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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*

Summary

The patient's tumor shows disomy 3. *GNAQ* sequencing confirms tumor was studied. When combined with the provided clinical and histomorphological data, multivariant analysis predicts the following:

Uveal Melanoma Survivorship Prediction[10, 11]

Survival	Year 3	Year 5	Year 10
Control*	97%	94%	85%
Patient	98%	77%	50%

*age and sex matched general population control group

Test results (actual test values attached)

- MLPA: Our lab tested a sample from the patient's uveal melanoma using the SALSA PO27.C1 Uveal Melanoma kit (MRC Holland) and determined that the patient's sample showed:
  - Chr 1p: disomy
  - Chr 3: disomy
  - Chr 6: disomy
  - Chr 8: disomy
- MSA: Our microsatellite assay (MSA) showed no loss of heterozygosity at any of the informative chromosome 3 loci tested, indicating a normal 2-copy result for chromosome 3.

Interpretation

- Survivorship percentages were calculated for this patient, based on this genetic result for chromosome 3 and the demographics provided, using the validated algorithm[10,11]. For additional information please see the appended 'Uveal Melanoma TNM Staging and Survivorship' report.
- Tumors with disomy 3 rarely progress to metastatic disease; the few metastasizing disomy 3 tumors showed larger basal tumor diameter (>15 mm) and mixed or epithelioid cell types[5].
- ***GNA11* exon 5 was sequenced for this patient's tumor, and showed the Q209L mutation, commonly seen in uveal melanoma tumors, confirming that tumor tissue was sampled**. There is still a possibility of tumor heterogeneity, with a small population of undetected tumor cells with monosomy 3.

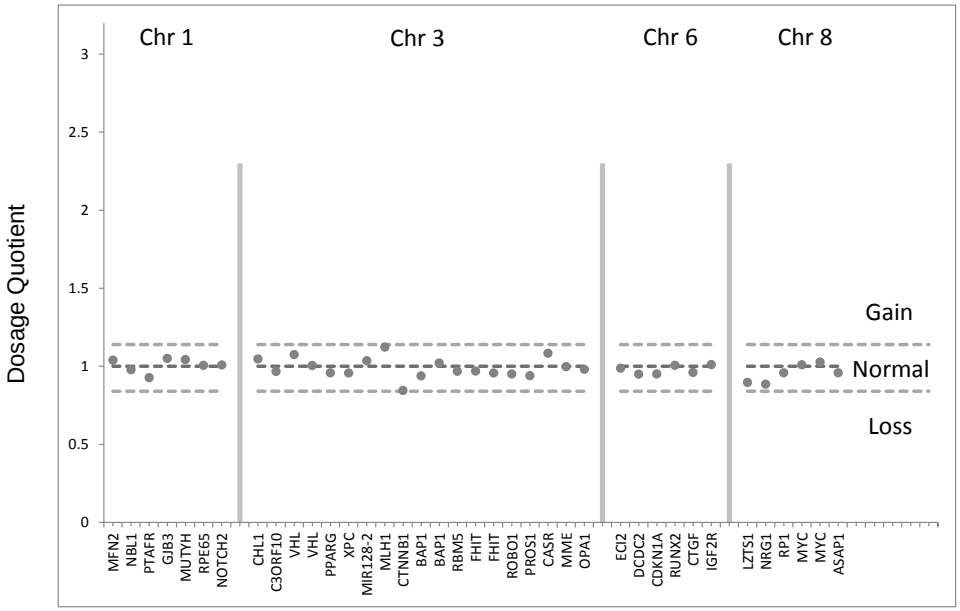
These results and the interpretation, including guidance and supplemental information, were reviewed and approved by:

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	*NI*	*NI*
D3S1263	11.5	3p25.3	1.03	Normal
D3S1481	60.6	3p14.2	1.30	Normal
D3S2406	73.3	3p13	1.00	Normal
D3S3045	108	3q13.12	1.18	Normal
D3S1744	148	3q25	1.17	Normal
D3S2421	176	3q26.3	1.26	Normal
D3S1311	198.5	3q29	1.26	Normal

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	*65, Female*	Predominant cellular classification	*Epitheloid*
Years since treatment	*0-1*	Epitheloid cells present	*Yes*
Largest basal diameter (LBD) (mm)	*17 mm*	Closed loops	*Yes*
Tumor thickness (mm)	*12 mm*	Mitotic count	*4 per HPF*
Anatomic sub-classification	*No*	Necrosis	*Not available*
Ciliary body involvement	*No*	AJCC TNM Stage	*Stage IIB*
Extraocular extension	*No*		

Details of Samples Tested

Patient Sample	Sample #	Collected	Received	Autorized/Test Started
Buccal	*##-####*	*MMM DD, YYYY*	*MMM DD, YYYY*	*MMM DD, YYYY*
Fresh 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.C1 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 mutations; mutations in *GNAQ* and *GNA11* occur in 83-92% of UM tumors[12,13], while mutations in *EIF1AX* and *SF3B1* occur less frequently and have been reported in approximately 20% of cases[16]. Mutations 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 validated Uveal Melanoma TNM staging and survivorship algorithm, Liverpool Uveal Melanoma Prognosticator Online (LUMPO: ocularmelanomaonline.org) using this patient's genetic result and 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 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, external validation with a cohort of patient's treated at the University of California – San Francisco revealed similar survival predictions suggesting that this tool may be applicable to other patient populations[20]. In situations where the presence of epithelioid cells in the tumor is not known/provided, we will provide a survivorship range by assessing both in the presence and absence of epithelioid cells. 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. It has not been cleared or approved by the Food and Drug Administration. Each of Impact Genetics' molecular tests use a direct method of mutation 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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