

References

- [1] **Merkel S, Mansmann U, Papadopoulos T, Wittekind C, Hohenberger W, Hermanek P.** (2001): The prognostic inhomogeneity of colorectal carcinomas Stage III: a proposal for subdivision of Stage III. *Cancer: Interdisciplinary International Journal of the American Cancer Society.* 92(11): 2754 – 2759.
- [2] **Kennedy RD, Bylesjo M, Kerr P, Davison T, Black JM, Kay EW, ... Goffard N.** (2011): Development and independent validation of a prognostic assay for stage II colon cancer using formalin-fixed paraffin-embedded tissue. *Journal of Clinical Oncology.* 29(35): 4620 – 4626.
- [3] **Croner RS, Merkel S, Papadopoulos T, Schellerer V, Hohenberger W, Goehl J.** (2009): Multivisceral resection for colon carcinoma. *Diseases of the Colon & Rectum.* 52(8): 1381 – 1386.
- [4] **Pedrazzani C, Lauka L, Sforza S, Ruzzenente A, Nifosi F, Delaini G, Guglielmi A.** (2015): Management of nodal disease from colon cancer in the laparoscopic era. *International journal of colorectal disease.* 30(3): 303 – 314.
- [5] **Yamamoto N, Daito M, Hiyama K, Ding J, Nakabayashi K, Otomo Y, ... Kato Y.** (2013): An optimal mRNA marker for OSNA (one-step nucleic acid amplification) based lymph node metastasis detection in colorectal cancer patients. *Japanese journal of clinical oncology.* 43(3): 264 – 270.
- [6] **Yamamoto H, Sekimoto M, Oya M, Yamamoto N, Konishi F, Sasaki J, ... Akamatsu H.** (2011): OSNA-based novel molecular testing for lymph node metastases in colorectal cancer patients: results from a multicenter clinical performance study in Japan. *Annals of surgical oncology.* 18(7): 1891 – 1898.
- [7] **Güller U, Zettl A, Worni M, Langer I, Cabalzar-Wondberg D, Viehl CT, ... Zuber M.** (2012): Molecular investigation of lymph nodes in colon cancer patients using one-step nucleic acid amplification (OSNA). A new road to better staging? *Cancer.* 118(24): 6039 – 6045.
- [8] **Croner RS, Gepper, CI, Bader FG, Nitsche U, Späth C, Rosenberg R, ... Stürzl M.** (2014): Molecular staging of lymph node-negative colon carcinomas by one-step nucleic acid amplification (OSNA) results in upstaging of a quarter of patients in a prospective, European, multi-centre study. *British journal of cancer.* 110(10): 2544.
- [9] **Aldecoa I, Atares B, Tarragona J, Bernet L, Sardon JD, Pereda T, ... Alonso GL.** (2016): Molecularly determined total tumour load in lymph nodes of stage I–II colon cancer patients correlates with high-risk factors. A multicentre prospective study. *Virchows Archiv.* 469(4): 385 – 394.
- [10] **Yamamoto H, Tomita N, Inomata M, Furuhashi T, Miyake Y, Noura S, ... Itabashi M.** (2016): OSNA-assisted molecular staging in colorectal cancer: a prospective multicenter trial in Japan. *Annals of surgical oncology.* 23(2): 391 – 396.
- [11] **Diaz Mercedes S, Aldecoa I, Bombi A, Tarragona J, Gonzalez-Farre B, Castillo P, ... Chic N.** (2018): Molecular Lymph Node Total Tumor Load Correlates Better With High Risk Factors Than pN Stage in Early colorectal carcinoma. A multicenter study. *USCAP conference.*
- [12] **Rakislova N, Montironi C, Aldecoa I, Fernandez E, Bombi JA, Jimeno M, ... Cuatrecasas M.** (2017): Lymph node pooling: a feasible and efficient method of lymph node molecular staging in colorectal carcinoma. *Journal of translational medicine.* 15(1): 14.
- [13] **Cuatrecasas M, Diaz-Mercedes S, Archilla I, Maurel J, Chic N, Bombi JA, Carrascosa C, Montironi C, de Lacy A, Almenara R, Castells A, Camps J, Balaguer F, Aldecoa I.** (2018): Budget impact of molecular lymph node staging in colorectal carcinoma. Should we invest more in diagnosis? *Poster at European Congress of Pathology.* Bilbao 2018.
- [14] **Matsuura N, Tomita N, Inomata M, Murata K, Hayashi S, Miyake Y, ... Furuhashi T.** (2017): Clinical impact of molecular positive lymph node status in colorectal cancer. *Annals of Oncology.* 28(suppl_5).

OSNA for colorectal cancer – Molecular detection of lymph node metastases for accurate staging

Current challenge in nodal staging

Lymph node (LN) positivity is among the most important prognostic factors in colorectal cancer (CRC) and is associated with increased recurrence as well as a lower rate of survival [1, 2, 3]. About 20% of early stage node negative patients show recurrence after surgery most likely due to undetected LN metastases and undertreatment [4]. One reason is that during hematoxylin-eosin (HE) histopathology, LNs are usually examined only on one level, leaving the rest of the tissue unanalysed. The use of more sensitive molecular methods for LN staging can help identify patients at risk of recurrence.

Increase accuracy with a molecular approach

OSNA is a molecular diagnostic assay that allows the detection of nodal metastasis by quantifying the expression of CK19 mRNA in the LN. The suitability of CK19 as a marker has been demonstrated in a pre-evaluation phase where CK19 was tested on histologically positive and negative LNs and in comparison to selected markers (Figure 1) [5]. Results showed that CK19 is a very sensitive and specific marker for LN metastasis in CRC. Moreover, 98.4% of the primary tumours express CK19, further confirming the validity and specificity of the marker for the detection of LN metastases [5].

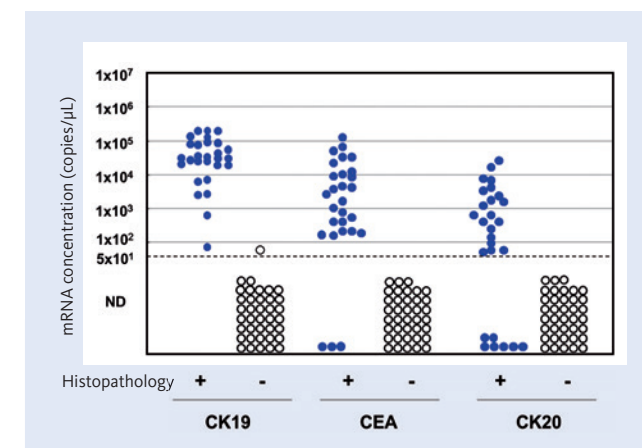
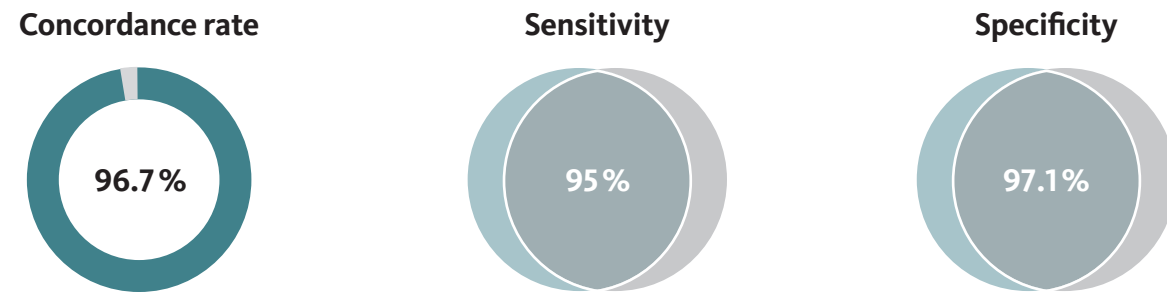


Figure 1: Analysis of 28 positive LNs from 19 patients and 38 negative LNs from 11 pN0 patients



The OSNA method has been compared to extensive histopathological examination in more than 1,000 nodes [3, 6, 7]. Each LN was cut into 4 slices and alternate slices were analysed either by OSNA or histological analysis. The resulting pooled data is:



The performance evaluation provided an initial indication for the method's upstaging potential. This was confirmed in three other studies looking at some 3,600 pN0 lymph nodes, where OSNA showed a higher sensitivity than histopathology, resulting in an upstaging rate of up to 25% [8]. Early stage patients who suffer from recurrent disease were most probably understaged by conventional HE, being in reality stage III [1, 2, 3].

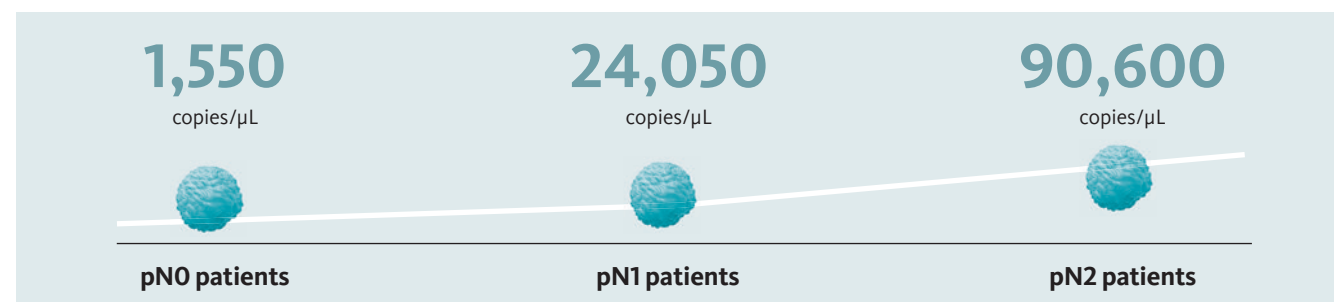
Prof. Peoc'h testimonial:

Knowing the difficulties of histology in nodal staging and, looking at the bad outcomes of some node-negative patients, makes it clear that with OSNA I can overcome a gap in CRC patient management by analysing the whole lymph node.

Identify patients with higher risk of recurrence by quantitative staging

OSNA does not only provide information about the presence or absence of metastasis in LNs, it also allows one to obtain the Total Tumour Load (TTL)* presents in the LNs of a given patient. Early stage patients are eligible to complete surgical resection, but patients at risk of disease recurrence are clinically and pathologically difficult to identify [9]. Therefore, a question may arise whether TTL can better select early stage patients at risk of recurrence. Several authors showed that TTL correlates better with high risk factors than pN stage and increases as the number of histologically positive LNs increases [9, 10, 11]. The biological significance of LN metastases detection is of leading importance because the sole presence of a small amount of tumour may indicate a risk of disease recurrence. Quantifying the amount of tumour burden in the whole node, not just the presence or absence of metastases is therefore important to foresee the patient's prognosis [9].

In CRC staging, TTL quantification can become an objective tool to stratify the risk of individual patients. Yamamoto *et al.* observed an increment in the TTL with the increase in the number of positive LNs, rising to 1,550 copies/μL in pN0 patients, 24,050 in pN1 patients and 90,600 in pN2. Hence, TTL represents a continuous value of the amount of tumour burden present in the LNs of a given patient, which may be more accurate than the number of positive LNs [12].



* TTL is defined as the total number of CK19 mRNA copies in all positive nodes.

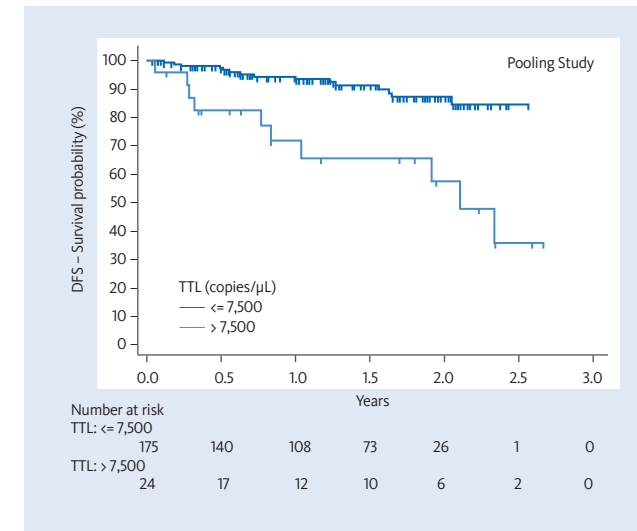


Figure 2: TTL cut-off established on Disease Free Survival [13]

Dr. Cuatrecasas testimonial:

Some histologically LN negative patients with positive LNs with the OSNA assay have recurred after 3 years. I believe that the biological value of TTL is different than the actual pN staging based on HE. TTL can provide additional information since its value may identify those patients at high risk of recurrence. We have estimated that with a TTL cut-off of 7,500 copies/μL (regardless of the pN stage) the relative risk of recurrence in that period was 4.3 (p = 0.0001) (Figure 2). Thus, TTL can have a positive influence on decision making in early stage CRC patients.

Expand the benefits of using molecular and quantitative staging

Recently, the OSNA result has shown prognostic value in early stage CRC patients [14]. The OSNA positive cases had a much lower 3-year disease-free survival (DFS) rate than the negative ones. Among various clinical and pathological parameters (see the list of high-risk factors below), only the OSNA status was a significant prognostic factor, which can help select early stage patients at risk of recurrence.

Stage II high-risk factors

- OSNA status in LNs
- Age
- Sex
- Tumour location
- Tumour size
- Differentiation
- Lymphatic invasion
- T stage
- Harvested LN number

The OSNA clinical evidence has evolved from a sensitive and accurate method to a quantitative and prognostic tool, which has shown to make staging more significant. Molecular lymph node staging and precision diagnostics make it possible to provide reliable information for decision-making. Finally, the successful OSNA clinical evidence should push forward the power of TTL, leading to an improvement of CRC management and patient benefits.