Abstract # 3525

Circulating Tumor and Endothelial Cells as Pharmacodynamic Biomarkers in a Phase I Clinical Trial of Intravenous Bevacizumab in Combination with Escalating Doses of Oral Cediranib for Patients with Advanced Malignancies

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Objective

Rare circulating tumor cells (CTCs) and endothelial cells (CECs) offer a feasible approach for studying the pharmacodynamic effects of investigational agents. We investigated the effects of Bevacizumab (B) and Cediranib (C) on CECs and CTCs, inhibition of VEGFR and correlated these changes with dose and clinical response.

Background

- Bevacizumab and Cediranib are inhibitors of angiogenesis; B is a humanized monoclonal antibody that inhibits VEGF interaction with its receptors[1]; C is an oral tyrosine kinase inhibitor of the three VEGF receptors[2]
- The angiogenic marker CD105 is upregulated in endothelial tumor cells and the detection of CECs in the blood has been correlated to metastatic activity[3]
- Drug inhibitors of angiogenesis induce apoptosis in mature tumor-associated endothelial cells (CD31)[4]
- The VEGFR family includes three receptors (VEGFR 1, 2 and 3). In cancerous cells, they can initiate a cellular response to VEGF that induces angiogenesis, tumor growth and metastasis[5].
- CTC enumeration at baseline can accurately predict progression-free survival (PFS) and overall survival OS in metastatic breast, colorectal, and prostate cancer[6,7,8].
- ERK is a MAP kinase that can modulate downstream cellular response to VEGF[9].

Hypothesis

- Treatment with B and C inhibits pVEGFR-2 and induces apoptosis in CD105+ and CD31+ CECs.
- Treatment with B and C reduces CTCs and overall tumor burden.

Methods

Peripheral blood was obtained at baseline, 24 hours and at C2D26-30 post-treatment from patients (n=18) undergoing dose escalation of intravenous B and oral C. CTCs and CECs (CD31+ or CD105+) were isolated and immunofluorescently stained. We used laser scanning cytometry (LSC)[10] to enumerate CTCs and quantify the expression levels of each biomarker.

- B was administered at an initial dose of 3mg/kg and then escalated to 5mg/kg. C was initiated at a dose of 20mg/day and escalated to 30mg/day, then 45mg/day.
- RECIST was assessed on 16 patients (4 withdrew prior to restaging). Patients were categorized as PR (n=2), SD (n=10), and PD (n=4). Clinical Benefit (CB) consists of PR and SD.

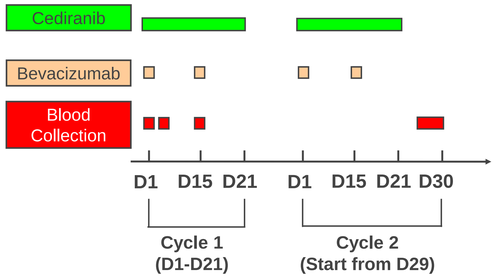

Figure 1: Two-cycle design with a dose escalation of Cediranib and Bevacizumab in the second cycle.

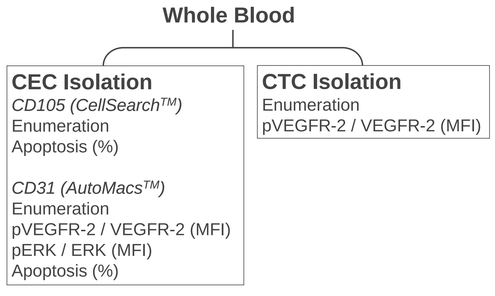

Figure 2: Pharmacodynamic endpoints measured from patient blood drawn at C1D1, C1D2, and C2D26-30.

- We used a custom CellSearch™ CEC modification to isolate CD105+ cells and the AutoMacs™ platform to isolate CD31+ cells.
- For CTC isolation, we compared the CellSearch™ CTC Kit (IVD) with a custom method of EpCAM+ enrichment using the CellSearch™ Profile Kit (RUO) and downstream enumeration of CTCs and quantification of biomarkers using LSC.

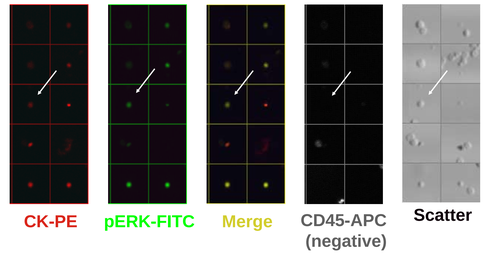

Figure 3: LSC-based enumeration of CTCs using the CK+ and CD45- markers (and scatter/nuclei) and the detection of pERK.

Results

CEC Enumeration

- B + C significantly decreased the number of CD105+ CECs in patients from the CB group compared to PD group at C2D26-30, and this observation correlated with changes in tumor size.

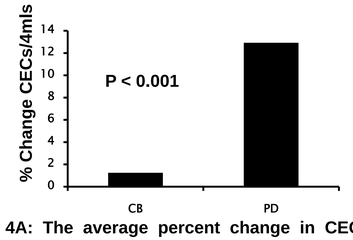

Figure 4A: The average percent change in CEC counts from C1D1 to C2D26-30 in patients categorized as CB and PD.

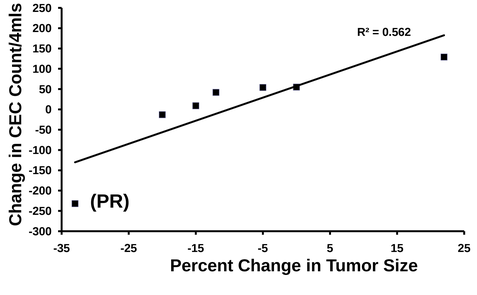

Figure 4B. Changes (post - pre) in CEC counts from C1D1 to C2D26-30 correlated with changes in tumor size.

CEC Apoptosis and Biomarker Analysis

- C+B treatment induced 3-fold and 2-fold increase in apoptosis in the CB and PD group, respectively, at 24hrs.

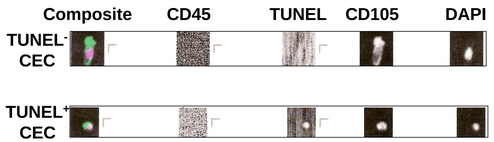

Figure 5. Representative images of the TUNEL assay as measured on the CellSearch™ device. Notice the top row shows a normal, elongated CEC whereas the bottom row depicts a TUNEL+, rounded-up CEC.

Effects on pVEGFR2

- Treatment with B significantly (p=0.011) inhibited pVEGFR-2 at lower doses (83%; 3mg/kg) compared to the higher doses (1.9%; 5mg/kg) in CD31+ CECs.

Effects on pERK/ERK

- A dose-dependant significant increase in pERK/ERK ratio was also observed at 24 hrs (avg. 34%; p=0.046) and at C2D26-30 (avg. 33%; p=0.043).

CTC Enumeration

- CTC detection is more sensitive using the CellSearch™ Profile Kit / LSC method.

Pat. No. (Time)	Tumor	CTC Count / 7.5mls (CTC Kit)	Pat. No. (Time)	Tumor	CTC Count / 7.5mls (Profile Kit & LSC)
1(C1D1)	Adenocarcinoma	2	5(C1D15)	CRC	0
1(C1D2)	Adenocarcinoma	1	6(C1D1)	RCC	18
1(C1D15)	Adenocarcinoma	0	6(C1D2)	RCC	18
1(C2D26-30)	Adenocarcinoma	0	6(C1D15)	RCC	0
2(C1D1)	CRC	0	7(C1D1)	Adenocystic	18
2(C1D2)	CRC	1	7(C2D26-30)	Adenocystic	90
2(C1D15)	CRC	0	8(C1D1)	RCC	18
3(C1D2)	Ovarian	3	8(C1D2)	RCC	18
4(C1D1)	Head/Neck	0	8(C1D15)	RCC	0

Table 1. Enumeration of CTCs from patient blood using the CellSearch™ CTC Kit and the Profile Kit with LSC.

- B + C treatment significantly (p<0.001) decreased CTCs in patients with CB (avg. -95%; pre=5 to post=0.25) compared to patients with PD (avg. 1,000%; pre=0.5 to post=5.5) at 24 hrs post-treatment, and this observation correlated with changes in tumor size.

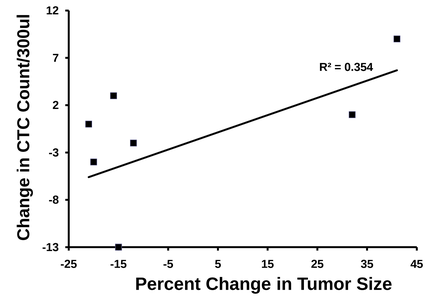

Figure 6. Changes in CTC counts from C1D1 to C1D2 as a function of change in tumor size.

Conclusions

- Circulating CD105+ and CD31+ endothelial cells are feasible surrogates for monitoring the effects of anti-angiogenic therapies.
- The CellSearch™ profile kit with LSC analysis offers a more sensitive method for CTC recovery and downstream molecular characterization.
- CTCs may provide an early quantitative measure of metastatic tumor burden.

References

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Acknowledgements

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