**Hypothesis:** Sex-based heterogeneity in the TME and antitumor response exists and contributes to more favorable outcomes in males after ICI therapy

**Analysis Plan**

* Focus on primary cutaneous melanoma (N=366):
  + Exclude: ocular (N=6), mucosal (N=6), unknown (N=1)
* Tumor microenvironment
  + Primary cutaneous melanoma with bulk RNA sequencing (N=?): TODO: Does primary cutaneous melanoma have bulk RNA sequencing if there is a corresponding row in clinical molecular linkage data with a value in column “RNA Batch”?
    - Immune cell infiltrate
    - CD8+ T cell phenotype
    - GSEA of immune-related gene signatures
    - TIDE assessment
  + Primary cutaneous melanoma with whole exome sequencing (N=352)
    - Tumor mutational burden
* Clinical outcomes

|  |  |  |  |
| --- | --- | --- | --- |
| **Characteristic, N (%)** | **Male (N=)5** | **Female (N=)5** | **Total (N=)** |
| **Sequencing data1** |  |  |  |
| **WES only1** |  |  |  |
| **RNAseq only1** |  |  |  |
| **WES and RNAseq1** |  |  |  |
| **Specimen collection site1** |  |  |  |
| **Skin and other soft tissues1,a** |  |  |  |
| **Lymph node1** |  |  |  |
| **Lung1** |  |  |  |
| **Abdominal viscera1,b** |  |  |  |
| **Brain1** |  |  |  |
| **Bone1** |  |  |  |
| **Melanoma driver mutations2** |  |  |  |
| **BRAF2** |  |  |  |
| **NRAS2** |  |  |  |
| **PTEN2** |  |  |  |
| **Agec (years)1** |  |  |  |
| **< 201** |  |  |  |
| **20-291** |  |  |  |
| **30-391** |  |  |  |
| **40-491** |  |  |  |
| **50-591** |  |  |  |
| **60-691** |  |  |  |
| **70-791** |  |  |  |
| **80-891** |  |  |  |
| **90+1** |  |  |  |
| **Mean** |  |  |  |
| **Median** |  |  |  |
| **Stage3,c** |  |  |  |
| **II3** |  |  |  |
| **III3** |  |  |  |
| **IV3** |  |  |  |
| **ICB Status**4,c |  |  |  |
| **Naïve4** |  |  |  |
| **Experienced4** |  |  |  |
| **Anti-PD1 only4** |  |  |  |
| **Anti-CTLA4 only4** |  |  |  |
| **Anti-PD1 and anti-CTLA44** |  |  |  |

**Table 1. Sequencing and clinicopathological characteristics of patient tumor specimens.**

1 Use `../../Clinical\_Data/24PRJ217UVA\_NormalizedFiles/24PRJ217UVA\_20241112\_ClinicalMolLinkage\_V4.csv`.

2 Use column `TMarkerTest` in `../../Clinical\_Data/24PRJ217UVA\_NormalizedFiles/24PRJ217UVA\_20241112\_TumorMarker\_V4.csv`.

3 Use column “EKN Assigned Stage” in `../pair\_clinical\_data\_and\_stages\_of\_tumors/output\_of\_pipeline\_for\_pairing\_clinical\_data\_and\_stages\_of\_tumors.csv`.

4 Use `../../Clinical\_Data/24PRJ217UVA\_NormalizedFiles/24PRJ217UVA\_20241112\_Medications\_V4.csv`.

5 Use column `PatientMaster` in `../../Clinical\_Data/24PRJ217UVA\_NormalizedFiles/24PRJ217UVA\_20241112\_PatientMaster\_V4.csv`.

a Includes muscle and chest wall: TODO: “chest wall” is in column `SpecimenSiteOfCollection` of clinical molecular data but not in list of keywords identifying a specimen site of collection of “Skin and other soft tissues”. Should we resolve this discrepancy?

b Includes organs of the retroperitoneum and genitourinary tract

c At tumor specimen collection

|  |  |  |  |
| --- | --- | --- | --- |
| **Characteristic, N (%)** | **Male (N=)** | **Female (N=)** | **Total (N=)** |
| **Age (years)** |  |  |  |
| **< 20** |  |  |  |
| **20-29** |  |  |  |
| **30-39** |  |  |  |
| **40-49** |  |  |  |
| **50-59** |  |  |  |
| **60-69** |  |  |  |
| **70-79** |  |  |  |
| **80-89** |  |  |  |
| **90+** |  |  |  |
| **Mean** |  |  |  |
| **Median** |  |  |  |
| **Race** |  |  |  |
| **[options]** |  |  |  |
| **Ethnicity** |  |  |  |
| **[options]** |  |  |  |
| **AJCC v8 Stagea** |  |  |  |
| **IA** |  |  |  |
| **IB** |  |  |  |
| **IIA** |  |  |  |
| **IIB** |  |  |  |
| **IIC** |  |  |  |
| **IIIA** |  |  |  |
| **IIIB** |  |  |  |
| **IIIC** |  |  |  |
| **IIID** |  |  |  |
| **IV** |  |  |  |
| **Primary diagnosis site** |  |  |  |
| **Head and neck** |  |  |  |
| **Upper extremity** |  |  |  |
| **Trunk** |  |  |  |
| **Lower extremity** |  |  |  |
| **Vulva** |  |  |  |
| **Skin, NOS** |  |  |  |
| **ECOG PS** |  |  |  |
| **0** |  |  |  |
| **1** |  |  |  |
| **2** |  |  |  |
| **3+** |  |  |  |

**Table 2. Patient baseline characteristics**. Demographic and clinical characteristics at the time of diagnosis.

*AJCC*, American Joint Committee on Cancer 8th edition; *NOS*, not otherwise specified; *ECOG* PS, Eastern Oncology Cooperative Group Performance Status

a Pathologic stage reported if known; otherwise, clinical stage reported

**Aim 1. Characterize the immune cell infiltrate in the melanoma tumor microenvironment.**

*1.1. Evaluate sex differences in immune cell infiltrate composition.*

* Include all tumor specimens with RNAseq data
* Immune cell enrichment scores using xCell
  + **Check with Dr. Shakeri - he wrote some technical details in the grant application for our plans that seem better than what I propose below, so you can edit this to be what you and Dr. Shakeri actually do**
    - Consider comparing enrichment scores by sex (FDR ≤ 0.05, two-sided Wilcoxon rank-sum test with Benjamini-Hochberg correction)
      * FDR ≤ 0.05 considered significant, but consider also showing 0.05 < FDR ≤ 0.20
      * Can also compare ICB naïve tumors to ICB experienced tumors within each sex
      * Consider adjusting for age and stage
* Summary publication of different methods (including xCell): 31510660
* Example publications for xCell: PMID 34016641, PMID 32286310, PMID 32102984
  + Can enter these PMID numbers into the search on Pubmed to see how others have used xCell and reported the results.
* <https://github.com/dviraran/xCell>
* User guide: <https://link.springer.com/protocol/10.1007/978-1-0716-0327-7_19>

*1.2. Evaluate sex differences in phenotypes of T cell populations*.

* CD8+ tumor infiltrating lymphocytes (TILs) using reference gene signatures published from single cell datasets of melanoma TILs
  + Sade-Feldman (Cell 2019; PMID 30388456): metastatic melanoma prior to or during ICB treatment (**this is the same gene signature set that you previously worked with; can also check with Dr. Shakeri on the technical details he wrote in the grant for this part, which may have been what you’ve already done; edit to what you and Dr. Shakeri actually do**)
    - CD8\_B (enriched in ICB non-responders) vs CD8\_G (enriched in ICB responders) by sex
      * CD8\_B = CD8\_1, CD8\_2, CD8\_3
      * CD8\_G = CD8\_4, CD8\_5, CD8\_6
      * Can also explore by fine clusters (CD8\_1 through CD8\_6)
    - Include all tumor specimens with RNAseq data
  + Can also consider Oliveira (Nature 2021; PMID 34290406) – *hold off for now*
    - 5 tumor-specific clusters
    - Include only ICB naïve tumor specimens with RNAseq data

**Aim 2. Characterize key features of the antitumor response in the melanoma tumor microenvironment.**

*2.1. Evaluate sex differences in immune signaling pathways*.

*2.2. Evaluate sex differences in immune evasion mechanisms*.

*2.3 Evaluate sex differences in the tumor mutational burden.*

**Aim 3**. **Investigate sex differences in clinical outcomes after immune checkpoint inhibitor therapy.**

*3.1. Evaluate sex as an independent predictor for clinical outcome after immune checkpoint inhibitor therapy.*

**Details for Table 1 (Specimens): only for AssignedPrimarySite = cutaneous**

**Sequencing Data**

* WES only = Counts of specimens [patients right now] with value for “WES” on Molecular Linkage file
* RNAseq only = Counts of specimens [patient right now] with value for “RNASeq” on Molecular Linkage file
* WES and RNAseq = Counts of specimens [patients right now] with values for both “WES” and “RNASeq” on Molecular Linkage file
* TODO: Should we include “neither WES nor RNAseq”?

**Specimen Collection Site**

* Counts for each category using the “SpecimenSiteOfCollection” on Molecular Linkage file
  + Skin and other soft tissues: contains “skin” OR [“soft tissue” and does not contain “lymph node”] OR “breast” OR “ear” OR “eyelid” OR [“head” and does not contain “lymph node”] OR “muscle” OR “thorax” OR “upper limb, NOS” OR “vulva”
  + Lymph node: contains “lymph node” OR “parotid”
  + Lung: contains “lower lobe” OR “lung” OR “trachea” OR “upper lobe”
  + Abdominal viscera: contains “anus” OR “adrenal” OR “colon” OR “gallbladder”, “ileum” OR “jejunum” OR “kidney” OR “liver” OR “retroperitoneum” OR “small intestine” OR “peritoneum” OR “spleen” OR “ureter” OR “vagina”
  + Brain: contains “brain” OR “cerebellum” OR “frontal lobe” OR “occipital lobe” OR “temporal lobe”
  + Bone: contains “bone” OR “spine” OR “vertebral”

**Melanoma Driver Mutations**

* I’m not sure how one identifies driver mutations from the available tumor and germline WES data, so you’ll need to discuss with Dr. Shakeri. TODO: Should we use column `TMarkerTest` in tumor marker data containing a mutation and `TMarkerResult` containing “Positive”? Common melanoma driver mutations that are of interest to us are *BRAF*, *NRAS*, and *PTEN*.

**Age**

* Counts for each age group and the mean and median using “Age At Specimen Collection” on Molecular Linkage file
  + For calculating the mean, will have to approximate by assigning age 90 for the age 90+ group

**Stage**

* Counts for each stage using the “AssignedStage” from your pipeline

**ICB Status**

* Naïve = “Age of Specimen Collection+0.005” on Molecular Linkage file is before any “AgeAtMedStart” entries for ICBs on the Medications file
  + Tumor collected prior to starting any ICB medications
* Experienced = “Age of Specimen Collection+0.005” on Molecular Linkage file is after any “AgeAtMedStart” entries for ICBs on the Medications file
  + Tumor collected after starting an ICB medication
* ICB Medications
  + Anti-PD1 = nivolumab (includes nivolumab-relatlimab-rmbw), pembrolizumab, atezolizumab
  + Anti-CTLA4 = ipilimumab

**Details for Table 2 (Diagnosis): only for AssignedPrimarySite = cutaneous**

* This table uses the fields associated with the diagnosis selected by your pipeline except for the race and ethnicity fields that are from the Patient Master file

**Age**

* Counts for each age group and the mean and median using “AgeAtDiagnosis”
  + For calculating the mean, will have to approximate by assigning age 90 for the age 90+ group

**Race**

* Counts for each category reported for “Race” on the Patient Master file

**Ethnicity**

* Counts for each category reported for “Ethnicity” on the Patient Master file

**Stage**

* Counts for each stage
  + Use “PathGroupStage” when known
  + If “PathGroupStage” = “Unknown/Not Reported” OR “Unknown/Not Applicable” OR “No TNM applicable for this site/histology combination”, then use “ClinGroupStage”
  + If “PathGroupStage” and “ClinGroupStage” are both unknown, list as a new category in table as “Unknown”
  + I started the table at stage IA with the assumption that none of our patients have a diagnosis of stage 0, but if there are some with that initial stage, just add as a row to the table.
* Pull these stages as they are, regardless of the associated “TNMEditionNumber”. This means you can ignore the header text AJCC v8. I can re-assign those that were previously classified under a prior version (before 8th edition) if there is enough information.
  + If possible, could you pull the list of patients with “TNMEditionNumber” that does not contain “Eighth Edition” into a separate Excel file for me to review (AvaterKey, Group (from your pipeline: A, B, C, D), ClinTStage, ClinNStage, ClinMStage, ClinGroupStage, PathTStage, PathNStage, PathMStage, PathGroupStage, TNMEditionNumber)

**Primary Diagnosis Site**

* Use “PrimaryDiagnosisSite”
  + Head and neck: contains "ear" OR "eyelid" OR "lip" OR "scalp" OR "face"
  + Upper extremity: contains "upper limb"
  + Trunk: contains "trunk"
  + Lower extremity: contains "lower limb"
  + Vulva: contains "Vulva, NOS"
  + Skin, NOS: contains "Skin, NOS"

**ECOG PS**

* Use “PerformStatusAtDiagnosisScale” and “PerformStatusAtDiagnosis” fields
* I haven’t seen what this data looks like, but the goal is to pull the Eastern Cooperative Oncology Group (ECOG) performance status (PS) score (range 0-5). It looks like ORIEN may also report a different score (Karnosfky), but my hope is that the majority have an ECOG status.
* Counts for each ECOG score (0, 1, 2, and then combine the others into 3+) if “PerformStatusAtDiagnosisScale” contains “ECOG” (you’ll have to see if they abbreviate or spell out the name)
* If “PerformStatusAtDiagnosisScale” does not contain “ECOG”, then add a row for Unknown.