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XXVIII CYCLE

# FAST-TRACK SURGERY IN ELDERLY PATIENTS: ENHANCED POSTOPERATIVE RECOVERY.

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# SUMMARY

# ABSTRACTS


## PAPER I

**Background** The elderly patients represent a potential target of enhanced recovery program (ERP) after surgery, even though effectiveness and feasibility of ERP in this population still remain unclear.

**Objectives** Evaluation of safety, feasibility of ERP enhanced by a rehabilitation device in patients 70 years of age or older after colorectal surgery, focusing on postoperative recovery and length-of-hospital-stay.

**Methods** Single-center, randomized, parallel-group trial comparing a multimodal ERP enhanced by a rehabilitation device vs. traditional care in elderly patients undergoing colorectal surgery. All patients underwent preoperative physiatric evaluation in preoperative setting and at time of hospital discharge.

**Results** Twenty-six patients were enrolled. ERP enhanced by a rehabilitation device in elderly patients was safe and feasible. Postoperative pain was lower in the ERP group even if not significant. Recovery of the bowel function was earlier for ERP patients (first flatus 1.8 vs. 2.4 days, $p<0.001$; defaecation 2.3 vs. 3.6 days, $p<0.001$). Early mobilization within postoperative day 2 was achieved in the 80% of ERP patients vs. 25% of controls ($p=0.010$), with a significant reduction of the rehabilitation time (sitting 1.7 vs.2.4 days, $p=0.068$; standing 1.9 vs.3.3 days, $p=0.003$; deambulation 2.6 vs.3.8 days, $p=0.013$). Recovery of physical performance was similar for the two groups albeit better for ERP patients (% variation 6MWD -26 vs. -38 $p=0.374$). Length-of-hospital-stay was similar although lower for ERP patients (LOS 9.00 vs. 9.75, $p=0.617$).

**Conclusions** ERP is feasible and safe, with a significant reduction in time to recovery of the bowel function and physical-performance even in the elderly patient.

**PAPER II**

**Objectives** Topoisomerase I (Topo I) and thymidylate synthase (TS) are essential enzymes for the replication, tran-scription and repair of DNA, and are potential biomarkers in colorectal cancer (CRC). The aim of the study was to correlate the tissue expression of Topo I and TS in sporadic CRCs with relevant pathological and molecular features and patients' outcome.

**Methods** Topo I and TS expression was assessed by immunostaining in 112 consecutive primary CRCs.

**Results** Increased expression of Topo I was found in 36% of tumors, preferentially rectal (50%) and with not otherwise specified (NOS) histology (44%). Topo I expression was associated with 18q allelic loss (LOH), ($p = 0.013$), microsatellite stable phenotype ($p = 0.002$) and normal expression of mismatch proteins hMLH1 and hMSH2 ($p = 0.0012$ and $p = 0.02$, respectively). High TS expression was found in 60% of tumors, more frequently in distal sites (62%) and with NOS histology (66%); no association with microsatellite instability was observed.

**Conclusions** Topo I seems to be involved in the chromosomal instability pathway of sporadic CRCs. Conversely, high TS expression is unlikely to affect the clinical behavior of microsatellite unstable CRCs.

**PAPER III**

**Objectives** Owing to the low incidence and the lack of a definition, many aspects of multiple sporadic colorectal cancers (MSCRC) remain to be cleared, in particular long-term survival and the prognostic influence of extent of surgical resection (multiple segmental resections vs. total/subtotal colectomy).

**Methods** A retrospective review was performed of patients diagnosed with multiple sporadic colorectal cancer from 1982 to May 2010. Clinical and pathologic data were collected and reviewed. Survival analysis was conducted comparing cases to 4790 controls resected with curative intent for single colorectal cancer, from the Parma Cancer Registry.

**Results** We identified 23 patients diagnosed with MSCRC, 8 with synchronous cancer (SC) and 15 with metachronous cancer (MC). Of the MC patients, 2 (13%) had the second cancer within 2 years, 4 (27%) in the time period of 2–5 years and 9 (60%) after 5 years. Twenty one patients underwent multiple segmental resections; 2 patients underwent subtotal colectomy. No total colectomy was performed. The 5-year overall survival rate of SC, MC and control patients was 100%, 87% and 55% ($p<0.001$) respectively; the 5-year cancer-free survival was 88% and 94% for SC and MC patients respectively.

**Conclusions** Synchronous and metachronous multiple sporadic colorectal cancer patients showed a better prognosis than single sporadic colorectal cancer patients. Multiple segmental colorectal resections seem appropriate from an oncologic point of view in the elective treatment of multiple sporadic colorectal cancer.

## LIST OF PUBLICATIONS

1. Role of topoisomerase I and thymidylate synthase expression in sporadic colorectal cancer: Associations with clinicopathological and molecular features.Azzoni C, Bottarelli L, Cecchini S, Ziccarelli A, Campanini N, Bordi C, Sarli L, Silini EM. *Pathol Res Pract. 2014* Feb;210(2):111-7. doi: 10.1016/j.prp.2013.11.004. Epub 2013 Nov 22.

2. Long-Term Survival Of Multiple Sporadic Colorectal Carcinoma. Cecchini S, Azzoni C, Bottarelli L, Marchesi F, Giulini L, Silini EM, Roncoroni L. *Submitted to International Journal of Colorectal Disease.*

# INTRODUCTION

## PAPER I

In the early 1990's surgery underwent revolutionary changes due to the introduction of ultra short acting anaesthetics, the development of regional anaesthetic techniques to control pain and the use of minimally invasive open or laparoscopic techniques in surgery. Combining these new anaesthetic and analgesic methods with these new surgical techniques in a treatment pathway shortened the time required for the early and intermediate postoperative recovery considerably. In ambulatory surgery, patients regained vital reflexes in the operating room and could directly be transferred to the day surgery unit, thereby bypassing the post anaesthesia care unit (PACU). This so termed "fast tracking" treatment path was aimed to streamline ambulatory surgery. Recovery times of patients who were fast tracked were significantly shorter compared to those for patients who were not fast tracked [1] and patients that were able to bypass the PACU were discharged home earlier than patients who were not [2,3] . Other arguments brought up to justify the "fast tracking" concept were reduced nursing workload, reduced costs and improved quality of care by focussing attention on getting patients back to their preoperative status as quickly as possible [4-6] The expansion of the fast track concept to more major surgical procedures resulted in an increasing number of surgical procedures being performed as short stay surgery. Prostatectomies, pneumonectomies, total hip and knee replacements and peripheral vascular reconstructions are procedures that are nowadays performed in a short stay setting [7] .

### *Fast-track colonic surgery*

The expansion of the fast track concept to colonic surgery has been pioneered by Henrik Kehlet, a surgeon of the Hvidovre University Hospital in Denmark. He reported on a median postoperative hospital stay of two days in 60 consecutive colectomy patients who were treated in a fast track surgery programme [8] , whereas colonic surgery usually requires a postoperative hospital stay of 5-10 days [9].

The median age of the patients was 74 years and most of the patients had comorbidity; only 18 patients were considered to have no significant concomitant illnesses and had normal preoperative mobility. Within two days, patients tolerated early nutrition, all but three of the patients had defecation and patients were spending between five and six hours out of bed. Kehlet emphasized that major improvements in recovery may require a multifaceted approach [10] .Stress induced organ dysfunction, pain, nausea and vomiting, ileus, hypoxemia and sleep disturbances, fatigue, immobilisation and semi-starvation and traditional perioperative care principles such as the use of drains, nasogastric tubes, fasting regimes and bed rest were identified as key factors that contribute to the postoperative functional deterioration.

Kehlet developed a multimodal rehabilitation programme that combined a number of interventions to reduce the stress of the surgical procedure, risk of organ dysfunction and loss of functional capacity. This multimodal programme involved the cancellation of traditional perioperative care principles such as immobilisation, drains, nasogastric tubes and fasting, while introducing innovations such as carbohydrate loaded liquids before surgery, regional anaesthetic techniques, minimally invasive open or laparoscopic surgical techniques, maintenance of normal temperature during surgery, optimal treatment of postoperative pain and prophylaxis for nausea and vomiting [11,12].

The application of this multimodal rehabilitation programme after colonic resections significantly reduced length of hospitalisation [8-13] and significantly improved postoperative recovery, physical performance, pulmonary function, body composition [14] and convalescence [15] .

Other single centre groups have subsequently confirmed that the application of such fast track multimodal perioperative care programmes in colorectal surgery patients results in a reduced length of hospital stay, less morbidity, reduced postoperative ileus, improved pulmonary function, less pain, and less fatigue [16,17]

***Enhanced recovery after surgery (ERAS)***

In 2001 the Enhanced Recovery After Surgery (ERAS) group was established as a collaboration of university or specialised departments of surgery from five northern European centres: Kehlet's group of the Hvidovre University Hospital, Hvidovre, Denmark, practicing fast track surgery and four centres practicing a more traditional pattern of care: the Royal Infirmary, Edinburgh, UK, the Karolinska Institutet at Ersta Hospital, Stockholm, Sweden, the University Hospital of Northern Norway, Tromsø, Norway and Maastricht University Medical Centre, Maastricht, the Netherlands. The ERAS group reviewed the case-mix, clinical management and clinical outcomes of colorectal surgery patients in these five participating centres [18] . A retrospective chart review was undertaken over a one year period between 1998 and 2001 and 451 consecutive patients undergoing an open colorectal resection above the peritoneal reflection were evaluated.

As expected, length of stay was significantly shorter in the "fast track" centre of Denmark compared to the centres practicing traditional care (a median of 2 days versus a median of 7-9 days). Based on patient demographics such as age, BMI, gender and P-Possum scores it was concluded that the surgical populations were comparable between the five centres and that case-mix did not account for the reduced length of hospitalisation. The reduction in length of stay in Denmark had no significant influence on morbidity and 30-day mortality, but was associated with increased readmission rates. The elements of perioperative care applied in each hospital varied widely, both within the traditional care centres and in comparison with the centre performing fast track surgery. With the Danish experience as a starting point, the ERAS group considered the evidence base for individual components of perioperative care and developed a new evidence based programme (the ERAS protocol) that presented recommendations on how surgical patients undergoing a colonic resection should be cared for. [19] Using a multidisciplinary team approach, with a focus on stress reduction and early return of function, the ERAS protocol aims to allow patients to recover more quickly from major surgery.

The key recommendations of the ERAS guideline comprise preoperative patient information and

counselling, no oral bowel preparation, no preoperative fasting, preoperative carbohydrate loading, no pre-anaesthetic medication, single dose antibiotic prophylaxis half an hour before surgery, short incisions, avoidance of long acting opioids, mid-thoracic epidural anaesthesia, paracetamol as baseline analgesic, avoidance of fluid overload, use of an upper-body forced-air heating cover, no drains, no nasogastric decompression tubes, prevention of postoperative nausea and vomiting in risk patients, standard laxatives, early removal of urinary bladder catheters, early oral nutrition and nutritional supplements and early mobilisation.

***Rationale***

Since age is a demonstrated risk factor for delayed recovery and consequently prolonged hospital stay after surgery, the elderly patients represent a potential target of ERP. However concerns exist regarding feasibility and efficacy of such programs on this specific population due to the high morbidity and limited compliance, even if results of recent studies appear encouraging. In a recent trial on ERP in elderly patients following colorectal resection, Pawa et al. reported tolerance for oral fluid intake and oral diet on first postoperative day in 89% and 77% of patients, respectively, whereas early mobilization on first postoperative day was achieved in about 20% of patients. [20] These results show how ERP in elderly patients could be feasible although physical rehabilitation remains the weakest link of this program. Recently, new strategies have been developed introducing innovative rehabilitation devices in clinical practice. Burtin et al. tested a cycle ergometer applied on the bed of intensive care unit (ICU) patients reporting enhanced recovery of functional exercise capacity, self-perceived functional status and muscle force at hospital discharge. [21] Elderly patients present a reduced functional capacity unable to tolerate early forced mobilization. Therefore rehabilitation devices could be the milestone of ERPs in elderly patients.

***Specific aims***

The primary aim is to evaluate the efficacy of a post-operative multimodal care program enhanced by a rehabilitation device in patients 70 years of age or older after colorectal surgery, focusing on recovery of functional exercise capacity and length of hospital stay.

The second aim is to estimate costs, examining impact on length of hospital stay, drop-out rate and clinical factors able to predict the drop-out or the failure of the program.

Finally the last aim is to evaluate the impact of ERP on pain perception and self-perceived functional status.

***Hypothesis***

The introduction of early exercise training on supine position starting on postoperative day one in ERP following colorectal surgery in patients 70 years of age or older could enhance recovery of functional exercise capacity without increasing morbidity, shorten hospital stay and improve the self-perceived functional status.

**PAPER II**

Colorectal cancer (CRC) is the third most common cancer in men and the second in women worldwide. According to WHO,there were 1.2 million new diagnoses of CRC in 2008, and more than 608,000 deaths [1,2]. CRC development follows two major pathways of genetic instability: chromosomal instability (CIN), observed in 85% of sporadic CRCs, and microsatellite instability (MSI), accounting for approximately 15% of cases [3]. CIN-related CRCs are characterized by gross chromosomal rearrangements, aneuploidy, defects in checkpoints for G1/S entry, and loss of heterozygosity (LOH) in particular at chromosomal arm 18q [3–5]. Conversely, MSI-related CRCs have diploid or nearly diploid karyotypes and show defects of the DNA mismatch repair system (MMR) genes [3]. CRCs referred to CIN or MSI pathway display relevant clinic-pathological differences as to tumor site, histology and response to adjuvant therapy [6]. Several studies have also demonstrated that the molecular phenotype may affect CRC outcome [7–10]. In particular, CRCs with MSI have been associated with improved survival [11], whereas 18qLOH is a molecular marker of adverse prognosis [7,8,12–21]. Several biological markers have been investigated for a prognostic role in CRC [22], among which are topoisomerase I (Topo I) and thymidylate synthase (TS), whose expression levels correlate with survival although this evidence is controversial among different studies [23–28]. Topo I is an essential enzyme in regulating the topology of supercoiled DNA by transiently cleaving of one of the two strands[29]. Antineoplastic drugs targeting Topo I, such as irinotecan and camptothecins, form stable Topo I-DNA cleavage complexes and inhibit Topo I activity, thus preventing DNA religation [30,31]. Topo I is expressed in primary CRCs and metastases, but it is debated whether its expression can predict the response to anti-Topo I treatments [32–35]. TS catalyzes the conversion of dUMP to dTMP and is essential for ‘de novo’ DNA synthesis [36,37]. The expression of TS may affect tumor sensitivity to fluoropyrimidines, such as 5-fluorouracil (5-FU) [38]. 5-FU-based treatment is the standard of care for adjuvant therapy of CRC in combination with oxaliplatin [39] and for the treatment of metastatic disease in association with oxaliplatin or irinotecan [40–43].

**PAPER III**

Multiple primary colorectal cancers account for 2-5% of all colorectal cancers. The incidence of multiple primary colorectal cancer increases to up to 20% in patients diagnosed with heredo-familial syndrome [1,2]. About 25% of patients with colorectal cancer have a family history of colorectal cancer that suggests a hereditary contribution, common exposures among family members, or a combination of both, whereas the majority of patients have a sporadic disease with no apparent evidence of having inherited the disorder. Multiple colorectal cancer can occur in the absence of a defined heredo-familial syndrome, presenting as metachronous carcinomas (MC) in patients with a history of sporadic colorectal cancer or two or more sporadic synchronous carcinomas (SC) at the diagnosis. A personal history of colorectal cancer is a well-known risk factor for developing a second colorectal cancer and it is estimated that in patients undergoing resection of a single colorectal cancer, metachronous colorectal cancer develops in 1.5% to 3% of cases within the first 5 years postoperatively. The risk remains high for up to ten years in some patients [3,4]. Over one-half of second primary colorectal cancers arise within 24 months of the initial resection and may represent SCs that were missed initially [5,6]. The prognosis of multiple colorectal cancer remains controversial, and although postoperative surveillance is highly recommended for detecting metachronous cancers or polyps, the optimal frequency and benefits of postoperative colonoscopy are still under debate [7-10]. Moreover, while in younger patients affected by heredo-familial colorectal cancer syndromes a prophylactic total colectomy is recommended instead of multiple segmental colorectal resections, in multiple sporadic colorectal cancer this indication seems unclear and not supported by current evidence [11]. The purpose of the present study is to better define MSCRC from the clinical, therapeutic, pathologic and prognostic points of view.

# METHODS

## PAPER I

### *Study design*

The present study is a single-center, randomized, parallel-group trial comparing a post-operative multimodal care program enhanced by a rehabilitation device, named "delettizzatore" vs. traditional care in elderly patients undergoing colorectal surgery with curative intent.

### *Exclusion criteria*

The exclusion criteria were the following: age <70 years, severe agitation or psychiatric disorders determing insufficient compliance to the protocol, preexisting diagnosis causing neuromuscular weakness unabling the patient to undergo passive rehabilitation of lower limbs, history of acute stroke, history of status epilepticus, severe obesity (BMI>35), serious bedsore or venous ulcers, coagulation disorders (INR>1.5 , PLT< 50*10^3/$mm^3$), vascular or cardiorespiratory instability ($FIO_2$>55%, $PaO_2$<65 torr, minute ventilation >150 mL/kg, RR>30 breaths per minute with adequate ventilatory support, need for vasopressor support). [21]

### *Pre-operative work-up*

Data were collected as to age, gender, BMI, ASA score, type of operation, operating technique (open vs VL), operating time, conversion rate, length of hospital stay, post-operative nausea and vomiting, post-operative pain, first flatus and first bowel movement, overall and specific (surgical and non-surgical) post-operative morbidity and mortality. The level of pain was measured by the visual analog scale (VAS 0–10). The patients were evaluated for the development of post-operative complications according to the Clavien-Dindo classification. [22] All patients underwent a preoperative physiatric evaluation, aimed to accurately evaluate the pre-operative performance

status, through the following tests: Berg Balance Scale [23], Short Form 36 Health Survey questionnaire (SF-36) [24], 6-min walking distance (6MWD) [25]. After one day, each patient underwent a training session on the sperimental rehabilitation device in clinostatic position, followed by a quantification of exercise capacity of the patient, named "3-minutes-test (3MT). Similarly to the 6MWD, the 3MT measures the number of the full range motion of the exercise that the patient is able to complete, defined as repetitions.

During the session a continuous monitoring of vital parameters, including hearth rate (HR), pre- and post-session systolic and dyastolic arterial pressure (SAP and DAP), trans-dermic oxygen saturation ($SpO_2$) and respiratory-rate (RR), was performed. The 3MT or the rehabilitative session on the device were interrupted in presence of at least one critical variation of the vital parameters observed during the clinical monitoring as increase equal or greater than 70% of the calculated maximum HR, decrease equal or greater than 20% of basal HR, SAP equal ore greater than 180 mmHg, decrease of DAP lower than 20%, $SpO_2$ lower than 90% or develop of signs/symptoms of cardio-respiratory distress.

***ERAS protocol (Tab.1)***

1. **Peri-operative nutrition**: normal diet till 10pm of the day before surgery. One carton of nutritional drink supplement of 250 mL (5% carbs) 2 hours before surgical procedure. Oral drink 30-50 mL di Ensure (400 g; Abbott, Chicago, IL, USA) dilued with water (1 Kcal/mL) every 1-2 hours starting from 6-12 hours after surgery and during PO day 1. Starting from PO day 2 administration of 100-200 mL every 2-3 hours of Ensure (400 g; Abbott, Chicago, IL, USA) with addtion of water (1 Kcal/mL).
2. **Bowel preparazion**: Administration of 16 tablets of Simeticone during the day (Simeticon cp). Phosphate enema at 7 am of the day before surgery.
3. **Anaesthesia and postoperative analgesia**: a thoracic epidural catheter was inserted at T7-T9 and 6-9 ml 0,5% bupivacaine was administered before operation. Thereafter general

anaesthesia was induced by midazolam 0,15 mg/kg, fentanyl 0,1-0,25 mg and enflurane and nitrous oxide-oxygen. The epidural blockade was mantained with 0,5% bupivacaine 4ml/h during operation and continued after operation for 48h with 0,25% bupivacaine 4ml/h. At hte end of surgery 20 ml 0,25% bupivacaine was injected beneath the fascia and subcutaneously in the largest incision. After operation, oral tenoxicam 20 mg or paracetamol 2 g 12 hourly was given daily for 6 days. Rescue analgesia included ibuprofen 600 mg every 8h. Opioids were not given routinely in order to avoid cerebral and bowel motility side-effects. Intraoperative intravenous fluid was restricted to a maximum of 1500 ml, unless bleeding indicated otherwise.

4. **Management of surgical devices**: naso-gastric tube was posistioned intraoperatively and removed at the end of the procedure or kept in selected cases of higher risk of postoperative ileum. The urinary bladder catheter was positioned intraoperatively and removed on the morning after operation. Intra-abdominal drain was removed as soon as possible depending on the operator indication.
5. **Early mobilization**: contrary to the other ERAS protocol in which mobilization is enforced starting on the the day of the operation, [26] we opted for a gradual rehabilitation program. An US of lower limbs was performed to rule out deep vein thrombosis. Subsequently, we administred an active physiscal exercise session of 15 minutes with the device "delettizzatore" twice a day, until the patient is able to walk independently.

***Perioperative traditional care program (Tab.2)***

1. **Peri-operative nutrition**: normal diet till 10pm of the day before surgery. Stop fluids intake at 12pm. NPT Clinimix 15% or glucosate solutions 2000 ml/day till normal diet is tolerated. Re-introduce fluids intake after the naso-gastric tube removal (between PO day 2 and 3).

2. **Bowel preparation**: laxative Polietilenglicole 2 liters 2 days before surgery and 2 liters the day before surgery. Administration of 16 tablets of Simeticone during the day (Simeticon cp).
3. **Postoperative analgesia**: NSAID and opioids on first line.
6. **Management of surgical devices**: naso-gastric tube was posistioned intraoperatively and removed at the po day of the first flatus or kept in selected cases of higher risk of postoperative ileum. The urinary bladder catheter was positioned intraoperatively and removed on po day 5. Intra-abdominal drain was removed the day before the discharge (po day 6-7).
4. **Mobilization**: starting from the po day 2 the patient is enforced to sit, then the patient is enforced to stand and walk with assistance in po day 3 and 4.

***Enrollement***

Every patient 70 years of age or older referred to our surgical unit, Clinica Chirurgica e Terapia Chirurgica, Maggiore Hospital, Parma, Italy diagnosed with colorectal cancer has been screened for inclusion/exclusion criteria. All potential candidates for the clinical enrollement underwent a counselling with an investigator of the protocol, aimed to inform the patient, define the eligibility for the enrollement and acquire informed consent.

***Randomization***

Patients were assigned to the two arms through a block randomization.

Using the web-based software *www.randomizer.org* was elaborated a list of random balanced allocation of 43 blocks of 2 cases (86 cases). Allocation list was kept at Secretary of Clinica Chirurgica dei Trapianti d'Organo, Prof. M.Sianesi, Department of Surgical Sciences, Parma

University, Parma, Italy. Treatment allocation was conducted following the numerical sequence of the list and comunicated by phone to the investigator at the time of the enrollement.

***Exercise with rehabilitation device***

The rehabilitation device “delettizzatore” allows an active exercise work-out of the lower limbs in clinostatic position, without involvement of the abdominal muscles. (fig.1)

- **3MT:** every patient underwent the 3MT (3 minute-test) with delettizzatore in pre-operative setting (6 days before surgery) and in post-operative setting at discharge (starting from PO day 6). The aim of the pre-operative test is the quantification of the performance of the patient (L0), under clinical monitoring of the vital paramters.
- **Post-operative application:** patients assigned to the sperimental arm underwent 2 exercise session per day of 15 minutes, with delettizzatore, under clinical monitoring of the vital paramters. Patients were encouraged to perform active exercise according to the patient’s capability. Ambulation was started when considered appropriate by the medical staff.

***Rehabilitation device "Delettizzatore"***

The "Delettizzatore" is a mobile experimental rehabilitation device. It can be positioned easily to the bed, locked into position and adjusted. This device allows to patients to perform active physical exercise in clinostatic position. (Fig.1)

The Delettizzatore is a registred trademark of **ADVANCED DISTRIBUTION S.p.A** - Via Peano, 70 – 10040 Leinì (TO) and it is officially registered as a medical aid of class I with attachment I e VII of direttiva 93/42 with CE registration (model: DLT ; n.inv: 001-01 ; year: 2012).

***Statistical analysis***

Statistical analysis was performed using the software package SPSS 23.0 (SPSS, Chicago, IL, USA). Descriptive data of continuous variables were expressed by the median or mean ± SD. We used Student's t test and the Mann– Whitney U test for comparing means when appropriate. General liner model for repeated measures was used for compared trend of postoperative pain between groups. A difference of $p \leq 0.05$ was considered statistically significant.

## PAPER II

### *Study design*

This study is a retrospective evaluation of 112 unselected, consecutive, primary CRCs that underwent curative resection between January 1997 and April 1999 at our institution, aimed to assess the expression of Topo I and TS in tumor tissue by immunostaining and to correlate it with pathological and clinical variables, patients' outcomes and molecular characteristics, such as MSI status, LOH at different loci and other markers implicated in CRC carcinogenesis.

### *Patients*

The study is a retrospective evaluation of 112 unselected, consecutive, primary CRCs that underwent curative resection between January 1997 and April 1999 at our institution (Table 1). There were 58 males (mean age: 69 years, range 27–94) and 54 females (mean age 69 years, range 45–91). Tumors were located in the proximal colon in 42 patients (37%), in the distal colon in 50 patients (45%), in the rectum in 20 patients (18%), and they were predominantly poorly differentiated (G3) adenocarcinomas (62%) with not otherwise specified (NOS) histology (66%). The tumor stage was determined according to the American Joint Committee on Carcinoma (AJCC) system [44]; most cases were stage II (53%). All cases were deemed sporadic, based on the absence of relevant family history as recorded prospectively at the initial patient interview. During the study period, a uniform surgical management protocol was adopted. Data on clinical and pathological characteristics and chemotherapy were obtained from surgical, pathological and oncological records. Recurrence and survival data were followed-up to September 2007 (censoring date) using databases on hospital admission and the National Central Registry on death recording. Overall recurrence rate was 16% (18 patients); 12 patients (11%) had local recurrence without metastases, 6 patients (5%) had local recurrence with metastases.

Patients with AJCC stage III colon cancer under 75 years old were eligible for adjuvant chemotherapy [45]; however, this treatment option was exerted at discretion of the patient and the oncologist. The standard drug regimen was 375 mg/m2/d 5-FU and 20 mg/m2/d levamisole, 5 days/week every 4 weeks for 6 months. Patients with rectal cancer received irradiation therapy administered in a dosage of 40 Gy, divided into 16 daily doses of 2.5 Gy each (4 doses/week for 4 weeks) before surgery [46]. Patients were observed at 3-month intervals for 24 months after the completion of therapy, every 6 months for 3 years, and then yearly. History and physical examination, complete blood cell and platelet count, liver chemistries, ultrasound and carcinoembryonic antigen measurement were performed at each visit; chest X-ray,colonoscopy and CT were performed once a year. All CRC specimens underwent histopathological analysis by the same gastrointestinal pathologists (B.C. and S.E.M.), who were unaware of the interim results of molecular genetic and immuno-histochemical analysis. The study protocol was approved by the Human Ethics Committee of the University of Parma.

***Immunohistochemistry***

For immunohistochemical analysis, tumors were routinely fixed in buffered 10% formalin immediately after surgery and embedded in paraffin. To avoid loss of immuno-reactivity due to prolonged storage, the sections were freshly cut, and all slides were processed simultaneously with antibodies from the same batch, including positive and negative controls. The following primary antibodies were used: anti-Topo I (clone1 D6, Sanbio, The Netherlands, working dilution: 1:100), anti-TS (clone TS106, Neomarkers, Fremont, CA, USA, working dilution:1:200). For antigen retrieval, sections were treated with 10 mM citrate buffer at pH 6.0, in a 750 W microwave oven for three 5-mincycles. The sections were developed with the streptavidin–biotin kit (LSAB2, Dako) in accordance with the manufacturer's specifications and counterstained with hematoxylin. Positive controls were CRCs previously assayed with strong positivity; negative controls consisted of substituting normal serum for the primary antibodies. The immunostains were concurrently

evaluated by two observers blinded to the clinical and pathological data. The scores of the two observers were averaged for every sample. In the vast majority of cases, the observers were in agreement. The expected/shown expression of Topo I or TS was very faint in normal colorectal epithelial cells that were used as internal reference to evaluate the intensity of expression in tumor cells. For Topo I, a score for intensity and distribution of nuclear staining was assigned according to a 4-tier system. Intensity ranged from 0 to 3 (0 = no staining, 1 = weakly positive, 2 = moderately positive, and 3 = strongly positive staining). The staining distribution considered the percentage of positive tumor cells and ranged from 0 to 3 (0 = 0 to 5%, 1 = 6% to 25%, 2 = 26% to 50%, 3 = 51% to 100%). An overall Topo I expression score was calculated as the sum of the intensity and distribution scores in each case. Cases with a total score of at least 4 were considered high expression tumors (with altered pattern), whereas cases with a total score of 0–3 were considered negative or low expression tumors (with normal pattern) [32]. TS expression levels were estimated semiquantitatively based on the intensity of cytoplasmic staining that has been previously validated as an accurate measure of protein levels [47]. In particular, TS staining considered both positive tumor cell percentage and staining intensity as follows: distribution score 0: <1% positive cells, score 1: 1–20%, score 2: 21–50%, score 3: 51–80%, score 4: >80%; intensity: score 1 (weak), score 2 (moderate), and score 3 (strong).

Where the neoplastic tissue showed a patchy, non-uniform staining intensity, the value was referred to the prevalent intensity. A finalscore was obtained by adding the two previous values as follows: negative (sum range 0–2), low (3–5), and high (6–7) expression, respectively. For the purpose of the study, scores 6–7 were considered high TS expression (altered pattern) whereas scores 0–5 were considered normal [48]. The immunostaining protocols and the semiquantitatively evaluations for hMLH1, hMSH2, FHIT, p27, p53, Cox-2, MGMT and p21were previously described [49].

***Molecular analysis***

Specimens of freshly resected CRCs were snap-frozen in liquid nitrogen and subsequently stored at −80◦C. In all cases, corresponding normal colon mucosa was collected and used as matching control. DNA was extracted using the QIAGEN DNeasy tissue kit (QIAGEN, Hilden, Germany) from 15 to 25 cryostat sections (20 mm-thick) of the tumor and matching normal samples. Only tumor samples containing at least 80% of neoplastic cells were considered. A panel of six polymorphic microsatellite markers located on chromosomal regions potentially involved in CRC development and progression was used to assess allelic imbalance: D18S58 (18q22-23), D18S61 (18q22) [12], BAT26 (2p16), BAT40 (1p13) [50], D8S254 (8p22) [51], D4S2397 (4p14-16) [52] and D5S346 [53]. The markers were selected from the Genome database (www.geneatlas.org) on the basis of chromosomal location and heterozygosity. The PCR conditions and fragment analysis have been described previously [49]. An imbalance factor was calculated as the ratio of relative allelic peak area in the tumor DNA to relative allelic peak area in the corresponding normal DNA on the basis of the following formula: (lowerallele/higher allele)tum/(lower allele/higher allele)norm. For informative markers, values of 0.6 (allelic imbalance of 40%) were considered as indicative of LOH [54]. The presence in the tumor DNA of one or more additional alleles, i.e. new peaks in the electropherogram, not present in its paired normal DNA, indicated MSI [55]. Tumors were classified as highfrequency MSI (MSI-H) when instability was detected in at least 40% of the informative microsatellite markers, or as low-frequency MSI (MSI-L) when instability was found in less than 40% of the markers; tumors without MSI were defined as microsatellite-stable (MSS) [53]. For the purposes of this study, MSS and MSI-L cases were grouped.

***Statistical Analysis***

The Chi-square test was used to correlate categories Topo I and TS expression with the clinicopathological and molecular variables. Correlations between Topo I and TS expression and patient age were evaluated using the Wilcoxon matched-pairs test. Univariate survival analysis was

performed by Kaplan Meier analysis, and differences between survival curves were studied by Log Rank test. The overall survival rate was stated as a five-year rate expressing the percentage of patients alive five years after surgery. All statistical analyses were performed using SPSS for Windows 95, version 9.0 (SPSS UK, Surrey, UK) at a significance level of $p < 0.05$.

## PAPER III

### *Study design*

This is a retrospective review of a series of patients diagnosed and resected with curative intent for multiple sporadic colorectal cancer from 1982 to May 2010.

### *Data collected*

The medical records of patients with the diagnosis of multiple synchronous or metachronous colorectal cancer treated at our Institution between 1982 and 2010 were retrospectively reviewed. A database of patients with a histological diagnosis of multiple colorectal adenocarcinoma and viable specimens was created. Follow-up data were obtained from the Parma Cancer Registry and patients' clinical charts.

Demographic and clinical data (age, gender, history of cancer, history of colonic polyposis, type of surgery, post-operative morbidity and mortality), pathological data (location of tumours, interval time between tumours, TNM Staging according to the American Joint Committee on Cancer, number of lymph nodes examined, number of lymph nodes positive, grade, histologic type) and data on adjuvant chemotherapy and radiotherapy were collected. If not clearly reported by clinical charts and registries, anamnestic data were additionally collected by telephone.

In order to focus the analysis only on sporadic colorectal cancers, familial adenomatous polyposis, Lynch syndrome cases and the presence of Amsterdam II criteria for hereditary non-polyposis CRC were considered as specific exclusion criteria [12]. Patients affected by inflammatory bowel disease were also excluded.

A metachronous cancer was defined as a second primary colorectal cancer occurring more than 6 months after the index cancer without evidence of local recurrence [13,14].

In order to summarize the location of the tumours, the large intestine was divided into 3 sectors based on the main feeding vessels: Right-section (R) from the caecum to the proximal transverse

colon fed by the ileo-colic and the right colic vessels, Transverse-section (T) from the middle transverse colon to the splenic flexure fed by the middle colic vessels and Left-section (L) from the descending colon to the rectum fed by the inferior mesenteric vessels. Thus, the location of MSCRC was classified into 3 groups based on the combination of the two colorectal sections involved for each patient: right and left sections (RL), right and transverse sections (RT), left and transverse sections (LT).

Since there were no cases of total colectomy, the extent of surgical resection was classified into two categories: multiple segmental colorectal resections in the case of preservation of at least one main pedicle with all its branches, and subtotal colectomy when one branch of the only preserved pedicle was ligated. Reconstruction of the bowel transit was achieved through 5 different procedures: ileo-transverse colo-rectal anastomosis (ITR), ileo-descending colonic anastomosis (ID), ileo-sigmoid colonic anastomosis (IS), ascending colo-rectal anastomosis (AR), or anti-peristaltic caeco-rectal anastomosis (aCR) [15].

***Statistical Analysis***

Data were analyzed using SPSS software (version 14.0; SPSS, Inc., Chicago, IL, USA). Survival analysis was performed utilizing the Kaplan–Meyer method comparing cases to 4790 controls resected with curative intent for a single colorectal cancer from the Parma Cancer Registry from 1992 to 2011. Follow up of metachronous CRC patients was considered as starting at the time of the first colorectal resection or alternatively as starting at the time of the second colorectal resection, when judged appropriate. Possible prognostic factors influencing survival were first evaluated by univariate analysis (log-rank test). Only parameters which showed significance in univariate analysis were further analyzed by multivariate analysis (Cox proportional hazards test, forward-conditional method). Statistical significance was determined by a $p$ value of less than $.05$.

# RESULTS

## PAPER I

A total of 26 patients were enrolled. Two patients dropped out for lack of compliance to the protocol, one patient was excluded after the randomization for intraoperative cardiovascular complication causing a 5 days of ICU stay, one patient was excluded after the randomization for the intraoperative finding of massive peritoneal carcinosis.

A total of 22 patients were included in the study, of which 10 patients were assigned to the experimental group and 12 patients to the control group. There were 8 female (36%) and 14 males (64%) with a mean age of 77 years (range: 70-90). The mean BMI was 25.6 (range: 21.4 - 34.9)

There was no difference in terms of age ($p=0.925$), gender ($p=0.204$), BMI ($p=0.978$), laboratory preoperative blood test and ASA score ($p=0.793$) between the two groups.

A laparoscopic colo-rectal resection was performed in 64% of patients, with a conversion rate of 9%. The mean operating time was 270 minutes (range: 80-470). There was only one intraoperative complication. The two groups were similar in terms of procedures performed, operating time, conversion rate and intra-operative complication.

### *Post-operative course*

Almost 32% of the patients was admitted to the intensive care unit (ICU) with a median lenght of ICU stay of 1 day. Postoperative morbidity rate was 45.5% with a 9% equal or greater than grade 3 complications of Clavien-Dindo classification. There were 2 cases of postoperative ileum, 2 cases of atrial fibrillation, 1 case od rectal bleeding and 1 case of anastomotic leak. The two groups were similar in terms of postoperative morbidity. The overall 30 days-mortality was 0%.

### *Enhanced recovery program with rehabilitation device*

ERP enhanced by a rehabilitation device in elderly patients was well-accepted by patients

randomized for experimental group, with a compliance rate of 86%. Patients of ERP group showed No adverse event was reported.

***Post-operative pain***

Postoperative pain was lower in the ERP group even if not significant (*p=0.371*) at general linear model for repeated measures. (Fig.2) Patients randomized for ERP group showed a tendency to an earlier postoperative reduction of VAS score.

***Recovery of the bowel function***

Postoperative nausea and vomiting were similar in both group with an overall rate of nausea in post-operative day 1,2 and 3 of 15%,5% and 25% respectively and an overall rate of vomiting in post-operative day 1,2 and 3 of 0%,0% and 20% respectively. In the ERP group, oral fluids were tolerated by 78% of patients within the first postoperative day, and 67% tolerated oral diet on the first postoperative day. The time to tolerate oral fluids and oral diets were both lower for ERP group (0.9 vs. 3.25 days, *p<0.001*; 2.2 vs.4.3 days, *p<0.001*). Recovery of the bowel function was earlier for ERP patients for either first flatus (1.8 vs. 2.4 days, *p<0.001*) and defaecation (2.3 vs. 3.6 days, *p<0.001*).

***Post-operative mobilization***

Early mobilization within postoperative day 2 was achieved in the 80% of ERP patients vs. 25% of controls (*p=0.010*), with a significant reduction of the rehabilitation time (sitting 1.7 vs.2.4 days, p=0.068; standing 1.9 vs.3.3 days, *p=0.003*; deambulation 2.6 vs.3.8 days, *p=0.013*). Recovery of physical performance was similar for the two groups albeit better for ERP patients (% variation 6MWD -26% vs. -38%, *p=0.374*).

***Length-of-hospital-stay***

The median lenght-of-hospital-stay was 8 days (range: 5-13). The median lenght-of-hospital-stay for patient to be defined dischargeable was 7 (range: 5-11). Length-of-hospital-stay was similar in the two groups although lower for ERP patients (LOS 9.00 vs. 9.75, *p=0.617*) as well as for lenght-of-hospital-stay for patient to be defined dischargeable (LOS to be defined dishargeable 7.00 vs. 7.82, *p=0.152*)

***Quality of life***

Comparison of pre- and post-operative of SF-36 health questionnaire showed similar results for all the eight sections. Only comparison of the mental health section revealed a tendency for a greater disability for ERP patients (*p=0.069*).

**PAPER II**

***Clinical and pathological variables***

High expression of Topo I was found in 34 (36%) of 94 CRC cases. The remaining 18 cases were considered inadequate due to poor immunostaining poor immunostaining likely caused by defect in fixation that impaired the interpretation of the results. No statistically significant differences in Topo I staining were found according to the clinicopathological variables analyzed (Table 2). However, increased Topo I expression occurred more frequently in tumors with rectal location (50%), AJCC stage I and III (45% and 41%, respectively) and adenocarcinoma, NOS (44%). All other variables were evenly distributed among the groups. High TS expression was found in 67 (60%) of 112 cases, and it was more frequent in distal CRCs (62%), AJCC stage I and II (67%and 63%, respectively) and in adenocarcinomas, NOS (66%). Gender and grade of differentiation were equally distributed among groups(Table 2). Representative immunostains are illustrated in Fig. 1.

***Molecular markers***

Among the chromosomal loci investigated by LOH analysis, increased expression of Topo I was significantly associated only with allelic losses at chromosome 18q ($p = 0.013$; Table 3). High Topo I expression also correlated with MSS phenotype ($p = 0.02$) and normal tissue staining for hMLH1 and hMSH2 ($p = 0.0012$and $p = 0.02$, respectively). No statistically significant differences in Topo I expression were found among the other markers examined, MGMT, FHIT, Cox-2, p21, p27 and p53 (Table 3).The expression of TS was not affected by LOH status at any chromosome locus. High TS expression was more frequent in MSS CRCs (63%) and in tumors with normal expression of hMLH1 (61%) and hMSH2 (58%), but differences did not reach the statistical significance. A significant correlation was found between the expression of TS and p27 (69%, $p = 0.0008$) and FHIT (63%, $p < 0.035$) staining. No association was observed with MGMT, Cox-2, p21 and p53 expression (Table 3).

***Survival***

Five-year OS was not affected by Topo I expression levels (99 months for normal, CI 81–116 months vs. 85 months for high Topo I expression, CI 61–108 months; log-rank p = 0.524). This result was confirmed when 5-FU/LV-treated patients were considered (60 months for normal, CI 32–88 months vs. 49 months for high Topo I expression, CI 12–87 months; log-rank p = 0.97). Five-year OS rates were also independent of TS staining intensity (95 months for normal, CI 75–114 months vs. 93 months for highTS expression, CI 76–111 months; log-rank p = 0.577). TS expression had no effect on survival of 5-FU/LV-treated patients (57 monthsfor normal, CI 28–85 months vs. 53 months for high TS expression,CI 16–90 months; log-rank p = 0.819).

**PAPER III**

***Population characteristics***

Of 28 patients diagnosed with multiple colorectal cancers 23 fulfilled the inclusion criteria, 8 (33%) with SC and 15 (67%) with MC. The median age at the time of diagnosis was 72 years (range: 63-80) for SC patients and 71 years (range: 50-85) and 78 years for index and metachronous cancers respectively in MC patients, with a mean interval time between the diagnosis of the first and the second cancer of 106 ± 97 months (range: 8-360). The majority of MC patients developed the second cancer after 5 years (60%), 2 (13%) within 2 years and 4 (27%) in the time period of 2–5 years .

No difference was found comparing the mean age at diagnosis of multiple vs. single sporadic colorectal cancer patients (69 vs. 70 years; *p=0.787*), although MC patients developed the metachronous cancer at a significantly later age than controls (77 vs. 70; *p=0.024*). The male-to-female ratio of SC and MC groups was 1.00 and 2.75 respectively, whereas the ratio of controls was 0.97.

***Location***

Cancer location is shown in Table 1. Eight, 11 and 4 patients were reported in group RL, LT and RT respectively. There were 5 patients with SC in the RL group, 1 in the LT group and 1 in the RT group. Only one patient, classified as LT, had three synchronous cancers, located at rectum, sigmoid colon and splenic flexure (Tab. 1). Twelve out of the 15 patients with MC (80%) had the first tumour located distally to the splenic flexure - 5 at the descending colon and 7 at the rectum; 3 of these subsequently developed a second cancer at the right colon, 8 at the transverse colon and 1 at the rectum. The remaining 3 MC patients had the first tumour located proximally to the hepatic flexure - 1 at the ascending colon and 2 at the hepatic flexure; these patients subsequently developed a second cancer at the transverse colon.

***Surgical resection and clinical outcomes***

The extent of surgical resection and reconstructive procedures are reported in Table 1. We performed a multiple segmental colorectal resection in 19 patients (83%). A subtotal colectomy was performed in 4 patients, 2 preserving the sigmoid colon fed by the sigmoid vessels and 2 preserving the caecum fed by the ileo-colic vessels, all due to a blood supply of the remnant colon judged as insufficient after multiple segmental colorectal resections. Bowel reconstruction was achieved through 9 AR (39%), 8 ITR (35%), 2 ID (9%), 2 IS (9%) and 2 aCR (9%) anastomoses. All the 8 patients with RL cancer location underwent ITR anastomosis with middle colic vessel preservation; the left colic vessels were preserved in 2 of those patients. The 4 patients with RT cancer location underwent 2 ID and 2 IS anastomoses respectively; the left colic vessels were consistently preserved in the 2 ID anastomoses while the middle and left colic vessels were both ligated in the 2 patients undergoing IS anastomosis. Nine out of the 11 patients with LT cancer location underwent AR; in 4 patients the middle colic vessels were preserved. The remaining 2 patients underwent aCR anastomosis.

Overall postoperative morbidity and mortality were 26% and 0% respectively. No difference in terms of morbidity and mortality between multiple segmental resection and subtotal/total colectomy was found (27% vs. 50%; $p=0.231$).

***Pathological Findings***

Stage, grade and histotype are reported in Table 1. Comparison of tumour stage in SC patients showed concordance for only 2 patients (25%) with a double stage I cancer. Four SC patients were diagnosed with stage II or stage III cancer associated with a stage I cancer. Only 1 SC patient was diagnosed with a combination of stage II and stage III cancers and only 1 patient was diagnosed

with a stage IVa multiple pT3N0 and pT1N0 colorectal cancer with a single synchronous colorectal liver metastasis. Only 2 SC patients had a nodal involvement (25%).

Comparison of tumour stages in MC patients showed a concordance in 9 patients out of 15 (60%), with double stage I, II and III colorectal cancers in 3, 3 and 3 patients respectively. There were 3 patients with stage I and stage III colorectal cancers, 2 patients with stage I and stage II colorectal cancers and 1 patient with stage II and stage III colorectal cancers. Overall 7 MC patients had a nodal involvement in at least one cancer (47%).

Comparison of grade of tumours in SC patients showed discordance in all but two patients, diagnosed with two G2 cancers. Five patients with SC had at least one poorly differentiated cancer (71%). On the other hand, grades of MC were mostly similar (67%), with a prevalence of poorly differentiated tumours higher than SC (14/30 vs. 5/16 ; *p=0.362*).

Mucinous histotype was found in 12 tumours with an overall prevalence of 26%. SC patients showed a higher prevalence of cancers with mucinous histotype, albeit not significant (31% vs. 23%; *p=0.726*). Four patients had both mucinous tumours whereas 4 patients had only one mucinous tumour.

***Adjuvant treatment***

Adjuvant treatment was administered to 4 patients. No patient received neo-adjuvant or adjuvant radiation therapy. The most common chemotherapeutic regimen administered was 5-fluorouracil with leucovorin. There was no significant improvement in overall survival for patients diagnosed with at least one stage III cancer treated with adjuvant therapy when compared with patients who were not treated.

***Survival Analysis***

The median follow-up of SC and MC patients was 78 and 132 months respectively. The 5-year and 10-year overall actuarial survival rates were 100% and 80% in SC patients, 87% and 78% from the first cancer in MC patients, and 55% and 41% in single sporadic cancer patients respectively (*p=0.002*). (Fig. 1)

No difference was found comparing overall survival of single colorectal cancer patients vs. MC patients after the second colorectal resection (5-year OS 41% vs. 66%; *p=0.259*). Interval time between the index and second cancer did not predict survival after the second cancer in MC patients (OR 1.005, CI 95%= 0.996 - 1.013; *p=0.284*). In fact, the 5-year overall survival after the second cancer of MC patients developing the second cancer before or after the 5-year-surveillance was 67% and 64% respectively (*p=0.*883). A recurrence was documented in 6 patients (26%). The first site of recurrence was loco-regional in 1 SC patient and distant in 5 patients: 3 MC patients and 2 SC patients. The site of distant failure was the liver in 2 patients, the lung in 2 patients and the peritoneum in 1 patient. The 5-year and 10-year cancer-free survival rates of SC patients were 88% and 47%, with a median time to tumour recurrence of 74 months and a median survival after the diagnosis of recurrent disease of 5.5 months. The 5-year and 10-year cancer-free survival rates of MC patients from the second tumour were 76% and 76%. Two MC patients had a cancer relapse over 10 years of follow-up, 1 with pulmonary and 1 with hepatic metastasis. (Fig. 2)

Univariate analysis demonstrated that later age at the first cancer diagnosis (*p=0.002*) and nodal involvement (*p=0.064*) were associated with a significant decrease in overall survival in MSCRC patients (Tab. 2). Multivariate analysis performed on MSCRC and controls identified the tumour stage (Stage II OR: 2.542; CI: 2.260 - 2.858; p<0.001; Stage III OR: 8.650; CI: 7.583 - 9.868; p<0.001), age at diagnosis (OR: 1.053; CI: 1.049 - 1.057; p<0.001) and single colorectal cancer (OR: 4.411; CI: 1.834 - 10.612; p=0.001) as independent predictors of poor prognosis. The Cox proportional hazards test, performed on the MSCRC patients, failed to identify independent predictors of prognosis.

# DISCUSSION

## PAPER I

Colorectal resections are increasingly performed on elderly or high-risk patients. Such surgery is frequently associated with high postoperative morbidity and mortality rates due to multiple co-morbidities and loss of cardiac and respiratory reserve. [27] Despite these issues surrounding the immediate postoperative period, long-term survival in elderly patients after colorectal resection is similar to survival in younger groups. [28,29] The enhanced recovery program following colorectal resection has been shown to offer a reduced total hospital stay for patients without an increase in morbidity or readmission rates. However, concerns have been raised regarding the use of such multimodal rehabilitation in selected groups of patients. [30] There has been a considerable increase in the age of surgical patients with colorectal cancer in the last three decades, with 22% of patients reported to be 80 years of age. [31,32] With the additional benefits reported using the laparoscopic approach, including reduced morbidity and mortality, further focus needs to be directed toward improving the perioperative management of this high-risk group of patients. [33] The present study examines the outcomes of a novel ERP enhanced by a rehabilitation device in a population of patients of 70 years of age diagnosed with colorectal cancer undergoing surgery with curative intent, further demonstrating the feasibility of this management approach and assessing adherence to the protocol.

ERP enhanced by a rehabilitation device in elderly patients was safe and feasible. All but two patients were compliant to the protocol. The information given to the patients during the preoperative counselling on the advantages of ERAS protocol and early mobilization, aroused enthusiasm in all the patients, enhancing a strong motivation for partecipating in the postoperative rehabilitation in both groups of treatment. As further proof of this enthusiasm, almost all the control patients demonstrated disappointment when they learned to be randomized for the control group.

however, concerns exist regarding compliance with the protocols. Maessen et al. reported on

compliance in a single-center study of 425 patients raising concerns about the adherence of elderly patients to the protocol and remarking the association between postoperative compliance and fast recovery [34].

Our ERP failed to demonstrated a reduction in postoperative pain, nausea and vomiting and the reasons should be addressed to the small size of the sample. In fact the earlier oral fluid and oral diet tolerance represent indirect proofs of reduced nausea and vomiting in ERP patients, as well as the earlier recovery of bowel function.

Early structured mobilization is central to any enhanced recovery protocol at any age. [35,36] Early upright mobilization does not only improve respiratory function but also has been suggested to reduce the length of hospital stay. [37,38] In multivariable analysis, Hendry et al. identified independent predictors of prolonged mobilization to be age of C 80 years and higher American Society of Anesthesiologists (ASA) score [39]. In literature, mobilization on the first postoperative day was achieved in 26-40% of elderly patients, [20,34], rising concerns on the feasibility of ERP in such population. Forcing an elderly patient to stand up after 24-48 hours from major abdominal surgery could be not-well tolerated, owing to poor physical performance, severe comorbidities and psycological opposition. In our opinion the elderly patients could not benefit from forcing rehabilitation protocol and they should be mobilized through a more tailored rehabilitation approach. Physical rehabilitation is a critical point of this study and our results demonstrated that ERP supported by a rehabilitation device could be successful in elderly patients, overcoming the traditional limitations of physical rehabilitation of these patients. The Delettizzatore allowed a more gradual administration of physical exercise, starting from the first postoperative day in supine position, leading to a reduction of rehabilitation time and postponing the target of the early mobilization of elderly patients at postoperative day 2. Unfortunately the small size of the sample didn’t permit to identify an earlier recovery of the physical performance at the time of discharge, as well as for lenght-of-hospital-stay and quality of life. We found a tendency to a shorter LOS for ERP patients but this results should be confirmed on larger series, controlling all the factors

influencing LOS.

**PAPER II**

We analyzed the tissue expression of Topo I and TS in a well-characterized series of primary CRCs and investigated their correlation with standard clinico-pathological variables, MSI status and other molecular markers implicated in colorectal carcinogenetic process. Topo I plays a pivotal role in key cellular processes, and it is the target of anti-neoplastic drugs used in clinical practice [29,56]. It has been suggested that higher levels of Topo I expression may predict the response to irinotecan containing neoadjuvant treatments in rectal cancer [57]. In addition, the results of the MRC FOCUS trial in metastatic CRC demonstrated that tumors with moderate or high levels of Topo I expression had the greatest benefit from adding irinotecan or oxaliplatin to 5-FU in the first-line setting [34].

In the current study, 36% of CRCs showed increased nuclear expression of Topo I. These data are in agreement with the results of Boonsong et al. [33] and Kostopoulos et al. [32], who found high expression in 24.4% and 48% of cases, respectively; conversely, Staley et al. [35] reported an elevated Topo I expression in 86% of cases. This variability may be attributed to the differences in the score systems used to evaluate the immunostains and in the sample sizes among studies [32,33,35]. No significant correlation was observed between Topo I staining and gender, age, tumor site, differentiation grade and AJCC stage, suggesting that Topo I inhibitors are active across different tumor histologies, stages and genders [33,59]. Interestingly, high expression of Topo I was significantly associated with CIN-related CRCs as shown by the correlation with LOH at the 18q locus, MSS phenotype and normal expression of MMR proteins hMLH1 and hMSH2. This finding has never been reported previously, to the best of our knowledge, and suggests that CIN-related sporadic CRC, in particular those with 18qLOH, may show a more favorable response to Topo I inhibitors treatment. This observation is clinically relevant as CRCs with 18qLOH and MSS phenotype seem to have a poorer prognosis as also confirmed by a non-significant trend in OS observed in our patients with high Topo I expression (85 months vs 99 months of normal protein). Several studies reported a significantly lower survival for CRCs with 18q LOH [15,60] with a two to seven fold increased risk for death [7,13,15] and recurrence [61]. However, other investigations

failed to demonstrate any prognostic effect [8,16,58,62]. The present results suggest that Topo I expression might confound the impact of 18qLOH on survival at least in those receiving anti-TopoI agents. Unfortunately, we could not test this hypothesis on the present series due to the small number of treated patients, but it certainly warrants further investigation.

TS is a key enzyme for DNA synthesis, and its competitive inhibition constitutes the major anti-tumoral mechanism of 5-fluorouracil therapy. In our series, high TS expression was observed in 60% of cases, mainly adenocarcinomas not otherwise specified. Previous studies have reported similar rates of TS positivity in CRCs, ranging from 53 to 72% [63–66]. No significant differences in TS expression were found in relationship to gender and age, tumor location, AJCC stage, histological grade and TS expression in agreement with other authors [66,67]. Among the many molecular features considered, high TS staining was found to correlate only with the normal expression of the cell cycle molecule p27 and FHIT. These results may suggest that the TS protein is not involved in the molecular pathways associated to the cyclin dependent kinase inhibitor p27 and to diadenosine hydrolase FHIT, involved in purine metabolism. Notably, no significant differences in TS expression were observed according to MSI status and MMR proteins immunostaining. Earlier reports on this issue have been conflicting, arguing either for a direct correlation [48,68,69] or no association [67,70]. Our results are in agreement with those authors [67,70] who reported that MSI status and TS expression are unrelated, and suggest that TS levels cannot explain the therapeutic resistance to 5-FU in MSI-H CRCs. Discrepancies among studies may partly relate to a lack of consistency in the criteria used to score TS immunostains [27,71].

**PAPER III**

MSCRC is a relatively rare disease. The reported incidence of multiple primary colorectal cancers is considerably variable, ranging from 0.6 to 10.6%; the probable reasons for this variability are the low incidence, the lack of routine screening for heredo-familial colorectal cancer syndromes and, most of all, the lack of distinction between sporadic and non-sporadic multiple colorectal cancer [16,17].

Several authors reported a lower mean age at diagnosis compared with that for single sporadic colorectal cancer, ranging from 47 to 71 years, supporting the hypothesis that MC patients tend to be of a younger age at the diagnosis of the first cancer. These results come from series including both sporadic and non-sporadic multiple colorectal cancer patients [18,19]. The age at diagnosis of MSCRC in our series was similar to that of single sporadic colorectal cancer patients, for both SC and MC patients. To date, younger age for index cancer remains a marker of heredo-familial cancer syndrome, in particular HNPCC, and therefore genetic counselling and testing are recommended in young patients [12].

The most relevant and surprising data of our series arose from survival analysis: both synchronous and metachronous multiple sporadic colorectal cancer patients showed a significantly better prognosis than single sporadic colorectal cancer patients. MC patients showed an even better 5-year trend than controls also when considering survival from the second cancer, despite a significantly higher median age (78 vs 70 years).

Survival data suggest the hypothesis of a different pathogenesis between MSCRC and single sporadic colorectal cancer, possibly resulting in a less aggressive biologic behaviour for MSCRC. The comparison of pathologic features of multiple cancers in each patient revealed a tendency of SC patients to develop cancers of differing stage and grade, whereas MC patients tend to develop cancers of similar stage and grade in the vast majority of cases. Mucinous histotype as a pathologic feature appears to have a similar distribution among SC and MC patients to that of the general population. Those data are surely not sufficient to account for such a difference in survival rate;

only a thorough pathological and genetic analysis on larger samples could yield more exhaustive answers. Moreover, SC and MC patients present important differences from a pathological and even clinical and therapeutic point of view, and thus can hardly be considered as a unique group.

There is a growing amount of data on the relationship between the anatomic sub-site of colorectal cancer and different pathogenic and clinical features. It has recently arisen that a different anatomic sub-site of a primary colorectal cancer determines a different risk of a second metachronous cancer, although the results are controversial [20,21]. In our series the most frequent cancer location pattern for SC patients was the right and left colon, while the most frequent cancer location of the index cancer in MC patients was distal, in accordance with Phipps et al. [20]. Moreover, in MC patients, the vast majority of metachronous cancers arose from the transverse colon, proximally to the resected primary colonic segment. These data suggest that the pathogenesis of SC could differ from that of MC and that metachronous cancer location could have been influenced by environmental promoting factors as well as by additional factors related to the type and the extent of the previous colorectal surgery.

From a clinical/therapeutic point of view, while SC patients represent a distinct subgroup of cancer patients with a possibly better prognosis, MC patients could be considered as a subgroup of patients with a new sporadic colorectal cancer after a curative treatment of the first one, thus reflecting both a higher tendency to develop colonic cancer and a positive response to oncologic treatment. If these hypotheses are confirmed in larger studies, they could lead towards a tailored therapeutic approach for synchronous MSCRC and a tailored surveillance program for metachronous MSCRC.

Unfortunately, the present predictors of metachronous cancer have a low sensitivity and they are not able to accomplish an effective risk stratification among patients resected for a sporadic colorectal cancer, raising questions as to the cost-effectiveness of endoscopic surveillance. Moreover, many authors reported an extremely varying average of interval to detection of a metachronous colorectal cancer [10,13,16,22]. In our series 63% of the MC patients developed the second cancer after the 5 years of surveillance and the interval time did not influence the prognosis of the second cancer.

Concerning the surgical approach, in our series it was constantly conservative, aimed at the preservation of bowel function, subtotal and total colectomy being performed only in the case of suboptimal or insufficient blood supply of the remnant colon [23]. Supported by our short- and long-term results, segmental or regional colonic resections seem appropriate in the elective treatment of MSCRC, and the indication for a total colectomy should not be based on oncologic purposes in either SC or MC patients.

## GENERAL DISCUSSION

More than 1.2 million patients are diagnosed with colorectal cancer every year, and more than 600 000 die from the disease. Incidence strongly varies globally and is closely linked to elements of a so-called western lifestyle. Incidence is higher in men than women and strongly increases with age; median age at diagnosis is about 70 years in developed countries. Colorectal resections are increasingly performed on elderly or high-risk patients. Such surgery is frequently associated with high postoperative morbidity and mortality rates. Despite these issues surrounding the immediate postoperative period, long-term survival in elderly patients after colorectal resection is similar to survival in younger groups. Therefore new strategies are needed to reduce perioperative stress of elderly patients, aimed to reduce lenght of hospital stay, postopertive morbidity rates and health care costs. The enhanced recovery program following colorectal resection demonstrated to offer a reduced total hospital stay for patients without an increase in morbidity or readmission rates, even in elderly patients but several limitations were identified, in particular, lack of compliance and low early mobilization rate. The results of the main project (paper I), a clinical prospective controlled trial, demonstrated that our ERP in the elderly patient supported by a rehabilitation device is feasible and safe, with a significant reduction in time to recovery of the bowel function, earlier tolerance to oral fluids and oral diet and improving the recovery of the physical performance, even if the small sample size did not allow definitive conclusions. More studies are needed to clear the potential benefit of such programs, including different type of surgical procedure.

The cornerstones of colo-rectal cancer therapy are surgery, neoadjuvant radiotherapy for patients with rectal cancer, and adjuvant chemotherapy. Indications for adjuvant chemotherapy are essentially based on cancer staging (stage III/IV and high-risk stage II), but tumor response to the available cytotoxic agents are rarely predictable. In spite of advances in systemic therapy, the 5-year survival rate is still a mere 12.5%, and the primary reason for treatment failure is believed to be acquired resistance to therapy which occurs in 90% of patients with metastatic cancer. Several

biological markers have been investigated for a prognostic role in CRC, among which are topoisomerase I (Topo I) and thymidylate synthase (TS), whose expression levels correlate with survival although this evidence is controversial among different studies. Antineoplastic drugs targeting Topo I, such as irinotecan and camptothecins, form stable Topo I-DNA cleavage complexes and inhibit Topo I activity, thus preventing DNA religation. Topo I is expressed in primary CRCs and metastases, but it is debated whether its expression can predict the response to anti-Topo I treatments. The expression of TS may affect tumor sensitivity to fluoropyrimidines, such as 5-fluorouracil (5-FU). In the first side-project (paper II), we analized retrospectively the expression of Topo I and TS in tumor tissue by immunostaining for comparing it with pathological and clinical variables, patients' outcomes and molecular characteristics (MSI, LOH and other markers implicated in CRC carcinogenesis). Interestingly, high expression of Topo I was significantly associated with CIN-related CRCs. This finding has never been reported previously, to the best of our knowledge, and suggests that CIN-related sporadic CRC, in particular those with 18qLOH, may show a more favorable response to Topo I inhibitors treatment. High TS staining was found to correlate only with the normal expression of the cell cycle molecule p27 and FHIT. These results may suggest that the TS protein is not involved in the molecular pathways associated to the cyclin dependent kinase inhibitor p27 and to diadenosine hydrolase FHIT, involved in purine metabolism. Notably, no significant differences in TS expression were observed according to MSI status and MMR proteins immunostaining.

About 25% of patients with colorectal cancer have a family history of colorectal cancer that suggests a hereditary contribution, common exposures among family members, or a combination of both, whereas the majority of patients have a sporadic disease with no apparent evidence of having inherited the disorder. Multiple colorectal cancer can occur in the absence of a defined heredo-familial syndrome, presenting as metachronous carcinomas in patients with a history of sporadic colorectal cancer or two or more sporadic synchronous carcinomas at the diagnosis.

The second side-project (paper III, see above), investigated retrospectively on patients curatively resected for multiple sporadic colorectal cancer, both synchronous and metachronous, comparing it with survival of single colo-rectal cancer patients from a large series of Parma Tumor Registry. The most relevant and surprising data of our series arose from survival analysis. Either synchronous and metachronous multiple sporadic colorectal cancer patients showed a significantly better prognosis than single sporadic colorectal cancer patients. Metachronous cancer patients showed an even better 5-year trend than controls also when considering survival from the second cancer, despite a significantly higher median age (78 vs 70 years). Survival data suggest the hypothesis of a different pathogenesis between MSCRC and single sporadic colorectal cancer, possibly resulting in a less aggressive biologic behaviour for MSCRC.

## CONCLUSIONS

In conclusion, our enhanced recovery program supported by a rehabilitation device is feasible and safe for the elderly patient, with a significant reduction in time to recovery of the bowel function, earlier tolerance to oral fluids and oral diet and improving the recovery of the physical performance. This study further supports the use of the enhanced recovery program after colorectal resection in elderly patients. Owing to the small size of the sample, these results must be confirmed on larger series of elderly patients.

The results of second paper (paper II) provide evidence that Topo I expression is linked to the CIN pathway of sporadic colorectal cancers. In particular, the association between high Topo I and 18qLOH may account for the poorer prognosis of 18q-deleted tumors. Conversely, high TS expression is unlikely to play any role in the behavior of colorectal cancers characterized by microsatellite instable pheno-type.

Retrospective analysis of the multiple sporadic colorectal cancer series treated at our institution (paper III) showed that multiple sporadic colorectal cancer have specific clinico-pathological features with a better prognosis than single sporadic colorectal cancer, both for synchronous and metachronous cancer patients. Segmental or regional colorectal resections seem appropriate from an oncologic point of view in the elective treatment of multiple sporadic colorectal cancer, and the indication for a total colectomy should not be based on oncologic purposes in these patients. Future research should confirm these results on larger series, possibly identifying a tailored therapeutic approach and surveillance for this subgroup of oncologic patients.

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# TABLES

## PAPER I

***TAB.1 ERAS program***

| | |
|---|---|
| 1 week before | • Physiatric evalutazione<br>• Questionnaire SF 36<br>• Berg-balance scale<br>• 6MWD |
| 6 days before | • Training ssession on delettizzatore, test 3MT (L0) |
| 2 days before | • Bowel prep with senna derivates laxative |
| 1 day before | • Low fiber diet till 10pm<br>• Phosphate enema<br>• 16 tablets of Simeticone per day<br>• Enoxaparina injection at 8pm |
| Day of operation | • One can of carbohydrate supplement drink 5% (250 mL) at 7am<br>• Anti-thrombotic stockings<br>• Epidural sited<br>• Urinary bladder catheter sited<br>• Surgical operation (open/laparoscopic)<br>• Minimal perioperative IV fluid<br>• Active perioperative warming with warming blanket<br>• Nasogastric drainage removal<br>• Abdominal drains only for surgery within the pelvis<br>• Drink in recovery ward 30-50 mL every 1-2 hours 6-12 hours after surgery<br>• Epidural analgesia supplemented with paracetamol 1 g qds<br>• Rescue opiate analgesia<br>• Enoxaparina injecction at 8pm |
| PO day 1 | • Drink in recovery ward 30-50 mL every 1-2 hours<br>• Removal of abdominal drain (if present)<br>• Epidural analgesia supplemented with paracetamol 1 g qds<br>• Rescue opiate analgesia<br>• Suspension of ev fluids if adequate oral diet<br>• US lower limbs veins<br>• Active physical exercise session with rehab device<br>• Enoxaparina injecction at 8pm |
| PO day 2 | • As above including<br>• Drink in recovery ward 30-50 mL every 1-2 hours<br>• Normal diet as tolerated<br>• Urinary catheter removed (men with prostatic symptoms)<br>• Active physical exercise session with rehab device |
| PO day 3 | • As above including<br>• Epidural infusion stopped at 6 a.m.<br>• Active physical exercise session with rehab device |
| PO day 4 | • As above including<br>• Paracetamol analgesia with opiate rescue<br>• Laxatives if necessary<br>• Thromboembolic deterrent stockings to be worn for 2 weeks<br>• Active physical exercise session with rehab device, if not yet mobilized |
| PO day 6-7 | • Physiatric evalutazione<br>• Questionnaire SF 36<br>• Berg-balance scale<br>• 6MWD<br>• 3MT test<br>• Discharge |

***TAB.2 Perioperative traditional care program***

| | |
|---|---|
| 1 week before | • Physiatric evalutazione<br>• Questionnaire SF 36<br>• Berg-balance scale<br>• 6MWD |
| 6 days before | • Training ssession on delettizzatore, test 3MT (L0) |
| 2 days before | • Low fiber diet<br>• Bowel prep woth polyethylene glycol 2L |
| 1 day before | • Low fiber diet<br>• Bowel prep woth polyethylene glycol 2L<br>• 16 tablets of Simeticone per day<br>• Enoxaparina injection at 8pm |
| Day of operation | • Antibiotics prophylaxis<br>• Anti-thrombotic stockings<br>• Epidural sited<br>• Urinary bladder catheter sited<br>• Surgical operation (open/laparoscopic)<br>• Minimal perioperative IV fluid<br>• Active perioperative warming with warming blanket<br>• Nasogastric drainage sited<br>• Abdominal drains sited<br>• Epidural analgesia supplemented with paracetamol 1 g qds<br>• Rescue opiate analgesia<br>• Enoxaparina injecction at 8pm |
| PO day 1 | • Totatl parenteral nutrition<br>• Nasogastric tube removal at the first flatus, in absence of nausea or output 300 mL/24h<br>• Epidural analgesia supplemented with Ketoprofene 160 mg x2<br>• Rescue opiate analgesia<br>• Enoxaparina injecction at 8pm |
| PO day 2 | • As above including<br>• Clear fluid diet, depending on patient tolerance<br>• Sitting mobilzation |
| PO day 3 | • As above including<br>• Interruption of epidural analgesia and catheter removal at 12 am<br>• Standing mobilization |
| PO day 4 | • As above including<br>• Urinary bladder catheter removal |
| PO day 5 | • Physiatric evalutazione<br>• Questionnaire SF 36<br>• Berg-balance scale<br>• 6MWD<br>• 3MT test<br>• Discharge |

## PAPER II

***TAB.1***

Main clinicopathological features of patients

| | |
|---|---|
| **Gender** | |
| Male | 58 (52) |
| Female | 54 (48) |
| **Age** | 69.2 |
| **AJCC stage** | |
| I | 12(11) |
| II | 60 (53) |
| III | 37 (33) |
| IV | 3 (3) |
| **Tumor site** | |
| Proximal | 42 (37) |
| Distal | 50 (45) |
| Rectum | 20 (18) |
| **Grade** | |
| G1-G2 | 43 (38) |
| G3 | 69 (62) |
| **Histotype** | |
| Adenocarcinoma,NOS | 74 (66) |
| Mucinous adenocarcinoma | 38 (34) |
| **Adjuvant therapy** | 12 (11) |

***TAB.2***

Main clinicopathological features of CRCs according to Topo I and TS expression

| | N | TOPO I expression | | *p* | N | TS expression | | *p* |
|---|---|---|---|---|---|---|---|---|
| | | Altered (%) (n=34) | Normal (%) (n=60) | | | Altered (%) (n=67) | Normal (%) (n=45) | |
| **Gender** | | | | | | | | |
| Male | 49 | 18 (37) | 31 (63) | *1.00* | 58 | 36 (62) | 22 (38) | *0.70* |
| Female | 45 | 16 (36) | 29 (64) | | 54 | 31 (57) | 23 (43) | |
| **Age** | | 70 (49-51) | 69 (33-94) | *0.64* | | 69 (27-91) | 69 (45-86) | *0.77* |
| **Tumor site** | | | | | | | | |
| Proximal | 31 | 10 (32) | 21 (68) | *0.39* | 42 | 24 (57) | 18 (43) | *0.89* |
| Distal | 45 | 15 (33) | 30 (67) | | 50 | 31 (62) | 19 (38) | |
| Rectum | 18 | 9 (50) | 9 (50) | | 20 | 12 (60) | 8 (40) | |
| **Grade** | | | | | | | | |
| G1-G2 | 34 | 12 (35) | 22 (65) | *1.00* | 43 | 29 (67) | 14 (33) | *0.24* |
| G3 | 60 | 22 (37) | 38 (63) | | 69 | 38 (55) | 31 (45) | |
| **AJCC stage** | | | | | | | | |
| I | 11 | 5 (45) | 6 (55) | *0.74* | 12 | 8 (67) | 4 (33) | *0.16* |
| II | 51 | 16 (31) | 35 (69) | | 60 | 38 (63) | 22 (37) | |
| III | 29 | 12 (41) | 17 (59) | | 37 | 21 (57) | 16 (43) | |
| IV | 3 | 1 (33) | 2 (67) | | 3 | 0 | 3 (100) | |
| **Histotype** | | | | | | | | |
| Adenocarcinoma,NOS | 52 | 23 (44) | 39 (56) | *0.82* | | 49 (66) | 25 (36) | *0.68* |
| Mucinous adenocarcinoma | 32 | 11 (32) | 21 (68) | | | 18 (47) | 20 (53) | |
| **Adjuvant therapy** | | | | | | | | |
| Yes | 11 | 5 (45) | 6 (55) | *0.52* | | 6 (50) | 6 (50) | *0.34* |
| No | 82 | 29 (36) | 53 (64) | | | 61 (62) | 38 (38) | |

***TAB.3***

Molecular features of CRCs according to Topo I and TS expressions. LOH, allelic loss; NI, not informative; ND, not available; MSI, microsatellite instability; HMSI, high microsatellite instability; MSS, microsatellite stable. Values in bold indicate statistical significance.

| | **N** | **TOPO I expression** | | ***p*** | **N** | **TS expression** | | ***p*** |
|---|---|---|---|---|---|---|---|---|
| | | Altered (%) (n=34) | Normal (%) (n=34) | | | Altered (%) (n=67) | Normal (%) (n=45) | |
| **18q** | | | | | | | | |
| LOH | 39 | 21 (54) | 18 (46) | | 45 | 29 (64) | 16 (36) | |
| NO LOH | 42 | 11 (26) | 31 (74) | *0.013* | 48 | 27 (56) | 21 (44) | *0.520* |
| NI/ND | 13 | 2 (15) | 11 (75) | | 19 | 11 (58) | 8 (42) | |
| **5q** | | | | | | | | |
| LOH | 20 | 9 (45) | 11 (55) | | 21 | 11 (52) | 10 (48) | |
| NO LOH | 37 | 13 (35) | 24 (65) | *0.57* | 43 | 24 (56) | 19 (44) | *1.000* |
| NI/ND | 37 | 12 (32) | 25 (68) | | 48 | 32 (67) | 16 (33) | |
| **4p** | | | | | | | | |
| LOH | 15 | 5 (33) | 10 (67) | | 17 | 13 (76) | 4 (24) | |
| NO LOH | 50 | 20 (40) | 30 (60) | *0.77* | 58 | 35 (60) | 23 (40) | *0.26* |
| NI/ND | 29 | 9 (31) | 20 (69) | | 37 | 19 (51) | 18 (49) | |
| **8p** | | | | | | | | |
| LOH | 13 | 5 (33) | 8 (67) | | 17 | 12 (70) | 5 (30) | |
| NO LOH | 29 | 10 (34) | 19 (66) | *1.000* | 35 | 23 (66) | 12 (34) | *1.000* |
| NI/ND | 52 | 19 (36) | 33 (64) | | 60 | 32 (53) | 24 (47) | |
| **MSI** | | | | | | | | |
| HMSI | 17 | 2 (12) | 15 (88) | ***0.02*** | 23 | 11 (48) | 12 (52) | *0.23* |
| MSS | 77 | 32 (41) | 45 (59) | | 89 | 56 (63) | 33 (37) | |
| **hMLH1** | | | | | | | | |
| Altered | 23 | 2 (9) | 21 (91) | ***0.001*** | 29 | 16 (55) | 13 (45) | *0.66* |
| Normal | 71 | 32 (45) | 39 (55) | | 83 | 51 (61) | 32 (39) | |
| ND | | | | | | | | |
| **hMSH2** | | | | | | | | |
| Altered | 9 | 0 | 9 (100) | | 10 | 6 (60) | 4 (40) | |
| Normal | 82 | 34 (41) | 48 (59) | ***0.02*** | 99 | 58 (58) | 41 (42) | *1.000* |
| ND | 3 | 0 | 3 (100) | | 3 | 3 (100) | 0 | |
| **MGMT** | | | | | | | | |
| Altered | 11 | 5 (45) | 6 (55) | *0.75* | 13 | 8 (61) | 5 (39) | |
| Normal | 63 | 25 (40) | 38 (60) | | 74 | 45 (61) | 29 (39) | *1.000* |
| ND | 20 | 4 (20) | 16 (80) | | 25 | 16 (56) | 11 (44) | |
| **FHIT** | | | | | | | | |
| Altered | 14 | 3 (21) | 11 (79) | *0.24* | 17 | 6 (35) | 11 (65) | ***0.035*** |
| Normal | 78 | 31 (40) | 47 (60) | | 93 | 59 (63) | 34 (37) | |
| ND | 2 | 0 | 2 (100 | | 2 | 2 (100) | 0 | |
| **COX-2** | | | | | | | | |
| Altered | 62 | 23 (37) | 39 (63) | *1.000* | 76 | 45 (59) | 31 (41) | |
| Normal | 26 | 9 (35) | 17 (65) | | 28 | 15 (53) | 13 (47) | *0.65* |
| ND | 6 | 2 (33) | 4 (67) | | 8 | 7 (87) | 1 (13) | |
| **P21** | | | | | | | | |
| Altered | 42 | 16 (38) | 26 (62) | *0.65* | 50 | 30 (60) | 20 (40) | *0.84* |
| Normal | 43 | 14 (32) | 29 (68) | | 51 | 29 (57) | 22 (43) | |
| ND | 9 | 4 (44) | 5 (56) | | 11 | 8 (73) | 3 (27) | |
| **P27** | | | | | | | | |
| Altered | 32 | 11 (34) | 21 (66) | *0.82* | 36 | 12 (33) | 24 (67) | ***<0.001*** |
| Normal | 57 | 22 (38) | 35 (62) | | 69 | 48 (69) | 21 (31) | |
| ND | 5 | 1 (20) | 4 (80) | | 7 | 7 (100) | 0 | |
| **P53** | | | | | | | | |
| Altered | 41 | 16 (39) | 25 (61) | *0.83* | 48 | 23 (48) | 25 (52) | *0.11* |
| Normal | 47 | 17 (36) | 30 (64) | | 57 | 37 (65) | 20 (35) | |
| ND | 6 | 5 (17) | 5 (83) | | 7 | 7 (100) | 0 | |

## PAPER III

***TAB.1***

Demographic, Clinical and Pathological Data of Multiple Sporadic Colorectal Cancer Patients.

| Code | Syn/Met | Gender | Age | Interval (months) | Tumour location | Extent surgery | Anastomosis | Stage | Grade | Mucinous tumour |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Syn | M | 69 | 0 | RL | Segmental | ITR | II - I | G2 - G3 | Both |
| 2 | Syn | F | 68 | 0 | RL | Segmental | ITR | I - I | G2 - G1 | None |
| 3 | Syn | M | 74 | 0 | RL | Segmental | ITR | II - III | G2 - G3 | Both |
| 4 | Syn | F | 70 | 5 | RL | Segmental | ITR | I - I | G2 - G3 | None |
| 5 | Met | M | 69 | 77 | RL | Segmental | ITR | III - I | G3 - G2 | None |
| 6 | Met | M | 79 | 104 | RL | Segmental | ITR | III - II | G2 - G3 | Second |
| 7 | Met | M | 71 | 33 | RT | STC | IS | III - III | G3 - G3 | Both |
| 8 | Syn | F | 80 | 3 | RT | Segmental | ID | II - I | G2 - G3 | None |
| 9 | Met | M | 48 | 215 | RT | Segmental | ID | I - III | G3 - G3 | None |
| 10 | Met | M | 73 | 62 | RT | STC | IS | II - II | G3 - G3 | First |
| 11* | Syn | M | 79 | 0 | LT | Segmental | AR | IV - IV | G2 - G2 | First |
| 12 | Met | F | 54 | 24 | LT | Segmental | AR | I - I | G3 - G2 | None |
| 13 | Met | M | 76 | 8 | LT | STC | aCR | I - I | G1 - G2 | Second |
| 14 | Met | M | 57 | 49 | LT | STC | aCR | II - II | G3 - G3 | Both |
| 15 | Met | M | 84 | 27 | LT | Segmental | AR | I - II | G2 - G2 | None |
| 16 | Met | F | 56 | 170 | LT | Segmental | AR | I - I | G2 - G2 | None |
| 17 | Met | F | 85 | 43 | LT | Segmental | AR | III - III | G3 - G3 | None |
| 18 | Syn | F | 63 | 2 | RL | Segmental | ITR | III - I | G2 - G3 | None |
| 19 | Met | M | 75 | 72 | LT | Segmental | AR | III - I | G3 - G2 | None |
| 20 | Met | F | 60 | 218 | LT | Segmental | AR | III - III | G2 - G2 | None |
| 21 | Syn | M | 73 | 2 | LT | Segmental | AR | I - II | G2 - G2 | None |
| 22 | Met | M | 76 | 133 | RL | Segmental | ITR | I - II | G2 - G2 | None |
| 23 | Met | M | 51 | 360 | LT | Segmental | AR | II - II | G2 - G2 | None |

**TAB.2**

Variable Influencing Survival in Multiple Sporadic Colorectal Cancer Patients

| | ***Univariate Analysis*** |
|---|---|
| Gender | 0.586 |
| Age | 0.041* |
| Interval (within 5 yrs) | 0.052* |
| Location | 0.660 |
| Extent of surgical resection | 0.655 |
| Stage | 0.281 |
| Grade | 0.730 |
| Nodal involvement | 0.064* |
| Mucinous histotype | 0.875 |
| Adjuvant therapy in stage III | 0.868 |

# FIGURES

## PAPER I

***FIG.1:***
The rehabilitation device named “Delettizzatore”.

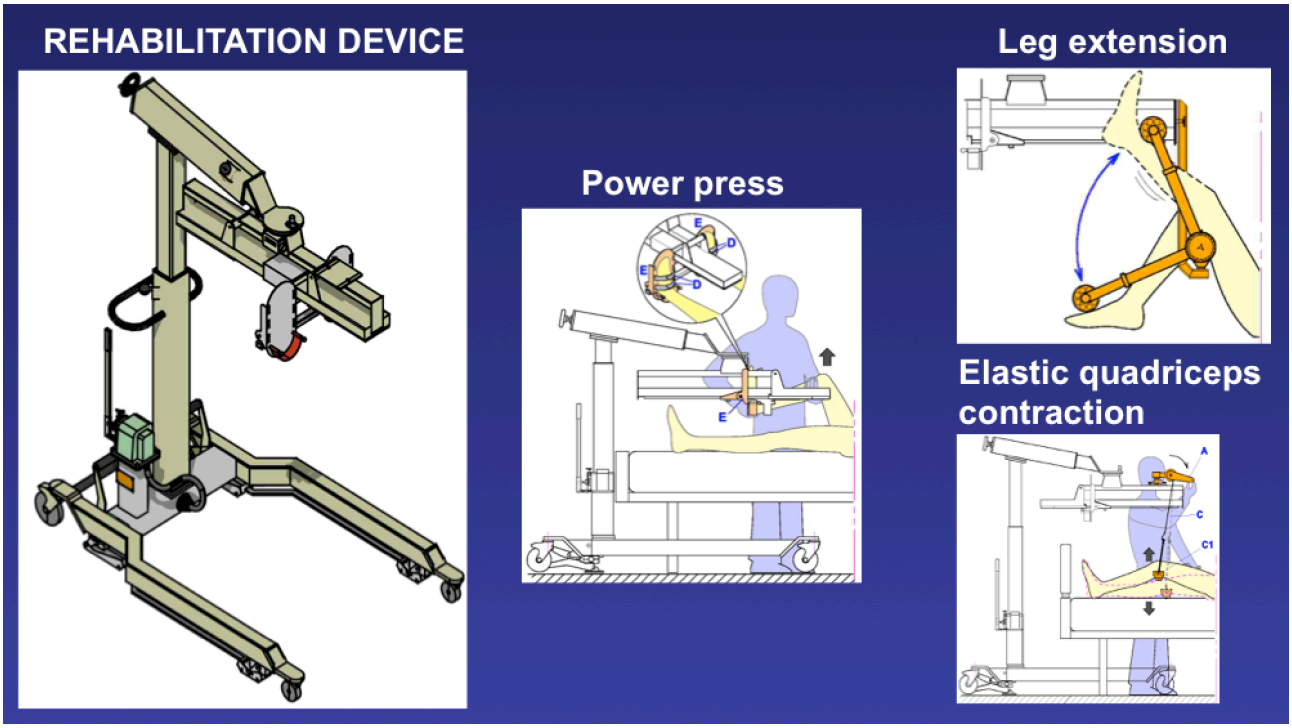
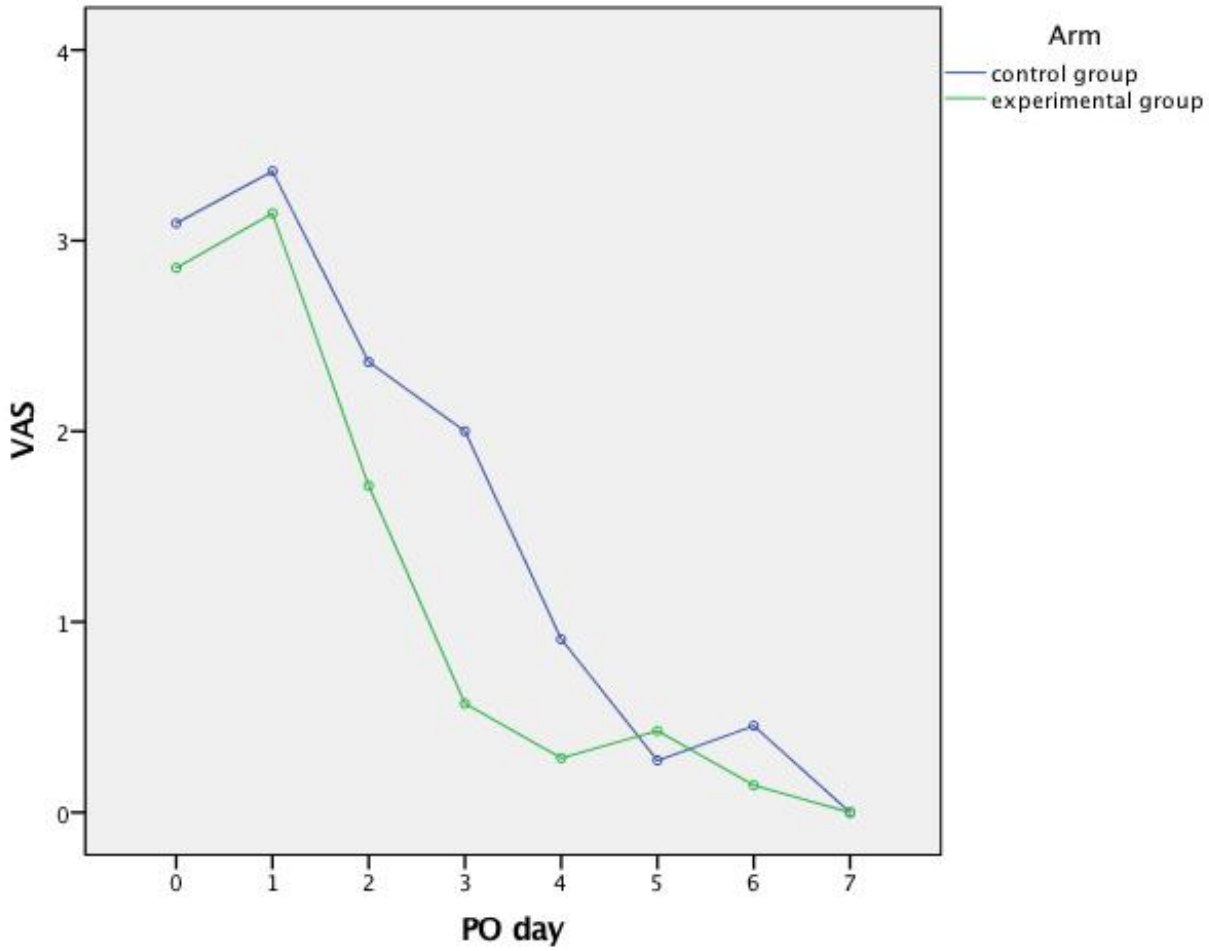


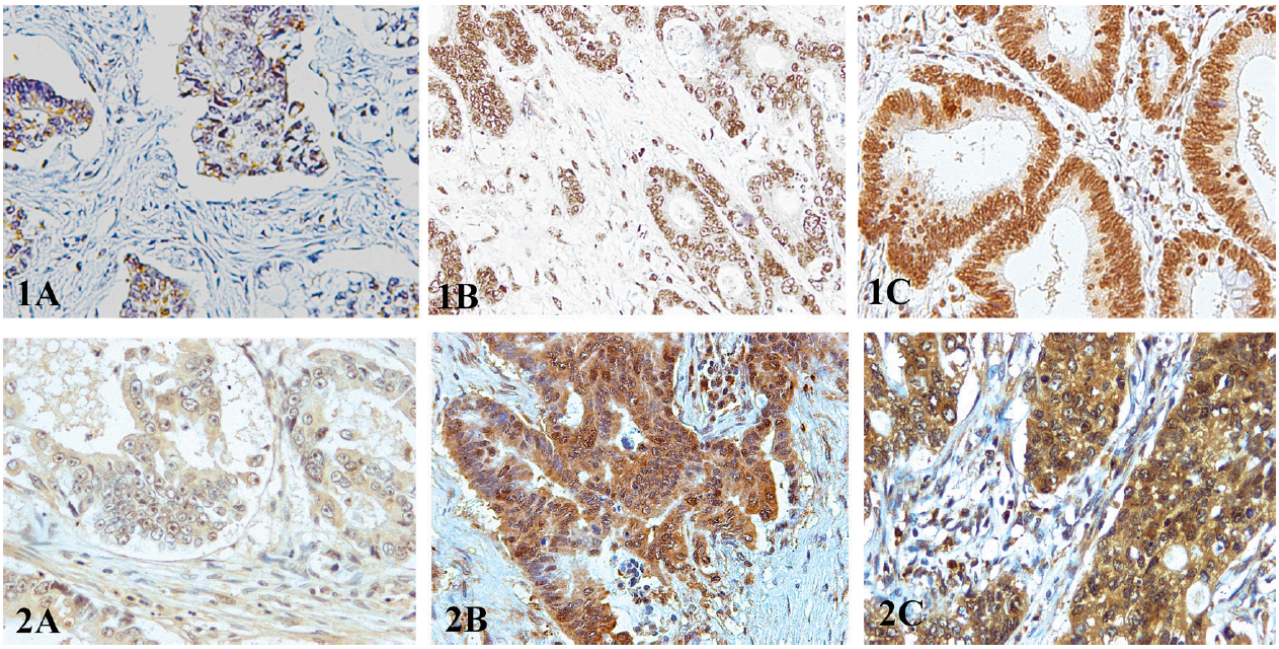
***FIG.2:***
Post-operative pain expressed with visual apperception scale (VAS).



**PAPER II**

***FIG.1***

Immunohistochemical detection of Topo I (Upper panel) and TS (Lower panel) in colorectal carcinoma cases. Upper panel: (1A) Mild nuclear Topo I staining graded as1/6 considered normal protein expression, (1B) moderate Topo I normal expression with a total score of 3/6, (1C) strong nuclear staining for Topo I graded as 6/6 evaluatedaltered expression. Lower panel: (1D) Mild cytoplasmic normal TS staining graded as 2/7, (1E) moderate, diffuse TS normal expression with a total score of 5/7, (1F) strongcytoplasmic staining for TS altered protein with a total score of 7/7. Magnification: 100×.



**PAPER III**

***FIG.1***

Kaplan-Meyer survival curves comparing overall survival of synchronous cancer patients (**Synch**), metachronous cancer patients from the first cancer (**Met1**), metachronous cancer patients from the second cancer (**Met2**) and single cancer patients (**Single**).

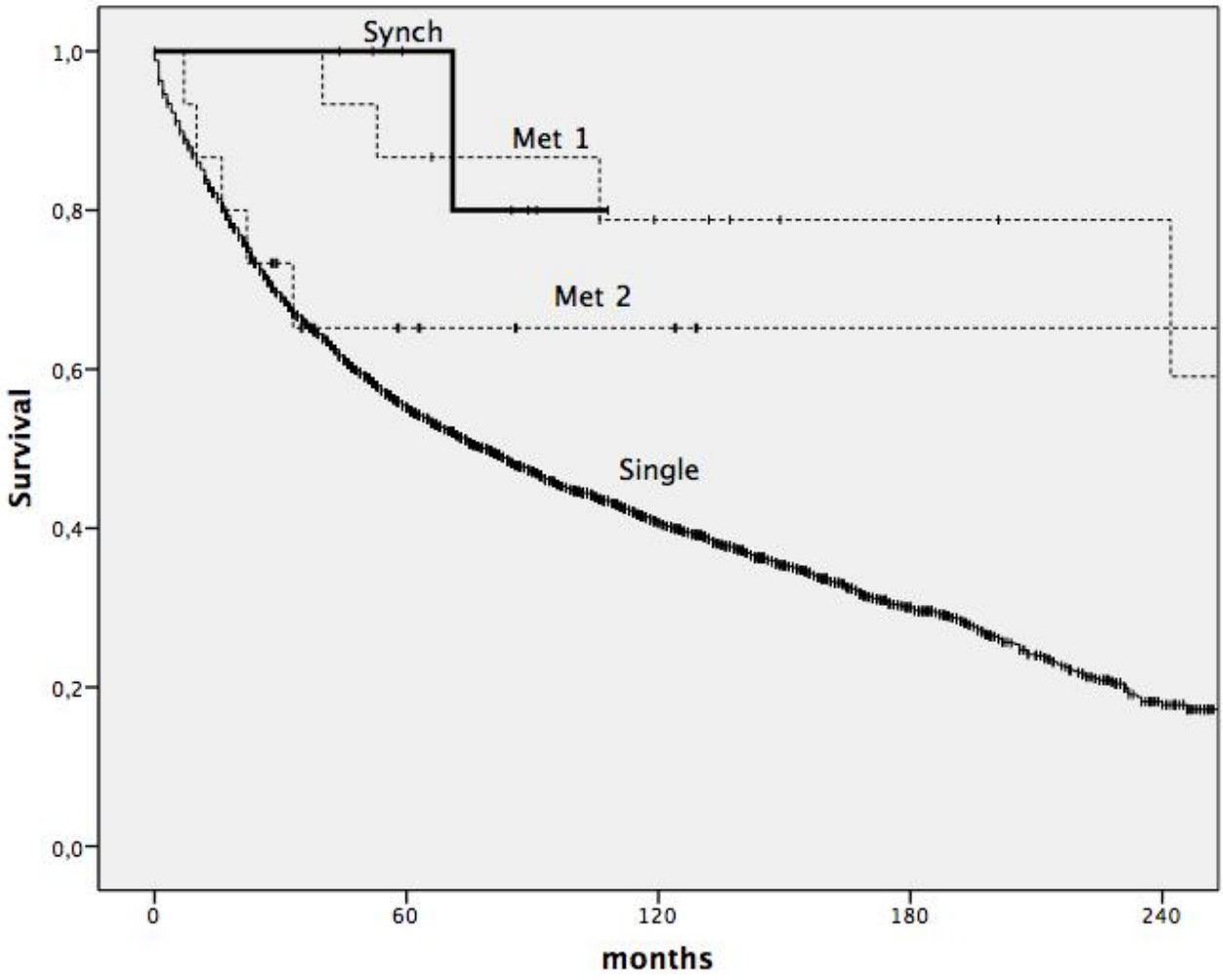


***FIG.2***

Kaplan-Meyer survival curves comparing synchronous cancer patients (**Synch**) and metachronous cancer patients from the second cancer (**Met2**) cancer-free survival.