

## FCGR2A Polymorphism Is Correlated With Clinical Outcome After Immunotherapy of Neuroblastoma With Anti-GD2 Antibody and Granulocyte Macrophage Colony-Stimulating Factor

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### ABSTRACT

#### Purpose

Anti-GD2 murine IgG3 antibody 3F8 kills neuroblastoma cells by antibody-dependent cell-mediated cytotoxicity (ADCC). Granulocyte macrophage colony-stimulating factor (GM-CSF) enhances phagocyte-mediated ADCC. The differential affinity of the human *FCGR* polymorphic alleles for 3F8 may influence the effectiveness of antibody immunotherapy.

#### Patients and Methods

The entire cohort of high risk neuroblastoma patients (N = 136) treated on protocol using 3F8 and GM-CSF were the subjects of this analysis. Tumor response was measured by standard clinical tools plus sensitive molecular monitoring using quantitative reverse transcription-polymerase chain reaction (qRT-PCR). Polymorphic alleles of *FCGR2A* and *FCGR3A* were determined by PCR plus direct sequencing using genomic DNA samples obtained from marrow or blood of patients.

#### Results

*FCGR2A* (R/R) genotype correlated with progression-free survival for the entire cohort ( $P = .049$ ) and for the subset of patients with no history of prior relapse ( $P = .023$ ). *FCGR2A* (R/R) also correlated with marrow remission 2.5 months after treatment initiation: by histology ( $P = .021$  and  $P = .036$ , for the entire cohort and the subset, respectively) and by qRT-PCR ( $P = .052$  and  $P = .033$ , respectively).

#### Conclusion

The favorable outcome associated with *FCGR2A* (R/R) genotype is consistent with the proposed role of *FCGR2A* and phagocyte-mediated ADCC in 3F8 plus GM-CSF immunotherapy.

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### INTRODUCTION

Monoclonal antibodies (MoAbs) show promise in cancer therapy. They can kill tumor cells through antibody-dependent cell-mediated cytotoxicity (ADCC), complement-mediated cytotoxicity (CMC), and apoptosis. ADCC and CMC are mediated by Fc $\gamma$  receptors (Fc $\gamma$ R), where individual Fc $\gamma$ Rs are suited for unique effector functions as well as unique effector cell types.<sup>1,2</sup> Since natural antibody responses to specific antigens or pathogens are polyclonal, their interaction with Fc $\gamma$ R can be quite complex. When MoAb is used for disease therapy, its preferential interaction with specific Fc $\gamma$ R alleles translates into distinct biologic effects. Thus, it is not surprising that polymorphic Fc $\gamma$ R alleles can influence and correlate with clinical efficacy of MoAb immunotherapy.

Fc $\gamma$ R2A has two polymorphic alleles at amino acid position 131. The Fc $\gamma$ R2A-H131 allelotype (histidine) has higher binding efficiency for human IgG2 and IgG3 antibodies when compared with Fc $\gamma$ R2A-R131 (arginine). On the other hand, Fc $\gamma$ R2A-V158 (valine) has higher affinity than Fc $\gamma$ R3A-F158 (phenylalanine) for human IgG1.<sup>3</sup> Greater binding affinity of Fc $\gamma$ R2A-H131 is expected to trigger stronger inflammatory responses predisposing patients to disease like Guillain-Barre syndrome.<sup>4</sup> However, ineffective Fc $\gamma$ R function may impair immune complex clearance, precipitating autoimmune processes (eg, Fc $\gamma$ R2A-R131 in lupus nephritis<sup>5</sup>). Impaired Fc $\gamma$ R function in clearing opsonized bacteria (eg, Fc $\gamma$ R2A-R131) has also been associated with infections, bacteremic pneumonia, periodontitis, and fulminant meningococcal septic shock.<sup>6</sup> The interaction of IgG with the inhibitory Fc $\gamma$ R2B on

B-cells, macrophages, and monocytes adds another dimension to antibody regulation of inflammatory responses.<sup>7</sup> Whether the polymorphic forms of this FCGR2B will translate into allele-specific outcomes remains to be elucidated.<sup>8</sup>

To date, almost all MoAbs in clinical trials are either chimeric or humanized antibodies bearing the human  $\gamma 1$  Fc domain. Antibodies of this subclass are known to interact preferentially with Fc $\gamma$ R3A-V158. Several reports have found a favorable association between this allele with clinical outcome following treatment with rituximab.<sup>9,10</sup> In contrast, there have been limited studies on the interaction of murine antibodies with human Fc $\gamma$ R. Mouse anti-CD3 MoAbs are used extensively as immunosuppressive agents for graft rejection; Fc $\gamma$ R-dependent mitogenic properties and cytokine release are believed to be responsible for the systemic adverse effects. Unlike murine IgG2a anti-CD3 antibodies, which bind both Fc $\gamma$ R2A allelotypes equally well, IgG1 subclass antibodies prefer the Fc $\gamma$ R2A-R131 over the -H131 allelotype, resulting in differential mitogenic potency and cytokine release,<sup>11</sup> believed to be responsible for the first-dose effect of anti-CD3 MoAb in patients,<sup>12</sup> characterized by systemic release of cytokines, chills, fever, nausea, headache, and diarrhea. Similar observations of Fc $\gamma$ R2A allelotype preference were made for the murine IgG1 antibody Leu2a specific for human CD8,<sup>13</sup> and platelet-activating murine IgG1 antibodies,<sup>14</sup> and cellular aggregation in the presence of IgG1 in flow cytometry.<sup>15</sup> The murine IgG3 anti-GD2 antibody 3F8 is being used in the clinic for its antitumor activity in high-risk neuroblastoma patients. In vitro studies using 3F8 have shown potent ADCC.<sup>16</sup> In the presence of granulocyte macrophage-colony-stimulating factor (GM-CSF), antitumor activity can be markedly increased. By enzyme-linked immunosorbent assay, 3F8 has preferential binding to Fc $\gamma$ R2A-R131 over Fc $\gamma$ R2A-H131. In this report, we correlate FCGR genotypes with antitumor response and survival among the entire cohort of 136 high-risk neuroblastoma patients who were treated with an immunotherapy regimen using murine MoAb 3F8 and GM-CSF.

## PATIENTS AND METHODS

### Patients

One hundred and thirty-six consecutive patients with neuroblastoma (38 with *MYCN* amplification of more than 10 *MYCN* copies per diploid human genome) treated on protocol IRB 9418 at Memorial Sloan-Kettering Cancer Center (MSKCC; New York, NY) were the subjects of this study. One hundred and thirty of 136 patients had metastatic stage 4 neuroblastoma, and six patients had high-risk stage 3 (four of six with *MYCN* amplification). Except for one infant with *MYCN*-amplified stage 4 neuroblastoma, 135 of 136 patients were diagnosed at older than 12 months of age, and 126 of 136 patients (93%) were diagnosed at older than 18 months of age, generally regarded as the highest-risk age group. This protocol utilized anti-GD2 MoAb 3F8 plus GM-CSF in children after chemotherapy.<sup>17</sup> Written informed consent was obtained from the patients and/or their parents/guardians. Their disease status at protocol entry was stratified into four categories:<sup>18</sup> complete remission/very good partial remission, primary refractory, second refractory, and progressive disease.<sup>17</sup> Of 136 patients, 124 had follow-up bone marrow (BM) samples after treatment cycle two, at a median of 2.5 months from protocol entry. There were 69 deaths and 90 relapses during follow-up. Median follow-up for survivors was 34 months and 36 months for overall survival (OS) and progression-free survival (PFS), respectively. Outcome at 2 years was correlated with disease status at the time of protocol entry. Patients who had prior relapse (either progressive disease or secondary refractory) fared worse; 97% pro-

gressed before 2 years compared with 62% of those with no prior relapse (ie, either complete remission/very good partial remission or primary refractory disease status) at protocol entry. Two of the 136 patients chose to go off protocol to enroll on other treatment studies.

### Histologic Examinations of BM Samples

All 136 patients had marrow studies under general anesthesia, before and after the second cycle of therapy. Each study consisted of six histopathologic examinations of two biopsy specimens and four aspirates from different sites, with a fresh needle for each site. After clearance of contaminating skin, bone, or endothelial cells, 2 mL to 2.5 mL of heparinized marrow from each aspiration site was pooled, mononuclear cells were then isolated and cryopreserved.

Real-time quantitative polymerase chain reaction (qRT-PCR) was performed on cryopreserved BM using the primers and probes for GD2 synthase and the endogenous reference glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as previously described.<sup>19</sup> The transcript levels of GD2 synthase and GAPDH were determined from their respective standard curves using serially diluted cDNA from neuroblastoma cell line NMB7. Normalized transcript levels were expressed as multiples of GAPDH expression.

### Fc Polymorphism Genotyping

Genomic DNA was purified from peripheral blood or marrow mononuclear cells using DNA extraction kit (Qiagen, Valencia, CA). Genotyping of *FCGR2A* 131-H/R and *FCGR3A* 158-V/F polymorphism was performed by PCR, followed by allele-specific restriction enzyme digestion.<sup>20,21</sup> One hundred and fifty ng genomic DNA was amplified using final concentrations of 0.5  $\mu$ mol/L each of forward and reverse primer (Table 1), 0.2 mmol/L dNTP Mix (Promega, Madison WI), 1.5 mmol/L MgCl<sub>2</sub>, 1X AmpliTaq Gold Buffer II (Applied Biosystems [ABI], Foster City, CA), and 1 U AmpliTaq Gold (ABI). All reactions included one 8-minute 94°C hot-start before cycling followed by 40 cycles of 30-second denaturation (94°C), 1-minute annealing (52°C or 60°C), 45-second extension (72°C), and one final 8-minute extension (72°C). *FCGR2A* polymorphism was detected by a subsequent restriction digest of PCR product with BstUI (New England Biolabs, Beverly, MA) where 15  $\mu$ L of *FCGR2A* PCR product was incubated overnight at 60°C with 5 units of BstUI. Digested products were run on a 2% agarose gel. Genotypes were represented by 322 base pairs (bp) alone (G/G), 343bp and 322 bp together (A/G), or 343 bp alone (A/A). *FCGR3A* polymorphisms were identified by direct sequencing of the PCR product. PCR products were cut from the agarose gel and purified using QIAquick PCR Purification Kit (Qiagen). Five  $\mu$ L of purified PCR product was sequenced using 0.625  $\mu$ mol/L of the forward PCR primer, 1  $\times$  BigDye Terminator sequencing buffer and 2  $\mu$ L BigDye Terminator v3.1 cycle sequencing mix (ABI) in a total volume of 20  $\mu$ L. After completion of sequencing reaction, products were ethanol-precipitated as follows: 16  $\mu$ L sterile water and 64  $\mu$ L 95% ethanol were added to each 20  $\mu$ L sample, incubated for 15 minutes at room temperature, then centrifuged at maximum speed (3,200  $\times$  g) at 4°C for 45 minutes. Supernatant was decanted and pellet was washed in 70% ethanol followed by a 10-minute centrifugation at 3200  $\times$  g at 4°C. Seventy percent ethanol supernatant was then poured off; to eliminate any residual ethanol, samples were inverted and centrifuged for 1 minute at 300  $\times$  g. Dried pellet was resuspended in 20  $\mu$ L Hi-Di Formamide (ABI). Samples

**Table 1.** FCGR Primer Sequences and PCR Conditions

Primer	Sequence (5' $\rightarrow$ 3')	Annealing Temperature	Product
<i>FCGR2A</i> (forward)	GGA AAA TCC CAG AAA TTC TCG C	52°C	343 and/or 322 bp
<i>FCGR2A</i> (reverse)	CAA CCT GAC TAC CTA TTA CGC GG		
<i>FCGR3A</i> (forward)	TCA CAT ATT TAC AGA ATG GCA ATG	60°C	177 bp
<i>FCGR3A</i> (reverse)	GTG GCA CAT GTC TCA CCT TG		

Abbreviations: PCR, polymerase chain reaction; bp, basepair.

were denatured at 94°C for 2 minutes and immediately placed on ice for 10 minutes and then loaded onto an ABI 3100-Avant Genetic Analyzer per manufacturer’s instructions. This analysis was carried out in accordance with the guidelines of the institutional review board of MSKCC.

**Statistical Analysis**

The upper limit of normal for GD2 synthase transcript in the qRT-PCR assay was 5 units.<sup>19</sup> Those patients whose post-treatment BM samples were marker negative by qRT-PCR were scored as achieving molecular remission. For our primary analysis, FCGR2A were dichotomized into R/R versus H/H or H/R and FCGR3A into F/F versus V/V or V/F. Additional analyses compared R/R or H/R versus H/H and estimated the additive effect of R. For the additive model, R/R was scored as 2 and H/R as 1; the hazard ratio can be interpreted as the change in risk of H/R compared with H/H, and of R/R compared with H/R. Proportional hazards Cox models were used to test if Fc polymorphism predicted PFS or OS. Fisher’s exact test was used to examine associations between Fc polymorphism and marrow response, defined by either histology or GD2 synthase transcript positivity. Analyses were conducted for the entire patient cohort, and separately for the more homogeneous subgroup of patients without prior relapse. There was no effect of sex or ethnic groups on the treatment outcome. For comparison, we used a retrospective cohort of 39 patients, all with stage 4 neuroblastoma diagnosed at older than 1 year of age and treated with dose-intensive chemotherapy,<sup>22</sup> but who did not receive GM-CSF. Twenty-three of 39 patients also received 3F8 immunotherapy as previously described.<sup>23</sup>

**RESULTS**

**Fc Polymorphism Among Patients With Neuroblastoma**

The genotype distribution of FCGR2A and FCGR3A among the 135 patients is detailed in Table 2. Samples from one patient were not assessable. The ethnic breakdown for the 135 patients was as follows: 78.5% white non-Hispanic, 7.4% black non-Hispanic, 5.2% Asian/Pacific Islander, 5.2% white-Hispanic, 3% South Asian, and 0.7% black-Hispanic. The allele frequency was 0.47 for FCGR2A-R131, 0.53 for FCGR2A-H131, 0.27 for FCGR3A-V158, and 0.73 for FCGR3A-F158. The genotype distributions for both FCGR2A and FCGR3A were as expected and they were in Hardy-Weinberg Equilibrium.<sup>24</sup>

**Association Between Fc Polymorphism and Survival**

The impact of FCGR polymorphism on survival was determined for the entire cohort and for the subgroup of patients with no prior relapse. Table 2 summarizes the median PFS for each genotype of FCGR2A and FCGR3A; patients with the FCGR2A-R/R genotype had the longest PFS of 47 months. In our primary analysis, FCGR2A (R/R)

was predictive of PFS ( $P = .049$  and  $P = .023$ , for the entire cohort and the subgroup, respectively; Table 3). There was no statistically significant association between FCGR3A and PFS ( $P = .7$  overall;  $P = .8$  for subgroup). Our exploratory analyses found no evidence that H/H predicted survival compared with combining H/R and R/R for FCGR2A, or V/V compared with V/F plus F/F for FCGR3A (Table 3). An additive model confirmed the strong association between FCGR2A and PFS, but not FCGR3A and PFS. We therefore restricted additional analysis to FCGR2A. To ensure that the proportional hazards assumption was met, a plot of predicted and actual duration of PFS showed good concordance (data not shown). PFS stratified by FCGR2A was analyzed by Kaplan-Meier analysis and results are shown in Figure 1 (entire cohort) and Figure 2 (subset of patients with no prior relapse). The association between FCGR2A and OS was weaker (hazard ratio, 0.67; 95% CI, 0.36 to 1.25;  $P = .2$ ) presumably because of the effects of salvage therapies after relapse after immunotherapy.

PFS probabilities at 5 years were 52% in the FCGR2A-131R/R group compared with 29% in the R/H or H/H group. The corresponding figures for patients with no prior relapse at protocol entry were 65% for R/R and 36% for R/H or H/H. There was no statistically significant association between FCGR2A genotype and the known predictors of response or outcome (ie, age, MYCN amplification [ $>10$  copies per cell], delay between diagnosis and immunotherapy, bony disease or marrow disease at diagnosis.) The hazard ratio for FCGR2A R/R, including these other predictors in a multivariable model, was not substantially altered: from 0.57 to 0.54 for the entire cohort and from 0.44 to 0.45 for the subgroup with no prior relapse.

We also tested the influence of FCGR2A and FCGR3A on PFS in a cohort of 39 patients similarly treated with intensive chemotherapy, where 23 of 39 also received 3F8 but without GM-CSF. There were 25 progression events, with a median follow-up for survivors of nearly 14 years. We found no statistically significant association between PFS and allelotypes. The hazard ratio for FCGR2A R/R compared with FCGR2A H/H and H/R was 1.62 (95% CI, 0.48 to 5.43). The lower bound of the 95% CI was only slightly lower than our central estimate (0.57; Table 3) if GM-CSF was added to the treatment. This suggests that the effects of FCGR2A on outcome are likely to be GM-CSF treatment-dependent.

**Fc Polymorphism and Marrow Remission**

The prognostic impact of Fc polymorphism on marrow remission by histology and molecular marker GD2 synthase was also analyzed. Post-treatment marrow histology data were available for 122 patients and GD2 synthase qRT-PCR data for 123 patients. As previously described,<sup>25</sup> patients were considered to be in molecular remission if the GD2 synthase transcript level of the post-treatment BM was  $\leq 5$  units. The relationships between FCGR2A and histologic plus molecular remission are shown in Table 4. FCGR2A-R/R genotype predicted histologic remission of post-treatment marrow for the entire cohort and for the subgroup of patients without prior relapse ( $P = .021$  and  $P = .036$ , respectively), and with molecular remission ( $P = .052$  and  $P = .033$ , respectively).

We last analyzed another subgroup of patients ( $N = 20$ ) with no prior relapse and whose marrows were in molecular remission by GD2 synthase. We have previously shown that although molecular remission defined a low-risk group, a subset of these patients still eventually relapsed.<sup>26</sup> FCGR2A was able to predict failures in this low-risk group:

**Table 2. FCGR Genotype Distribution**

Genotype	White Genotype Distribution	Black Genotype Distribution	Genotype Distribution		Median Progression-Free Survival (months)
	Expected (%) <sup>24</sup>	Expected (%) <sup>24</sup>	No.	%	
<b>FCGR2A–131</b>					
H/H	26	24	39/135	29	6.8
H/R	50	47	66/135	49	10.2
R/R	24	29	30/135	22	47.0
<b>FCGR3A–158</b>					
F/F	44	42	69/135	51	8.0
F/V	44	50	59/135	44	11.3
V/V	12	8	7/135	5	5.4

**Table 3.** Association Between *FCGR* Genotype and Progression-Free Survival

Genotype	All Patients (N = 135)					Patients With No Prior Relapse (n = 106)				
	Frequency		Hazard Ratio*	95% CI*	P	Frequency		Hazard Ratio*	95% CI*	P
No.	%	No.				%				
<i>FCGR2A</i> H/H or H/R	105	78	1	Reference		82	77	1	Reference	
<i>FCGR2A</i> R/R†	30	22	0.57	0.33 to 1.00	.049	24	23	0.44	0.22 to 0.89	.023
<i>FCGR2A</i> H/H	39	29	1	Reference		31	29	1	Reference	
<i>FCGR2A</i> R/R or H/R	96	71	0.73	0.47 to 1.14	.16	75	71	0.67	0.40 to 1.14	.14
Additive model			0.74	0.55 to 0.99	.040			0.66	0.47 to 0.94	.022
<i>FCGR3A</i> F/F	69	51	1	Reference		55	52	1	Reference	
<i>FCGR3A</i> F/V or V/V†	66	49	0.91	0.60 to 1.39	.7	51	48	0.94	0.57 to 1.55	.8
<i>FCGR3A</i> F/F or F/V	128	95	1	Reference		101	95	1	Reference	
<i>FCGR3A</i> V/V	7	5	1.63	0.71 to 3.74	.3	5	5	2.16	0.78 to 6.00	.14
Additive model			1.01	0.70 to 1.44	1			1.06	0.68 to 1.65	.8

\*"Reference" indicates the relationship between the row that contains the word and the row immediately below it.

†Principal analyses.

seven of 20 patients (35%) had molecular relapse within the R/R group versus 38 of 59 patients (64%) within the R/H or H/H group (risk difference, 29%; 95% CI, 5% to 54%;  $P = .035$  by Fisher's exact test).

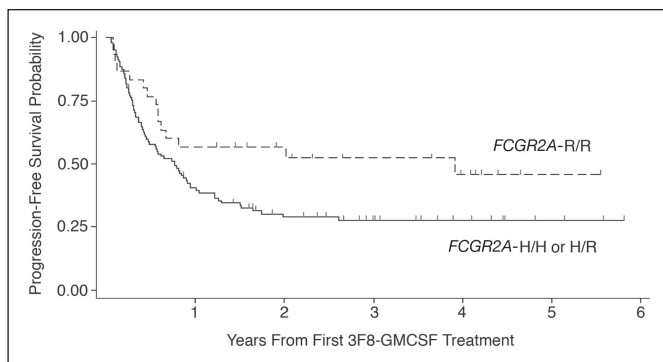
## DISCUSSION

Treatment success following immunotherapy with 3F8 plus GM-CSF is highly correlated with patients having the *FCGR2A*-R/R genotype. Since FcγR2A is expressed on human neutrophils, macrophages, and antigen-presenting cells, these findings are consistent with a critical role of phagocyte-mediated antitumor response. Whether the effect is direct tumor cytotoxicity,<sup>16,27</sup> or immune activation through enhanced antigen presentation, will require a more detailed analysis.

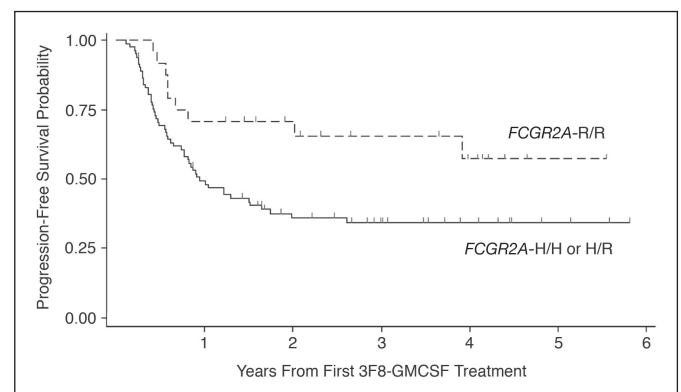
There are well known pitfalls encountered in molecular discovery,<sup>28,29</sup> which include reproducibility, adequate case delineations, false discovery, and adherence to the general guidelines for biomarker studies.<sup>30</sup> We chose the entire cohort of patients treated on the protocol MSKCC IRB 9418, in which 135 of 136 patients had samples assessable for polymorphism. No patient was excluded from analysis

for other reasons. In terms of reproducibility of the assay, the entire 135 samples were reanalyzed using the ABI Taqman SNP allelic discrimination assay. There was 100% agreement between restriction digest (used in this report) and the Taqman single-nucleotide polymorphism findings. Although there were only two principal analyses, the potential risk for false discovery still exists. Clearly, further validation in prospectively collected patient samples will be needed. A preliminary analysis of the first 45 patients in a follow-up study using 3F8 plus GM-CSF again showed that *FCGR2A*-R/R was highly correlated with PFS (data not shown).

The ability of phagocytes to mediate efficient ADCC against human tumors in vitro is well known.<sup>16,31-34</sup> However, defects in neutrophil ADCC have been found in cancer patients,<sup>35</sup> and reversal with cytokines, such as GM-CSF and G-CSF, is possible.<sup>16,36,37</sup> In vivo tumor models have also demonstrated the antitumor effects of neutrophil-mediated ADCC, especially when activated by cytokines.<sup>38,39</sup> In vitro studies with neuroblastoma indicated that neutrophil ADCC was particularly effective<sup>16,40,41</sup> in the presence of anti-GD2 MoAb 3F8. Similar observations were reported for another murine IgG3 anti-GD2 MoAb 7A4,<sup>42</sup> and the human-mouse chimeric



**Fig 1.** Kaplan-Meier plot of progression-free survival with respect to *FCGR2A* genotype among 135 high-risk neuroblastoma patients treated with an immunotherapy protocol using anti-GD2 antibody 3F8 plus granulocyte macrophage-colony-stimulating factor. Progression-free survival (PFS) of two patients were censored when they chose to go off protocol. PFS in the *FCGR2A*-131R/R group was superior to that in the R/H or H/H group ( $P = .049$ ).



**Fig 2.** Kaplan-Meier plot of progression-free survival with respect to *FCGR2A* genotype among patients with no history of prior relapse (N = 106). Progression-free survival in the *FCGR2A*-131R/R group was superior to that in the R/H or H/H group ( $P = .023$ ).

**Table 4.** Association Between *FCGR2A* and Histologic and Molecular Remission at First Post-Treatment Follow-Up Marrow

Patients	Histology Negative					GD2 Synthase Negative				
	Frequency		Risk Difference (%)	95% CI	P*	Frequency		Risk Difference (%)	95% CI	P*
	No.	%				No.	%			
All patients										
H/H and H/R	66/95	69	23	9 to 37	.021	63/105	60	20	3 to 37	.052
R/R	24/26	92				24/30	80			
Patients with no prior relapse										
H/H and H/R	60/73	82	18	9 to 27	.036	56/82	68	23	8 to 38	.033
R/R	21/21	100				22/24	92			

\*By Fisher's exact test.

anti-GD2 MoAb ch14.18.<sup>42-44</sup> The exact tumoricidal mechanism is still unclear, but probably involves granular proteases<sup>43</sup> and independent of oxidative intermediates.<sup>41</sup>

Despite the compelling evidence in preclinical models, the clinical role of phagocyte mediated ADCC in human cancer is controversial. Since FcγR2A is preferentially expressed on neutrophils/macrophages, and not on lymphocytes, dependence of clinical outcome on *FCGR2A* genotype provides indirect evidence of a pivotal role of phagocyte ADCC. A recent report described improved clinical outcome for patients with the *FCGR2A*-H/H genotype after rituxan (chimeric IgG1)<sup>9</sup>; although, an earlier study failed to find such an association.<sup>10</sup> Furthermore, this association was unexpected since the H/H genotype was not known to have preferential affinity for human IgG1.<sup>45</sup> The preferential binding of FcγR2A-R131 ectodomain to mouse IgG and the correlation with patient outcome strongly suggests that *FCGR2A* is mediating an antitumor effect in vivo. However, we should be cautious not to over interpret the published literature or our results since they are relatively small studies.

In addition to its expression on phagocytes and platelets, FcγR2A is also found on dendritic cells and langerhans cells.<sup>1,2</sup> Although neutrophil is a major myeloid population activated in patients receiving GM-CSF, and is a likely cell type through which FcγR2A polymorphism exerts its biologic effects, it remains possible that 3F8 helps

target tumors to macrophages or antigen-presenting cells. In neuroblastoma, BM metastasis is a common event. In addition to being the site of minimal residual disease after induction therapy, marrow is also an organ conducive to phagocyte ADCC since the trafficking of MoAb or effector cells is not a limiting factor. Here, FcγR2A may exert its effect by enhancing ADCC and/or adaptive immunity through positive antibody feedback.<sup>46,47</sup> An in depth analysis of induced tumor specific immunity will be needed to understand the underlying mechanisms.

In vitro binding studies of individual MoAb to recombinant FcγR may help define the importance of different polymorphic alleles in the antitumor mechanism of MoAb in vivo. An accurate prediction of outcome based on *FCGR* allelotype can help identify patients most likely to benefit from a specific form of MoAb. Historically, *FCGR2A* polymorphism is particularly relevant among patients with complement deficiency where phagocytosis is the only intact defense for clearing infections. Thus, one might expect *FCGR2A* genotype to be most critical if the antitumor mechanism is complement dependent. Identification of the clinically important FcγR for each MoAb will help focus various strategies on Fc to improve their affinities for specific FcγR. Since FcγR is only one of the many receptors important in ADCC, CMC, and APC function, a better understanding of gene polymorphism in antibody-based therapies may provide insight in making this modality even more effective.<sup>48</sup>

REFERENCES

- van Sorge NM, van der Pol WL, van de Winkel JG: FcγR polymorphisms: Implications for function, disease susceptibility and immunotherapy. *Tissue Antigens* 61:189-202, 2003
- Ravetch JV, Bolland S: IgG Fc receptors. *Annu Rev Immunol* 19:275-290, 2001
- Shields RL, Namenuk AK, Hong K, et al: High resolution mapping of the binding site on human IgG1 for Fc gamma RI, Fc gamma RII, Fc gamma RIII, and FcRn and design of IgG1 variants with improved binding to the Fc gamma R. *J Biol Chem* 276:6591-6604, 2001
- Vedeler CA, Raknes G, Myhr KM, et al: IgG Fc-receptor polymorphisms in Guillain-Barre syndrome. *Neurology* 55:705-707, 2000
- Karassa FB, Trikalinos TA, Ioannidis JP: The role of FcγRIIA and IIIA polymorphisms in autoimmune diseases. *Biomed Pharmacother* 58:286-291, 2004
- Emonts M, Hazelzet JA, de Groot R, et al: Host genetic determinants of Neisseria meningitidis infections. *Lancet Infect Dis* 3:565-577, 2003

- Ravetch JV, Lanier LL: Immune inhibitory receptors. *Science* 290:84-89, 2000
- Su K, Wu J, Edberg JC, et al: A promoter haplotype of the immunoreceptor tyrosine-based inhibitory motif-bearing FcγRIIIb alters receptor expression and associates with autoimmunity, I: Regulatory *FCGR2B* polymorphisms and their association with systemic lupus erythematosus. *J Immunol* 172:7186-7191, 2004
- Weng WK, Levy R: Two immunoglobulin G fragment C receptor polymorphisms independently predict response to rituximab in patients with follicular lymphoma. *J Clin Oncol* 21:3940-7394, 2003
- Cartron G, Dacheux L, Salles G, et al: Therapeutic activity of humanized anti-CD20 monoclonal antibody and polymorphism in IgG Fc receptor FcγRIIIa gene. *Blood* 99:754-758, 2002
- Tax WJ, Willems HW, Reekers PP, et al: Polymorphism in mitogenic effect of IgG1 monoclonal antibodies against T3 antigen on human T cells. *Nature* 304:445-447, 1983
- Tax WJ, Tamboer WP, Jacobs CW, et al: Role of polymorphic Fc receptor Fc gammaRIIIa in cytokine release and adverse effects of murine IgG1

anti-CD3/T cell receptor antibody (WT31). *Transplantation* 63:106-112, 1997

- Wee SL, Colvin RB, Phelan JM, et al: Fc-receptor for mouse IgG1 (Fc gamma RIII) and antibody-mediated cell clearance in patients treated with Leu2a antibody. *Transplantation* 48:1012-1017, 1989
- Denomme GA, Warkentin TE, Horsewood P, et al: Activation of platelets by sera containing IgG1 heparin-dependent antibodies: An explanation for the predominance of the Fc gammaRIIIa "low responder" (his131) gene in patients with heparin-induced thrombocytopenia. *J Lab Clin Med* 130:278-284, 1997
- Gratama JW, van der Linden R, van der Holt B, et al: Analysis of factors contributing to the formation of mononuclear cell aggregates ("escapes") in flow cytometric immunophenotyping. *Cytometry* 29:250-260, 1997
- Kushner BH, Cheung NK: GM-CSF enhances 3F8 monoclonal antibody-dependent cellular cytotoxicity against human melanoma and neuroblastoma. *Blood* 73:1936-1941, 1989

17. Kushner BH, Kramer K, Cheung NKV: Phase II trial of the anti-GD2 monoclonal antibody 3F8 and granulocyte-macrophage colony-stimulating factor for neuroblastoma. *J Clin Oncol* 19:4189-4194, 2001
18. Brodeur G, Pritchard J, Berthold F, et al: Revisions of the international criteria for neuroblastoma diagnosis, staging and response to treatment. *J Clin Oncol* 11:1466-1477, 1993
19. Cheung IY, Cheung NK: Quantitation of marrow disease in neuroblastoma by real-time reverse transcription-PCR. *Clin Cancer Res* 7:1698-1705, 2001
20. Koene HR, Kleijer M, Algra J, et al: Fc gammaRIIIa-158V/F polymorphism influences the binding of IgG by natural killer cell Fc gammaRIIIa, independently of the Fc gammaRIIIa-48L/R/H phenotype. *Blood* 90:1109-1114, 1997
21. Jiang XM, Arepally G, Poncz M, et al: Rapid detection of the Fc gammaRIIIa-H/R 131 ligand-binding polymorphism using an allele-specific restriction enzyme digestion (ASRED). *J Immunol Methods* 199:55-59, 1996
22. Kushner BH, Kramer K, LaQuaglia MP, et al: Reduction from seven to five cycles of intensive induction chemotherapy in children with high-risk neuroblastoma. *J Clin Oncol* 22:4888-4892, 2004
23. Cheung NK, Kushner BH, Cheung IY, et al: Anti-GD2 antibody treatment of minimal residual stage 4 neuroblastoma diagnosed at more than 1 year of age. *J Clin Oncol* 16:3053-3060, 1998
24. Lehrnbecher T, Foster CB, Zhu S, et al: Variant genotypes of the low-affinity Fc gamma receptors in two control populations and a review of low-affinity Fc gamma receptor polymorphisms in control and disease populations. *Blood* 94:4220-4232, 1999
25. Cheung IY, Lo Piccolo MS, Kushner BH, et al: Quantitation of GD2 synthase mRNA by real-time reverse transcriptase polymerase chain reaction: Clinical utility in evaluating adjuvant therapy in neuroblastoma. *J Clin Oncol* 21:1087-1093, 2003
26. Cheung IY, Lo Piccolo MS, Kushner BH, et al: Early molecular response of marrow disease to biologic therapy is highly prognostic in neuroblastoma. *J Clin Oncol* 21:3853-3858, 2003
27. Munn DH, Cheung NK: Antibody-dependent antitumor cytotoxicity by human monocytes cultured with recombinant macrophage colony-stimulating factor: Induction of efficient antibody-mediated antitumor cytotoxicity not detected by isotope release assays. *J Exp Med* 170:511-526, 1989
28. Ntzani EE, Ioannidis JP: Predictive ability of DNA microarrays for cancer outcomes and correlates: An empirical assessment. *Lancet* 362:1439-1444, 2003
29. Ioannidis JP: Microarrays and molecular research: Noise discovery? *Lancet* 365:454-455, 2005
30. Bossuyt PM, Reitsma JB, Bruns DE, et al: Towards complete and accurate reporting of studies of diagnostic accuracy: The STARD Initiative. *Ann Intern Med* 138:40-44, 2003
31. Gale RP, Zigelboim J: Polymorphonuclear leukocytes in antibody-dependent cellular cytotoxicity. *J Immunol* 114:1047-1051, 1975
32. Dallegri F, Patrone F, Frumento G, et al: Antibody-dependent killing of tumor cells by polymorphonuclear leukocytes: Involvement of oxidative and nonoxidative mechanisms. *J Natl Cancer Inst* 73:331-339, 1984
33. Barker E, Mueller BM, Handgretinger R, et al: Effect of a chimeric anti-ganglioside GD2 antibody on cell-mediated lysis of human neuroblastoma cells. *Cancer Res* 51:144-149, 1991
34. Ottonello L, Morone P, Dapino P, et al: Monoclonal Lym-1 antibody-dependent lysis of B-lymphoblastoid tumor targets by human complement and cytokine-exposed mononuclear and neutrophilic polymorphonuclear leukocytes. *Blood* 87:5171-5178, 1996
35. Dallegri F, Ballestrero A, Ottonello L, et al: Defective antibody-dependent tumour cell lysis by neutrophils from cancer patients. *Clin Exp Immunol* 77:58-61, 1989
36. van der Kolk LE, de Haas M, Grillo-Lopez AJ, et al: Analysis of CD20-dependent cellular cytotoxicity by G-CSF-stimulated neutrophils. *Leukemia* 16:693-699, 2002
37. Ottonello L, Epstein AL, Mancini M, et al: Chimaeric Lym-1 monoclonal antibody-mediated cytotoxicity by neutrophils from G-CSF-treated patients: Stimulation by GM-CSF and role of Fc gamma receptors. *Br J Cancer* 85:463-469, 2001
38. Stockmeyer B, Valerius T, Repp R, et al: Preclinical studies with Fc(gamma)R bispecific antibodies and granulocyte colony-stimulating factor-primed neutrophils as effector cells against HER-2/neu overexpressing breast cancer. *Cancer Res* 57:696-701, 1997
39. Hernandez-Ilizaliturri FJ, Jupudy V, Ostberg J, et al: Neutrophils contribute to the biological antitumor activity of rituximab in a non-Hodgkin's lymphoma severe combined immunodeficiency mouse model. *Clin Cancer Res* 9:5866-5873, 2003
40. Kushner BH, Cheung NK: Absolute requirement of CD11/CD18 adhesion molecules, FcRII and the phosphatidylinositol-linked FcRIII for monoclonal antibody-mediated neutrophil antihuman tumor cytotoxicity. *Blood* 79:1484-1490, 1992
41. Kushner BH, Cheung NK: Clinically effective monoclonal antibody 3F8 mediates nonoxidative lysis of human neuroectodermal tumor cells by polymorphonuclear leukocytes. *Cancer Res* 51:4865-4870, 1991
42. Michon J, Moutel S, Barbet J, et al: In vitro killing of neuroblastoma cells by neutrophils derived from granulocyte colony-stimulating factor-treated cancer patients using an anti-disialoganglioside/anti-Fc gamma RI bispecific antibody. *Blood* 86:1124-1130, 1995
43. Barker E, Reisfeld RA: A mechanism for neutrophil-mediated lysis of human neuroblastoma cells. *Cancer Res* 53:362-367, 1993
44. Metelitsa LS, Gillies SD, Super M, et al: Antidialoganglioside/granulocyte macrophage-colony-stimulating factor fusion protein facilitates neutrophil antibody-dependent cellular cytotoxicity and depends on Fc gamma RII (CD32) and Mac-1 (CD11b/CD18) for enhanced effector cell adhesion and azurophil granule exocytosis. *Blood* 99:4166-4173, 2002
45. Parren PW, Warmerdam PA, Boeijs LC, et al: On the interaction of IgG subclasses with the low affinity Fc gamma RIa (CD32) on human monocytes, neutrophils, and platelets: Analysis of a functional polymorphism to human IgG2. *J Clin Invest* 90:1537-1546, 1992
46. Diaz de Stahl T, Dahlstrom J, Carroll MC, et al: A role for complement in feedback enhancement of antibody responses by IgG3. *J Exp Med* 197:1183-1190, 2003
47. Rossi M, Young JW: Human dendritic cells: Potent antigen-presenting cells at the crossroads of innate and adaptive immunity. *J Immunol* 175:1373-1381, 2005
48. Erichsen HC, Chanock SJ: SNPs in cancer research and treatment. *Br J Cancer* 90:747-751, 2004

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### Authors' Disclosures of Potential Conflicts of Interest

The authors indicated no potential conflicts of interest.

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## ERRATA

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The June 1, 2006, correspondence by Gruenberger et al, entitled “Neoadjuvant Therapy With Bevacizumab” (J Clin Oncol 24:2592-2593, 2006) contained an error.

In the References section, reference 11 was given as:

Gruenberger T, Schuell B, Puhalla H, et al: Changes in liver surgery for colorectal cancer liver metastases under neoadjuvant treatment strategies. Acta Med Austriaca 36: 317-321, 2004

While it should have read:

Gruenberger T, Schuell B, Puhalla H, et al: Changes in liver surgery for colorectal cancer liver metastases under neoadjuvant treatment strategies. Eur Surg 36:317-321, 2004

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The June 20, 2006, article by Cheung et al entitled, “*FCGR2A* Polymorphism Is Correlated With Clinical Outcome After Immunotherapy of Neuroblastoma With Anti-GD2 Antibody and Granulocyte Macrophage Colony-Stimulating Factor” (J Clin Oncol 24:2885-2890, 2006) contained an error.

In the Introduction, the third sentence of the second paragraph was given as, “On the other hand, FcγR2A-V158 (valine) has higher affinity than FcγR3A-F158 (phenylalanine) for human IgG1.” While it should have read, “On the other hand, **FcγR3A**-V158 (valine) has higher affinity than FcγR3A-F158 (phenylalanine) for human IgG1.”

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