The Journal of Molecular Diagnostics, Vol. ■, No. ■, ■ 2016

the Journal of Molecular Diagnostics
jmd.amjpathol.org

Validation of a Gene Expression Test for Mesothelioma Prognosis in Formalin-Fixed Paraffin-Embedded Tissues

Q39 Assunta De Rienzo,* Robert W. Cook,† Jeff Wilkinson,‡ Corinne E. Gustafson,* Waqas Amin,§¶ Clare E. Johnson,† Kristen M. Oelschlager,† Derek J. Maetzold,† John F. Stone,‡ Michael D. Feldman,§‖ Michael J. Becich,§‖ Beow Y. Yeap,** William G. Richards,* and Raphael Bueno*

Q18 Q19 *From the Thoracic Surgery Oncology Laboratory and the International Mesothelioma Program,* Division of Thoracic Surgery, Brigham and Women's Hospital and Harvard Medical School, Boston, Massachusetts; Castle Biosciences, Inc.,† Friendswood, Texas; DNA Diagnostics,‡ St. Joseph's Hospital and Medical Center, Phoenix, Arizona; the National Mesothelioma Virtual Bank,§ Pittsburgh, Pennsylvania; the Department of Biomedical Informatics,¶ University of Pittsburgh, Pittsburgh, Pennsylvania; the Perelman School of Medicine,‖ University of Pennsylvania, Philadelphia, Pennsylvania; and the Department of Medicine,** Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts*

Accepted for publication July 28, 2016.

Address correspondence to Raphael Bueno, Division of Thoracic Surgery, Brigham and Women's Hospital, 75 Francis Q20 St., Boston, MA 02115. E-mail: rbueno@partners.org.

A molecular test performed using fresh-frozen tissue was proposed for use in the prognosis of patients with Q21 pleural mesothelioma. The accuracy of the test and its properties was assessed under Clinical Laboratory Improvement Amendments—approved guidelines using FFPE tissue from an independent multicenter Q22 patient cohort. Concordance studies were performed using matched frozen and FFPE mesothelioma samples. The prognostic value of the test was evaluated in an independent validation cohort of 73 mesothelioma patients who underwent surgical resection. FFPE-based classification demonstrated overall high concordance (83%) with the matched frozen specimens, on removal of cases with low confidence scores, showing sensitivity and specificity in predicting type B classification (poor outcome) of 43% and 98%, respectively. Concordance between research and clinical methods increased to 87% on removal of low confidence cases. Median survival times in the validation cohort were 18 and 7 months in type A and type B cases, respectively ($P = 0.002$). Multivariate classification adding pathologic staging information to the gene expression score resulted in significant stratification of risk groups. The median survival times were 52 and 14 months in the low-risk (class 1) and intermediate-risk (class 2) groups, respectively. The prognostic molecular test for mesothelioma can be performed on FFPE tissues to predict survival, and can provide an orthogonal tool, in combination with established pathologic parameters, for risk evaluation. *(J Mol Diagn 2016, ■: 1—7; http://dx.doi.org/10.1016/j.jmoldx.2016.07.011)*

Q10 Supported by National Cancer Institute grant 2 R01 CA120528 (R.B.), Q11 by the International Mesothelioma Program, and by the Centers for Disease Control and Prevention and the National Institute for Occupational Safety Q12 and Health for funding NMVB grant U24 OH009077-06. Castle Biosciences, which has licensed the prognostic test described herein from Brigham and Women's Hospital (BWH), supported some of the work Q13 Q14 through a grant to BWH and the laboratory of R.B.

Disclosures: The research described herein was partially funded by Castle Bioscience through a research grant to Brigham and Women's Hospital (BWH) and the laboratory of R.B. Castle Biosciences licensed the patent for the molecular tests described herein from BWH. R.W.C., C.E.J., K.M.O., D.J.M. are employees of, may hold stock options in, Castle Biosciences Inc. M.J.B. has received consulting fees from Empire Genomics, the scientific advisory board of NinePoint Medical, Omnyx (a joint Venture with University of Pittsburgh Medical Center and GE), and De-ID Q15 Data Corp. deidentification software (licensing agreement); and corporate support from API, Strategic Summit and Pathology Informatics 2014 Today, ASCP, Definiens, LabVantage, PathCentral—Aperio, Atlas Medical, Q16 Q17 Aurora Interactive, Data General, Halfpenny Technologies, Hamanatsu, HistoIndex, Knome, LifePoint Informatics, LigoLab Information Systems, Milestone, mTuitive, NinePoint Medical, NovoPath, Odin, Orchard Software, Phillips, Softek Illuminate, Software Testing Solutions, StarLIMS, Ventana, and Voicebrook.

http://dx.doi.org/10.1016/j.jmoldx.2016.07.011

Malignant pleural mesothelioma (MPM) is a rare and aggressive cancer that arises in the pleura and grows relentlessly into adjacent structures. Approximately 3200 new cases are diagnosed in United States each year, and the incidence worldwide is estimated to rise in the next 2 decades.[1,2] The World Health Organization classifies MPM into three major histologic types: epithelioid, sarcomatoid, and biphasic (composed of both epithelioid and sarcomatoid cells).[3] Prognosis is generally poor in most MPM patients, with a median survival time between 4 and 12 months.[4,5] Patients with localized tumors may undergo surgical resection, such as extrapleural pneumonectomy or extended pleurectomy, followed by a combination of adjuvant chemotherapy and/or radiation therapy[6,7]; however, only a subset of patients is most likely to have sufficient survival benefit from this approach, with a 5-year survival rate of ~20%.[8]

A clinically and pathologically accurate and internationally accepted staging system is essential for selecting patients for treatment and for assessing the benefit of new therapies.[9] However, the validity of the current MPM staging system is questionable because it was derived from analyses of small-scale, retrospective surgical series that are difficult to apply to clinical staging, and it because it uses descriptors for lymph node involvement, which may not be relevant to MPM.[9] In the current clinical practice, several cancer-specific risk factors have been associated with survival; however, some of them, such as histologic examination, resection margins, stage, and lymph node status, can be accurately determined only after resection.[10–12] Therefore, it is clinically important to identify prognostic factors to classify patients into groups with distinctly different outcomes before embarking on extirpative surgery.

We developed a molecular algorithm (the *gene ratio method*) that translates comprehensive RNA expression profiling data into simple clinical tests that are based on the expression levels of a relatively small number of genes.[13] Genes differentially expressed between two clinically different conditions are identified in RNA-based expression profiling and are used in combination to calculate ratios of gene expression capable of predicting the clinical state associated with arbitrary patient samples.[14–16] In particular, a four-gene, three-ratio *gene expression score* (GES) has been reported and subsequently validated in prospectively collected frozen tissue samples to predict overall survival in patients with MPM.[17–19] In a multivariate model, the GES was independently significant after adjustment for lymph node status, tumor stage, and histologic subtype, indicating that this test appears to provide prognostic information additional to current pathologic staging methods. It has been suggested that the use of this molecular test, coupled with a multiplatform prognostic strategy, which includes lymph node status and histologic subtype determination, may be a predictor of overall survival and may lead to the selection of patients more likely to benefit from surgery.[17] Patients whose prognosis is predicted, using this algorithm, to have limited overall cancer survival may avoid futile operation.

In the current study, this MPM-prognostic GES has been adapted for use with formalin-fixed, paraffin-embedded (FFPE) tissue on a high-throughput microfluidics real-time quantitative PCR (qPCR) platform to assess its accuracy when performed under Clinical Laboratory Improvement Amendments (CLIA)-approved guidelines. The accuracy of the high-throughput FFPE test in classifying risk has been investigated in comparison to the published test using standard qPCR on frozen samples derived from the same tumor specimen. In addition, the high-throughput protocol has been further validated using an independent multicenter cohort of MPM tumor cases provided by the National Mesothelioma Virtual Bank (NMVB).

Materials and Methods

Patients and Sample Acquisition

MPM tumor specimens from cases included in the concordance study were collected at Brigham and Women's Hospital (BWH) (Boston, MA), from patients undergoing surgical extirpation with intent to cure, between 1999 and 2010. In this cohort, the survival time was calculated from the debulking surgery. Cases included in the independent validation study were collected at University of Pittsburgh School of Medicine (Pittsburgh, PA) and the University of Pennsylvania (Philadelphia, PA) between 1996 and 2013. These validation cases were obtained through the NMVB at University of Pittsburgh School of Medicine. All de-identified specimens and matched clinical data were obtained under Institutional Review Board–approved protocols (University of Pittsburgh School of Medicine NMVB protocol REN13070160/IRB0608194; University of Pennsylvania NMVB protocol 804329). The NMVB MPM validation set included 73 patients with FFPE biopsy specimens and clinically documented evidence of surgical resection of the tumor with diagnosis of histologic subtype. In this case, the survival time was calculated from diagnosis.

Sample Preparation and Quantitative PCR

Specimens processed at BWH were collected at surgery as discarded specimens and were fresh-frozen, stored, and annotated by the BWH tumor bank (Dana Farber/BWH Institutional Review Board protocol 98-063). Five thick tumor sections from each sample were homogenized in Trizol (Thermo Fisher Scientific, Carlsbad, CA), and RNA was extracted using the RNeasy kit (Qiagen, Valencia, CA). DNase I (Thermo Fisher Scientific) treatment was conducted according to the manufacturer's instructions to remove any genomic DNA contamination. RNA was quantified using an ND-1000 spectrophotometer (NanoDrop; Thermo Scientific, Wilmington, DE) and the ratio

FLA 5.4.0 DTD ■ JMDI552_proof ■ 15 November 2016 ■ 7:25 pm ■ EO: JMD16_0034