Quantitative Analysis of Immune Infiltrates in Primary Tumors and Liver Metastases of Colorectal Cancer

„from research to clinical application“

Niels Halama, MD
Focus
NCT: Key features

**Medical Oncology**

**Translational Oncology**

**Preventive Oncology**

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**Research in oncology**

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**Medical treatment / Patient care**

- **Interdisciplinary cancer outpatient clinic**
- **Unit NCT1**
- **Outpatient treatment clinic 1**
- **Outpatient treatment clinic 2**
- **Consulting services**

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**Other departments**

- **NCT registry**
- **NCT tissue bank**
- **NCT trial coordination and study evaluation**

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**Other services**

- Cancer information service, social services, diet & nutrition counseling, psycho-oncology, genetic counseling, medical sports, phone services (‘quit smoking’)

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[Diagram of NCT key features with sections for Medical Oncology, Translational Oncology, Preventive Oncology, Research in oncology, Medical treatment/Patient care, Other departments, and Other services]
Immune infiltrates and response to chemotherapy?
Previous Work

Intraepithelial CD8$^+$ T-cell-count becomes a prognostic factor after a longer follow-up period in human colorectal carcinoma: possible association with suppression of micrometastasis

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Prognostic Role of CD8+ Tumor-Infiltrating Lymphocytes in Stage III Colorectal Cancer With and Without Microsatellite Instability

FRIEDRICH PRALL, MD, THOMAS DÜHRKOP, VOLKER WEIRICH, MD, CHRISTIANE OSTWALD, PhD, PETER LENZ, MD, HORST NIZZE, MD, AND MALTE BARTEN, MD

HUMAN PATHOLOGY Volume 35, No. 7 (July 2004)
Type, Density, and Location of Immune Cells Within Human Colorectal Tumors Predict Clinical Outcome

Jérôme Galon,¹† Anne Costes,¹ Fatima Sanchez-Cabo,² Amos Kirilovsky,¹ Bernhard Mlecnik,² Christine Lagorce-Pagès,³ Marie Tosolini,¹ Matthieu Camus,¹ Anne Berger,⁴ Philippe Wind,⁴ Franck Zinzindohoué,⁵ Patrick Bruneval,⁶ Paul-Henri Cugnenc,⁵ Zlatko Trajanoski,² Wolf-Herman Fridman,¹,⁷ Franck Pagès¹,⁷†

The role of the adaptive immune response in controlling the growth and recurrence of human tumors has been controversial. We characterized the tumor-infiltrating immune cells in large cohorts of human colorectal cancers by gene expression profiling and in situ immunohistochemical staining. Collectively, the immunological data (the type, density, and location of immune cells within the tumor samples) were found to be a better predictor of patient survival than the histopathological methods currently used to stage colorectal cancer. The results were validated in two additional patient populations. These data support the hypothesis that the adaptive immune response influences the behavior of human tumors. In situ analysis of tumor-infiltrating immune cells may therefore be a valuable prognostic tool in the treatment of colorectal cancer and possibly other malignancies.

Science 2006
Immunohistochemistry in colorectal primary tumors

IHC: CD3, CD8, Granzyme B and CD45RO

Galon, Pages, Fridman et al. in Science 2006
Local immune response versus UICC-TNM stage

UICC-TNM stadium as reference

CD3\textsubscript{CT}CD3\textsubscript{IM} evaluation

plus

CD45RO\textsubscript{CT}CD45RO\textsubscript{IM} evaluation

Survival (months)

Disease-Free Survival

CD3\textsubscript{CT}^{Hi}CD3\textsubscript{IM}^{Hi}
CD45RO\textsubscript{CT}^{Hi}CD45RO\textsubscript{IM}^{Hi}

ns

CD3\textsubscript{CT}^{Lo}CD3\textsubscript{IM}^{Lo}
CD45RO\textsubscript{CT}^{Lo}CD45RO\textsubscript{IM}^{Lo}

ns

Galon et al. in Science 2006

Better local immune response = better chemotherapy response?
Sample analysis I: *localization*  
(colorectal cancer)

**Primary colorectal tumor**

- **Invasive margin**
- **Center**

**Liver metastasis of colorectal cancer**

- **Invasive margin**
- **Center**
Sample analysis II: *numbers* (colorectal cancer)

Step I: Manual cell counts

Step II: Relative cell counts

Step III: automated large scale cell counting
Sample analysis III: *sampling size*
(colorectal cancer)

Tissue microarray analyses (TMA): 2 cores (each ~1 mm²)
Virtual microscopy: 10 to 12 mm²
Results: metastases, invasive margin

Patients' characteristics / Data acquisition

Timeline

- Liver metastasis removed
- Evaluation / staging (RECIST)
- chemotherapy

Clustering

Generation of a score system (2+1+1')
Results: metastases, invasive margin

* partial or complete remission
Results: metastases, invasive margin
Results: *primary tumors*

Problem: heterogeneity of the tumor (center)
From many to just one...

TMA-Analyses

Individual patient
Heterogeneity of immune infiltrate density (I)

Measurements:

Median number of positively stained cells (across all fields)

Single field evaluation
Lower and upper (maximum) deviation in cell counts / mm² as observed in samples from 20 different patients presented in percentage deviation from median (horizontal bars, negative percentage represents lower deviation, positive percentage represents upper deviation, bar length indicates maximum deviation) for CD3 staining.
Heterogeneity of immune infiltrate density (III)

Lower and upper (maximum) deviation in cell counts / mm² as observed in samples from 20 different patients presented in percentage deviation from median (horizontal bars, negative percentage represents lower deviation, positive percentage represents upper deviation, bar length indicates maximum deviation) for CD8 staining.
Visualization of Heterogeneity

CD3

- real cell count
- individual lower limit
- individual upper limit

CD45RO

- real cell count
- individual lower limit
- individual upper limit

observed cell count / mm² (single field)

real cell count / mm² (possible range of measurements)
Immune infiltrates and response to chemotherapy?
Response to chemotherapy…a clinical example

CT scans, pre- and post-treatment, Irinotecan-based regimen, four cycles chemotherapy (8 weeks)

Patient A has had a complete remission of all liver metastases…
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Can we use Virtual Microscopy to identify (immunologic) parameters in patient cohorts AND make predictions for individual patients?
YES WE CAN

OBAMA

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