

Supplementary Materials

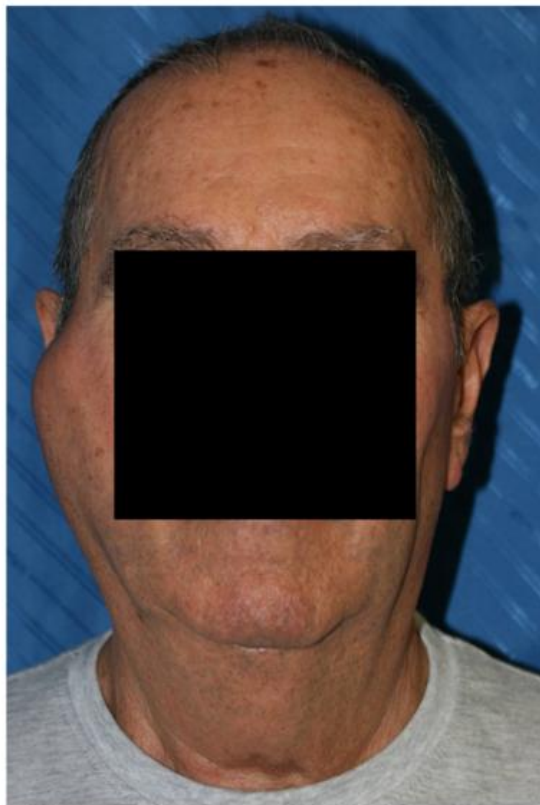
Table S1. Primers and probes used for Real-time PCR analysis.

SYBR™ Green Assays		
Gene	Forward primer (5'-3')	Reverse primer (5'-3')
<i>TP53I3</i>	AAT-GCT-TTC-ACG-GAG-CAA-ATT-C	TTC-GGT-CAC-TGG-GTA-TCT
<i>LAPTM4A</i>	GCC-TGT-GTT-CTT-TTT-CCC-GTC-T	GGA-ATC-AGC-CAA-CCC-ACT-TGA
<i>RAB22A</i>	CAC-CTT-ACC-GTC-TCT-GTG-ATT-G	GTC-TGT-GGC-CAT-TCT-TTA
<i>S100P</i>	TGC-AGA-GTG-GAA-AAG-ACA-AGG-AT	CCA-CCT-GGG-CAT-CTC-CAT-T
<i>CD109</i>	CCT-GTG-ACC-TTT-GCA-GTG-ATG-T	GAG-TGA-TGG-GAG-CCT-GAA
<i>LAPTM4B</i>	GTT-ACC-AGC-AAT-GAC-ACT-ACG-G	CTC-CTT-GGC-AGC-ACC-ATT
<i>GAPDH</i>	ACC-ACA-GTC-CAT-GCC-ATC	TCC-ACC-ACC-CTG-TTG-CTG-TA
TaqMan® Assays		
Gene	Assay identification number	
<i>VIM</i>	Hs_00185584_m1	
<i>CDH1</i>	Hs_01023894_m1	
<i>TGFb</i>	Hs_00998133_m1	
<i>SNAIL1</i>	Hs_00195591_m1	
<i>MMP2</i>	Hs_01548727_m1	
<i>MMP9</i>	Hs_00234579_m1	
<i>SLUG</i>	Hs_00161904_m1	
<i>HPRT</i>	Hs_99999909_m1	

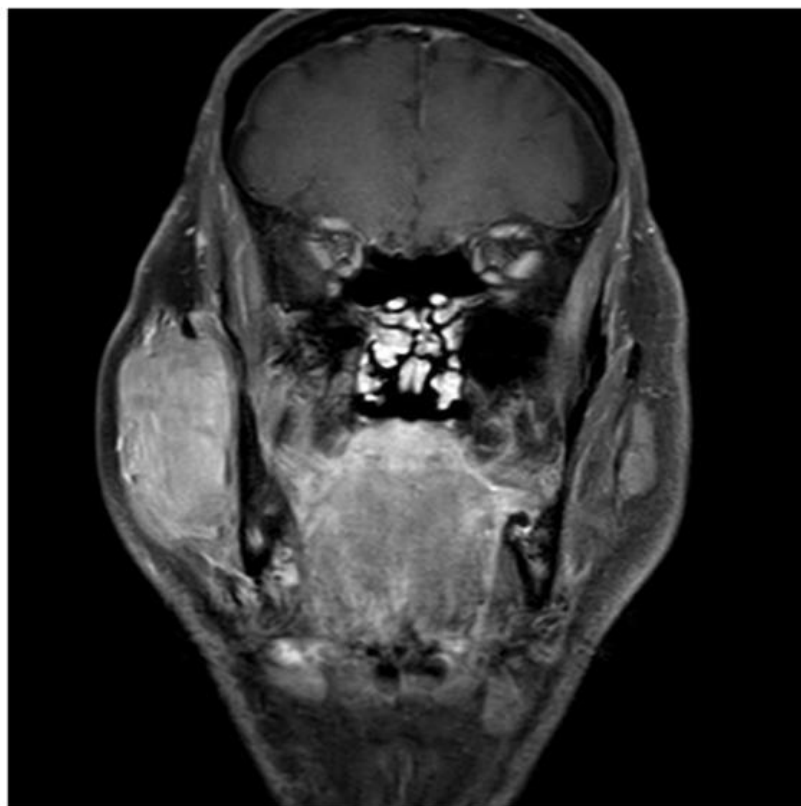
Table S2. Target gene analyzed for microsatellite instability (MSI) status.

Marker	Gene	NCBI Ref. Seq. (GRCh 38.p12)	Choromosom
BAT25	<i>cKIT</i>	NC_000004.12	4(4q12)
BAT26	<i>MSH2</i>	NC_000002.12	2(2p21-p16.3)
NR21	<i>SLC7A8</i>	NC_000014.9	4(14q11.2)
NR22	<i>STT3A</i>	NC_000011.10	11(11q24.2)
NR24	<i>ZNF2</i>	NC_000002.12	2(2q11.1)
NR27	<i>BIRC3</i>	NC_000011.10	11(11q22.2)
CAT25	<i>CASP2</i>	NC_000007.14	7(7q34)
MONO27	<i>MAP4K3</i>	NC_000002.12	2(2p22.1)

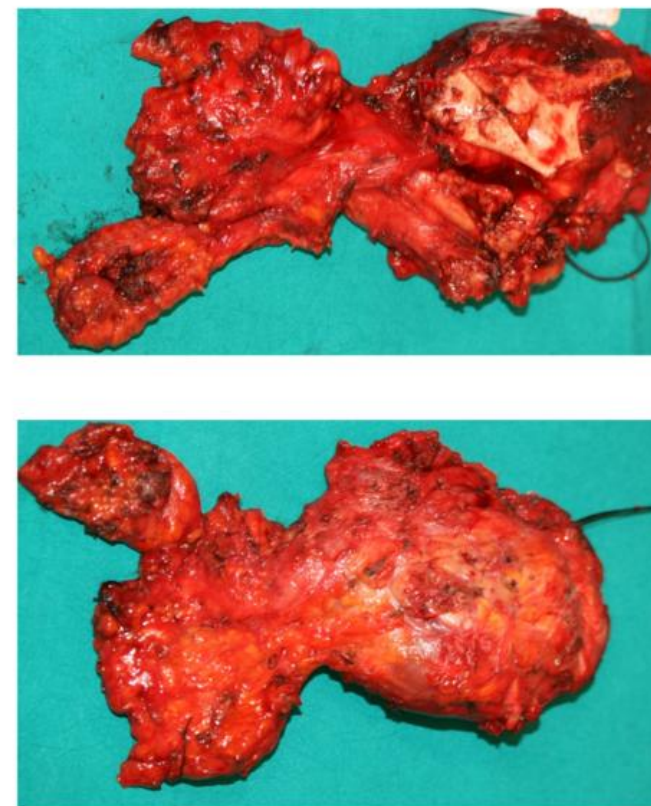
(a)
Patient



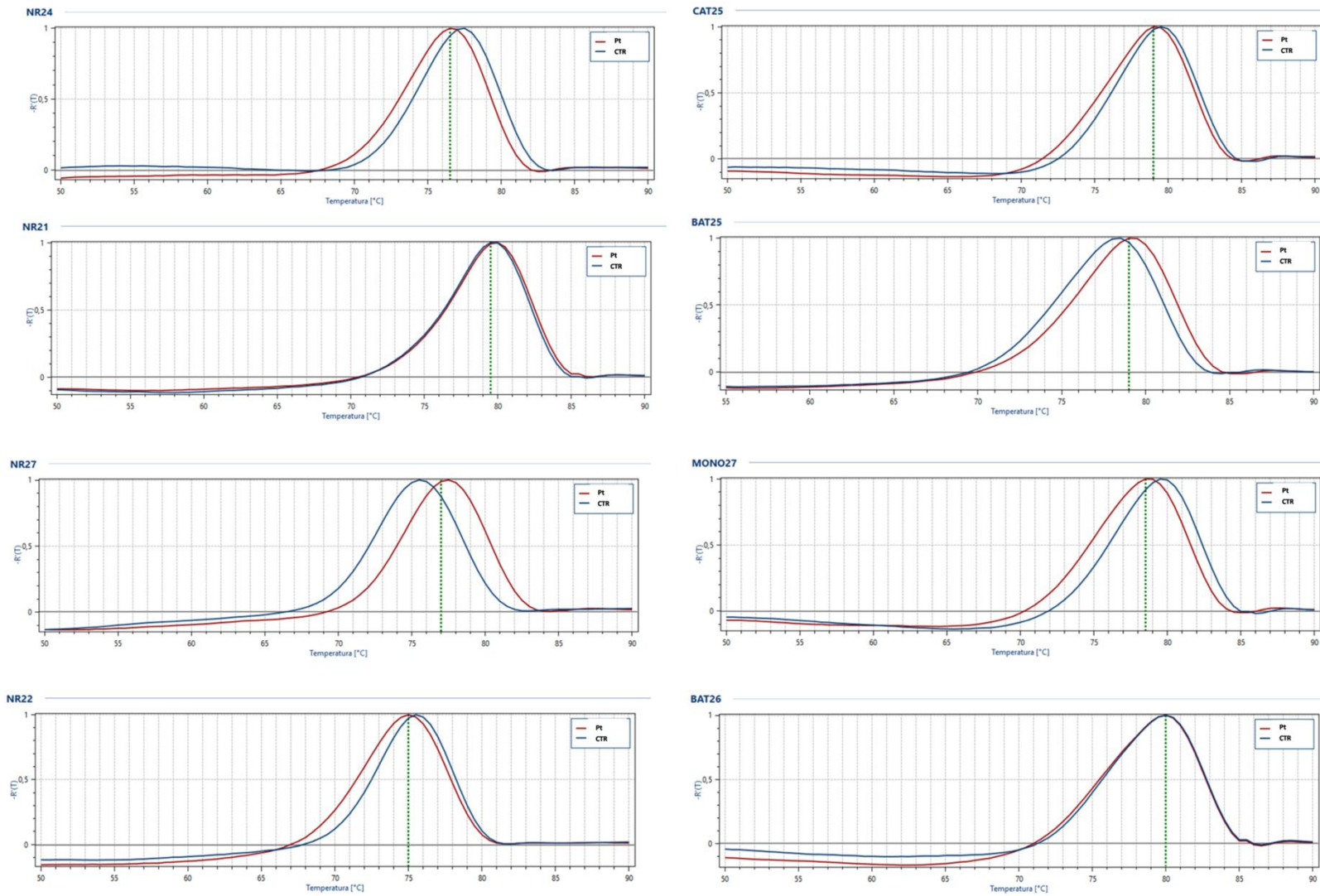
(b)
MRI



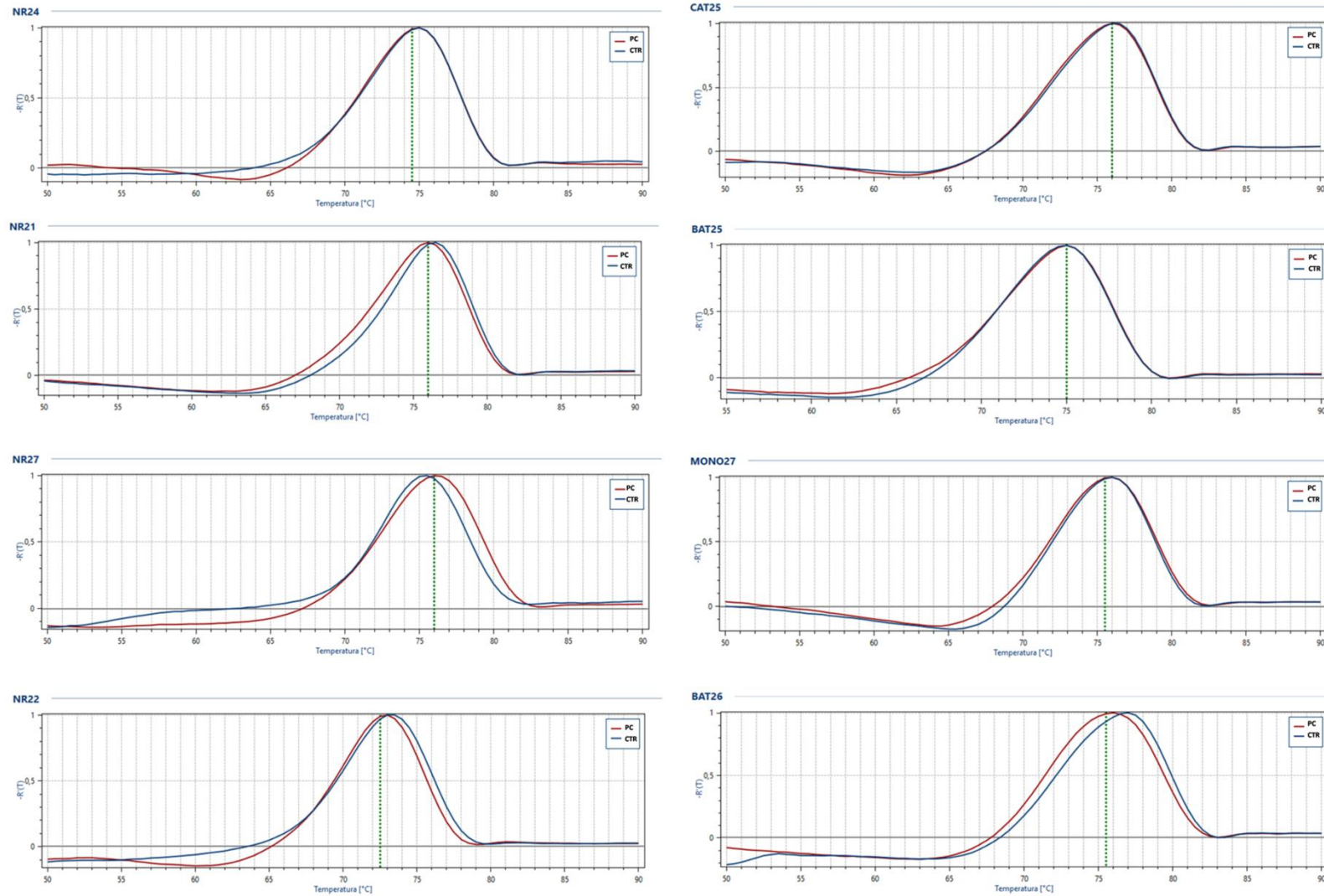
(c)
Lesion



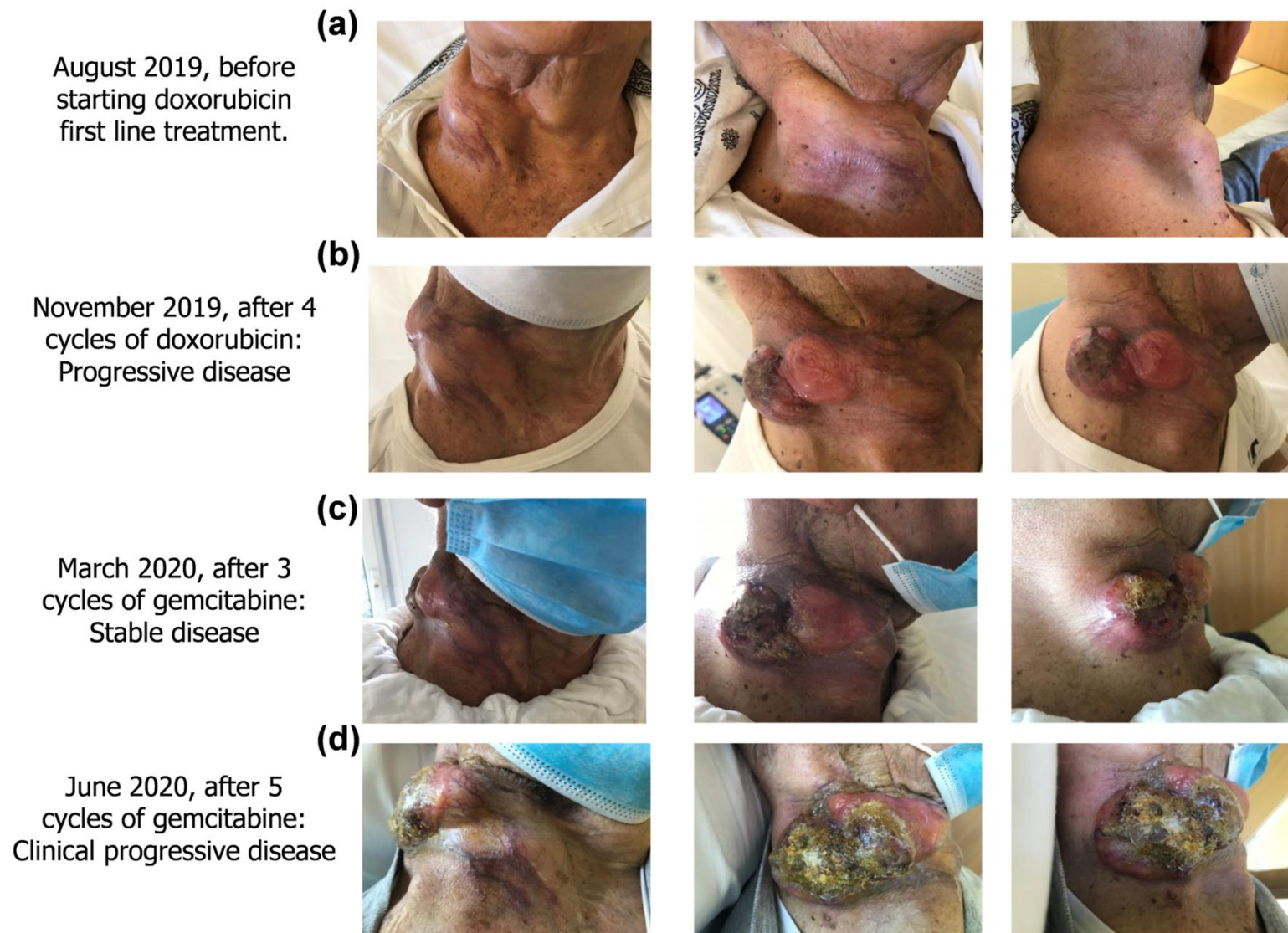
Supplementary Figure S1. (a) Frontal view of patient face. (b) Coronal MRI. (c) Lesion surgically excised.



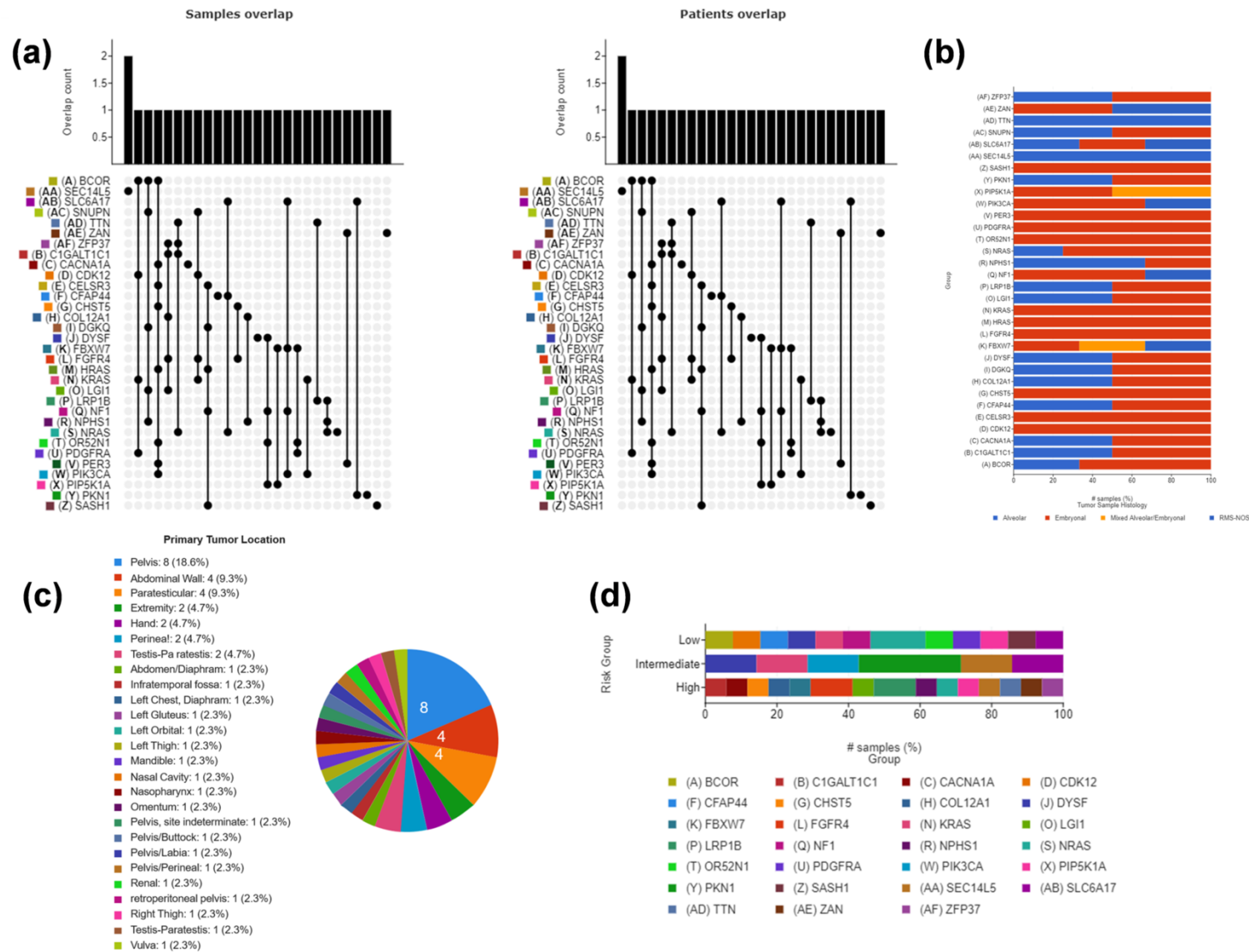
Supplementary Figure S2. Representative melt curves of BAT-25, BAT-26, NR-21, NR-22, NR-24, NR-27, CAT-25, MONO-27 (see complete list of gene in Supplementary Table S1) in the RMS patient tumor sample (Pt, red line) compared to the stable positive control (CTR, blue line).



Supplementary Figure S3. Representative melt curves of BAT-25, BAT-26, NR-21, NR-22, NR-24, NR-27, CAT-25, MONO-27 (see complete list of gene in Supplementary Table S1) in the RMS primary culture (PC, red line) compared to the stable positive control (CTR, blue line).



Supplementary Figure S4. Clinical and radiological evaluation of disease showing: **(a)** clinical and radiological presentation of local relapse three months after adjuvant radiotherapy. **(b)** clinical and radiological progression of disease after four cycles of doxorubicin. **(c)** clinical and radiological stable disease after 3 cycles of gemcitabine. **(d)** clinical progression of disease that led to treatment discontinuation.



Supplementary Figure S5. (a) In silico analysis among 43 RMS patient showing the overlapping among tumor and patient samples of the most mutated genes in case study. (b) Correlation between the most mutated genes and RMS tumor histology. (c) Primary tumor location in the in silico case study. (d) Correlation between the most mutated genes and RMS risk group.