Lobsterpot
CA19138

Collection of samples for the ring-study

A recent worldwide survey conducted with Lobsterpot (De Schepper *et al.*, Mod Pathol 2022) demonstrated that there is a great variety between different pathology laboratories concerning the use or not of immunohistochemistry (IHC) for invasive lobular breast cancer (ILC) diagnosis and concerning the antibody clones and their concentrations that are used. Indeed, 11 different antibody clones were reported, without clear continental differences. Also, it has become clear that false positive E-cadherin staining could be observed, as there exist different somatic mutations in the gene coding for E-cadherin, *CDH1* (frameshift mutation, extracellular truncating mutation, extracellular missense mutation, truncating mutation), and each antibody may have its own specificity for these mutations. Additional IHC, under form of ß-catenin or p120 catenin, can be used as additional adjunct to assess E-cadherin functionality. These proteins are bound to the cytoplasmic domain of E-cadherin and have a membranous staining when E-cadherin is present and fully functional. However, when the E-cadherin function is compromised, ß-catenin and p120 catenin lose their localization at the plasma membrane and translocate to the cytosol. This process is reflected with IHC, as ß-catenin and p120 catenin will display cytoplasmic or para-nuclear staining pattern. This additional IHC support the diagnosis of ILC in case of aberrant E-cadherin staining, however, is not used by all pathology laboratories.

The consortium has thus decided to perform a ring study, called Cad-E-lac (Characterization of E-cadherin antibody staining pattern in invasive lobular (adeno)carcinoma) in which they will map the staining patterns of the most used antibodies worldwide, and evaluate them according to different types of *CDH1* mutations. This study will demonstrate the differences and possible pitfalls associated with some of the commercially available antibodies. The final goal of the study is thus to improve diagnostics of ILC in clinical practice as well as for clinical trials (WG6), by reducing errors in E-cadherin staining interpretation and set the standards for the use of a better informed functional IHC in the context of breast cancer diagnostics. We expect that the results of this study might even impact the future WHO guidelines for pathological ILC diagnosis.

In practice, the protocol has been written and was approved by the central EC of UZ/KU Leuven on 14/10/2022 (S66045). Waiver has been asked and was granted for informed consent. The legal department of UZ/KU Leuven has drafted MTA which has been sent

and signed by the different participating centers of the consortium which are (in addition to UZ/KU Leuven) 1) Medizinische Hochschule Hannover, Hannover, Germany; 2) Universitair Medisch Centrum Utrecht, Utrecht, The Netherlands. The contract with Institut Curie is ongoing. Formalin fixed paraffin embedded (FFPE) tissue blocks from around 60 and 40 patients diagnosed locally with primary ILC with and without known somatic *CDH1* mutations and treated in Leuven or in one of the German trials have been collected by UZ/KU Leuven and the Medizinische Hochschule from Hannover, retrospectively. The clinical data of the patients, transferred between the centers, are the reported histology at time of diagnosis, the status of the tumor block used for the study and the somatic *CDH1* status. Pseudonymized blanco FFPE slides obtained from all the selected patients will be IHC stained at UZ/KU Leuven for the most frequently used antibodies in clinical practice worldwide (NCH38, EP700Y, Clone 36, ECH-6, Clone 36B5). Additional IHC staining for P-cadherin (BD clone 56), p120-catenin and ß-catenin will be considered as well. The specifications of IHC (antigen retrieval and concentrations) will be performed according to the recommended protocol by NordiQC. The histological type confirmation and if ILC, the subtype, the different E-cadherin stains and the additional ß-catenin or p120-catenin staining will be evaluated by a group of 6 worldwide experts pathologists and based on a consensus diagnosis. Full details on the exact procedure are being described in the protocol. Currently, FFPE blocks are being processed. This first step of consensus scoring will be held in March 2023 with all the worldwide expert pathologists at UZ/KU Leuven. The second step (validation of the selected antibodies) will be planned later based on tissue micro-arrays (TMA's) and/or FFPE samples from The Netherlands and from Institut Curie.