Complete Response to Avelumab and IL-15 Superagonist N-803 with Abraxane in Merkel Cell Carcinoma: A Case Study

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SUPPLEMENTARY MATERIAL

Morphology and biomarker labeling in CNB

MCC and small cell lung cancer (SCLC) may be morphologically very similar, but is SCLC is typically negative for CK20 and positive for TTF-1; thus this case did not meet criteria for SCLC. As described in Kervarrec *et al* 2018 [1] MCC differs from lymph node metastasis from other neuroendocrine tumors by 7 discriminative criteria: elderly age, location of the tumor, extent of the disease, cytokeratin expression, TTF-1 expression, histologic type, and MCPyV detection (when positive). IHC data revealed frequent positivity for TTF-1 in MCC (and therefore this is not a definitive marker) and cytokeratin 7 (positive), either the absence or overexpression of p53, and frequent lack of neurofilament expression in virus-negative cases. By contrast, CK8, 18 and 20 and a CD99 with a dot pattern as well as high EMA expression are characteristic features of virus-positive MCC. In particular, the CD99 positivity is strongly

Drusbosky L, *et al. J Immunother Cancer* 2020; 8:e001098. doi: 10.1136/jitc-2020-001098

associated with MCPyV. CD99 was positive in the CNB here, but the tissue was negative for MCPyV.

Mutations and frequencies in the CNB

A total of 446 somatic variants were identified in the tumor, including three known pathogenic variants, four likely pathogenic variants, and 46 variants of unknown significance (**Supplementary Table 1**). Microsatellite status was stable (MSS), with the tumor sample measuring only 0.79% more unstable than normal tissue. No expression of the APOBEC/AID family of cytosine deaminases [2] and no mutations in the exonuclease domain of DNA polymerase (POLE) [3, 4] were detected in the tumor. The mutational signature was consistent with failure of double-strand break-repair by homologous recombination, caused by *BRCA1*/*BRCA2* mutations.[5]

Supplementary Table 1. *A subset of 53 variants in the CNB for the MCC patient*. Variant allele frequency (VAF).

Category	Gene	Variant	Class	VAF
Pathogenic	DMD	p.W1660C	Missense	0.2963
Pathogenic	FBXO11	p.P49Q	Missense	0.0104
Pathogenic	PABPC1	p.K312Nfs*10	Frame Shift	0.0595
Likely Path.	ZNF117	p.E189G	Missense	0.0106
Likely Path.	ZNF117	p.K168E	Missense	0.0145
Likely Path.	ZNF181	p.S287T	Missense	0.0088
Likely Path.	ZNF479	p.R295K	Missense	0.0115
Unkn. Sig.	ANK3	p.D839Ifs*32	Frame Shift	0.1395
Unkn. Sig.	ANTXR2	p.A357del	In-Frame Del.	0.0263
Unkn. Sig.	ARHGEF10	p.D215G	Missense	0.0130
Unkn. Sig.	ARMC3	c.733-1G>A	Splice Site	0.0879
Unkn. Sig.	ATP13A1	p.C515*	Nonsense	0.0422

Unkn. Sig.	ATXN2L	p.P84Q	Missense	0.0058
Unkn. Sig.	C15orf59	p.D177G	Missense	0.0065
Unkn. Sig.	CATSPER1	p.H250Sfs*13	Frame Shift	0.2665
Unkn. Sig.	CCDC64	p.R548M	Missense	0.0466
Unkn. Sig.	CFAP46	p.E1551*	Nonsense	0.0402
Unkn. Sig.	DEPDC5	p.R1425Q	Missense	0.0083
Unkn. Sig.	DHRS2	p.E83G	Missense	0.0052
Unkn. Sig.	DOCK5	p.D328Efs*4	Frame Shift	0.1046
Unkn. Sig.	FAM196B	p.C470*	Nonsense	0.2798
Unkn. Sig.	FLII	p.R623Vfs*32	Frame Shift	0.1181
Unkn. Sig.	FOXD1	p.R297Afs*169	Frame Shift	0.0140
Unkn. Sig.	GDF6	p.A401D	Missense	0.0510
Unkn. Sig.	GDF7	p.R51Gfs*43	Frame Shift	0.0291
Unkn. Sig.	IRS2	p.P1036del	In-Frame Del.	0.0204
Unkn. Sig.	ISLR2	p.E675G	Missense	0.0049
Unkn. Sig.	LRFN5	p.R52I	Missense	0.1353
Unkn. Sig.	MAFA	p.H207Pfs*229	Frame Shift	0.0256
Unkn. Sig.	MLLT3	p.S190del	In-Frame Del.	0.0064
Unkn. Sig.	MROH8	p.K31Tfs*1023	Frame Shift	0.0067
Unkn. Sig.	NANOS1	p.R283Pfs*69	Frame Shift	0.0049
Unkn. Sig.	NEFM	p.D197G	Missense	0.0628
Unkn. Sig.	NGFR	p.C128Wfs*93	Frame Shift	0.0037
Unkn. Sig.	NOL4	p.Q456K	Missense	0.1164
Unkn. Sig.	PABPC1	p.Q558E	Missense	0.0240
Unkn. Sig.	PHLDA1	p.Q203_Q204del	In-Frame Del.	0.0073
Unkn. Sig.	PLAC4	p.I82T	Missense	0.0165
Unkn. Sig.	PLAC4	p.L26_T27insV	In-Frame Ins.	0.0078
Unkn. Sig.	PLEKHG2	p.Q981Pfs*10	Frame Shift	0.1270
Unkn. Sig.	SDSL	p.E109G	Missense	0.0062
Unkn. Sig.	SLC16A3	p.F97Cfs*59	Frame Shift	0.0057
Unkn. Sig.	SYNE1	p.I8281T	Missense	0.0058
Unkn. Sig.	SYNM	p.R235Kfs*1332	Frame Shift	0.0056
Unkn. Sig.	TGFB1	p.P10del	In-Frame Del.	0.0113
Unkn. Sig.	UBR5	p.M2688L	Missense	0.2185
Unkn. Sig.	ZFHX4	p.P2057Tfs*82	Frame Shift	0.0140

Unkn. Sig.	ZNF493	p.E681G	Missense	0.0075
Unkn. Sig.	ZNF91	p.K1138E	Missense	0.0043
Unkn. Sig.	ZNF91	p.G1109E	Missense	0.0073
Unkn. Sig.	ZNF91	p.L853P	Missense	0.0086
Unkn. Sig.	ZNF91	p.T694A	Missense	0.0128
Unkn. Sig.	ZNF91	p.R333H	Missense	0.0066

Neoantigen prediction and expression

For this patient, neoepitopes predicted from the CNB sample did not reveal any for Class 2 MHC peptides. The Class 1 MHCs peptides were at low allele frequency and RNA read analysis did not provide evidence they were expressed. The one potential neoantigen that was present within the RNA appeared to be expressed in less than 2% of the tumor.

Expression analysis

The case was compared to a wide range of other cancers and MCC cases from TCGA RNA-Seq FASTQ files. Transcript quantification was computed as an average number of reads per base within each transcript. To assess gene expression, we obtained transcript per million (TPM) from RSEM output of RNA sequencing data. Per-gene expression was presented as a sum of TPM for all isoforms of a gene. All genes that have at least one isoform that begins with NM_ (mRNA RefSeq category) were quantified to compose the final expression matrix of protein coding genes. To allow uniform and interpretable comparison of expression levels across samples, we normalized all quantifications by rescaling to gene-wise TPM values in sample-wise manner, therefore each sample's TPMs sum up to 1 million.

Mapping TCGA data into the FFPE RNA-Seq space for the MCC case

We obtained public RNA sequencing data for 10,471 poly-A capture samples from the TCGA project for 33 different cancer types and mapped them into the space of the FFPE samples for the case represented here. We utilized quantile normalization 40 procedure, with FFPE samples'

expression quantiles as the target distribution; this quantile normalization procedure was performed separately for each gene. Zero expression values were excluded from both source and target datasets (per-gene) and attached those after the mapping was completed. In order to account for differences in cancer type composition between the two datasets, we implemented this quantile normalization in two steps. We first broke down TCGA dataset into two subsets: in the first, cancer type composition was matched (number of samples in each cancer type comprises at least the same percent of total samples as in the target FFPE dataset); and in the second, with the remaining samples that were not included into the first subset. We then quantile normalize the first subset as described above. The second subset was normalized using quantiles of the first subset as a source.

SUPPLEMENTARY REFERENCES

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