

thrombocytopenia, it is very sensitive and specific for the detection of antibodies associated with this condition.¹ The diagnosis of heparin-induced thrombocytopenia is almost untenable in patients with a negative or a very weak positive result on PF4 ELISA.¹ Therefore, it is very unlikely that heparin-induced thrombocytopenia was present in any of the 29 patients with vancomycin-dependent antibodies and thrombocytopenia described in our report. The patient whose clinical course is shown in Figure 1 of our article had an initial rise in the platelet count 3 days after a second intravenous infusion of immune globulin but did not have a count of 100,000 per cubic millimeter until day 6. It is certainly possible that intravenous immune globulin shortened the duration of thrombocytopenia in this patient. However, an equally rapid platelet response after the discontinuation of vancomycin occurred in 12 other patients who did not receive intravenous immune globulin.

In response to Okamoto's question, we note that clinically significant vancomycin-dependent antibodies almost certainly persisted for at least 6 months in two patients who had a recurrence of severe thrombocytopenia when reexposed to van-

comycin at a later time. We did not perform sequential studies to determine how long antibodies remained detectable in the other patients.

In response to Naina and colleagues, none of the patients described in our report had thrombosis in conjunction with vancomycin-induced thrombocytopenia. To our knowledge, an association between immune thrombocytopenia and thrombosis has been described only in patients with sensitivity to heparin^{1,2} and platelet-function inhibitors of the fiban class.³

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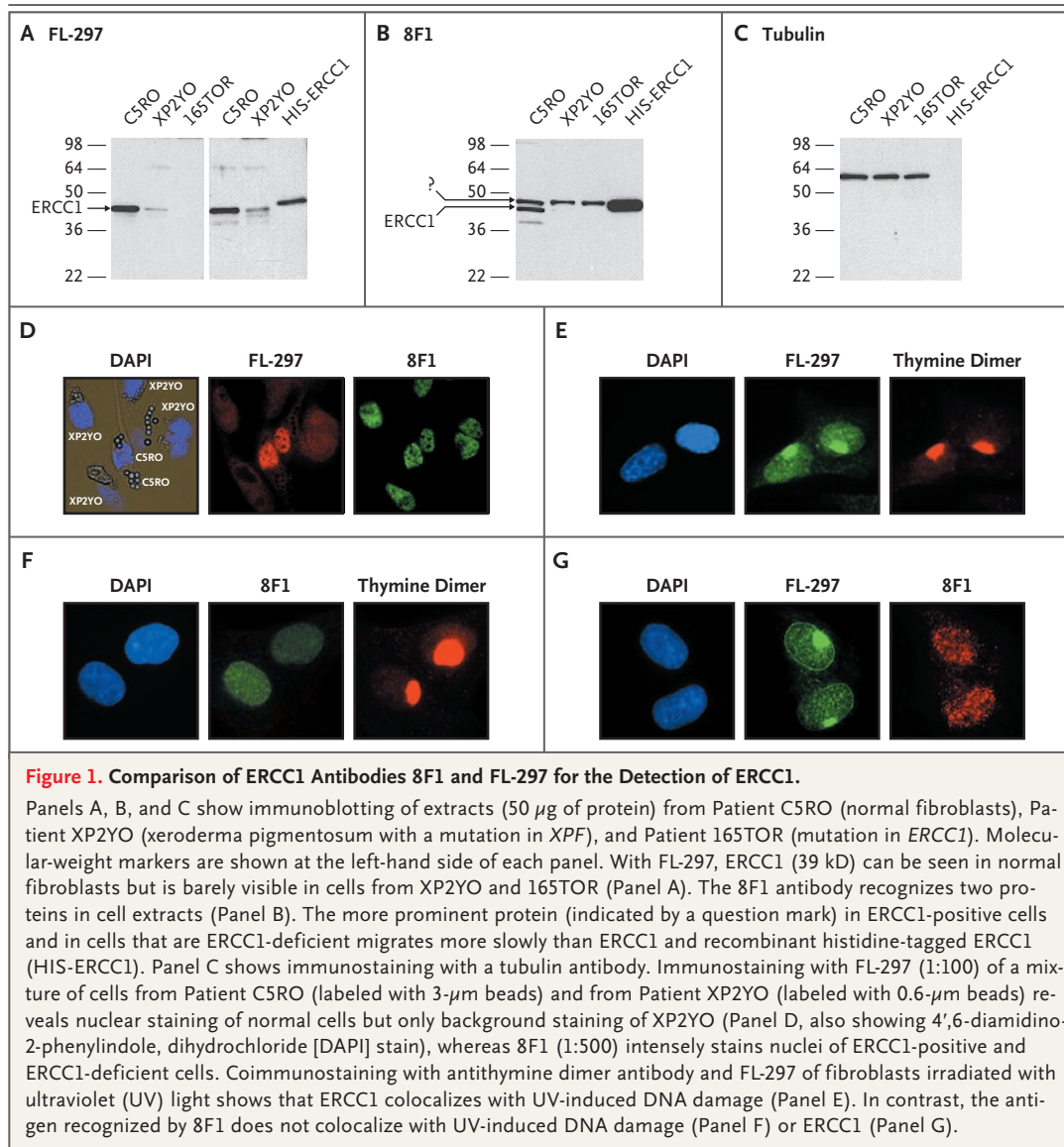
ERCC1 and Non-Small-Cell Lung Cancer

TO THE EDITOR: Zheng et al. (Feb. 22 issue)¹ conclude that the excision repair cross-complementation group 1 (ERCC1) protein is a determinant of survival after surgical treatment of early-stage non-small-cell lung cancer. The ERCC1 protein associates with the xeroderma pigmentosum group F (XPF) protein to form a nuclease that functions in DNA repair.² The level of ERCC1 protein was proposed as a useful predictor of response to cisplatin-based chemotherapy and clinical outcome,³ on the basis of immunohistochemical staining of tumors with the use of the monoclonal antibody 8F1. To our knowledge, however, no controlled experiments demonstrating the specificity of this antibody have been reported.

We examined 8F1 and a second commercially available antibody (FL-297) for specificity in detecting ERCC1, using ERCC1-positive normal human fibroblasts and cells from patients with

inherited mutations in *ERCC1* (Patient 165TOR) and *XPF* (Patient XP2YO) causing a deficiency of ERCC1-XPF nuclease.^{2,4} Immunoblotting of cell lysates with FL-297 revealed a single band of appropriate molecular weight in normal fibroblasts and confirmed that the level of ERCC1 protein was reduced in cells from Patients XP2YO and 165TOR (Fig. 1A). The 8F1 antibody detected ERCC1 and at least one other protein in whole-cell extracts (Fig. 1B). Tubulin antibody confirmed equal loading of cell extracts (Fig. 1C). The cross-reacting protein is present in normal and ERCC1-deficient cell extracts.

ERCC1-deficient cells from Patient XP2YO and normal human fibroblasts were differentially labeled with cytoplasmic beads and cocultured. Immunostaining with FL-297 discriminated between ERCC1-positive and ERCC1-deficient cells (Fig. 1D). In contrast, 8F1 strongly stained the nuclei of all cells. Irradiation of cells with ultra-



violet (UV) light through a filter containing 8- μ m pores causes subnuclear domains of UV-induced DNA damage that can be identified with an antibody recognizing thymine dimers.⁵ ERCC1-XPF repair nuclease accumulates at these sites of DNA damage.⁵ Immunostaining of irradiated fibroblasts with antithymine dimer and FL-297 yielded signals that colocalized (Fig. 1E). In contrast, the antigen recognized by 8F1 does not preferentially accumulate at sites of DNA damage (Fig. 1F) or give a signal that colocalizes with that of FL-297 (Fig. 1G).

These experiments show that ERCC1 is not the principal antigen recognized by the 8F1 an-

tibody on immunostaining of human cells. Furthermore, 8F1 does not discriminate between ERCC1-positive and ERCC1-deficient nuclei. The identity of the antigen recognized by 8F1 and the reason that 8F1 stains some tumors more strongly than others are unknown. These results underscore the point that even monoclonal antibodies raised against recombinant protein are not guaranteed to be specific.

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TO THE EDITOR: Zheng et al. appear to have oversimplified the relationship of regulatory subunit of ribonucleotide reductase (*RRM1*) and *ERCC1* expression levels with prognosis in non-small-cell lung cancer. The authors did not stratify their patients with regard to smoking and other potential covariables and genotyping analysis of *RRM1* and *ERCC1*. To date, 226 and 92 single-nucleotide polymorphisms (SNPs) have been reported in *RRM1* and *ERCC1*, respectively, and many of these SNPs have a functional effect (details are available at www.ncbi.nlm.nih.gov/SNP/). For instance, the SNP in *RRM1* (2464G→A) is associated with resistance to gemcitabine therapy,¹ whereas the SNP in *ERCC1* (118C→T) influences the outcome of cisplatin therapy in patients with non-small-cell lung cancer.² Smoking is also an important factor that has interplay with the SNP of *ERCC1*.²

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TO THE EDITOR: Zheng et al. suggest that using a combination of the gene expression of both *ERCC1*

and *RRM1* can isolate a subgroup of patients with excellent survival. In particular, Figure 5 of the article shows that the subgroup with a high expression of both genes has an overall survival of more than 120 months. It does not appear that the authors analyzed this particular subgroup with regard to whether the patients' tumors were largely in stage pIA. If this is the case, it would be interesting to know what the average size of the tumor was among these 55 patients with high expression of *ERCC1* and *RRM1*. If the tumors were small, it may be that these two markers were just identifying patients who presented with small tumors and thus had a relatively better prognosis.

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THE AUTHORS REPLY: Niedernhofer et al. show two distinct proteins with slightly different molecular masses interacting with 8F1, *ERCC1* and an unidentified protein. This difference may explain the reported lack of association between *ERCC1* messenger RNA (mRNA) and protein expression. Since the identity and pattern of expression of the larger band are obscure, its effect on the prognostic usefulness of *ERCC1* levels measured by 8F1 is unknown.

We did not observe a significant difference in *RRM1* and *ERCC1* levels between tumors measuring less than 3 cm and those measuring more than 3 cm. In our data set, tumor size was not significantly associated with either overall survival ($P=0.07$ by the log-rank test) or disease-free survival ($P=0.09$) when categorized into four groups (≤ 2.0 cm, 2.1 to 3.5 cm, 3.6 to 5.0 cm, and >5.0 cm). We believe that the observed survival advantage for patients with high *RRM1* and *ERCC1* expression cannot be explained by tumor size.

The Entrez database contains a total of 272 SNPs for *RRM1* and 109 for *ERCC1*. Nine are in the coding region of *RRM1*; four result in amino acid alterations, and SNP 2464A→G does not (Table 1). For *ERCC1*, five coding-region SNPs are described, and two result in amino acid alterations. We sequenced the genomic region of *RRM1* and deposited the data in GenBank (AF107045), which provided the reference for some of the reported SNPs.¹ In addition, we described SNPs in the *RRM1* promoter region that have a substantial effect on in vitro reporter gene transcrip-

Table 1. *RRM1* Coding-Region Variations in Non–Small-Cell Lung Cancers.

Patient No.	249 G→A Arg/Gln	568 C→T His/His	733 A→C Pro/Pro	768 A→C His/Pro	821 T→G Trp/Gly	871 A→G Pro/Pro	1082 A→C Arg/Arg	2455 A→G Thr/Thr	2464 A→G Ala/Ala	2565 T→C Val/Ala
D234	cGa	caC	ccA	cAc	Tgg	ccA	Aga	acG	gcA	gTg
D236	cGa	caC	ccA	cAc	Tgg	ccA	Aga	acG	gcA	gTg
D245	cGa	caC	ccA	cAc	Tgg	ccA	Aga	acG	gcA	gTg
D247	cGa	caC	ccA	cAc	Tgg	ccA	Aga	acG	gcA	gTg
D314	cGa	caC	ccA	cAc	Tgg	ccA	Aga	acG	gcA	gTg
D352	cGa	caC	ccA	cAc	Tgg	ccA	Aga	acG	gcA	gTg
D356	cGa	caC	ccA	cAc	Tgg	ccA	Aga	acG	gcA	gTg
D358	cGa	caC	ccA	cAc	Tgg	ccA	Aga	acG	gcA	gTg
D366	cGa	caC	ccA	cAc	Tgg	ccA	Aga	acG	gcA	gTg
D372	cGa	caC	ccA	cAc	Tgg	ccA	Aga	acG	gcA	gTg
D374	cGa	caC	ccA	cAc	Tgg	ccA	Aga	acG	gcA	gTg
D376	cGa	caC	ccA	cAc	Tgg	ccA	Aga	acG	gcA	gTg
D378	cGa	caC	ccA	cAc	Tgg	ccA	Aga	acG	gcA	gTg

tion; however, we were unable to show their effect on *in vivo* gene expression.²

We sequenced the coding region of *RRM1* in fresh-frozen specimens from 13 white men and women with non–small-cell lung cancer; at least 70% of the cells in the specimens were tumor cells. Sequences of good quality were compared with NM_001033 (Table 1) and all patient sequences were identical to one another.

We had previously reported an A at position 2455. In all specimens, G was the only nucleotide found at this position. For all other SNPs, we found only the nucleotide reported in the reference sequence. We have noted that the sequence chromatograms for the regions containing the SNPs frequently display low nucleotide signal values. The automated base assignment is often ambiguous in particular when double cytosines

or guanines precede or follow the referenced SNPs. These technical limitations may account for the reported SNPs and call their existence into question. Given these limitations, it is our opinion that investigations of the correlations between reported SNPs and clinical outcomes are premature.

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Prepubertal Gynecomastia Linked to Lavender and Tea Tree Oils

TO THE EDITOR: The study by Henley et al. (Feb. 1 issue)¹ raises many questions. Product names were not provided. Did the authors contact manufacturers to report concerns or ask about constituents? The variability, adulteration, and contamination of herbal products have been widely reported,^{2,3} as have discrepancies between labels

and contents.⁴ Plastic containers may contain phthalates, known endocrine disruptors.⁵ What was actually in the products cited in this report?

None of the hormonal testing showed abnormal results, except in Patient 2, who had elevated levels of testosterone (not estrogen). There was no report on ultrasound examination or needle