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ORIGINAL PAPER

A biomarker signature to predict complete response to itacitinib and corticosteroids in acute graft-versus-host disease

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Summary

A broad proteomic analysis was conducted to identify and evaluate candidate biomarkers potentially predictive of response to treatment with an oral selective Janus kinase 1 (JAK1) inhibitor, itacitinib, in acute graft-versus-host disease (GVHD). Plasma samples from 25 participants (identification cohort; NCT02614612) were used to identify novel biomarkers that were tested in a validation cohort from a placebo-controlled, randomised trial (n = 210; NCT03139604). The identification cohort received corticosteroids plus 200 or 300 mg itacitinib once daily. The validation cohort received corticosteroids plus 200 mg itacitinib once daily or placebo. A broad proteomic analysis was conducted using a proximity extension assay. Baseline and longitudinal comparisons were performed with unpaired t-test and one-way analysis of variance used to evaluate biomarker level changes. Seven candidate biomarkers were identified. Monocyte-chemotactic protein (MCP)3, pro-calcitonin/calcitonin (ProCALCA/CALCA), together with a previously identified prognostic acute GVHD biomarker, regenerating islet-derived protein (REG)3A, stratified complete responders from non-responders (participants with progressive disease) to itacitinib, but not placebo, potentially representing predictive biomarkers of itacitinib in acute GVHD. ProCALCA/CALCA, suppressor of tumorigenicity (ST)2, and tumour necrosis factor receptor (TNFR)1 were significantly reduced over time by itacitinib in responders, potentially representing response-to-treatment biomarkers. Novel biomarkers have the potential to identify patients with acute GVHD that may respond to itacitinib plus corticosteroid treatment (NCT02614612; NCT03139604).

KEYWORDS
acute graft-versus-host disease, biomarkers, itacitinib, JAK inhibitors

INTRODUCTION

Acute graft-versus-host disease (GVHD) is a serious complication occurring in 50%–70% of participants who undergo allogeneic haematopoietic cell transplantation.[1] Diagnosis and severity grading are primarily based on clinical assessment of key organ systems including skin, gastrointestinal tract, and liver.[2] However, the scoring system is often imprecise, requiring histological confirmation that can be inconclusive or subjective. Therefore, acute GVHD is difficult to distinguish from other causes of organ dysfunction.[3,4]

Corticosteroids are the preferred first-line systemic therapy[5]; however, only 40%–60% of participants respond.[6–8]

Non-responders have a dismal outlook, with mortality approaching 80%.[8–11] Adverse events associated with prolonged steroid use are well documented and include immunosuppression, osteopenia, hyperglycaemia, and cataracts.

Acute GVHD response to therapy has traditionally been measured by changes in clinical symptoms and reduction in overall grade after 4 weeks of therapy; however, this approach is imprecise with poor predictive value. Therefore, biomarkers have been pursued for acute GVHD because of their ability to predict mortality,[12,13] to measure and predict severity,[14] and to potentially predict responses to new therapies. The first validated panel of acute GVHD biomarkers consisted of interleukin 2 receptor alpha chain (IL2RA), tumour necrosis factor receptor (TNFR)1, hepatocyte growth factor, and IL8 and confirmed an acute GVHD diagnosis in 85% of participants at symptom onset.[13] Improved risk stratification for treatment-resistant GVHD and death without relapse was observed by measuring suppressor of tumorigenicity (ST)2 levels at therapy initiation and the first month after transplant.[15] The Mount Sinai Acute GVHD International Consortium (MAGIC) has reported biomarkers predicting 6-month non-relapse mortality and risk of treatment failure based on levels of TNFR1, ST2, and regenerating islet-derived protein (REG)3A.[12] More recently, ST2 and REG3A were shown to predict long-term outcomes in steroid-resistant (SR) GVHD better than clinical criteria; additionally, these markers may be used in clinical practice to help guide how aggressively to treat acute GVHD at initial presentation.[14,16,17]

Recent efforts have focused on development of new therapies that enable steroid taper or discontinuation.[18] Although multiple agents have been studied in combination with corticosteroids as both first-line[19–24] and SR-acute GVHD[5,25–27] treatment, most combined therapies evaluated have provided modest or no benefit over corticosteroids alone.[19–24,28] One exception is a sirolimus trial (NCT02806947), which was the first prospective study using biomarkers to stratify participants with standard-risk acute GVHD prior to treatment.[18] Recent success through Janus kinase (JAK) inhibition was reported using an oral, broad-spectrum JAK1/JAK2 inhibitor in patients with SR-acute GVHD.[29] Similar success was also reported using an oral, selective JAK1 inhibitor, itacitinib, plus corticosteroids in a phase I trial (NCT02614612).[30] A phase III study (NCT03139604) evaluated itacitinib plus corticosteroids as an initial treatment for acute GVHD.[31] Although the study did not meet its primary end-point (Day 28 observed response), assumptions used in powering the study led to over enrolment of patients with standard-risk or Grade II acute GVHD and over performance of the placebo group based on historical data of corticosteroid monotherapy.[31] Thus, additional data will be required to determine if itacitinib provides clinical benefit for patients with acute GVHD.

Part 1 of the present study aimed to identify candidate predictive biomarkers and response-to-treatment biomarkers of itacitinib (plus corticosteroids) from patient samples obtained from a phase I clinical trial (NCT02614612; identification cohort).[32] In part 2, samples from the phase III trial (NCT03139604[31]; validation cohort) were used to validate biomarkers identified in part 1. Other than one study that was performed on retrospective sets[15] and another study that was performed on an interventional prospective set using previously identified proteomic profiles,[33] to our knowledge, this is the first study performing discovery proteomics in interventional prospective sets.

METHODS

Part 1 (identification cohort)

A total of 10 steroid-naive and 18 steroid-refractory participants with acute GVHD were enrolled in a phase I clinical trial (NCT02614612). Plasma samples were collected from 27 participants at screening/baseline (Day 1) and Days 7, 14, and 28 after treatment. Based on the Center for International Blood and Marrow Transplant Research (CIBMTR) Response Criteria at Day 28, participants were separated into responders (including complete responders [CR], $n = 10$; very good partial responders [VGPR], $n = 1$; and partial responders [PR] $n = 8$) and non-responders (including mixed responders [MR], $n = 2$; and progressive disease/death [PD], $n = 6$; Table S1). The MRs were not included for further analysis.

Identification cohort participants ($n = 25$) were treated with corticosteroids plus either 200 mg ($n = 13$) or 300 mg ($n = 12$) itacitinib once daily. Clinical response did not vary significantly between the two doses[30]; thus, data were combined. Because of limited sample size, steroid-naive (9) and steroid-refractory (16) participants were also combined.

Part 2 (validation cohort)

An interim analysis from the phase III clinical trial (NCT03139604)[31] was used in part 2 as a validation cohort including the first 237 participants (210 evaluable: $n = 101$, itacitinib 200 mg; $n = 109$, placebo). Serum samples were collected at screening/baseline (Day 1) and Days 7, 14, and 28 after treatment. Based on CIBMTR Response Criteria at Day 28, participants were separated into responders (CR [$n = 52$ itacitinib, $n = 46$ placebo], VGPR [$n = 13$ itacitinib, $n = 17$ placebo], and PR [$n = 9$ itacitinib, $n = 17$ placebo]) and non-responders (MR [$n = 6$ itacitinib, $n = 4$ placebo] and PD [$n = 20$ itacitinib, $n = 23$ placebo]; Table S1). The MRs were not included for further analysis.

Day 28 response was chosen as a key efficacy end-point in both trials because it has been reported to predict long-term outcomes.[34] Overall, 74.0% of participants with treatment-naive acute GVHD responded to itacitinib at Day 28.[31]

Proteomic analysis (identification cohort)

Broad proteomic analysis of plasma samples was conducted using a proximity extension assay (PEA) as described by the manufacturer (OLINK Proteomics, Watertown, MA, USA).[35] The protein library consists of >1000 proteins. Data

are presented as normalised protein expression (NPX) in $\log_2$ scale. Fold change (FC) was calculated based on NPX values in CR (n = 10) versus PD (n = 6), with a negative FC reflecting proteins downregulated and a positive FC reflecting proteins upregulated in CR relative to PD.

Biomarker quantitation (identification and validation cohorts)

For each biomarker, a commercial source of a recombinant protein used as standard and detection antibodies were identified. PEAs were developed for each biomarker to quantitate levels in biological fluids after extrapolation from a standard curve developed in the same biological matrix.

Biomarker comparison to commercial platform

Comparison of the quantitative PEA to a commercial platform (Protein Simple Ella) was based on quantitation of several biomarkers in baseline samples from the identification cohort (ST2, REG3A, TNFR1, pro-calcitonin/calcitonin [ProCALCA/CALCA] and monocyte-chemotactic protein [MCP]3), per manufacturer recommendations.[36]

The calcitonin antibody used in the quantitative PEA is directed against amino acids 26–117, suggesting it will detect both CALCA and ProCALCA. According to the manufacturer, the corresponding ProCALCA antibody used in the Protein Simple assay should theoretically detect both the pro and active forms.

Statistical approach

All biomarker data in picograms per millilitre are presented as log-transformed values (natural log). For baseline comparisons, statistical analysis comparing protein levels between CR and PD participants in the identification cohort was performed using an unpaired t-test and corrected for multiple comparisons by false discovery rate.[37]

Proteins with a FC > 1.2 and $p < 0.05$ between CR and PD participants were selected as candidates for the quantitative assay. Proteins were assessed for importance using lasso logistic regression and decision tree analyses. A one-way analysis of variance (ANOVA) was used to determine significant changes of each quantitated candidate biomarker among CR, VGPR/PR, and PD/death cohorts.

For comparisons within each treatment arm of the validation cohort, a one-way ANOVA was used to determine significant changes between baseline and post-treatment. For longitudinal comparisons between treatment arms, a repeated-measures mixed-model was conducted. Additionally, an unpaired t-test was used to compare differences between treatment arms at each time point.

Correlation between detection platforms was calculated using Pearson correlation. A $p < 0.05$ was deemed statistically significant.

RESULTS

Part 1: Identification of candidate predictive biomarkers

Baseline plasma samples from the identification cohort were used to identify candidate predictive biomarkers differentially expressed in CR (n = 10) or PD (n = 6) in response to itacitinib and corticosteroid combined therapy to identify levels of protein expression with the greatest difference. Based on standard risk score,[7] 36% of patients were standard risk and 64% were high risk. A total of 130 differentially expressed proteins between the CR and PD groups were identified. In all, 55 proteins were increased and 75 were decreased in CR compared with PD (Figure 1; Table S2).

The list of predictive biomarker candidates was further refined based on (i) correlation to known acute GVHD biomarkers, including ST2, REG3A, and TNFR1, to identify independent pathways from previously reported biomarkers; (ii) response to treatment at Day 28 to identify predictive biomarkers that may also represent response-to-treatment biomarkers (i.e., ProCALCA/CALCA); (iii) availability of commercial sources of recombinant proteins and antibodies representing the candidate biomarkers to ensure reagents were available to develop the quantitative PEA and (iv) successful quantitation from a standard curve to ensure accurate quantitation of the native candidate biomarker based on a recombinant protein standard. Seven biomarkers were selected (Table S3).

Quantitation of candidate predictive biomarkers

The seven candidate predictive biomarkers from the identification cohort were measured in a quantitative PEA. Table S3 lists the range at baseline of each biomarker in CR versus PD participants. A significant difference between CR versus VGPR/PR and/or PD was observed for MCP3, C-X-C motif chemokine 10 (CXCL10), TNFR superfamily member 6b (TNFRSF6B), ProCALCA/CALCA, paraoxonase 3 (PON3), and c-Kit ligand (Stem Cell Factor; Figure 2). Detection of REG3A, ST2, TNFR1, and IL2RA was also included to allow for comparison to previously validated prognostic biomarkers,[16] and IL6 and IL8 represent cytokines on JAK/signal transducer and activator of transcription (STAT)-mediated pathways. Although interferon-γ was also measured, levels were below the limit of detection (data not shown).

Part 2: Validation of candidate predictive biomarkers

A total of 13 candidate biomarkers, listed in Figure 2, were validated against serum samples taken at baseline from a validation cohort representing the first 210 participants enrolled in a phase III trial.[31] Responses for the validation cohort were classified per protocol (Figure 3). Based on

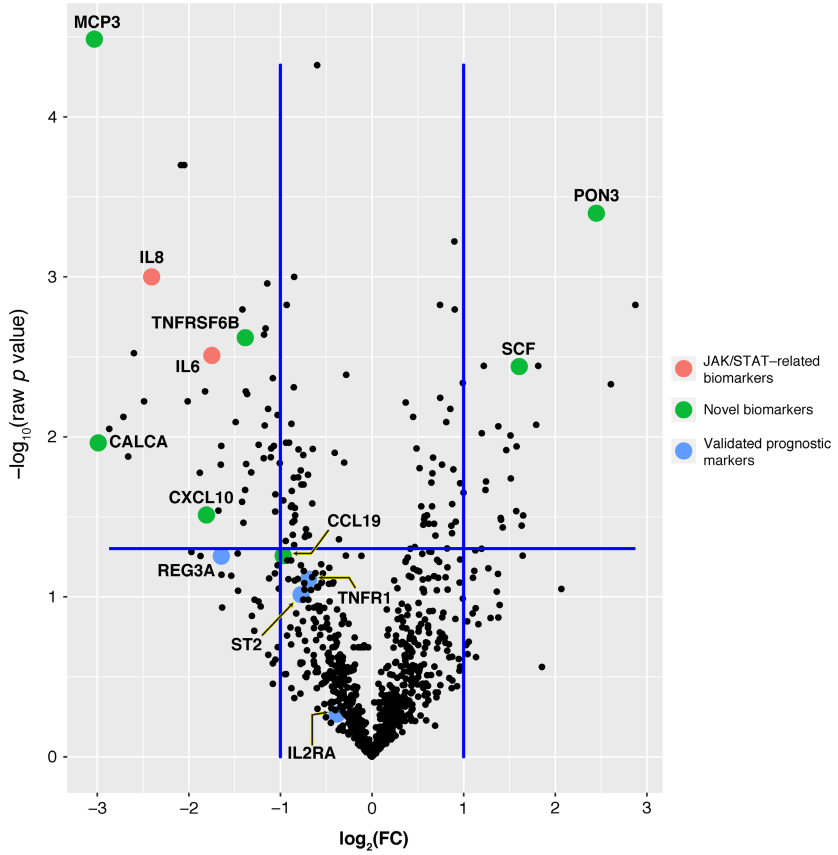

FIGURE 1 Volcano plot representing differentially expressed proteins at baseline in plasma of CR compared with PD groups in identification cohort. Broad proteomic analysis of plasma samples was conducted by OLINK proteomics (Watertown, MA, USA) using a proximity extension assay as described by the manufacturer. The protein library consists of >1000 proteins. Data are presented as NPX in $\log_2$ scale. FC was calculated based on NPX values in CR ($n = 10$) and PD ($n = 6$). A negative FC represents proteins downregulated in CR relative to PD; a positive FC represents proteins upregulated in CR relative to PD. In the volcano plot shown, the x-axis is the $\log_2$ transformed FC, calculated as $\log_2$(FC), and y-axis is the negative log p value, calculated as $-\log_{10}$(raw p value). Identity of the novel predictive candidate biomarkers is shown in green, JAK/STAT-related biomarkers are shown in red, and previously validated prognostic biomarkers are shown in blue. Identity of all other proteins illustrated is listed in Table S2. CALCA, calcitonin; CCL19, C-C motif chemokine 19; CR, complete responder; CXCL10, C-X-C motif chemokine 10; FC, fold change; IL2RA, interleukin 2 receptor alpha chain; IL6, interleukin-6; IL8, interleukin-8; JAK, Janus kinase; MCP3, monocyte-chemotactic protein 3; NPX, normalised protein expression; PD, progressive disease or death; PON3, paraoxonase 3; REG3A, regenerating islet-derived protein; SCF, kit ligand; ST2, suppressor of tumorigenicity 2; STAT, signal transducer and activator of transcription; TNFR1, tumour necrosis factor receptor 1; TNFRSF6B, TNF receptor superfamily member 6b.

standard-risk score,[7] 78% of participants were considered standard risk and 22% were considered high risk. Two treatment arms, itacitinib (plus corticosteroid) versus placebo (corticosteroid alone), were compared. Participants were stratified based on CR or PD within each treatment arm to identify levels of protein expression with the greatest difference. Participants with PRs (VGPR, PR) or MR were not included in the baseline analyses. Baseline serum biomarker levels of the subset of participants with CR or PD from the validation cohort are listed in Table S4.

The levels of several biomarkers in CR and PD for each treatment arm are shown in Figure 4. Two of the novel candidate biomarkers identified, MCP3 and ProCALCA/CALCA, stratified CR and PD in the itacitinib arm exclusively ($p < 0.05$), suggesting they may represent candidate predictive biomarkers of CR to JAK inhibition. Furthermore, this finding supports validation of these candidate predictive biomarkers initially selected from the identification cohort.

Interestingly, one of the previously validated prognostic biomarkers, REG3A, also stratified CR and PD exclusively in the itacitinib arm. ST2 represents a candidate predictive biomarker that stratified CR and PD cohorts in both the itacitinib and placebo groups, suggesting it is a systemic marker of inflammation (Figure 4; Table S4). A third group, including TNFRSF6B and IL6, stratified CR and PD only in the placebo group, suggesting they represent candidate predictive biomarkers of corticosteroid response (Table S4).

Longitudinal analysis of candidate predictive biomarkers in validation cohort

Longitudinal data were plotted over time (i) to compare the treatment effect of itacitinib versus placebo on biomarker levels at specific time points (Figure 5) and (ii) to compare

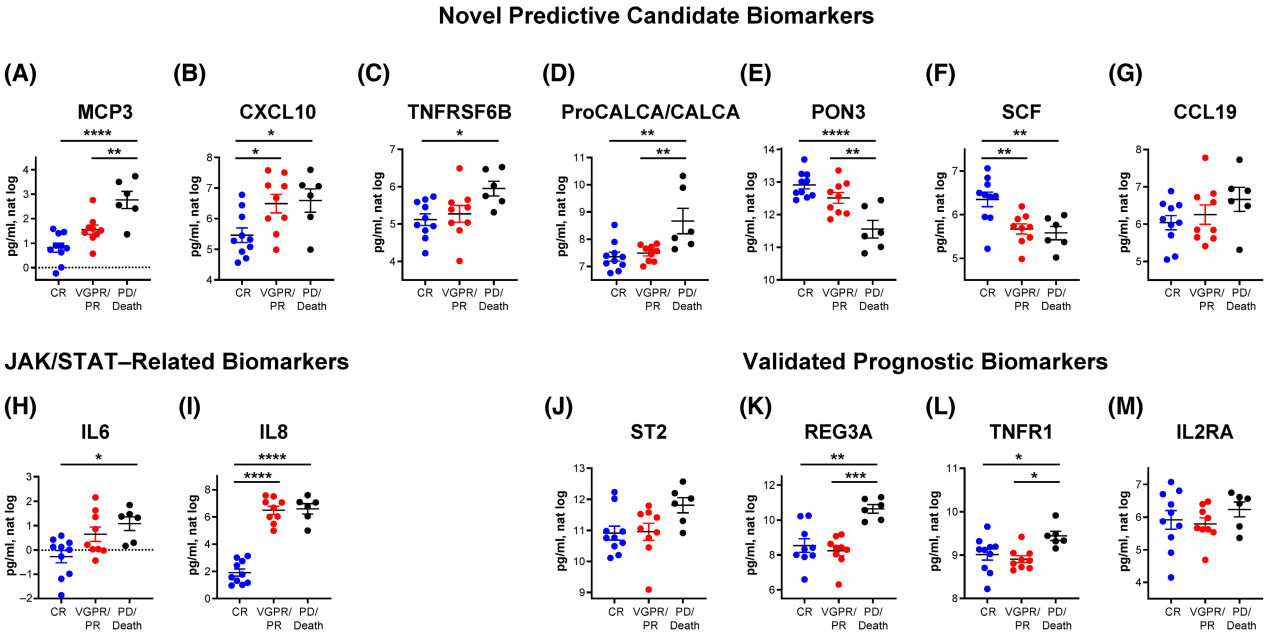

FIGURE 2 Baseline levels of novel predictive candidate biomarkers, JAK/STAT-related biomarkers, and previously validated prognostic biomarkers in identification cohort. Baseline plasma samples from CR and PD participants (or participants who died) and from participants with an intermediate response, including VGPR and PR, were assayed for biomarker levels by proximal extension assay. Statistical analysis was by one-way ANOVA. Significance was conferred when $p < 0.05$. The lines over particular cohorts indicate comparison of those cohorts with p values indicated. ANOVA, analysis of variance; CCL19, C-C motif chemokine 19; CR, complete responder; CXCL10, C-X-C motif chemokine 10; IL2RA, interleukin 2 receptor alpha chain; IL6, interleukin 6; IL8, interleukin 8; JAK, Janus kinase; MCP3, monocyte-chemotactic protein 3; nat, natural; PD, progressive disease; PON3, paraoxonase 3; ProCALCA/CALCA, pro-calcitonin/calcitonin; PR, partial responder; REG3A, regenerating islet-derived protein; SCF, kit ligand; ST2, suppressor of tumorigenicity 2; STAT, signal transducer and activator of transcription; TNFR1, tumour necrosis factor receptor 1; TNFRSF6B, TNF receptor superfamily member 6b; VGPR, very good partial responder; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$; CR, blue; PD/death, black; VGPR/PR, red.

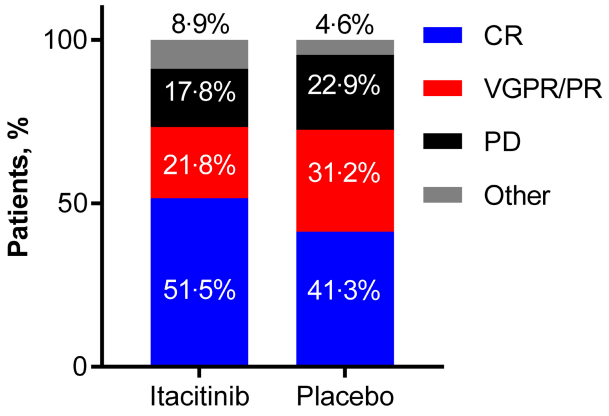

FIGURE 3 Comparison of day 28 treatment response between itacitinib versus placebo in validation cohort. Day 28 treatment responses from itacitinib ($n = 101$) and placebo ($n = 109$) treatment arms from the validation cohort are presented. PD includes participants whose disease progressed during treatment and/or who died before day 28. The 'other' category includes mixed responders, non-responders, and unclassified participants. CR, complete responder; PD, progressive disease/death; VGPR/PR, very good partial responder/partial responder.

to relative baseline biomarker levels (Table S5). By 7 days post-treatment, the levels of ProCALCA/CALCA were significantly reduced by itacitinib versus placebo (Figure 5). ProCALCA/CALCA levels were also significantly reduced versus baseline with itacitinib treatment, but not placebo (Table S5).

Although ST2 levels did not change versus baseline (Table S5), there were significant differences between treatment arms by Day 7 (Figure 5). TNFR1 had a similar profile to ProCALCA/CALCA, with significant changes exclusively in the itacitinib arm versus baseline (Table S5). TNFR1 also demonstrated significant changes longitudinally exclusively in the itacitinib arm (Figure 5). Longitudinal analysis of the other candidate predictive biomarkers, MCP3 and REG3A, suggest minimal changes in response to itacitinib versus placebo.

Baseline comparison of identification and validation cohorts

The overlaid distribution of each biomarker at baseline from identification and validation cohorts is shown in Figure 6. Although baseline levels of several proteins between the two cohorts were similar (ProCALCA/CALCA, SCF, REG3A, TNFR1), baseline levels of most proteins (9 of 13) were statistically different between the two trials.

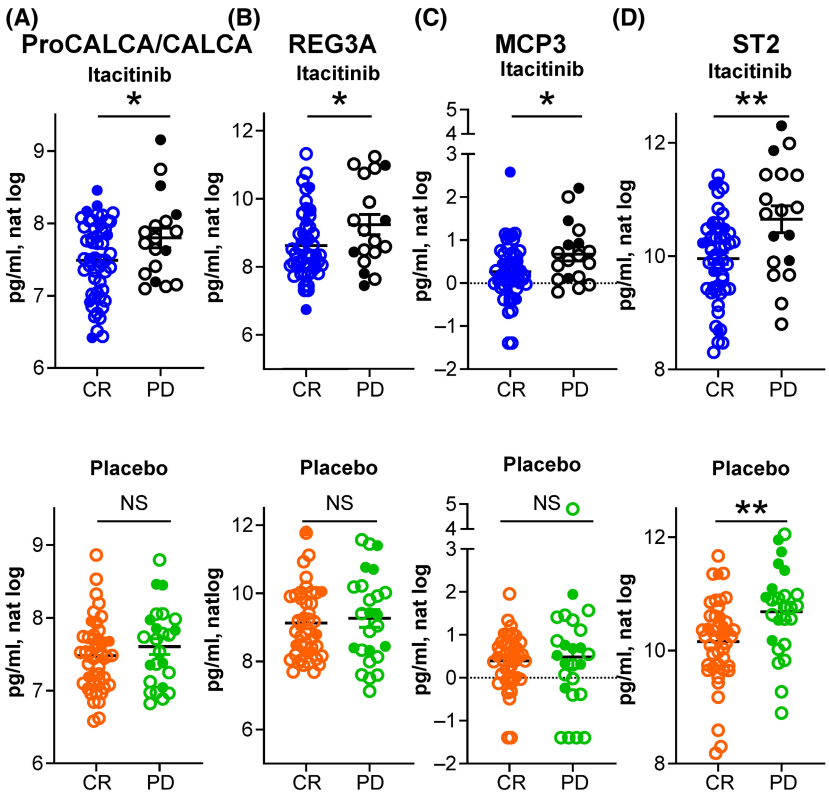

FIGURE 4 Baseline levels of candidate predictive biomarkers (A) ProCALCA/CALCA; (B) REG3A; (C) MCP3; (D) ST2 in the validation cohort. Baseline serum samples from itacitinib versus placebo arms of validation cohort were assayed for biomarker levels by proximal extension assay. Statistical analysis was by unpaired *t*-test. Significance was conferred when $p < 0.05$. Itacitinib CR: Blue; Itacitinib PD: Black; placebo CR: Orange; placebo PD: Green. Open symbols represent participants with standard risk; closed symbols represent participants with high-risk acute GVHD. CR, complete response; GVHD, graft-versus-host disease; MCP3, monocyte-chemotactic protein 3; nat, natural; NS, not significant; PD, progressive disease/death; ProCALCA/CALCA, pro-calcitonin/calcitonin; REG3A, regenerating islet-derived protein; ST2, suppressor of tumorigenicity 2; $^{*}p < 0.05$; $^{**}p < 0.01$.

Comparison of PEA to commercial multiplex platform

Plasma samples from the identification cohort were evaluated for levels of ST2, REG3A, ProCALCA/CALCA, MCP3, and TNFR1 using the PEA and were compared to identical samples evaluated in a commercially available platform, the Protein Simple Ella (Figure 7).[36] Although the levels of each biomarker differed between the two methods, possibly because of the different standards used for each platform, the overall trends within each sample population were similar, resulting in a strong positive correlation between PEA and Protein Simple Ella measurements.

DISCUSSION

Despite a growing understanding of acute GVHD, many patients are not adequately treated. This study identified novel candidate predictive biomarkers and response-to-treatment biomarkers of itacitinib. From seven biomarkers identified, mean baseline levels of MCP3 and ProCALCA/CALCA, together with the previously identified prognostic biomarker, REG3A, were significantly higher in PD versus CR participants exclusively in the itacitinib, but not placebo arm. These findings provide initial validation of these candidate biomarkers selected from a relatively small identification cohort. Although there was no apparent difference in response between itacitinib versus placebo in responders in the validation cohort, there was a higher CR percentage in the itacitinib arm versus placebo (51.5% vs. 41.3%) that may correlate with ProCALCA/CALCA, REG3A, and MCP3 levels.

Development of the critical panel of predictive candidate biomarkers was performed within a limited acute GVHD population represented by a mixture of steroid-naive (9) and steroid-refractory (16) participants. This relatively small and imbalanced subset of participants may influence selection of predictive biomarkers for itacitinib, because the pathobiology of the prevailing majority of 16 participants with steroid-refractory acute GVHD might be different from the mainstream front-line population treated in the validation cohort. Additionally, involvement in the phase III trial used for validation was only allowed within the first 2 days of high-dose steroid initiation for acute GVHD.[31] Altogether, this may serve as a source of

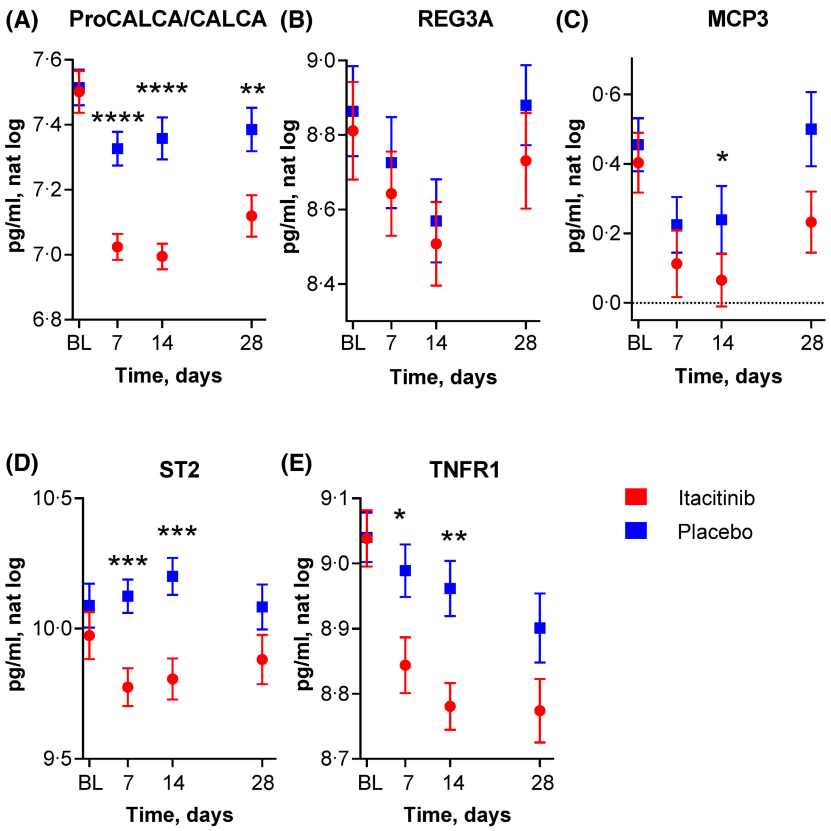

FIGURE 5 Longitudinal analysis of candidate biomarkers in responders from validation cohort. Serum levels of candidate biomarkers identified in the identification cohort were measured at baseline and at days 7, 14, and 28 (where available) from responders (CR, VGPR, PR) in the validation cohort, and mean ± SEM values are plotted from the itacitinib/corticosteroid combination ($n = 74$; red circles) versus corticosteroid alone (placebo $n = 79$; blue squares). Data are shown for each biomarker showing a significant difference between treatment arms. Data were compared at each time point by unpaired *t*-test, and significant differences between treatment arms are shown (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$). BL, baseline; CR, complete responder; MCP3, monocyte-chemotactic protein 3; nat, natural; PR, partial responder; ProCALCA/CALCA, pro-calcitonin/calcitonin; REG3A, regenerating islet-derived protein; SEM, standard error of the mean; ST2, suppressor of tumorigenicity 2; TNFR1, tumour necrosis factor receptor 1; VGPR, very good partial responder.

bias and imprecision in conducting predictive biomarker analyses within an initial small cohort of participants that are essentially different from the mainstream validation cohort.

Consistent with this hypothesis, baseline levels of several biomarkers were significantly different between the identification and validation cohorts, suggesting that enrolled populations may have been different. Specifically, only 22% of participants in the validation cohort were considered high-risk per standard risk score.[7] Conversely, in the identification cohort, 64% of participants in the pooled biomarker analysis were high risk. Assumptions used in powering the study of the validation cohort may have led to over enrollment of participants with standard-risk or Grade II acute GVHD.[31] Thus, the lower percentage of high-risk participants in the validation cohort may explain the lower levels of several inflammatory-associated markers, including ST2, IL8, and MCP3.

It is therefore not surprising that only three of the seven selected novel biomarkers were deemed to be validated in the itacitinib/steroid subset according to the ability to differentiate CR from those with PD. An alternative study design would include selecting participants from the identification cohort who are similar to those treated in the phase III clinical trial used for validation. Unfortunately, there were only nine participants in the identification cohort who were steroid-naive, similar to the phase III clinical trial, and the limited number of participants made it impossible to identify statistically valid predictive biomarkers. Consequently, the participants in the identification cohort were pooled.

From the validation cohort, longitudinal data were plotted over time to identify itacitinib candidate response-to-treatment biomarkers. ProCALCA/CALCA, ST2, and TNFR1 represent candidate response-to-treatment biomarkers based on a significant reduction in the itacitinib arm by Day 7 following treatment. Longitudinal effects were limited to responders, as levels of ProCALCA/CALCA, ST2, and TNFR1 did not significantly change in PD participants on treatment (data not shown), although survival bias could also influence interpretation of the PD results.

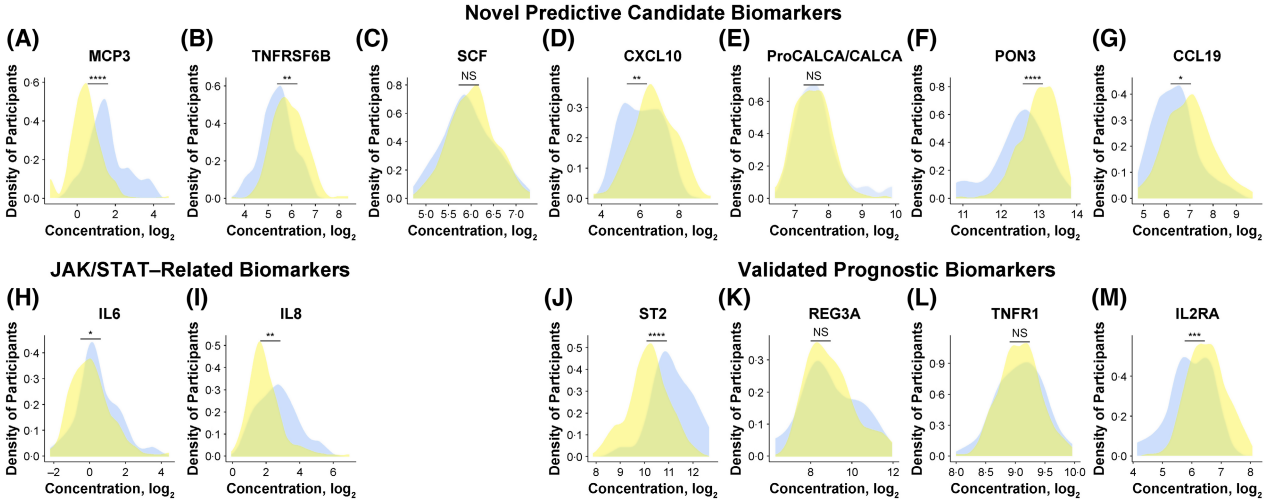

FIGURE 6 Density plots comparing baseline levels of each candidate biomarker in the identification and validation cohorts. Baseline levels of each candidate biomarker are shown in density plots, where $\log_2$ of biomarker concentration was compared with the density of participants at a corresponding biomarker concentration. Baseline levels from identification cohort are shown in blue; baseline levels from the validation cohort are shown in yellow. The markers were classified according to novel predictive candidate biomarkers, JAK/STAT-related biomarkers, and previously validated prognostic biomarkers. Statistical analysis was by unpaired *t*-test. Significance was conferred when $p < 0.05$. The lines over particular cohorts indicate comparison of those cohorts with *p* values indicated. CCL19, C-C motif chemokine 19; CXCL10, C-X-C motif chemokine 10; IL2RA, interleukin-2 receptor alpha chain; IL6, interleukin 6; IL8, interleukin 8; JAK, Janus kinase; MCP3, monocyte-chemotactic protein 3; NS, not significant; PD, progressive disease; PON3, paraoxonase 3; ProCALCA/CALCA, pro-calcitonin/calcitonin; REG3A, regenerating islet-derived protein; SCF, kit ligand; ST2, suppressor of tumorigenicity 2; STAT, signal transducer and activator of transcription; TNFR1, tumour necrosis factor receptor 1; TNFRSF6B, TNF receptor superfamily member 6b. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$.

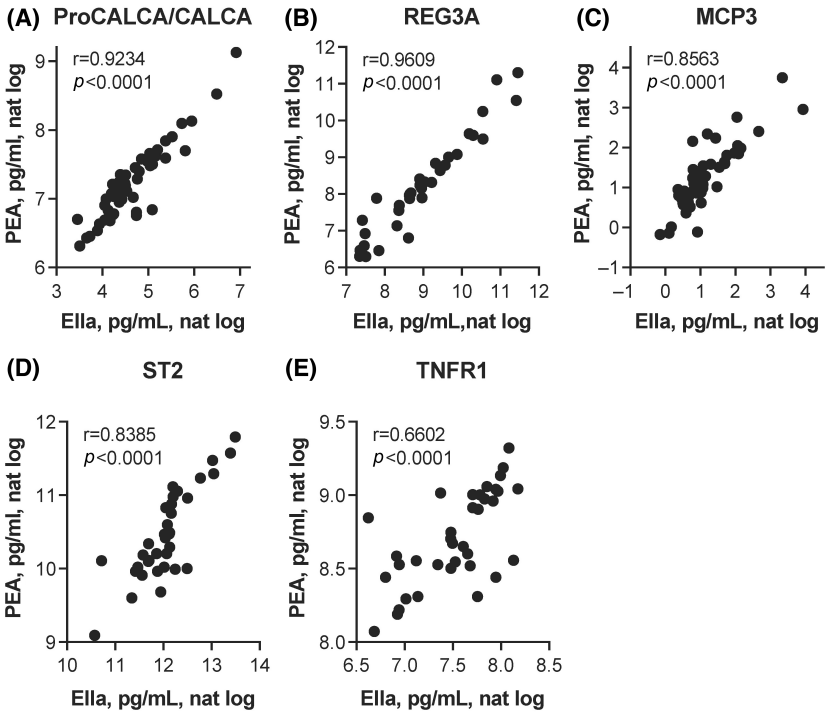

FIGURE 7 Correlation between protein simple Ella and proximal extension assay in the detection of candidate biomarkers in samples from identification cohort. Plasma samples from CR and PD participants from the identification cohort were tested for (A) ProCALCA/CALCA; (B) REG3A; (C) MCP3; (D) ST2; and (E) TNFR1 by protein simple Ella (*x*-axis) and by PEA (*y*-axis). Correlation between the two assay platforms is shown for each biomarker. Statistical analysis was performed using Pearson correlation, and *r* and *p* values are shown. Significance was conferred when $p < 0.05$. CR, complete responder; MCP3, monocyte-chemotactic protein 3; nat, natural; PD, progressive disease/death; PEA, proximal extension assay; ProCALCA/CALCA, pro-calcitonin/calcitonin; REG3A, regenerating islet-derived protein; ST2, suppressor of tumorigenicity 2; TNFR1, tumour necrosis factor receptor 1.

Stratification of responders from the validation cohort into standard groups (itacitinib 59 vs. placebo 63) and high-risk (itacitinib 14 vs. placebo 14) and comparison of baseline and longitudinal levels of each candidate biomarker were performed to determine whether a candidate biomarker was predictive in a particular risk population per standard risk score.[7] At baseline, ST2, REG3A, and SCF levels were significantly different between CR and PD in the standard-risk but not in the high-risk population. Longitudinally, most of the candidate biomarkers that distinguished response to itacitinib versus placebo in the combined-risk populations had a similar effect in the standard-risk population. However, only ProCALCA/CALCA showed a significant difference between itacitinib and placebo in the high-risk population. Conclusions from a high-risk population comprising only 14 participants in each treatment arm should be drawn cautiously.

Because protein expression can be influenced by demographic traits, correlations between protein expression and various demographic traits were assessed in responders from the validation cohort. TNFR1 was associated with age and high-risk status (per standard risk score),[7] whereas IL2RA was associated with both age and sex. Other correlations included REG3A with age, ProCALCA/CALCA with sex, IL8 with high-risk and SCF with standard-risk strata.

The identification cohort used to initially select the 13 novel candidate biomarkers was based in plasma, and the validation cohort was based in serum. Differences in biomarker levels between plasma and serum have been reported previously[38] and should be taken into account. Levels of MCP3 and ProCALCA/CALCA have been measured in paired serum and plasma samples from 10 healthy volunteers, and there were minimal differences in the levels of each marker observed, as demonstrated by a high correlation between the two matrices with r values >0.9 (data not shown).

Many of the biomarkers identified in this report may not be associated with acute GVHD but associated with JAK inhibition. For example, ProCALCA/CALCA is a transcriptional target of cyclic AMP-dependent transcription factor (ATF) 1, which has been reported to activate JAK1 transcription.[39] REG3A has been shown to induce JAK1 expression in a pancreatic cell line.[40]

Additional studies are needed to evaluate these markers in patients with acute GVHD treated with other potential treatments for acute GVHD, including other JAK inhibitors such as ruxolitinib. A multiplex platform consisting of ProCALCA/CALCA, MCP3, REG3A, ST2, and possibly TNFR1 would represent both predictive and response-to-treatment biomarker candidates in patients with acute GVHD treated with itacitinib. A strong positive correlation was demonstrated between the PEA and a commercially available platform, the Protein Simple Ella, suggesting that Protein Simple Ella could be used to further validate these candidate biomarkers.

In conclusion, multiple novel candidate acute GVHD biomarkers identified from a phase I study could successfully stratify complete responders treated with itacitinib versus placebo before treatment in a larger validation cohort. These candidate biomarkers could be used to predict response to itacitinib before treatment initiation, thus avoiding weeks of determining whether treatment is efficacious.

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CONFLICT OF INTEREST

Sophie Paczesny has a patent, 'Methods of detection of graft-versus-host disease' (US 20130115232A1, WO 2013066369A3), licensed to Viracor-IBT Laboratories, and received honoraria from Incyte, Genentech, and Omeros for advisory board meetings. Gerard Socie received lecture fees from Incyte Corporation and Novartis and honoraria from Novartis for advisory board meeting. Mark A. Schroeder received research funding paid to his institution by companies for which he is a Principal Investigator for a company-sponsored research study including Genentech Inc., Incyte, Cellect Inc., Fortis, Seattle Genetics, Amgen, Celgene, PDB Incorporated, Genzyme Sanofi, and Janssen; served on advisory boards, and received honoraria and consultant fees from Amgen, Astellas, Dova Pharmaceuticals, FlatIron Inc., Incyte, Partners Therapeutics, Pfizer, and Sanofi Genzyme; and served on the speakers bureau and received honoraria as a consultant for AbbVie, Merck, and Takeda. Michael Pratta, Natalie Barkey, and Michael C. Arbushites are employees of Incyte Research Institute, Incyte Corporation. Hao Liu, Sherry Owens, and Michael D. Howell were employees of Incyte Corporation at the time these studies were undertaken.

DATA AVAILABILITY STATEMENT

Access to individual patient-level data is not available for this study. For original data requests, please contact data-sharing@incyte.com.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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