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Genotypes of NK cell KIR Receptors, Their Ligands, and Fcγ Receptors in the Response of Neuroblastoma Patients to Hu14.18-IL2 Immunotherapy:

A Children's Oncology Group Report#

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Abstract

Response to immunocytokine (IC) therapy is dependent on natural killer (NK) cells in murine neuroblastoma (NBL) models. Furthermore, killer immunoglobulin-like receptor (KIR) / KIR-ligand mismatch is associated with improved outcome to autologous stem cell transplant for NBL. Additionally, clinical anti-tumor response to monoclonal antibodies has been associated with specific polymorphic-FcγR alleles. Relapsed/refractory NBL patients received the hu14.18-IL2 IC (humanized anti-GD2 monoclonal antibody linked to human IL2) in a Children's Oncology Group (COG) Phase II trial. In this report, these patients were genotyped for KIR, HLA and FcR alleles to determine if KIR receptor-ligand mismatch or specific FcγR alleles were associated with anti-tumor response. Thirty-eight of 39 patients enrolled had DNA available for analysis; 24 were found to have autologous KIR/KIR-ligand mismatch; 14 were matched. Seven of 24 mismatched patients experienced either complete response or improvement of their disease after IC therapy.

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There was no response or comparable improvement of disease in patients who were matched. Thus KIR/KIR-ligand mismatch was associated with response/improvement to IC ($p = 0.03$). There was a trend towards patients with the Fc γ R2A 131-H/H genotype showing a higher response rate compared to other Fc γ R2A genotypes ($p = 0.06$). These analyses indicate that response or improvement of relapsed/refractory neuroblastoma patients after IC treatment is associated with autologous KIR/KIR-ligand mismatch, consistent with a role for NK cells in this clinical response.

Keywords

Natural killer cells; Killer immunoglobulin-like receptor; Fragment c gamma-receptor; Neuroblastoma; Immunocytokine

Introduction

The therapeutic role of natural killer (NK) cells in allogeneic hematopoietic stem cell transplant (HSCT) has been well described. Ruggeri et al. first reported on the phenomenon of killer immunoglobulin-like receptor (KIR)/KIR-ligand incompatibility and response to HLA-haploidentical HSCT, primarily in adult patients with acute myeloid leukemia (AML) (1). According to this analysis, a difference in HLA between the donor and recipient such that the donor KIR receptors lack their cognate ligand in the recipient results in improved leukemia control. Leung et al. defined the principle of missing KIR ligand (designated here as KIR/KIR-ligand mismatch), in which the HSCT recipient lacks one or greater HLA class-I ligands for the HSCT donor's inhibitory KIRs(2). The KIR/KIR-ligand mismatch principle posits that a difference in HLA between the donor and recipient is not necessary for the benefit of KIR-HLA mismatching. They found that response of pediatric patients with AML and acute lymphoid leukemia (ALL) to haploidentical HSCT could be predicted by the presence of this KIR/KIR-ligand mismatch. An analysis of results of HLA-identical T cell depleted sibling HSCT also revealed a benefit of KIR/KIR-ligand mismatch(3). KIR/KIR-ligand mismatch also extends to the autologous transplant setting. Since the genes encoding for KIR and HLA class I KIR ligands are inherited independently, it is possible for an individual to be KIR/KIR- ligand mismatched with himself. This scenario has been implicated as a favorable prognostic factor in pediatric solid tumor patients following autologous HSCT (ASCT) (4- 5). To date, there have been no reports relating KIR/KIR-ligand mismatch and response of cancer patients to immunotherapy or other treatments outside of the HSCT or allogeneic NK cell infusion settings(6).

Several groups have investigated the role of Fragment c gamma receptor (Fc γ R) polymorphisms in the response of cancer patients to rituximab, the chimeric anti-CD20 immunoglobulin G1 (IgG1) monoclonal antibody. Some have found an association between Fc γ R genotypes for higher affinity Fc γ R alleles (Fc γ R2A-131 H/H, and Fc γ R3A-158 V/V genotypes) and response to rituximab-based therapy(7-9). Association between Fc γ R genotype and response, for both Fc γ R2A (found largely on neutrophils and monocytes/macrophages) and Fc γ R3A (found predominantly on NK cells) genotypes, has now been observed in cancer patients receiving other monoclonal antibodies (mAbs) (i.e.: cetuximab and trastuzumab) as well (10- 11). Cheung et al. reported a correlation between Fc γ R2A polymorphism and response of neuroblastoma (NBL) patients to the anti-GD2 murine IgG3 mAb, 3F8, plus granulocyte macrophage colony-stimulating factor (GM-CSF) (12). In that study, progression-free survival (PFS) was associated with the Fc γ R2A 131-R/R alleles with the highest affinity for the 3F8 murine mAb.

GD2 is a disialoganglioside that is expressed on human melanoma and NBL cells. Our recent Children's Oncology Group (COG) phase III study has shown a significant

improvement in overall survival (OS) and event-free survival (EFS) for children receiving an immunotherapy regimen of a chimeric anti-GD2 mAb (ch14.18-IL2) in combination with interleukin 2 (IL2) and granulocyte-macrophage colony stimulating factor (GM-CSF)(13). The hu14.18-IL2 immunocytokine (IC) is a fusion protein linking a molecule of interleukin 2 (IL2) to the carboxyl terminus of each of the IgG1 heavy chains of the humanized anti-GD2 monoclonal antibody hu14.18. Tumor response to this IC in mice is superior to the antitumor effects of comparable amounts of anti-GD2 mAb infused together with comparable amounts of IL2 (14). Antitumor effects in mice are primarily dependent on NK cells in a murine model of NBL (15), with better responses seen in the face of less established disease (16). Furthermore, augmented major histocompatibility complex (MHC) class I expression on murine NBL was associated with escape from IC-mediated immunotherapy, implying a role for MHC-induced inhibition of NK cell function(17). Our recent phase II COG trial has demonstrated antitumor activity for hu14.18-IL2 in children with relapsed or refractory neuroblastoma(18).

In this study we evaluated the role of genotypes for KIR, KIR ligands (HLA class I), and FcγR polymorphisms, in the response of relapsed/refractory neuroblastoma patients to hu14.18-IL2 from our phase II trial(18).

Materials & Methods

Patients

Patients included in this analysis were eligible for and enrolled on a COG Phase II trial of hu14.18-IL2 (ANBL0322); the clinical findings from that study have been reported separately(18). In brief, children (1–21 years) with refractory or recurrent NBL who met all study criteria were enrolled in 1 of 2 strata. Stratum 1 included 15 patients with measurable disease (using standard radiographic criteria). Stratum 2 included 24 patients with disease that was not measurable by standard imaging, but was evaluable by ¹²³I-metaiodobenzylguanidine (MIBG) scan and/or bone marrow histology. DNA samples were available for 38 of the 39 enrolled patients. Informed consent was obtained from IRB approved clinical protocols for all patients and/or their families.

Hu14.18-IL2

The clinical grade hu14.18-IL2 IC (EMD 273063) was generously provided by Merck Serono (Darmstadt, Germany) via Dr. Toby Hecht and the NCI-Biological Resources Branch (Frederick, MD).

KIR/KIR-ligand genotyping

KIR genotyping was performed on patient DNA samples by PCR sequence-specific primer technique (SSP Unitray assay, Invitrogen Corporation, Carlsbad, CA). KIR-ligand typing was performed at low-resolution on the same samples for HLA-B & -C loci by reverse PCR-SSO methodology (LifeMatch assay, Gen-Probe, Inc, Stamford, CT) and for high-resolution HLA-C alleles by direct sequencing (AlleleSEQR assay, Abbott Labs, Des Plaines, IL).

FcγR genotyping

Genotyping of FcγR2A 131-H/R (rs1801274) and FcγR3A 158-F/V (rs396991) polymorphisms were detected using pyrosequencing and TaqMan (19). Forward and reverse primers are in Table I. PCR was performed in 25 μl reactions using 2X PCR Master Mix (Promega, Madison, WI) with 5 pmole forward, 0.5 pmole reverse, and 4.5 pmole of universal biotin primer. The biotinylated PCR products were isolated with Streptavidin-Sepharose HP (GE Healthcare, Piscataway, NJ) and Sepharose beads. Allele quantification was performed using a Pyrogold reagent kit (dATP, dCTP, dGTP, dTTP, enzyme and

substrate mixtures) on the PSQ HS96A system (Biotage AB, Uppsala, Sweden).. Negative controls were included on each plate. Plates were read on a PSQ HS pyrosequencer using PSQ HS 96A SNP analysis software version 2.1 in AQ mode.

To account for variation in the accuracy of FcγR3A 158-V/F allele frequency determination, standard curves were generated by using synthesized biotin-labeled DNA oligonucleotides. Two oligonucleotides with either a “T” or “G” in the mutation position (representing F and V alleles, respectively) were combined in ratios (10:0, 9:1, ...1:9, 0:10) and underwent pyrosequencing. The expected and observed allele frequencies were plotted. Backwards fitting was used to find the most parsimonious fit.

Genotyping of FcγR3A 158-V/F mutation polymorphisms was confirmed using TaqMan^R SNP genotyping assay (C_25815666-10, Chr-1, DIS484) in accordance with the manufacturer’s instructions on a 7300 Real-Time PCR system (Applied Biosystems, Foster City, CA). This TaqMan Assay uses primers and probes that are pre-designed and validated by the company. The probes are conjugated with VIC-MGB or FAM-MGB dyes, one to each allele. The amount of volume required for each 25 μl reaction was TaqMan Genotyping PCR Master Mix 12.5 μl and TaqMan SNP Genotyping Assay 1 μl. The reaction conditions were; 95°C for 10 minutes, and 50 cycles of 92°C for 15 seconds and 60°C for 1 minute., Plates were read by the 7300 Real-Time PCR system according to manufacturer protocols.

Statistical methods

Fisher’s exact test was used to examine the association between two categorical variables (i.e., KIR mismatch vs. response/improvement, genotype vs. response/improvement, and KIR mismatch vs. stratum). Chi-square test of homogeneity was used to determine whether there was a difference in distributions of KIR mismatch/match and FcγR genotypes between our study population and others(5· 12). For the analysis of the association between genotype and response/improvement, FcγR2A were dichotomized into H/H vs. H/R or R/R, and FcγR3A into V/V vs. V/F or F/F. Statistical significance was defined as a two-tailed p-value < 0.05, and a p-value from 0.05 to 0.1 was regarded as marginally statistically significant. Due to the exploratory nature of this study, no multiplicity adjustment was made for significance tests.

Results

KIR/KIR-ligand genotyping

Table II lists the 4 inhibitory KIR genes (KIR2DL1, KIR2DL2, KIR2DL3, KIR3DL1) evaluated in this study and their corresponding KIR-ligands (HLA-C1, HLA-C2, or HLA-Bw4). KIR/KIR-ligand mismatch was defined as absence of one or more HLA alleles known to be ligands for the inhibitory KIR genes present, using previously published criteria (2). The specific KIR and KIR-ligand genotypes for all 38 patients (with DNA available) are shown in Table III. Two patients showed evidence for only 2 of the 3 inhibitory KIR gene specificities evaluated in this study; the other 36 patients had all 3 inhibitory KIR gene specificities present. KIR/KIR-ligand mismatch was observed in 24/38 patients while 14/38 patients were matched (Table IV). This ratio of matched to mismatched genotypes, when using this set of KIR genes and KIR ligand genes, is not significantly different ($p = 0.93$) from that reported for the set of NBL patients undergoing ASCT reported by Venstrom et al (5).

FcγR genotyping

FcγR genotyping data for all patients tested are also included in Table III, and is summarized in Table IV. The genotype distributions for both FcγR2A and FcγR3A were in

Hardy-Weinberg Equilibrium (HWE). The FcγR2A 131-H/R genotype was more prevalent (16/38 patients) than the H/H genotype (10/38) and R/R genotype (12/38) (p of HWE=0.34). Two of 38 patients (patients 8 and 35) were excluded from the analysis of FcγR3A genotyping (and relevant analyses of associations) due to discrepant results of pyrosequencing and TaqMan; both patients were F/V by pyrosequencing and F/F by TaqMan. If patients 8 and 35 are included in the analyses as either F/F or F/V the relevant statistical analyses, as do their conclusions, remain virtually unchanged. The FcγR3A 158-F/F and F/V genotypes were equally prevalent (both 17/36 patients) in this patient population while the V/V genotype was present in only 2 patients (p of HWE=0.39). These distributions of the FcγR2A ($p = 0.5$) and FcγR3A ($p = 0.92$) genotypes were not significantly different from those reported for the population of NB patients reported by Cheung et al. (12).

Clinical response to hu14.18-IL2

The clinical details for this Phase II trial have been reported separately(18). Individual response and stratum data are included in Table III. For the purposes of the associations examined in this report, 5 patients (2, 10, 22, 27 and 29) showed a complete response (CR). These CRs lasted 13, 9, 20, 30 and >35 months, respectively. Two additional patients (3 and 22) were scored as showing stable disease, but showed clear clinical signs of improvement (clearing of all marrow disease and MIBG improvement not quite meeting partial response criteria for patient 3, and resolution of all MIBG detectable disease with near clearing of marrow disease for patient 22). All 5 patients with CR and both with “improved” status were in Stratum 2.

Associations between clinical response and genotype data

KIR—The analyses of KIR/KIR ligand mismatch and FcγR genotyping data for clinical associations are summarized in Tables V and VI. All 7 patients who responded (5/38) or showed improvement (2/38) following IC therapy were mismatched for their KIR/KIR-ligand genotypes (i.e. lacked HLA ligand for at least one of their KIR genes, Table V-A). In contrast, none of the 14 KIR/KIR-ligand matched patients demonstrated any response or improvement. Thus, KIR/KIR ligand mismatch was associated with clinical response/improvement following IC treatment ($p = 0.03$). When this same analysis was done for the 24 Stratum 2 patients, there was a trend (that did not meet criteria for statistical significance) towards a similar association of mismatch and response/improvement (7 of 7) vs. non-improvement (11 of 17) ($p = 0.13$, Table V-B). In addition, there appeared to be a greater fraction of KIR/KIR-ligand mismatched patients enrolled in Stratum 2 (18 of 24, 75%) than in Stratum 1 (6 of 14, 43%) with marginal statistical significance ($p = 0.08$, Table V-C).

FcγR—The FcγR2A genotype data analysis for association with response suggests, with marginal statistical significance, that patients with the H/H genotype showed a higher likelihood of response/improvement (Table VI-A, $p = 0.06$). In contrast, there was no suggestion of association between FcγR3A genotype and response/improvement to IC ($p = 1.0$, Table VI-B).

Discussion

NK-mediated lysis of neuroblastoma cell lines *in vitro* is augmented by the addition of IL2 (20), especially when using NK cells from patients receiving IL2 (21). Furthermore, NK-mediated antibody dependent cell-mediated cytotoxicity (ADCC) is augmented when the NK cells are obtained following *in vivo* administration of IL2 (22). Initial studies with chimeric anti-GD2 antibody, ch14.18, fused with human IL-2 (ch14.18-IL2), demonstrated NK-mediated regression of local and disseminated murine neuroblastoma (15). Similar results were later seen in murine neuroblastoma models with administration of hu14.18-

IL2(16). As escape from this NK-mediated response to hu14.18-IL2 was associated with up-regulation of MHC-class I on NBL cells (known to induce inhibitory responses via Ly-49 receptors on murine NK cells) (17), we hypothesized that similar relationships may influence the clinical response to hu14.18-IL2. In order to test this hypothesis, it was first necessary to identify a population that shows some clinical response to hu14.18-IL2.

Our recently reported Phase II study of hu14.18-IL2 in patients with relapsed or refractory NBL demonstrated CR or improved disease in 7 of 38 treated patients(18). All 7 of these responding/improved patients were in Stratum 2 (evaluable but not radiographically measurable disease), consistent with our preclinical data showing greater detection of anti-tumor activity in animals with less tumor burden (16). Although the role of KIRs has been evaluated in the setting of autologous and allogeneic stem cell transplantation and infusions of allogeneic NK cells following lymphodepletive chemotherapy (1⁶), it has not been studied for association with antitumor response in patients receiving only cytokines or monoclonal antibodies for immunotherapy. We hypothesized that children with recurrent/refractory NBL who received the hu14.18-IL2 in our Phase II COG study would demonstrate greater response to IC in the presence of KIR/KIR -ligand mismatch. When the data were analyzed for all 38 patients that provided DNA samples, 24/38 patients were found to be KIR/KIR -ligand mismatched, and all 7 of the responding/improved patients were found in this group ($p = 0.03$, Table V-A). Since the KIR/KIR-ligand interaction is primarily a mechanism controlling NK cell activity, this result is consistent with the murine data showing that the anti-neuroblastoma effect of ch14.18-IL2 and hu14.18-IL2 is primarily mediated by NK cells (15¹⁷). Even prior to the administration of hu14.18-IL2, there is a trend towards greater KIR/KIR-ligand mismatch in those patients that enter Stratum 2 than Stratum 1 ($p = 0.08$, Table V-C). If additional data validate this trend, it would suggest that a child's endogenous KIR/KIR-ligand status may play a role in the clinical pattern of relapse; namely, those patients that are KIR-mismatched may be less likely to relapse with "bulky" (measurable/Stratum 1) disease. Similarly, if additional data validate the trend ($p = 0.13$) that KIR/KIR-ligand mismatch is associated with response within stratum-2 patients (Table V-B), the presence of stratum-2 status and KIR/KIR-ligand mismatch might be considered as eligibility criteria for future treatment with hu14.18-IL2 for children with relapsed or refractory neuroblastoma.

The roles of the activating Fc receptors involved in ADCC, Fc γ R2A and Fc γ R3A, have been demonstrated in response to rituximab, cetuximab and trastuzumab (7¹¹). Cheung et al have found an association between Fc γ R2A polymorphism and outcome of neuroblastoma patients receiving the murine anti-GD2 IgG3 antibody, 3F8, but only when given in combination with GM-CSF. This result suggests that when neutrophils or monocytes/macrophages are activated with GM-CSF, they facilitate clinically meaningful ADCC via the high affinity alleles of Fc γ R2A for 3F8(12). This response was unlikely to be NK-mediated, as Fc γ R2A is not present on NK cells. As preclinical data and our current results (Table V-A) suggest that NK cells are playing a role in the clinical response to hu14.18-IL2 in NB patients, we initially hypothesized that the response/improvement of neuroblastoma patients to the IgG1-containing IC might be associated with the presence of the high-affinity Fc γ R3A 158-V/V genotype (influencing NK function), and not the high-affinity Fc γ R2A 131-H/H genotype reflecting neutrophil and macrophage ADCC. However, we found that patients with the H/H genotype for Fc γ R2A did show a trend toward higher response rate, which was of marginal statistical significance ($p = 0.06$). Clearly these comparisons are underpowered. Even so, the near significant association of improvement with the Fc γ R2A 131-H/H genotype would be consistent with the activation of some neutrophil or macrophage-mediated ADCC. IL2 treatment is known to induce release of GM-CSF by IL2 responsive cells (23), and it is possible that activation of ADCC by these Fc γ R2A-bearing cells is resulting from GM-CSF production stimulated by the IL2 component of the IC.

Given the infrequency of the Fc γ R3A 158-V/V genotype in the general population, with only 2 of our 36 genotyped patients identified with this genotype, it is no surprise that a statistically significant association was not seen between this genotype and response. However, this lack of statistical significance cannot be equated with the lack of proof in this study due to inadequate sample size with a highly unbalanced design. Even so, it may be surprising that none of the 7 patients that showed response or improvement had this V/V genotype. This very preliminary result would suggest that there may not be an association between the V/V genotype and improvement in this population when treated with hu14.18-IL2. The lack of an association with Fc γ R3A alleles and response (even if confirmed in a larger sample) does not necessarily indicate that NK cells are uninvolved in mediating ADCC. Rather, it could suggest that there is no advantage for high vs. lower affinity Fc γ R3A alleles in this setting of IC-mediated ADCC. We have recently shown that some NK cells can use their IL2 receptors to recognize the membrane-bound IL2 on tumor cells coated with tumor-reactive IC (Gubbels et al submitted). This results in NK adhesion to the IC-coated tumor cells, activation of an immune synapse and subsequent tumor cell destruction, apparently without requiring Fc receptors (Buhtoiarov et al, submitted). In the face of this IL2 receptor-facilitated IC-mediated killing, high affinity Fc γ R3A on the NK cells might not be needed. In other words, while high affinity Fc γ R3A play an important role in the clinical effects of FcR-mediated ADCC using conventional mAbs, they might not be as important for NK mediated antitumor effects of ICs. This might enable NK cells with the F/V or F/F Fc γ R3A alleles to mediate comparable *in vivo* destruction to that mediated by cells with V/V alleles. Clearly, these are speculative hypotheses as the results were obtained from a limited number of subjects and must be extended to a larger population to draw firmer conclusions.

The COG plans to perform a follow-up study of hu14.18-IL2 in Stratum 2 patients, to further characterize these responses. That follow-up study should provide additional data to better test the associations analyzed in this report. Furthermore, the KIR/KIR-ligand mismatch might also be further tested by genotyping of subjects participating in larger trials of clinically effective mAbs that mediate ADCC, such as ch14.18, rituximab, cetuximab and trastuzumab(7-11).

In summary, we conclude that response or improvement of relapsed or refractory neuroblastoma patients following treatment with hu14.18-IL2 is associated with KIR receptor-ligand mismatch, consistent with a role for NK cells in this clinical response. While there appears to be no statistical significance of Fc γ R genotype in response of neuroblastoma patients to IC in this COG trial, further clinical and *in vitro* data are needed to better clarify the potential roles of Fc γ R2A and Fc γ R3A in these clinical responses.

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Table I

Forward and reverse primer sequences for FcγR2A 131-H/R & FcγR3A 158-V/F polymorphism genotyping

Primers	Sequences
FcγR2A 131-H/R: Forward	TTT TGC TGC TAT GGG CTT TC
FcγR2A 131-H/R: Reverse	/5Biosg/CCA GAA TGG AAA ATC CCA GAA A
FcγR2A 131-H/R: Sequencing	AAG GTG GGA TCC AAA
FcγR3A 158-V/F: Forward	/5Biosg/CAC TCA AAG ACA GCG GCT CCT
FcγR3A 158-V/F: Reverse	ATT CCA GGG TGG CAC ATG TC
FcγR3A 158-V/F: Sequencing	AGT CTC TGA AGA CAC ATT TTT

Table II

KIR receptors and their MHC class I ligands that were determined by genotyping

Receptor	Ligand
KIR2DL1 (CD158a)	HLA-C2 (Cw2, Cw4, Cw5, Cw6, Cw15, Cw1602, Cw17, Cw18)
KIR2DL2/KIR2DL3 (CD158b)	HLA-C1 (Cw1, Cw3, Cw7, Cw8, Cw12, Cw13, Cw14, Cw1601)
KIR3DL1 (CD158e)	HLA-Bw4

Table III

KIR, KIR ligand, FcγR and response data for each patient

Pt#	Stratum	KIR Genotype	KIR Ligand	Match vs. Mismatch	Fc R2 A-131	Fc R3A -158	Response
1	1	2DL1, 2DL2/2DL3, 3DL1	Cw1, Cw5, Bw4	M	R/R	F/V	PD
2	2	2DL1, 2DL2/2DL3, 3DL1	Cw1, Cw3, Bw4	MM	R/R	F/V	CR
3	2	2DL1, 2DL3, 3DL1	Cw4, Cw15	MM	H/H	F/V	I
4	2	2DL1, 2DL2/2DL3, 3DL1	Cw2, Cw8, Bw4	M	R/R	F/V	SD
5	2	2DL1, 2DL2/2DL3, 3DL1	Cw7, Cw16, Bw4	MM	R/R	F/V	PD
6	2	2DL1, 2DL3, 3DL1	Cw6, Cw7	MM	H/H	F/V	PD
7	2	2DL1, 2DL2/2DL3, 3DL1	Cw7	MM	H/H	F/V	PD
8	2	2DL1, 2DL3, 3DL1	Cw12, Bw4	MM	R/R	**	PD
9	2	2DL1, 2DL2/2DL3, 3DL1	Cw6, Cw7, Bw4	M	R/R	F/V	PD
10	2	2DL1, 2DL3, 3DL1	Cw4, Cw7	MM	H/H	F/F	CR
11	1	2DL1, 2DL3, 3DL1	Cw5, Bw4	MM	R/R	F/F	PD
12*							
13	1	2DL1, 2DL3, 3DL1	Cw2, Cw7, Bw4	M	H/R	F/V	NE
14	2	2DL1, 2DL2/2DL3, 3DL1	Cw3, Cw6, Bw4	M	H/R	F/F	PD
15	2	2DL1, 2DL3, 3DL1	Cw7	MM	H/H	F/F	PD
16	2	2DL1, 2DL3, 3DL1	Cw12, Cw16, Bw4	MM	R/R	F/V	PD
17	1	2DL1, 2DL2/2DL3, 3DL1	Cw2, Cw16	MM	H/R	V/V	SD
18	1	2DL1, 2DL3,	Cw4, Bw4	MM	H/R	F/F	PD
19	1	2DL1, 2DL2/2DL3, 3DL1	Cw2, Cw7, Bw4	M	H/H	F/V	SD
20	1	2DL1, 2DL2/2DL3, 3DL1	Cw7, Bw4	MM	R/R	V/V	PD
21	2	2DL1, 2DL2/2DL3, 3DL1	Cw4, Cw6, Bw4	MM	H/R	F/V	I
22	2	2DL1, 2DL2/2DL3, 3DL1	Cw5, Bw4	MM	H/H	F/F	CR
23	2	2DL1, 2DL3, 3DL1	Cw8, Cw15	MM	H/R	F/F	PD
24	1	2DL1, 2DL2/2DL3, 3DL1	Cw2, Cw3, Bw4	M	H/R	F/F	NR
25	2	2DL1, 2DL3, 3DL1	Cw5, Bw4	MM	R/R	F/F	SD
26	2	2DL1, 2DL2/2DL3, 3DL1	Cw2, Cw6, Bw4	MM	H/R	F/V	PD
27	2	2DL1, 2DL3, 3DL1	Cw12, Cw16, Bw4	MM	H/H	F/V	CR

Pt#	Stratum	KIR Genotype	KIR Ligand	Match vs. Mismatch	Fc R2 A-I31	Fc R3A -I58	Response
28	1	2DL1, 2DL2/2DL3, 3DL1	Cw6, Cw8, Bw4	M	H/R	F/V	PD
29	2	2DL1, 2DL3, 3DL1	Cw5, Cw7	MM	R/R	F/V	CR
30	1	2DL1, 2DL2/2DL3, 3DL1	Cw12, Cw15, Bw4	M	H/H	F/F	PD
31	1	2DL1, 2DL3, 3DL1	Cw4, Cw7	MM	H/R	F/F	PD
32	2	2DL1, 2DL3, 3DL1	Cw6, Cw7, Bw4	M	H/R	F/V	PD
33	2	2DL1, 2DL3, 3DL1	Cw6, Cw7, Bw4	M	R/R	F/F	PD
34	2	2DL2, 3DL1	Cw12, Cw17	MM	H/R	F/F	PD
35	2	2DL1, 2DL2, 3DL1	Cw6, Cw7	MM	H/R	**	PD
36	2	2DL1, 2DL2/2DL3, 3DL1	Cw3, Cw7, Bw4	M	H/H	F/F	PD
37	1	2DL1, 2DL3, 3DL1	Cw3, Cw14, Bw4	MM	H/R	F/F	PD
38	1	2DL1, 2DL3, 3DL1	Cw4, Cw7, Bw4	M	H/R	F/F	PD
39	1	2DL1, 2DL2/2DL3, 3DL1	Cw6, Cw7, Bw4	M	H/R	F/F	PD

* Pt 12 not included. DNA was not available.

** FcγR3A genotyping results from patients 8 and 35 excluded from analysis due to discrepancy in results of pyrosequencing and TaqMan, M = Match, MM = Mismatch, I = (clinically) improved, SD = stable disease, PD = progressive disease, CR = complete response.

Table IV

Distribution of genotypes for KIR mismatch/match and FcγR genotypes among the 38 patients evaluated.

KIR Mismatch	KIR Match		Statistical Analysis
24/38 (63%)	14/38 (37%)		p = 0.93 [*]
FcγR2A 131-H/H	FcγR2A 131-H/R	FcγR2A 131-R/R	
10/38 (26%)	16/38 (42%)	12/38 (32%)	p = 0.5 ^{**}
FcγR3A 158-F/F	FcγR3A 158-F/V	FcγR3A 158-V/V	
17/36 (47%)	17/36 (47%)	2/36 (6%)	p [#] = 0.92 ^{**}

* p value for the difference in distribution of KIR Match/Mismatch for this population vs. comparable population of NB patients evaluated for KIR Match/Mismatch by Venstrom et al (4).

** p value for the difference in distribution of FcγR genotypes for this population vs. that of comparable NB patients described by Cheung et al (11).

p value remains 0.79–1.0 regardless of whether patients 8 and 35 are FF or FV and included

Table V

Tests of association of Mismatch vs. A) Response/Improvement of all patients; B) Response/Improvement for patients in Stratum 2 only; C) Stratum 1 and Stratum 2

Table V-A: Mismatch vs. Response/Improvement (Stratum 1 & 2)

	KIR-Mismatch	KIR-Match	Total
Response/improvement	7 (29%)	0 (0%)	7
No Response/No improvement	17 (71%)	14 (100%)	31
Total	24	14	38

P = 0.03

Table V-B

Mismatch vs. Response (Stratum 2 only)

	KIR-Mismatch	KIR-Match	Total
Response/improvement	7 (39%)	0 (0%)	7
No Response/No improvement	11 (61%)	6 (100%)	17
Total	18	6	24

P = 0.13

Table V-C

Mismatch vs. Stratum (Stratum 1 & 2)

	Stratum 1	Stratum 2	Total
KIR-Mismatch	6 (43%)	18 (75%)	24
KIR-Match	8 (57%)	6 (25%)	14
Total	14	24	38

P = 0.08

Table VI

A) Response/Improvement for patients with higher-affinity receptor genotype (H/H) for FcγR2A-131 vs. both other genotypes (H/R and R/R).

Table VI-A: FcγR2A

	HH	HR + RR	Total
Response/improvement	4 (40%)	3 (11%)	7
Non-Response/Non-improvement	6 (60%)	25 (89%)	31
Total	10	28	38

P = 0.06

Table VI-B

B) Response/Improvement for patients with higher-affinity receptor genotype (V/V) for FcγR3A-158 vs. both other genotypes (V/F and F/F).

FcγR3A

	VV	VF + FF	Total
Response/improvement	0 (0%)	7 (21%)	7
Non-Response/Non-improvement	2 (100%)	27 (79%)	29
Total	2	34*	36

p* = 1.0

* [p value remains 1.0 regardless of whether patients 8 and 35 (both FF or FV) are included]