# Is CD10 a reliable marker of invasive colorectal cancer?

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**Is CD10 a reliable marker of invasive colorectal cancer?**

AIM: *Previous studies reported that CD10 positive Colorectal Cancer Cells (CRC) characterized by deeply invasive neoplasia.*
MATERIALS AND METHODS: *We have examined 50 pts surgically treated for colorectal cancer on at least 5 years follow up. TNM, grading score and survival have been compared to CD10 expression.*
RESULTS: *Thirty- four out of fifty cases have been analyzed (18 males and 16emale) of whom nineteen were CD10 positive and fifteen were CD10 negative. The remaining 16 cases were droping out. No difference in survival rate between CD10 positive and negative in N0,N1,N2. No difference on survival rate and grading 1,2,3. We have then analyzed CD10 positive and CD10 negative cases , according to neoplasia grading, in patients with positive linphonodes N1 and N2. We showed a statistical difference between the CD10 positive/N2 (grading 1.66 ±0.5) and the CD10 negative/N2 (grading 3) (p<0.005).*
CONCLUSIONS: *We can hypothesize that CD10 positive neoplasia display a more invasive behaviour, independently from the N score and the G score, compared to CD10 negative neoplasia.*

KEY WORDS: CD10, Colorectal cancer, Grading, Survival, TNM

## Introduction

A molecular understanding of oncogenesis is assuming increasing importance in Oncology and is affecting current standards in the clinical approaches to neoplasia
On the light of developing new medications targeted to specific neoplastic markers, several studies aimed to better understand natural history and clinical behaviour of specific neoplastic diseases are currently ongoing.

Early Identification of invasive cancer might lead to different clinical approach, both for clinical and/or for surgical implications.
In our study we are testing the hypothesis that early identification of CD10, a metal-protein detected on CRC cells by immunohistochemistry, might lead to predict invasive behaviour of colorectal cancer (CRC).
Previous studies reported that CD10 positive CRC characterized by deeply invasive neoplasia according to survival rate, grading, and metastases.
An early report about CD10 as a marker of adenoma developing CRC at an early stage has been carried out by our group;
CD10 (named also neprilisine, CALLA, gp100 o NEP) is a 100 kDa trans-membrane idrolase protein.
Specifically CD10 is a zinc-dependent metalloproteasis enabling degradation of bioactive peptides by catalyzing a separation between phenylalanine and tyrosine;

CD10 is one of few antigens, together with CD38, whose extracellular part has a catalytic effect; it is expressed on the surfaces of several cells:
– Spinal cord stem cell;
– Myeloid cells (including neutrophils);
– Adult B lymphocytes • Parafollicular cortex T cells;
– Enterocites
– Bile ducts
– Renal glomerulal cells end proximal contort tubule cells;
– Pulmonary alveolar cells;
– Mioepithelial cells of the breast ;
– Prostatic cells;
– Trophoblasts;

Il CD 10 is expressed in various neoplastic disorders of the haemopoietic system as in the common acute lymphoblastic leukaemia antigen (CALLA), in the follicular lymphoma and in the Burkitt lymphoma. CD10 is used as marker for classification of leukaemia and B cells lymphoma [1]. This antigen is expressed also in other non-hematologic neoplasia as:
– Endometrial sarcoma
– Cholangiocarcinoma;
– Renal cells carcinoma
– Urothelial carcinoma;
– Prostatic carcinoma;
– Pancreatic carcinoma;
– Colorectal carcinoma;
– Breast cancer;
– Melanoma;
– Sarcoma, leiomiosarcoma, rabdomiosarcoma;
– Schwannoma.

Among some of the above mentioned neoplasia CD10 is assuming an increasingly important role on the ground of classification and prognosis. It is used for identification of mesenchimal cells neoplasia in gynaecology [2]. Recent studies (2006) have highlighted that CD10 expression in neoplastic cells lead to a worse prognosis. It has been displayed as CD10 expression in breast cancer correlate with invasive behaviour (high grading score and estrogens receptor- negative breast carcinoma cells) and fewer therapeutic options [3] as well as in urinary tract carcinoma [4]. As far as for gastrointestinal neoplasia a recent study from Japan in 2005 showed a correlation between invasive gastric cancer (earlier metastases and deeper vascular and lymphatic infiltration) [5].
Over recent years several studies aimed to confirm correlation between CD10 and invasive colorectal cancer have been carried out. A significant correlation between colorectal cancer score and CD10 expression on cells of lesions characterized by worse prognosis has been shown. The first hypothesis aiming to confirm the value of considering CD10 expression as predictive marker of invasive colorectal cancer was the correlation between CD10 positive colorectal adenoma and earlier transformation to CRC.

A recent (2008) study [5], conducted on sessile and peduncolated adenoma, confirmed that CD10 and Beta-Catenina expressing adenoma were predictive of early transformation to CRC. In this study evaluating CD10 expression in 60 metastatic colorectal cancer and 60 colorectal cancers without metastases CD10 positive neoplasia had a positive statistically significant difference in metastasis expression then CD10 negative neoplasia.
These dates have been confirmed in our recent study where we analyzed CD10 expression in colorectal adenoma with mild and moderate dysplasia and with severe dysplasia and/or cancer [6].
A majority of CD10 expression has been reported in colorectal adenoma with severe dysplasia and/or cancer, in colorectal cancer with metastatic linphonodes and in colorectal cancer with higher Astler end Coller score.
In conclusion a strong correlation between CD10 and invasive neoplasia has been highlighted. Moreover CD10 positive neoplasia has been shown to be expressed in metastatic colorectal cancer, in particular with liver metastases and low survival rate. Only few studies addressing these issues have been carried out so far and only correlation with liver metastases has been appropriately studied. A recent study from Japan has been focused on identification of clinical and immunohistochemical risk factors for liver metastases [7]. In This study sixty patients with colorectal cancer and liver metastases and sixty patients with colorectal cancer without liver metastases have been analyzed. CD10 expression has been showed at a higher rate in patients with liver metastases and deep vascular and lymphatic infiltration. This is why we are looking at this new biomarker with extreme interest given its potential use in the selection of highly invasive adenoma from low risk adenoma as well as in the classification of colorectal cancer with different degree of invasiveness. Another interesting Japanese study in 2009 evaluated CD10 expression in invasive colorectal carcinoma with liver metastases. The molecular effect of metionine-encefaline (MENK) inhibiting colorectal cancer cells growth, invasion and survivor within the hepatic parenchyma has been highlighted by this study. It has been hypothesised that CD10 inhibit MENK to consent neoplastic cells invasion [8].
In a recent study carried out in 2010 the role of another biomarker CD133 has been evaluated on the light of correlation with invasive colorectal cancer. However the results of this study are still rather controversial. A positive correlation between invasive neoplasia and CD133 expression has been showed in cells culture [9]. This correlation has been demonstrated especially when CD10 positive fibroblasts were also present in the culture. On the light of all the above mentioned scientific evidence we can conclude that CD10 expression in colorectal carcinoma should be considered as a reliable marker of invasive neoplasia in colorectal cancer with both clinical and biologic implications.

## Material and Methods

In our study a correlation between CD10 expression in colorectal cancer cells and lower survival rate has been reported leading to the consideration of CD10 positive neoplasia as a very invasive colorectal cancer. Fifty patients on at least 5 years follow up for operated CRC at our Surgical Unit have been randomized for the study. TNM staging, grading, and survival rate have been compared to CD10 expression in collaboration with the Histopathology Unit which carried out Immunohistochemistry on tissue slices.

CD10 has been studied on tissues extracted from surgical specimens to perform Immunohistochemitry.

Formalin-fixed, paraffin-embedded (FFPE) colon mucosa tissue samples underwent Immunohistochemistry by using biotin and streptovidin. Polymeric chains (Dako, ADVANCE, HRP) have been used for incubation of tissue sections with anti CD10 primary monoclonal antibody (clone 56C6, diluizione 1:50, Novocastra). Sections underwent antigen unmasking by treatment in a thermostatic bath at 98°C for 30 minutes with EDTA buffer pH 9. The binding sites have been identified with 3, 3 diaminobenzidine (DAB) as chromomeric substrate. Finally, sections have been counterstained with Harris haematoxylin. On the other hand, the negative controls have been treated with normal serum to confirm antibody specificity.

To evaluate survival rate a data base has been set up to analyse all the available information obtained from patients and family contacts. A long rank test and t-student test have been applied to the available information. Statistical significance has been fixed at $p < 0.05$.

All patients have been divided in different groups according to CD10 expression and classified on N score (N0, N1, N2) and G grading (G1, G2, G3);

## Results

Thirty- four out of fifty cases have been analyzed (18 males and 16 female) of whom nineteen were CD10 positive end fifteen were CD10 negative. The remaining 16 cases were droping out.

Patients with CD10 positive neoplasia and patients with CD10 negative neoplasia have been compared for surveillance rate and divided according to the N score (Tab. I). No statistically significant difference in survival rate

TABLE I - *CD10 positive and CD10 negative survival rate and linphonodes metastases (N) scor.*

| CD 10 + | CD 10 - | P = 0.957 |
|---|---|---|
| CD 10 ± N0 | CD 10 - /N0 | P = 0.438 |
| CD 10 ± N1 | CD 10 - /N1 | P = 0.867 |
| CD 10 + /N2 | CD 10 -/ N2 | P = 0.495 |

TABLE II - *CD10 positive and CD10 negative histology grading.*

| CD 10 + /G1 | CD 10 - /G1 | P = 0.386 |
|---|---|---|
| CD 10 +/ G2 | CD 10 - /G2 | P = 0.138 |
| CD 10 +/ G3 | CD 10 - /G3 | P = 0.84 |

TABLE III - *CD10 negative and CD10 positive histology grading (G) and linphonodes score (N)*

| | CD 10 + | CD10- |
|---|---|---|
| **N 1** | GRADING = 2.93 +/- 0.4 | GRADING = 2.33 +/- 0.5   P = 0.170 |
| **N 2** | GRADING = 1.66+/- 0.5 | = 3 P = 0.002 GRADING |

has been shown in N0 (p=0.438), N1 (p=0.867) and N2 (p=0.495) neoplasia in comparing CD10 positive and CD10 negative patients. No statistically significant difference on survival rate has been found between CD10 positive and CD10 negative patients (p=0.957).

Survival rate has been sought on the light of CD10 expression according to G grading as index of neoplasia invasiveness (Tab. II).

No statistically significant difference in survival rate has been shown in G1 (p=0.386), G2 (p=0.138), G3 (p=0.848) comparing CD10 positive and CD10 negative patients.

We have then analyzed CD10 positive and CD10 negative cases , according to neoplasia grading, in patients with positive linphonodes N1 and N2 (Tab. III). In the group CD10 positive/ N1 the G grading was 2.93±0.4; in the group CD10 negative/N1 the G grading was 2.33 ± 0.5. In the CD10 positive/N2, the G grading was 1.66 ±0.5; in the CD10 negative/N2 the G grading was 3 (p<0.005).

Our results, supported by previous studies, lead to hypothesize that invasive colorectal cancer with early metastases express CD10 positive cells as biomarker of invasiveness.

## Conclusions

On the light of the reported results we can hypothesize that CD10 positive neoplasia display a more invasive behaviour, independently from the N score and the G score, compared to CD10 negative neoplasia. Survival rate was not significantly different between the groups. However it should be acknowledged that the numbers were too small for obtaining statistical significance.

These observations might lead to implement the use of Immunohistochemistry as a reliable and easy to perform tool, to predict more invasive colorectal cancer. Previous about CD10 expression leading to early adenoma transformation to colorectal cancer supporte this hypothesis. In conclusion CD10 should be considered a reliable biomarker of invasive neoplasia with clinical and biological implications both in benign disease and in colorectal cancer enabling to predict early metastasis.

## Riassunto

AIM: Precedenti studi hanno riportato la positività, delle cellule da carcinoma colorettale, al CD10 come caratteristica di maggiore aggressività.

MATERIALI E METODI: Abbiamo esaminato 50 pazienti trattati chirurgicamente per carcinoma colorettale con almeno 5 anni di follow up.Il TNM, il grading score,la sopravvivenza sono state correlate alla positività al CD10.

RISULTATI: 34 casi su 50 sono stati analizzati (18 femmine e 16 maschi) dei quali 19 erano CD10 positivi e 15 CD10 negativi. I rimanenti 16 casi sono stati persi al follow up.Non abbiamo registrato differenze nella sopravvivenza tra pazienti CD10 positivi e negativi in rapporto a N0,N1,N2. Nessuna differenza tra sopravvivenza e grading. Abbiamo allora analizzato i casi C10 positivi e negativi in accordo al grading ed in rapporto alla positività dei linfonodi N1 e N2. Abbiamo evidenziato una differenza statisticamente significativa tra CD10 positivi/N2 (grading 1.66+/-0.5) e i casi CD10 negativi/N2(grading 3) (p<0.005).

CONCLUSIONI: Possiamo ipotizzare che le cellule che presentano una positività al CD10 dimostrino una tendenza ad essere maggiormente aggressive indipendentemente dal valore di N e dal grading, se confrontate con le cellule CD10 negative.

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