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Tumor *HER3* mRNA expression as a biomarker for panitumumab efficacy in advanced colorectal cancer: towards further personalization of anti-EGFR therapy

Jenny F. Seligmann PhD[1], Ace J Hatch PhD [2], Susan D. Richman PhD[1], Faye Elliott MSc[1], Bart Jacobs PhD, Sarah Brown PhD[4], Herbert Hurwitz MD[2], Jennifer H Barrett PhD[1], Philip Quirke PhD[1], Andrew B. Nixon PhD*[2], Matthew T. Seymour MD*[1]

*ABN and MTS are joint senior authors

Affiliations

1. Leeds Institute of Cancer and Pathology, University of Leeds, St. James's University Hospital, Beckett St., Leeds, United Kingdom, LS9 7TF
2. Duke University Medical Center, Durham, NC, US
3. Digestive Oncology Unit, University Hospital Gasthuisberg, Herestraat 49, B-3000 Leuven, Belgium
4. Clinical Trials Research Unit, University of Leeds, United Kingdom

Corresponding Author

Matthew T. Seymour
St James Institute of Oncology
St James's Hospital
Beckett St
Leeds
LS9 7FT

Matt.seymour@nihr.ac.uk

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Key Points

Question: Can tumor mRNA expression of HER3 predict response to anti-EGFR agents in *RAS* wild-type advanced colorectal cancer?

Findings: In this study of patients in a large phase 3 trial of irinotecan with/without panitumumab, *RAS* wild-type patients whose tumors had high HER3 mRNA expression benefitted markedly from panitumumab, but patients with *RAS* wild-type low HER3 tumors gained no benefit. The biomarker/ treatment interaction was significant.

Meaning: Tumor HER3 mRNA expression may be a useful predictive biomarker for anti-EGFR therapy in *RAS* wild-type colorectal cancer: further study is urgently warranted.

Abstract

Importance:

EGFR (HER1) signaling depends on ligand binding and dimerization with itself or other HER receptors. We previously showed in a randomized trial that high EGFR ligand expression is predictive of panitumumab benefit in advanced colorectal cancer. Tumor expression of HER3 may further refine the *RAS* wt population benefitting from anti-EGFR agents.

Objective:

To examine HER3 mRNA expression as a prognostic and predictive biomarker for anti-EGFR therapy in a randomized clinical trial of panitumumab.

Design, setting and participants:

Prospectively planned retrospective biomarker study from the PICCOLO trial which tested the addition of panitumumab to irinotecan therapy in patients with *KRAS* wt aCRC who experienced failure with prior fluoropyrimidine treatment. HER3 was assessed as a prognostic marker, then as a predictive biomarker in *RAS*-wt patients, first as a continuous variable then as a binary (high vs low) variable. Relationship with MEK-AKT pathway mutations and EGFR ligands (EREG/AREG) were also assessed.

Main outcomes and measures:

Primary end point was progression-free survival (PFS); secondary end points were response rate (RR) and overall survival (OS).

Results:

Higher HER3 was weakly prognostic for OS (HR per 2-fold change 0.91[0.83-0.99],p=0.04), but not PFS (HR=0.93[0.83-1.05],p=0.25). Higher HER3 was strongly predictive, being associated with prolonged PFS on IrPan (HR=0.71[0.61-0.82],p< 0.001), but not irinotecan (HR=0.96[0.82-1.13],p=0.65) in *RAS* wt patients; biomarker/treatment interaction (p=0.001). Similar biomarker/treatment interaction was seen for OS (p=0.004).

In an exploratory binary model, dividing the population at the 66thcentile, HER3 was strongly predictive of panitumumab benefit: in HER3-high patients, median PFS was 8.2 (IrPan) vs 4.4 months (irinotecan), HR=0.33[0.19-0.58],p<0.0005; HER3-low patients gained no benefit: 3.3 (IrPan) vs 4.3 months (Ir), HR=0.96[0.67-1.38],p=0.84; interaction p=0.002. The binary model was also predictive for OS: interaction p=0.01.

Combining HER3 and ligand data, patients with HER3-high, AREG/EREG-high tumors gained markedly from panitumumab: PFS HR =0.24[0.11-0.51],p<0.005; OS HR=0.36[0.18-0.73],p=0.004; conversely HER3-low, AREG/EREG-low patients did not benefit (PFS HR=1.14[0.73-1.79],p=0.57; OS HR=1.44[0.92-2.26],p=0.11).

Conclusions and Relevance:

High HER3 identified *RAS*-wt patients who gained markedly from panitumumab, and those who did not, with statistically significant biomarker/treatment interactions for PFS and OS. This finding provides insight into the mechanism of anti-EGFR agents and is of potential clinical utility.

Background

Whilst *RAS*-mutations identify advanced colorectal cancer (aCRC) patients who will not benefit from anti-EGFR agents,[1] *RAS*-wt status does not reliably predict who will.[2] We recently published clinical validation of combined tumor mRNA overexpression of the EGFR ligands amphiregulin (AREG) and epiregulin (EREG) as a predictive biomarker for panitumumab in *RAS*-wt aCRC[3] in a large randomized trial (PICCOLO: irinotecan/panitumumab [IrPan] vs irinotecan for second-line treatment of *KRAS*-wt aCRC[2]).

The activity of agents targeting EGFR (HER1) may also depend upon interactions with other HER receptors. HER family interdependence is well documented;[4] HER3, unlike other HER receptors, cannot generate signaling through homodimerization but is an obligate heterodimer, predominantly with EGFR or HER2.[5] The equilibrium between HER3 heterodimers may be a key factor in downstream EGFR family signaling.[6,7,8].

We investigate the role of HER3 tumor mRNA expression in aCRC in pre-treatment samples from the PICCOLO trial, first as a prognostic biomarker, then as a predictive biomarker for panitumumab efficacy. We further investigate its relationship with AREG and EREG, with a view to future development as a clinically applicable selection tool.

Patients and Methods

The results of the PICCOLO trial (ISRCTN93248876) have been reported previously.[2] This study includes all patients with adequate stored tumor material. RNA was extracted from formalin-fixed, paraffin-embedded tissue sections and HER3 expression measured by reverse transcription polymerase chain reaction (primer sequences are shown in Supplementary Table 1).

The primary endpoint was PFS; secondary endpoints were OS and 12-week response rate (RR). The primary analysis assessed HER3 expression as a continuous variable (log-transformed to base 2). In a planned exploratory analysis HER3 expression was assessed as a dichotomous variable, using a range of cut-points to achieve optimum discrimination for biomarker/treatment interaction. Further details are provided in the Supplementary material.

Results

331 patients had tumor available for RNA; HER3 expression measurement was successful in 308. Baseline characteristics by treatment arm were well balanced (Supplementary Table 2), with no significant differences from the overall trial population.

RAS/RAF genotype data ($KRAS_{c.12\text{-}13,61,146}$ $NRAS_{c.12\text{-}13,61}$ and $BRAF_{V600E}$) was available for all cases. 209/308 (67.8%) patients were wt for all *KRAS/NRAS* codons and form the primary analysis population for this study (Supplementary Figure 1). HER2 expression data was available for 210/308 (68%) patients;[9] however, overexpression was found in only 3/210 (1.4%), precluding meaningful analysis.

HER3 was not significantly associated with *RAS* mutation status (p=0.46), but was lower in *BRAF*-mutant and *PIK3CA*-mutated groups compared with wt (p<0.05 and p=0.02 respectively). HER3 levels were weakly positively correlated with both AREG and EREG levels (Spearman's rho 0.26 for each, p<0.001). In *RAS* wt patients, HER3 levels were lower in right-sided compared with left-sided tumors (p=0.02), but no association in the whole population (p=0.09).

Higher $\log_2$ HER3 was prognostic for improved OS (HR per 2-fold change 0.91,[0.83–0.99],p=0.04), but not PFS (HR=0.93,[0.83-1.05],p=0.25) in the whole population(Supplementary Table 3).

In *RAS*-wt patients, increasing HER3 expression was significantly predictive for panitumumab benefit on PFS (IrPan: HR=0.71[0.61-0.82] per 2-fold increase, p<0.0005; Ir: HR=0.96[0.82-1.13],p=0.65; interactions: unadjusted p=0.001 (Table 1), estimates were similar after adjusting for performance status (PS) and previous response, interaction p=0.003)(data not shown). HER3 was also significantly predictive for panitumumab benefit on OS in *RAS* wt patients (IrPan:

HR=0.73[0.64-0.83],p<0.0005; Ir: HR=0.93[0.83-1.05],p=0.25; interactions: unadjusted p=0.004 (Table 1); adjusted p=0.006 (data not shown)). This effect was independent of *BRAF*-mutation status and primary tumour location (PTL)(Supplementary Table 4).

Preplanned predictive analyses of HER3 expression dichotomized at the 50th, 66th, 80th and 90th centile in *RAS* wt patients (Supplementary Table 5) showed that at the 66th centile, there was no PFS benefit from panitumumab in low expressers (median PFS 3.3 months (IrPan) vs 4.3 months (irinotecan); HR=0.96[0.67-1.38],p=0.84), clear benefit in high expressers (HR=0.33[0.19-0.58],p<0.0005)(Figure 1) and strong evidence for interaction (p=0.002). Secondary analyses adjusting for PS and previous response showed similar estimates (interaction p=0.008, data not shown). This cut-point was therefore selected for all subsequent analyses to define "HER3-high" and "HER3-low" populations. Dichotomized HER3 was also predictive for OS in *RAS* wt patients, though here the interaction was driven in part by a negative impact of panitumumab in HER3-low patients. In *RAS*-wt, HER3-high patients, median OS was 14.6 (IrPan) vs 13.2 months (irinotecan) (HR=0.66[0.40-1.10],p=0.11), while in *RAS*-wt, HER3-low patients, median OS was 8.3 (IrPan) vs 10.3 months (irinotecan); HR=1.56[1.09-2.23],p=0.02); interaction p=0.01 (Table 2 and Figure 1). Secondary analyses adjusting for PS and previous response showed similar estimates, interaction p=0.02 (data not shown). The dichotomized HER3 model was also independent of *BRAF*-mutation status and PTL (Supplementary Table 4).

Dichotomized HER3 was not significantly prognostic for OS (HR=0.85[0.60-1.20],p=0.35) or PFS (HR=1.03[0.73-1.45],p=0.86) (Supplementary Table 3 and Supplementary Figure 2).

In *RAS*-wt, HER3-high patients, 12-week response rate (RR) was 48.6% (IrPan) vs 12.1% (irinotecan) (Relative risk=4.01[1.50-10.68]), while in *RAS*-wt, HER3-low patients RR was 24.6% (IrPan) vs 11.1% (irinotecan) (Relative risk=2.21[1.03-4.75]) (interaction p=0.34) (Supplementary Table 6).

We previously reported that in a dichotomous ligand mRNA expression model, "EREG-high and/or AREG-high" was strongly predictive of panitumumab PFS benefit in *RAS*-wt patients, and that as a continuous variable AREG was the more predictive of the two ligands.[3]

As continuous variables in a joint model, HER3 and AREG were independent predictors of IrPan PFS benefit (interaction HER3*treatment p=0.03; AREG*treatment p=0.05), while for OS the evidence was equivocal (interaction HER3 p=0.07; AREG p=0.21) (Supplementary Table 7).

In an exploratory dichotomous model, the *RAS*-wt population was divided into four groups: HER3-high/, AREG/EREG-high[n=42]; HER3-high/, AREG/EREG-low[n=27]; HER3-low/, AREGEREG-high[n=53]; HER3-low, AREG/EREG-

low[n=87]. Marked panitumumab benefit was observed in the HER3-high, AREG/EREG-high group (PFS HR=0.24[0.11-0.51],p<0.005; OS HR=0.36[0.18-0.73],p=0.004). Conversely, the HER3-low, AREG/EREG-low group showed no evidence of benefit (PFS HR=1.14[0.73-1.79],p=0.57; OS HR=1.44[0.92-2.26],p=0.11). Patients in the two intermediate groups had intermediate effects for PFS (Supplementary Table 8).

Discussion

The work described here represents the largest dedicated analysis of HER3 expression in CRC to date. We found no good evidence for HER3 as a prognostic marker but found HER3 overexpression to be significantly associated with benefit from panitumumab.

Overall in PICCOLO panitumumab produced a significant improvement in PFS but not OS.[2] It is therefore encouraging that HER3 was significantly predictive for OS as well as PFS. Furthermore, in our combined HER3-and-ligands model, patients with HER3-high, AREG/EREG-high tumors achieved marked and significant OS benefit with panitumumab (HR=0.36[0.18-0.73], p=0.004). These findings are interesting, but must be treated with caution, especially given that the HER3 cut-point for dichotomization was derived internally, from this dataset.

The interaction between HER3 expression and anti-EGFR agent efficacy in aCRC has been studied previously in 2 smaller series.[9,10] Instead HER3 overexpression was associated with inferior clinical outcomes with anti-EGFR agents. The present study is a dedicated comprehensive analysis in a mature, large, randomized trial, allowing for adjustments for likely confounders. Preclinical work supports both hypotheses: heregulin activation of HER3 might trigger an alternate signaling pathway circumventing EGFR blockade,[8,11] supporting HER3 overexpression as a negative predictive marker. Alternatively, given its role as an obligate heterodimer, HER3 expression could identify those

tumors most reliant on EGFR signaling through autocrine feedback loops, and more likely to respond to EGFR-targeted agents.[7]

In conclusion, this study suggests that high HER3 expression is related to anti-EGFR agent activity in aCRC, and may offer a clinically useful selection biomarker. Prior to clinical application, re-validation of the findings and refining of the cut-point is required, in well-designed hypothesis-based studies using other randomized datasets.

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Legend to Tables and Figures

Figure 1 i. PFS KM curves for *RAS* wt patients for high HER3 expressers and low HER3 expressers treated with IrPan vs Ir (interaction p=0.002); ii. OS KM curves for high HER3 expressers and low HER3 treated with IrPan vs Ir (interaction = 0.01)

Table 1. Estimated crude Hazard Ratios (HRs) and 95% CIs for the effect of $\log_2$ HER3 on PFS and OS in all patients, *RAS* wt, *RAS* mut, *BRAF* mut and *PIK* mut patients separately

Table 2. Estimated crude HRs and 95% CIs for the effect of treatment (IrPan vs Ir) on PFS and OS in low HER3 expression and high HER3 expression stratifying by *RAS* and *BRAF* mutation status, including likelihood ratio tests for HER3*treatment interactions.

Table 1

		All patients		Ir		IrPan		
	Mutation subgroup	**Events/Patients, Nos.**	**Unadjusted HR for log_2 HER3[a] (95% CI)**	**Events/Patients, Nos.**	**Unadjusted HR for log_2 HER3 (95% CI)**	**Events/ Patients, Nos.**	**Unadjusted HR for log_2 HER3 (95% CI)**	**P Value for Interaction[b]**
PFS	**RAS wt**	190/208	0.82 (0.74-0.90),p<0.0005	106/114	0.96 (0.82-1.13),p=0.65	84/94	0.71 (0.61-0.82),p<0.0005	0.001
	RAS mut	95/99	0.95 (0.87-1.05),p=0.34	48/49	0.88 (0.72-1.07),p=0.19	47/50	0.99 (0.88-1.12),p=0.91	0.37
	BRAF mut	44/47	0.79 (0.66-0.94),p=0.009	18/19	1.03 (0.70-1.53),p=0.88	26/28	0.77 (0.63-0.93),p=0.006	0.11
	PIK mut	32/35	0.87 (0.69-1.10), p=0.26	22/23	0.95 (0.72-1.27), p=0.74	10/12	0.53 (0.28-1.02), p=0.06	0.01
	All patients	285/307	0.88 (0.83-0.94),p<0.0005	154/163	0.93 (0.83-1.05),p=0.25	131/144	0.87 (0.81-0.94),p<0.0005	0.21
OS	**RAS wt**	192/209	0.86 (0.80-0.94),p<0.0005	106/115	0.93 (0.83-1.05),p=0.25	86/94	0.73 (0.64-0.83),p<0.0005	0.004
	RAS mut	97/99	0.94 (0.86-1.03),p=0.17	47/49	0.83 (0.70-0.98),p=0.03	50/50	1.01 (0.89-1.14),p=0.93	0.07
	BRAF mut	45/47	0.81 (0.70-0.94),p=0.005	18/19	0.89 (0.61-1.31),p=0.56	27/28	0.80 (0.68-0.95),p=0.009	0.67
	PIK mut	33/35	0.96 (0.79-1.16), p=0.67	21/23	0.93 (0.74-1.17), p=0.54	12/12	0.65 (0.37-1.15), p=0.14	0.31
	All patients	289/308	0.90 (0.85-0.95),p<0.0005	153/164	0.91 (0.83-0.99),p=0.04	136/144	0.89 (0.83-0.96),p=0.001	0.74

[a] HR per 2-fold increase in HER3

[b] P-value is from a likelihood ratio test comparing a model including the main effects for log_2 Her3 and treatment (IrPan versus Ir) plus the log_2 Her3*treatment interaction term with a model including only the main effects

Table 2

		All patients		Low HER3 expression (<66th centile)		High HER3 expression (>66th centile)		
	Mutation subgroup[a]	**Events/Patients, Nos.**	**Unadjusted HR for IrPan vs Ir (95% CI)**	**Events/Patients, Nos.**	**Unadjusted HR for IrPan vs Ir(95% CI)**	**Events/Patients, Nos.**	**Unadjusted HR for IrPan vs Ir (95% CI)**	**P Value for Interaction**
PFS	**RAS wt**	190/208	0.67 (0.50-0.90) p=0.008	128/139	0.96 (0.67-1.38) p=0.84	62/69	0.33 (0.19-0.58) p<0.0005	0.002
	RAS mut	95/99	1.18 (0.78-1.77) p=0.43	60/63	1.17 (0.70-1.97) p=0.54	35/36	1.26 (0.63-2.52) p=0.51	0.89
	BRAF mut	44/47	1.07 (0.57-1.98) p=0.84	33/35	1.29 (0.60-2.74) p=0.51	11/12	0.29 (0.06-1.40) p=0.12	0.06
	All patients	285/307	0.81 (0.64-1.03) p=0.09	188/202	1.05 (0.78-1.40) p=0.76	97/105	0.55 (0.36-0.84) p=0.006	0.008
OS	**RAS wt**	192/209	1.10 (0.82-1.46) p=0.52	130/140	1.56 (1.09-2.23) p=0.02	62/69	0.66 (0.40-1.10) p=0.11	0.01
	RAS mut	97/99	1.36 (0.91-2.04) p=0.14	63/63	1.02 (0.62-1.69) p=0.92	34/36	2.09 (1.0-4.37) p=0.05	0.09
	BRAF mut	45/47	1.33 (0.73-2.43), p=0.35	33/35	1.41 (0.67-2.98), p=0.36	12/12	0.41 (0.11-1.57), p=0.19	0.11
	All patients	289/308	1.17 (0.93-1.48) p=0.18	193/203	1.36 (1.02-1.81) p=0.03	96/105	0.95 (0.64-1.42) p=0.80	0.15

[a]*PIK3CA mutant cases excluded due to small patient numbers*