impact genetics inc.
advancing genetic diagnostics

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Patient name:	--	**Referring specialist:**	--
		Contact person:	--
Date of birth:	--		
Medical record #:	--	**Institution:**	--
			Address:
Patient history:	*Uveal melanoma*		
			Fax:
Date of report:	--		
Patient #:	--	**Copies to:**	*Name*
			Fax:

Test requested: *Uveal melanoma screen for prognostic genomic copy number changes*

Test results summary (actual test values page 2)

TCGA Classification[21]	D
Chromosome 3	Monosomy
Chromosome 8	8q multiple gain, 8p loss
Chromosome 6	Disomy
Chromosome 1p	Disomy
GNAQ exon 5	Sequencing not performed
GNA11 exon 5	Sequencing not performed
SF3B1 exon 14	Sequencing not performed
EIF1AX exon 1 & 2	Sequencing not performed

Survivorship prediction

When combining this patient's tumor genetics with the provided clinical and histomorphological data, multivariant analysis predicts the following:

Liverpool Uveal Melanoma Prognosticator Online (LUMPO) III [11, 22]

Survival	**Year 3**	**Year 5**	**Year 10**
all causes	79%	63%	36%
Mortality	**Year 3**	**Year 5**	**Year 10**
Metastasis	13%	24%	37%
Other causes	8%	13%	27%

Interpretation

- Survivorship percentages were calculated for this patient, based on this genetic result for chromosome 3 and 8q, as well as the demographics provided, using the multicenter externally validated algorithm[11,22]. For additional information please see the appended 'Liverpool Uveal Melanoma Prognosticator Online (LUMPO) III' report. .
- Monosomy 3 with 8p loss and 8q multiple gains is strongly associated with metastasis, poor survival prognosis, and a high disease-specific mortality.

On direction of the referring physician, patients can speak with a certified genetic counsellor to review the results of this report. Book online at impactgenetics.com/genetic-counseling **or by calling 1-855-422-2557.**

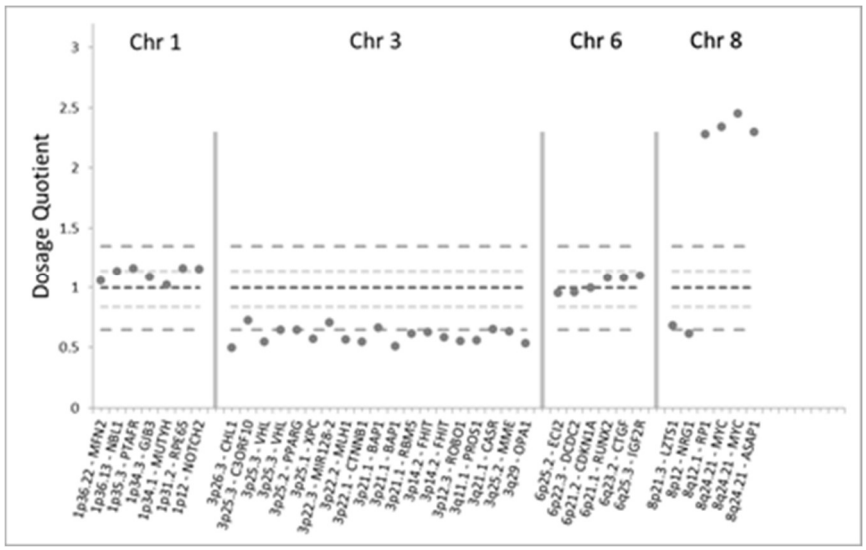
These results and the interpretation, including guidance and supplemental information, were reviewed and approved by:

Electronically signed by Hilary Racher, PHD FCCMG, Laboratory Director, on <DATE> at <TIME>
Electronically signed by Brenda Gallie, MD FRCSC, Medical Director, on <DATE> at <TIME>

Test values

MLPA results

Tumor sample # ##-####



Interpretation

DQ= Dosage Quotient
DQ<0.65 = Loss
DQ 0.65-0.84 = Borderline loss
DQ 0.85-1.14 = Normal
DQ 1.15-1.35 = Borderline gain
DQ 1.35 = Gain

Microsatellite analysis results

Tumor sample #			##-####	
Marker	**Chr 3 position (Mb)**	**Locus**	**AIR**	**Result**
D3S3050	3.3	3p26	7.12	LOH
D3S1263	11.5	3p25.3	9.33	LOH
D3S1481	60.6	3p14.2	9.24	LOH
D3S2406	73.3	3p13	6.19	LOH
D3S3045	108	3q13.12	8.32	LOH
D3S1744	148	3q25	ni	NI
D3S2421	176	3q26.3	9.49	LOH
D3S1311	198.5	3q29	5.89	LOH

LOH: Loss of heterozygosity
AIR: Allele Imbalance Ratio
AI: Allelic Imbalance
NI: Not Informative
NA: Not Applicable

Interpretation
AIR<1.3 = no LOH
AIR>2.5 = LOH
AIR 1.3-2.5 = AI

Histology results (available to our lab at the time of this report)

Age, Sex	*79 yrs, M*	Predominant cellular classification	*Epitheloid*
Years since treatment	*0-1*	Epitheloid cells present	*Yes*
Largest basal diameter (LBD) (mm)	*22 mm*	Closed loops	*Yes*
Tumor thickness (mm)	*16 mm*	Mitotic count	*31 per HPF*
Anatomic sub-classification	*Ciliary body involvement*	Necrosis	*Not available*
Ciliary body involvement	*Yes*	AJCC TNM Stage	*Stage IIB*
Extraocular extension	*No*		

Details of Samples Tested

Patient Sample	Sample #	Collected	Received	Authorized/Test Started
Buccal	*##-####*	*MMM DD, YYYY*	*MMM DD, YYYY*	*MMM DD, YYYY*
Tumor	*##-####*	*MMM DD, YYYY*	*MMM DD, YYYY*	*MMM DD, YYYY*

Supplemental Information

Test Methods (not all samples employ every method)**:**

- Multiplex ligation-dependent probe amplification, (MLPA) SALSA PO27.C2 Uveal Melanoma kit (MRC Holland) contains probes for 1p, 3, 6, and 8 to detect genomic copy number changes in representative regions of these chromosomes. Impact Genetics accepts the results of an MLPA run if ≥6 control probes are within the normal range and if the SD is <0.2.
- Analysis of 8 microsatellite markers (MSA) located along the length of chromosome 3 [1,5] is used to detect loss of a copy or loss of heterozygosity on chromosome 3. Loss of heterozygosity (isodisomy) occurs when a portion of a chromosome is lost, and the other copy (possibly defective) is re-duplicated to produce two identical copies. MSA can detect isodisomy (present in ~6% of UM[4]), which is believed to be functionally equivalent to monosomy 3, and is associated with a high risk of metastatic disease.
- In order to provide confirmation that tumor was sampled, in cases with disomy 3 and no chromosomal gains or losses by MLPA, sequencing of *EIF1AX* (NM_001412.3) exon 1 and 2, *SF3B1* (NM_012433.2) exon 14 and exon 5 in *GNAQ* (NM_002072.4) and *GNA11* (NM_002067.4) is performed to identify recurrent UM tumor variants; variants in *GNAQ* and *GNA11* occur in 83-92% of UM tumors[12,13], while variants in *EIF1AX* and *SF3B1* occur less frequently and have been reported in approximately 26-40% of cases[16]. Variants are described using HGVS (v15.11) guidelines. For coding DNA sequences, the A of the ATG initiator codon is denoted as nucleotide 1.

Notes on Interpretation of Results:

- About 50% of patients with uveal melanoma (UM) die of the disease usually, as a result of liver metastases.
- **Chromosome 3 loss** found in approximately 50% of UM is strongly associated with (liver) metastases, poor prognosis, and a high disease-specific mortality. In one study, monosomy 3 correlated with a reduction of 5-year survival from almost 100% to less than 50%[3].
- Tumors with **Disomy 3** rarely progress to metastatic disease; the few metastasizing Disomy 3 tumors showed larger basal tumor diameter (>15 mm) and were more frequently of mixed or epithelioid cell types[5].
- **Chromosome 8q gain or amplification** occurs in about 40% of UMs and is associated with metastasis when found with or without monosomy 3 but has a worse prognosis when occurring together with chromosome 3 loss. One study reported a ten-year mortality of 0% for 133 tumors with disomy 3, 55% for tumors with monosomy 3 but no 8q gain, and 71% for tumors with both chromosome 3 loss and 8q gain[7].
- **Chromosome 6p gain** and isochromosome 6p occur preferentially in tumors with disomy 3, and appear protective. When both 6p gain and 3 loss occur together, the survival time is longer than in patients whose tumor shows only chromosome 3 loss[7].
- If chromosome 3 loss is present, chromosome **1p loss** may correlate with decreased disease-free survival.
- **Partial loss** means that only some but not all tested loci on a particular chromosome are abnormal. At present, there is mixed evidence regarding the clinical significance of a partial loss of chromosome 3 (partial monosomy 3); metastatic disease has been observed is some cases[7], while other studies show an association with partial monosomy 3 and good prognosis[19].
- **Borderline abnormality by MLPA:** the dosage quotient has a value of between 0.65 and 0.84 for loss, or between 1.15 and 1.35 for gain. Borderline chromosome 3 loss and borderline chromosome 8q gain are attributed to tumor heterogeneity, and imply poor prognosis.
- When the tumor shows **chromosome 3 loss**, the time to metastatic death shortens with **increasing basal tumor diameter** and with **higher histological grade**, as indicated by presence of epithelioid cells, closed loops and higher mitotic count. In the absence of chromosome 3 loss, high histological grade may increase suspicion that the MLPA has missed detection of chromosome 3 loss.
- Fresh or flash frozen tumor samples in cell lysis buffer are recommended.

Analytic Sensitivity: MLPA and MSA can detect chromosome 3 loss if monosomy 3 is present in at least 40% of cells of a heterogeneous sample.

UM TNM Staging and survivorship disclaimer: A printout from the multicenter externally validated Uveal Melanoma TNM staging and survivorship algorithm, Liverpool Uveal Melanoma Prognosticator Online (LUMPO) III (https://mpcetoolsforhealth.liverpool.ac.uk/matsoap/lumpo3cr.htm), using this patient's genetic result from chromosome 3 and 8q, as well as demographics provided, has been included as an additional prognostication tool and should not be used as a replacement to sound clinical judgement. Note: this algorithm was initially created using data compiled from mainland Britain UM patients and may not accurately reflect the diagnostic and treatment practices for UM patients in other countries. However, a multicenter external validation was conducted and showed an agreement between observed and predicted survival probabilities[22]. Permission has been granted to include the Uveal Melanoma TNM staging and survivorship report with the Impact Genetics report. Impact Genetics takes no responsibility for these survival predictions.

General disclaimer: This test was developed and its performance characteristics determined by Impact Genetics Inc. (Ontario, Canada), a wholly- owned subsidiary of Dynacare Gamma Laboratory Partnership, a subsidiary of Laboratory Corporation of America Holdings. This test has not been cleared or approved by the Food and Drug Administration or by the Therapeutic Products Directorate (TPD) of Health Canada. Each of Impact Genetics' molecular tests use a direct method of variant detection and analysis is based on current knowledge of the genes. For small tumors, and FNABs showing disomy 3, there is a possibility of sampling error, which may occur when cells with disomy 3 are mixed with tumor cells with monosomy 3. Such heterogeneity is estimated to occur in 14-18% of uveal melanomas.

Additional Information regarding the test performed and uveal melanoma is available at impactgenetics.com.

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