Abstract # C165

Predictive markers of response in a Phase I/II pharmacodynamic (PD) study of erlotinib and bevacizumab for recurrent or metastatic head and neck cancer (HNC)

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Abstract

Purpose: It is known that EGFR activation up-regulates VEGF which has been correlated with resistance to anti-EGFR agents. The purpose of this PD study was to investigate effects of the EGFR inhibitor erlotinib (E) with the VEGF antibody bevacizumab (B) in recurrent or metastatic HNC.

Patients and Methods: Phase I/II trial of fixed dose erlotinib (E) given orally (150 mg) daily with escalation of bevacizumab (B) to a maximum of 15 mg/kg for 3 weeks and continued at 15 mg/kg in the phase II portion. Patients were randomized to receive the initial bevacizumab dose on either day 1 or day 15. Serum VEGF and TGFα levels were quantified by ELISA. Pre-treatment (n=12) and post-treatment (n=5) biopsies taken after 2 weeks of treatment (E alone or E + B) were analyzed by immunofluorescence and laser scanning cytometry (LSC).

Results: Serum VEGF levels were decreased following E or E + B treatments (p=0.057), but TGFα levels were increased (p=0.097) and no correlation with clinical response or progression-free survival (PFS) was observed. Paired tissue samples showed that E or E + B treatments reduced expression of pERK by 47% (p=0.05) and pERK/ERK ratio by 53% (p=0.04). Furthermore, E or E +B treatments increased apoptosis in tumor cells (pre: 1.05%, post: 7.98%; p=0.03) and in endothelial cells (pre: 0%, post: 14.43%; p=0.09). At baseline, higher pEGFR/EGFR and pKDR/KDR ratios correlated with a complete response (CR) (CR>non-CR; p=0.09 and p=0.002, respectively). Using a second anti-pKDR antibody, we confirmed that higher pKDR/KDR ratio also correlated with CR (p=0.02). Of note, higher pKDR/KDR ratio correlated with a complete tumor response (p=0.04) and better PFS (p=0.18).

Conclusions: Compared to erlotinib (E) alone, erlotinib + bevacizumab (E+B) treatment showed an increased inhibition of survival factors, e.g., pEGFR/EGFR and pKDR/KDR ratios appear to predict a complete response to E + B. The promising E + B efficacy and predictive biomarkers warrant further validation in larger patient cohorts.

Background

- More than 40,000 patients per year are diagnosed with metastatic squamous cell carcinoma of the head and neck (SCCHN).
- Almost 100% of SCCHN overexpress EGFR.
- EGFR inhibitors such as gefitinib and cetuximab only had a response rate of 7.6-12.6% and a stable disease rate of 40-50%.
- Preclinical studies suggest a synergism between EGFR inhibition and antiangiogenic therapy by overcoming resistance to EGFR inhibitors.
- A phase I/II SCCHN trial with combined treatment of an EGFR inhibitor, erlotinib (150 mg daily), and an anti-VEGF mAb, bevacizumab (15 mg/kg every 3 weeks) showed the combined therapy to be well-tolerated with a 15% overall response rate and a median survival rate of 6-8 months.

Study Design

- Phase I: Fixed standard dose of erlotinib (150 mg daily), escalating dose of bevacizumab to 15 mg/kg.
- Phase II: 1st stage (n=22) and 2nd stage (n=24) design, and correlative laboratory studies (Fig. 1, below).

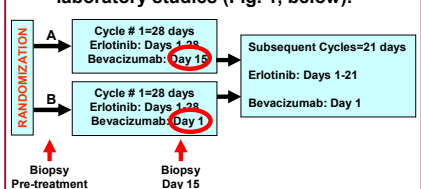

Figure 1. Two-stage design in Phase II studies. Patients received erlotinib daily (150 mg) and bevacizumab on day 15 (A) or day 1 (B).

Patients and Methods

- Pre-treatment (n=12) and pre-/post-treatment (day 15) of paired biopsies (n=5) were obtained (Fig. 1).
- Out of 12 pre-treatment biopsies, 2 showed a complete response (CR), 1 a partial response (PR), 7 a stable disease (SD), and 2 a progressed disease (PD).

Table 1. Clinical response and treatment options of the pre-/post-paired biopsies.

	Erlotinib alone		Erlotinib + Bevacizumab
Status	Pre only	Pre & Post Pair	Pre & Post Pair
CR	0	1	1
PR	0	0	1
SD	5	2	0
PD	2	0	0

CR/Complete and PR/Partial Response, SD/Stable and PD/Progressive Disease

- Serum VEGF and TGFα levels were determined by ELISA.
- Expression of pEGFR, EGFR, pKDR, KDR, pERK, ERK and TUNEL was determined by immunofluroescence and quantified by laser scanning cytometry (LSC).

Results

Serum Markers

- *Serum VEGF (n=28) decreased (p=0.057) and TGFα increased (p=0.097) after treatment. There was no correlation with RR, survival, or PFS.*

Tissue Markers

- *Decreased pERK/ERK ratio (53%) was significantly associated with erlotinib ± bevacizumab treatment (Table 2).*
- *Apoptosis increased in tumor (TC) and endothelial cells (EC) with significant levels detected in TC (Table 2 and Figure 2).*

Table 2. Decreased pERK/ERK ratio and TC apoptosis significantly associated with erlotinib ± bevacizumab treatment.

Marker	Pre (n=5)	Post (n=5)	% Target Inhibition	p value
pEGFR/EGFR ratio	0.86	1.01	-17	0.17
pKDR/KDR ratio	0.53	0.58	-9	0.34
pERK/ERK ratio	0.59	0.28	53	0.04*
TC apoptosis (%)	1.05	7.98	-660	0.03*
EC apoptosis (%)	0	14.43	NA	0.09

TC: tumor cells; EC: endothelial cells (CD31-positive); * p ≤ 0.05

Figure 2

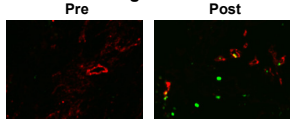

Figure 2: Increased apoptosis in SCCHN patient tumors after erlotinib + bevacizumab treatment. Red: endothelial cells as stained with CD31. Green: apoptotic cells stained by TUNEL.

- *PD analysis of biomarkers in SCCHN patients treated with E or E ± B (Table 3).*

Table 3. PD analysis of biomarkers in SCCHN patients: Significance of pERK/ERK ratio by Erlotinib.

Marker	Erlotinib alone (n = 3)				Erlotinib + Bevacizumab (n = 2)			
	Pre	Post	% Inhibition	P value	Pre	Post	% Inhibition	P value
pEGFR/EGFR	0.65	0.78	-20.13	0.34	0.98	1.36	-39.88	0.08
pKDR/KDR	0.53	0.60	-13.46	0.36	0.54	0.56	-2.96	0.46
pERK/ERK	0.76	0.28	62.56	0.04*	0.35	0.28	20.29	0.25
TC Apoptosis	0.95	9.10	860.92	0.06	1.20	6.29	426.36	0.26
EC Apoptosis	0.00	17.12	NA	0.19	0.00	10.40	NA	0.25

* p < 0.05

- *pKDR/KDR ratio predicts CR (p=0.002) (Table 4 and Figure 3).*

Table 4. pEGFR/EGFR and pKDR/KDR predicts CR.

Baseline Marker	CR (n = 2)	non-CR (n = 10)	p value (CR vs. non-CR)
pEGFR (MFI)	107136	72931	0.320
EGFR (MFI)	158498	105772	0.360
pEGFR/EGFR ratio	1.04	0.83	0.089
pKDR (MFI)	58529	54177	0.390
KDR (MFI)	83278	173357	0.110
pKDR/KDR ratio	0.70	0.38	0.002*
pERK (MFI)	115717	61048	0.200
ERK (MFI)	218506	131291	0.450
pERK/ERK ratio	1.96	0.56	0.280
EC Apoptosis (%)	0.00	1.50	0.131
TC Apoptosis (%)	1.65	1.82	0.430

* p < 0.05

Figure 3

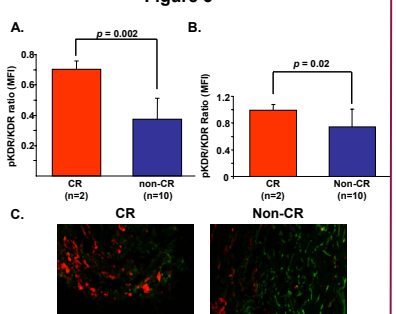

Figure 3: High pKDR/KDR ratio predicts CR using anti-pKDR antibodies PC460 (A) or ACCL-14 (B). Higher pKDR expression was observed in CR than non-CR (C.). Red: pKDR. Green: total KDR.

- *Higher pKDR/KDR ratio correlated with changes in tumor size and better PFS (Figure 4).*

FIGURE 4

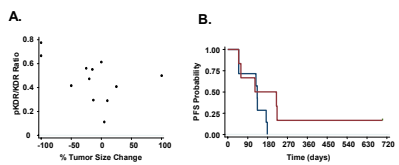

Figure 4. Higher pKDR/KDR ratio correlated with changes in tumor size (p=0.04) (A.) and better PFS (p=0.18 by Cox regression analysis) (B.).

Conclusions

- Serum levels of VEGF and TGFα did not correlate with RR, survival, or PFS.
- Decreased pERK/ERK ratio was associated with erlotinib or erlotinib ± bevacizumab treatment with significance found only in erlotinib-treated patients.
- Apoptosis increased in tumor (TC) and endothelial cells (EC) with significant levels detected in TC.
- Higher pEGFR/EGFR and pKDR/KDR ratios in baseline tumor biopsies predicts CR with significance found for pKDR/KDR.
- Higher pKDR/KDR ratio in baseline tumor biopsies significantly predicts tumor size changes and better PFS.
- The promising clinical efficacy of erlotinib + bevacizumab and predictive biomarkers warrant further validation studies with larger patient cohorts.

Acknowledgements

This study was supported by funding from NIH (N01 CM-57018-16 and NCI U01-63187) to EEC.