# CHARACTERIZATION AND QUANTIFICATION OF MACROPHAGES IN COLORECTAL CANCER BY AN AUTOMATED CELL DETECTION SYSTEM

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**INTRODUCTION:** Macrophages represent around 10% of mononuclear cells in intestinal lamina propria and participate in maintaining the peculiar intestinal homeostasis balancing protective immunity against pathogens and preventing pathological inflammation under normal conditions.

Colorectal tumours very often show strong infiltration of immune cells. Among these, macrophages are the major cellular components. The connection between inflammation and colorectal cancer (CRC) is now well established, but the role of macrophages in this process is controversial. Recently, it was proposed that the macrophages have different roles in tumorigenesis depending on their state of activation. In chronic inflammation macrophages can fuse in multinucleated giant cells (MGC)

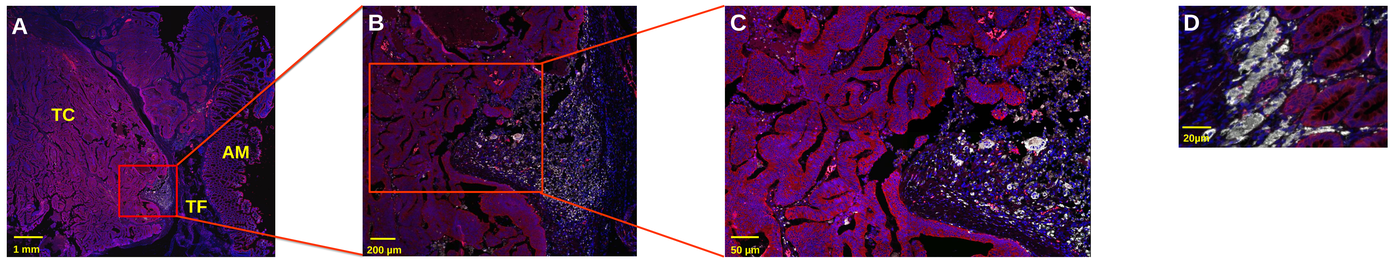
In order to understand the role of Tumour Associated Macrophages (TAMs) in CRC progression, we investigated the distribution of total macrophages (CD68+) and alternatively activated macrophages (CD163+) in colorectal tumours.

Our **hypothesis** is that infiltration of total macrophages (CD68+) and alternatively activated macrophages (CD163+) correlates with the presence of liver metastasis in patients with CRC.

## RESULTS

This study was carried out using microscopic analysis of tissue in combination with automatic evaluation. This technique called cytomics technology gives the opportunity to analyse large areas of tissue in more detail and with more accuracy. Automated cell detection by recognition of cell-associated marker provides new qualitative and quantitative information at a cellular and subcellular level.

**Fig1: CD68 and Keratin-8 double immunostaining of colon tumour section**




**A**: Overview of colon tumour tissue : Macrophages are detected by CD68 antibody (white) and epithelial area by Keratin-8 staining (red). It is possible to recognize different colon areas: AM=Adjacent Mucosa; TF=Tumour Front; TC=Tumour Centre; **B** and **C**: Larger magnification of TF with several CD68+ multinucleated giant cells.

The scanning of large areas of tissue shows the presence of CD68+ MGC. They are mostly located at the basal side of the crypt in the adjacent mucosa (Fig1D) and in the stroma at tumour front (Fig1C). MGC are negative for CD163.

MGC play a central role in chronic inflammatory reactions. Although the presence of isolated MGC was described in the past in human colitis, this is the first time that MGC are shown in colorectal tumour. Their expression is inversely correlated with the presence of tumour metastasis (Table 1).

**Table 1: Expression of MGC in different areas of colorectal tumour from patient without and with liver metastasis**

| without liver metastasis | | | with liver metastasis | | |
|---|---|---|---|---|---|
| Patient # | Mucosa | Tumour | Patient # | Mucosa | Tumour |
| 1 | + | - | 5 | - | - |
| 2 | + | + | 6 | - | + |
| 3 | - | + | 7 | - | - |
| 4 | - | + | 8 | - | - |

MGC are present in colorectal tumour from patients without tumour metastasis. Only one patient with tumour metastasis shows expression of MGC.

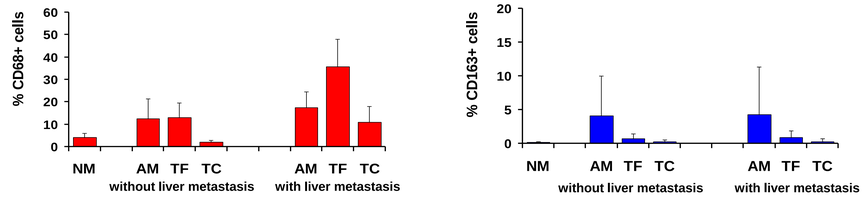
## CONCLUSIONS

**1) Colorectal tumours in patients with liver metastasis show a strong increase in CD68+ infiltration.**

**2) The number of CD163+ cells does not change in relation of the presence of liver metastasis.**

**3) The function of multinucleated CD68+ giant cells has not been described in colorectal tumour. Their involvement in tissue repair might be ipothesised and we plan to analyse it further.**

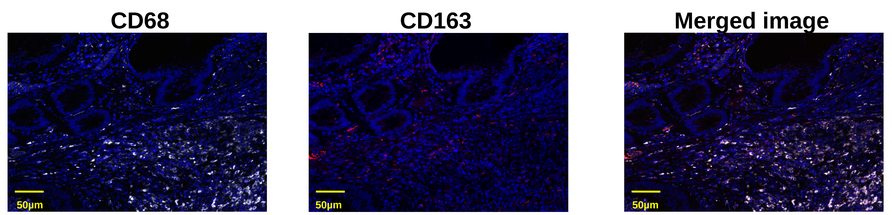
**Fig2: Percentage of cells expressing CD68+ or CD163+ in normal mucosa and in different tumour areas**




**NM: Normal mucosa; AM: Mucosa adjacent to tumour; TF: Tumour Front; TC: Tumour Centre**

Increased expression of CD68+ cells is already seen in AM. In patients with liver metastasis the percentage of infiltrated CD68+ cells is strongly increased at TF when compared with patients without metastasis. Infiltration of CD163+ cells increases in AM with a reduction in tumour area. No differences are observed between patients with or without liver metastasis.

**Fig3: CD68/CD163 double staining at tumour front**




Cells positive for CD68 marker show cytoplasmatic staining and very different phenotypes (white). Cells positive for CD163 are small and express the marker on the membrane (red).

**METHODS:** Paraffin sections from 8 colorectal G2 tumours and adjacent mucosae were used for immunofluorescent staining. Four patients already developed liver metastasis. In addition 3 healthy mucosae were examined.
The following primary antibodies were used: CD68 clone KP1 (Thermo Fisher Scientific); CD163 (Akris); Keratin-8 clone EP1628Y (Thermo Fisher Scientific). Alexa Fluor 568 and 647 (Invitrogen) were used as secondary antibodies. Single (CD68, CD163) and double staining (CD68/Keratin-8 and CD163/CD68) were performed using classical immunofluorescent technique.
Images were recorded by the automatic slide scanner TissueFAXS (TissueGnostics GmbH, Austria) using a 20x objective and analysed by TissueQuest (TissueGnostics) analysis software using classical image processing algorithms optimized for large images.

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