# Weak Cytotoxic T Cells Activation Predicts Low-Grade Dysplasia Persistence in Ulcerative Colitis

Andromachi Kotsafti, MS, PhD[1], Renata D'Incà, MD[2], Melania Scarpa, MS, PhD[1], Matteo Fassan, MD, PhD[3], Imerio Angriman, MD[4], Claudia Mescoli, MD[3], Nicolò Bortoli, MD[4], Paola Brun, MS, PhD[5], Romeo Bardini, MD[4], Massimo Rugge, MD[3], Edoardo Savarino, MD, PhD[4], Fabiana Zingone, MD[4], Carlo Castoro, MD[6], Ignazio Castagliuolo, MD[5] and Marco Scarpa, MD, PhD[7]

INTRODUCTION: **In patients with ulcerative colitis (UC), dysplasia develops in 10%–20% of cases. The persistence of low-grade dysplasia (LGD) in UC in 2 consecutive observations is still an indication for restorative proctocolectomy. Our hypothesis is that in the case of weak cytotoxic activation, dysplasia persists. We aimed to identify possible immunological markers of LGD presence and persistence.**

METHODS: **We prospectively enrolled 112 UC patients who underwent screening colonoscopy (T0) who had biopsies taken from their sigmoid colon. Ninety of them had at least a second colonoscopy (T1) with biopsies taken in the sigmoid colon and 8 patients had dysplasia in both examinations suggesting a persistence of LGD in their colon. Immunohistochemistry and real time polymerase chain reaction for CD4, CD69, CD107, and CD8β messenger RNA (mRNA) expression and flow cytometry for epithelial cells expressing CD80 or HLA avidin-biotin complex were performed. Non-parametric statistics, receiver operating characteristic curves analysis, and logistic multiple regression analysis were used.**

RESULTS: **Thirteen patients had LGD diagnosed at T0. The mucosal mRNA expression of CD4, CD69, and CD8β was significantly lower than in patients without dysplasia ($P = 0.033$, $P = 0.046$ and $P = 0.007$, respectively). A second colonoscopy was performed in 90 patients after a median follow-up of 17 (12–25) months and 14 of the patients were diagnosed with LGD. In these patients, CD8β mRNA expression at T0 was significantly lower in patients without dysplasia ($P = 0.004$). A multivariate survival analysis in a model including CD8β mRNA levels and age >50 demonstrated that both items were independent predictors of dysplasia at follow-up (hazard ratio [HR] = 0.47 [95% confidence interval [CI]: 0.26–0.86], $P = 0.014$, and HR = 13.32 [95% CI: 1.72–102.92], $P = 0.013$).**

DISCUSSION: **These data suggest a low cytotoxic T cell activation in the colonic mucosa of UC patients who do not manage to clear dysplasia. Thus, low level of CD8β mRNA expression in non-dysplastic colonic mucosa might be considered in future studies about the decision making of management of LGD in UC.**

## INTRODUCTION

Ulcerative colitis (UC) patients are at risk of colorectal cancer (1) with a cumulative risk at approximately 8% at approximately 20 years after the initial diagnosis and up to 18% 30 years after (2,3). However, adenocarcinoma of the colon develops from a dysplastic precursor lesion, which may occur even early in the course of the disease (4). Pre-malignant histological alterations in UC patients are broadly referred to as dysplasia, rather than adenoma, since dysplasia is frequently not polypoid (5). While current guidelines clearly recommend colectomy for high-grade dysplasia (HGD) because of the risk of a concomitant or future colorectal cancer, the indications for the management of flat low-grade dysplasia (LGD) in UC are less definite (6). In 3 studies with a small number of patients operated on for LGD, 20%, 27%, and 19% of the patients, respectively, were found to have colorectal cancer (CRC) (7–9). In a meta-analysis of 20 studies, when dysplasia is detected during surveillance, the risk of developing CRC is 9-fold for LGD and 12-fold for HGD, and the positive

[1]Laboratory of Advanced Translational Research, Veneto Institute of Oncology IOV - IRCCS, Padova, Italy; [2]Gastroenterology Unit, Azienda Ospedaliera di Padova, Padova, Italy; [3]Department of Medicine, University of Padova, Padova, Italy; [4]Department of Surgery, Oncology and Gastroenterology, University of Padova, Padova, Italy; [5]Department of Molecular Medicine, University of Padova, Padova, Italy; [6]Upper GI Surgery Unit, Istituto Humanitas, Rozzano, Italy; [7]General Surgery Unit, Azienda Ospedaliera di Padova, Padova, Italy. **Correspondence:** Marco Scarpa, MD, PhD. E-mail: marcoscarpa73@yahoo.it.

predictive value for progression from LGD to HGD or CRC was 14.6% (10). High rates of progression have generally been reported in retrospective studies (8,11). In contrast, prospective studies reported no increased progression rates in patients with LGD compared to patients without dysplasia (12,13). However, in a recent study investigating 172 UC patients diagnosed with LGD in the St Mark's Hospital surveillance cohort, the cumulative incidence of HGD or CRC development after 5 years was 6.0% for polypoid dysplasia and 65.2% for non-polypoid dysplasia with a high degree of multifocal localization (14). These data support findings from earlier studies, where there was a strong association of metachronous or synchronous carcinoma with non-polypoid dysplasia, ranging from 38% to 83%. For this reason, it is generally recommended that patients with UC and endoscopically unresectable non-polypoid dysplasia should undergo immediate colectomy, regardless of the grade of dysplasia detected by biopsy analysis (15). Therefore, current evidence is insufficient to assess the balance of risks and benefits of colectomy for flat LGD (6). Thus, the clinical question here is how to predict LGD persistence because LGD persistence in 2 consecutive colonoscopies might be the indication to restorative proctocolectomy.

Cancer immunoediting, mediated by CD8 and CD4 T cells, macrophages, and natural killer cells, may lead to cancer cell destruction (cancer immunosurveillance) with complete extrinsic tumor elimination resulting in definitive protection (16). It is well-known, nonetheless, that some tumor cells escape immunosurveillance leading to unrestrained neoplastic cell growth and metastatic diffusion. The immune escape mechanism is thought to be facilitated by both the mechanisms of tumor cell defense and/or immune system failure (17,18). Activation of tumor-specific and cytotoxic activity of CD8 T cells and the tumor-selective migration of CD4 T helper cells take place during the early stages of colorectal cancer (19). In UC patients, immunogenic proteins, such as the products of oncogenes or oncosuppressor-mutated proteins, are potentially expressed by mutated colorectal epithelial cells, and they are usually rejected by the intraepithelial T cells through the CD80-CD28 cross talk (20). This interplay had been previously documented in other cancer cascade (21,22). The first event occurring in colonic carcinogenesis driven by inflammation is due to increased DNA oxidative damage and this kind of damage is associated to costimulatory molecule CD80 expression (23). Our hypothesis was that the immunological status of the mucosal microenvironment might favor either LGD persistence (immune surveillance failure) or LGD elimination (complete immune surveillance), depending on its effectiveness (16).

The aim of our study was, then, to identify immunological markers of LGD presence and persistence in the case of LGD detection during surveillance colonoscopy at random biopsies.

## METHODS
### Patients
A prospective cohort study of UC patients (n = 112) who underwent colonoscopy for screening was designed. The study, which received institutional review board (Ethical Committee of the Veneto Institute of Oncology) approval (project MICCE1 IOV 2011/53), was performed according to the principles of the Declaration of Helsinki, and all those participating signed informed consent forms. The patients were, therefore, grouped in UC patients without dysplasia and UC patients with LGD. UC was diagnosed on the basis of clinical, laboratory, and

endoscopical features (24,25). None of the patients had concomitant primary sclerosing cholangitis.

### Study design
Six 3-mm mucosa samples were taken from the sigmoid region (20–25 cm from the anal verge) during colonoscopy prescribed for surveillance purposes in UC patients. The detection of LGD anywhere in the colon categorized the patient as having LGD. Specimens were frozen in liquid nitrogen and then stored at −80 °C for molecular analysis, preserved in 10% formalin solution for histological analysis, or immediately processed for flow cytometry. The patients' medical records were reviewed and their demographic and clinical data (including duration and disease extension, symptoms, therapy, colonoscopy findings, colonic biopsies, surgery and its indication, findings and histological grading, and the dates of follow-up examinations and vital signs) were collected.

### Histology
After fixation in 10% neutral buffered formalin, the specimens were dehydrated and embedded in paraffin wax; sections of 3 mm were produced and stained with hematoxylin-eosin. Vienna classification of gastrointestinal epithelial neoplasia was adopted: negative for neoplasia/dysplasia, indefinite for neoplasia/dysplasia, non-invasive low-grade neoplasia/LGD, non-invasive high-grade neoplasia/HGD, and invasive carcinoma (26–28).

### Gene expression analysis
Total RNA was extracted using the RNeasy Plus Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. Then 0.5 μg total RNA was converted to complementary DNA using the Applied Biosystems complementary DNA Synthesis kit (Applied Biosystems, Foster City, CA), again, according to the manufacturer's instructions. Specific messenger RNA (mRNA) transcripts were quantified with SYBR Green polymerase chain reaction (PCR) Master Mix in a ABI PRISM 7000 Sequence Detection System (Applied Biosystems). Gapdh expression was used as reference gene for normalization. Primer sequences and PCR conditions are outlined in Supplementary Table 1 (Supplementary Digital Content 1, http://links.lww.com/CTG/A62).

**Table 1. Patients' characteristics**

| Timing | Characteristics | |
|--------|-----------------|---|
| T0 | Patients | 112 |
| | Gender | 45 female/67 male |
| | Age | 51 (41–61) yr |
| | Age at diagnosis | 35 (27–43) yr |
| | UC duration | 13 (8–22) yr |
| | Harvey-Bradshaw activity index | 3 (2–5) |
| | Mayo endoscopic subscore | 1 (0–3) |
| | History of dysplasia | 28 patients |
| | Current diagnosis of LGD | 13 patients |
| T1 | 1st follow-up (patients) | 90 |
| | Follow-up | 17 (12–25) mo |
| | Diagnosis of LGD at follow-up | 14 patients |

UC = ulcerative colitis; LGD = low-grade dysplasia.
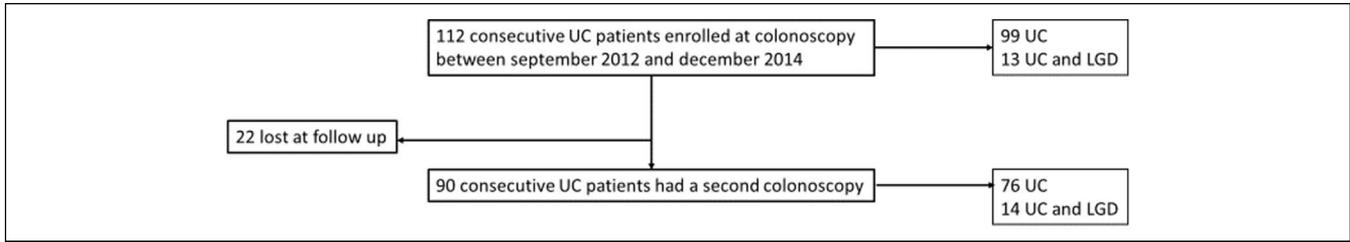
**Figure 1.** Patients' enrolment flow chart. LGD = low-grade dysplasia; UC = ulcerative colitis.

## Immunohistochemistry

Once fixed on a slide, the samples were deparaffinized in xylol and subsequently treated with H2O2. To release the molecule from the remaining formalin bonds, the slides was submerged in citrate buffer and incubated in a microwave oven at 90 °C. Normal horse serum was used to reduce non-specific binding. The primary antibody, a murine IgG1, specific for the CD80, CD4, CD8, CD107, and CD8β was added and incubated for 30 minutes at room temperature. After several washes, the secondary antibody (a horse immunoglobulin conjugated with biotin, directed against
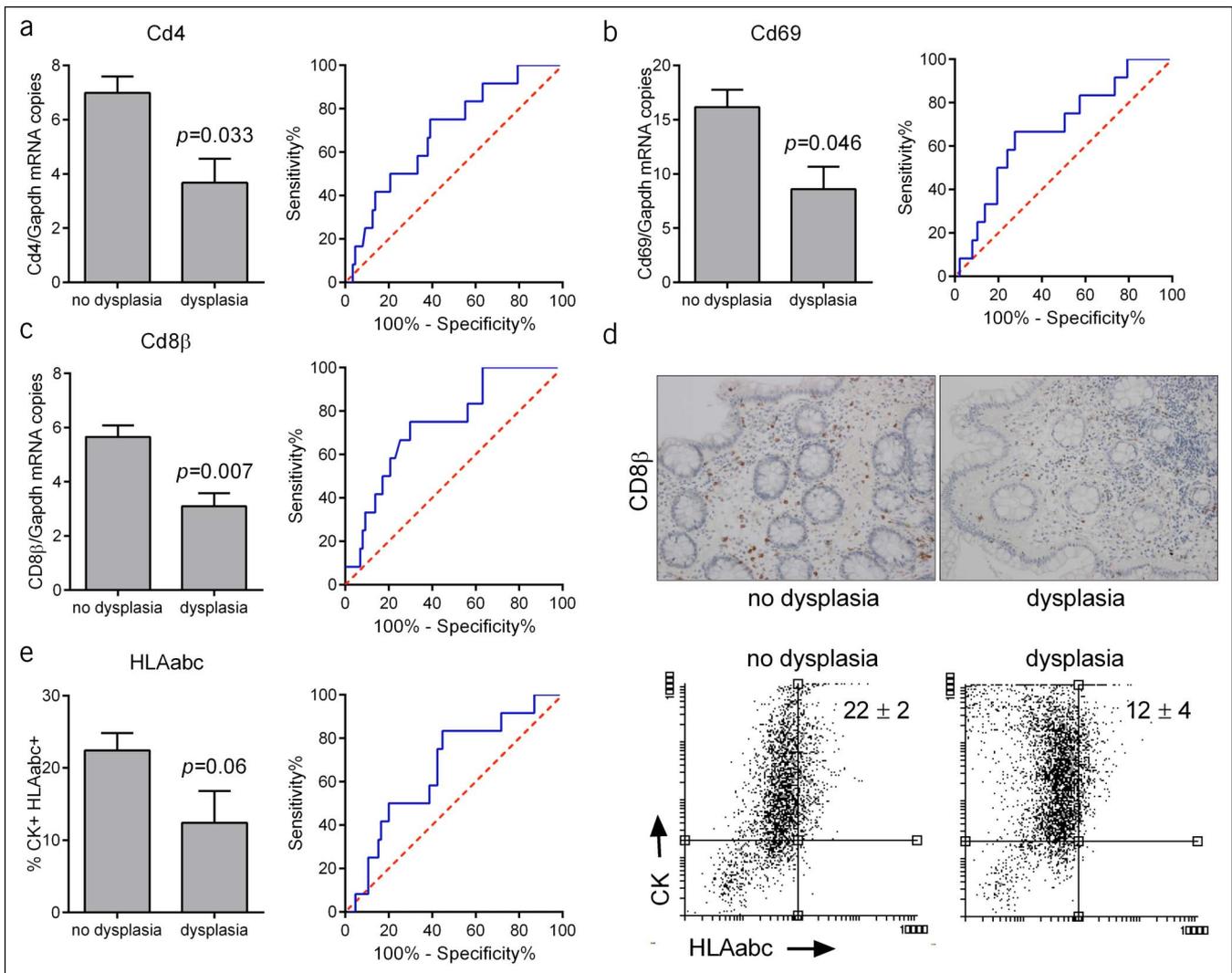


**Figure 2.** Immunological markers of LGD observation at colonoscopy. Mann-Whitney *U* test and receiver operating characteristic (ROC) curves analysis were performed ($P < 0.05$). (**a**) CD4 mRNA expression is compared in patients with LGD vs non-dysplastic patients and a ROC curve to show the accuracy of CD4 mRNA to predict LGD presence. (**b**) CD69 mRNA expression (naive T cell activation marker) is compared in patients with LGD vs non-dysplastic patients and a ROC curve to show the accuracy of CD69 mRNA to predict LGD presence. (**c**) CD8β mRNA expression is compared in patients with LGD vs non-dysplastic patients and a ROC curve to show the accuracy of CD8β mRNA to predict LGD presence. (**d**) CD8β + T cell infiltration is compared in patients with LGD vs non-dysplastic patients. (**e**) HLA-ABC+ epithelial cells (epithelia cells acting as non-professional antigen presenting cells) is compared in patients with LGD vs non-dysplastic patients. ABC = avidin-biotin complex; LGD = low-grade dysplasia; mRNA = messenger RNA.
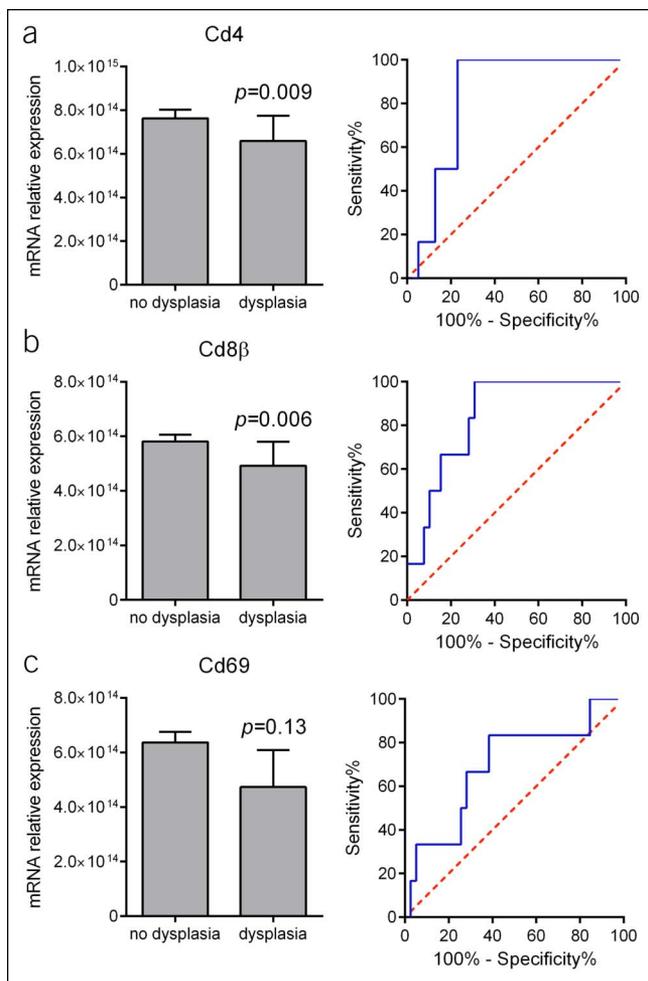
**Figure 3.** Immunological markers of LGD observation at colonoscopy: a validation cohort on GSE47908 dataset (29). Mann-Whitney *U* test was performed (*P* < 0.05). (**a**) CD107 mRNA expression (degranulation marker) is compared in patients with LGD vs non-dysplastic patients and a receiver operating characteristic (ROC) curve to show the accuracy of CD107 mRNA to predict LGD presence. (**b**) CD8β mRNA expression is compared in patients with LGD vs non-dysplastic patients and a ROC curve to show the accuracy of CD8β mRNA to predict LGD presence. (**c**) HLA-ABC mRNA expression is compared in patients with LGD vs non-dysplastic patients. ABC = avidin-biotin complex; LGD = low-grade dysplasia; mRNA = messenger RNA.

murine immunoglobulins) was added and incubated for further 30 minutes. The slides were washed in phosphate buffered saline (PBS) with a final wash of 30 minutes with the avidin-biotin-peroxidase complex. The peroxidase of the detecting system reacted with 3′3′-diaminobenzidine, which were added to the slides and allowed to incubate for 5 minutes, giving the cells a brown stain. In order to quantify the number of positive cells, we counted the percentage of leukocytes stained by the avidin-biotin complex (ABC) system immediately below the epithelium. From each sample, we examined 10 random fields at ×60 magnification. Antibodies details are outlined in Supplementary Table 2 (Supplementary Digital Content 1, http://links.lww.com/CTG/A62).

**Flow cytometry**
Mucosal biopsies were mechanically dissected and passed through a sterile Nylon Filter (BD Falcon, Heidelberg, Germany). The single cell suspension was pelleted, suspended in fluorescence-activated

cell sorting (FACS) buffer (PBS/2% FACS/0.02% sodium azide), and stained with fluorochrome-conjugated antibodies. Single-cell suspensions were subjected to flow cytometry to determine the proportion of epithelial cells (Cytokeratin+) acting as antigen-presenting cells (expressing HLA-ABC or CD80). Flow cytometric analysis was performed using a FACSCalibur based on CellQuest software (Becton Dickinson, Franklin Lakes, NJ).

**External validation series**
Validation series consisted of gene expression data from 96 samples from Hungary accessed from the Gene Expression Omnibus databank (dataset ID: GSE47908 (29)). According to the Gene Expression Omnibus entries, total RNA was extracted from colonic biopsy samples of UC patients (n = 54) and of patients with colonic dysplasia in UC (n = 6) and were hybridized on Affymetrix HGU133 Plus 2.0 microarrays. Our selected gene panel was tested on the downloaded dataset, and comparison between dysplasia vs non-dysplasia was done using non-parametric Mann-Whitney *U* test adjusted for multiple comparison (*P*-value < 0.001).

**Statistical analysis**
Considering an effect size of 20% and a standard deviation of 20%, the subsequent standardized effect size was 1.0. Then we assumed a level of statistical significance (α) of 0.05 and a power (1 − β) of our tests of 0.20. Consequently, the sample size required per group when using the 2-tailed *t* test to compare means of continuous variables was 16 patients for each group. Statistical analysis was performed with Windows Microsoft Excel (Redmond, WA) and STATISTICA 7.1 software (Statsoft, Tulsa, OK). Non-parametric Mann-Whitney *U* 2-tailed test was used for comparison where appropriate. Receiver operating characteristic (ROC) curve analysis was used to assess the accuracy and the threshold values of the potential immune markers in order to provide estimated values to plan future validation studies. Cox proportional hazards models were used to define the role of the different possible covariates. Statistical significance was set at *P* < 0.05.

## RESULTS
**Patient characteristics**
The mucosal immunological markers were prospectively assessed in the colonic mucosa of 112 consecutive patients in the Gastroenterology Unit of the Azienda Ospedaliera di Padova (45 female/67 male) undergoing colonoscopy from September 2012 to December 2014. Patient and disease characteristics are shown in Table 1. Patient disposal is shown in Figure 1.

**Immunological markers of LGD observation at colonoscopy**
Within the whole study group, 13 patients had the diagnosis of current LGD at colonoscopy at T0. In patients who were diagnosed with LGD, the mucosal mRNA expression of CD4 (Figure 2a), CD69 (Figure 2b), and CD8β (Figure 2c) was significantly lower than in patients with a colonoscopy negative for dysplasia (*P* = 0.033, *P* = 0.046 and *P* = 0.007, respectively). The number of CD80+ and CD107+ cells within the colonic mucosa nor the number of CD8+ and CD8β+ cells (Figure 2d) within the colonic mucosa was significantly different between the 2 groups (see Supplementary Figure 1, Supplementary Digital Content 2, http://links.lww.com/CTG/A63). Moreover, in these patients, the rate of epithelial cells expressing HLA-ABC tended to be lower (*P* = 0.06) (Figure 2e). ROC curve analysis showed that CD4, CD69, and
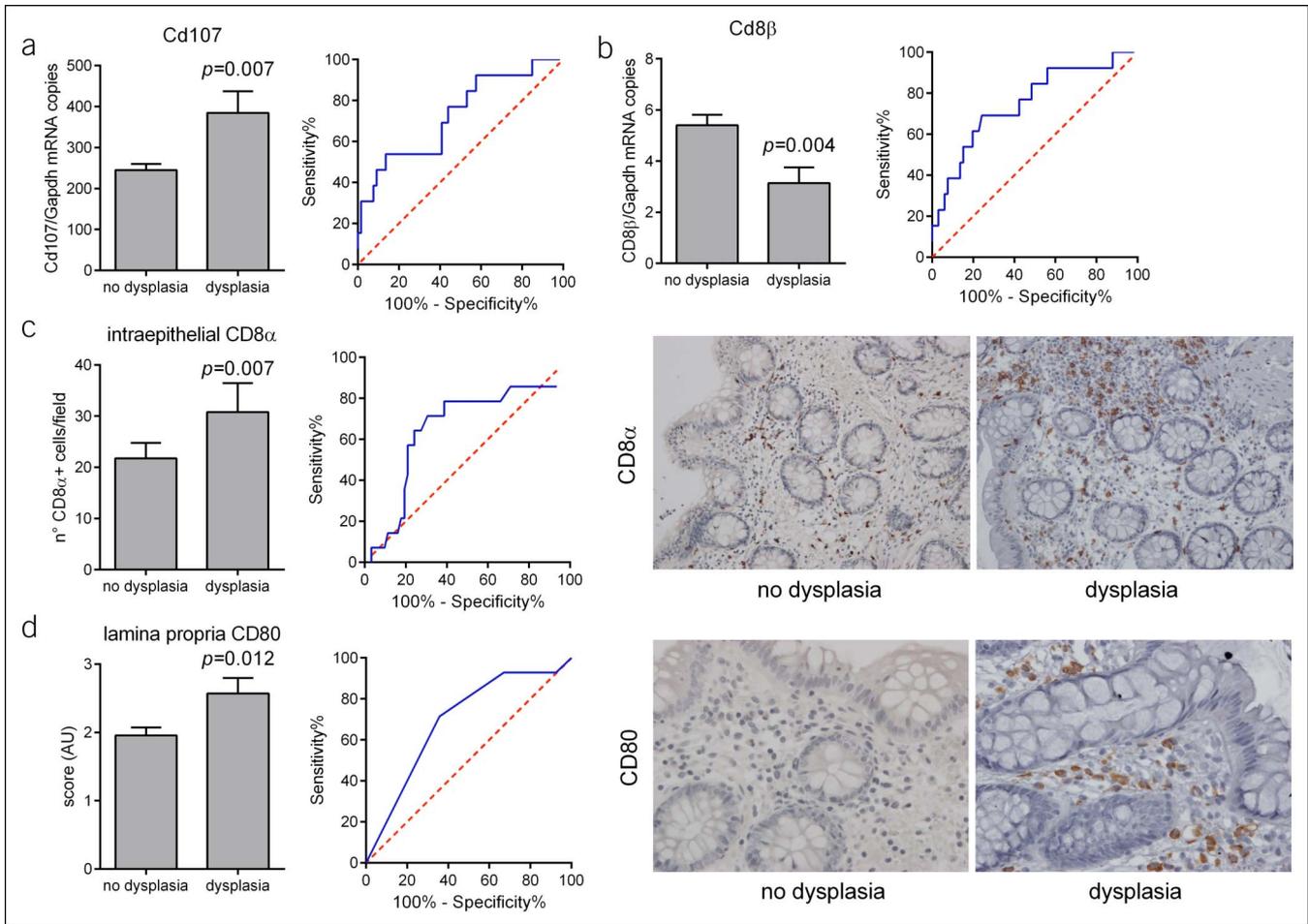
**Figure 4.** Immunological predictors of LGD at second colonoscopy. Mann-Whitney $U$ test and receiver operating characteristic (ROC) curves analysis were performed ($P < 0.05$). (**a**) CD107 mRNA expression (degranulation marker) is compared in patients had LGD at second colonoscopy vs patients who did not had it, and a ROC curve is shown to show the accuracy of CD107 mRNA to predict LGD presence at second colonoscopy. (**b**) CD8β mRNA expression is compared in patients had LGD at second colonoscopy vs patients who did not had it, and a ROC curve is shown to show the accuracy of CD8β mRNA to predict LGD presence at second colonoscopy. (**c**) CD8α+ T cell infiltration is compared in patients with LGD at second colonoscopy vs non-dysplastic patients and a ROC curve to show the accuracy of CD8α infiltration to predict LGD presence at second colonoscopy. (**d**) CD80+ antigen presenting cell infiltration is compared in patients with LGD at second colonoscopy vs non-dysplastic patients and a ROC curve to show the accuracy of CD80 infiltration to predict LGD presence at second colonoscopy. ABC = avidin-biotin complex; LGD = low-grade dysplasia; mRNA = messenger RNA.

CD8β mRNA levels and the rate of epithelial cells expressing HLA-ABC had an accuracy in predicting the presence of LGD in the UC colon of 0.69 (95% confidence interval [CI]: 0.58–0.78, $P = 0.009$), 0.68 (95% CI: 0.57–0.77, $P = 0.016$), 0.74 (95% CI: 0.64–0.83, $P < 0.001$), and 0.66 (95% CI: 0.56–0.75, $P = 0.032$), respectively.

No clinical features and no type of therapy were associated with LGD presence. No difference in CD4 and CD8 mRNA expression was observed according Mayo severity score or according to the histological disease severity (see Supplementary Figure 2b and c, Supplementary Digital Content 3, http://links.lww.com/CTG/A64). However, even if CD8β+ T cell rate did not correlate with the microscopic disease severity, the CD8α+ T cell rate positively correlated with the histological disease severity (rho = 0.19, $P = 0.05$) (see Supplementary Figure 2d, Supplementary Digital Content 3, http://links.lww.com/CTG/A64).

The external validation of immune surveillance related genes as predictors of LGD in UC on the GSE47908 dataset (25) showed that CD4 (Figure 3a) and CD8β (Figure 3b) mRNA levels were significantly lower in LGD patients than in those without dysplasia ($P = 0.009$, and $P = 0.006$, respectively) while CD69 (Figure 3c) tended to be lower ($P = 0.13$). The ROC curve analysis on the GSE47908 dataset showed a good accuracy for LGD diagnosis of both 2 markers (AUC = 83% and AUC = 84%, respectively).

## Immunological predictors of LGD observation at second colonoscopy

A second colonoscopy was performed in 90 patients after a median follow-up of 17 (12–25) months while 22 patients were lost at follow-up. In 14 patients, LGD was diagnosed at random colonic biopsies at the second colonoscopy. In patients who would be diagnosed with LGD at the second colonoscopy, mucosal mRNA expression of CD107 (Figure 4a) at T0 was significantly higher ($P = 0.009$) while CD8β (Figure 4b) mRNA expression at T0 was significantly lower than in patients with a colonoscopy negative for dysplasia ($P = 0.004$). Moreover, in these patients, CD8α+ T cells (Figure 4c) and CD80+ antigen
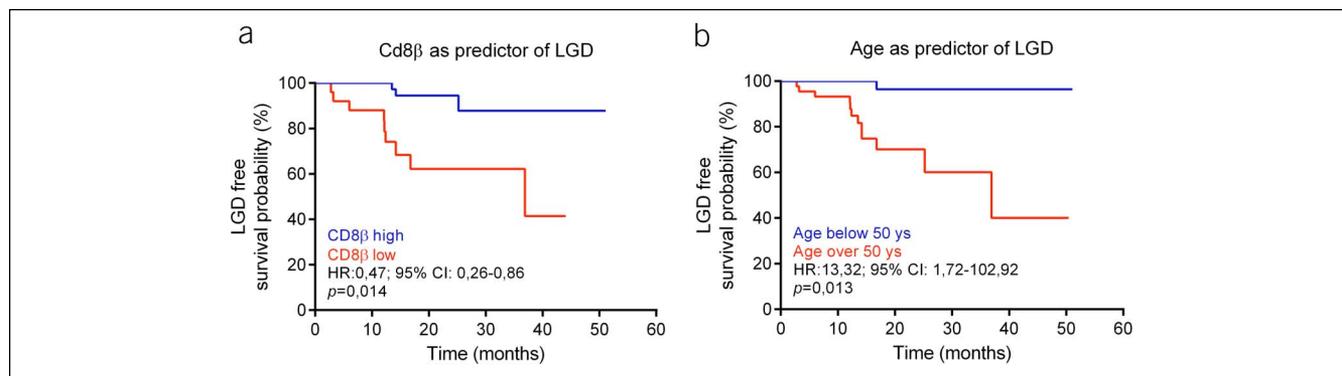
**Figure 5.** LGD free survival was depicted with Kaplan-Meier curves and log rank test was performed to compare clinical and immunological markers ($P <$ 0.05). A multivariate survival analysis in a model including CD8β mRNA levels (**a**) and age >50 yr (**b**) demonstrated that both items were independent predictors of dysplasia at follow-up. ABC = avidin-biotin complex; LGD = low-grade dysplasia; mRNA = messenger RNA.

presenting cell (Figure 4d) infiltration in the lamina propria were significantly higher than patients without dysplasia at the second colonoscopy ($P = 0.007$ and $P = 0.012$, respectively). The ROC curve analysis showed that intraepithelial CD8+ cell rate, CD107, and CD8β mRNA levels and CD80 expression in the lamina propria had an accuracy in predicting the presence of LGD at a second colonoscopy of 0.74 (95% CI: 0.62–0.84, $P = 0.004$), 0.73 (95% CI: 0.61–0.82, $P = 0.007$), 0.75 (95% CI: 0.64–0.84, $P < 0.001$), and 0.76 (95% CI: 0.59–0.88, $P = 0.009$), respectively. At multivariate survival analysis in a model including CD8β mRNA levels (Figure 5a) and age >50 years (Figure 5b) demonstrated that both items were independent predictors of dysplasia at follow-up (hazard ratio [HR] = 0.47 [95% CI: 0.26–0.86], $P = 0.014$ and HR = 13.32 [95% CI: 1.72–102.92], $P = 0.013$, respectively). Intraepithelial CD8+ cell rate, CD107 mRNA expression, and CD80 expression in the lamina propria did not result to be independent predictors of LGD at the second colonoscopy if adjusting for patients' age.

Finally, we analyzed the subgroup of patients with exclusively de novo LGD at T1 and the subgroup of patients who had never had LGD and we found no statistically significant difference between the 2 groups in terms of immune markers within the colonic mucosa (see Supplementary Figure 3, Supplementary Digital Content 4, http://links.lww.com/CTG/A65).

### Immunological markers of LGD persistence at second colonoscopy

LGD was confirmed in 8 patients in 2 separate colonoscopies: CD107 (Figure 6a) mRNA expression was higher than in patients without dysplasia ($P = 0.02$) while CD8β (Figure 6b) mRNA levels were significantly lower in patients with LGD than in patients without dysplasia ($P = 0.009$). Moreover, in these patients, the rate of epithelial cells expressing HLA-ABC (Figure 6c) tended to be lower ($P = 0.07$). The ROC curve analysis showed that CD8β mRNA levels had a good accuracy in predicting the persistence of LGD at the second colonoscopy (AUC = 0.80 [95% CI: 0.70–0.88, $P = 0.0001$]). On the contrary, CD107 mRNA expression and rate of epithelial cells expressing HLA-ABC showed a lower accuracy, (AUC = 0.64 [95% CI: 0.54–0.72, $P = 0.23$] and AUC = 0.70 [95% CI: 0.59–0.80, $P = 0.023$], respectively).

## DISCUSSION

UC patients are at increased risk of colorectal cancer (1) that usually develops from dysplasia (4) not necessarily polypoid (5). Therefore, surveillance with multiple biopsies is suggested, every 1–3 years based on the risk (6,15,26). UC mucosa may be irregular due to chronic inflammation or inflammatory polyps; therefore, markers of possible evolution toward dysplasia will be welcomed especially if detectable in any colonic segment l (30). The diagnostic value of gene mutation, gene methylation, or single nucleotide polymorphisms (31–34) has been evaluated but the heterogeneity of the mutational load and the rarity of the event made these measurements inapplicable. On the other hand, the paucity, and, possibly, the heterogeneity of the mutational load at the very early carcinogenesis step suggested exploring different possible markers that might predict the evolution of LGD towards cancer. Thus, we aimed to identify immunological markers of LGD presence and persistence in the case of LGD detection during surveillance colonoscopy at random biopsies.

In our series, within the whole study group, 13 patients had the diagnosis of current LGD at colonoscopy. The characteristics of these patients were similar to the remaining group of patients; in particular, no difference in terms of therapy was observed. In patients who were diagnosed with LGD, the mucosal mRNA expression of CD4, CD69, and CD8β was significantly lower than in patients with colonoscopy without dysplasia. These results did not seem to be influenced to UC disease activity. Moreover, in these patients, the rate of epithelial cells expressing HLA-ABC tended to be lower. However, the number of CD8+ and CD8β+ cells was not different between the 2 groups. These data suggest that the grade of activation and not the number of activated cytotoxic lymphocytes determines the fate of LGD in UC colonic mucosa. In fact, the ROC curve analysis showed that CD8β mRNA levels were the best predictors of the presence of LGD. The external validation of the analysis on the GSE47908 dataset (29) showed that CD38, CD4, CD80, and CD8β mRNA levels were significantly lower in those with LGD than in those without dysplasia. The ROC curve analysis on the GSE47908 dataset showed a good accuracy for the 3 markers. These data suggest that inactivating immune surveillance opens the way to LGD. In previous studies, we have observed in the mouse model of colonic carcinogenesis with azoxymethane-dextran sulfate sodium that the inhibition of CD8 leaded to a significant increase of LGD extension (20). All
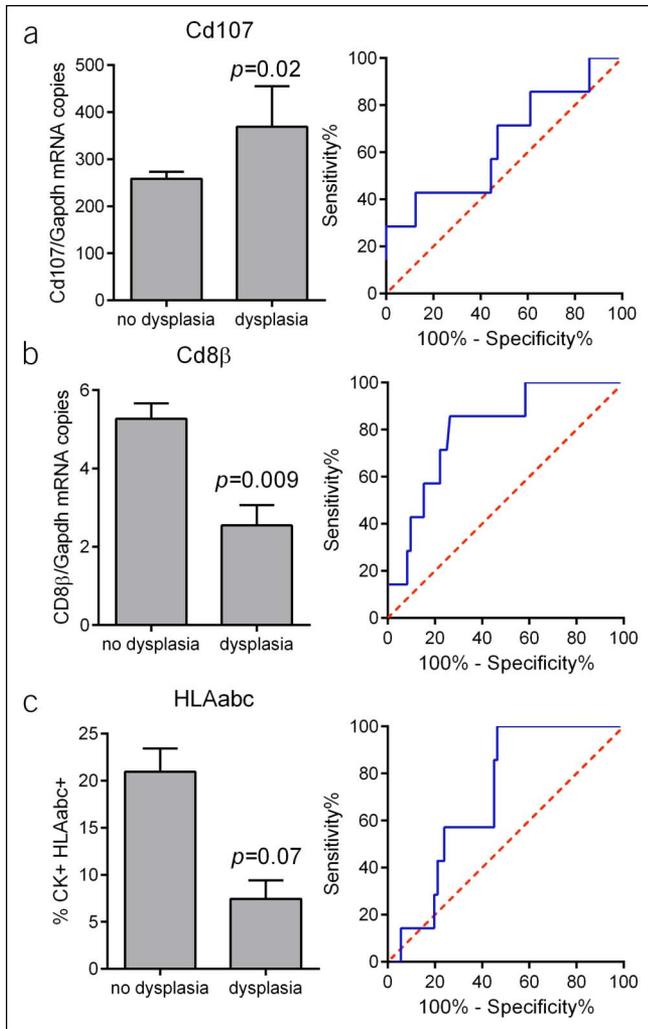
**Figure 6.** Immunological predictors of LGD persistence at second colonoscopy. Mann-Whitney *U* test and receiver operating characteristic (ROC) curves analysis were performed ($P < 0.05$). (**a**) CD107 mRNA expression (degranulation marker) is compared in patients with LGD persistence vs patients who did not had LGD, and a ROC curve is shown to show the accuracy of CD107 mRNA to predict LGD persistence at second colonoscopy. (**b**) CD8β mRNA expression is compared in patients with LGD persistence vs patients who did not had LGD, and a ROC curve is shown to show the accuracy of CD8β mRNA to predict LGD persistence at second colonoscopy. (**c**) HLA-ABC mRNA expression is compared in patients with LGD persistence vs patients who did not had LGD, and a ROC curve is shown to show the accuracy of HLA-ABC mRNA to predict LGD persistence at second colonoscopy. ABC = avidin-biotin complex; LGD = low-grade dysplasia; mRNA = messenger RNA.

these data confirm the role of cytotoxic lymphocytes in the prevention of the progression to LGD in the colonic mucosa of UC patients.

Currently, the decision to undergo colectomy vs continued surveillance in patients with LGD without visible lesions is still controversial (6). Colectomy will eradicate the risk of CRC, but if a patient is unwilling to undergo colectomy, tight surveillance is strongly recommended (35). In our series, a second colonoscopy was performed in 90 patients and LGD was found in 15.5% at random colonic biopsies. The intraepithelial CD8+ cell rate and mucosal mRNA expression of CD107 at T0 were significantly

higher while CD8β mRNA expression was significantly lower in patients who had LGD at follow-up colonoscopy. At multivariate survival analysis, both CD8β mRNA levels and age >50 years independently predicted dysplasia at the follow-up. On the other hand, intraepithelial CD8+ cell rate, CD107 mRNA expression, and CD80 expression in the lamina propria did not result to be independent predictors of LGD at the second colonoscopy if adjusting for patients' age. In the group of patients who had LGD at T0, 8 patients had LGD confirmed at the second colonoscopy. In this subgroup of patients, CD8β mRNA levels were significantly lower than in patients who did not have LGD. Thus, a low mucosal expression of CD8β, representing a weak cytotoxic T cells activation, could be considered a marker of LGD presence and persistence. These data might be potentially relevant for the clinical management of UC patients: a validation study on the role of CD8β as a marker of LGD persistence is warranted to assess its potential clinical value in the decision making between colectomy and endoscopic surveillance in the case of LGD detection.

On the other hand, the lack of significant difference in terms of immune markers in the subanalysis of patients with de novo LGD at T1 compared to patients who had never had LGD is in part due to the small sample size of the de novo LGD group and, in part, due to the different timings of the somatic mutations that might not have induced a complete immune response yet.

Intestinal intraepithelial lymphocytes reside within the epithelium of the intestine forming one of the main branches of the immune system and most of them express CD8α homodimer together with other molecules associated with immune regulation (36). In fact, in our series, CD8α+ T cell rate positively correlated with the histological disease severity but CD8β+ T cell rate did not correlate with the microscopic disease severity. These data seem to suggest that CD8β expression is more specific for reacting against LGD than for the inflammatory mucosal infiltration. High affinity receptor CD8β might be devoted to reacting against mutated cells while low affinity receptor CD8α might prevail in the case of chronic inflammation.

The main limitation of this study is that the marker we found was obtained through Real time-PCR and not through simple immunohistochemistry. This method is less diffuse, more expensive, and needs definite expertise. We tested the diagnostic yield of immunohistochemistry but the data we obtained were different: the number of CD8β+ cells that infiltrate the colonic mucosa was the same in both groups suggesting that the level of mRNA expression probably reflects the actual activation of the cytotoxic lymphocytes. On the other hand, western blot could be attempted but the low protein levels made this method questionable.

In conclusion, our data suggest that weak cytotoxic T cells activation is associated to LGD presence and persistence in the colonic mucosa of UC patients. Low CD8β expression in the colonic mucosa of UC patients can be considered a marker of LGD presence. Moreover, the occurrence of LGD together with low levels of CD8β mRNA expression might foresee LGD persistence. These data might be considered in future studies about the decision making in a patient affected by UC and LGD.

## CONFLICTS OF INTEREST

**Guarantor of the article:** Marco Scarpa, MD, PhD.
**Specific author contributions:** Andromachi Kotsafti, Renata D'Incà, Ignazio Castagliuolo and Marco Scarpa, MD, PhD, equally

## Study Highlights

**WHAT IS KNOWN**

✓ In patients with UC, dysplasia develops in 10%–20% of cases.
✓ The persistence of LGD in 2 consecutive observations is an indication for restorative proctocolectomy.

**WHAT IS NEW HERE**

✓ The mucosal mRNA expression of CD4, CD69, and CD8β is significantly lower than that in patients without dysplasia.
✓ In patients with persisting LGD, CD8βmRNA expression at T0 is significantly lower than that in patients without dysplasia.

**TRANSLATIONAL IMPACT**

✓ Lowlevel of CD8βmRNA expression in nondysplastic colonic mucosa might be used in the decision-making of UC management as a predictor of persistence of dysplasia.

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