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CORRESPONDENCE

Failure to Validate Toxicity and Outcome Biomarkers From the FFCD 2000-05 Trial in the MRC CR08 FOCUS Trial

TO THE EDITOR: We read with great interest the article by Boige et al,[1] who investigated whether germline polymorphisms in genes suspected to be involved in chemotherapeutic pathways were associated with toxicity and clinical outcome in patients with advanced metastatic colorectal disease. Using blood samples from 349 patients enrolled onto the FFCD (Fédération Francophone de la Cancérologie Digestive) 2000-05 randomized phase III trial, the authors genotyped 20 polymorphisms. Of particular interest to our group, they reported that only patients with the *TS-5′UTR 2R/2R* or *2R/3R* genotype benefited in terms of progression-free survival (PFS) from a first-line fluorouracil (FU), leucovorin, and oxaliplatin regimen (hazard ratio [HR], 0.39; 95% CI, 0.23 to 0.68, and HR, 0.59; 95% CI, 0.42 to 0.82, respectively). Conversely, patients with the *3R/3R* genotype gained no benefit from the addition of oxaliplatin to FU plus leucovorin (HR, 0.96; 95% CI, 0.66 to 1.40). At the request of the authors, we attempted to validate their findings using material collected by our group from patients in the MRC (Medical Research Council) CR08 FOCUS (FU, Oxaliplatin and CPT11 [irinotecan]: Use and Sequencing) trial. This data set was of particular interest because of the similarities in treatment regimens between the two trials in question.

The FOCUS trial has been reported elsewhere.[2] Subsequent to ethical approval, 2,135 consenting patients with advanced colorectal cancer were randomly assigned between 2000 and 2003. Separate consent was obtained for retrieval of surplus pathologic material from tissue archives. The MRC Clinical Trials Unit managed the trial, overseen by an independent trials steering committee. FOCUS was designed to compare different sequences of cytotoxic agents. Patients were randomly assigned equally among three treatment strategies. In strategies A and B, first-line therapy was FU alone, followed on progression by either single-agent irinotecan (A) or combination therapy (B), whereas in strategy C, combination therapy was administered as first-line treatment. In strategies B and C, the choice of combination therapy was also randomized equally between FU plus irinotecan and FU plus oxaliplatin. Regions of normal bowel mucosa were identified using hematoxylin and eosin–stained sections; DNA extractions were then carried out from macrodissected areas using proteinase-K digestion, phenol/chloroform/isoamyl alcohol (25:24:1), and ethanol precipitation.

We designed our thymidylate synthase enhancer region primers on the basis of sequences obtained from the National Center for Biotechnology Information database. The resultant *2R* polymerase chain reaction product measured 214 bp, with the *3R* product measuring 242 bp. These were distinguished by running on a 3% agarose gel. Nine hundred thirty-eight FOCUS samples were available for analysis. A majority of the remaining FOCUS samples were received as biopsies and therefore too small for DNA extraction. We saw strong agreement in the genotype frequencies obtained between samples from the FOCUS trial and those of Boige et al[1] (Table 1).

Table 1. Comparison of Genotype Frequencies Detected by Boige et al[1] and in the FOCUS Trial

	Frequency			
	Boige et al		FOCUS	
Genotype	No.	%	No.	%
2R/2R	119	35.4	330	35.2
2R/3R	156	46.4	430	45.8
3R/3R	61	18.2	178	19.0

Abbreviation: FOCUS, Fluorouracil, Oxaliplatin and CPT11 [irinotecan]: Use and Sequencing.

The prognostic analysis of the FOCUS trial demonstrated a weak effect of the polymorphism on PFS independent of treatment regimen, the *2R* allele being protective (per-allele HR, 0.9; 95% CI, 0.82 to 0.99; $P = .03$), based on 910 patients with complete data (Fig 1). Furthermore, this effect persisted in a model adjusted for treatment and other prognostic factors (per-allele HR, 0.86; 95% CI, 0.77 to 0.95; $P = .005$). In contrast, Boige et al[1] saw no overall prognostic effect on PFS ($P = .98$). In neither trial population was there any evidence of an effect on overall survival (per-allele HR, 1.00; $P = .94$ [FOCUS trial]).

In our predictive analysis, we failed to confirm the predictive effect seen in the FFDC 2000-05 trial. HRs for PFS with first-line FU plus oxaliplatin versus FU alone were 0.68 (95% CI, 0.48 to 0.96), 0.79 (95% CI, 0.60 to 1.04), and 0.53 (95% CI, 0.35 to 0.80) for patients with *3R/3R*, *2R/3R*, and *2R/2R* genotypes, respectively. Thus, benefit from the addition of oxaliplatin is seen irrespective of genotype; patients with the *3R/3R* genotype showed clear evidence of benefit. (A similar finding was seen for FU plus irinotecan *v* FU alone, with HRs of

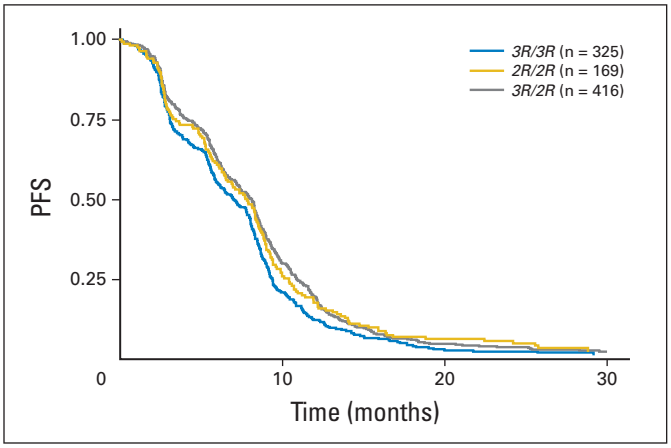

Fig 1. Kaplan-Meier plot of progression-free survival (PFS) in the FOCUS (Fluorouracil, Oxaliplatin and CPT11 [irinotecan]: Use and Sequencing) trial population.

0.85, 0.65, and 0.71 for patients with *3R/3R*, *2R/3R*, and *2R/2R* genotypes, respectively, demonstrating again that each genotype group gained similar benefit from addition of irinotecan).

As in the Boige et al[1] study, we repeated the above analyses, this time grouping the genotypes into *3R/3R* versus carriage of *2R* (*2R/2R* plus *2R/3R*), but again, this produced similar HRs of 0.68 and 0.69, respectively. Finally, we examined the contrast between combination therapy with either oxaliplatin or irinotecan versus deferring the introduction of combination therapy until after the failure of FU alone. HRs for PFS were 0.78 (95% CI, 0.62 to 0.98), 0.72 (95% CI, 0.58 to 0.89), and 0.60 (95% CI, 0.43 to 0.83) for the *3R/3R*, *2R/3R*, and *2R/2R* genotypes, respectively. Similarly, there was no suggestion of an effect of genotype on differential benefit of treatment in terms of overall survival.

Thus, to summarize, using material from the FOCUS trial, we failed to validate in a larger data set the findings of Boige et al,[1] who suggest that genotyping of *TS-5′ UTR* may be a valuable tool in identifying patients who will and will not benefit from first-line combination therapy. No genotype-treatment interactions were statistically significant, and indeed, the pattern of HR estimates did not match that seen in the FFCD trial. Our data show that patients with the *3R/3R* genotype also benefit from this treatment. This work highlights the need for strong validation of any molecular findings before they are used as the basis of clinical trials.

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author(s) indicated no potential conflicts of interest.

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