Neuropilin-1 as a potential biomarker of progression-free survival benefit for tivozanib + mFOLFOX6 versus bevacizumab + mFOLFOX6 in metastatic colorectal cancer: post-hoc biomarker analysis of BATON-CRC Phase 2 trial

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Background

- Tivozanib is a selective oral vascular endothelial growth factor tyrosine kinase inhibitor (VEGF TKI),[1] with a long half-life and activity against all 3 VEGF receptors (VEGFRs)[2]
  - In clinical studies, tivozanib has shown activity when combined with temsirolimus in patients with renal cell carcinoma,[3] paclitaxel in patients with metastatic breast cancer,[4] and everolimus in patients with metastatic colon cancer[5]
- BATON-CRC (Biomarker Assessment of Tivozanib in Oncology-colorectal cancer) was a randomized, open-label, Phase 2 trial of tivozanib + mFOLFOX6 vs bevacizumab + mFOLFOX6 in patients with previously untreated metastatic CRC (mCRC) initiated to expand on results observed in a Phase 1b study
  - In the Phase 2 interim analysis of efficacy, progression-free survival (PFS) and overall response rate (ORR) were comparable between the two arms and there were no significant associations between serum/tumor biomarkers and outcomes[6]
- The identification of biomarkers in targeted VEGFR cancer therapy has been challenging; biomarker analysis was included in the Phase 2 study and neuropilin-1 (NRP-1) is a possible target candidate[7,8]
  - NRP-1, expressed on both endothelial and tumor cells, regulates cell migration (branching angiogenesis) and tumor growth
    - Membrane-bound NRP-1 is a receptor for both semaphorins (Sem3A, B, C, E, F) and VEGFs (VEGF-A$_{(165,121)}$, VEGF-B$_{(167)}$, VEGF-C, VEGF-D, PlGF-2)
  - NRP-1 is a VEGFR-2 co-receptor and is involved in regulation of VEGFR-2–mediated angiogenesis
    - Soluble NRP-1 binds to VEGF-A165 and appears to prevent VEGFR-2 binding
    - Blocking NRP-1 function is additive to anti-VEGF therapy in preclinical models

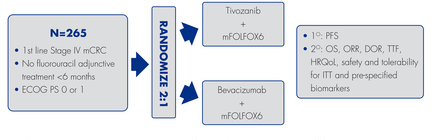
Objective

- The objective of this study was to provide final results of BATON-CRC Phase 2 trial of tivozanib + mFOLFOX6 (Arm A) vs bevacizumab + mFOLFOX6 (Arm B), including results from predefined biomarker analyses

Methods

- Eligible patients
  - No prior systemic chemotherapy, no fluorouracil-containing adjuvant therapy in the previous 6 months, and an Eastern Cooperative Oncology Group performance status (ECOG PS) ≤1
  - No prior VEGF therapy (including bevacizumab) was permitted, nor a history of significant thromboembolic or vascular disorders within 6 months of study entry
- Study design
  - Patients were randomized 2:1 and stratified by lactate dehydrogenase (LDH), origin of cancer, and number of metastatic sites (**Figure 1**)
  - Patients received either tivozanib 1.5 mg once daily for 21 days followed by 7 days off treatment or bevacizumab 5 mg/kg every 2 weeks on days 1 and 15
  - All patients received mFOLFOX6 every 2 weeks of each 28-day cycle
    - Oxaliplatin: Days 1 and 15: 85 mg/m^2 IV bolus in 500 mL of D5W over 2 hours
    - Leucovorin calcium: Days 1 and 15: 400 mg/m^2 IV bolus in 500 mL of D5W over 2 hours (may be given concurrently with oxaliplatin through a separate IV line)
    - Fluorouracil bolus: Days 1 and 15: 400 mg/m^2 IV bolus over 5–15 minutes or infused per institutional guidelines
    - Fluorouracil infusion: Days 1–3 and 15–17: 2400 mg/m^2 continuous IV infusion via infusion pump
- End points
  - Primary end point was PFS by investigator radiologic assessment
  - Secondary end points included PFS by independent radiological review, overall survival (OS), ORR, duration of response (DOR), time to treatment failure (TTF), and biomarker subgroup analysis of LDH; VEGF A, C, D; CD68; myeloid-derived gene signature; NRP-1; and serum soluble cytokines
- Biomarker analysis
  - Serum biomarker analysis was performed using Myriad Rules Based Medicine (RBM) assay, which measures multiple serum proteins in multiplex fashion
    - Assay is based on the capture-sandwich format using antibodies attached to fluorescently encoded microspheres to capture the antigen from a biological sample such as serum
- A Cox proportional hazard model was used to assess the association between potential serum biomarkers and PFS, OS

Figure 1. Study Design of BATON-CRC



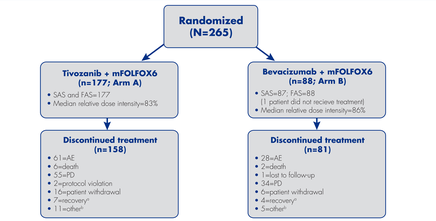
DOR, duration of response; ECOG PS, Eastern Cooperative Oncology Group performance status; HRQoL, health-related quality of life; ITT, intent-to-treat; mCRC, metastatic colorectal cancer; PFS, progression-free survival; ORR, objective response rate; OS, overall survival; TTF, time to treatment failure.

Results

Patients

- Between 12/20/11 and 4/28/13, 265 subjects were randomized (**Figure 2, Table 1**)

Figure 2. Patient Disposition



*The "recovery" category consisted of patients who had tumor shrinkage sufficient to discontinue treatment in order to have resection/curative surgery.
†"Other" included physician decision, complete treatment response, patient wanted to pursue other treatment options, etc.
AE, adverse event; FAS, full analysis set; PD, progressive disease; SAS, safety analysis set.

Table 1. Baseline Patient Characteristics

	Tivozanib + mFOLFOX6 (n=177)	Bevacizumab + mFOLFOX6 (n=88)	Total (n=265)
Sex, n (%)			
Male	118 (66.7)	55 (62.5)	173 (65.3)
Age			
Mean (SD)	61.9 (9.6)	62.6 (11.2)	62.2 (10.1)
Race, n (%)			
White	169 (95.5)	85 (96.6)	254 (95.8)
Black	2 (1.1)	0	2 (0.8)
Asian	3 (1.7)	2 (2.3)	5 (1.9)
ECOG PS, n (%)			
0	95 (53.7)	58 (65.9)	153 (57.7)
1	82 (46.3)	30 (34.1)	112 (42.3)
LDH status, n (%)			
<1.5 x ULN	127 (71.8)	64 (72.7)	191 (72.1)
≥1.5 x ULN	50 (28.2)	24 (27.3)	74 (27.9)
Origin of cancer, n (%)			
Rectal	53 (29.9)	24 (27.3)	77 (29.1)
Colon	124 (70.1)	64 (72.7)	188 (70.9)
No. of metastatic sites/organs, n (%)			
1	56 (31.6)	30 (34.1)	86 (32.5)
2	80 (45.2)	34 (38.6)	114 (43.0)
3	29 (16.4)	21 (23.9)	50 (18.9)
≥4	12 (6.8)	3 (3.4)	15 (5.7)

ECOG PS, Eastern Cooperative Oncology Group performance status; LDH, lactate dehydrogenase; SD, standard variation; ULN, upper limit of normal.

Safety

- The overall safety profile was comparable between treatment arms
  - For both, the most common all-grade treatment-emergent adverse event (TEAE) was diarrhea (58.2% Arm A and 57.5% Arm B) and the most common grade 3/4 TEAE was neutropenia (39.5% Arm A and 24.1% Arm B) (**Table 2**)

Table 2. All-Grade Treatment-Emergent AEs ≥20% of Patients in Either Treatment and Grade 3/4 Treatment Emergent AEs

	Tivozanib + mFOLFOX6 (n=177)		Bevacizumab + mFOLFOX6 (n=88)	
AE, n (%)	**All-Grade**	**Grade 3/4**	**All-Grade**	**Grade 3/4**
Diarrhea	103 (58.2)	19 (10.7)	50 (57.5)	9 (10.3)
Nausea	99 (55.9)	5 (2.8)	47 (54.0)	2 (2.3)
Fatigue	97 (54.8)	20 (11.3)	46 (52.9)	8 (9.2)
Neutropenia	95 (53.7)	70 (39.5)	37 (42.5)	21 (24.1)
Hypertension	79 (44.6)	29 (16.4)	25 (28.7)	9 (10.3)
Peripheral neuropathy	75 (42.4)	18 (10.2)	34 (39.1)	11 (12.6)
Decreased appetite	64 (36.2)	2 (1.1)	25 (28.7)	2 (2.3)
Vomiting	60 (33.9)	10 (5.6)	24 (27.6)	1 (1.1)
Thrombocytopenia	54 (30.5)	10 (5.6)	13 (14.9)	2 (2.3)
Constipation	50 (28.2)	1 (0.6)	32 (36.8)	1 (1.1)
Paresthesia	46 (26.0)	2 (1.1)	20 (23.0)	3 (3.4)
Abdominal pain	45 (25.4)	7 (4.0)	17 (19.5)	5 (5.7)
Dysphonia	42 (23.7)	1 (0.6)	13 (14.9)	0
Mucosal inflammation	40 (22.6)	5 (2.8)	29 (33.3)	6 (6.9)
Asthenia	39 (22.0)	5 (2.8)	17 (19.5)	1 (1.1)
Stomatitis	37 (20.9)	5 (2.8)	14 (16.1)	2 (2.3)
Epistaxis	34 (19.2)	0	25 (28.7)	0
Dysgeusia	26 (14.7)	0	18 (20.7)	0

  - For both, the most common treatment-related AEs were hypertension (39.5% Arm A and 25.3% Arm B)
  - Discontinuation of treatment due to an AE occurred in 41.2% of patients in Arm A and 34.5% of patients in Arm B
    - Pulmonary embolism was the most common AE leading to discontinuation for Arm A and deep vein thrombosis for Arm B
- Serious AEs (SAEs) were reported for 46.3% of patients in Arm A compared with 48.3% in Arm B
  - The 2 most common SAEs in Arm A were diarrhea (4.0%) and pulmonary embolism (4.0%), and in Arm B were pyrexia (8.0%) and diarrhea (5.7%)
  - Serious treatment-related AEs were reported in 21.5% of patients for tivozanib (most common being pulmonary embolism) and in 17.2% of patients for bevacizumab (most commonly abdominal pain at 3.4%)
- A total of 9 patients died while on treatment or within 30 days of last dose
  - 7 (4.0%) patients in Arm A, 3 of whom had at least 1 fatal AE considered to be either probably related to tivozanib (pulmonary hemorrhage, gastrointestinal hemorrhage, and duodenal neoplasm) or possibly related (asthenia)
  - 2 (2.3%) patients in Arm B, both of which were due to AEs considered to be probably related to bevacizumab (hepatic hemorrhage and large intestine perforation)

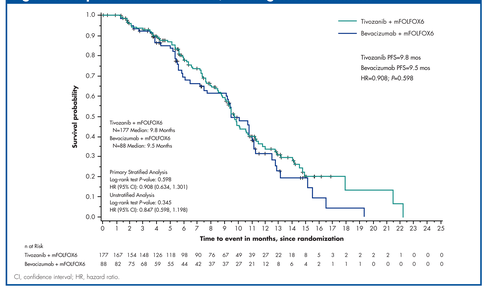
Overall Efficacy

- In the BATON-CRC final PFS analysis, tivozanib performed similar to bevacizumab in the intent-to-treat (ITT) population (**Figure 3**)
  - ORR was 49.6% (39.4%, 54.5%) tivozanib vs 43.2% (32.7%, 54.2%) bevacizumab

NRP-1–Related Efficacy

- Of the biomarkers analyzed, NRP-1 is the only biomarker that predicts a treatment effect (**Figure 4**)
  - NRP-1 low and high were defined as above and below the median of 298.5 pg/mL
- In both arms, patients with NRP-1 low showed an improved PFS vs patients with NRP-1 high (**Figures 5A and 5B**)

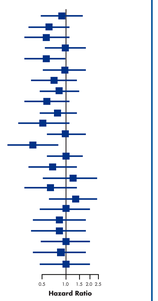
Figure 3. Kaplan-Meier Plot of PFS; Investigator Assessment



CI, confidence interval; HR, hazard ratio.

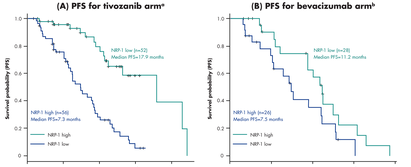
Figure 4. Forest Plot for Biomarker[a] Subgroup Analysis of PFS

Subgroups	Tivozanib Event/N	Bevacizumab Event/N	HR (95% CI)
Serum VEGF-A < Median	27/54	16/27	0.88 [0.47, 1.65]
Serum VEGF-A ≥ Median	29/54	18/27	0.62 [0.34, 1.12]
Serum VEGF-C < Median	23/52	16/26	0.57 [0.3, 1.1]
Serum VEGF-C ≥ Median	33/56	18/28	0.99 [0.55, 1.81]
Serum VEGF-C/A < Median	31/56	15/25	0.56 [0.3, 1.05]
Serum VEGF-C/A ≥ Median	25/52	19/29	0.96 [0.52, 1.79]
Serum sVEGFR-2 < Median	23/53	14/28	0.71 [0.36, 1.4]
Serum sVEGFR-2 ≥ Median	33/55	20/26	0.81 [0.46, 1.43]
Serum sVEGFR-3 < Median	20/51	16/30	0.58 [0.3, 1.14]
Serum sVEGFR-3 ≥ Median	36/57	18/24	0.78 [0.44, 1.38]
Serum IL-8 < Median	22/53	14/26	0.52 [0.26, 1.04]
Serum IL-8 ≥ Median	34/55	20/28	0.97 [0.55, 1.71]
Serum Neuropilin < Median	15/52	16/28	0.38 [0.18, 0.79]
Serum Neuropilin ≥ Median	41/56	18/26	1 [0.58, 1.75]
Tumor VEGF-A < Median	18/38	13/18	0.67 [0.33, 1.37]
Tumor VEGF-A ≥ Median	24/37	7/16	1.21 [0.51, 2.87]
Tumor VEGF-C < Median	20/39	11/16	0.63 [0.3, 1.34]
Tumor VEGF-C ≥ Median	22/36	9/18	1.33 [0.61, 2.9]
Tumor VEGF-C/A < Median	22/37	9/15	0.99 [0.45, 2.18]
Tumor VEGF-C/A ≥ Median	20/38	11/19	0.83 [0.39, 1.73]
Tumor VEGF-D < Median	23/41	8/13	0.82 [0.36, 1.85]
Tumor VEGF-D ≥ Median	19/34	12/21	1.01 [0.49, 2.09]
Tumor PlGF < Median	20/38	9/16	0.85 [0.38, 1.89]
Tumor PlGF ≥ Median	22/37	11/18	0.98 [0.47, 2.04]



[a]Serum values indicate protein levels in circulation; for tumor biomarkers, the categories indicate RNA expression.
IL-8, interleukin-8; PFS, progression-free survival; PlGF, placental growth factor; sVEGFR, serum vascular endothelial growth factor receptor; VEGF, vascular endothelial growth factor.

Figure 5. PFS of Tivozanib- (A) and Bevacizumab-Treated (B) Patients With High vs Low NRP-1 Levels Based on a Median Cutoff

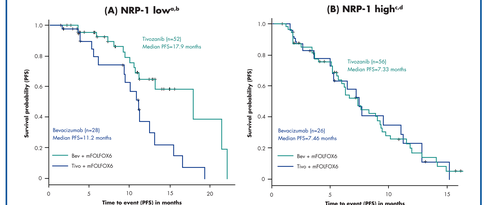


[a]Unstratified PFS analysis: (n=108; events=56); log rank test *P* value=2.88e-7; HR (95% CI): 0.21 (0.11, 0.41).
CI, confidence interval; HR, hazard ratio; PFS, progression-free survival.

[b]Unstratified PFS analysis: (n=54; events=34); log rank test *P* value=0.059; HR (95% CI): 0.506 (0.246, 1.04).

- Patients with low NRP-1 treated with tivozanib + FOLFOX6 had an increased PFS compared with patients treated with bevacizumab, whereas PFS was comparable for both treatments in patients with high NRP-1 (**Figures 6A and 6B**)

Figure 6. PFS of Patients With NRP-1 Low (A) and NRP-1 High (B) Levels Based on a Median Cutoff



[a]Unstratified analysis: (n=80; events=31); log rank test *P* value=0.0075; HR (95% CI): 0.38 (0.183, 0.794).
[b]Multivariate analysis (cancer origin, met sites, LDH); Wald test *P* value=0.0034; HR (95% CI): 0.30 [0.133, 0.671].

[c]Unstratified analysis: (n=82; events=59); log rank test *P* value=0.99; HR (95% CI): 1.00 (0.997, 0.573).
[d]Multivariate analysis (cancer origin, met sites, LDH); Wald test *P* value=0.68; HR (95% CI): 1.12 (0.639, 1.980).

Bev, bevacizumab; CI, confidence interval; HR, hazard ratio; LDH, lactate dehydrogenase; met, metastatic; PFS, progression-free survival; Tivo, tivozanib.

Conclusions

- Tivozanib and bevacizumab have comparable PFS and ORR when used in combination with mFOLFOX6 in unselected patients with untreated mCRC
- Patients with low NRP-1 showed an improved PFS vs patients with high NRP-1 in both treatment arms, supporting the value of NRP-1 as a potential prognostic marker for angiogenesis inhibitors
- Data suggest that in patients with advanced CRC and low NRP-1, treatment with tivozanib in combination with mFOLFOX6 may be superior to treatment with bevacizumab with mFOLFOX6
  - Differential activity observed with tivozanib vs bevacizumab in NRP-1 low patients is potentially due to the broader VEGF pathway inhibitory activity of tivozanib
- A potential hypothesis for the NRP-1 effect may be that:
  - In the presence of high serum NRP-1, VEGF-A164 is bound and VEGFR-2 is not activated, making the method of VEGFR blockade less important
  - In the presence of low serum NRP-1, VEGFR activation is high and modality of blockade can affect the response (tivozanib blocks all 3 VEGFRs)
- The effect of therapy on patients with low serum NRP-1 levels was not seen at the interim analysis due to a paucity of progressions
- A prospective randomized trial comparing tivozanib with bevacizumab in patients with low NRP-1 is warranted

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Acknowledgments

This study was sponsored by AVEO Oncology. Editorial assistance was provided by Scientific Connexions, an Ashfield Company, and was funded by AVEO Oncology.

POSTER PRESENTED AT THE AACR ANGIOGENESIS MEETING; MARCH 6, 2015; ORLANDO, FL