**Prognostic biopsy of choroidal melanoma after proton beam radiation therapy.**

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No conflicting relationship exists for any authors.Choroidal melanoma is fatal in almost 50% of patients (1). A large majority of patients with this cancer want to know their prognosis, even if there is no effective treatment for metastatic disease (1). Those with a good prognosis are reassured. Special measures, such as more intensive surveillance, can be targeted at high-risk patients, who may ultimately either undergo liver surgery or be given systemic or localized hepatic chemotherapy (2).

Metastatic disease develops almost exclusively in patients whose tumor shows chromosome 3 loss, with/without changes in chromosome 8q, and/or a class 2 gene expression profile (1). Genetic tumor analysis greatly enhances estimation of survival probability, especially if clinical and histologic predictors are included in the multivariate analysis (1). Most patients are treated with proton beam radiotherapy or plaque brachytherapy, so that biopsy is required for prognostication. Complications, such as vitreous hemorrhage can complicate the radiotherapy treatment; furthermore, there are concerns that the biopsy might seed the tumor to other parts of the eye and extraocularly. For these reasons, for several years we have offered patients prognostic tumor biopsy after radiotherapy. The aim of this study was to correlate metastatic mortality with multiplex ligation-dependent probe amplification (MLPA) or microsatellite analysis (MSA) performed on choroidal melanoma biopsy samples obtained *soon after* *completion* of PBR.

 Patients were included if they resided in the UK and had a successful transretinal biopsy of choroidal melanoma within a month of PBR completion. The National Health Service Cancer Registry is populated with every patient with a staged tumor diagnosis; the registry is commissioned to report to the referring oncology unit at the time of death, along with the death certificate issued by local physicians; it is from this that survival data were obtained.

Proton beam radiotherapy (56Gy over four consecutive days) was administered at the Clatterbridge Cancer Center, located 14 Km from the Liverpool Ocular Oncology Center, where all ophthalmic care was delivered. Biopsy was performed as soon as possible after PBR. Tumor samples were obtained with a 25-gauge vitreous cutter (2), the cytospin stained with May Grunewald Giemsa (MGG) and assessed histologically by an experienced ophthalmic pathologist (SEC) for cell-content and type. DNA extraction, DNA quality assessment and quantification and identification of chromosome aberrations by MLPA or MSA were performed as described previously (3). This study was conducted in accordance with the tenets of the Declaration of Helsinki and Good Clinical Practice Guidelines. The service evaluation was approved by the Royal Liverpool and Broadgreen University Hospital Trust (RLBUHT) (Reference number: TA0517).

The study cohort included 102 patients, who comprised 47 females and 55 males with a mean age of 57.3 yrs (range 25-82) (Supplemental Table 1). The tumors had a mean largest basal diameter 12.0 mm (range 5.4-19.3) and a median tumor thickness of 3.5mm (range 0.9-10.3). Twenty-four tumors involved the ciliary body and eight extended to anterior chamber and angle. Tumor biopsy was performed on the last day of PBR treatment in 70 patients (69%), after 1-7 days in 28 patients, and 8-20 days in four patients. Diagnosis of melanoma was confirmed cytologically in all cases (Supplemental Figure 1 ). Genetic analysis was performed by MLPA in 74 patients and MSA in 28 cases. Chromosomal analysis demonstrated monosomy 3 in 39 (38%) and disomy 3 in 63 (62%). Other chromosomal alterations included chr.6p gain in 49%, chr.8q gain in 40%, and chr.1p loss in 16%. The median follow-up was 3.6 years (range 0.3–8.6). By study close, 12 patients died (11.8%), nine from metastatic disease. Actuarial rates of metastatic death at 7 years were 0% in disomy 3 patients and 35% in monosomy 3 patients (Figure 1).

To our knowledge, this is the largest study yet performed on genetic typing of choroidal melanoma samples obtained after radiotherapy. We previouslycompared pre- and post-radiotherapy MLPA/MSA data from the same tumors in four patients, showing concordance in all tumors (3). Similarly, array CGH of five tumors pre- and post-radiotherapy showed no significant change in chromosome 3 status because of treatment (4). Dogrusoz *et al* performed karyotyping and/or fluorescence in-situ hybridization in 36 enucleated eyes with previously irradiated choroidal melanoma and found frequent, complex and extensive chromosomal abnormalities in irradiated tumors (5). Their genetic studies were performed many months after radiotherapy, when tumors had developed necrosis and inflammation and when clonal expansion of any surviving melanoma cells may have occurred; therefore, their results *cannot* be extrapolated to our tumors, which were biopsied within a month of PBR.

There is scope for further studies with larger sample sizes and longer follow-up, after brachytherapy and other forms of radiotherapy and evaluating gene expression profiling and other methods of genetic tumor analysis. There is also a need for evaluating the reliability of genetic tumor analysis months and years after radiotherapy when results may be influenced by necrosis and/or clonal expansion. We expect that the random genetic alterations caused by radiation should not result in detectable monosomy 3. Nevertheless, we investigated this possibility by multivariate analysis, which excluded prior-radiation as an independent factor associated with chromosome 3 loss (*unpublished data*). We plan to publish this study soon.

In conclusion, genetic analysis of choroidal melanoma by MLPA or MSA following completion of PBR distinguishes between disomy 3 and monosomy 3 tumors and produces results that are predictive of metastasis-free survival.

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**Legends**:

Figure 1: Metastatic mortality according to chromosome 3 status.

Supplemental Table 1: Baseline patient and tumor characteristics of the examined cohort.

Supplemental Figure 1: Cytospin of samples stained with MGG