CHAPTER 15
Adjuvant therapy

General principles

Systemic adjuvant therapy consists of chemotherapy administered after surgical treatment to prevent postoperative recurrence and improve the prognosis of patients after R0 resection. It is also indicated to treat unresectable colon cancer.[1,2]

The rationale for adjuvant therapy is to treat tumor disease at a time when it is of smaller size and most likely to be eradicated. If a regimen is effective in reducing tumor size or progression, it can also effectively treat residual microscopic disease after surgery. Curability is greatest when the number of cells that make up the tumor population is smaller. A cycle of chemotherapy allows this population to be reduced, although it is recovered by cell growth between cycles. Adjuvant therapy for CRC began in the 1970s with regimens based on 5-fluorouracil (5FU), a drug useful in the treatment of advanced disease.[3,4]

The high incidence of lymph node involvement at the time of surgery and recurrence rates of over 50%, support the need to add therapy after removal of macroscopic disease. Micrometastatic disease exists in operated patients considered free of lymph node disease by traditional pathology techniques.

Adjuvant therapy is based on the treatment of residual microscopic disease, theoretically considered a preventive treatment, depending on the risk of disease persistence or recurrence.

The American cooperative group National Surgical Adjuvant Breast and Bowel Project (NSABP) published the first prospective study showing a significant difference in survival of patients operated on for CRC who received postoperative chemotherapy.[5]

Most drugs are common to all countries but immune or molecular therapy varies according to each national health institute, or drug approval system. The main drugs for the chemotherapy treatment of colon cancer are:

- Cytotoxic drugs: 5FU, leucovorin (LV), Capecitabine (Cape), Irinotecan (Iri), Oxaliplatin (OXA).
- Molecular targeted drugs: bevacizumab (Bev), cetuximab (Cetu), panitumumab (Pani), regorafenib (Reg), aflibercept (AFL), ramurizumb (Ram).
- Immune checkpoint inhibitors: pembrolizumab (Pembro).

Chemotherapy regimens

The most commonly used regimens with proven benefits are:

- Monotherapy with fluoropyrimidine: Cape, 5FU + LV.
- Combined therapy with OXA: FOLFOX (5FU ± infusional LV + OXA), CapeOX or CAPOX: (Cape + OXA)
- Combined therapy with Iri: FOLFIRI (5FU + LV + Iri)

Other regimens are:

- FLOX: 5FU ± weekly infusional LV + biweekly OXA
- UFT+LV - tegafur uracil + LV- S-1: tegafur-gimeracil-oteracil potásico

The usual treatment period is 6 months, but it is adapted to each case and each patient.

The guidelines used in different regions of the world can be seen in Table 16.1.

Indications for adjuvant therapy

The principles of adjuvant therapy indication include:

- Stage II colon cancer with increased risk of recurrence.
- Stage III colon cancer with R0 resection: (T1-4 N1-2 M0 of the TNM classification and T1-4 N1-3 M0 of the Japanese classification).
- Stage IV colon cancer after surgical resection.
- Patient recovered from a postoperative complication.
- Patient with performance status PS 0-1.
- Patient without alteration of organ function.
- Patient without other associated complications.

Table 16.1. Chemotherapy regimens by region: Japan (a), USA (b), Europe (c).

JSCCR (a)[6]	NCCN (b)[7]	ESMO (c)[3]
UFT ± LV	FOLFOX	FOLFOX
Cape	CapeOX	CapeOX
S-1	FLOX	Cape
5FU ± LV	Cape	5FU ± LV
FOLFOX	5FU ± LV	
CapeOX		

Elderly patients or those over 70 years of age

Elderly patients or those over 70 years of age with high-risk stage II have not had benefits in OS and DFS with the addition of OXA to the treatment regimen.[1,3,4]

Timing of adjuvant chemotherapy initiation

The timing of adjuvant treatment initiation after surgery remains a matter of debate. Based on the evidence, it is relevant to initiate chemotherapy treatment as soon as possible, ideally no later than 8 weeks after surgery. Evidence IIB.[1,4]

A meta-analysis of 14 studies demonstrated that a delay of more than 8 weeks in the initiation of adjuvant chemotherapy is associated with a higher relative risk of death (HR 1.02; 95% CI 1.15-1.26; p = 0.001).8 Likewise, Japanese guidelines recommend starting adjuvant chemotherapy within 4 to 8 weeks of surgery and continuing it for 6 months.[6]

Although other studies have shown that adjuvant therapy may be useful even if started at 5 or 6 months, the benefit is apparently minimal or virtually nonexistent if treatment is started after 6 months postoperatively.[9]

According to ASRCS, adjuvant chemotherapy should begin within 8 weeks of colon resection. Evidence IB.[4]

Recommendations from Asian guidelines[2]

- The combination of fluoropyrimidine, either 5FU or Cape with OXA, constitutes the basis for adjuvant treatment of stage III colon cancer. Evidence IA.
- Adjuvant treatment of stage III colon cancer based on OXA, can be indicated for 3 to 6 months in the CAPOX regimen and 6 months for the FOLFOX regimen, following the evidence from the IDEA study. Evidence IA.
- According to the IDEA study, adjuvant treatment can be individualized according to 3 risk subgroups: 1) in T1-3 N1: 3 months of CAPOX, 2) in any T4 or any N2: 6 months of

CAPOX and 3) in any of the previous scenarios: 6 months of FOLFOX.
- For patients with intolerance to OXA, Cape or 5FU + LV for 6 months constitute acceptable regimens. Evidence IA.
- It is important to start chemotherapy as soon as possible after surgery, ideally not later than 8 weeks. Evidence IA.

Evaluation of recurrence risk and benefits of adjuvant therapy

TNM staging remains the relevant criterion for postoperative oncologic risk assessment. Five-year survival after surgical resection is 99% for stage I, 68-83% for stage II, 45-65% for stage III, and about 20% for stage IV.[1,2,4]

Recurrence risk assessment is relevant and is estimated taking into account clinical and histological findings and the MMR/MSI status of the tumor.

Adjuvant systemic therapy with 5FU decreases the risk of death by 3-5% in high-risk stage II tumors and by 10-15% in stage III tumors. Adding OXA to the treatment adds 4-5% improvement. Evidence IA.[4]

Stage I tumors usually have a cure rate of more than 90% with surgery, so they do not require adjuvant treatment. The risk is so low that the absolute value of the potential benefit is negligible and the risk/benefit equation due to treatment complications contraindicates therapy.[4]

In stage II and stage III tumors, the risk of residual microscopic disease increases depending on the depth of tumor invasion and the involvement of regional lymph nodes. The risk is variable and in some cases, greater than 50%, so adjuvant treatment is especially relevant.[4]

The particular situation of patients with stage II is highlighted, as they are divided into two groups of low and high risk of recurrence. The definition of the risk of patients with stage II includes other parameters that greatly influence their prognosis. The major prognostic parameters are lymphatic mapping with less than 12 nodes and pT4, including perforation. The minor prognostic parameters are: high grade of tumor differentiation, vascular, lymphatic, or perineural invasion, tumor obstruction and high preoperative CEA level.

In a Memorial Sloan Kettering Cancer Center multivariate analysis of patients with stage II, only 3 factors had independent prognostic influence: T4, elevated preoperative CEA, and lymphovascular or perineural invasion. The 5-year specific survival rate for patients with 1, 2, or more than 2 factors was 95%, 85%, and 57%, respectively.[10]

There is a subgroup of patients with stage II tumors (10-15%) with a very low risk of recurrence, in whom the benefits of fluoropyrimidine have not been demonstrated, and who should therefore not receive adjuvant chemotherapy. Evidence IA.[4]

Molecular aspects in risk assessment

According to NCCN guidelines, all new colon cancers should be evaluated for expression of DNA repair proteins and to rule out MSI (MMR) status.[7]

Five to fifteen percent of colon tumors have this mutation mechanism as the only one related to their pathogenesis, while 3-5% are associated with Lynch syndrome, the most common form of hereditary cancer. They tend to be tumors with greater chemoresistance and their preferred treatment is based on immunotherapy. Another peculiarity is that the incidence decreases as the stage is more advanced; while it is 15% in stage II, it is only 5% in metastatic disease.

Clinically, these tumors are located in the right colon, occur in younger patients (under 45 years of age), are poorly differentiated, mucinous, with signet ring cells and present peritumoral lymphocytic infiltration (marker of MSI-H). They have a better prognosis in stage II, but do not benefit from chemotherapy with 5FU, have a worse prognosis and chemoresistance in stage IV.

MMR/MSI status is the most validated prognostic molecular marker for deciding on adjuvant therapy, in association with other clinical prognostic factors. The dMMR status can be identified by immunohistochemistry (IHC), detecting the loss of MMR protein expression (MLH1, MSH2, MSH6, PMS2), or by polymerase chain reaction (PCR) that determines the MS status and microsatellite mutation. The determination of the MMR/MS status in localized colon tumors has two objectives: to characterize the prognosis and predict the benefit of adjuvant therapy, and on the other hand, to determine the genetic predisposition.[11]

MSI/dMMR is more prevalent in stage II than in stage III (21 vs. 14%). dMMR tumors are typically located in the right colon, have a mucinous histology with tumor-infiltrating lymphocytes, and have a better prognosis than tumors with microsatellite stability (MSS).[12,13]

Determination of MMR/MS status is important to rule out Lynch syndrome. Loss of MSH2 and/or MSH6 protein on IHC indicates suspicion of Lynch syndrome, whereas loss of MLH1 and PMS2 requires investigation of the BRAF mutation or hypermethylation of the MLH1 promoter region, since the identification of some of these mutations suggests a high probability of an acquired alteration of the somatic MLH1 gene rather than Lynch syndrome.[14] In addition to the implication of the diagnosis of Lynch syndrome, dMMR/MS status defines a subgroup of patients with a better prognosis and less benefit from chemotherapy.[15]

MMR/MS status determination should be requested in all cases based on the following premises according to the stage:
- Stage I: The determination of instability does not modify the approach. Its advantage is to perform individual and family genetic screening.
- Stage II: Unlike what happens in stable patients, the prognosis of unstable patients worsens with fluoropyrimidine-based chemotherapy, so to decide on adjuvant chemotherapy, especially in patients with a high risk of recurrence (less than 12 nodes examined, perineural or lymphovascular invasion), it is necessary to know the MMR status. dMMR patients do not benefit from chemotherapy with 5FU.
- Stage III (pT3N+M0): The standard is surgery followed by adjuvant therapy with FOLFOX or CAPOX/XELOX. The determination of MSI does not modify the indication for chemotherapy. However, early studies such as NICHE (neoadjuvant therapy in early colon cancer) showed an almost complete pathological response and minimal residual disease in unstable patients, so it could be a validated future therapy, even in localized tumors.
- Stage IV: According to the Keynote 177 study, determining stability first should be the standard, reserving immunotherapy with pembrolizumab for unstable tumors due to the better outcomes achieved.

Biomarkers

Currently, for routine assessment of resistance risk in non-metastatic patients, the study of MSI/MMR status and other genetic markers such as RAS and BRAF and their mutations is recommended.

Other biomarkers, such as genetic signature, Immunoscore, CD-X2 and postoperative circulating DNA, have shown some benefits in determining prognosis.[16–18]

Genetic signatures

Genetic signatures have emerged for prognostic stratification in locoregional disease, i.e. for an accurate and personalized assessment of the risk of relapse and the benefits of chemotherapy. The best documented validated tool is Oncotype DX and the FxColon gene. They are obtained from formalin-fixed, paraffin-embedded tumor samples.

The Oncotype DX is a test that quantifies the expression of 5 reference genes and 7 recurrence risk genes, with a prognostic classification that establishes the low, intermediate and high probability of recurrence of colon cancer. It is used in tumor samples from patients with stage II. The Cancer and Leukemia Group B (CALGB) study showed an average recurrence index of 31.4 (range: 2-78). An increase of 25 points in the index was significantly associated with the risk of recurrence.[19]

In a similar analysis from the National Surgical Adjuvant Breast and Bowel Project (NSABP C-07), in patients with stage II, a high recurrence index was associated with a higher recurrence rate (HR 2.11; $p < 0.001$), and lower OS and DFS when compared with a low recurrence index, with an increased benefit of OXA in the chemotherapy regimen.[20]

This was corroborated by the QUASAR study (13-gene study) which reported a 3-year recurrence of 12, 18 and 22% in stage II patients classified as low, intermediate and high risk, respectively.[21]

ColorPrint is a multigene assay that studies the expression of 18 genes and quantifies the high or low probability of cancer recurrence. In a study of 206 patients with stage II-III colon cancer, the 5-year recurrence-free survival rate was 88% for those with low probability and 67% for those with high probability.[22]

ColDx is a multigene assay that uses 634 helper tests to identify patients with stage II colon cancer at high risk of recurrence. In one study, those identified by this assay had a high risk of recurrence and decreased recurrence-free survival compared to those at low risk.[23]

Following the above-mentioned studies, genetic signature analysis could be considered to complement histopathological findings in order to determine chemotherapy treatment in stage II patients. For example, treating T3N0 patients, classified as high risk based on their genetic signature, and avoiding chemotherapy in T4N0 patients classified as low risk. Evidence IIC. However, this has not been validated by any regulatory entity and according to ASCO, NCCN and ESMO there is insufficient data to recommend the use of multigene panels to determine adjuvant therapy.[1,7,24]

Immunoscore

The Immunoscore involves quantification of the T cell population, particularly CD3+ and CD8+, in the center of the tumor and at the invasion margins, using histopathology. It is both a prognostic and predictive test.

The Immunoscore was recently validated in a prospective cohort of over 2500 patients with stage I-III. It was a strong predictor of time to recurrence, OS, and DFS, independently of age, sex, MSI status, and other prognostic factors.[25]

A study from the Society for Immunotherapy for Cancer, in an international consortium of 14 centers in 13 countries, retrospectively evaluated a standardized Immunoscore assay in patients with stage I-III. stage I-III. primary colon tumors, demonstrating that it provides reliable evidence on the risk of recurrence.[26]

A comparative evaluation of the outcome of patients receiving adjuvant chemotherapy vs. observation demonstrated a survival benefit of chemotherapy only in patients with a high Immunoscore.[27]

Retrospective analysis of evidence from the French study, which included patients with stage III colon cancer at low and high clinical risk, demonstrated that an intermediate and high Immunoscore significantly predicts the benefit of administering 6 vs. 3 months of adjuvant therapy with FOLFOX regimen.[28]

Retrospective analysis of the French study, which included patients with stage II colon cancer at low and high clinical risk, demonstrated that an intermediate and high Immunoscore significantly predicts the benefit of administering 6 vs. 3 months of adjuvant therapy with FOLFOX regimen.[2]

The Immunoscore could be used as an additional prognostic information to identify high-risk stage II and stage III subgroups of patients and redefine the benefit of adjuvant chemotherapy. Evidence IIIC-IVB.[2]

CD X2

CDX2 is a transcription factor that has recently been shown to be important in identifying high-risk stage II colon cancer patients who may benefit from adjuvant chemotherapy. Patients with CDX2-negative tumors had a significantly lower 5-year DFS than those with CDX2-positive tumors. In turn, the incidence of 5-year DFS was higher in CDX2-negative patients treated with adjuvant chemotherapy than in those without treatment (91 vs. 56%; $p = 0.006$; respectively).[16]

According to the ASCRS in its latest publication of practice parameters, multigene assays, CDX2 expression analysis and circulating tumor DNA (ctDNA) or liquid biopsy, should be used to complement the multidisciplinary decision in stage II and stage III colon cancer patients. Evidence IB.[4]

Circulating tumor DNA (ctDNA) or liquid biopsy

Liquid biopsy is the determination of tumor DNA fragments that are in the bloodstream and can be used as markers of residual or recurrent disease. The presence of ctDNA can be used both for risk assessment and to identify patients at high risk of recurrence.

Liquid biopsy may also be useful in follow-up after surgical resection and adjuvant chemotherapy, to detect recurrences earlier than with current follow-up procedures.

A correlation between decreased ctDNA and tumor response has been demonstrated during systemic therapy for metastatic colon cancer. Thus, ctDNA is being studied to determine whether it could be a useful marker for monitoring adjuvant treatment.[29]

There are some studies worth highlighting, such as a prospective analysis of patients with stage II colon cancer, in which the detection of ctDNA immediately after completing adjuvant chemotherapy was associated with a lower recurrence-free survival.

In a similar study, patients with stage III colon cancer with detectable ctDNA after completing adjuvant treatment had a recurrence-free survival of 30% compared with 77% in those without detectable ctDNA.[30] An additional study reported a 17-fold increased risk of recurrence if ctDNA remained detectable after completing adjuvant chemotherapy.[17]

In another study, in patients not treated with chemotherapy, positive ctDNA was detected in 7.9%, with a recurrence rate of 79%. Recurrence occurred in only 9.8% of patients with negative ctDNA, with a statistical difference in patients treated with chemotherapy. Positive ctDNA after completion of chemotherapy was associated with a lower DFS.[31]

Postoperative determination of ctDNA in stage II patients has been shown in these early studies to provide direct evidence of residual disease, and to identify patients at very high risk of recurrence. Two trials (CIRCULATE-IDEA and CIRCULATE-EUROPE) are under development to determine the role of liquid biopsy.

In the recently published study by Hofste et al.[32] on 53 patients with colorectal metastases resected with curative intent, mutation analysis of 15 specific tumor genes and determination of ctDNA were performed preoperatively and one week after surgery. ctDNA was detected in preoperative samples in 88% of patients who did not receive preoperative systemic treatment, 55% of patients with chemotherapy, 75% of patients with no pathological response, and 0% of patients with good pathological response ($p < 0.06$). Postoperatively, ctDNA was found in 80% of patients with incomplete resection and 0% of patients with complete resection ($p = 0.003$).

The DYNAMIC study was based on the premise that postoperative ctDNA predicts very low recurrence-free survival, whereas its absence predicts a low risk of recurrence. Patients with a positive postoperative liquid biopsy received adjuvant chemotherapy, whereas those with a negative result did not receive treatment. The 3-year recurrence-free survival was 86.4 and 92.5%, respectively. Liquid biopsy reduced the use of adjuvant chemotherapy in patients with stage II, without compromising recurrence-free survival.[33]

There are numerous trials in development (9NCT04068103 COBRA, NCT 04120701 CIRCULATE, ACTRN12615000381583 or DYNAMIC-II) that may answer whether ctDNA can be successfully used as a marker of survival, recurrence, or effectiveness of adjuvant therapy.

Current NIH guidelines through NCCN state that these assays can report the risk of cancer recurrence on other risk factors, but consider that there is insufficient evidence to recommend the use of liquid biopsy in daily clinical practice to determine adjuvant treatment.[7] The same situation occurs with the guidelines of the European Society of Medical Oncology (ESMO).[1,34] The Japanese guidelines only include the determination of the MSI/MMR status.[6]

Recommendations

- The risk of recurrence after surgery for colon cancer should be assessed by integrating the TNM system, MMR/MS status, and the number of lymph nodes examined. Evidence IIIA.
- To redefine the risk of recurrence in patients with stage II, other additional clinical-pathological factors should be used, such as the histological subtype and its grade of differentiation, venous, lymphatic, or perineural invasion, lymphoid inflammatory response, involvement of resection margins, and CEA level. Evidence IIIA.
- The patient's age has no predictive value for indicating or not adjuvant therapy; other situations such as life expectancy and comorbidities should be considered. However, it can be generalized that the combination of fluoropyrimidine and OXA seems to have a more limited benefit with a greater possibility of toxicity in elderly patients.
- MS/MMR status is the only molecular marker validated and used in the decision of adjuvant treatment in patients with stage II. In stage III, its use is limited to identifying Lynch syndrome. Evidence IVA.
- Determination of dihydropyrimidine dehydrogenase deficiency, both genotypically and phenotypically, is recommended before adjuvant fluoropyrimidine-based treatment, in order to avoid adverse effects. Evidence IIIA.
- Gene expression signature is not routinely recommended in practice given its lack of predictive value for the benefit of chemotherapy. However, it can be used to complement the clinical-pathological evaluation in patients with intermediate-risk stage II. Evidence IIC.
- The Immunoscore could be considered together with the TNM to redefine the prognosis of early colon cancer and adjust the decision of adjuvant treatment in patients with stage II, or low-risk stage III. Evidence IIIC.

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