Title

Neuroblastoma Patients' KIR and KIR-Ligand Genotypes Influence Clinical Outcome for Dinutuximab-based Immunotherapy: A Report from the Children's Oncology Group

Journal

Clinical Cancer Research, 24(1)

ISSN

1078-0432

Authors

Erbe, Amy K
Wang, Wei
Carmichael, Lakeesha
et al.

Publication Date

2018

DOI

10.1158/1078-0432.ccr-17-1767

Peer reviewed

HHS Public Access

Author manuscript

Clin Cancer Res. Author manuscript; available in PMC 2018 July 01.

Published in final edited form as:

Clin Cancer Res. 2018 January 01; 24(1): 189–196. doi:10.1158/1078-0432.CCR-17-1767.

Neuroblastoma Patients' KIR and KIR-ligand Genotypes Influence Clinical Outcome for Dinutuximab-based Immunotherapy: A Report from the Children's Oncology Group

Amy K Erbe[#,1], **Wei Wang**[#,1], **Lakeesha Carmichael**[2], **KyungMann Kim**[2], **Eneida A Mendonça**[2,3], **Yiqiang Song**[2], **Dustin Hess**[1], **Patrick K Reville**[1], **Wendy B London**[4], **Arlene Naranjo**[5], **Jacquelyn A Hank**[1], **Mitchell B Diccianni**[6], **Ralph A Reisfeld**[7], **Stephen D Gillies**[8], **Katherine K Matthay**[9], **Susan L Cohn**[10], **Michael D Hogarty**[11], **John M Maris**[11], **Julie R Park**[12,13], **M. Fevzi Ozkaynak**[14], **Andrew L Gilman**[15], **Alice L Yu**[6,16,#], and **Paul M Sondel**[1,3,#]

[1]Department of Human Oncology, University of Wisconsin, Madison, WI, USA

[2]Department of Biostatistics and Medical Informatics, University of Wisconsin, Madison, WI, USA

[3]Department of Pediatrics, University of Wisconsin, Madison, WI, USA

[4]Dana–Farber / Boston Children's Cancer and Blood Disorders Center, Harvard Medical School, Boston, MA, USA

[5]COG Statistics and Data Center, Department of Biostatistics, University of Florida, Gainesville, FL

[6]Department of Pediatrics, Hematology/Oncology & Moores Cancer Center, University of California, San Diego, CA, USA

[7]Scripps Research Institute, La Jolla, CA, USA

[8]Provenance Biopharmaceuticals, Carlisle, MA, USA

[9]UCSF Benoiff Children's Hospital and University of California School of Medicine, San Francisco, CA, USA

[10]Department of Pediatrics, University of Chicago, Chicago, IL, USA

[11]Children's Hospital of Philadelphia and University of Pennsylvania School of Medicine, Philadelphia, PA, USA

[12]Seattle Children's Hospital, Seattle, WA, USA

[13]University of Washington, Seattle, WA, USA

[14]New York Medical College, Valhalla, NY, USA

Correspondence to: Paul M. Sondel, MD PhD, University of Wisconsin-Madison, 1111 Highland Avenue, 4159 WIMR Bldg., Madison, WI 53705, Phone Number: 608-263-9069, Fax Number: 608-263-4226, pmsondel@humonc.wisc.edu.

[*]Co-first authors

[#]Co-senior authors.

Conflict of Interest Statement:

Dr. Steve Gillies discloses equity ownership in Provenance Biopharmaceuticals of Carlisle, MA and ownership of intellectual property related to certain anti-GD2 related mAb-based agents. All other authors declare no competing financial interests.

[15]Levine Children's Hospital, Charlotte, NC, USA

[16]Institute of Stem Cell & Translational Cancer Research, Chang Gung Memorial Hospital, Taoyuan, Taiwan

Abstract

PURPOSE—In 2010, a Children's Oncology Group (COG) phase III randomized trial for high-risk neuroblastoma patients (ANBL0032) demonstrated improved event-free survival (EFS) and overall survival (OS) following treatment with an immunotherapy regimen of dinutuximab, GM-CSF, IL-2, and isotretinoin compared to treatment with isotretinoin alone. Dinutuximab, a chimeric anti-GD2 monoclonal antibody, acts in part via NK cells. Killer Immunoglobulin-like Receptors (KIRs) on NK cells and their interactions with KIR-ligands can influence NK cell function. We investigated whether KIR/KIR-ligand genotypes were associated with EFS or OS in this trial.

PATIENTS AND METHODS—We genotyped patients from COG study, ANBL0032, and evaluated the effect of KIR/KIR-ligand genotypes on clinical outcomes. Cox regression models and log-rank tests were used to evaluate associations of EFS and OS with KIR/KIR-ligand genotypes.

RESULTS—In this trial, patients with the "all KIR-ligands present" genotype, as well as patients with inhibitory KIR2DL2 with its ligand (HLA-C1) together with inhibitory KIR3DL1 with its ligand (HLA-Bw4) were associated with improved outcome if they received immunotherapy. In contrast, for patients with the complementary KIR/KIR-ligand genotypes, clinical outcome was not significantly different for patients that received immunotherapy vs. those receiving isotretinoin alone.

CONCLUSIONS—These data show that administration of immunotherapy is associated with improved outcome for neuroblastoma patients with certain KIR/KIR-ligand genotypes, while this was not seen for patients with other KIR/KIR-ligand genotypes. Further investigation of KIR/KIR-ligand genotypes may clarify their role in cancer-immunotherapy, and may enable KIR/KIR-ligand genotyping to be utilized prospectively for identifying patients likely to benefit from certain cancer immunotherapy regimens.

Keywords

Immunotherapy; KIR; Personalized Medicine; Neuroblastoma; NK cells

INTRODUCTION

Neuroblastoma is the most common extracranial solid tumor in children, accounting for 10% of childhood cancer mortality. High-risk neuroblastoma patients have less than 40% 5-year survival when treated with traditional chemotherapeutic agents (1). The Children's Oncology Group (COG) ANBL0032 phase III clinical trial enrolled high-risk neuroblastoma patients following initial treatment with multi-agent chemotherapy, surgical resection, local radiation therapy and autologous stem cell transplant. This randomized trial compared an immunotherapy regimen [consisting of the combination of dinutuximab (chimeric 14.18 monoclonal anti-GD2 antibody), aldesleukin (IL-2), sargramostim (GM-CSF) and

isotretinoin (herein this treatment regimen is referred to as “immunotherapy”)] to treatment with isotretinoin alone. Those treated with immunotherapy showed significant clinical benefit in both event-free survival (EFS) and overall survival (OS) (2). Further advances are still needed for these high risk patients; treatment with anti-GD2 immunotherapy is expensive, has toxic side effects, and many treated patients still relapse (2).

The variability in clinical presentation of neuroblastoma and the response to immunotherapy may, in part, reflect patient-to-patient differences in immune function. Some immune functions are genetically inherited and can be assessed by genotyping (3). NK cells contribute to antibody-dependent cellular cytotoxicity (ADCC). Killer-Immunoglobulin-like Receptors (KIRs) are a family of highly polymorphic receptors which regulate NK cell function via balanced transmission of activating or inhibitory signals. Most inhibitory KIRs have ligands that belong to the HLA class-I family. NK cell development and effector function are influenced by the specific inherited KIR and KIR-ligand repertoires, and their interactions. This study focuses on 4 inhibitory KIRs: KIR2DL1 is a receptor for HLA-C2; KIR2DL2 and KIR2DL3 are receptors for HLA-C1; and KIR3DL1 is a receptor for HLA-Bw4 epitopes (4–9).

Mature NK cells expressing inhibitory KIRs mediate reduced tumor-directed ADCC when KIRs encounter their respective KIR-ligands on tumor (10,11). We and others have previously found that neuroblastoma patients that have at least one KIR-ligand missing for their inhibitory KIRs (“KIR-ligand missing”, Supplementary Table 1) have improved outcomes compared to those that inherit all of the KIR-ligands for their inhibitory KIRs (“KIR-ligands present”) when receiving anti-GD2 mAb-based therapies (12–14).

In this study, we asked if KIR and KIR-ligand genotypes of the neuroblastoma patients in ANBL0032 were associated with clinical outcome. We also asked whether the clinical outcome for certain KIR/KIR-ligand genotypes could be influenced by the administration of this immunotherapy (2).

MATERIALS AND METHODS

Patients

The phase III neuroblastoma clinical trial ANBL0032 (ClinicalTrials.gov # NCT00026312) evaluated the efficacy of isotretinoin alone compared to immunotherapy. Of the 226 patients randomized, 174 individual patients had DNA available (immunotherapy: 88; isotretinoin: 86), allowing evaluation of KIR/KIR-ligand genotype association with updated clinical outcome (>5 years of follow up) (Supplemental Methods). Clinical characteristics for the COG patients, and for those genotyped in this report, are found in Supplemental Table 4. Appropriate IRB approved consent forms, detailing the therapy involved in the randomized study and the collection of blood/DNA samples for correlative immune-related studies, were obtained for all patients. The clinical trial was conducted in accordance with the Helsinki Declaration of 1975.

KIR/KIR-ligand Analyses

Genotyping—KIR gene status was determined for 15 separate KIR genes for each patient by a SYBR green real time PCR reaction, which uses the melt curve to determine the presence or absence of the gene (15). As KIR2DL1, KIR2DL2, KIR2DL3 and KIR3DL1 are the best studied inhibitory KIR genes, with known ligands, in prior studies of cancer immunotherapy (9–14,16), they are the focus of this study. The genotypes of these known KIR-ligands for the KIRs of interest in this study [including HLA-C1, HLA-C2, and the three known HLA-Bw4 epitopes (HLA-Bw4T80, HLA-Bw4I80, and HLA-A-Bw4)] were determined by PCR-SSP reactions using the KIR HLA Ligand SSP typing kit (Olerup, West Chester, PA) with GoTaq DNA polymerase (Promega, Madison,WI).

KIR2DL2 and KIR2DS2 are in linkage disequilibrium. In this study, of the 89 KIR2DS2+ patients, 86 (97%) were also KIR2DL2+ and of the 85 KIR2DS2− patients, 83 (98%) were also KIR2DL2−.

All KIR/KIR-ligand genotyping was conducted in a blinded manner, whereby individuals that determined the genotype of the patients did not have access to the randomization and clinical outcome data. “KIR-ligands present” is defined as all the KIR-ligands present for each inhibitory KIR gene present. “KIR-ligand missing” is defined as having at least one of the KIR-ligands absent for the inhibitory KIR genes present (Supplemental Table 1).

Statistical Methods—The primary objective was to evaluate the association of EFS and OS with treatment and KIR-ligand status (KIR-ligands present compared with KIR-ligand missing). All other analyses were exploratory. All analyses reported here utilized patient data based on intent to treat. Cox proportional hazards regression models and log-rank tests were used to compare EFS/OS curves by treatment and genotype. The proportional hazards assumption was tested, and when the assumption was not met, adjustments were made by incorporating time-dependent covariates into the model. Statistical analyses were performed using SAS v9.4 (SAS Institute, Cary, NC).

EFS was defined as the time from study enrollment until the first occurrence of relapse, progressive disease, secondary cancer, or death or until the last contact with the patient if none of these events occurred (censored). OS was defined as the time from study enrollment until death or the last contact with the patient if death did not occur during the study (censored). Only patients who were randomized were included in these analyses.

With the exception of Table 1, analyses were performed without corrections for multiple comparisons. For Table 1, due to the complexity of assessing KIR2DL2 and its ligand together with KIR3DL1 and its ligand, the comparisons of treatment groups were performed within specific KIR2DL2/ligand and KIR3DL1/ligand subgroups with p-values adjusted using the Bonferroni method.

RESULTS

Immunotherapy treatment improved outcome for patients with KIR-ligands present

Since patients in this COG study were randomized to receive immunotherapy or isotretinoin alone, we could assess how individual genotype groups were influenced based on the treatment they received. For patients with a KIR-ligands present genotype, treatment with immunotherapy improved both EFS and OS as compared to those that were treated with isotretinoin alone (EFS p=0.03, Figure 1A; OS p=0.01, Figure 1B). In contrast, for patients with KIR-ligand missing, there was no significant improvement in EFS or OS for immunotherapy treatment (Figure 1).

KIR-ligand missing was not associated with improved clinical outcome in the Immunotherapy group

In contrast to some previous reports where the KIR-ligand missing genotype was associated with improved clinical outcome with anti-GD-2 therapy (11–14), amongst the immunotherapy patients here we found no association of KIR-ligand missing compared with KIR-ligands present for either EFS or OS (Figures 1A and 1B). Patients in the isotretinoin alone group did show a trend towards improved OS if they were KIR-ligand missing vs. KIR-ligands present (OS p=0.06; Figure 1B).

Immunotherapy treatment improved outcome for patients dependent upon KIR2DL2/KIR-ligand status

Unlike KIR2DL1, KIR2DL3 and KIR3DL1, which are found in ≥92% of these neuroblastoma patients, KIR2DL2 is found in only 51% of this study population (Supplemental Table 2), which are similar frequencies as others have reporte for these genes (11,16). Several groups reported that the status of the inhibitory KIR2DL2 (and/or a KIR gene closely linked to KIR2DL2, the activating receptor KIR2DS2) influence patient outcome, and some of these assessed the impact of KIR2DL2 with or without its ligand (17–19). KIR2DL2 is also of interest as both KIR2DL2, and KIR2DL2 with its HLA-C1 ligand, are more common in patients with neuroblastoma than in healthy individuals (16). Thus, we investigated the influence of KIR2DL2 and its ligand HLA-C1 on patient outcomes in this study.

For patients treated with isotretinoin alone, individuals that possessed KIR2DL2 (“KIR2DL2+”) along with its ligand C1 (“ligand+”) had significantly worse EFS and OS as compared to those individuals that were *not* KIR2DL2+/C1+ (Supplementary Table 3: those KIR2DL2+ with HLA-C2/C2, or those KIR2DL2− with HLA-C1/C1, C1/C2 or C2/C2) (EFS p=0.04; OS p= 0.004, Figure 2A–2B). For those patients treated with immunotherapy, there were no significant differences in EFS or OS for patients that were KIR2DL2+/C1+ compared to those that were *not* KIR2DL2+/C1+ (Figure 2A–2B). For patients that were KIR2DL2+/C1+, treatment with immunotherapy significantly improved both EFS and OS as compared to treatment with isotretinoin alone (EFS p=0.02; OS p =0.002, Figure 2A–2B). In contrast, for patients that were *not* KIR2DL2+/C1+, the EFS and OS were similar for patients receiving immunotherapy compared to those receiving isotretinoin alone (Figure 2A–2B).

We did not observe any significant associations between the presence or absence of KIR2DL1 and its HLA-C2 ligand or between the presence/absence of KIR2DL3 and its HLA-C1 ligand with either EFS or OS in this study (data not shown).

Immunotherapy treatment significantly improved outcome for patients dependent upon KIR3DL1/KIR-ligand status

In our previous evaluation of follicular lymphoma patients, we found that maintenance rituximab treatment in patients that had KIR3DL1 along with its ligand, HLA-Bw4, resulted in improved duration of response over those that were *not* KIR3DL1+/Bw4+ (20). Forlenza et al. recently reported that neuroblastoma patients treated with a mouse anti-GD2 mAb, 3F8, in combination with GM-CSF, had improved OS and progression-free survival if they were HLA-Bw4− compared to those with HLA-Bw4+ (21).

In this study, for those patients that possess KIR3DL1 with its ligand ("KIR3DL1+/Bw4+"), treatment with immunotherapy resulted in significant improvements in both EFS and OS as compared to treatment with isotretinoin alone (EFS p=0.03; OS p=0.03, Figure 2C–2D). In contrast, for patients that were *not* KIR3DL1+/Bw4+ (Supplementary Table 3: those KIR3DL1+/− and HLA-Bw4−; KIR3DL1− with HLA-Bw4+) the EFS and OS were similar for patients receiving immunotherapy compared to those receiving isotretinoin alone (Figure 2C–2D).

Patients that are both KIR2DL2+/C1+ as well KIR3DL1+/Bw4+ had improved clinical outcome if treated with immunotherapy vs. isotretinoin alone

Recently, Lode et al. reported that neuroblastoma patients that were KIR2DS2+ treated with a similar anti-GD2 chimeric antibody had improved clinical response as compared to patients that were both KIR2DS2− and KIR3DL1+ with the KIR3DL1+/Bw4 present (i.e. those KIR2DS2+ vs. KIR2DS2−, KIR3DL1+, Bw4+) (22). Since we found that both KIR2DL2 and its ligand status, as well as KIR3DL1 and its ligand status (Figure 2), influence outcome dependent upon treatment type, we investigated whether these KIR/KIR-ligand subsets together could further influence patient outcomes. We thus compared the group of patients with KIR2DL2 with its HLA-C1 ligand that also have KIR3DL1with its HLA-Bw4 ligand (designated: KIR2DL2+/C1+/KIR3DL1+/Bw4+) to the remaining patients, those lacking KIR2DL2, HLA-C1, KIR3DL1, or HLA-Bw4 ("*not* KIR2DL2+/C1+/KIR3DL1+/Bw4+"). The distinct genotypes comprising these 2 groups are detailed in Supplemental Table 3. We found that patients treated with isotretinoin alone that were *not* KIR2DL2+/C1+/KIR3DL1+/Bw4+ had significantly improved EFS and OS as compared to those that were KIR2DL2+/C1+/KIR3DL1+/Bw4+ (EFS p=0.04; OS p=0.007, Figure 3). For those patients treated with immunotherapy, there were no significant differences in either EFS or OS when comparing these genotype groupings (EFS p=0.42; OS p=0.06, Figure 3).

Similar to what we found in Figures 1 and 2, we found that, based on this grouping of KIR/KIR-ligand genotypes, a subset of patients appear to significantly benefit from immunotherapy treatment as compared to isotretinoin alone. Specifically, KIR2DL2+/C1+/KIR3DL1+/Bw4+ patients had significantly improved EFS and OS if treated with

immunotherapy vs. isotretinoin alone (EFS p=0.02; OS p=0.007, Figure 3). In contrast, for the counter to this genotype, those *not* KIR2DL2+/C1+/KIR3DL1+/Bw4+, outcome was similar if treated with immunotherapy vs. isotretinoin alone (Figure 3). To determine if one genotypic factor (either KIR2DL2/ligand status *or* KIR3DL1/ligand status) was driving this influence on clinical benefit, we analyzed the different possible genotypic combinations of KIR2DL2/ligand or *not* KIR2DL2+/C1+ and KIR3DL1/ligand or *not* KIR3DL1+/Bw4+, and used a Bonferonni adjustment of the p-values. Only the subgroup that was both KIR2DL2+/C1+ **and** KIR3DL1+/Bw4+ showed statistically significantly higher EFS and OS for patients treated with immunotherapy compared to those receiving isotretinoin alone (EFS p=0.04; OS p=0.01, Table 1). For the 3 other subgroupings of KIR2DL2 and its HLA-C1 ligand and KIR3DL1 and its HLA-Bw4 ligand, there was no evidence of differences in EFS or OS when receiving immunotherapy vs. isotretinoin alone.

DISCUSSION

In this study of high-risk neuroblastoma patients who had responded to initial induction and consolidation therapy, we assessed potential associations of KIR/KIR-ligand genotype with clinical outcome. Unlike some prior reports (11–14), in the immunotherapy group, we found no evidence of improved outcome for patients with the KIR-ligand missing genotype compared to patients with KIR-ligands present. We also had the opportunity to analyze the potential associations of KIR/KIR-ligand genotypes on the outcome of patients in the isotretinoin alone group. For the patients in the isotretinoin group, we saw a trend for improved OS in the patients with KIR-ligand missing vs. those with KIR-ligands present. We hypothesize that this may be, in part, due to the increased inhibition burden on NK cells from a KIR-ligands present genotype as compared to a KIR-ligand missing genotype, such that those patients with a KIR-ligand missing genotype are less inhibited and thus more able to reduce the tumor load without the presence of immunotherapy.

All four prior published studies of KIR/KIR-ligand genotypes for neuroblastoma patients receiving anti-GD2 mAb-based treatment have reported better outcome for patients with KIR-ligand missing vs. KIR-ligands present (11–14). However, this study of neuroblastoma patients with minimal residual disease does not recapitulate those findings. One of those studies was a COG phase-II trial for patients with relapsed or refractory neuroblastoma treated with a humanized anti-GD2 mAb molecularly-linked to IL-2, instead of a chimeric anti-GD2 antibody in combination with IL2, GM-CSF and isotretinoin which was given in this present trial (ANBL0032) (12). In this prior report for patients with relapsed/refractory disease, neither OS or EFS were reported; instead disease response was the reported outcome. It is possible that differences in the treatment regimen (humanized anti-GD2 mAb linked to IL2 vs. chimeric anti-GD2 antibody in combination with IL2, GM-CSF and isotretinoin), the disease state (minimal residual disease vs. refractory/recurrent neuroblastoma), or the measure of outcome (response vs. EFS/OS) might modify the clinical biology, potentially accounting for the differences between the KIR/KIR-ligand results reported here and in that study (12).

The other reports are from Memorial Sloan Kettering Cancer Center (MSKCC), and all involve administration of the murine 3F8 anti-GD2 mAb to patients after completing

chemotherapy (11,13,14). These three reports present accumulated data from MSKCC with significant overlap of patients in each report (patients from NCT00072358, NCT00037011, NCT00002634 and NCI-V90-0023 clinical trials).

Two major differences between these MSKCC studies and our study is their use of murine-derived monoclonal antibody (3F8) vs. a chimeric monoclonal antibody (dinutuximab), as well as the addition of the cytokine, IL-2, to all patients in the COG immunotherapy regimen and only a few in the MSKCC trial. The structural or immunological differences between these two antibody constructs could contribute to differences in response to treatment. Murine-based monoclonal antibodies are more immunogenic than chimeric antibodies, as only about 25% of the chimeric mAb is mouse-derived, and 75% of the backbone is human-derived. Human anti-mouse antibody (HAMA) responses against murine mAbs can reduce the efficacy of the antibody immunotherapy by neutralizing the antibody, not allowing for effective recruitment of immune cells to the tumor site. It is possible that the frequent induction of a neutralizing (HAMA) response to 3F8 vs. the infrequent induction of a human anti-chimeric antibody (HACA) response to ch14.18 (23,24), or the use of IL2, may somehow account for the differential association of KIR-ligand missing status with better outcome for anti-GD2 treated patients in the MSKCC regimen but not the COG regimen.

Due to the randomized design of this study, we also could compare outcomes for patients receiving immunotherapy vs. isotretinoin alone. This provided the unique opportunity to assess whether the observed improved outcome following immunotherapy, as compared to isotretinoin, was associated with certain KIR/KIR-ligand genotypes. We found that patients with a KIR-ligands present genotype had a statistically significant benefit in EFS and OS if they received immunotherapy instead of receiving isotretinoin alone. In contrast, for those with KIR-ligand missing, there was no evidence of improved outcome from immunotherapy.

Prior studies have shown that having a population of unlicensed NK cells (having at least one KIR-ligand missing) enhances tumor cell killing when the tumor microenvironment expresses KIR-ligands (7–10). This suggests that individuals with at least one KIR-ligand missing have NK cells better equipped to kill HLA-expressing tumor cells. We hypothesize these individuals might not require the COG immunotherapy regimen to further boost their NK capability. We also hypothesize that patients with all KIR-ligands present may have NK cells that are more inhibited upon encountering their own HLA-expressing tumor cells; as such they may require an additional “boost of function” provided by this COG immunotherapy regimen. Caution is needed, since these hypotheses require the tumor cells to express their inherited ligands; in this study, we have only assessed genotype. Even so, if these genotype/outcome associations are validated, they would suggest that for some patients, depending on the functional implications that are based on ones’ genotype, immunotherapy overcomes these genotype-restraints. In other words, for patients whose genotype predicts worse NK ADCC function (namely those with all KIR-ligands present) (11), the administration of immunotherapy is associated with outcome comparable to that seen for patients with favorable genotype that receive immunotherapy.

To further elucidate the KIR/KIR-ligand genotype influence on which patients have improved outcome associated with immunotherapy (vs. isotretinoin alone), we analyzed

additional KIR/KIR-ligand genotypes. These were selected based on prior reports. We identified certain KIR/KIR-ligand genotypes that were significantly associated with benefit from this immunotherapy regimen, which may have future actionable clinical relevance. Given previous studies assessing the role of KIR2DL2/S2 and KIR2DL2-ligand status (16–19), KIR3DL1 and its HLA-Bw4 ligand (20,21), and KIR2DL2/S2 and KIR3DL1 status simultaneously (22), we assessed how these inhibitory KIR/KIR-ligand interactions may influence outcome for patients receiving immunotherapy vs. isotretinoin alone. In this study, we found that patients with KIR2DL2+/C1+ treated with immunotherapy had significantly improved outcome compared to those receiving isotretinoin alone. There was no evidence of such a difference for those patients that are *not* KIR2DL2+/C1+. Similarly, we demonstrated that KIR3DL1+/Bw4+ patients treated with immunotherapy had significantly improved outcome as compared to those treated with isotretinoin alone. Conversely, in a study by Forlenza et al. of neuroblastoma patients treated with a different anti-GD2 regimen (21), which involved a more recent analysis of many of the same neuroblastoma patients previously reported on by MSKCC's neuroblastoma research team (11,13,14); they demonstrated worse outcome for HLA-Bw4+ patients when treated with 3F8 than HLA-Bw4− patients. HLA-Bw4 interactions with KIR3DL1 causes inhibition of NK cell activity, but this interaction is also a component of NK cell licensing (6,7). It is possible that distinct combinations of immunotherapeutic treatments can differentially influence either the licensing effect or the inhibitory potential of KIR3DL interactions with its HLA-Bw4 ligand, potentially accounting for the differences in these studies.

We identified a subset of our patient population, those KIR2DL2+/C1+/KIR3DL1+/Bw4+, that have clear clinical benefit with immunotherapy as compared to isotretinoin alone. In contrast, the complementary genotype groups showed no apparent difference in outcome if treated with immunotherapy or isotretinoin alone. Patients with KIR2DL2+/C1+/KIR3DL1+/Bw4+ make up ~30% of the patients in our study (49 out of 174), yet seem to account for the majority of clinical benefit that the entire population experiences from immunotherapy treatment in this study.

In summary, regardless of patient KIR/KIR-ligand genotype, the overall group of patients that received immunotherapy had improved outcome compared to patients receiving isotretinoin alone (2). Our evaluation of KIR/KIR-ligand genotypes suggests that patients with certain KIR/KIR-ligand genotypes significantly benefit from the COG immunotherapy regimen. As these findings have not been validated independently in other studies, it is premature to classify them as clinically actionable. However, if this strategy were to be validated, it could enable administration of this regimen of immunotherapy to those that would best benefit, and allow avoiding this somewhat toxic 5-month regimen (or using a different strategy) for those who might not benefit from this regimen. Enhancements to anti-GD2 mAb-based therapy, based on preclinical and early clinical data are being evaluated in efforts to improve its efficacy (22,25–27). Since we cannot be certain that the benefit in the immunotherapy group observed for patients with KIR-ligands present or for patients with KIR2DL2+/C1+/KIR3DL1+/Bw4+ will be applicable for newer generations of anti-GD2 immunotherapeutic regimens, further studies of KIR/KIR-ligand associations with outcome in subsequent trials of immunotherapeutic regimens for children with neuroblastoma will be needed to determine the potential clinical utility of these findings.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Financial Support: This research was supported by Hyundai Hope on Wheels Grant; Midwest Athletes Against Childhood Cancer; The Stand Up To Cancer – St. Baldrick's Pediatric Dream Team Translational Research Grant (SU2C-AACR-DT1113). Stand Up To Cancer is a program of the Entertainment Industry Foundation. Research Grants are administered by the American Association for Cancer Research, the Scientific Partner of SU2C; University of Wisconsin Carbone Cancer Center; NCTN Operations Center Grant U10CA180886; and supported in part by Public Health Service Grants CA014520, CA166105, CA164132 and CA197078, from the National Cancer Institute, the National Institutes of Health, the Department of Health and Human Services. Its content is solely the responsibility of the authors and does not necessarily represent the official views of the National Cancer Institute.

References

1. Bosse KR, Maris JM. Advances in the translational genomics of neuroblastoma: From improving risk stratification and revealing novel biology to identifying actionable genomic alterations. Cancer. 2016; 122(1):20–33. DOI: 10.1002/cncr.29706 [PubMed: 26539795]
2. Yu AL, Gilman AL, Ozkaynak MF, London WB, Kreissman SG, Chen HX, et al. Anti-GD2 antibody with GM-CSF, interleukin-2, and isotretinoin for neuroblastoma. N Engl J Med. 2010; 363(14):1324–34. DOI: 10.1056/NEJMoa0911123 [PubMed: 20879881]
3. Koehn TA, Trimble LL, Alderson KL, Erbe AK, McDowell KA, Grzywacz B, et al. Increasing the clinical efficacy of NK and antibody-mediated cancer immunotherapy: potential predictors of successful clinical outcome based on observations in high-risk neuroblastoma. Front Pharmacol. 2012; 3:91 d.doi: 10.3389/fphar.2012.0009 [PubMed: 22623917]
4. Moesta AK, Norman PJ, Yawata M, Yawata N, Gleimer M, Parham P. Synergistic polymorphism at two positions distal to the ligand-binding site makes KIR2DL2 a stronger receptor for HLA-C than KIR2DL3. J Immunol. 2008; 180(6):3969–79. [PubMed: 18322206]
5. Almeida CR, Ashkenazi A, Shahaf G, Kaplan D, Davis DM, Mehr R. Human NK cells differ more in their KIR2DL1-dependent thresholds for HLA-Cw6-mediated inhibition than in their maximal killing capacity. PLoS One. 2011; 6(9):e24927 d.doi: 10.1371/journal.pone.0024927 [PubMed: 21949790]
6. Litwin V, Gumperz J, Parham P, Phillips JH, Lanier LL. NKB1: a natural killer cell receptor involved in the recognition of polymorphic HLA-B molecules. J Exp Med. 1994; 180(2):537–43. [PubMed: 8046332]
7. Yokoyama WM, Kim S. Licensing of natural killer cells by self-major histocompatibility complex class I. Immunol Rev. 2006; 214:143–54. DOI: 10.1111/j.1600-065X.2006.00458.x [PubMed: 17100882]
8. Jonsson AH, Yokoyama WM. Natural killer cell tolerance licensing and other mechanisms. Adv Immunol. 2009; 101:27–79. DOI: 10.1016/S0065-2776(08)01002-X [PubMed: 19231592]
9. Kim S, Poursine-Laurent J, Truscott SM, Lybarger L, Song YJ, Yang L, et al. Licensing of natural killer cells by host major histocompatibility complex class I molecules. Nature. 2005; 436(7051): 709–13. DOI: 10.1038/nature03847 [PubMed: 16079848]
10. Wang W, Erbe AK, Hank JA, Morris ZS, Sondel PM. NK Cell-Mediated Antibody-Dependent Cellular Cytotoxicity in Cancer Immunotherapy. Front Immunol. 2015; 6:368 d.doi: 10.3389/fimmu.2015.00368 [PubMed: 26284063]
11. Tarek N, Le Luduec JB, Gallagher MM, Zheng J, Venstrom JM, Chamberlain E, et al. Unlicensed NK cells target neuroblastoma following anti-GD2 antibody treatment. The Journal of clinical investigation. 2012; 122(9):3260–70. DOI: 10.1172/JCI62749 [PubMed: 22863621]
12. Delgado DC, Hank JA, Kolesar J, Lorentzen D, Gan J, Seo S, et al. Genotypes of NK cell KIR receptors, their ligands, and Fcgamma receptors in the response of neuroblastoma patients to Hu14. 18-IL2 immunotherapy Cancer research. 2010; 70(23):9554–61. DOI: 10.1158/0008-5472.CAN-10-2211 [PubMed: 20935224]

13. Cheung NK, Cheung IY, Kushner BH, Ostrovnaya I, Chamberlain E, Kramer K, et al. Murine anti-GD2 monoclonal antibody 3F8 combined with granulocyte-macrophage colony-stimulating factor and 13-cis-retinoic acid in high-risk patients with stage 4 neuroblastoma in first remission. J Clin Oncol. 2012; 30(26):3264–70. DOI: 10.1200/JCO.2011.41.3807 [PubMed: 22869886]

14. Venstrom JM, Zheng J, Noor N, Danis KE, Yeh AW, Cheung IY, et al. KIR and HLA genotypes are associated with disease progression and survival following autologous hematopoietic stem cell transplantation for high-risk neuroblastoma. Clin Cancer Res. 2009; 15(23):7330–4. DOI: 10.1158/1078-0432.CCR-09-1720 [PubMed: 19934297]

15. Alves LGT, Rajalingam R, Canavez F. A novel real-time PCR method for KIR genotyping. Tissue Antigens. 2009; 73(2):188–91. DOI: 10.1111/j.1399-0039.2008.01184.x [PubMed: 19140829]

16. Keating SE, Ni Chorcora C, Dring MM, Stallings RL, O'Meara A, Gardiner CM. Increased frequencies of the killer immunoglobulin-like receptor genes KIR2DL2 and KIR2DS2 are associated with neuroblastoma. Tissue Antigens. 2015; 86(3):172–7. DOI: 10.1111/tan.12608 [PubMed: 26202659]

17. Middleton D, Diler AS, Meenagh A, Sleator C, Gourraud PA. Killer immunoglobulin-like receptors (KIR2DL2 and/or KIR2DS2) in presence of their ligand (HLA-C1 group) protect against chronic myeloid leukaemia. Tissue Antigens. 2009; 73(6):553–60. DOI: 10.1111/j.1399-0039.2009.01235.x [PubMed: 19493232]

18. Velarde-de la Cruz EE, Sanchez-Hernandez PE, Munoz-Valle JF, Palafox-Sanchez CA, Ramirez-de Los Santos S, Graciano-Machuca O, et al. KIR2DL2 and KIR2DS2 as genetic markers to the methotrexate response in rheumatoid arthritis patients. Immunopharmacol Immunotoxicol. 2016; 38(4):303–9. DOI: 10.1080/08923973.2016.1194429 [PubMed: 27251940]

19. Wisniewski A, Jankowska R, Passowicz-Muszynska E, Wisniewska E, Majorczyk E, Nowak I, et al. KIR2DL2/S2 and HLA-C C1C1 genotype is associated with better response to treatment and prolonged survival of patients with non-small cell lung cancer in a Polish Caucasian population. Hum Immunol. 2012; 73(9):927–31. DOI: 10.1016/j.humimm.2012.07.323 [PubMed: 22836042]

20. Erbe AK, Wang W, Reville PK, Carmichael L, Kim K, Mendonca EA, et al. HLA-Bw4-I-80 Isoform Differentially Influences Clinical Outcome As Compared to HLA-Bw4-T-80 and HLA-A-Bw4 Isoforms in Rituximab or Dinutuximab-Based Cancer Immunotherapy. Front Immunol. 2017; 8:675 d.doi: 10.3389/fimmu.2017.00675 [PubMed: 28659916]

21. Forlenza CJ, Boudreau JE, Zheng J, Le Luduec JB, Chamberlain E, Heller G, et al. KIR3DL1 Allelic Polymorphism and HLA-B Epitopes Modulate Response to Anti-GD2 Monoclonal Antibody in Patients With Neuroblastoma. J Clin Oncol. 2016; doi: 10.1200/JCO.2015.64.9558

22. Lode HN, Troschke-Meurer S, Valteau-Couanet D, Garaventa A, Gray J, Castel V, et al. Correlation of killer-cell Ig like receptor (KIR) haplotypes and Fcγ- receptor polymorphisms with survival of high-risk relapsed/refractory neuroblastoma patients treated by long-term infusion of anti-GD2 antibody ch14.18/CHO. 2016 ASCO Annual Meeting (abstract). Journal of Clinical Oncology. 34

23. Kushner BH, Ostrovnaya I, Cheung IY, Kuk D, Kramer K, Modak S, et al. Prolonged progression-free survival after consolidating second or later remissions of neuroblastoma with Anti-GD2 immunotherapy and isotretinoin: a prospective Phase II study. Oncoimmunology. 2015; 4(7):e1016704 d.doi: 10.1080/2162402X.2015.1016704 [PubMed: 26140243]

24. Siebert N, Eger C, Seidel D, Juttner M, Zumpe M, Wegner D, et al. Pharmacokinetics and pharmacodynamics of ch14.18/CHO in relapsed/refractory high-risk neuroblastoma patients treated by long-term infusion in combination with IL-2. MAbs. 2016; 8(3):604–16. DOI: 10.1080/19420862.2015.1130196 [PubMed: 26785755]

25. Morris ZS, Guy EI, Francis DM, Gressett MM, Werner LR, Carmichael LL, et al. In Situ Tumor Vaccination by Combining Local Radiation and Tumor-Specific Antibody or Immunocytokine Treatments. Cancer Res. 2016; 76(13):3929–41. DOI: 10.1158/0008-5472.CAN-15-2644 [PubMed: 27197149]

26. Shusterman S, London WB, Gillies SD, Hank JA, Voss SD, Seeger RC, et al. Antitumor activity of hu14.18-IL2 in patients with relapsed/refractory neuroblastoma: a Children's Oncology Group (COG) phase II study. J Clin Oncol. 2010; 28(33):4969–75. DOI: 10.1200/JCO.2009.27.8861 [PubMed: 20921469]

27. Furman WL, Federico SM, McCarville MB, Davidoff AM, Krasin MJ, Wu J, et al. Improved clinical responses with the concomitant use of an anti-GD2 monoclonal antibody and chemotherapy in newly diagnosed children with high-risk (HR) neuroblastoma (NB): Preliminary results of a phase II study. 2016 ASCO Annual Meeting. Journal of Clinical Oncology. 34

STATEMENT OF TRANSLATIONAL RELEVANCE

The use of anti-GD2 mAb as tumor targeted immunotherapy has improved the outcome for high-risk neuroblastoma patients, but not all patients benefit from this immunotherapy. Preclinical data suggest that an important mechanism of anti-tumor action is antibody dependent cell-mediated cytotoxicity (ADCC) by NK cells. Prior clinical trials have demonstrated that genotypic polymorphisms in KIR and KIR-ligand genotypes are associated with NK function and clinical outcome. We evaluated KIR/KIR-ligand genotypes in patients from a randomized Phase-III trial of anti-GD2 based immunotherapy, comparing results for patients randomized to immunotherapy or no immunotherapy. We identified KIR/KIR-ligand genotypes that were associated with improved outcome if immunotherapy was given. These results confirm a role for NK cells in this effect, and could provide a biomarker for prospectively personalizing care.

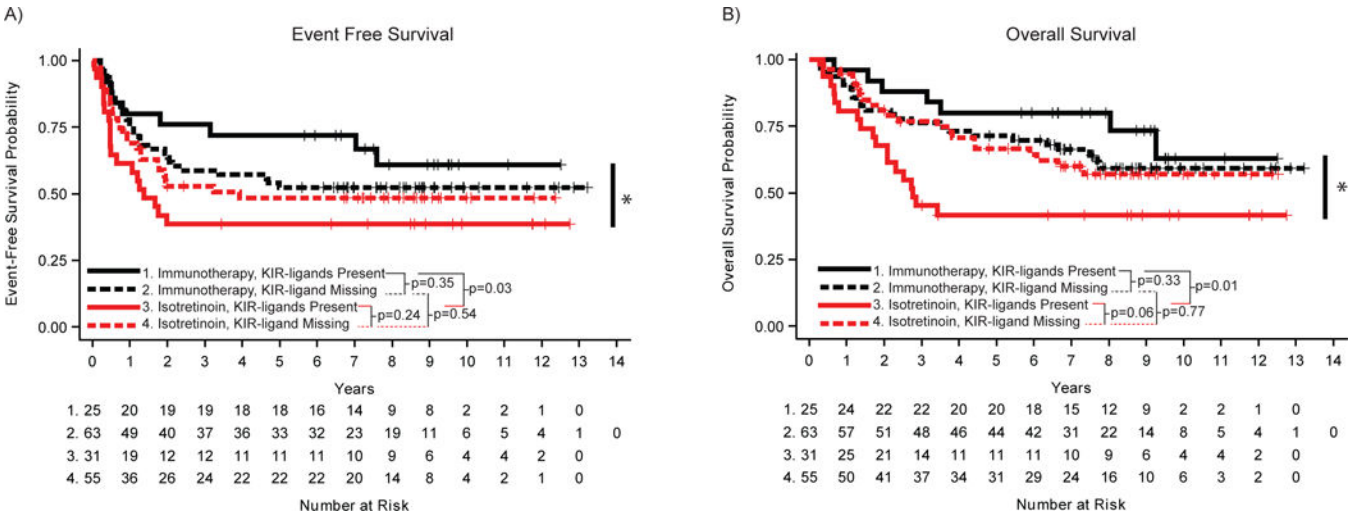

Figure 1. Associations of overall KIR/KIR-ligand status with clinical outcome

Figure 1A – EFS; Figure 1B – OS. For immunotherapy patients, those with KIR-ligands present (Line 1: solid-black line) were compared to those with KIR-ligand missing (Line 2: dashed-black line). For isotretinoin patients, those with KIR-ligands present (Line 3: solid-red line) were compared with those with KIR-ligand missing (Line 4: dashed-red line). In addition, comparisons by treatment group were performed. For both EFS and OS, the assumption of proportional hazards was upheld, and p-values are reported from Cox regression analyses. (“*” indicates $p<0.05$)

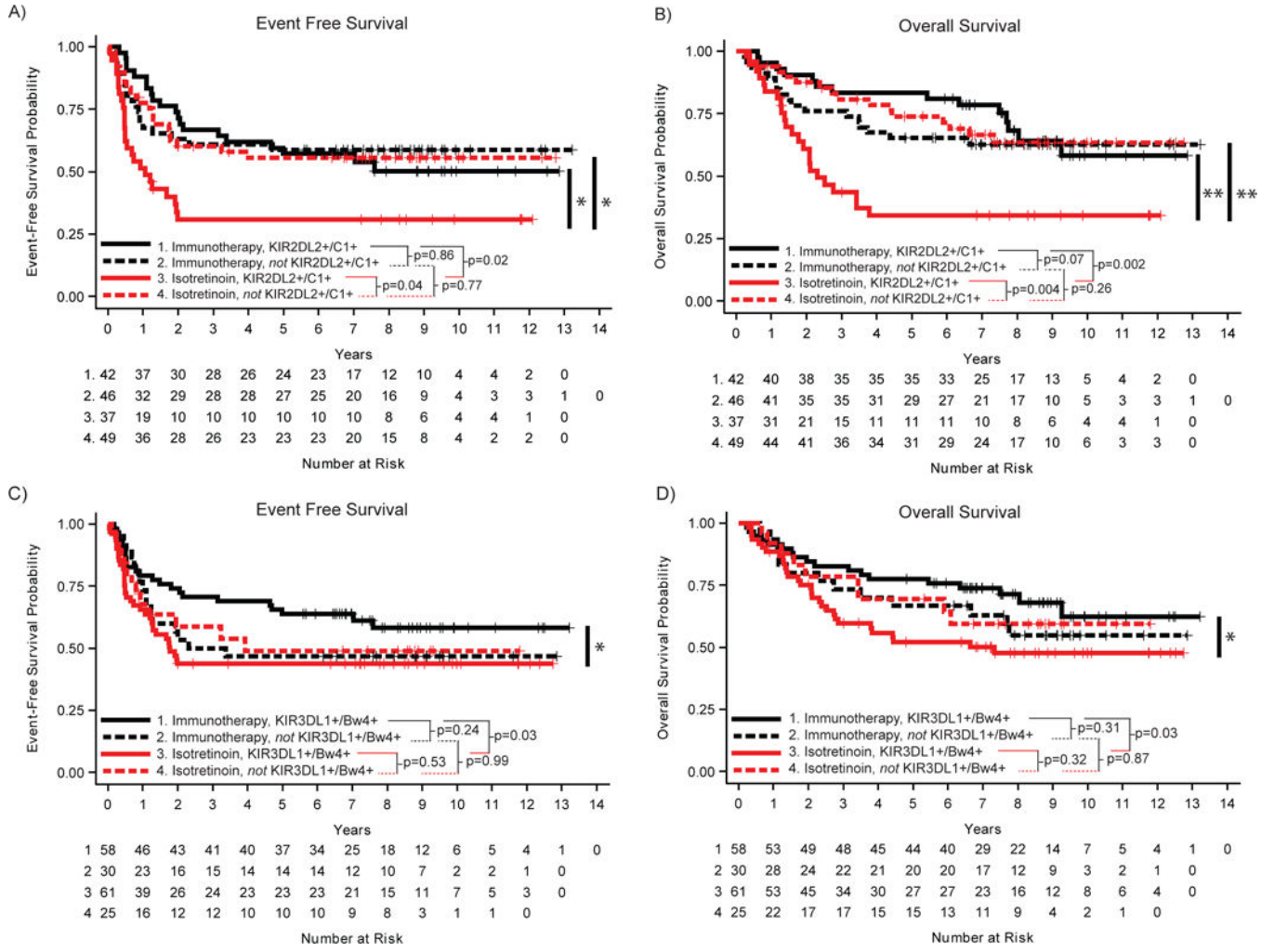

Figure 2. Associations of KIR2DL2+/C1+ status and KIR3DL1+/Bw4+ status with clinical outcome

Figure 2A – EFS; Figure 2B – OS: For immunotherapy patients, KIR2DL2+/C1+ (Line 1: solid-black line) were compared to *not* KIR2DL2+/C1+ (Line 2: dashed-black line). For isotretinoin patients, KIR2DL2+/C1+ (Line 3: solid-red line) were compared with those *not* KIR2DL2+/C1+ (Line 4: dashed-red line). In addition, comparisons by treatment group were performed. Figure 2C – EFS; Figure 2D – OS: For immunotherapy patients, those KIR3DL1+/Bw4+ (Line 1: solid-black line) were compared to those *not* KIR3DL1+/Bw4+ (Line 2: dashed-black line). For isotretinoin patients, KIR3DL1+/Bw4+ (Line 3: solid-red line) were compared with those *not* KIR3DL1+/Bw4+ (Line 4: dashed-red line). For KIR2DL2+/C1+ status, for both EFS and OS, the proportional hazards assumption was violated, so p-values are reported from the Cox model after adjustment by incorporating time-dependent covariates. For KIR3DL1+/Bw4+ status, both EFS and OS, the proportional hazards assumption was upheld, and p-values are reported from Cox regression analyses. ("*" indicates $p<0.05$; "**" indicates $p<0.01$)

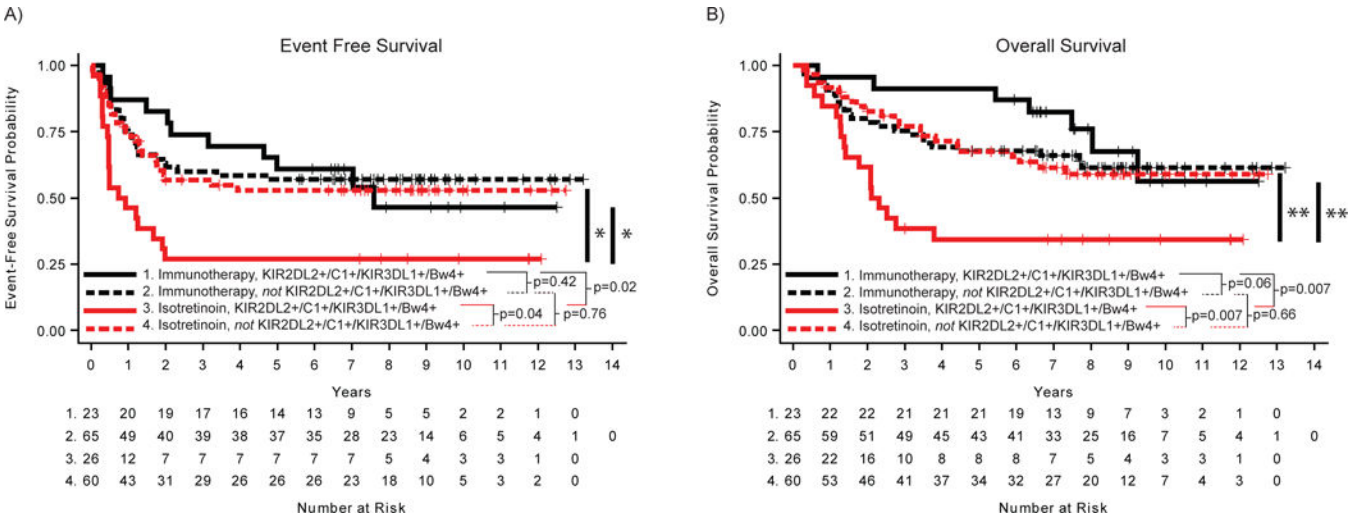

Figure 3. Associations of KIR2DL2+/C1+/KIR3DL1+/Bw4+ with clinical outcome

Figure 3A – EFS; Figure 3B – OS. For immunotherapy patients, KIR2DL2+/C1+/KIR3DL1+/Bw4+ (solid-black line) were compared to those *not* KIR2DL2+/C1+/KIR3DL1+/Bw4+ (dashed-black line). For isotretinoin patients, KIR2DL2+/C1+/KIR3DL1+/Bw4+ (solid-red line) were compared with those *not* KIR2DL2+/C1+/KIR3DL1+/Bw4+ (dashed-red line). In addition, comparisons by treatment group were performed. For both EFS and OS, the proportional hazards assumption was violated, so p-values are reported from the Cox model after adjustment by incorporating time-dependent covariates. (“*” indicates $p<0.05$; “**” indicates $p<0.01$).

Table 1

KIR2DL2+/C1+/KIR3DL1+/Bw4+ influence patient EFS and OS depending on treatment group (immunotherapy vs. isotretinoin alone).

		EFS				OS			
		n (#Events)[A]	**2-yr % rate (95% CI)[B]**	**5-yr % rate (95% CI)[B]**	**p-value[C]**	**n (#Events)[A]**	**2-yr % rate (95% CI)[B]**	**5-yr % rate (95% CI)[B]**	**p-value[C]**
KIR2DL2+/C1+ and KIR3DL1+/Bw4+	**Immunotherapy**	23 (11)	83 (60–93)	61 (38–77)	0.04	23 (7)	96 (73–99)	91 (69–98)	0.01
	Isotretinoin	26 (19)	27 (12–44)	27 (12–44)		26 (17)	62 (40–77)	34 (17–52)	
KIR2DL2+/C1+ and *not* KIR3DL1+/Bw4+	**Immunotherapy**	35 (12)	69 (50–81)	66 (48–79)	1.00	35 (11)	77 (59–88)	69 (50–81)	1.00
	Isotretinoin	35 (15)	56 (38–71)	56 (38–71)		35 (13)	86 (69–94)	66 (47–80)	
KIR3DL1+/Bw4+ and *not* KIR2DL2+/C1+	**Immunotherapy**	19 (9)	58 (33–76)	53 (29–72)	1.00	19 (7)	84 (59–95)	74 (48–88)	0.67
	Isotretinoin	11 (6)	41 (12–69)	41 (12–69)		11 (6)	58 (23–82)	35 (8–64)	
***not* KIR2DL2+/C1+ and *not* KIR3DL1+/Bw4+**	**Immunotherapy**	11 (7)	45 (17–71)	36 (11–63)	1.00	11 (6)	73 (37–90)	55 (23–78)	0.39
	Isotretinoin	14 (6)	71 (39–88)	55 (26–77)		14 (3)	92 (57–99)	92 (57–99)	

A n = number of individuals, "#Events" = number of individuals that had an event throughout the duration of the study [median follow-up among all patients: 6.7 years (0.2–13.2 years)];

B 95% Confidence Interval;

C p-value adjusted using Bonferonni method

The combination of both KIR2DL2 with its ligand, together with KIR3DL1 with its ligand (top line in this table, and corresponding to the genotype evaluated as solid lines in Figure 3), has a statistically significant effect on both EFS and OS for patients in the immunotherapy group as compared to the isotretinoin alone group. All other combinations of these genotypes (those KIR2DL2+/C1+ **but** *not* KIR3DL1+/Bw4+; those KIR3DL1+/Bw4+ **but** *not* KIR2DL2+/C1+; and those *not* KIR2DL2+/C1+ **and also** *not* KIR3DL1+/Bw4+) had no significant difference in EFS or OS for treatment group comparisons.