Figure S1.

A. Metastasis 1 (A&B) (Left axilla)
   Metastasis 2 (Right chest)

B. Mutational overlap

<table>
<thead>
<tr>
<th>Metastasis 1A</th>
<th>Metastasis 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>3%</td>
<td>0%</td>
</tr>
<tr>
<td>0%</td>
<td>8%</td>
</tr>
<tr>
<td>2%</td>
<td>9%</td>
</tr>
<tr>
<td>28</td>
<td>47</td>
</tr>
<tr>
<td>30%</td>
<td>50%</td>
</tr>
</tbody>
</table>

C. Mutational signatures

D. Neoantigens

E. Flow cytometry

F. Immunohistochemistry

G. Clone frequency

H. Hive Plot
Figure S2.

A. Metastasis 1 
   (Left abdomen) 
   Metastasis 2 
   (Right abdomen)

B. Mutational overlap

Metastasis 1 Metastasis 2
48 32% 80 53% 23 15%

D. Neoantigens

IC_{50} (nM)
500
400
300
200
100
0
M1
M2

F. Immunohistochemistry

Counts/mm² or H-score
500
400
300
200
100
0
Metastasis 2

G. Clone frequency

% of total population
10
1
0.1
0.01
0.001

H. Hive Plot

CD8
Clonality

E. Flow cytometry

% of CD45+
75
50
25
0
M1
M2

C. Mutational signatures

Frequency

M1
M2

Patient 37

0
100
200
300
400
500

Frequency

C>A C>G C>T T>A T>C

Clone frequency

% of total population

M1 M2

α-CTLA-4/Paclitaxel BRAFi BRAFi/MEKi

Baseline

6 weeks

PD (+90%) PD (+153%)
Figure S3.

A. Metastasis 1 (Right flank) and Metastasis 2 (Right shoulder).

B. Mutational overlap: Metastasis 1 and Metastasis 2.

IC 50 (nM)

M1

M2

IC 50 (nM)

M1

M2

23% 60% 17%

46 121 35

C. Mutational signatures: M1 and M2.

Frequency

M1

M2

C>A C>G C>T T>A T>C T>G

D. Neoantigens: Unique and Shared.

E. Flow cytometry: % of CD45+

% of CD45+

M1

M2

% of CD45+

M1

M2

F. Immunohistochemistry: Counts/mm² or H-score.

Counts/mm² or H-score

Metastasis 2

G. Clone frequency: % of total population.

% of total population

M1

M2

H. Hive Plot.
Figure S4.

A. Metastasis 1 (Right axillary lymph node) → α-CTLA-4/α-PD-1 → BRAFi/MEKi → Metastasis 2 (Right neck lymph node)

B. Mutational overlap

<table>
<thead>
<tr>
<th>Metastasis 1</th>
<th>Metastasis 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>20%</td>
<td>307%</td>
</tr>
<tr>
<td>5%</td>
<td>75%</td>
</tr>
</tbody>
</table>

C. Mutational signatures

D. Neoantigens

E. Flow cytometry

F. Immunohistochemistry

G. Clone frequency

H. Hive Plot
Figure S5.

A. Metastasis 1 (Left inguinal lymph node) and Metastasis 2 (Left popliteal fossa)

B. Mutational overlap

Metastasis 1  Metastasis 2

\[
\begin{array}{ccc}
277 & 79 & 303 \\
42\% & 12\% & 46\% \\
\end{array}
\]

D. Neoantigens

IC50 (nM)

\[
\begin{array}{c}
M1 \\
M2 \\
\end{array}
\]

F. Immunohistochemistry

G. Clone frequency

H. Hive Plot

Metastasis 1  Metastasis 2

Baseline 11 weeks

SD (+1%)  PD (+71%)

Counts/mm²

CD4 T cells
CD8 T cells
γδ T cells
NK cells
Dendritic cells
Neutrophils
Mast cells
Basophils
B cells
Macrophages
B cells
Other CD45+

M1
M2

IC50 (nM)

Frequency

C>A  C>G  C>T  T>A  T>C  T>G

M1
M2

% of CD45+

% of total population

NSEM

Clonality

CD8
Figure S6.

A. Metastasis 1 (Left abdominal mass)  
Metastasis 2 (Right abdominal mass)

B. Mutational overlap

Metastasis 1 Metastasis 2

522 36%  422 29%  521 35%

D. Neoantigens

 IC 50 (nM)  
M1  M2

F. Immunohistochemistry

Counts/mm² or H-score

G. Clone frequency

% of total population

H. Hive Plot

CD8
CD4
CD57
CD45RO
GzmB
FoxP3
CD68
OX40

M1 M2

α-CTLA-4  α-PD-1

HD IL-2

E. Flow cytometry

% of CD45+

CD4 T cells  CD8 T cells  γδ T cells  NK cells  Dendritic cells  Neutrophils  Mast cells  Basophils  B cells  Macrophages  Eosinophils  Other CD45+
Figure S7.

A. 

Metastasis 1 
(Mid abdominal mass)

Metastasis 2 
(Mid abdominal mass)

Adjuvant IFN-α  α-CTLA-4/α-PD-1

B. Mutational overlap

Metastasis 1 Metastasis 2

34 21%
95 60%
30 19%

C. Mutational signatures

D. Neoantigens

E. Flow cytometry

F. Immunohistochemistry

G. Clone frequency

H. Hive Plot

Count/mm² or H-score

PD-L1
PD-1
CD3
CD4
CD8
CD57
CD45RO
GzmB
FoxP3
CD68
OX40

M1 M2 M1 M2 M1 M2 M1 M2

IC 50 (nM)

Frequency

% of CD45+

% of total population

Clonality

Unique
Shared

Flow cytometry

Clone frequency

Hive Plot

M1 M2

CD8

CD8 T cells
CD8 T cells
γδ T cells
NK cells
Dendritic cells
Neutrophils
Mast cells
Basophils
B cells
Macrophages
Eosinophils
Other CD45+

PD-L1
PD-1
CD3
CD4
CD8
CD57
CD45RO
GzmB
FoxP3
CD68
OX40
Figure S8.

A. Mutational overlap

B. Mutational signatures

D. Neoantigens

F. Immunohistochemistry

C. Mutational signatures

E. Flow cytometry

G. Clone frequency

H. Hive Plot
Figure S9.

A. Metastasis 1 (Right breast)
Metastasis 2 (Left breast)
Metastasis 3 (Left breast)
Metastasis 4 (Right breast)

B. Mutational overlap

Metastasis 4

Metastasis 1

17
9%

4
2%

11
6%

6
3%

C. Mutational signatures

D. Neoantigens

E. Flow cytometry

F. Immunohistochemistry

G. Clone frequency

H. Hive Plot
Figure S10.

A. Metastasis 1 (A-F) (Spleen)
Metastasis 2 (A&B) (Right abdominal wall)

B. Mutational overlap

C. Mutational signatures

D. Neoantigens

E. Flow cytometry

F. Immunohistochemistry

G. Clone frequency

H. Hive Plot
**Figure S11.**

A. Metastasis 1 (Left axillary mass)
   Metastasis 2 (Left axillary mass)

---

**B. Mutational overlap**

Metastasis 1 Metastasis 2

<table>
<thead>
<tr>
<th></th>
<th>M1</th>
<th>M2</th>
</tr>
</thead>
<tbody>
<tr>
<td>C&gt;A</td>
<td>28</td>
<td>5%</td>
</tr>
<tr>
<td>C&gt;G</td>
<td>431</td>
<td>77%</td>
</tr>
<tr>
<td>C&gt;T</td>
<td>99</td>
<td>18%</td>
</tr>
</tbody>
</table>

---

**C. Mutational signatures**

---

**D. Neoantigens**

---

**E. Flow cytometry**

---

**F. Immunohistochemistry**

---

**G. Clone frequency**

---

**H. Hive Plot**

---
Figure S12.

A. Metastasis 1 (Left axillary lymph node)
   Metastasis 2 (Left axillary lymph node)

   Treatment-naive

B. Mutational overlap

   Metastasis 1
   Metastasis 2

   211 41%
   246 48%
   56 11%

D. Neoantigens

   IC_{50} (nM)

   M1 M2

   Unique Shared

F. Immunohistochemistry

   Counts/mm^2 or H-score

   PD-L1 PD-1 CD3 CD4 CD8 CD57 CD45RO GzmB FoxP3 CD68 OX40

C. Mutational signatures

E. Flow cytometry

   % of CD45+

   CD4 T cells CD8 T cells γδ T cells NK cells Dendritic cells Neutrophils Mast cells Basophils B cells Macrophages Eosinophils Other CD45+

G. Clone frequency

   % of total population

   M1 M2

H. Hive Plot

   NSEM CD8 Clonality
Figure S13.

A. Metastasis 1 (Left axillary mass)  
Metastasis 2 (Right supraclavicular mass)

B. Mutational overlap

Metastasis 1 Metastasis 2

13 7%  
149 81%  
23 12%

D. Neoantigens

IC_{50} (mM)

M1  
M2

F. Immunohistochemistry

Counts/mm² or H-score

Metastasis 1  
Metastasis 2

G. Clone frequency

% of total population

H. Hive Plot

CD8  
Clonality
Figure S15.

A. Metastasis 1 (A-D) (Right thigh)

B. Mutational overlap

C. Mutational signatures

D. Neoantigens

E. Flow cytometry

F. Immunohistochemistry

G. Clone frequency

H. Hive Plot

Dendritic Cells/ Bacillus Calmette-Guerin/IFN-α
α-FGFR3
IFN-α/IL-2 Arginine Deaminase Polyethylene Glycol/ α-PD-1
Cisplatin

Mutational signatures

Counts/mm² or H-score

PD-L1
PD-1
CD3
CD4
CD8
CD57
CD45RO
GzmB
FoxP3
CD68
OX40

CD4 T cells
CD8 T cells
γδ T cells
NK cells
Dendritic cells
Neutrophils
Mast cells
Basophils
B cells
Macrophages
Eosinophils
Other CD45+

Frequency

% of CD45+

% of total population

Hive Plot

NSEM

Clonality
Figure S17.

Patient #2
Top 100: $r=-0.150$, $p=0.136$
Top 10: $r=-0.111$, $p=0.759$

Patient #3
Top 100: $r=0.023$, $p=0.817$
Top 10: $r=0.073$, $p=0.838$

Patient #4
Top 100: $r=-0.057$, $p=0.568$
Top 10: $r=-0.055$, $p=0.892$

Patient #6
Top 100: $r=-0.090$, $p=0.380$
Top 10: $r=0.576$, $p=0.088$

Patient #7
Top 100: $r=0.045$, $p=0.660$
Top 10: $r=0.196$, $p=0.584$

Patient #11
Top 100: $r=0.068$, $p=0.503$
Top 10: $r=-0.042$, $p=0.918$

Patient #12
Top 100: $r=-0.490$, $p<0.0001$
Top 10: $r=-0.394$, $p=0.263$

Patient #13
Top 100: $r=-0.041$, $p=0.684$
Top 10: $r=0.778$, $p=0.010$

Legend:
- Red circle: Targeted Therapy
- Blue circle: Immunotherapy
- Green square: Treatment-Naïve
Figure S18.

A

% of Lesions Above Median

NSEM CD8 Clonality

p=0.009

n.s.

n.s.

B

Worst Responding Metastasis

Best Responding Metastasis

Mutational Burden Neoantigen Load TCR Clonality OX40 FoxP3 GzmB CD45RO PD-1 PD-L1 CD68 CD57 CD8 CD4 CD3 CD8/Treg Treg Eosinophils Neutrophils Mast cells Basophils DC Macrophages B NK γδ T CD8 CD4

Flow cytometry Immunohistochemistry TCR Sequencing Genomic Analysis

p-value
Figure S19.

A

Random Forest Analysis
(All complete variables)

Contrast
Dissimilarity
Homogeneity
TCR Clonality
Energy
CD4 T cells
NK cells
CD8 T cells
γδ T cells
Entropy
B cells

Importance score

Texture Feature
Genomic Factor
Immune Factor

B

Random Forest Analysis
(Selected variables)

Contrast
CD8 T cells
TCR Clonality
Neoantigens
NSEM

Importance score

Texture Feature
Genomic Factor
Immune Factor

C

Hierarchical Clustering
(All complete variables)

Worst Synchronous Response
Best Synchronous Response

Contrast
Dissimilarity
Energy
Homogeneity
Entropy
γδ T cells
B cells
CD4 T cells
TCR Clonality
CD8 T cells
NK cells

Texture Feature
Genomic Factor
Immune Factor

Z-score

-3 0 3
Figure S20.

Flow cytometry
Immunohistochemistry
TCR sequencing

Immune profiling
Flow cytometry
Immunohistochemistry
NanoString Analysis
TCR sequencing

Molecular profiling
Whole Exome Sequencing
Neoantigen Prediction

Metastasis 1
Metastasis 2