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My name is Christian Steidl from the Department of Pathology and Laboratory Medicine at UBC (University of British Columbia), and I am also a scientist at the Department of Experimental Therapeutics in the Center for Lymphoid Cancer at the British Columbia Cancer Agency.

Today, I want to give you a summary of a talk that I gave in the biomarker workshop at the 9th International Symposium on Hodgkin lymphoma entitled, "Prognostic markers from gene expression profiling in formalin-fixed paraffin-embedded tissues." To give you a little bit of background on the topic, classical Hodgkin lymphoma has been labeled the success of chemotherapy and radiotherapy. Dramatic improvements and outcomes over the last half century have been highlighted in various reports; 10-15% of patients with advanced-stage classical Hodgkin lymphoma still succumb to the disease, and lack of reliable biomarkers that identify a substantial proportion of patients at significant risk of death is an unmet need to further improve outcomes in this disease. Current predictors in advanced stage classical Hodgkin lymphoma is the International Prognostic Score called IPS which is the gold standard at present to assign risks to patients that are used for risk stratification in classical Hodgkin lymphoma. To improve on the IPS, in the past, several biomarker studies have been conducted. Primarily, these were studies using immunohistochemistry in formalin-fixed paraffin-embedded tissues. These studies encompassed biomarkers that were derived from the malignant Hodgkin Reed-Sternberg cells, from the tumor microenvironment, or from whole biopsies. However, some of these markers are not easily reproduced and they are not really suitable for direct application in clinical workflows. Our group has reported on the use of immunohistochemistry-based biomarkers, namely CD68 immunohistochemistry that was used to risk-stratify patients and was proposed as a biomarker that can be used for informing on treatment in subsequent validation studies. However, open questions and challenges remain. First, reproducibility and thresholds that have to be established to develop a test, and second are multigene predictors superior to single-gene predictors such as CD68 immunohistochemistry alone. Therefore, our research questions were related to feasibility of a standardized gene expression profiling-based test using archival formalin-fixed paraffin-embedded tissue and the predictive value of intermediate density digital gene expression profiling in advanced-stage classical Hodgkin lymphoma.

Lastly, we were focusing on the clinical utility of a predictive model for overall survival. Towards these goals, we sought to develop gene expression based predictor of overall survival in advanced-stage classical Hodgkin lymphoma treated with standard-intensity treatment. We trained on data from a phase III randomized controlled clinical trial and validated our predictor in an independent cohort treated with ABVD enriched for primary treatment failure and treated at the British Columbia Cancer Agency. In detail, we used 306 patients of a phase III randomized controlled trial of Intergroup E2496 comprising of locally extensive and advanced-stage Hodgkin

lymphoma treated with ABVD or Stanford V and the validation cohort comprised of 82 patients that were enriched for treatment failure and were treated at the British Columbia Cancer Agency, all advanced-stage classical Hodgkin lymphoma. We extracted RNA from tissue blocks of archival formalin-fixed paraffin-embedded tissue and used the gene expression data to train a model using penalized COX regression and validated our model in the validation cohort of 82 patients. In the training cohort, we observed 94% 5-year overall survival for the good-risk group and 75% 5-year overall survival for the bad-risk group as defined by the predictor score. The training cohort had a median follow up of 5.3 years. The next question was if this test is clinically useful. This of course is dependent on external validation and demonstration that identification of a high-risk group changes management. The power of the predictor was nicely shown in the validation cohort when the predictive model and the thresholds were applied.

The predictive model comprised of 23 genes that produced a predictive score. With a threshold that was optimized, we were able to dichotomize the patient cohort into high risk and low risk. When applying the model and the thresholds to the validation cohort, we could separate and stratify the cohort into high risk and low risk and could reproduce the results in the training cohort. In the validation cohort, the median follow up was 5.8 years, and the high-risk stratum identified roughly 25% of the patients. The difference between the high-risk and low-risk group with respect to survival was highly significant. We next performed a multivariate analysis with the predictive score, clinical variables that were present in the IPS, pathology data including CD68+ cells. In this multivariate analysis, only the predictive score was significant in multivariate analysis highlighting the superiority of the predictive score over the other parameters.

In conclusion, NanoString is a very robust platform for intermediate-density gene expression profiling of archival formalin-fixed paraffin-embedded tissues. The 23-gene predictor discriminates between patients that can be safely treated with standard intensity upfront treatment and those at high risk. Further study will determine whether this increased risk can be overcome by dose-intense chemotherapy or novel agents. We believe that this predictor is the first step to truly personalize treatment in advanced-stage Hodgkin lymphoma.

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