

**“Integrating morphology and molecular pathology to manage the diagnosis and treatment of colon cancer.”**

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Summary (handout)

By integrating morphology and molecular pathology, pathologists can play a role in three crucial issues that may improve the colorectal cancer management: the assessment of an individual's colorectal cancer risk, indication and/or evaluation of neoadjuvant therapies and the assessment of individual's molecular targeted therapies.

**a) INDIVIDUAL'S COLORECTAL CANCER RISK:**

One out of each 17 persons will develop a colorectal cancer (average risk) but 25% of cases of colorectal cancer (CRC) occur in individuals who have above average or high risk for colorectal cancer. Individuals with one or more first-degree relatives with colorectal cancer have a relative risk for colorectal cancer of 2.25 and 4.25, respectively. This risk is even higher for familial and hereditary colon cancer syndromes. Patients with inherited germline mutations of hereditary nonpolyposis colorectal cancer (HNPCC) syndrome (accounts for approximately 5% of cases of colorectal cancer) have an 80% lifetime cancer risk and patients with Familial Adenomatous Polyposis (FAP) a 100% cancer risk. To identify the 6-8% of all colorectal cancers occurring in a familial or hereditary onset is crucial prevention and early detection of other tumours associated with these syndromes and for genetic testing of the families to detect mutation carriers that should undergo rigorous cancer screening, starting at 20 years of age.

HNPCC is the most frequent hereditary syndrome associated with colorectal cancer. Affected individuals carry a germline mutation of mismatch repair (MMR) genes (*MLH1, MSH2, MSH6 or PMS2*) and a somatic genetic second hit originates loss of expression of the MMR gene and microsatellite instability (MSI). Molecular studies to identify HNPCC can be recommended with a meticulous family history and the early onset of tumours, but still some cases

may be missed. Some authors have proposed screening of all colorectal cancer tumors for microsatellite instability. There is a good correlation between the loss of MMR protein expression by immunohistochemistry and microsatellite instability identification by PCR. A cost effective strategy for HNPCC screening is the analysis of MMR gene expression by IHC and/or microsatellite instability and only positive cases undergo genetic testing for mutations in mismatch repair genes *MLH1* and *MSH2*. Pathologists can integrate clinical data, IHC results and MSI data to identify those patients. MMR genes function as heterodimers (*MLH1*-*PMS2* and *MLH2*-*MSH6*). Loss of protein expression usually affects two MMR genes: the protein of the mutated MMR gene and its heterodimer protein. Positive staining for inflammatory and stromal cells can be used as an internal IHC control.

Gross examination of colorectal cancer specimen can detect more than 100 adenomatous polyps characteristic of FAP, although attenuated forms of FAP may have fewer polyps and a later onset (mean age 35 years-old for classic FAP and 50 years-old for attenuated forms).

Individuals with inflammatory bowel disease, adenomas with dysplasia and traditional or sessile serrated adenomas also have a higher CRC risk. During the past decade, major advances have occurred in our understanding of the molecular basis of CRC and its precursor lesions. There is increasing evidence for an alternative pathway of sporadic colorectal tumorigenesis other than the adenoma-carcinoma sequence. A serrated polyp pathway that is associated with DNA microsatellite instability (MSI), due to methylation and loss of expression of the mismatch repair gene *MLH1* with activating mutations in *BRAF* has been highlighted. However, for many pathologists the lack of consensus criteria in morphologic parameters makes difficult to distinguish hyperplastic polyps and the spectrum of serrated mucosal lesions.

#### **b) THE TNM STAGING SYSTEM:**

The TNM was established as a prognostic system but now determines individual treatment. A same patient can receive or not neoadjuvant therapy or

may enter into a clinical trial depending on which version of the TNM system is being used.

- Number of identified lymph nodes. In most institutions, CRC surgical resection specimens with less than 12 identified lymph nodes will receive chemotherapy even if no lymph node metastases are found. Recent large scale studies show that increased fixation time (over 36 hours with formalin) can increase between 10-15% the number of specimens with more than 12 lymph nodes. A fat clearance technique for the detection of lymph nodes in colorectal cancer is been used at some institutions. The fixatives used in this technique can interfere with DNA extraction and when used, we recommend procuring frozen or formalin fixed tumour and normal tissues for molecular studies.
- Tumoural deposits in the fat: The TNM version 6 distinguishes independent deposits with a round contour that should be considered as lymph node metastasis from irregularly shaped deposits that should be considered an extension of the T category (V1 as microscopic vascular invasion and V2 as gross vascular invasion), although some discrepancies have been reported about the prognostic significance of these deposits in some series.
- Micrometastasis: groups measuring less than 0.2 mm are considered as non relevant.

### **c) CIRCUMFERENTIAL RESECTION MARGIN (CRM) AND TOTAL MESORECTAL EXTIRPATION (TME) EVALUATION IN RECTAL ADENOCARCINOMA:**

The surgical margin of resection is crucial for determining the prognosis and survival in rectum. A 2008 review showed in a series of 17,568 patients that the assessment of CRM margin has a greater prognostic influence than the depth of infiltration of the primary tumor, regardless of neoadjuvant therapy. Pathological evaluation of the CRM was described by Quirke et al. and more recently by Nagtegaal et al. It is defined as the margin of soft tissue or perineal adventicia

closer to the point of maximum penetration of the tumour or lymph node metastasis. It is considered to be positive if it is <1 mm. Despite these data, the report of the CRM is not a routine practice in many laboratories. The evaluation of the circumferential margin is even more important after preoperative treatment when the radiological study shows a positive margin.

Related to circumferential margin is the evaluation of the level of quality of surgery over the usual total mesorectal excision (TME), which is valued from grade 1 to 3 (3= complete resection). There are controversies over its usefulness in some recent clinical trials.

#### **d) RESPONSE TO PRE-SURGICAL CHEMO-RADIOTHERAPY:**

Several clinical studies have highlighted the importance of a grading system to assess tumour regression in response to preoperative chemotherapy or radiotherapy treatment in rectal cancer. Four degrees of tumour regression (TRG) can be determined based on the proportion of viable tumour versus fibrosis: TRG 4 there is no viable tumour, TRG 3: regression greater than 50%, TRG 2 less than 50% and TRG 1: tumour unchanged.

#### **e) INDIVIDUAL'S MOLECULAR TARGETED THERAPIES:**

K-ras mutations and advanced CRC treatment with EGFR inhibitors: Most colorectal cancers (up to 80%) have increased expression of Epidermal Growth Factor receptors (EGFR) and use this molecular pathway as a mechanism for tumour growth. Blocking EGFR with monoclonal antibodies is a useful cancer therapy only when K-ras oncoprotein is "wild type". Kras is located downstream in the EGFR growth stimulation molecular pathway (ras / MAPK pathway) and when kras is mutated it will continue to activate this pathway even if EGFR are blocked. Kras mutations are present in 40-45% of all colorectal cancers (47% in our first 200 advanced CRC cases analyzed for EGFR inhibitors treatment). Clinical trials results presented at the ASCO in June 2008 and the World Congress of Gastro-intestinal cancer in Barcelona 2008 demonstrated in large multicenter clinical trials that metastatic CRC with wildtype Kras (non-mutated) can benefit from therapy with anti-EGFR monoclonal antibodies such as Amgen

Vectibix (panitumumab) and Erbitux from Imclone / Bristol-Myers Squibb (cetuximab), as a second line treatment. Results of clinical trials using EGFR inhibitors as a first line treatment alone or associated with chemotherapy or angiogenesis inhibitors will be presented in ASCO 2009.

Currently, a network to use EGFR inhibitors as a second line treatment in all advanced CRC has been set up in Spain as a multi-level, European pioneer for cancer treatment. Endoscopy material, tumour paraffin blocks or cytological FNAB are submitted to a few reference hospitals as the Hospital San Carlos to assess the kras mutation status. The pathologist can play an important role in the selection of tumour material for DNA extraction, valuing the percentage of neoplastic cells or the need for microdissection. Each molecular technique that can be used to analyze kras status has a different tissue requirement and a different mutation sensibility.

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