Translating Uveal Melanoma Cytogenetics Into Clinical Care

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Objective: To report our experience in translating uveal melanoma cytogenetics to routine clinical practice.

Methods: In 1998, we confirmed that monosomy 3 in uveal melanomas correlates with mortality. In 1999, we started offering all patients treated by enucleation or local resection the possibility of monosomy 3 testing, using fluorescence in situ hybridization. In 2006, we started analyzing tumors with multiplex ligation-dependent probe amplification, which provided more information from smaller samples. In 2007, we extended this service to patients undergoing radiotherapy or phototherapy.

Results: Initial expectations regarding the sensitivity and specificity of monosomy 3 tests in predicting metastatic death were found to be overoptimistic. Cytogenetic classification of uveal melanomas into "lethal" and "nonlethal" types was insufficient for estimating risk of metastasis. Prognostication also required consideration of (1) clinical tumor stage; (2) histologic grading of malignancy; and (3) influence of age and sex on life expectancy. We developed a neural network for such multivariate analysis. Metastatic deaths occurred without monosomy 3, possibly because small deletions were missed or because of tumor heterogeneity.

Conclusion: Genomic typing of uveal melanomas proved more complex than we had anticipated but enabled us to reassure patients with a good prognosis while targeting systemic screening at high-risk cases.

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ABOUT 50% OF PATIENTS with uveal melanoma die of their disease, usually as a result of hematogenous metastatic spread to the liver.[1] This outcome occurs despite apparently successful treatment of the primary ocular tumor, raising questions about the natural history of the disease and how it is influenced by treatment, if at all. Such lack of knowledge has limited our ability to interpret survival data and has obliged us to base treatment on speculation.[2]

In the 1990s, it was discovered that uveal melanomas tend to develop several chromosomal abnormalities, the most important of which are monosomy 3, isochromosome 6p, trisomy 8, and isochromosome 8q.[3,4] A landmark article by Prescher et al[5] showed monosomy 3 to correlate strongly with metastatic death, data indicating a reduction in the 5-year survival from 100% to less than 50%. Such dramatic results prompted us to start performing research into uveal melanoma cytogenetics for the purposes of individualizing ocular treatment, screening for metastasis, and, eventually, administering systemic adjuvant therapy according to the lethality of the disease.

Initially, we naively believed that it would be a simple matter to type uveal melanomas according to their chromosome 3 status and to manage patients accordingly. In practice, the deployment of uveal melanoma cytogenetics in the clinical arena proved more difficult than expected because of technical difficulties, problems harvesting tumor samples, psychological concerns, and ethical dilemmas.

In this article, we review our experience in translating uveal melanoma cytogenetics into clinical practice, highlighting the hurdles and discussing the benefits that have accrued.

METHODS

BASIC RESEARCH

Our initial priority was to validate the Prescher et al results. Using microsatellite analysis, we confirmed in 105 cases that monosomy 3 was a strong predictor of metastatic death (log-rank, $P<.001$) (**Figure 1**).[6] Although this chromosomal abnormality correlated strongly with largest basal tumor diameter (Mann-Whitney, $P=.002$) and presence of epithelioid cells (χ^2 test, $P<.001$), it was not

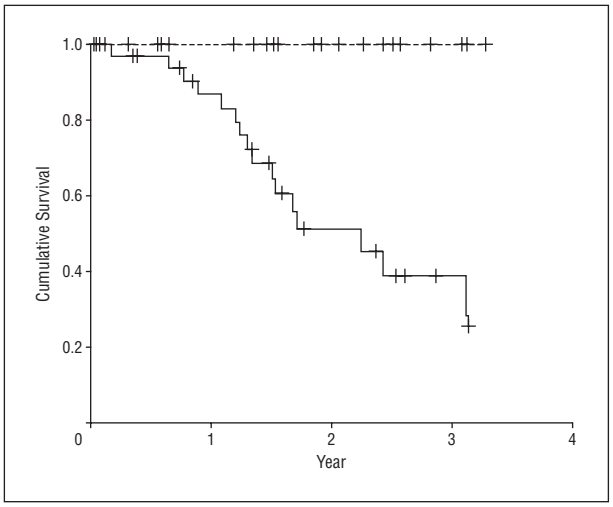


Figure 1. Kaplan-Meier curves showing actuarial rates of metastasis-related death according to presence of monosomy 3. Absence and presence of the adverse factor are represented by dashed and solid lines, respectively. The ticks represent censored patients. Reproduced from Scholes et al.[6]

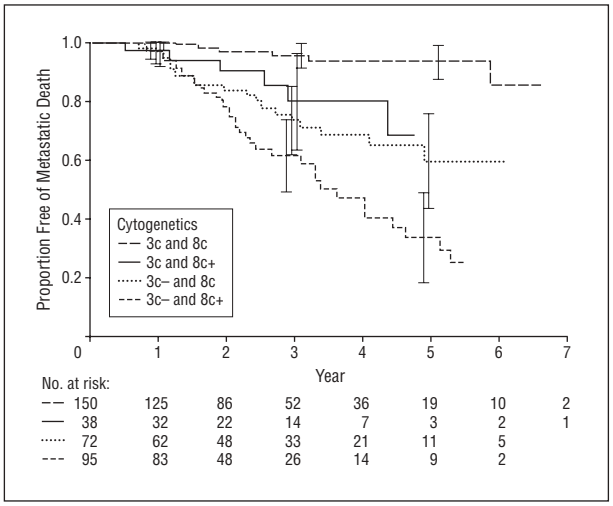


Figure 2. Kaplan-Meier survival curves showing disease-specific mortality according to combined chromosome 3 and chromosome 8 status. Reproduced from Damato et al.[9]

possible to predict the presence of monosomy 3 according to these factors in most cases.[6] Cytogenetic analysis of chromosome 3 was therefore essential for accurate prognostication.

INTRODUCING FLUORESCENCE IN SITU HYBRIDIZATION TO CLINICAL PRACTICE

In 1999, we felt there was enough evidence to justify clinical application of cytogenetic analysis of uveal melanomas, even though, to our knowledge, this had not been done previously. The National Health Service agreed to fund cytogenetic studies when it was persuaded that a single laboratory test would be cost-effective in the long run by preventing patients with "nonlethal" uveal melanoma from having 6-monthly liver imaging studies for many years.

Because monosomy 3 indicated almost inevitable death from metastatic disease, we were concerned that patients might be harmed by receiving such a bad prognosis. We therefore modeled our practice on human immunodeficiency virus testing, developing a protocol consisting of counseling the patient (1) before obtaining informed consent for cytogenetic testing; (2) before revealing the result of the test, also confirming that the patient still wanted to know the result; and (3) providing advice and support after informing the patient of the prognosis. The large majority of patients decided without hesitation that they wanted to know their prognosis, with only a few declining.

Most patients lived a long distance from our hospital so that it was difficult for them to travel all the way to our clinic just to be informed of the result. We arranged for an oncologist at Clatterbridge Centre for Oncology, near Liverpool, England, to invite by mail all patients with monosomy 3 to his clinic to (1) be informed of their prognosis; (2) receive counseling; and (3) be offered 6-monthly hepatic imaging and liver function tests at his hospital. Patients who were unable to attend our oncologist's clinic were referred by him to an oncologist at their local hospital, who was provided with an information pack. Patients with a disomy 3 melanoma were informed of this result by one of us (B.D.) directly by letter, copies of which were mailed to their referring ophthalmologist and general practitioner.

Because we were uncertain of the risks and benefits of cytogenetic testing, we initially confined this analysis to patients whose tumor was being removed for therapeutic purposes, that is, by enucleation, transscleral local resection, or endoresection.

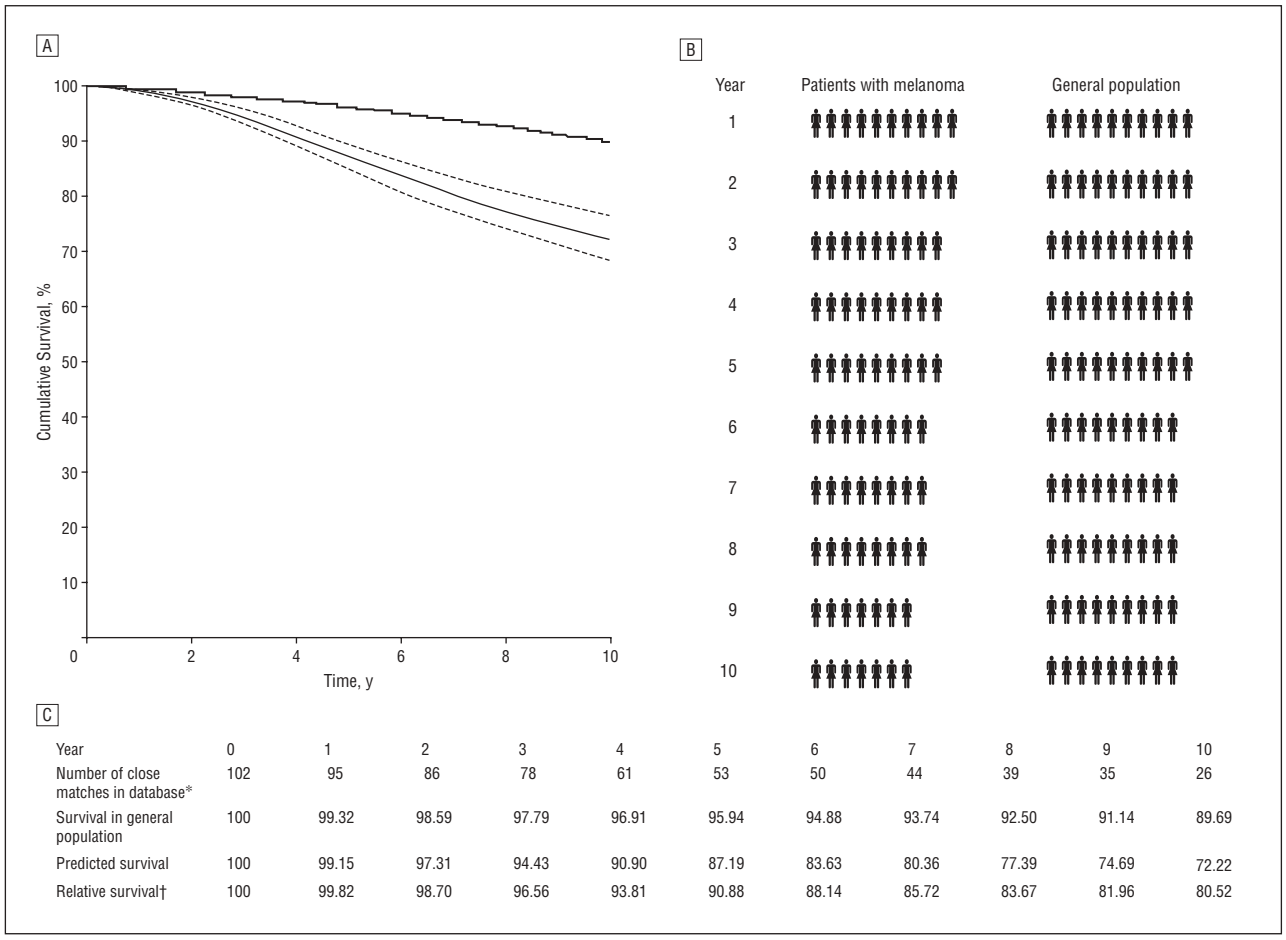
LIMITATIONS OF FLUORESCENCE IN SITU HYBRIDIZATION

Initially, we performed fluorescence in situ hybridization (FISH) using only a single centromeric probe for chromosome 3. Some published literature suggested that both chromosome 3 loss and chromosome 8 gain were required for uveal melanomas to have metastatic potential.[7] This prompted us to perform FISH on chromosome 8q as well as chromosome 3. Several studies and our own unpublished data indicated that chromosome 6p abnormalities were associated with a good prognosis[7,8]; however, we were unable to find a suitable probe for FISH until 2006.

Our outcomes analyses eventually revealed that in a cohort of 356 patients, between 5% and 20% of patients with no apparent monosomy 3 died of metastasis within 4 years of treatment, according to whether chromosome 8q gains were present (**Figure 2**).[9] Published studies suggested that death was related to partial deletions of chromosome 3, which were missed by FISH (Figure 2).[10,11] Our study showed that cytogenetic testing for monosomy 3 alone was insufficient and it was also necessary to take account of largest basal tumor diameter and tumor cell type (ie, presence or absence of epithelioid cells).[9] In that study, chromosome 8 gains were excluded by multivariate Cox analysis.[9]

MULTIVARIATE ANALYSIS

We developed neural networks predicting all-cause mortality according to age, sex, largest basal tumor diameter, ciliary body involvement, extraocular spread, presence of epithelioid cells, closed connective tissue loops, mitotic rate, and monosomy 3.[12] This tool was validated with a data set that was distinct from the one used for its training. Survival curves were generated both for patients with uveal melanoma and the age- and sex-matched British population, using census data (**Figure 3**). This enabled the risk of melanoma-related death to be calculated (by subtracting mortality in the general population from that of the patient population). We considered this to be preferable to relying on death certificates, which are known to be inaccurate. Insights from our neural network studies confirmed to us that prognostication was a 3-step process involving (1) genomic tumor typing to determine whether the melanoma had any metastatic potential; (2) clini-



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Year	0	1	2	3	4	5	6	7	8	9	10
Number of close matches in database*	102	95	86	78	61	53	50	44	39	35	26
Survival in general population	100	99.32	98.59	97.79	96.91	95.94	94.88	93.74	92.50	91.14	89.69
Predicted survival	100	99.15	97.31	94.43	90.90	87.19	83.63	80.36	77.39	74.69	72.22
Relative survival†	100	99.82	98.70	96.56	93.81	90.88	88.14	85.72	83.67	81.96	80.52

Figure 3. A, Curves showing 10-year all-cause survival of a 60-year-old woman with a low-grade 13-mm choroidal melanoma (thin solid line) as compared with survival of the age-matched female population in Britain (thick solid line). Dashed lines represent the 95% credibility interval. B, Pictogram showing survival of patients with choroidal melanoma and of the matched British population. C, Number of close matches in database, survival in general population, survival in patients with melanoma, and relative survival. *Close matches are determined by grouping age to the nearest decade and largest tumor diameter into less than 10, 10 to 15, and more than 15 mm categories. †Calculated as (predicted from patient population)/(observed from general population). Reproduced from Damato et al.[12]

cal staging, based mostly on basal tumor diameter, to adjust for lead-time bias; and (3) histological grading of malignancy to take account of the rate of growth of any metastases.

Our neural network produced an individualized survival curve for each patient, with credibility intervals (similar to confidence intervals) indicating the reliability of the prognostication, which varied according to the number of patients in our database with a similar combination of risk factors. A printout of this survival curve was mailed to each patient's referring ophthalmologist and general practitioner and our oncologist, together with copies of histopathological and cytogenetics reports. These practitioners also received a covering letter dictated by the ocular oncologist (B.D.) explaining the significance of the laboratory results and suggesting directions for further patient care.

MULTIPLEX LIGATION-DEPENDENT PROBE AMPLIFICATION

Multiplex ligation-dependent probe amplification (MLPA) is a recently developed technique that involves (1) hybridization of 43 probes, each of unique length, to tumor DNA; (2) amplifying the probes ligated using polymerase chain reaction; (3) separating the MLPA polymerase chain reaction products by capillary electrophoresis; and (4) comparing tumor results with controls to identify gains and losses of relevant genomic sequences.[13] Multiplex ligation-dependent probe amplification has several advantages over FISH in that it (1) simultaneously tests 31 genomic sequences on chromosomes 1, 3, 6, and 8; (2) discriminates sequences differing by only a single nucleotide; (3) requires a smaller sample, comprising only 80 ng of DNA; (4) is successful with paraffin-embedded, formalin-fixed tissue; and (4) easily allows quality-control studies.

We validated the application of MLPA to uveal melanoma by analyzing 73 DNA samples that were used 10 years previously for our microsatellite analysis studies.[6,14] Briefly, 28 patients died of metastatic disease. The 10-year survival probability was approximately 35% in 35 patients with unequivocal (ie, statistically significant) loss of any of 13 loci tested on chromosome 3. Four of 31 tumors without any unequivocal chromosome 3 losses were fatal. These tumors showed low chromosome 3 values that did not reach statistical significance. These findings suggested that MLPA results from monosomy 3 tumor cells were diluted by disomy 3 cells in heterogenous tumors (**Figure 4**). Interestingly, gains in chromosome 8q correlated with metastasis, unlike our previous studies. These findings revealed to us that the genomic analysis of only chromosome 3 was insufficient. We now use MLPA routinely in the evaluation of chromosomal abnormalities in patients with uveal melanoma treated at the Liverpool Ocular Oncology Centre. As with other molecular biological techniques, the data obtained by MLPA are considered together with clinical and histomorphological risk factors.

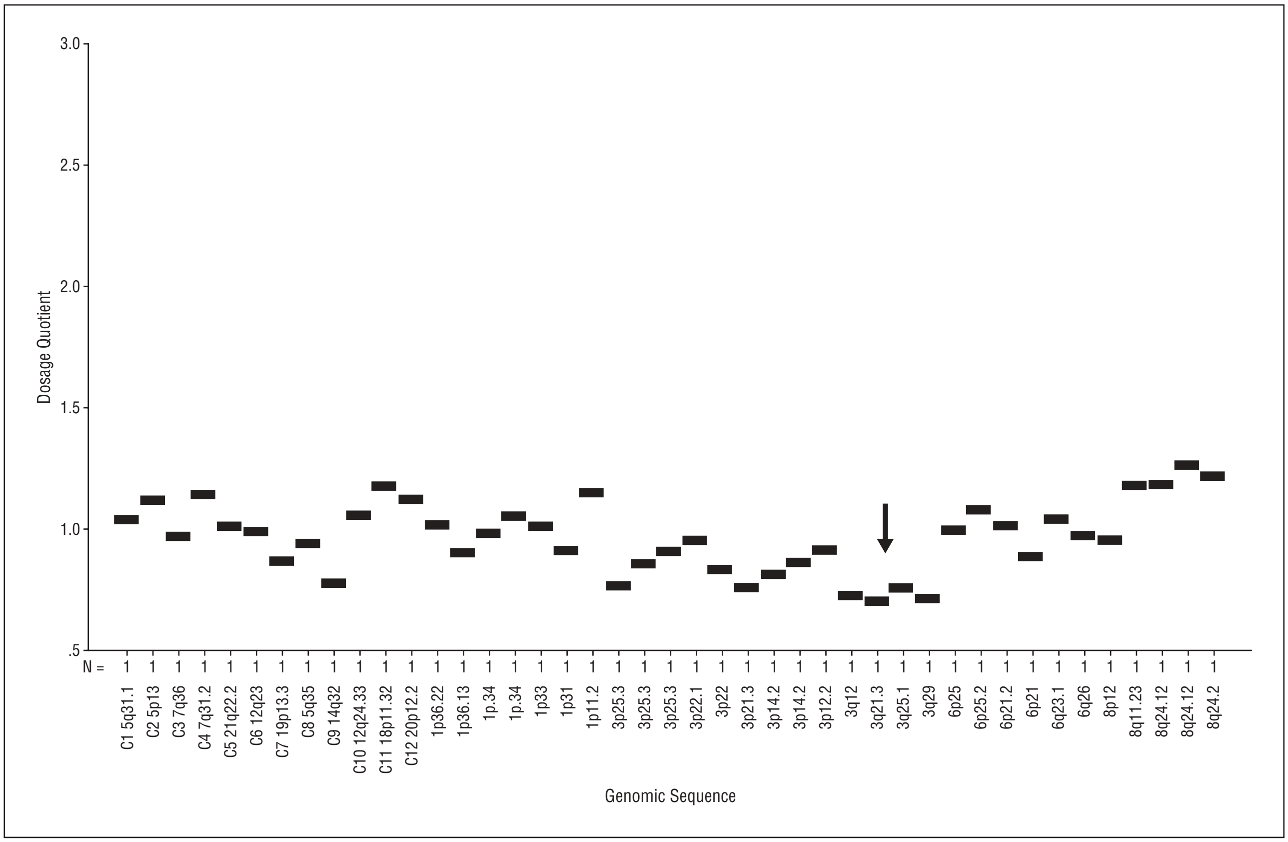

Figure 4. Multiplex ligation-dependent probe amplification results showing "nonsignificant" loss of chromosome 3q in a fatal melanoma (arrow) (courtesy of J. Dopierala, BSc, Liverpool, England, unpublished data).

TUMOR BIOPSY

In early 2007, we started offering cytogenetic profiling to all patients having radiotherapy or phototherapy for uveal melanoma. This was considered appropriate because (1) smaller specimens were required for MLPA; (2) we were confident that we could perform the biopsies without causing ocular complications; and (3) psychological studies indicated that patients benefited from knowing their prognosis, even if this was bad. We also felt that it was the patients with a small tumor who were most likely to have a disomy 3 melanoma with an excellent prognosis and, therefore, to gain most from the genomic profiling. Ironically, these were the very patients who were not being offered the test.

The biopsy technique varied according to the type of radiotherapy and the location of the tumor. Biopsy was usually performed transvitreally with a 25-G vitreous cutter.[15] If, however, a pre-equatorial tumor was treated with brachytherapy, then transscleral fine-needle aspiration biopsy was performed. Others have used a similar technique.[16] The sampling was done at the time of plaque insertion or on the last day of proton beam radiotherapy. The most common complication was failure to obtain an adequate sample and this was most likely to happen with tumors less than 2 mm thick. A small number of patients required treatment for rhegmatogenous retinal detachment and/or severe vitreous hemorrhage and this will be the subject of a future audit.

The genomic interpretation of the biopsy results was more difficult than with enucleation or local resection specimens. First, the tissue samples were of course too small to assess mitotic rate and closed loops. Second, with small tumors, the neural network suggested that monosomy 3 did not significantly worsen the 7-year survival probability (prognostication was limited to 7 years because we started cytogenetics only 7 years before the neural network was trained). Further studies are therefore needed to determine whether such good survival with small monosomy 3 tumors was because of lead-time bias. We may need to retrain the neural network once we have more data from patients with a small melanoma.

RESULTS

BIOLOGICAL INSIGHTS PROVIDED BY GENOMIC STUDIES

The results of our genomic studies, together with the published literature, have provided several insights into the biology of uveal melanomas. First, we found that the prevalence of monosomy 3 in small tumors (ie, with a basal diameter <10 mm) was as high as 35%.[6,9] Others report similar findings.[17] This taught us that small melanomas are not less malignant than large tumors but only less likely to be of the malignant monosomy 3 type.

IMPACT OF GENOMIC STUDIES ON TREATMENT OF OCULAR DISEASE

Genomic studies have changed the way in which we manage our patients with uveal melanoma. Because of the high prevalence of chromosome 3 loss in small tumors, we have become more aggressive in treating such cases, especially as it is with small tumors that any opportunities for preventing metastatic spread are perhaps greatest. We have recently started performing biopsy of some small

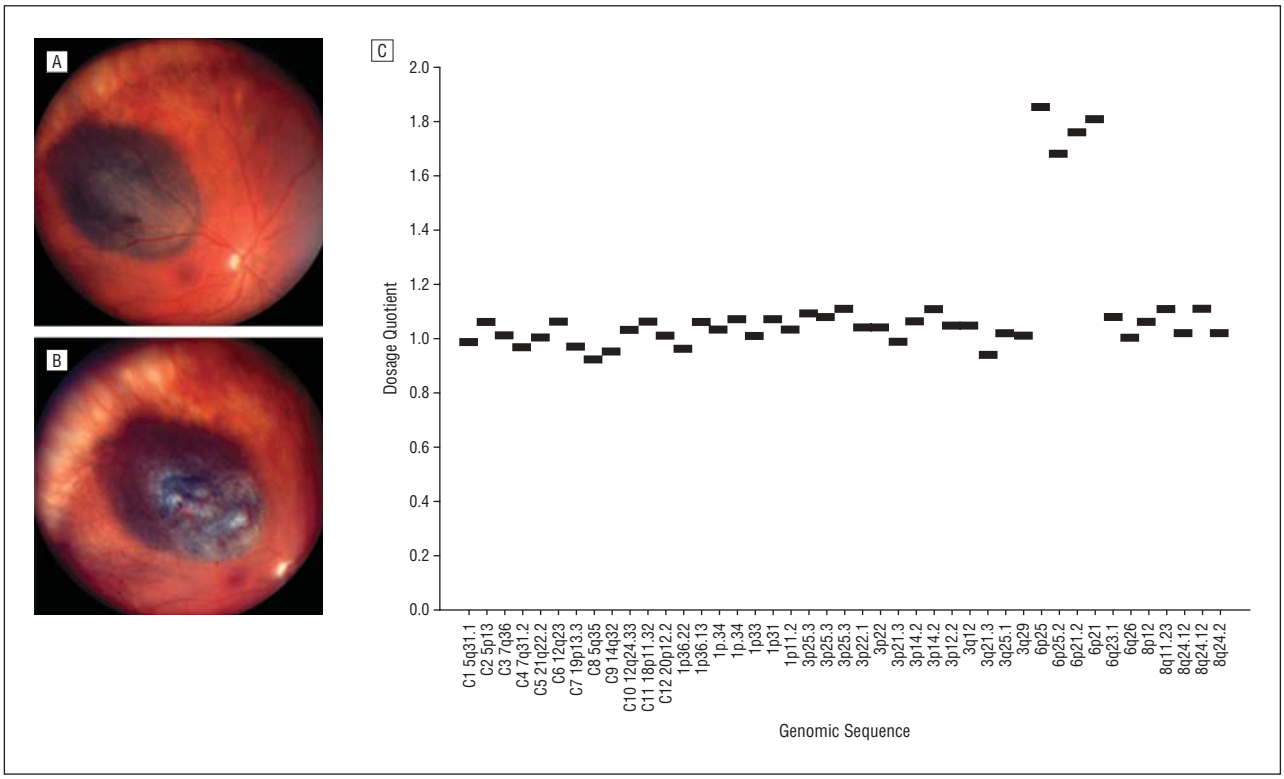

Figure 5. A 51-year-old woman with a superotemporal choroidal melanoma in the right eye. A, Pretreatment photograph showing a superotemporal tumor, measuring 14.1 × 10.6 × 2.6 mm and reducing the visual acuity to 6/24. B, Fundus appearance 6 months after proton beam radiotherapy, transpupillary thermotherapy, and tumor biopsy. The visual acuity had improved to 6/6 and the tumor thickness had diminished to 1.2 mm. C, Multiplex ligation-dependent probe amplification result showing gains in chromosome 6p with no loss of chromosome 3, no gains in chromosome 8, and, hence, minimal risk of metastasis.

melanomas to plan treatment of the ocular tumor (eg, to decide whether to administer photodynamic therapy or proton beam radiotherapy, according to whether the tumor shows disomy or monosomy 3, respectively). We are conscious, however, that with a small tumor a disomy 3 result (1) may occur because of a sampling error and (2) may not preclude subsequent development of monosomy 3. Some have suggested that monosomy 3 and disomy 3 melanomas are distinct "classes" from tumor inception[18]; however, the existence of mosaic tumors suggests that one "type" can develop from the other.

IMPACT OF GENOMIC STUDIES ON MANAGEMENT OF METASTATIC DISEASE

With the exception of resectable, isolated hepatic metastases, which are rare, the treatment of metastases from uveal melanoma only rarely seems to prolong life.[19] Nevertheless, it has become standard practice for all patients with uveal melanoma to undergo hepatic imaging and biochemical liver function tests every 6 or 12 months for many years.[20] Whether such screening is beneficial is debatable. We have therefore delegated decisions about screening to the oncologist who would need to treat the patient should metastatic disease ever develop. Our genomic studies have enabled screening to be targeted at high-risk patients, thereby sparing those with nonlethal melanoma from the stress and inconvenience of repeated examinations.

Studies on systemic adjuvant therapy have not been shown to prolong life significantly in patients with uveal melanoma.[21] We suspect that this is because patients were included in such studies on the basis of tumor size alone (ie, without genomic studies). Our hypothesis is that systemic adjuvant therapy is most likely to be effective if started early, when the metastases are not only undetectable but also very small and relatively few in number. Any randomized studies on the impact of ocular or systemic treatment on the development of metastatic disease would require genomic tumor typing to avoid "statistical noise" caused by the inclusion of many patients with a nonlethal tumor (as happened with the Collaborative Ocular Melanoma Study).[22]

COMMENT

PSYCHOLOGICAL IMPLICATIONS

Several ocular oncologists have criticized us for predicting metastasis when such disease is essentially untreatable. We therefore investigated this formally, using standard psychological techniques.[23] Briefly, we found that patients did not regret being given a poor prognosis, which they felt was less stressful than not being given a prognosis at all. Several patients reported that the information they were given enabled them to get their affairs in order while they still felt well. The patients with monosomy 3 seemed to be less distressed by their poor prognosis than expected, either because they had not fully grasped the implications or because they hoped that screening would prolong life or because of various other

Table. Expectations and Experiences in Cytogenetics of Uveal Melanoma

Expectation	Experience
Loss of entire chromosome 3 predicts metastatic death	Partial deletions of chromosome 3 also predict metastatic death
Chromosome 8 gains required for tumors to have metastatic potential	Chromosome 8 gains not required for tumors to metastasize
Chromosome 6p gains protect against metastatic disease	Chromosome 6p gains do not guarantee metastasis-free survival
Chromosome 3 status is sufficient for prognostication	Chromosome 3 status must be considered together with clinical tumor staging and histologic grading of tumor malignancy
MLPA supersedes FISH	MLPA requires FISH in selected cases to differentiate monosomy 3 from tumor hyperploidy and to interpret significance of borderline results (which can occur in mosaic [heterogenous] tumors)
Patients with monosomy 3 melanoma are severely depressed by poor prognosis	Patients with monosomy 3 melanoma develop several coping mechanisms to maintain their own well-being
Patients with disomy 3 melanoma are greatly relieved by their good prognosis	Patients with disomy 3 melanoma need convincing that their good prognosis is based on high-quality data
Intensive screening for hepatic metastases would enhance prospects for partial hepatic resection and significant prolongation of life	Despite intensive screening, few patients have resectable hepatic metastases

Abbreviations: FISH, fluorescence in situ hybridization; MLPA, multiplex ligation-dependent probe amplification.

coping mechanisms. Patients given a good prognosis were obviously greatly relieved (**Figure 5**). Most needed convincing, however, that the reassurance they received was well founded. The most unhappy patients were the ones in whom biopsy failed, which is why we are now more circumspect about recommending this procedure if the tumor is small.

ETHICAL IMPLICATIONS

Some might question the ethics of performing genomic studies in the absence of sufficient evidence regarding the sensitivity and specificity of any predictions.[24] We felt that even if not infallible, genomic studies were justified as long as they improved any chances of providing an accurate prognosis. We now believe that it may be unethical to deprive patients of genomic studies, especially with small tumors, when the chances of providing reassurance are greatest.

CONCLUSIONS

Our experiences in translating uveal melanoma cytogenetics to clinical practice were different from our expectations (**Table**). Nevertheless, there have been several benefits (**Figure 6**). Genomic tumor typing is now becoming routine in a growing number of ocular oncology services.

We have gained awareness of the prevalence of metastatic potential of "small" uveal melanomas and reappraised our management of patients with such tumors. We now adopt a less paternalistic approach to treatment selection and involve patients more in decision making.

We have reinterpreted the significance of survival curves, taking account of tumor lethality and hopefully improving our treatment selection thanks to a better understanding of the influence of treatment on survival.

Patients with a small disomy 3 spindle-cell melanoma can be reliably informed that they have a "nonlethal melanoma" or a "good melanoma." Such patients can be spared the expense, stress, and inconvenience of 6- or 12-monthly screening for metastatic disease, which usually involves hepatic imaging and biochemical liver function tests.

Patients with a monosomy 3 melanoma can organize their affairs while they still feel well so that personal matters can be sorted out by the time illness from metastatic disease develops.

Patients with a monosomy 3 melanoma can be referred to an oncologist for personalized care. At present, in most centers, such care consists only of systemic screening. In the future, such management will probably include possible enrollment into a trial of systemic adjuvant therapy.

Immunohistochemical, proteomic, and other predictors of metastatic disease can be evaluated according to their correlation with monosomy 3 when data on actuarial survival are insufficient (ie, using monosomy 3 as a surrogate end point).

With genomic tumor typing, studies on the impact of ocular or systemic treatment on survival can exclude patients with nonlethal melanoma, greatly enhancing their statistical power and reducing the number of patients needing to be enrolled.

Genomic typing of uveal melanoma may influence choice of treatment of the primary tumor, so that monosomy 3 melanomas are treated more aggressively. Although it is assumed that, besides being more likely to metastasize, monosomy 3 melanomas are also more destructive to the eye, this may not be the case. Further studies are required.

Figure 6. Benefits of genomic typing of uveal melanoma

We do not wish to imply that we have yet "arrived" at the final destination in the application of cytogenetics to uveal melanoma management. It would be ideal if uveal melanomas were subtyped not only by their metastatic potential but also according to signaling pathway abnormalities so that treatment could be individualized to each tumor, as has already been achieved with other cancers.

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From the Archives of the ARCHIVES

140 Years Ago . . .

After a broad vertical incision of the conjunctiva in the neighborhood of the insertion of the muscles, or better somewhat behind it, I burrow beneath the conjunctiva with the scissors, both toward the cornea and the opposite directions so as to separate it completely from the subjacent Tenon's capsule. Afterward I make the tenotomy and cut the capsule above and below, in the direction of the insertion of the muscle, so far that the muscle and the part of the capsule that lies upon it are completely movable, and may easily be brought forward to the border of the cornea. I pass two fine needles, attached to the two ends of the same thread, above and below, at a distance of about one line from each other; first through the capsule and the end of the muscle, and then from behind forward through the conjunctiva, and tie the loop over the conjunctiva.

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