inflammation could hypothetically be beneficial in the setting of immunotherapy.

Given that obesity was associated with improved outcomes in metastatic melanoma patients treated with both targeted and immune therapy, it was important to examine whether obesity was simply prognostic in advanced melanoma or if the effect was specific to these therapies.

Chapter 4: Association of BMI with outcomes in patients treated with chemotherapy

Introduction

Metastatic melanoma is relatively resistant to chemotherapy and there is only one chemotherapeutic that has been approved in this disease, dacarbazine (DTIC). The ORR with DTIC is 5-10% and the median PFS is <2 months.(2, 23)

The reason why melanoma is so chemo-resistant is not well understood.(74) The rewiring of molecular pathways that were purported to make melanoma "bullet-proof" are not unique to this disease. There are clinical factors associated with outcome in patients with melanoma treated with chemotherapy including age, sex, stage, and LDH. However, these are prognostic factors rather than predictive to this specific therapy. Given the very low activity of chemotherapy in melanoma, the association of BMI with outcomes in metastatic melanoma patients with chemotherapy can be used to approximate the impact of obesity on the natural history of this disease.

Thus, I next examined the associations of BMI in metastatic melanoma patients treated with DTIC, a control arm in multiple RCTs(2, 23) to explore whether BMI was prognostic in metastatic melanoma or if the effect was specific to targeted and immune therapy.

Results

Metastatic melanoma patients with available BMI data treated with dacarbazine (DTIC) in the control arms of CA 184-024(23) (n=221) and the Phase III BRIM3 randomized controlled trial (2) (n=309) were analyzed. Clinical characteristics of patients treated with DTIC are shown in Table 13.

BMI was not significantly associated with PFS (CA 184-024, HR 0.83, 95% CI 0.59-1.18; BRIM-3, HR 0.86, 95% CI 0.62-1.18) or OS (CA 184-024, HR 0.95, 95% CI 0.66-1.37;

BRIM-3, HR 0.94, 95% CI 0.62-1.41) in either cohort (Table 14, Figure 9). Further, BMI was

not significantly associated with outcomes in either males or females. Importantly, CA 184-024

randomized patients to DTIC alone or DTIC + IPI which allowed me to statistically examine

treatment interaction. In the OS analysis, the $p_{interaction}$ for BMI, treatment, and sex was 0.035,

indicating that BMI associations were sex- and treatment-specific.

Table 13: Characteristics of patients treated with dacarbazine (DTIC)

Characteristic	DTIC (CA 184-024, n=320)			DTIC (BRIM3, n=221)		
	BMI 18.5-24.9 No. (%)	BMI 25-29.9 No. (%)	BMI ≥30 No. (%)	BMI 18.5-24.9 No. (%)	BMI 25-29.9 No. (%)	BMI ≥30 No. (%)
Patients, No. (%)	143 (45)	107 (33)	70 (22)	74 (33)	88 (40)	59 (27)
Age, Mean, y (range)	49 (17-86)	56 (22-84)	53 (31-78)	55 (23-83)	60 (24-88)	56 (32-88)
Male, No. (%)	70 (49)	73 (68)	31 (44)	38 (51)	66 (75)	36 (61)
Stage, No. (%)						
III/M1a/M1b	42 (29)	29 (27)	31 (44)	24 (32)	37 (42)	33 (56)
M1c	101 (71)	78 (73)	39 (56)	50 (68)	51 (58)	26 (44)
LDH >ULN, No. (%)[a]	68 (48)	44 (41)	23 (33)	37 (50)	36 (41)	23 (39)
ECOG PS, No. (%)						
0	95 (66)	77 (72)	46 (66)	55 (74)	63 (72)	38 (64)
≥1	48 (34)	30 (28)	24 (34)	19 (26)	25 (28)	21 (36)
Mutation, No. (%)[b]						
BRAF mutant	143 (100)	107 (100)	70 (100)	-	-	-
V600E	132 (92)	94 (88)	62 (89)	-	-	-
Other V600	8 (5)	10 (9)	5 (7)	-	-	-

[a]Data missing for 1 patient in DTIC-G cohort. [b]Specific BRAF mutation data (V600E vs other V600) missing for 9 patients in DTIC cohort.

Table 14: Outcomes for patients treated with dacarbazine (DTIC)

Population	BMI	Patient No. (%)	PFS		OS	
			Univariate Adjusted HR (95% CI)	Multivariate Adjusted HR (95% CI)	Univariate Adjusted HR (95% CI)	Multivariate Adjusted HR (95% CI)
DTIC (BRIM3)[a]						
All patients (n=320)	18.5-24.9	143 (45)	1	1	1	1
	25-29.9	107 (33)	0.93 (0.70-1.23)	0.94 (0.70-1.25)	1.05 (0.79-1.39)	0.98 (0.73-1.31)
	≥30	70 (22)	0.86 (0.62-1.25)	0.91 (0.64-1.26)	0.92 (0.66-1.28)	0.94 (0.66-1.32)
Male (n=174)	18.5-24.9	70 (40)	1	1	1	1
	25-29.9	73 (42)	0.87 (0.61-1.25)	0.91 (0.63-1.32)	1.09 (0.76-1.57)	0.97 (0.66-1.41)
	≥30	31 (18)	0.75 (0.46-1.20)	0.73 (0.43-1.17)	1.05 (0.64-1.68)	0.92 (0.56-1.51)
Female (n=146)	18.5-24.9	73 (29)	1	1	1	1
	25-29.9	34 (23)	0.97 (0.60-1.53)	0.84 (0.49-1.40)	1.00 (0.61-1.60)	0.85 (0.50-1.40)
	≥30	39 (27)	0.97 (0.60-1.53)	1.02 (0.63-1.65)	0.82 (0.51-1.29)	0.94 (0.57-1.52)
DTIC (CA 184-024)[b]						
All patients (n=221)	18.5-24.9	74 (33)	1	1	1	1
	25-29.9	88 (40)	0.73 (0.52-1.01)	0.81 (0.58-1.14)	0.85 (0.61-1.19)	0.91 (0.64-1.28)
	≥30	59 (27)	0.83 (0.59-1.18)	0.96 (0.67-1.39)	0.95 (0.66-1.37)	1.16 (0.79-1.70)
Male (n=140)	18.5-24.9	38 (27)	1	1	1	1
	25-29.9	66 (47)	0.62 (0.41-0.94)	0.77 (0.50-1.18)	0.72 (0.48-1.10)	0.89 (0.58-1.36)
	≥30	36 (26)	0.70 (0.44-1.12)	1.06 (0.63-1.78)	0.97 (0.60-1.56)	1.55 (0.91-2.66)
Female (n=81)	18.5-24.9	36 (44)	1	1	1	1
	25-29.9	22 (27)	1.08 (0.62-1.90)	1.13 (0.63-2.01)	1.06 (0.60-1.88)	1.29 (0.70-2.36)
	≥30	23 (28)	1.04 (0.61-1.77)	1.02 (0.58-1.77)	0.88 (0.50-1.55)	0.91 (0.51-1.64)

[a]Adjusted for age, gender, stage, LDH, mutation, and ECOG performance status. [b]Adjusted for age, gender, stage, LDH, and ECOG performance status
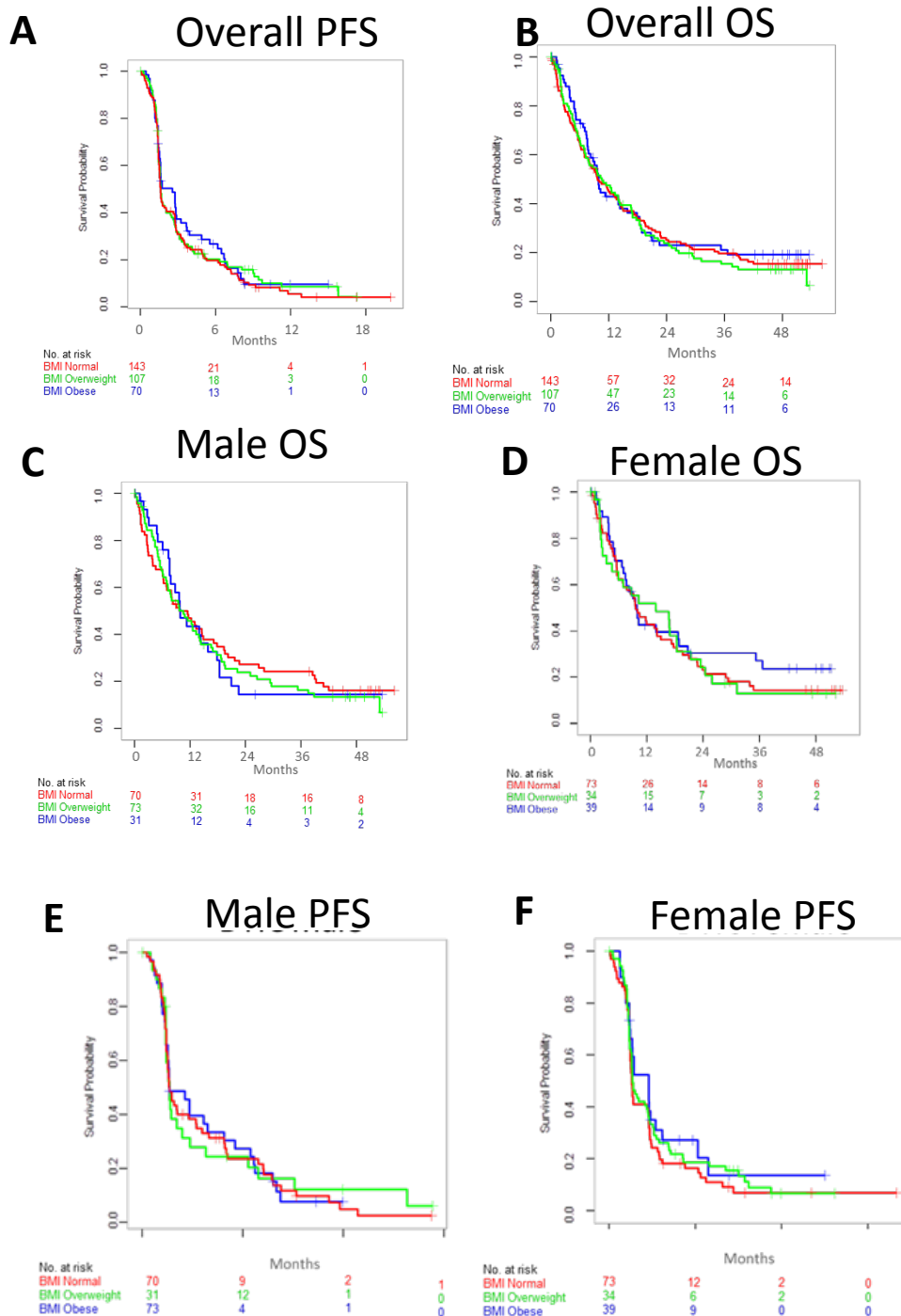
Figure 9. Outcomes by BMI in metastatic melanoma patients treated with DTIC. A. All patients progression-free survival. B. All patients overall survival C. Male overall survival. D. Female overall survival. E. Male progression-free survival. F. Female progression-free survival. Red lines, normal BMI; Green lines, overweight; Blue lines, obese

Discussion

In contrast to the results observed with targeted and immune therapy, BMI was not associated with outcomes in patients treated with chemotherapy. Interestingly, in some other malignancies, obesity has been associated with improved survival and one posited mechanism is via improved metabolic reserve in obese patients to allow them to better withstand chemotherapy which commonly causes anorexia, weight loss, nausea and vomiting, and diarrhea.(75) In contrast, in metastatic melanoma the paradoxical association is observed in patients treated with immunotherapy and targeted therapy which do not commonly cause weight loss, and not in those treated with chemotherapy.

One potential explanation for why a BMI association is not seen with chemotherapy is that the activity is so low that it would in essence be difficult to "move the needle." However, the activity of ipilimumab is low as well and in males treated with that therapy, the multivariable HR for overall survival was 0.4. Moreover, a test for interaction in the trial that randomized patients to ipilimumab + DTIC vs DTIC alone, a 6-way interaction for BMI, sex, and treatment was 0.35, supporting that these effects are predictive of response to contemporary therapies and not merely prognostic.

Chapter 5: Functional characterization of obesity in preclinical models of melanoma

Introduction

In order to functionally test the impact of obesity on melanoma tumor, I utilized a mouse model of diet-induced obesity. This model, in which male C57/Bl6 mice are fed a 60% high-fat diet, is well-validated in the literature and commonly used to test the impact of obesity on cancer initiation and progression.(76, 77) Compared to age-matched controls who have been fed a matched purified ingredient low-fat diet, diet-induced obesity (DIO) mice have higher body weight and adiposity by body composition analysis, as well as altered levels of obesity related cytokines such as insulin, IGF-1, leptin, and adiponectin. In other cancers, this model has been used to show that system-level metabolism can affect tumor biology and lead to increased tumor growth.(14-16, 78) Previously, models of B16 melanoma had demonstrated that diet-induced obesity can increase melanoma tumor growth and progression though the mechanism has not been worked out. (52-54) Moreover, B16 melanoma is not a genetically relevant model with a defined driver mutation. Newer models now exist that are based on the driver mutations found in metastatic melanoma, specifically the BRAF mutation.

Thus, in order to test the impact of energy balance in melanoma, I used a BRAF-mutant mouse melanoma cell lines injected into DIO and control mice. As the hypothesis was that energy balance would impact melanoma tumor growth and therapeutic sensitivity via the PI3K pathway, cell lines were chosen that were PTEN intact so that the PI3K pathway would not be constitutively activated. As this model was BRAF-mutant and was in an immunocompetent mice, I was also able to examine the effect of obesity on response to both MAPK-pathway directed targeted therapy and immune therapy.

Cell line characterization

Two BRAF mutant, PTEN intact mouse melanoma cell lines were obtained from the lab of Dr.

Marcus Bosenberg at Yale University, YUMM 3.1 (BrafV600E::Cdkn2a-/-) and YUMM 5.2

(BrafV600E::Cdkn2a+/-::p53-/-). Reverse-phase protein array (RPPA) was performed on

protein lysates extracted from the cell lines to quantitatively measure the expression levels of

217 total- and phospho-proteins. Using a paired t-test with a cut-off of $p<0.05$, 141 proteins

were identified which were differentially expressed between the two cell lines (Figure 10A). To

further drill down on the most significantly changed proteins, I introduced a new filter and

examined those proteins with a fold change in mean protein expression >2.0 and identified 38

differentially expressed proteins (Figure 10B). This analysis revealed Yumm 3.1 has significant

higher expression of insulin-like growth factor ($p<0.0001$, average ratio YUMM 3.1/YUMM 5.2 =

2.6) and insulin receptor (IR) ($p<0.001$, average ratio YUMM 3.1/YUMM 5.2 = 2.4) compared to

YUMM 5.2. To validate these findings, I next performed western blot for IR and IGF1-R using

the cell line Mel624 as a positive control and A375 as a negative control for IGF1-R. This

analysis confirmed that YUMM 3.1 had high levels of both IR and IGF1-R and YUMM 5.2 had

low levels (Figure 11).
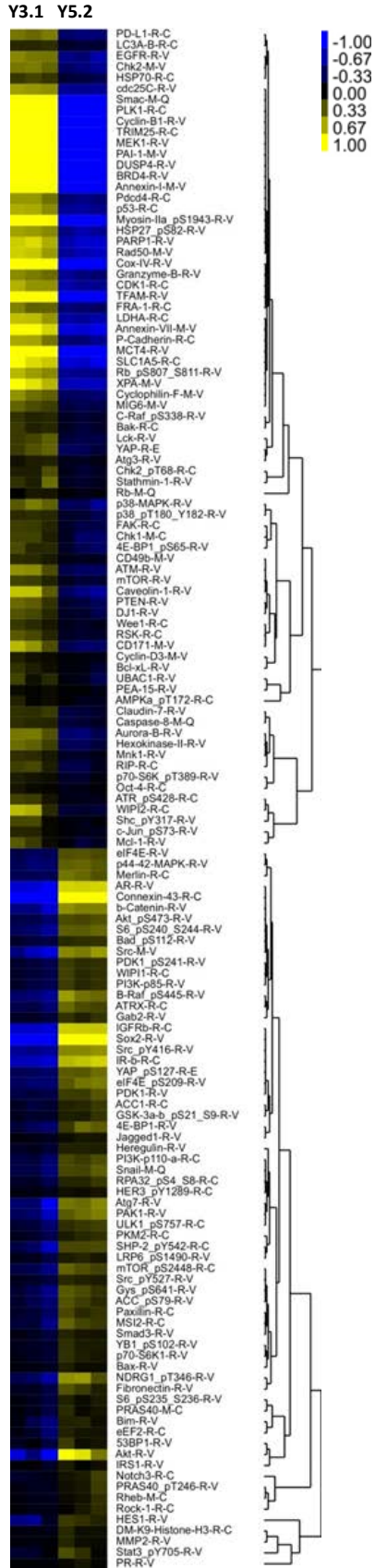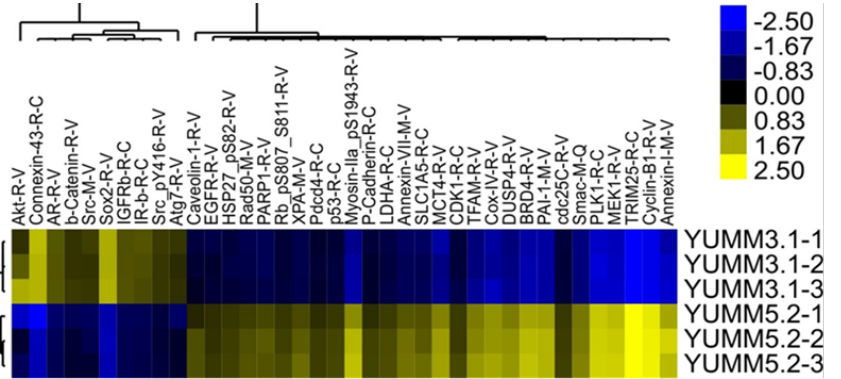
Figure 10. YUMM 3.1 and YUMM 5.2 cell line reverse phase protein array

Supervised clustering of RPPA data of 217 proteins in YUMM3.1 and YUMM 5.2 cell lines. A. Using a threshold of $p<0.05$, 141 proteins were identified which differentially expressed between the two cell lines. B. Applying an additional filter of > 2-fold change in mean protein expression between the two lines identified 38 proteins that were the most differentially expressed. Blue=decreased expression. Yellow=increased expression.
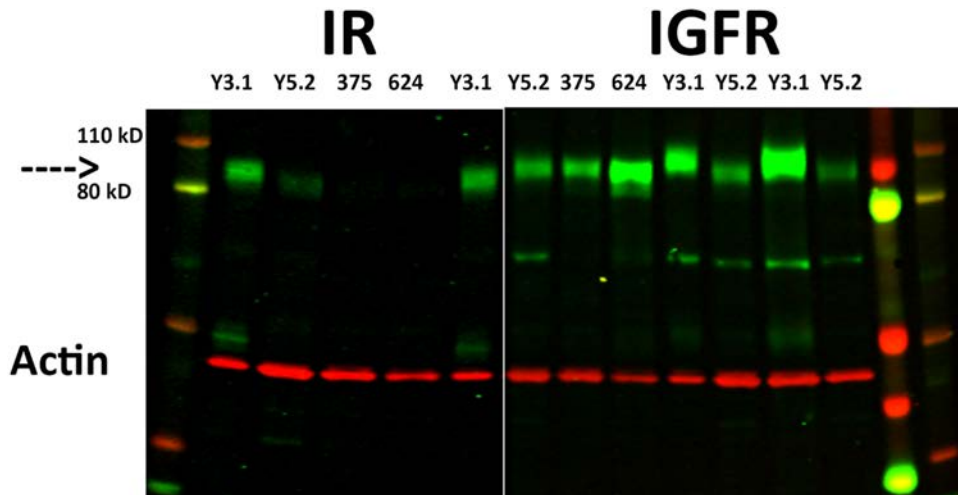
Figure 11. Western blot for IR and IGF1R

Western blotting for IR on left and IGF1-R demonstrating high levels of both proteins in YUMM

3.1 cells. Y3.1=YUMM 3.1, Y5.2=YUMM 5.2, 375=A375 (negative control), 624=Mel 624.

Mouse model characterization

Mouse models of diet-induced obesity (DIO) and matched control mice were obtained from Jackson laboratories. Male C57/Bl6 mice are fed a 60% high fat diet (Research Diets Inc) since weaning at 6 weeks of age to produce a validated DIO phenotype.[77] Control mice of the same background were fed a matched purified ingredient 10% fat diet (Research Diets Inc). 18 week old DIO and control mice were received and then acclimated for 3 weeks before experiments were started. Throughout the experiment, they were continued on the same diets as fed at Jackson laboratories.

In order to characterize and validate the metabolic phenotypes of the DIO vs control mice, baseline body weight, and fasting serum IGF-1, IGFBP-1 and glucose were measured (Figure 12). DIO mice were found to have significantly higher body weight (DIO 39.5 g vs. control 28.1 g, $p < 0.001$) serum IGF-1 (DIO 454.5 ng/ml vs. control 322.6 ng/ml, $p < 0.001$), and fasting glucose (DIO 153.8 mg/dl, vs. control 120.9 mg/dl, $p = 0.01$) and lower IGFBP-1 (DIO 6.7 ng/ml vs. control 25.3 ng/ml. $p = 0.0029$). These values are consistent with the published literature on this model and indicate the expected phenotypes were achieved.
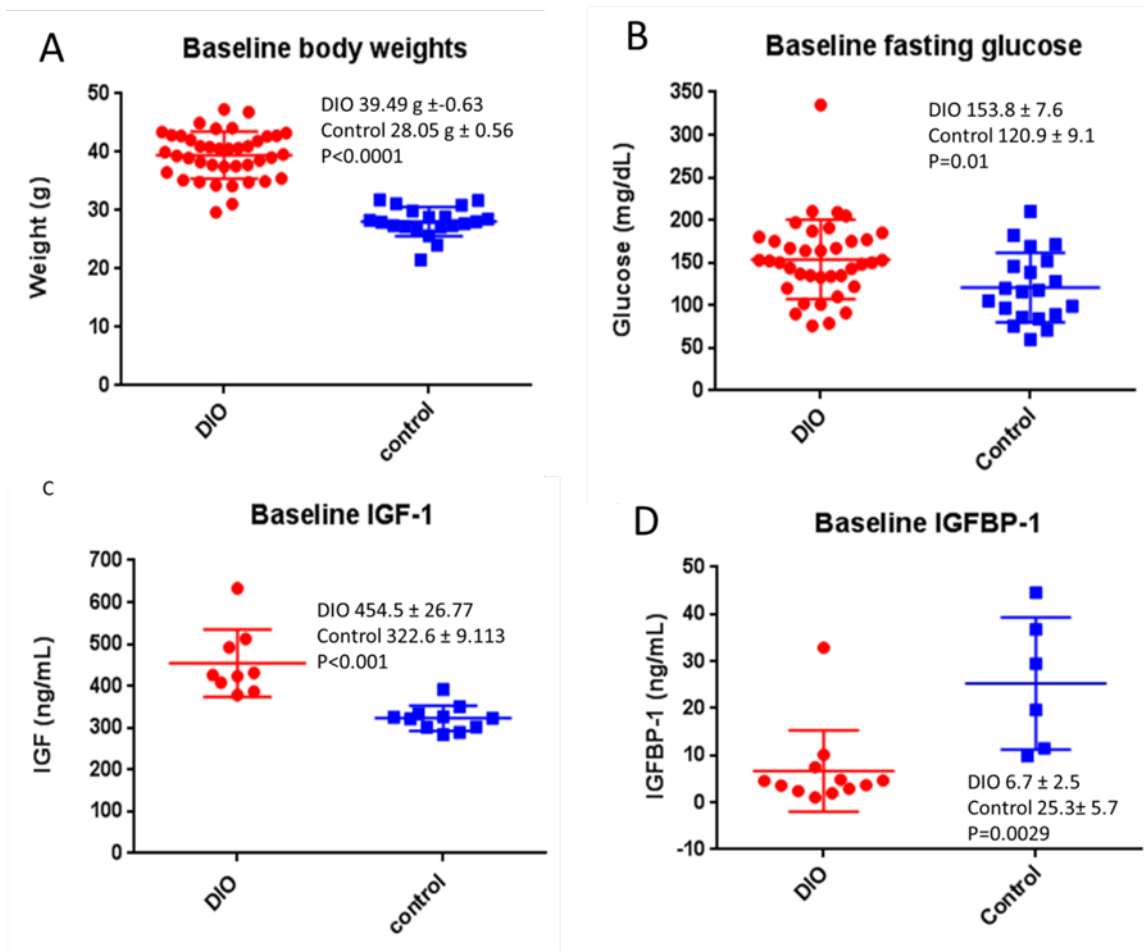
Figure 12. Baseline metabolic characteristics of DIO and control mice.

DIO mice have higher A. body weight, B. serum IGF-1, and C. fasting glucose and lower D. IGFBP-1 compared to control mice.

Tumor growth experiments

 In the initial pilot study on melanoma tumor growth, 21 week old DIO and control mice were injected subcutaneously into the left flank with $1x10^6$ YUMM 3.1 cells or YUMMM 5.2 murine melanoma cells. Tumor size was measured 3X weekly and followed until tumor size reached 1.5 cm, or mice became moribund and were sacrificed. DIO increased tumor growth in both the YUMM 3.1 (Day 15 tumor size DIO 1059 ± 146 mm^3 vs. control 282 ± 86 mm^3, p=0.01) and YUMM 5.2 models (DIO 717.2 ± 229 mm^3 vs control 62.13 ± 62.13 mm^3, p=0.04) (Figure 13). As tumor growth was more consistent in the YUMM 3.1 model and our cell line characterization had shown that this line had higher levels of IR and IGF-R, follow-up studies were conducted with this model. A validation study conducted in 10 DIO and 10 control mice showed that tumors grew faster in DIO mice, with significantly greater day 14 tumor weight (p=0.012) (Figure 14).
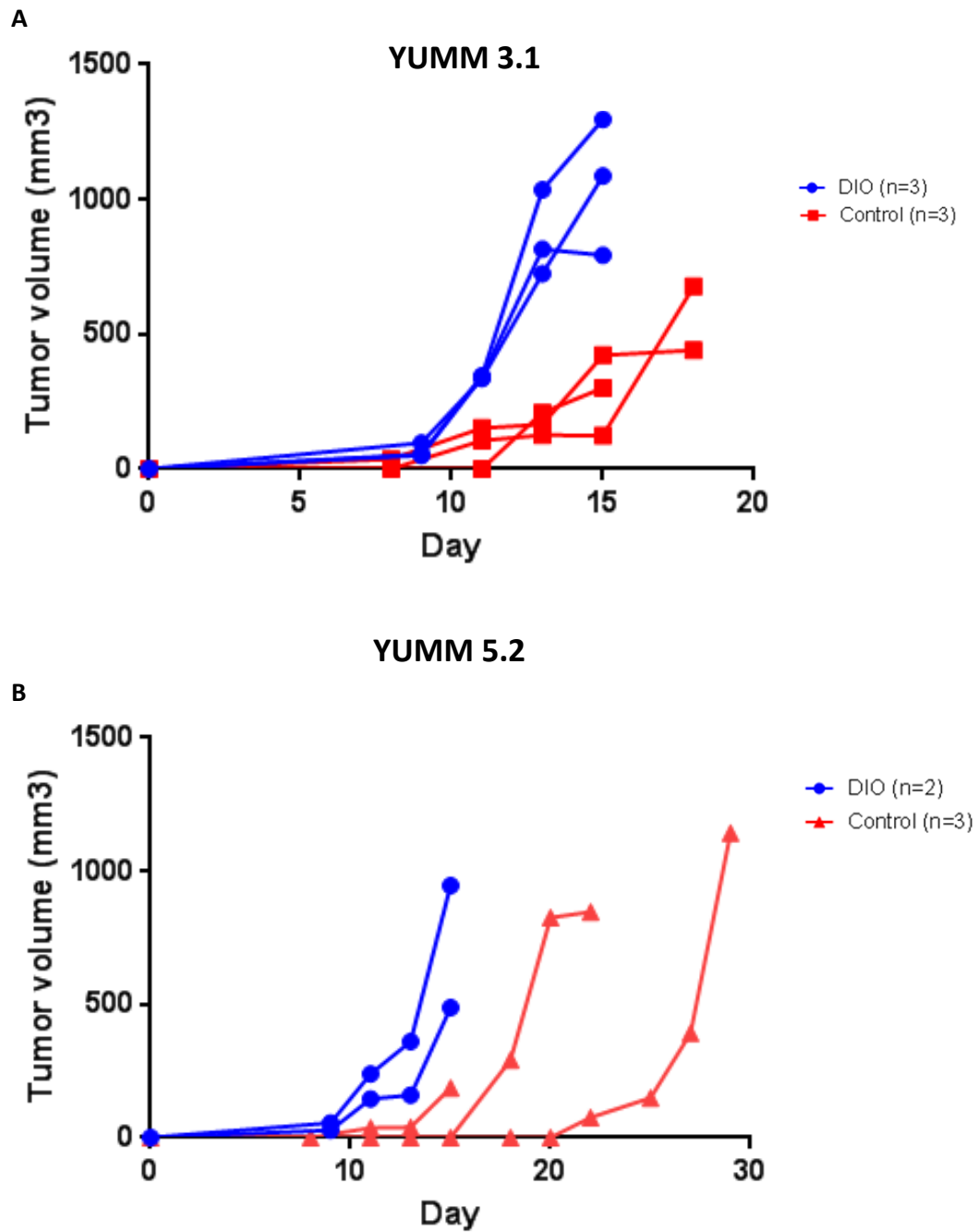
Figure 13. Tumor growth in DIO vs. control mice

1.0 x 10^6 cells were injected subcutaneously into the flank of DIO or control mice.

Increased tumor growth was observed in the DIO mice with both a. YUMM 3.1 cells

and b. YUMM 5.2 cells
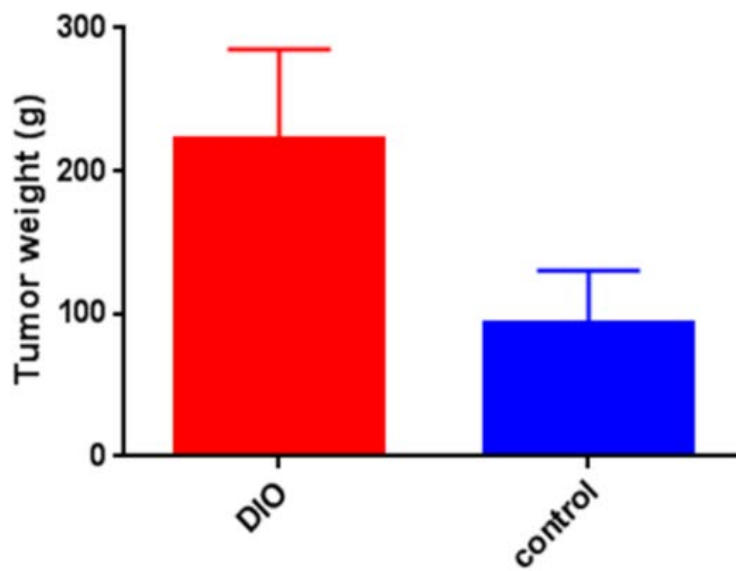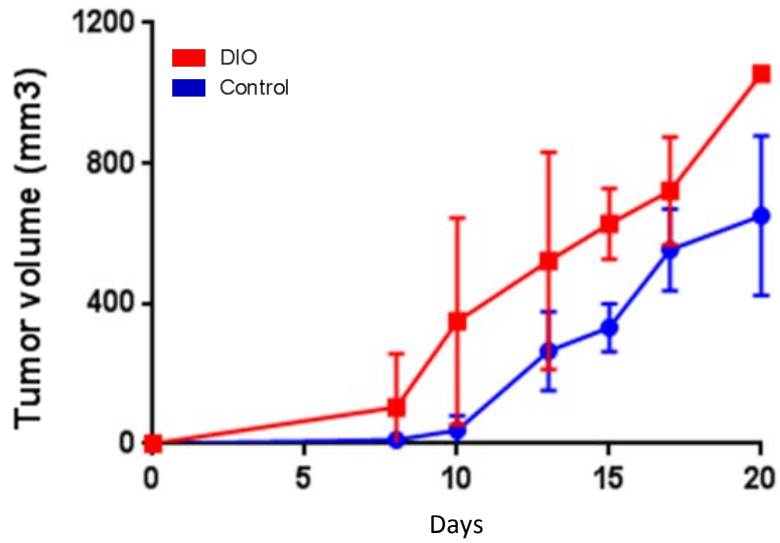
Figure 14. Tumor growth in DIO vs. control mice with YUMM 3.1 model. DIO led to

increased day 14 tumor weight (p=0.012).

Molecular and immunological correlates of obesity

On reverse phase protein array analysis comparing the tumor samples from the DIO xenografts to those of the control mice, there were significant differences in 99 of the 298 proteins assessed, demonstrating that obesity impacts the molecular biology of melanoma (Figure 15A). Two of the most differentially expressed proteins were pS6 residues, highly sensitive markers of PI3K pathway activation (Figure 15B). This was confirmed by immunohistochemistry (Figure 15C). Significant differences were also seen in pAMPK, a key marker of cellular metabolism, between the DIO and control mice (Figure 15B).

By flow cytometry, there was decreased immune infiltration in DIO xenografts compared to the control mice. However, there were no significant differences in spleen immunocytes indicating that this was specific to tumor infiltration and not a global decrease in immune populations (Figure 16).
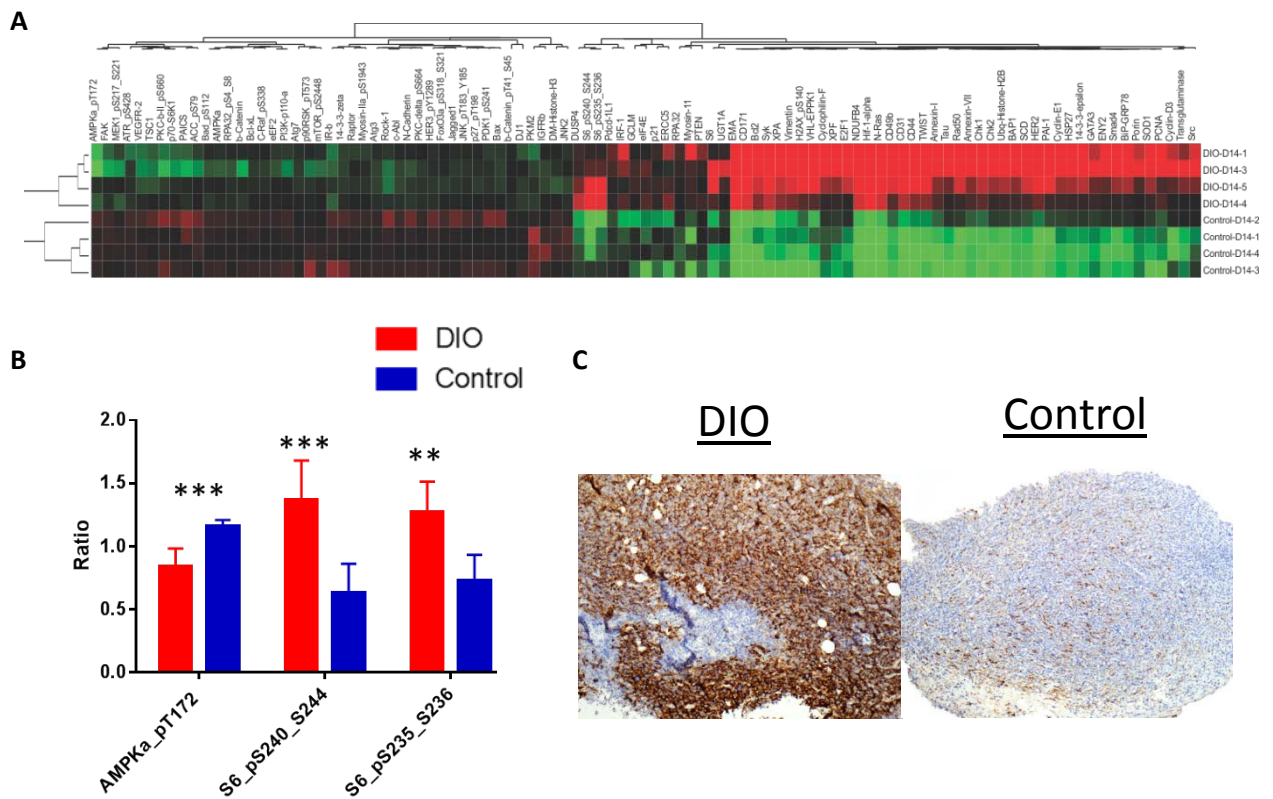
Figure 15. Diet induced obesity and melanoma molecular signaling.

A. Heat map shows 99 proteins differentially expressed between DIO and control mice. B. pS6 residues significantly increased and pAMPK significantly decreased in DIO vs. control mice. C. Immunohistochemistry of representative samples demonstrating increased pS6 staining in DIO tumor samples.
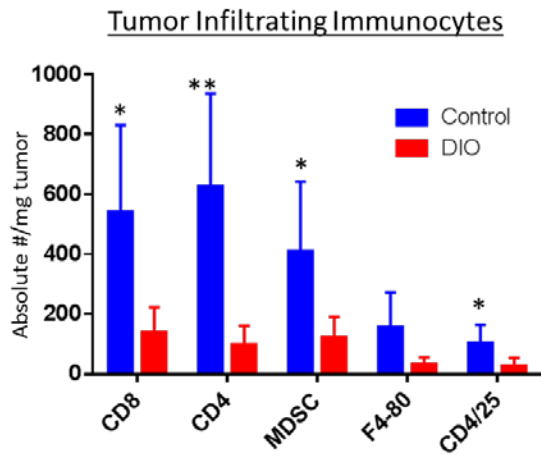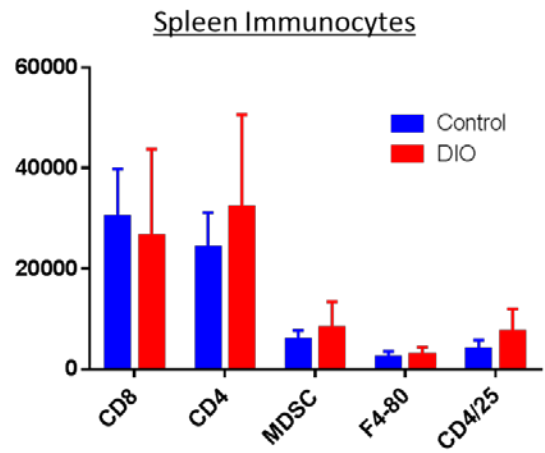
Figure 16 Effects of DIO on immune cell populations.

A. Tumors from DIO mice exhibit decreased CD8, CD4, MDSC, and CD4/25 cells. B. splenic immunocytes do not differ by dietary group

Therapeutic response

Finally, I examined the impact of DIO on response to targeted and immune therapy in the subcutaneous YUMM 3.1 model. 21 week old DIO and control mice were injected subcutaneously into the left flank with $1x10^6$ YUMM 3.1 cells murine melanoma cells. When tumors were measurable in >75% of mice, treatments were started.

In the first experiment, mice were treated with twice weekly IP injections of anti-PD1 antibody or vehicle control. Tumors in both the DIO and control mice were resistant to PD1 immunotherapy with no tumor regression seen in either model and there was no significant difference in tumor kinetics (Figure 17 A&B).

In the next experiment, mice were treated with daily oral gavage of either dabrafenib + trametinib or vehicle control. Tumors in both the DIO and the control mice were rapidly eradicated by the targeted therapy combination, again with no significant differences by diet group (Figure 17 C and D)

Discussion

In a genetically relevant subcutaneous mouse melanoma model, diet-induced obesity increases melanoma tumor growth. Importantly, the phenotype of the mice was validated prior to initiating therapy and I demonstrated that these mice did have higher fasting glucose and IGF-1 in addition to higher body weight. Tumors from DIO mice exhibited increased PI3K pathway activation and decreased pAMPK, consistent with the initial hypothesis. Interestingly, the tumors of the DIO mice had decreased tumor infiltrating immune cells. To my knowledge, this is the first time that RPPA and tumor flow cytometry have been used to examine the molecular and immunological impact of obesity. These studies support the clinical association of higher BMI with worse outcomes in patients with early stage melanoma. To definitively demonstrate that PI3K pathway activation underlies this relationship, future studies should

examine whether the effect of DIO can be abolished with PI3K inhibitors, fasting, and/or fasting mimetics.

In this subcutaneous model, there were no differences in sensitivity to either PD1 immunotherapy or dabrafenib + trametinib targeted therapy. This cell line (YUMM 3.1) was resistant to immunotherapy and exquisitely sensitivity to combination targeted therapy which makes it very difficult to be able to show differences in sensitivity to therapy between two organism metabolic phenotypes. Further, this subcutaneous model may not reflect the biology of metastatic melanoma. Finally, the implantation of a tumor into a host that has been fattened for 15 weeks may not reflect the biology of a tumor that develops in a patient with a lifetime of obesity.
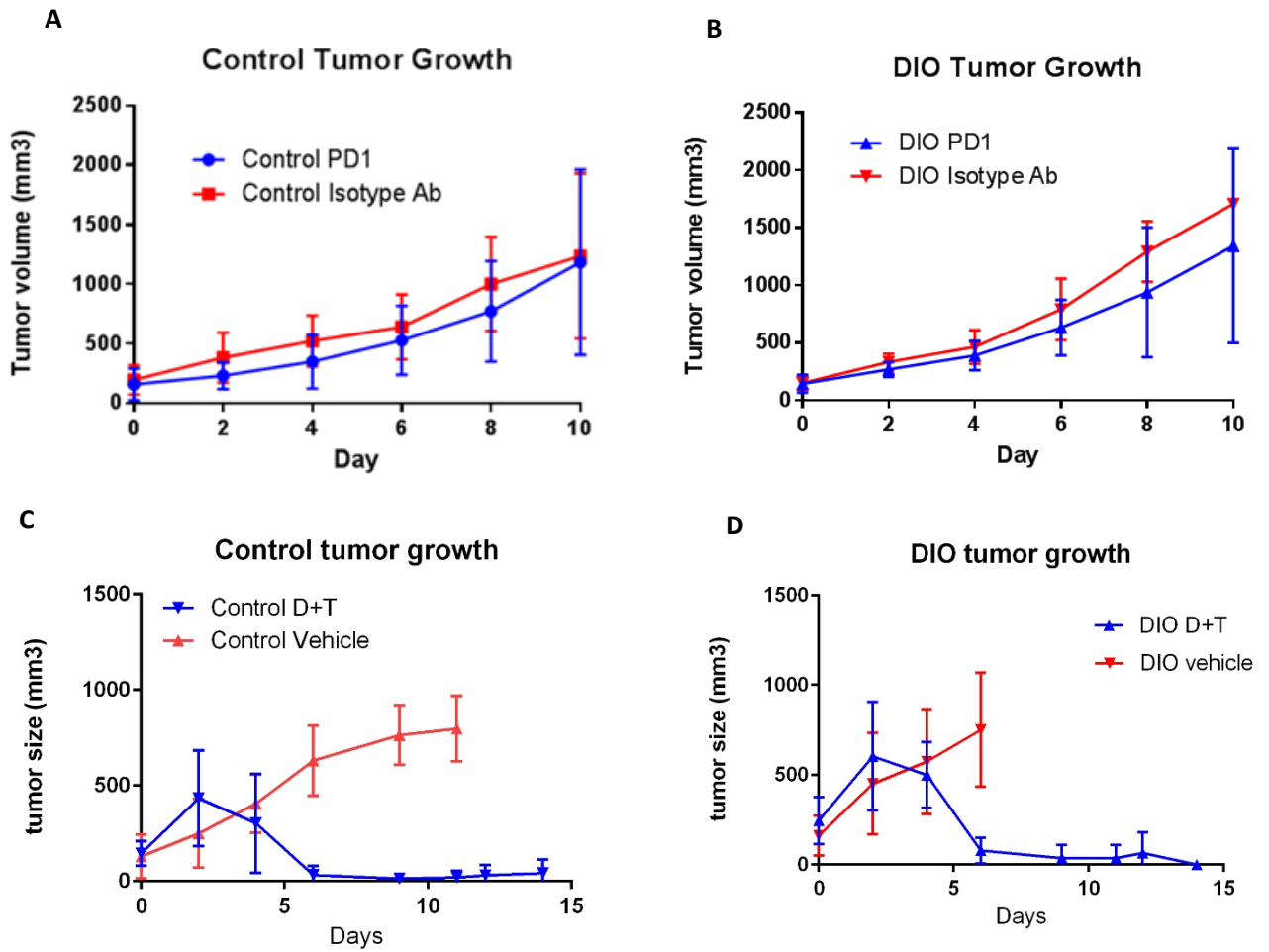
Figure 17. Effects of diet-induced obesity on response to immune and targeted therapy.

YUMM 3.1 tumors are resistant to PD1 in both A. control and B. DIO mice. Dabrafenib +

trametinib combination results in rapid tumor elimination in both C. control and D. DIO mice.

Chapter 6: Summary and Future Directions

Little is known about the clinical and therapeutic significance of obesity in melanoma. Our recent analysis of melanoma patients with clinically localized disease demonstrated that obesity is associated with worse outcomes in this population. (50) My findings in a preclinical mouse melanoma subcutaneous model that diet-induced obesity increases melanoma tumor growth support these epidemiological associations. Further, the findings that diet-induced obesity leads to increased PI3K pathway activation and decreased tumor infiltrating immunocytes in this model suggest a biological basis for these findings.

In contrast, my analysis of multiple large, independent cohorts of metastatic melanoma patients treated with contemporary targeted and immune therapies unexpectedly revealed that obesity was associated with significantly improved outcomes. In aggregate, these data support the presence of an "obesity paradox" across the spectrum of melanoma development, progression, and treatment response. Future work will be directed at understanding the biological basis of this paradox. In the subcutaneous mouse model, I did not observe differences in sensitivity to either targeted or immune therapy between obese and non-obese mice.

There are several possible explanations for why the mouse findings on therapeutic response do not reflect those observed in humans. First, targeted and immune therapies are used in humans in the metastatic setting and the mouse experiments were conducted using a subcutaneous model with rapid tumor growth which does not allow time for the development of metastases. Second, the cell lines used have the advantage of being genetically relevant (BRAF-mutant) and as they are mouse cell lines are able to be used in immunocompetent models and thus this model can be used to test sensitivity to both MAPK pathway directed targeted therapies and immune therapies. However, these driver-mutation driven cell lines do not reflect the many stochastic mutations that occur in UV-induced human melanoma.

Therefore, they are poorly immunogenic as reflected by the complete lack of sensitivity to PD1 inhibition with either diet. To address these issues, future studies will be conducted using metastatic models (tail-vein injection and survival surgery) in which BRAF-mutant cell lines are first irradiated to make them more immunogenic and a GEMM model to try to recapitulate the biology of a tumor that develops in the setting of obesity rather than being injected into a model of obesity.

The other possibility for the discrepancy between the findings in humans and mice on the effect of obesity on sensitivity to therapy is that the associations observed in humans are spurious or artificial and represent a methodological issue rather than a true clinical association. Indeed, an "obesity paradox" has been demonstrated in other diseases but remains controversial for this reason. Possible methodological explanations for an obesity paradox include BMI as an imperfect measure of adiposity, confounding, selection bias, collider stratification bias, detection bias, or reverse causality.(75) However, several features of the current study suggest a biological role of adiposity in the survival advantage associated with obesity with metastatic melanoma. In other diseases in which the obesity paradox has been observed, the survival advantage is often limited to overweight or only mildly obese patients. In several diseases, either the cancer or its treatment (i.e., chemotherapy) often cause weight loss, raising the possibility that this association may represent reversal causality.(75, 79) However, in the current study, there was a dose-dependent effect of BMI with modestly improved outcomes in overweight patients and strong, consistent associations in obese patients, and a nearly linear association between increasing BMI and PFS that extended to morbid obesity in the large D+T cohort. Further, the magnitude of the benefit seen with obesity, particularly in men, was equal to or greater than that seen in the registration trials that led to drug approval, and were seen in both test and validation cohorts for targeted and immune therapy, again supporting this association is unlikely to be spurious. I also accounted for the potential contribution of traditional prognostic factors and the use of concomitant medications

which may have anti-cancer activity, including metformin,(80, 81) statins,(82) beta blockers,(83) and aspirin.(84) To interrogate other potential causes of the observed differences, I examined rates of adverse events and available pharmacokinetic data (cobimetinib). These analyses again showed no significant differences by BMI category, supporting that treatment tolerance and drug exposure are unlikely to explain the observed associations. Differences in drug absorption are also an unlikely cause given that associations were seen with agents given orally at a fixed dose (targeted therapies) and with weight-based intravenous dosing (immunotherapies). As BMI was analyzed at a single time point (therapy initiation), I cannot rule out potential antecedent weight loss. However, the BMI distribution of each cohort mirrored that of the general population,(55) with over 60% of patients classified as overweight or obese, and <2% underweight. ECOG PS and albumin levels (PD-1/PDL-1 cohort) also did not differ by BMI category. Further, the targeted and immune therapy regimens examined are not usually associated with significant weight loss, which instead is more typical of chemotherapy, for which no associations with BMI were detected.

The strength and consistency of these associations support the need for focused investigations into their biological basis. Although targeted and immune therapy are fundamentally different modalities, cross-talk between oncogenic signaling pathways and immune response has been implicated in response and resistance to both treatments in melanoma.(70, 71, 85-87) The tumor that develops in an obese individual could have a fundamentally different, more indolent, biology. In order to examine differences in molecular signaling pathways between obese and non-obese individuals, we will analyze the molecular data from the Melanoma The Cancer Genome Atlas (TCGA) by BMI. The TCGA project includes comprehensive molecular profiling (whole exome sequencing (WES), RNA sequencing, and DNA methylation) on 471 melanoma patients. Through collaborations with other institutions I have been able to get BMI data for about 2/3 of these patients. We will examine this data using an unbiased approach but also in a directed fashion to investigate

specific hypotheses. For example, whole exome sequencing data will be used to examine mutation burden as high mutation load has been associated with response to therapy as these tumors are more immunogenic.(88, 89) Alternatively, obesity may impact tumor gene expression profiles or epigenetics. As previously noted, PI3K pathway alterations have been found to mediate resistance to both targeted and immune therapy.(25, 28) Given the fact that, consistent with my initial hypothesis, obesity was found to increase PI3K pathway activation in the mouse model, it is necessary to assess the association of BMI and PI3K pathway in humans.

Our approach of examining BMI associations in the melanoma TCGA is supported by a recent investigation in renal cell carcinoma (RCC) (another cancer in which an obesity paradox exists) where an analysis of BMI in the RCC TCGA found that alterations in fatty acid metabolism were associated with both high BMI and improved outcomes.(36)   Given emerging evidence implicating tumor metabolism in melanoma therapeutic response,(90-92) the relationship between tumor metabolism and clinical metabolic phenotype should also be explored in this disease.

Alternatively, rather than impacting the tumor biology, obesity could impact the host response to the tumor, namely the immune response. The immune response is important to response not only to immune therapy, but to targeted therapy as well.(68, 93) The presence of tumor infiltrating lymphocytes (TILs) has long been recognized as a favorable prognostic factor in melanoma.(94) The initial melanoma TCGA analysis also identified an "immune signature" that was associated with improved survival.(95) Immune signatures have also been shown to correlate with response to both targeted and immune therapy.(68, 96, 97) The impact of obesity on tumor immunology is unknown. Obesity is a state of chronic inflammation and this is one of the key mechanisms linking obesity to increased risk of many malignancies.(37) Obesity has been shown to impair response to vaccines and infections. However, the impact of obesity on anti-tumor immunology, and specifically in the setting of immune and targeted therapy, has not

been examined. Thus, I will examine the association of BMI with tumor infiltrating lymphocytes, and PD-L1 expression. In addition, I will examine BMI associations with peripheral blood cell populations and cytokines as these have also been associated with response to therapy.(98, 99) Given recent data implicating the microbiome in response to immunotherapy in melanoma(100-102), and prior evidence linking the microbiome with diet and obesity,(103) I will also examine the microbiome as a potential mediator of the observed effects.

The striking differences in BMI and outcome associations by sex observed here suggest a potential hormonal mediator. Strong and consistent associations of higher BMI with improved outcomes was seen in male metastatic melanoma patients treated with both targeted and immune therapy while BMI was not associated with outcomes in females (Figure 18). Female sex has long been recognized as a predictor of improved outcomes in melanoma.(104, 105) Intriguingly, higher BMI seems to overcome the survival disadvantage among males, as the outcomes of obese males were similar to females of any BMI in the targeted therapy and immunotherapy cohorts, whereas normal BMI males had significantly inferior outcomes. Interrogation of TCGA data in other malignancies has demonstrated gender-specific differences in tumor biology, notably in metabolic and immune pathways.(57) In melanoma, there is emerging evidence of differential tumor immunology by gender, with female melanoma patients exhibiting improved autologous tumor infiltrating lymphocytes (TIL) expansion(106) and increased tumor-antigen specific T cells compared to their male counterparts.(107) Preclinical data showed increased sensitivity to immune checkpoint blockade in female vs male mice, and pointed towards an estrogen-mediated functional reduction in T regulatory cells as the mechanism.(108) This data is consistent with the key role estrogen has been shown to play in sex differences in autoimmune disease and immune response to vaccination and infections.(109)
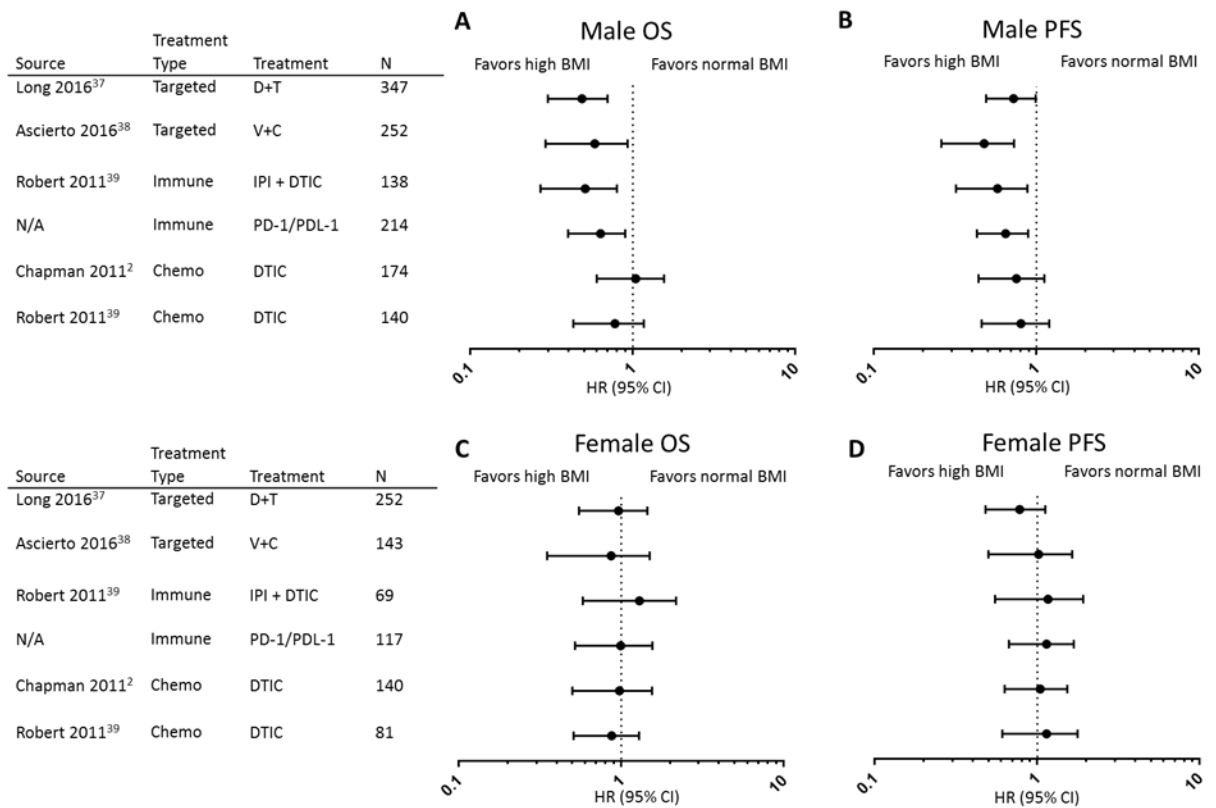
Figure 18. Forest plot of hazard ratios for OS and PFS for high vs. low BMI by sex.

Forest plots of hazard ratios for highest BMI group in comparison to normal BMI by cohort and

sex for A. overall survival (OS) and B. progression-free survival (PFS) in males, and C. OS and

D. PFS in females with metastatic melanoma receiving the indicated therapies.

Obesity leads to higher levels of circulating estrogen due to adipocyte aromatase conversion of androgens to estrogen compounds, though this effect is only significant in men and postmenopausal females.(110) Combined with our findings, these data provide a strong rationale to examine the role of estrogen signaling on immune and/or tumor cells as a possible mediator of the gender-specific associations of obesity in this disease. Notably, although a randomized controlled trial of DTIC +/- tamoxifen, a selective estrogen receptor modulator, showed no benefit in metastatic melanoma patients overall, higher BMI in men was predictive of benefit from the addition of tamoxifen to chemotherapy, but not with chemotherapy alone.(111) Interestingly, the rationale for testing tamoxifen in melanoma was based on flawed studies showing high expression of estrogen receptor alpha (ERα) based on non-specific binding.(112) It has now been demonstrated that melanomas have very low (ERα) expression. However, melanoma does have high expression of estrogen-receptor beta (ERβ). (113, 114) ERβ has been shown to be anti-proliferative in other malignancies and recently in melanoma as well.(115-119) In order to test this hypothesis, we will examine the TCGA data for correlations between BMI and sex and hormonal signaling and also perform functional testing of hormone signaling modulation.

In conclusion, I have found a strong and consistent association of obesity with improved outcomes in patients with metastatic melanoma treated with targeted and immune therapies across multiple large independent cohorts. These associations appear to be driven predominantly by markedly improved outcomes in obese compared to normal BMI males. In contrast, I did not observe any significant associations of BMI with outcomes in female patients. As obesity is associated with worse outcomes in early stage melanoma, these findings support the presence of an obesity paradox in melanoma. The magnitude of the survival benefit associated with obesity in patients with metastatic melanoma support the need to include BMI as a stratification factor in the design of clinical trials. However, the implications for patient guidance at this point is unclear. While obesity at treatment initiation is associated with

improved outcomes, we do not know if deliberate change in weight after diagnosis can shift outcomes. It should be noted that we also do not know if deliberate weight changes can change the outcomes of other malignancies as well. Specifically, though it has now been very well-established that obesity is associated with worse outcomes in early stage breast cancer patients undergoing adjuvant therapy,(12) we currently lack prospective data examining the impact of deliberate weight loss after diagnosis on breast cancer patient outcomes. There are currently ongoing randomized clinical trials assessing this question. These types of trials are critical to establish whether BMI and outcome associations are reversible or whether these are non-reversible fixed biological effects, i.e. that the host metabolic phenotype has determined a tumor biology that cannot be changed.

However, while in breast cancer we may lack data supporting that weight loss will decrease recurrence risk, there is abundant preclinical and correlative data supporting this strategy.(15, 16, 62, 73, 120-123) Moreover, most patients with early stage breast cancer, which has a very high cure rate, will ultimately die from other causes. The data linking obesity with increased risk of heart disease, stroke, and diabetes as well as second cancers is clear. Thus, even lacking the randomized clinical trial data supporting weight loss interventions in breast cancer survivors, there is compelling data both for supporting the rationale for trials testing this approach and even for supporting weight loss efforts pending these results in breast cancer patients as this will reduce their burden of other major causes of mortality and plausibly decrease their risk of breast cancer recurrence.

In contrast, without an understanding of the mechanism underlying the association of obesity with improved outcomes in metastatic melanoma, preclinical evidence that obesity may increase cancer growth, and the impact of obesity on other potential causes of death, the epidemiological evidence presented in this thesis is not sufficient to support a weight gain intervention trial in metastatic melanoma patients. Instead, future research should be focused on understanding the biological basis for the association of obesity with improved outcomes so

that was can design rational therapeutic interventions (e.g. hormonal therapy, metabolic

modulators) to improve the outcomes of all patients.

obes

Vita

Jennifer Leigh McQuade was born in Beckley, West Virginia on November 22, 1976, the daughter of Thomas Alan McQuade and deAnna Sue McQuade. After graduating from Midlothian High School in Midlothian, Virginia in 1995, she entered the University of Virginia in Charlottesville. Jennifer graduated with an Echols Interdisciplinary Bachelor of Arts with concentrations in Biology and International Studies in 1998. She then moved to Taipei, Taiwan where she spent two years teaching English and studying Chinese at national Taiwan University. She moved to Houston, Texas in 2000 and worked as an analyst at Enron and then UBS Warburg until 2002. In 2001, she enrolled in the American College of Acupuncture in Oriental Medicine and graduated with a degree in Oriental Medicine in 2007. In 2004, Jennifer was awarded a Fulbright Fellowship from the US Department of State and spent one year in Shanghai, China studying the integration of traditional Chinese medicine and western cancer care and conducting collaborative research with Dr. Lorenzo Cohen, Director of MD Anderson's Integrative Medicine program. In 2005, she enrolled in Baylor College of Medicine and earned her medical degree in 2009. She subsequently completed an internship and residency in Internal Medicine at the University of Pennsylvania in 2012. She spent one year as an Internist with Southpark Medical Group in Charlotte, North Carolina before returning to Houston to begin her fellowship in Medical Oncology at the University of Texas MD Anderson Cancer Center in 2013. Dr. McQuade began her work in the University of Texas Graduate School of Biomedical Sciences in 2014 under the mentorship of Michael Davies MD PhD. She completed her oncology fellowship in 2016 and is currently an Instructor in Melanoma Medical Oncology at the University of Texas MD Anderson Cancer Center through the Advanced Scholar Program.

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