HHS Public Access

Author manuscript

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

Published in final edited form as:

Clin Cancer Res. 2017 May 01; 23(9): 2159–2168. doi:10.1158/1078-0432.CCR-16-1874.

FCGR Polymorphisms Influence Response to IL-2 in Metastatic Renal Cell Carcinoma

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Abstract

Background—Fc-gamma receptors (FCGRs) are expressed on immune cells, bind to antibodies, and trigger antibody-induced cell-mediated anti-tumor responses when tumor-reactive antibodies are present. The affinity of the FCGR/antibody interaction is variable and dependent upon *FCGR* polymorphisms. Prior studies of cancer patients treated with immunotherapy indicate that *FCGR* polymorphisms can influence antitumor response for certain immunotherapies that act via therapeutically administered mAbs or via endogenous tumor-reactive antibodies induced from tumor antigen vaccines. The previously published "SELECT" trial of high-dose aldesleukin (HD-IL2) for metastatic renal cell carcinoma (mRCC) resulted in an objective response rate (ORR) of 25%. We evaluated the patients in this SELECT trial to determine whether higher affinity *FCGR* polymorphisms are associated with outcome.

Methods—Single nucleotide polymorphisms (SNPs) in *FCGR2A*, *FCGR3A*, and *FCGR2C* were analyzed, individually and in combination, for associations between genotype and clinical outcome.

Results—When higher affinity genotypes for *FCGR2A*, *FCGR3A* and *FCGR2C* were considered together, they were associated with significantly increased tumor shrinkage and prolonged survival in response to HD-IL2.

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Conclusions—While associations of higher affinity *FCGR* genotype with clinical outcome have been demonstrated with mAb therapy and with idiotype vaccines, to our knowledge, this is the first study to show associations of *FCGR* genotypes with outcome following HD-IL2 treatment. We hypothesize that endogenous anti-tumor antibodies may engage immune cells through their FCGRs, and HD-IL2 may enhance antibody-induced tumor destruction, or antibody-enhanced tumor antigen presentation, via augmented activation of innate or adaptive immune responses; this FCGR-mediated immune activity would be augmented through immunologically favorable FCGRs.

Keywords

FCGR; IL2; Immunotherapy; Renal Cell Carcinoma; Endogenous Antibody

INTRODUCTION

Patients with metastatic renal cell carcinoma (mRCC) show a 14%–22% response to the standard high-dose regimen of aldesleukin [interleukin-2 (IL2)]. Patients with mRCC were entered into the “SELECT” clinical trial of high-dose IL2 (HD-IL2) to prospectively determine if certain clinical and pathological criteria are associated with response to IL2. As noted in the clinical report, this study produced a response rate of 25% (1). In an effort to further identify genetic markers that might associate with efficacy of the HD-IL2 treatment for patients with mRCC, and potentially identify immunologic mechanisms involved in the response, we sought to identify genotypic factors that may influence the immune activity of HD-IL2 therapy. In this study we genotyped single nucleotide polymorphisms (SNPs) found in certain activating Fc-gamma Receptor (*FCGR*) genes (*FCGR2A*, *FCGR3A* and *FCGR2C*).

Variably expressed on immune cells, FCGRs bind the Fc fragment of IgG antibodies (2–4). Upon engagement and crosslinking, activating FCGRs transmit signaling within the immune cell and initiate immune activation (5–8). FCGR2A (expressed on dendritic cells, macrophages, monocytes, neutrophils, and eosinophils), FCGR3A (expressed on NK cells and macrophages), and FCGR2C (also expressed on NK cells) are all activating FCGRs (4, 9). The SNPs found in both *FCGR2A* and *FCGR3A* genes convey differential binding affinities for the Fc portion of antibody. The *FCGR2A* SNP encodes amino acids of either histidine (H) or arginine (R) at position 131 of the FCGR2A protein (FCGR2A-H131R, rs1801274), and the *FCGR3A* SNP encodes either valine (V) or phenylaline (F) at amino acid 158 of FCGR3A (FCGR3A-V158R, rs396991) (10–13). The FCGR2A-H and FCGR3A-V receptors each have higher binding affinities to human IgG than do the FCGR2A-R and FCGR3A-F receptors, respectively (2, 4, 12). This stronger binding affinity results in more potent *in vitro* antibody-dependent cell-mediated cytotoxicity (ADCC) and tumor cell death (14, 15). In some clinical trials involving various chimeric or humanized monoclonal antibodies (mAbs) specific for head and neck, colorectal, or B-cell malignancies, both FCGR2A-H and FCGR3A-V SNPs are associated with improved clinical response (14–17). Similarly, in a trial of an idiotypic vaccine for B-cell lymphoma, designed to induce endogenous anti-idiotypic antibody, better outcome was seen for patients with the higher affinity FCGR2A-H and FCGR3A-V SNPs (17). Alternatively, other studies have

found no association of FCGR2A-H/R or FCGR3A-V/F SNP genotype with patient response to immunotherapy (18–20).

The *FCGR2C* gene has a SNP in exon 3 (c.169 C<T, rs759550223) that influences the expression of FCGR2C on NK cell surfaces (21–23). The presence of a “C” nucleotide in this SNP leads to an open reading frame, enabling the expression of the FCGR2C receptor. In contrast, a “T” nucleotide creates a stop codon, resulting in lack of expression, for that allele (21, 22, 24). A minority of individuals (20–40%) have the “C” allele (either FCGR2C-C/C or C/T genotype), and thus have FCGR2C expressed on their NK cells (24–27). When expressed, FCGR2C is capable of inducing ADCC after receptor crosslinking (24, 25, 27). While the SNP of *FCGR2C* genotype has been correlated with patient response to immunomodulatory therapy for autoimmune-based diseases (25, 28–32), little has been published regarding the role of FCGR2C expression in cancer immunotherapy.

In this study of patients with mRCC who received HD-IL2, we looked for associations of patient *FCGR2A*, *FCGR3A* and *FCGR2C* genotypes with clinical outcome. We found that higher affinity *FCGR* genotypes resulted in improved tumor shrinkage and overall survival (OS). These findings suggest a potential role for cells expressing these FCGRs in the clinical response of patients with mRCC to HD-IL2 therapy.

METHODS

DNA

A total of 106 patients from the SELECT trial had DNA available for genotyping, along with clinical data for correlative analyses. DNA was isolated from peripheral blood mononuclear cells (PBMCs) following the manufacturer’s protocol of the DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA). DNA was kept at 4C during the time of analyses, and later was transferred to −80C for long term storage after completion of the analyses.

Genotyping

All SNP genotyping was performed on a StepOnePlus quantitative PCR machine (ABI/Life Technologies, Grand Island, NY). The *FCGR2A* SNP was determined using Taqman primer/probes available from ABI/Life Technologies and used per the manufacturers protocol. For both *FCGR3A* and *FCGR2C*, Rnase H primers and probes for each gene were developed in our lab to allow for specific amplification of each gene. For genotyping the *FCGR3A* SNP, Rnase H primers were developed to specifically amplify *FCGR3A* while not co-amplifying *FCGR3B*. These primers were paired with specific probes to determine the SNP (33). For genotyping the *FCGR2C*-C/T SNP, Rnase H primers were developed to specifically amplify this gene while not co-amplifying *FCGR2B*. Primers and probes for both *FCGR3A* and *FCGR2C* were designed through Integrated DNA Technologies (IDTDNA, Coralville, Iowa). Specific method details can be found in Erbe AK et al., 2016 (33). Genotyping was conducted in a blinded manner, where those individuals that determined the genotype of the patients did not have access to the clinical outcome data. Specific genotype results can be found in Supplementary Table 1.

Clinical data

The clinical results of the SELECT trial have been published (1). Clinical data for % tumor shrinkage and for OS were obtained from the clinical data set. Data were updated through October 31, 2013. The clinical characteristics of the patient subset we analyzed (106 patients of the 120 patients in the original trial) are similar to the clinical characteristics of those observed in the original study (Table 1). For our analysis of % tumor shrinkage, 2 patients did not have % tumor shrinkage clinical data available, and thus were excluded from the % tumor shrinkage analyses (104 for % tumor shrinkage).

Statistical methods

The clinical outcomes assessed included % tumor shrinkage and overall survival. The % tumor shrinkage was defined as the percent change in tumor size from baseline to maximum shrinkage. OS was defined as the time in months from the date of treatment initiation to the date of death, or was censored at the date of last contact with the patient. The association between % tumor shrinkage and genotyping predictors was evaluated using two-sample t-tests. The Kaplan-Meier method was used for estimation of the survival distribution for OS. For the survival plots, the tick marks along each line indicate patients censored; each drop of the line indicates a clinical event (i.e. patient death). Log-rank tests were used to assess the association between genotyping predictors and OS. The association between FCGR genotypes was assessed using Fisher's exact test. Changes in tumor size were represented using box plots, which show the 25th percentile (Q1) (bottom of box), the 50th percentile (Q2) (bolded black line), the 75th percentile (Q3) (top of box), and the mean (red cross inside the box). The lower and upper short horizontal red lines represent the minimum and maximum values, excluding the outlying high and low values. Outlying values [i.e.: those that are a distance of more than 1.5x(Q3-Q1) from the box], are shown as circles outside the horizontal lines. No adjustments in reported p-values were made for multiplicity of testing.

RESULTS

Individual FCGR3A, FCGR2A, and FCGR2C genotypes show associations with clinical outcome

In these mRCC patients treated with HD-IL2, we found that individuals homozygous for the high-affinity FCGR3A-V/V allele had significantly prolonged OS compared to those having only 1 or no copy of this high affinity allele (FCGR3A-V/V: 73.4 months vs. FCGR3A-V/F or F/F: 40.6 months; p=0.03, Table 2). Additionally, although not significant, the % tumor shrinkage for FCGR3A-V/V patients was greater than that for FCGR3A-V/F or F/F patients, (FCGR3A-V/V: 32.8% vs. FCGR3A-V/F or F/F: 9.4%; p=0.21, Table 2). For FCGR2A, those with the higher-affinity-genotype compared to lower-affinity receptors (H/H vs. H/R or R/R, respectively) had increased tumor shrinkage, although these differences were not significant (FCGR2A-H/H: 25.8% vs. FCGR2A-H/R or R/R: 7.1%; p=0.18, Table 2). There was no difference in OS based on FCGR2A genotype. Patients that express the FCGR2C receptor [those with at least one copy of the C allele (C/C or C/T)] had prolonged OS as compared to those that did not express FCGR2C on their NK cell surface (those with an *FCGR2C* genotype of T/T), however these differences were not significant (FCGR2C-C/C

or C/T: 73.3 months vs. FCGR2C-T/T: 40.6 months; p=0.12, Table 2). There were no differences in % tumor shrinkage based on FCGR2C genotype (Table 2).

Higher affinity FCGR2A and FCGR3A genotypes influence tumor shrinkage

Upon antibody recognition and binding, there may be crosstalk between cells that express FCGR2A and cells that express FCGR3A that can influence NK cell response (34). Prior studies of patients treated with mAb have reported associations between FCGR SNPs and clinical outcome when both genotypes of *FCGR2A* and *FCGR3A* were combined for the analyses (35–37). We compared individuals that were homozygous for either the H allele of higher-affinity FCGR2A or for the V allele of higher-affinity FCGR3A (Group-1 in Fig. 1A) with individuals that were not homozygous for either the higher affinity allele of FCGR2A or FCGR3A (Group-2 in Fig. 1A). We found significantly improved tumor shrinkage in Group-1 vs. Group-2 (Fig. 1B, $p<0.05$). Additionally, Group-1 also showed prolonged OS vs. Group 2, but this was not significant (p=0.17, Fig. 1C).

Higher affinity FCGR3A and expression of FCGR2C genotypes influence OS

NK cell ADCC capabilities can be enhanced if FCGR2C is expressed on the cell surface (26). Since NK cells can express both FCGR3A and FCGR2C, we considered whether patient outcome was influenced by the combined genotypes for FCGR3A and FCGR2C. Patients that have 2 copies of the high affinity FCGR3A allele (V/V) or one copy of the high affinity FCGR3A allele (V/F) and at least one copy of FCGR2C (C/C or C/T), or 2 copies of FCGR2C (C/C) are identified as Group-3 (boxes I, II, III, IV, V and VII) in Fig. 2A. All other patients are identified as Group-4, and include those with genotypes that have only one copy of the high affinity FCGR3A allele (V/F) and have no copy of FCGR2C (T/T), and those that have no copy of the high affinity FCGR3A allele (F/F) and have only 1 or no copy of FCGR2C (C/T or T/T), (boxes VI, VIII and VIIII in Fig. 2A). While there was no difference between Group-3 and Group-4 for % tumor shrinkage (Fig. 2B), Group-3 showed significantly prolonged OS compared to Group-4 (Fig. 2C, p=0.01).

Favorable overall FCGR3A/2A/2C genotypes influence clinical outcome

Based on our findings that patients with FCGR2A and FCGR3A genotypes in homozygous form resulted in prolonged OS (although not statistically significant) and significantly improved % tumor shrinkage (Fig. 1), as well as our finding that high-affinity FCGR3A-V in combination with the expression of FCGR2C resulted in significant improvement in the length of OS (Fig. 2), we further assessed the combined influence of all three of these FCGR genotypes on patient response. In order to simultaneously consider the genotype combinations for all three FCGRs studied here, we categorized patients into “favorable” and “unfavorable” groups (Fig. 3A) based on the genotypic patterns presented in Figs. 1A and 2A. The favorable group (shaded in Fig. 3A) included all patients homozygous for FCGR3A V/V or FCGR2A H/H, as well as patients with at least 2 higher affinity alleles of FCGR3A or FCGR2A (at least one copy of FCGR3A-V and at least one copy of FCGR2A-H) with FCGR2C expression (C/C or C/T), namely V/F-H/R patients, if they also expressed FCGR2C (C/C or C/T). This corresponded to 42 favorable-genotype patients. The remaining 64 patients (unshaded in Fig. 3A) are designated as unfavorable genotype.

Patients in the favorable FCGR genotype group had a significantly improved % tumor shrinkage as compared to those with “unfavorable” FCGR genotype (Fig. 3B, 28.5% vs. 1.7%; p=0.03). As depicted in the waterfall plot of % tumor shrinkage (Fig. 3C), those patients in the favorable group (red bars) are more prominent than those in the unfavorable group (blue bars) amongst those that showed tumor shrinkage rather than growth. Patients in the favorable group also showed a trend towards improved OS (Fig. 3D; 56.0 vs. 37.4 months for favorable vs. unfavorable groups; p=0.07).

DISCUSSION

While both the genotypes of the SNPs on *FCGR2A* and *FCGR3A* have been implicated in some analyses of the clinical anti-tumor response to tumor-reactive mAb immunotherapy, we believe this is the first study to show a potential association of favorable FCGR genotype with clinical outcome in the anti-tumor use of single-agent HD-IL2, without mAb administration. Moreover, FCGR2C expression based on SNP status has not yet been shown to influence clinical response to immunotherapeutics in cancer patients, in particular in patients not treated with mAb.

The data presented in Figs. 3B, C and D, show a significant association with % tumor shrinkage and a trend with OS when simultaneously considering genotypes for all 3 of these loci (*FCGR2A, 3A* and *2C*). The finding that there are associations of *FCGR* genotype with the clinical outcome parameters of both tumor shrinkage and OS appears to involve all 3 of these *FCGR* genes. This is consistent with data in Table 2, showing a significant role for FCGR3A in OS, as well as trends in improved % tumor shrinkage for FCGR2A, and improved OS for FCGR2C (although not statistically significant).

Although we found significant associations of *FCGR* genotype with both of these clinical parameters (% tumor shrinkage and overall survival), we did not see significant associations of patient *FCGR2A, 3A* and *2C* SNP genotype with patient overall response rate (data not shown). Since the overall response rate is based on data for % tumor shrinkage, but it is evaluated in binary form [responders (those with >50% tumor shrinkage) vs. non-responders (those with <50% tumor shrinkage)], it does not take into consideration the quantitative amount of tumor shrinkage. The waterfall analysis (Fig. 3C), which scores each patient based on their maximum amount of % tumor shrinkage, is based on quantitative measures and it is known to be more sensitive compared to overall response rate. This may account for the genotypic associations with % tumor shrinkage, but not with overall response status, found in this study.

Such associations of favorable *FCGR* genotypes and clinical outcome with HD-IL2 treatment do not prove a causal link. McDermott et al., 2015, reported that in the original cohort of patients treated with HD-IL2, in addition to the HD-IL2, 80 patients also received VEGF-targeted therapy. This additional VEGF-targeted therapy may have contributed to the OS length found in those individuals that were treated with it. Beyond the additional treatment measures (i.e. VEGF-targeted treatment) that may have influenced clinical response differences, genotypes that show an association with a clinical condition may do so because of their linkage disequilibrium to nearby loci that were not directly genotyped, yet

influence the clinical associations seen. The *FCGR* genes are located in close proximity to each other on chromosome 1q23, with *FCGR2A* located upstream [with genomic coordinates (GRCh38): 1:161,505,41–161,524,048] of *FCGR3A* [with genomic coordinates (GRCh38): 1:161,541,759–161,550,623] followed by *FCGR2C* [with genomic coordinates: Genomic coordinates (GRCh38): 1:161,581,339–161,601,220] (38). Using the genotype data for this population (Supplementary Table 1), we found a trend towards linkage disequilibrium between *FCGR3A* and *FCGR2A* (p=0.08), a significant disequilibrium between *FCGR3A* and *FCGR2C* (p<0.01), and no significant disequilibrium between *FCGR2A* and *FCGR2C* (p=0.70) (data not shown). This linkage disequilibrioum involving these 3 genes could contribute to the favorable FCGR genotype grouping found in this study, as shown in Fig. 3. Furthermore, although unlikely, these favorable *FCGR* gene alleles that are associated with better outcome in this study could potentially be in linkage disequilibrium with a favorable allele for some separate (non-*FCGR*) gene that might actually be responsible for the improved outcome we observe with the favorable *FCGR* genotype. The fact that some of the associations that we have observed are significant while others are trends, suggests that the effect of the favorable *FCGR* genes is one of several factors involved in the anti-tumor activity of HD-IL2 in some patients (but not others) with mRCC.

This association of outcome with favorable FCGRs suggests that greater functionality of FCGRs, due to higher-affinity (for FCGR2A and FCGR3A) and expression of FCGR2C, may be playing a role in at least part of the anti-tumor activity of HD-IL2. Our current understanding of these FCGRs is that they function primarily through engaging antigen-bound IgG, transmitting an activating signal, and inducing cellular responses, such as the induction of ADCC (by NK cells, neutrophils and monocytes/macrophages) or antibody dependent cellular phagocytosis (ADCP), and the uptake of antigens by antigen presenting cells through immobilized, bound IgG molecules, resulting in antigen processing and presentation (3, 4, 6, 8, 26). In each of these settings, an antigen-reactive antibody (either endogenous or passively administered) is needed for antibody/FCGR-facilitated ADCC, ADCP or antigen processing. The data presented here, showing that HD-IL2 treated mRCC patients with more functional FCGR genotypes showed increased tumor shrinkage and prolonged OS compared to those with less-functional FCGR genotypes supports the hypothesis that some of these patients may have formed endogenous antibodies, reactive with their autochthonous mRCC that were capable of mediating ADCC, ADCP and/or antigen presentation. The *in vivo* antitumor activity of such endogenous anti-tumor antibodies would potentially be enhanced by the presence of more favorable FCGRs.

In 1955, Graham and Graham suggested that some gynecologic oncology patients formed endogenous antibodies recognizing autologous tumor antigens, but these endogenous antibodies did not recognize the tumor antigens derived from tumors of similar histology from other patients (39). Since that time, several endogenous antibodies that are reactive against well-described and conserved shared tumor antigens have been identified, including antigens on RCC (40–44). For example, Knutson et al. 2016 recently showed that for HER2+ breast cancer patients, a combination therapy that included chemotherapy together with trastuzumab (mAb against HER2) induced, in 69% of patients, endogenous IgG-antibodies directed against HER2 and a subset of endogenous shared tumor-associated

antigens; this endogenous antibody response was associated with improved disease outcome (45). However, for most tumor types, methods to readily demonstrate and quantify the presence and functional activity of endogenous antibodies against the unique neo-antigens present on patients' autochthonous tumors, for the full cohort of patients enrolled in a trial such as this one, remain elusive. Thus, in this retrospective study, with no access to patient sera or to patient tumor tissue, we have not attempted to evaluate the presence or functionality of endogenous antibody to autochthonous tumor.

The interplay of several immune cell types, through engagement of their FCGRs via antibody-bound-antigen recognition, creates the potential for a successful immunotherapeutic response following treatment with mAb (46). Based on the associations reported here, of more functional FCGRs being associated with improved outcome with HD-IL2 therapy, we hypothesize the following immunological pathways may be involved. First, the presence of pre-existing endogenous tumor-reactive IgG antibodies might enable IL2 to induce augmented ADCC and ADCP, which would be enhanced by the presence of more functional FCGR alleles through crosstalk of NK cells (expressing FCGR3A and potentially FCGR2C) and monocytes (expressing FCGR2A) (34). Alternatively, the pre-existing anti-tumor antibodies might facilitate tumor-antigen presentation and induction of an adaptive (dendritic cell, T-cell and potentially B-cell) response, which could be augmented by the IL2 treatment and more functional FCGR. Finally, in some patients, more than one of these mechanisms could be at work simultaneously. The FCGR genotype combinations identified here have the potential to serve as biomarkers to personalize immunotherapeutics for cancer treatment (47). Future studies validating this association of favorable FCGR genotype with outcome, as well as prospective efforts to evaluate sera from all treated patients for functional antibody reactive to tumor (particularly autochthonous tumor), will be needed to test these hypotheses, and determine whether they lead to actionable clinical modifications in this approach towards immunotherapy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Financial Support: This research was supported by The Institute for Clinical and Translational Research; Hyundai Hope on Wheels Grant; Midwest Athletes Against Childhood; Stand Up To Cancer – St. Baldrick's Pediatric Dream Team Translational Cancer Research Grant, Grant Number SU2C-AACR-DT11-13 – Stand Up To Cancer is a program of the Entertainment Industry Foundation administered by the American Association for Cancer Research; University of Wisconsin-Madison Carbone Cancer Center; by NCI-Cytokine Working Group and supported in part by Public Health Service Grants CA014520, CA021115, CA023318, CA066636, CA180820, CA180794, CA021076, CA180799, CA14958, CA180816, CA166105, and CA197078, from the National Cancer Institute; the National Institutes of Health and 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.

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Statement of Translational Relevance

Associations with clinical outcome were found in this study in individuals that have a "favorable" *FCGR* genotype (higher-affinity alleles of FCGR2A and FCGR3A with expression of FCGR2C), suggesting that greater functionality of FCGRs plays a role in the anti-tumor activity of high-dose IL2 for patients with metastatic renal cell cancer (mRCC). The data presented in this report suggest that FCGRs may play a role in the *in vivo* antitumor effect seen in mRCC patients receiving high-dose IL2, raise important hypotheses for future research that may focus on the potential role of endogenous anti-tumor antibody, and indicate that future work should be pursued to test whether the combined analyses of FCGR3A/2A/2C genotypes may become a useful biomarker for prospective clinical planning and retrospective outcome analyses for other clinical trials of cancer immunotherapy that may involve NK cells or other FCGR-bearing immune cells.

A.

		FCGR3A		
		V/V	V/F	F/F
FCGR2A	H/H	I	II	III
	H/R	IV	V	VI
	R/R	VII	VIII	VIIII
	n's			
		Group 1: I, II, III, IV, VII	Group 2: V, VI, VIII, VIIII	Total
	3A and 2A OS n (% Tumor Shrinkage n)	35 (34)	71 (70)	106 (104)

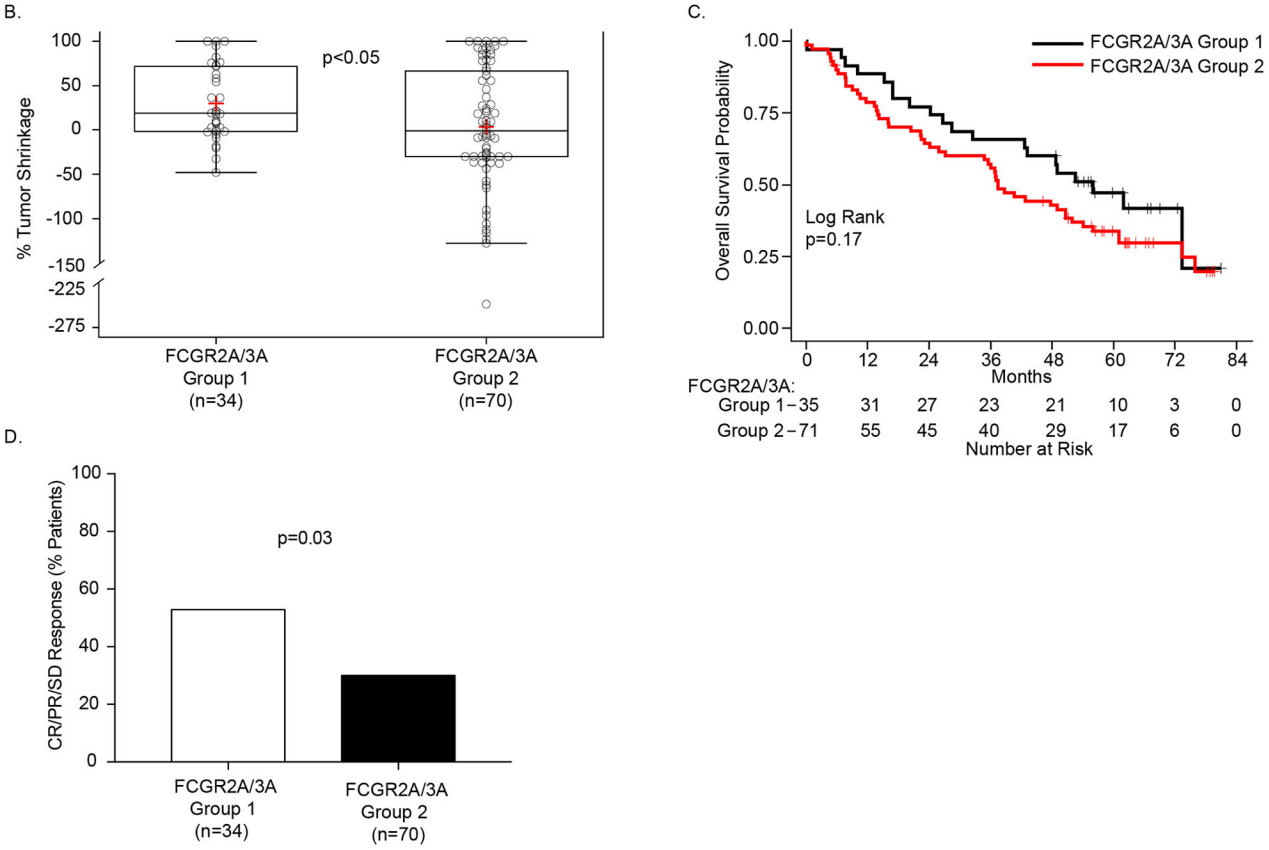

Figure 1. FCGR2A and FCGR3A higher-affinity genotypes resulted in improved % tumor shrinkage

A) The 3 separate genotypes for FCGR3A (V/V, V/F and F/F), when combined with the 3 separate genotypes for FCGR2A (H/H, H/R and R/R), yield 9 separate genotypes, each in a separate box designated: I–VIIII. Those patients with homozygous expression of either V/V or H/H (cells I, II III, IV, and VII) are included in Group-1 (n=35 patients for OS, n=34 for % Tumor Shrinkage), all remaining patients (neither V/V nor H/H) are included in Group-2 (n=71 patients for OS, n=70 for % Tumor Shrinkage). **B)** Patients in Group-1 (homozygous for either V/V or H/H) show a significant increase in the % tumor shrinkage as compared to those in Group-2 (not homozygous for either V/V or H/H). **C)** OS for Group-1 was prolonged vs. that for Group-2, although not significant.

A.

		FCGR3A		
		V/V	V/F	F/F
FCGR2C	C/C	I	II	III
	C/T	IV	V	VI
	T/T	VII	VIII	VIIII
	n's			
		Group 3: I, II, III, IV, V, VII	Group 4: VI, VIII, VIII	Total
	3A and 2C OS n (% Tumor Shrinkage n)	27 (27)	79 (77)	106 (104)

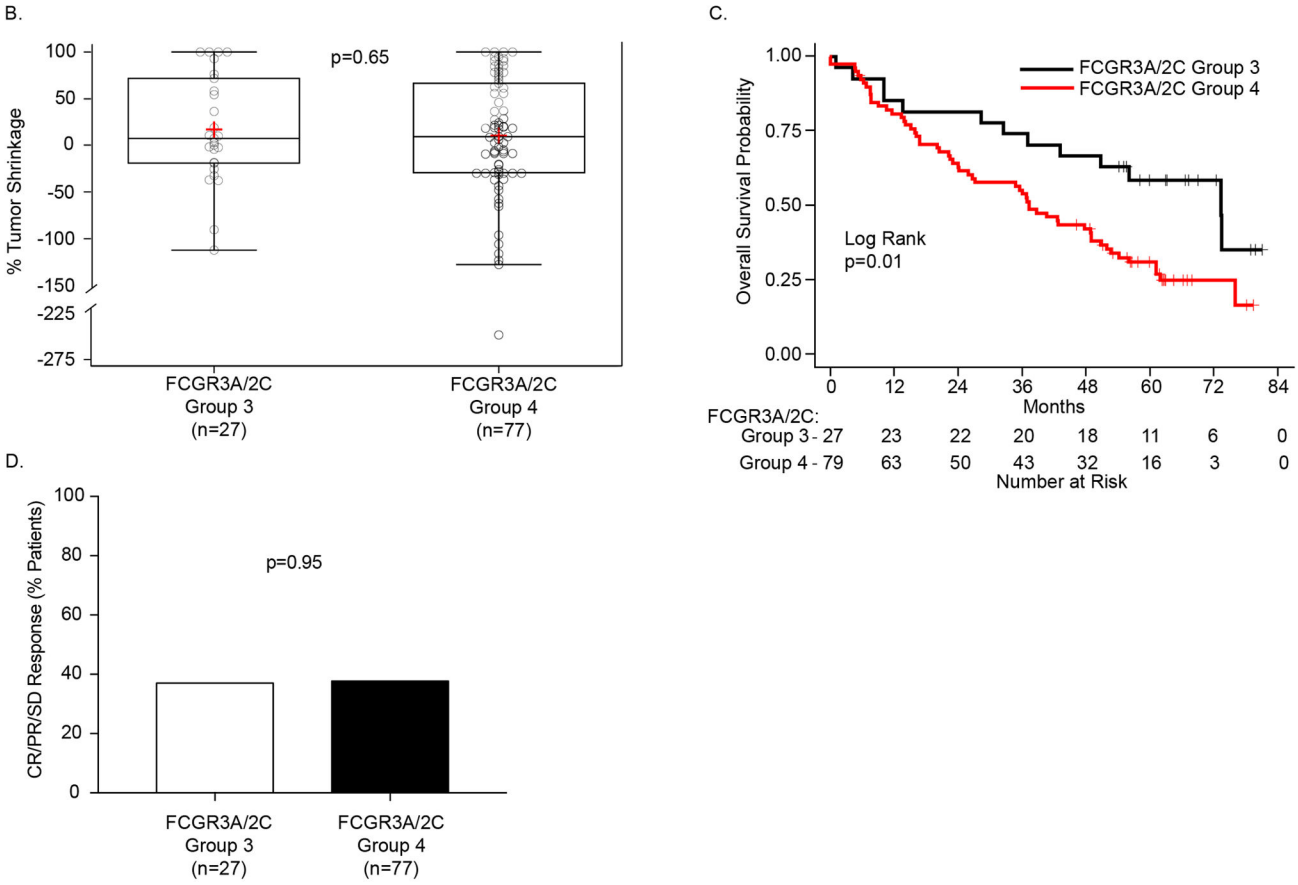

Figure 2. FCGR3A and FCGR2C are associated with OS

A) The 3 separate genotypes for FCGR3A (V/V, V/F and F/F), when combined with the 3 separate genotypes for FCGR2C (C/C, C/T and T/T), yield 9 separate genotypes, each in a separate box designated: I–VIIII. Since expression of FCGR2C (via C/C or C/T genotypes) can influence FCGR3A (2), we included those heterozygous for both FCGR2C and FCGR3A (box V). Thus, those patients with homozygous expression of either V/V or C/C (cells I, II III, IV, and VII) or with heterozygous expression for both (cell V) are included in Group-3 (n=27 patients for both OS and % Tumor Shrinkage), all remaining patients are included in Group-4 (n=79 patients for OS, n=77 for % Tumor Shrinkage). **B)** Patients in

Group-3 show no difference in the % tumor shrinkage as compared to those in Group-4, however **C)** OS for Group-3 was significantly prolonged vs. that for Group-4.

A.

				FCGR3A		
				V/V	V/F	F/F
FCGR2A	H/H	FCGR2C	C/C	A.I	A.II	A.III
			C/T	A.IV	A.V	A.VI
			T/T	A.VII	A.VIII	A.VIIII
				FCGR3A		
	H/R			V/V	V/F	F/F
		FCGR2C	C/C	B.I	B.II	B.III
			C/T	B.IV	B.V	B.VI
			T/T	B.VII	B.VIII	B.VIIII
				FCGR3A		
	R/R			V/V	V/F	F/F
		FCGR2C	C/C	C.I	C.II	C.III
			C/T	C.IV	C.V	C.VI
			T/T	C.VII	C.VIII	C.VIIII

		n's		
		"Favorable": A.I-A.VIIII; B.I-B.V, B.VII; C.I, C.IV, C.VII	"Unfavorable": B.VI, B.VIII, B.VIIII; C.II, C.III, C.V, C.VI, C.VIII, C.VIIII	Total
	2A, 3A and 2C OS n (% Tumor Shrinkage n)	42 (41)	64 (63)	106 (104)

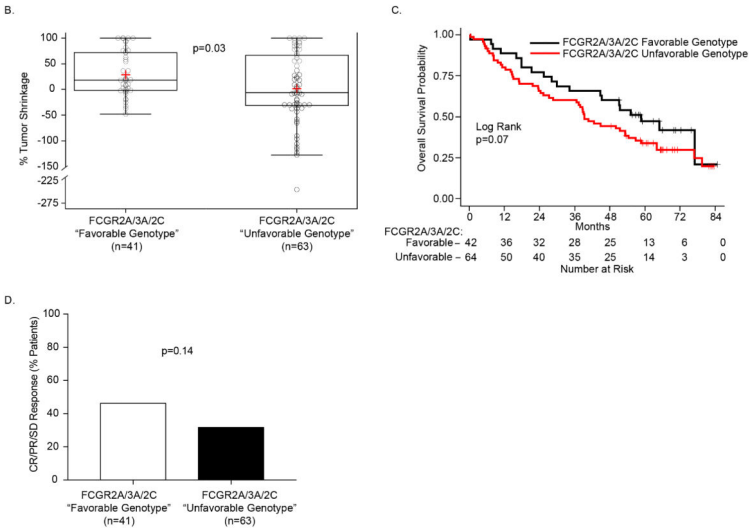

Figure 3. The combination of FCGR2A, FCGR3A and FCGR2C SNPs is associated with % tumor shrinkage and OS

A) The 3 separate genotypes for FcgR3a (V/V, V/F and F/F), when combined with the 3 separate genotypes for FCGR2c (C/C, C/T and T/T) yield 9 separate genotypes. Here these are combined with the 3 separate genotypes for FcGR2A: H/H (upper panel); H/R (center panel); and R/R (lower panel). When genotypes for all 3 of these loci are combined (27 separate boxes), we divided them into favorable (shaded in Fig. 3A) vs. unfavorable (unshaded) genotypes. The favorable group includes all patients homozygous for H/H or V/V, as well as patients heterozygous for H/R if also expressing V/V or at least one copy of FCGR2C-"C", and patients negative for H (ie: R/R patients) if they are V/V homozygotes, corresponding to 42 patients for OS, 41 patients for % Tumor Shrinkage. All others, namely those patients that do not have at least 2 copies of either high affinity allele (F/F-R/R, V/F-R/R or F/F-H/R), and those patients heterozygous for V/F and H/R but lacking any expression of FCGR2C are unshaded and labeled as unfavorable (n=64 patients for OS, n=63 patients for % Tumor Shrinkage). **B)** Patients with a favorable genotype show improved % tumor shrinkage as compared to those in the unfavorable group (p=0.03). **C)** This predominance of patients with favorable genotype (red) vs. unfavorable genotype (blue) amongst those with tumor shrinkage is also seen in the right side of the waterfall plot for % tumor shrinkage for all 105 patients evaluable for this analysis. **D)** A trend towards better OS was also seen for patients with favorable genotype (p=0.07)

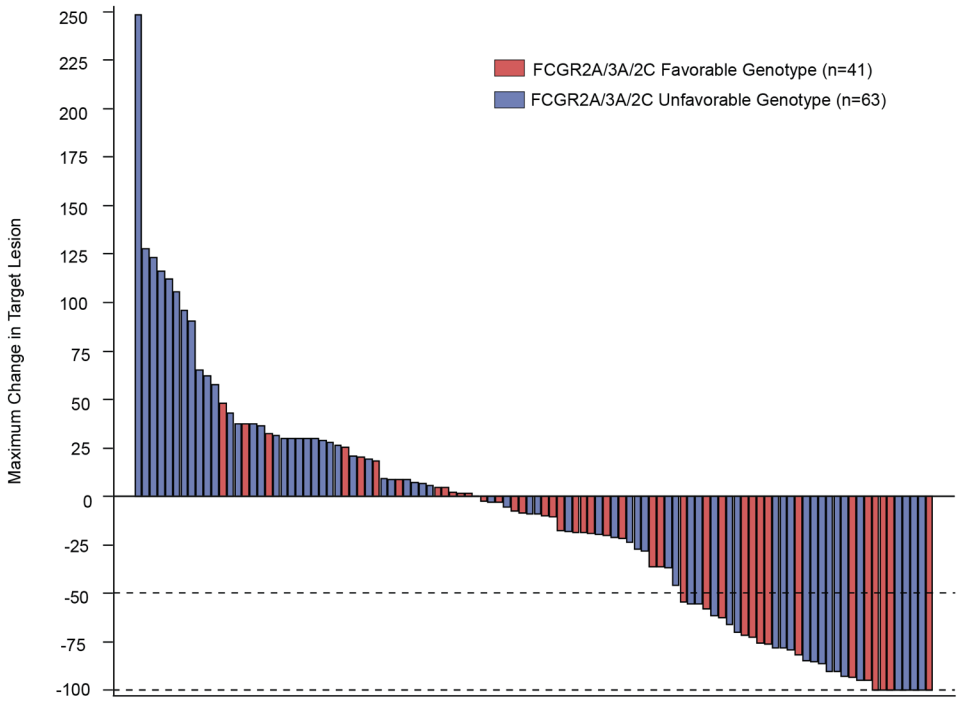

Figure 4.

Table 1

Patient characteristics from original SELECT Trial and the subset of patients analyzed in this study.

	Total Patients Enrolled	FCGR Genotyped Pts
Characteristics	**n = 120**	**N = 106**
Median age, y (range)	56 (28–70)	56 (28–70)
ECOG performance status (0/1), %	72/24	71/25
Prior nephrectomy, %	99	99
MSKCC risk factor, n (%)		
0 (favorable)	23 (19)	22 (21)
1–2 (intermediate)	84 (70)	72 (68)
≥3 (poor)	13 (11)	12 (11)
UCLA SANI Score, n (%)		
Low	10 (8)	10 (9)
Intermediate	102 (85)	88 (83)
High	8 (7)	8 (8)

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

Table 2

Clinical Outcome by FCGR2A, FCGR3A and FCGR2C SNP. The amount of % tumor shrinkage and the duration of the OS for each FCGR were compared for individual FCGRs.

		Tumor Shrinkage		Overall Survival		Disease Control Rate		
	Genotype Group	Mean (%)[Std Dev]	p-value	Median (months) [95% CI]	p-value	n	CR/PR/SD	p-value
FCGR3A SNP	**VV**	32.8 [45.5] (n=13)	0.21	73.4 [32.5-NR][A] (n=13; #Events=5)	0.03	13	53.8%	0.23
	VF or FF	9.4 [64.7] (n=91)		40.6 [27.2–50.7] (n=93; #Events=65)		91	35.2%	
FCGR2A SNP	**HH**	25.8 [39.8] (n=29)	0.18	48.8 [26.7–61.9] (n=30; #Events=19)	0.85	29	51.7%	0.07
	HR or RR	7.1 [69.4] (n=75)		40.6 [34.8–56.0] (n=76; #Events=51)		75	32.0%	
FCGR2C SNP	**CC or CT**	12.0 [61.2] (n=31)	0.97	73.3 [32.5-NR][A] (n=31; #Events=17)	0.12	31	35.5%	1.00
	TT	12.4 [64.1] (n=73)		40.6 [26.7–50.7] (n=75; #Events=53)		73	38.4%	

[A]The value “NR” is reported where the Median Overall Survival is “Not Reached”.