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# CD133 expression in patients with rectal adenocarcinoma selected to chemoradiotherapy treatment: a preliminary study of clinical implications

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**Running title**: CD133 expression in rectal adenocarcinoma

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## Abstract

**Background**: CD133 positive cancer stem cells have been correlated with resistance to preoperative chemoradiotherapy (CRT) in rectal adenocarcinoma. We analyse CD133 expression in rectal adenocarcinoma selected to CRT to determine it clinical relevance. **Materials and Methods**: CD133 expression was determined in 29 rectal adenocarcinoma patients by immunohistochemistry. Demographic and clinicopathological variables including treatment regression grade, lymph node invasion and tumor stage were determined. **Results**: Most of the rectal adenocarcinoma (72,4%) showed high CD133 expression. However, no significant association between CD133 protein expression and clinicopathological findings was found including treatment regression grade. Conclusions: Future research will be necessary to determine CD133 utility as biomarker in the treatment of patients with rectal adenocarcinoma.

**Key words**: CD133, chemoradiotherapy, Rectal adenocarcinoma

## Introduction

Patients with rectal adenocarcinoma stage II-III are usually treated with preoperative chemoradiotherapy (CRT) based on 5-fluorouracil (5-FU) or capecitabine. However, few data on molecular biomarkers for the rectal adenocarcinoma prognosis and treatment-response have been obtained1. Cancer stem cells (CSC) characterized by chemo-radio/resistance, self-renewal/capacity and multipotency, showed expression of CD133 (Prominin)2. CD133-positive cells correlated with CRT-resistance and poor prognosis3 although CD133-negative cells were also able to induce tumors in vivo4. Thus, the role of CD133 in

rectal adenocarcinoma has not yet been elucidated. In this study, CD133 protein expression was evaluated in rectal adenocarcinoma patients selected to CRT treatment in order to determine their status and relevance as prognostic biomarkers.

## Materials and Methods

### Clinical history and tissue samples

Rectal-adenocarcinoma patients (n=29) from stage II-III were recruited after obtaining informed consent (Biomedical Investigation Ethic Committee; Servicio Andaluz de Salud). All patients were evaluated before treatment (physical examination with digital rectal examination, colonoscopy and biopsy, chest X-ray, abdominopelvic scan and/or endorectal ultrasound and magnetic resonance image of the pelvis). All patients were treated with preoperative CRT using pelvic radiotherapy (46-50 Gy in 2 Gy fractions) and intravenous capecitabine (4 cycles of 1250 mg/m2 capecitabine every 12 h for 14 days) or 5-FU (5 day cycles of 500 mg/m2 5-FU every 21 days). Surgery (total mesorectal excision) was carried out 6 weeks after CRT. Tumor samples were obtained from each patient from endoscopic biopsy before CRT. CRT response was histopathologically staged based on tumor regression grade (Mandard's classification: grade I and II = complete/partial regression and grade III, IV or V = no regression)5. Evaluation was made by two expert pathologists in an intra-operatively sample after CRT. Data of the age, sex, and tumor stage were obtained. Clinicopathologic variables including treatment response and lymph node invasion was analysed in the patients (Table 1).

### Immunohistochemistry

CD133 expression can be determined in 93.1% specimens (27/29). Immunohistochemical analysis of samples (n=27) was performed with a Dako Autostainer EnVision™ FLEX System kit (Agilent Technologies) and evaluated by two experienced pathologists. CD133 (1:50, Miltenyi biotec., Bergisch Gladbach, Germany) mAb and 3.3'-diaminobenzidine as substrate chromogen were used. Hematoxylin (blue) was used as counterstaining. CD133 staining on the apical and/or endoluminal surface of tumor glands and/or on cell debris was considered positive, in accordance with previous studies6 and grouped as low (≤5% stained glands) and high expression (>5%) following Coco et al.7.

### Statistical analysis

SPSS version 15.0 (IBM, Chicago, IL) was used for the data analyses. Association between CD133 expression and clinicopathologic variables were analyzed by Fisher's exact test. Results were considered statistically significant if $p<0.05$.

**Table 1**: Characteristics of rectal cancer patients

| | All patients (n = 29) |
|---|---|
| Age | |
| ≥50 yrs | 27 (93.1%) |
| <50 yrs | 2 (6.9%) |
| Sex | |
| Male | 22 (75.9%) |
| Female | 7 (24.1%) |
| Tumor stage | |
| II | 10 (34.5%) |
| III | 19 (65.5%) |
| Lymph node metastasis | |
| Yes | 19 (65.5%) |
| No | 10 (34.5%) |
| Treatment regression grade | |
| I, II | 11 (38%) |
| III, IV, V | 18 (62%) |

## Results and discussion

CD133 protein expression has been correlated with colorectal cancer histological grade, infiltrative depth, metastasis, tumor stage and survival8. Our group of patients was a mean age of 64.43±12.24 years (range, 33-83 yrs) with a 24.1% female and 75.9% male and a median follow up of 20.53±9.07 months. Of the 27 patients analysed, the greatest proportion showed high CD133 expression (72.4%) while only the 27.6% showed low marker expression. However, CD133 expression (Fig. 1) was not significantly associated with any of the clinicopathological features analysed including tumor regression grade, lymph node invasion and tumor stage (Table 2). Coco et al.7 correlated this protein only with disease-free survival, tumor stage and recurrence and Reggiani et al.9 with colorectal cancer budding, micrometastasis and disease-free survival. By contrast, Huh et al.1 showed a no significant correlation between mRNA CD133 and colorectal cancer response after CRT. Kawamoto et al.10 reported that higher CD133 mRNA levels in colorectal cancer were associated with vascular invasion, recurrence, and disease-free survival. Despite our results in a group of rectal adenocarcinoma a more extensive research will be needed to elucidate the relationship between these biomarker and the rectal cancer treatment.

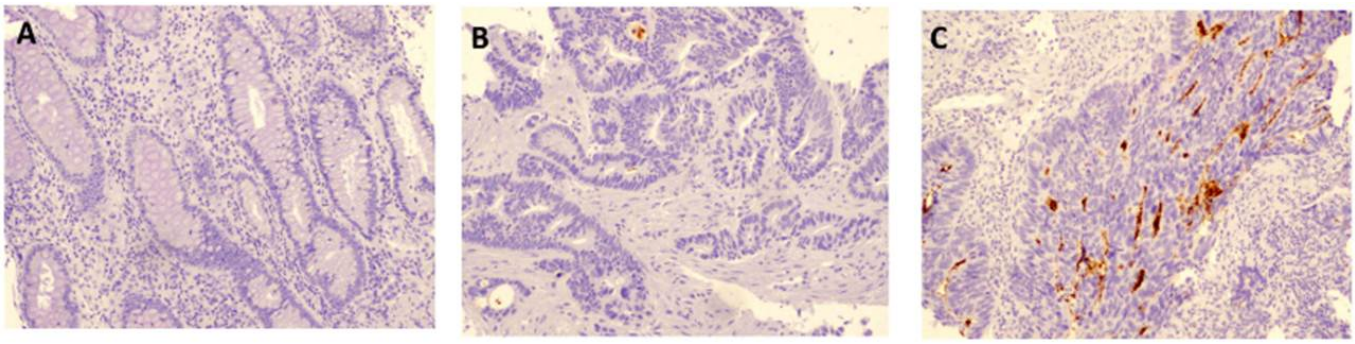


**Figure 1.** Immunohistochemical staining of rectal adenocarcinoma tissue samples with a mouse monoclonal antibody against human CD133 protein. Formalin-fixed paraffin-embedded of rectal cancer samples with an antibody against CD133 (see Methods) were used. The figure show representative photomicrographs of slides illustrating different percentages of CD133 expression. A) A tumor with no detectable CD133 expression; B) Positive tumor to CD133 expression (<5%). C) Positive tumor to CD133 expression (≥5%). (20x Magnification).

**Table 2**: Correlation between CD133 expression and clinicopathologic variables

| **Clinicopathological features** | **CD133 expression**: n=27 (% of patients) | |
|---|---|---|
| | Low | High |
| Tumor stage | | |
| II | 0 (0) | 9 (33,3) |
| III | 4 (14,8) | 14 (51,8 ) |
| Lymph node invasion | | |
| Yes | 4 (14,8 ) | 14 ( 51,8) |
| No | 0 ( 0) | 9 (33,3) |
| Treatment regression grade | | |
| I, II | 1 (3,7) | 9 (33,3) |
| III, IV, V | 3 (11,1) | 14 (51,8) |

## Conclusion

Although some studies reported the CD133 utility as biomarker in patients with rectal adenocarcinoma treated with CRT, our results showed no clinical value, suggesting that more researchs are needed.


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## Conflicts of Interest

There are no conflcts of interest

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