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## 1551P Epithelial to mesenchymal transition induces autophagy in renal cell carcinoma: Implications in cancer therapy

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**Background:** Epithelial to mesenchymal transition (EMT) contributes to the metastatic and invasive potential of tumors including renal-cell carcinoma (RCC). Various preclinical and clinical results have indicated that dysregulated elements leading to EMT can be a potential target in RCC. We assessed the expression profile of EMT associated genes in surgically resected tumor tissue and targeted the survival mechanism of EMT-induced cells.

**Methods:** We studied the expression of epithelial marker (E-cadherin), mesenchymal markers (Snail, Slug, Vimentin, Twist) and cancer stem cell marker (ALDH1) in 71 patients of histologically proven RCC. These were analysed in both tumor section and the adjoining normal looking parenchyma by real time PCR and Western immunoblot. In vitro studies were carried out in primary cultures and RCC cell line (A498) where EMT was induced pharmacologically using tumor growth factor-beta (TGF-β, 10ng/ml). Autophagy was assessed in EMT induced cells by acridine orange staining, Tetrazolium dye, (MTT) 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay and propidium iodide (PI)-Annexin staining were done to evaluate post-treatment cell survival in EMT cells with temsirolimus and autophagy inhibitor, choloroquine.

**Results:** Epithelial marker, E-cadherin was significantly down-regulated in tumor tissue while expression of mesenchymal and cancer stem cell markers increased in tumor tissue as compared to adjoining normal tissue. EMT signature proteins showed an increase in in vitro culture of primary cells from tumor tissue as well as in EMT-induced A498 cells. We also observed increased invasiveness and autophagy in EMT induced A498 cells and primary tumor cells as compared to the normal cells. It was observed that addition of autophagy inhibitor (chloroquine) with temsirolimus to EMT-induced cells decrease their viability. Annexin-PI assay showed a significant cell death in combination of chloroquine and temsirolomus on EMT induced cells.

**Conclusions:** Our study shows that the process of EMT is involved in the metastatic spread of RCC and autophagy helps in survival of the EMT-induced cells. Thus, inhibition of autophagy might represent a future therapeutic option.

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## 1552P Toward therapeutic drug monitoring of everolimus? Results of an exploratory study of the dose-exposure relationships

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**Background:** The efficacy of everolimus (EVR) has been demonstrated in the treatment of (i) hormone receptor-positive advanced breast cancer (ii) metastatic renal cell cancer and (iii) neuroendocrine tumours of pancreatic origin. The recommended dosage of EVR is 10 mg once daily but contrary to transplantation, therapeutic drug monitoring (TDM) of EVR is not mandatory and no blood trough levels ($C_0$) have been defined. The aims of this study were (i) to determine $C_0$ that could predict the occurrence of toxicities and (ii) to investigate the relationship between polymorphisms of candidate genes and $C_0$.

**Methods:** This monocentric retrospective observational study was carried out over 4 months in 54 patients on EVR for breast, renal or neuroendocrine cancer. Clinical, biological and radiologic data were collected from the patients' medical records. Toxicity was defined by temporary interruption and/or dose reduction of EVR. Patients' exposure to EVR was dichotomized by ROC curve. The association between exposure and toxicity was then determined using a Cox model for repeated events. The impact of *CYP3A4*22* and *CYP3A5*3* SNPs on $C_0$ was investigated by generalized estimating equation.

**Results:** Forty-two patients (77.8%) had breast cancer, 10 (18.5%) had renal cell cancer and 2 (3.7%) had neuroendocrine cancer. Toxicity (all grades) was reported in 75.9% of the patients (EVR discontinuation in 25.9% patients). Haematological disorders were observed in the majority of toxicity cases (22%). The EVR $C_0$ threshold determined by ROC analysis was 26.3 µg/L (Sen = 0.38,Spe = 0.88). The risk of toxicity was increased 4-fold for $C_0$ > 26.3 µg/L (HR= 4.12, IC95% = [1.48-11.5], p = 0.0067). $C_0$ was significantly lower in carriers of at least one CYP3A5*1 allele ($intercept_{(0 \text{ expressors})}$ = 10.72 ± 1.45, $\beta_{\text{from expressors}}$ = +6.32 ± 2.22, p = 0.0044). No association between carriers of *CYP3A4*22* variant and blood trough $C_0$ was found.

**Conclusions:** An EVR $C_0$ > 26.3 µg/L was associated with an increased 4-fold risk of toxicity, with a specificity of 88%. The genetic polymorphism *CYP3A5*3* has an important impact on EVR exposure. These results provide elements of proof in favour of using EVR TDM in oncology.

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## 1553P The role of immune system on the efficacy of bevacizumab in patients with metastatic colorectal cancer (mCRC)

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**Background:** We aimed to investigate the role of anti-bevacizumab antibodies, regulatory T, CD4+ and CD8+ cells on the efficacy of bevacizumab plus chemotherapy combination in patients with mCRC.

**Methods:** Thirty consecutive patients treated with bevacizumab plus either irinotecan or oxaliplatin based chemotherapy regimens were included. The levels of Tregs (CD4 + CD25(hi)FoxP3+), CD4+ and CD8+ cells from peripheral blood were assayed by flow cytometry before the onset and after 4 cycles of bevacizumab. The anti-bevacizumab antibody levels were assayed by ELISA 3 months after the onset of bevacizumab.

**Results:** The median age was 59 years. The majority of the patients had metastatic disease at the time of diagnosis. The chemotherapy backbone was FOLFIRI in 75% of the patients. The median number of treatment cycles was 7. The objective response (OR) and disease stabilization rates were 30% and 73.3%, respectively. The median progression-free survival (PFS) and overall survival times were 8.0 and 16.0 months. Four patients had measurable anti-bevacizumab levels. There was no OR in patients with measurable anti-bevacizumab antibody levels. The levels of Treg cells were between 0.15 and 4.82% (median 0.40%) and the ratio of CD4 + /CD8+ cells between 0.91 and 4.30 (median 1,9) on peripheral blood before the treatment. There were no significant changes in the levels of Tregs and the ratio of CD4 + cell/CD8+ cells on bevacizumab treatment. The patients with higher CD4 + /CD8+ cells before the bevacizumab had favorable PFS times (14 vs 6 mos, P = 0.065). The patients having low Treg levels after 4 cycles of bevacizumab had favorable PFS time (14 vs 7 mos, p = 0.061). The chemotherapy backbone had no significant effect on trial parameters.

**Conclusions:** The Tregs and the ratio of CD4 + /CD8 + cells could be predictors of PFS for bevacizumab treatment in patients with mCRC. The pre-treatment ratio of CD4+ /CD8+ cells, the levels of Tregs and anti-bevacizumab antibodies might influence the efficacy of long term use of bevacizumab in patients with mCRC. The improved PFS in patients having lower Tregs after bevacizumab treatment may provide a rationale for the combination of bevacizumab and immune checkpoint inhibitors.

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**Disclosure:** All authors have declared no conflicts of interest.

## 1554P The role of Krüppel-like factor (KLF5) and its mechanism for treatment resistance in preoperative chemoradiation therapy for rectal cancer

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**Background:** The aim of this study was to determine whether Krüppel-like factor 5 (KLF5) expression in pre-irradiation tumor biopsies is a useful predictive marker of tumor response in patients with rectal cancer

**Methods:** This study included 60 human colon tumor pre-irradiation specimens. Expression was studied by immunohistochemistry(IHC) using scoring system(0-15). Functional roles of KLF5 were analysed by over-expression of the protein in colon cancer cell line. Protein interactions were studied by stress induction such as chemo or radiation and MTT assays.

**Results:** Complete remission was achieved by 9(18%) patients. Tumor regression was significantly related with p53 and KLF5( p = 0.021,p = 0.004,respectfully). The KLF5 IHC score significantly correlated with KRAS mutation status(5.92 ± 2.54 vs 8.44 ± 1.94,p = 0.006),and pCR(4.11 ± 2.61 vs 6.68 ± 2.43,p = 0.005). In HCT 116 cell line, KLF5 protein was significantly increased after radiation therapy, suggesting that KLF5 via cydin D1, b-catenin. HCT 116 with KLF5 overexpression exhibited significantly better cell viability compared to control cells in MTT assay.

# The role of immune system on the efficacy of bevacizumab in patients with metastatic colorectal cancer (mCRC)

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## INTRODUCTION

Angiogenesis is one of the key steps in the progression and metastasis of cancer. The addition of bevacizumab to chemotherapy in patients with metastatic colorectal cancer (mCRC) has significantly improved overall survival (1). VEGF is not only important for angiogenesis, but is also a key factor produced by solid tumors to mediate immunosuppression in the tumor microenvironment (2,3).
In the current study we aimed to investigate the role of immune cells, including regulatory T cells (Tregs), CD4+ cells and CD8+ cells on the efficacy of bevacizumab plus chemotherapy combination in patients with metastatic colorectal cancer. We also aimed to test the development of anti-bevacizumab antibodies in those patients.

## PATIENTS & METHODS

Forty patients with metastatic colorectal cancer whom treated with bevacizumab plus either irinotecan or oxaliplatin based chemotherapy regimens were included. The levels of Tregs (CD4+CD25hiFoxP3+), CD4+ and CD8+ cells from peripheral blood were assayed by flow cytometry (Becton-Dickinson) before the onset of the treatment and after 4 cycles of bevacizumab. The anti-bevacizumab antibody levels were assayed by a sandwich ELISA method (SHIKARI® S-ATB, Matrix Biotechnology) 3 months after the onset of bevacizumab.

## RESULTS

The results of the forty consecutive potentially non-resectable patients with ECOG performance status 0-2, whom completed at least 4 cycles of bevacizumab, were presented. The patients' characteristics are outlined on the Table 1. The median age was 61 years old (range: 31-79). The majority of the patients (84%) had metastatic disease at the time of diagnosis and the others (16%) had recurrent disease.
The chemotherapy backbone was FOLFIRI in 78% of the patients. The median number of treatment cycles was 6.
The treatment outcomes were outlined on Table 2. The objective response and disease stabilization rates were 28% and 72%, respectively. The median progression-free survival (PFS) and overall survival (OS) times were 6.0 and 15 months.
Four patients (10%) had measurable anti-bevacizumab levels. There was no objective response in patients with measurable anti-bevacizumab antibody levels.
The levels of Treg cells were between 0.04% and 4.82% (median 0.40%) and the ratio of CD4+ /CD8+ cells between 0.91 and 4.30 (median 2) on peripheral blood before the bevacizumab treatment.

**Table 1.** Patients' characteristics

| Parameter | |
|---|---|
| Median age (Range) | 61 (31-70) |
| Disease involvement sites | |
| Liver (liver only) | 83.3% (33.3%) |
| Lung | 33.3% |
| Peritoneum | 16.7% |
| Lymph node | 9.5% |
| Performance status (ECOG) | |
| 0 | 6 |
| 1 | 22 |
| 2 | 12 |
| RAS status | |
| K-RAS mutant | 16/40 (40%) |
| N-RAS mutant | 2/40 (5%) |
| Chemotherapy regimens | |
| FOLFIRI | 78% |
| FOLFOX | 22% |
| FOLFIRINOX | 2% |
| Line of bevacizumab combination | |
| First | 8 (20%) |
| Second | 32 (80%) |
| Number of Chemo cycles (median-range) | 6 (4-12) |
| Number of Bevacizumab cycles (median-range) | 6(4-15) |

**Table 2.**Treatment outcomes

| | |
|---|---|
| Objective response rate | 28% |
| Disease stabilization rate | 72% |
| Median response duration (months) | 4,5 |
| Median follow-up time (months) (range) | 14 (3-25) |
| Median PFS (95 CI%) | 6,0±1,0 (4,1-7,9) |
| Median OS (95 CI%) | 15±1,4 (12,3-17,7) |

There were no significant changes in the levels of Tregs and the ratio of CD4+/CD8+ cells on bevacizumab treatment. The patients with higher CD4+/CD8+ cells (CD4/CD8-hi: >2.0) before the bevacizumab had significantly favorable PFS times (13,0±2,1 vs. 6,0±1,0 months, P=0.0092) (Figure 1).

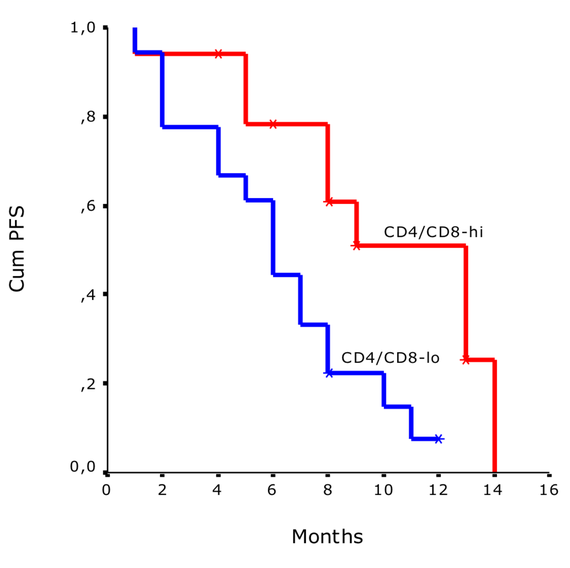


**Figure 1.** The patients with higher CD4+/CD8+ ratio (>2.0) before the bevacizumab had significantly favorable PFS times (13,0±2,1 vs. 6,0±1,0 months, P=0.0092).

The pre-treatment Treg levels were not significant for PFS; however, the patients having decreased Treg levels (<0.40%) after 4 cycles of bevacizumab treatment had more favorable PFS time (8,0±1,2 vs. 6±1,8 months, P=0.0385) (Figure 2). No significant correlations between the immunological parameters and overall survival were found. The chemotherapy backbone had no significant effect on any parameter studied in the current trial.

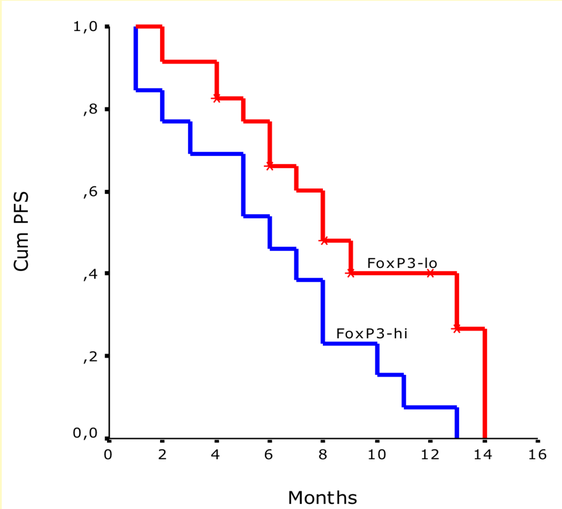


**Figure 2.** The patients having decreased Treg levels (FoxP3-lo: <0.40%) after 4 cycles of bevacizumab treatment had more favorable PFS times (8,0±1,2 vs. 6,0±1,8 months, P=0.0385).

## CONCLUSIONS

Our preliminary results show that the Tregs and the ratio of CD4+ / CD8+cells could be predictors of PFS in patients with advanced colorectal cancer whom treated with bevacizumab.
The immunological parameters including the pre-treatment ratio of CD4+ cells/CD8+ cells, the levels of Tregs and anti-bevacizumab antibodies might influence the efficacy of long-term use of bevacizumab in patients with advanced colorectal cancer.
The improved PFS in patients having lower Tregs after bevacizumab treatment may provide a rationale for the combination of immune checkpoint inhibitors and bevacizumab.

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