**Flotetuzumab as Salvage Immunotherapy for Refractory Acute Myeloid Leukemia**

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**Key Point 1:** Flotetuzumab is associated with acceptable safety and evidence of activity in AML patients with primary induction failure/early relapse.

**Key Point 2:** A 10-gene immune signature predicts response to flotetuzumab with greater accuracy than the ELN risk classifier.


## ABSTRACT

Despite recent advancements, approximately 50% of patients with acute myeloid leukemia (AML) do not respond to induction therapy (primary induction failure, PIF) or relapse after <6 months (early relapse, ER). We have recently shown an association between an immune-infiltrated tumor microenvironment (TME) and resistance to cytarabine-based chemotherapy but responsiveness to flotetuzumab, a bispecific DART® antibody-based molecule to CD3ε and CD123.

This study reports the results of a multicenter, open-label, phase 1/2 study of flotetuzumab in adults with relapsed/refractory AML. Eighty-eight AML patients were enrolled, 42 in dose-finding and 46 at the recommended phase 2 dose (RP2D) of 500ng/kg/day. Consistent with flotetuzumab's mode of action, the most frequent adverse events were infusion-related reactions (IRR)/cytokine release syndrome (CRS), the majority as grade 1-2. Stepwise dosing during week 1, pre-treatment dexamethasone, prompt use of tocilizumab and temporary dose reductions/interruptions successfully prevented severe IRR/CRS, resulting in acceptable tolerability. Clinical benefit accrued to PIF/ER AML patients, who showed an immune-infiltrated TME. Among 30 PIF/ER patients treated at the RP2D, the CR/CRh rate was 26.7%, with an overall response rate (CR/CRh/CRi) of 30.0%. In PIF/ER patients who achieved CR/CRh, median OS was 10.2 months (range 1.87-27.27), with 6- and 12-month survival rates of 75% (95%CI, 0.450-1.05) and 50% (95%CI, 0.154-0.846). Bone marrow transcriptomic analysis showed that a parsimonious 10-gene signature predicted complete responses to flotetuzumab (AUROC=0.904 *versus* 0.672 for the ELN risk classifier).

Flotetuzumab represents an innovative experimental approach associated with acceptable safety and encouraging evidence of activity in PIF/ER AML patients.

**Trial registration number**: NCT02152956.


## INTRODUCTION

Acute myeloid leukemia (AML) is a highly heterogeneous disease.[1] Despite the recent approval of several new drugs, cure rates remain largely unsatisfactory, with the majority of patients being either refractory to currently available therapeutics or relapsing after achieving remission.[1,2] Primary refractory and relapsed (R/R) AML are usually treated as one single clinical entity, even though the probability of success of subsequent salvage therapy differs substantially between affected individuals.[3-8] Particularly poor outcomes have been observed in patients with primary induction failure (PIF) or those with an initial remission duration of <6 months (early relapse, ER). Differences in outcomes have been recently associated with immune transcriptomic profiles of the tumor microenvironment (TME) that stratifies AML cases into an immune-infiltrated and immune-depleted subgroup, with the former being enriched in interferon (IFN)-γ-related mRNA profiles and showing resistance to cytotoxic chemotherapy but an enhanced probability of response to immunotherapy.[9]

Given the success of bispecific antibodies, including the T cell-engaging single-chain antibody construct blinatumomab, in the treatment of B-cell malignancies,[10,11] similar therapeutic approaches are being developed in AML. CD123, the low affinity interleukin-3 receptor α subunit (IL3RA), is expressed in 60-80% of patients with AML.[12-15] What makes CD123 particularly appealing as a therapeutic target in AML is not only that high expression of CD123 is enriched in PIF/ER AML patients but also that high CD123 on AML blasts is associated with poor outcomes.[16-18] Although CD123 is not specifically expressed in putative AML progenitor and stem cells, its targeting could lead to the eradication of leukemia stem cell pools.[16]

Flotetuzumab (MGD006) is an investigational bispecific antibody-based molecule to CD3ε and CD123 engineered in a DART® format.[19] CD3-engaging bispecific molecules bind both tumor and effector cells to promote an immunologic synapse and redirect polyclonal effectors to kill tumor cells in an MHC-independent fashion. In preclinical models, flotetuzumab mediated target-effector cell association, T-cell activation and proliferation, and potent killing of $CD123^+$

AML blasts *in vitro* and *in vivo*.[20,21] Herein, we report the results of a phase 1/2 study of flotetuzumab in R/R AML. Furthermore, given the preliminary evidence of an association between an immune-infiltrated TME and responsiveness to flotetuzumab that we recently reported,[9] we explored this agent's activity specifically in the PIF/ER AML subset.

## METHODS

### Study design

This open-label, multi-dose, single-arm, multi-center, phase 1/2, dose escalation study was designed by the Sponsor (MacroGenics Inc.) in collaboration with the investigators to evaluate the toxicity profile, maximum tolerated dose (MTD), immunogenicity, pharmacokinetic (PK), and potential anti-tumor activity of flotetuzumab in patients with R/R AML or intermediate-2/high risk MDS. The trial (NCT02152956) was approved by the Institutional Review Boards of participating centers and was conducted according to the International Conference on Harmonization (ICH) Guideline for Good Clinical Practice (ICH E6), all applicable local and national regulations, and in accordance with the ethical principles of the Declaration of Helsinki. All participants provided written informed consent prior to enrollment.

The trial was conducted as a standard 3+3 dose escalation study with expansion of the studied population at the protocol defined MTD (500ng/kg/day administered as a continuous infusion) (**Fig. S1**).

Two dosing schedules were tested during the dose escalation part of the trial, intermittent dosing of 4 days on/3 days off per week and continuous 7-day infusion (CIV), at each target dose. The highest dose that was tested in the clinic was 700 ng/kg/day, at which dose 8 AML patients were treated, 5 on a 4 days on/3 days off per week schedule and 3 on a CIV schedule. Overall, this dose level exceeded the MTD, as evidenced by dose limiting toxicity: 1 event of Grade 3 delirium, 1 event of Grade 3 acute confusional state and 1 event of Grade 3 CRS. At this dose, the high frequency and severity of the observed CRS and other AEs was in part managed by frequent dose reductions and dose interruptions, which had a significant impact on the dose intensity (DI, defined as the fraction of flotetuzumab delivered relative to the intended dose over time). The DI at the 700 ng/kg/day target dose level (post the lead-in dose [LID] phase) was 64.5% and 50.2%, for intermittent and CIV dosing, respectively, compared

with 85.7% and 57.2-64.5%, respectively, for the 500 ng/kg/day target dose after LID phase. PK and exposure-response analyses indicated that MGD006 exposure plateaus following the dose of 500 ng/kg/day (**Fig. S2**), with exposure at doses above 500 ng/kg/day remaining equivalent to the exposure observed at the 500 ng/kg/day dose level, owing to a disproportional decreased DI in the 700 ng/kg/day cohort. The goal of the LID step-up dosing was to mitigate CRS and, consequently, improve the DI throughout the dosing period. Once the dose of 500 ng/kg/day was determined as the MTD and specified as the recommended phase 2 dose (RP2D), a "multi-step LID" (MS-LID) scheme was introduced during Week 1 Cycle 1. The MS-LID comprised the following dosing: 30, 60, 100, 200, 300, 400 ng/kg/day each for 24 hours, and on Day 7 the dose increased to 500 ng/kg/day and was administered as a continuous infusion for the remainder of Cycle 1.

Overall the three LID schedules during Week 1 were as follows: 1-step LID (100 ng/kg/day on days 1-4, Cohort 2), 2-step LID (30 ng/kg/day on days 1-3 followed by 100 ng/kg/day), and MS-LID (**Table S1**). At the RP2D (500 ng/kg/day), 30 patients received the 2-step LID and 20 patients received the MS-LID.

The dosing regimens tested were intermittent dosing (4 days on/3 days off per week, Cohort 2a) and continuous infusion dosing (Cohorts 7, expansion, and ruxolitinib). Impact of LID on CRS (**Fig. 1B**) and DI (**Fig. 1C** and **Fig. 1D**) is notably improved with multiple small step-up dosing.

Consistent anti-leukemic activity and clinical responses were observed at the 500 ng/kg/day dose compared to doses below 500 ng/kg/day, where no MLFS, CRi, CRh or CR were noted. Balancing CRS incidence and severity with DI and anti-leukemic activity, the RP2D and schema was henceforth defined as: MS-LID of 30, 60, 100, 200, 300, 400 ng/kg/day for 24 hours each for Days 1 through 6, followed by 500 ng/kg/day CIV from Days 7 to 28 (Cycle 1), with subsequent additional 28-day cycles not requiring a LID phase, and was used in the expansion phase of the study.

The primary objective was to determine the MTD and schedule of flotetuzumab and to characterize its dose-limiting toxicities (DLT). Secondary objectives included characterization of the PK and pharmacodynamic profile and clinical activity of flotetuzumab. Eligible patients were diagnosed with non-promyelocytic, R/R AML (according to WHO criteria) unlikely to benefit from cytotoxic chemotherapy defined as a) refractory to ≥ 2 induction attempts (primary induction failure; PIF); b) first relapse with an initial complete remission (CR) duration <6 months (early relapse; ER); c) first relapse following an unsuccessful salvage attempt; d) 2$^{nd}$ relapse or higher, or e) prior failure of hypomethylating agents (HMA), defined as no evidence of response following a minimum of 4 cycles. Late relapse (LR) was defined as patients that achieved remission lasting ≥6 months following prior therapy. Documentation of CD123 expression was not required for study inclusion. Details on study design and participants, including inclusion/exclusion criteria, study assessments, safety and efficacy criteria, and gene expression profiling are provided in the **Supplementary Appendix** and in previous publications.[9,22]

**Study assessments**

Results are analyzed for three populations: pharmacokinetic population, safety population, and response-evaluable population. The pharmacokinetic population includes all patients who received flotetuzumab and provided at least one quantifiable flotetuzumab concentration value. The population of patients for the assessment of safety included all AML patients who received at least 1 dose of flotetuzumab in any portion of the study. The primary safety analysis is based on pooled analysis of all diagnoses and dosing cohorts. Safety was also summarized for AML patients in the dose expansion phase treated at the RP2D of 500 ng/kg/day, and for patients treated at RP2D that fit the criteria of PIF/ER. The response-evaluable population includes all AML patients who received at least 1 dose of flotetuzumab in any portion of the study, had baseline BM assessment, and had at least 1 post-baseline

disease response assessment or discontinued treatment due to documented disease progression or death.

Disease status was assessed by modified International Working Group (IWG) criteria.[23] Complete response was defined as CR, CR with partial hematological recovery (CRh), CR with incomplete hematological recovery (CRi) or morphological leukemia-free state (MLFS) at the end of cycle 1. Partial responses (PR) were defined as > 50% decrease in BM blasts from baseline to 5-25% at the end of cycle 1. Thus, if the pretreatment BM blast percentage was 50% to 100%, the percentage of blasts must decrease to a value between 5% and 25%; if the pretreatment BM blast percentage was 20% to less than 49%, the percentage of blasts must decrease by at least half to a value of more than 5%.

Within the response-evaluable population, subgroup analyses were conducted based on disease status at study entry: PIF, early relapse (CR <6 months), and late relapse (CR >6 months); number of lines of prior therapy (2, 3, 4, >4); age (≤70, > 70); primary *versus* secondary AML (treatment-related or history of antecedent hematological malignancy); and 2017 European Leukemia-Net (ELN) risk classification.[2]

**Statistical methods**

Population PK (nonlinear mixed effects) modeling was used to evaluate flotetuzumab PK. Logistic regression was used to describe the relationship between flotetuzumab exposure (predicted mean cycle 1 concentration, $C_{mean}$) and probability of complete response. The rates of survival function estimates at 6 months and 1 year were also calculated. Binary logistic regression was used to ascertain the relative contribution of immune subtypes and other pretreatment covariates toward the predicted likelihood of anti-leukemic activity of flotetuzumab. A two-sided *P* value < 0.05 was considered to reflect statistically significant differences.

## RESULTS

### Patient characteristics

Eighty-eight AML patients were enrolled as of November 1st, 2019: 42 in the dose-escalation/dose-finding segment, including 4 patients treated at the RP2D, and 46 in the RP2D expansion phase (**Table 1**). Of the 50 patients treated at the RP2D, 30 (60%) met criteria for PIF, of which 24/30 (80%) were refractory to ≥2 induction attempts and 6/30 (20%) were ER. The majority of PIF/ER patients (18/30, 60%) had adverse risk by ELN 2017 criteria and 12/30 (40%) had secondary AML. Patients were heavily pretreated, with a median of 4 lines of prior therapy (range 1-9). The distribution of flotetuzumab cycles received is summarized in **Table S2**.

### Pharmacokinetic and dose-response analysis

Flotetuzumab PK is linear and is described by a two-compartment model, albeit with inter-individual and inter-occasion variability (**Table S3**). Anti-drug antibodies, which were measured on day 1 pre-infusion (cycle 1), on day 1 pre-infusion or prior to dose change, as applicable, during subsequent cycles and at end of treatment visit, were detected in only 1/88 (0.9%) subjects initially enrolled, indicating little, if any, immunogenicity of flotetuzumab. Flotetuzumab exposure was optimal at 500 ng/kg/day (**Fig. S2A**), with a higher nominal dose of 700 mg/kg/day resulting in actual lower exposure due to dose reductions or interruptions to mitigate incidence and severity of IRR/CRS, which were graded as recommended by Lee and coworkers.[24] Consistent with this observation, dose intensity decreased in the 700 ng/kg/day cohort compared to the 500 mg/kg/day cohort (**Fig. S2B-D**).

### Safety

In the primary safety analysis (n=88), irrespective of dose, the most common treatment-emergent adverse events were IRR/CRS. Non-IRR/CRS treatment-emergent adverse events included peripheral edema (40.9%) and nausea (30.7%). The most common non-IRR/CRS treatment-emergent adverse events ≥ Grade 3 were cytopenias including anemia (28.4%), and decreased platelets (20.5%), lymphocytes and leukocytes (18.2%). The most prominent nonhematologic treatment-emergent adverse events ≥ Grade 3 included hypophosphatemia (14.8%) and hypokalemia (13.6%). IRR/CRS was the predominant treatment-related adverse event (TRAE) observed. The most common non-IRR/CRS treatment-related ≥ Grade 3 adverse events (>10%) were cytopenias including decreased platelets (12.5%), lymphocytes, neutrophils and total white blood cell count (10.2%; **Table 2**). Seventy-seven percent of the patients (68/88) were neutropenic at baseline (absolute neutrophil count [ANC] < $1.0\times10^3/\mu L$. The median absolute neutrophil count and platelet count at baseline were $0.27\times10^3/\mu L$ (range, 0-12) and $36\times10^3/\mu L$ (range, 2-452), respectively. Fourteen treatment-emergent adverse events of neutropenia were reported in 3/88 (3.4%) patients. Four events in 1 patient were deemed to be possibly related to flotetuzumab administration. Most prominent nonhematologic treatment related ≥ Grade 3 events included transient dyspnea (6.0%), myalgias (4.0%), C-reactive protein increase (4.0%) and alanine transaminase increase (4.0%).

Three DLTs were observed among 8 AML patients receiving the highest dose (700 ng/kg/day) administered (1 event of Grade 3 delirium, 1 event of Grade 3 acute confusional state, and 1 event of Grade 3 CRS). The MTD and RP2D was thus defined as 500 ng/kg/day administered continuously following a step-up LID during Cycle 1 Week 1.

In RP2D treated patients in dose expansion (n=50), the most commonly observed treatment related event was IRR/CRS (96.0%), and nausea (26%). The most common treatment related ≥ Grade 3 (>10%) in the RP2D population included anemia, decreased lymphocyte, white

blood cell, and platelet counts (14.0%; **Table 2**). The most commonly observed treatment-emergent adverse events were comparable to the overall patient population, with IRR/CRS being the most common treatment-emergent adverse events and cytopenias being the most common treatment-emergent adverse events ≥ Grade 3.

Neurologic AEs have been infrequent, with the most prominent event being grade 1 or grade 2 headache in 10% (5/50) treated at the RP2D. Two grade 3 neurologic AEs were reported, 1 headache and 1 episode or delirium, both were of short duration (1-4 days) and fully reversible with no clinical sequelae.

*Infusion-related reaction/cytokine release syndrome*

Of the patients with IRR/CRS, the majority (71/88, 81%) had mild to moderate signs/symptoms. Seven patients (8%) experienced Grade 3 events; no Grade 4/5 events were observed. A single episode of Grade 3 IRR/CRS was observed in each of 4 patients (4/50, 8%) treated at the RP2D. Most IRR/CRS events (32%) occurred in the first week of treatment during step-up dosing and their incidence decreased each week during continuous dosing at 500 ng/kg/day (27% in week 2, 11% in week 3, and 7% in week 4). IRR/CRS events decreased with ongoing treatment, irrespective of dose and LID scheme (**Table S4**).

*Mitigation of infusion-related reaction/cytokine release syndrome (IRR/CRS)*

Strategies employed to mitigate the incidence and severity of IRR/CRS included: step-up lead-in dose (LID) schedules (**Table S1**), temporary dose reduction or interruption (**Table S5**), and the prompt use of tocilizumab. Optimal CRS mitigation was achieved through the implementation of gradual step-up in dose through a multi-step LID (MS-LID), which not only decreased the severity of IRR/CRS in the RP2D population but also improved mean dose intensity by minimizing dose reductions and interruptions (**Fig. 1B, 1C, 1D**). The median

duration of drug interruptions for IRR/CRS was 7.2 hours (range, 0.2-129 hours; n=102 events overall).

Of the 78 doses of tocilizumab administered in 40 patients, 25 (32%) doses were given for Grade 1 IRR/CRS in 14 patients, 51 (65%) for Grade 2 IRR/CRS in 29 patients, and 2 (3%) doses for Grade 3 IRR/CRS in 2 patients. While tocilizumab use was recommended, some investigators opted not to use the drug promptly due to evidence of resolving CRS within two hours, as indicated in the guidelines for management of IRR/CRS (**Table S5**). A detailed comparison between patients that did and did not receive tocilizumab only for CRS management is shown in **Table S6**. Tocilizumab usage was associated with a decreased duration of IRR/CRS events with an average of 1.3 (range 1-3) *versus* 1.8 days (range 1-5; *P* = 0.0202; **Fig. 1E**). Consistent with previous observations and its mechanism of action, higher levels of IL-6 were observed for up to 6 days in tocilizumab-treated patients.

Fifty-three out of 88 (60%) patients experienced IRR/CRS events that led to dose interruption, including 31/50 (62%) patients that received flotetuzumab at the RP2D. Most IRR/CRS events that resulted in dose interruptions in the RP2D population occurred in the first week of treatment, with fewer dose interruptions beyond the second week after reaching the target drug dose. Fewer dose adjustments were necessary with MS-LID than the 2-step LID (dose interruptions: 35% *versus* 67%; dose reductions: 69% *versus* 95%), contributing to the higher dose intensity observed with MS-LID. Notably, there were no Grade 3 IRR/CRS events with MS-LID compared to 11% patients treated with 2-step LID who experienced Grade 3 IRR/CRS. A total of 12 patients, 8 at the RP2D, received a total of 19 doses (median of 1 dose [range 1-3]) of corticosteroids for the treatment of CRS, 2 for Grade 1 events, 10 for Grade 2 events, and 1 for a Grade 3 event.

*Myelosuppression*

Preclinical data suggest that effective targeting of $CD123^+$ cells may lead to profound myelosuppression.[25] However, flotetuzumab, at concentrations equivalent to Cmax levels at 500 ng/kg/day, had minimal *in vitro* effect on normal hematopoietic stem cells, megakaryocyte erythroid progenitors, granulocyte-myeloid progenitors or common myeloid progenitors (**Fig. S3**), consistent with previous *in vivo* reports in cynomolgus monkeys.[21] In line with these preclinical data, we observed that 57% and 67% of patients who achieved response of CR or CRh, respectively, experienced recovery of peripheral blood counts even with continued flotetuzumab treatment (**Table S7** and **Table S8**), suggesting that flotetuzumab as dosed in this study does not result in significant and prolonged suppression of normal hematopoiesis.

*Dose-limiting toxicities and recommended phase 2 dose and schema*

Three DLTs (Grade 3 delirium, Grade 3 acute confusion, Grade 3 CRS) were observed among 8 AML patients receiving the highest dose administered (700 ng/kg/day), while a dose of 500 ng/kg/day demonstrated manageable safety, with no DLTs observed among 13 patients treated during dose escalation. The RP2D dosing schema was defined as 500 ng/kg/day administered by continuous infusion following step-up lead-in dosing during first week of Cycle 1 (detailed in **Fig. 1A**).

**Clinical efficacy**

*Response in patients with primary induction failure and early relapse AML*

Of 88 AML subjects enrolled to receive any dose of flotetuzumab, 9 (10%) patients discontinued treatment within the first 2 weeks of treatment initiation, 2 patients withdrew consent, and 7 patients were withdrawn for non-treatment related adverse event (**Table S9**). The rate of CR or CRh was 11.7% (10/88), with an overall response rate (ORR: CR, CRh, CRi) of 13.6% (12/88). Circulating and BM blast reductions were noted at all doses tested; however, complete responses were observed among patients treated at the RP2D of 500

ng/kg/day (n=50) or higher. At the RP2D, a CR/CRh rate of 18% (9/50) and an ORR of 24% (12/50) were documented (**Fig. 3A** and **Table 3**). Furthermore, subgroup analysis of evaluable patients based on AML status at study entry showed increased flotetuzumab activity (≥50% BM blast reduction) in PIF/ER (43%, 12/28) as compared to LR (14%, 1/7) or HMA failure (30%, 3/10; **Fig. 3A**, **Table 3** and **Fig. S4**). This observation is consistent with an increased probability of response to immunotherapy in patients with infiltrated/inflamed TME,[9] a biomarker coincidental with the PIF/ER AML subgroup (see below). Indeed, with the exception of a single CR in a patient that failed HMA, all remaining 9 clinical responses (5, 3 and 1 CR, CRh and CRi, respectively, 30% ORR) occurred among the 30 patients that met criteria for PIF (n=24) or ER (n=6), while no responses were seen in 12 LR patients. Within the evaluable patients in the PIF/ER subgroup, ORR was 28.6% (8/28) for patients with non-favorable risk by ELN 2017 criteria (6/19 in patients with adverse and, 2/7 in patients with intermediate risk), and 40.0% (4/10) for secondary AML. Notably, more limited exposure to prior therapies was associated with greater likelihood of response to flotetuzumab, with an ORR of 56% (5/9) among patients with 2 lines of prior treatment as compared to an ORR of 0% among 7 patients with ≥5 lines of prior therapy (**Table S10**). While there was no relation between IRR/CRS severity and efficacy, the latter was associated with incidence of IRR/CRS events (**Fig. 2**). In line with previously published data,[26] CRS severity did not correlate with disease burden (absolute AML blasts, % CD123 AML blasts), CD123 expression on AML blasts, monocyte levels or effector-to-target ratio in the peripheral blood. However, the frequency of circulating $CD4^+$ T cells was significantly higher at baseline in patients who experienced Grade 2 or higher CRS compared with Grade 1 CRS (median 73% compared with median 47%, respectively; $P = 0.0082$). Response rate in post-HMA treated patients was 10.0% (1/10), with a very short duration of response.

*Time to response, duration of response and survival*

Among RP2D patients, median follow-up on study was 0.8 months (range 0-25), median time-to-first response was 0.84 months (range 0.8-2.1), and median overall survival (OS) was 3.2 months (95% confidence interval [CI]: 2.10-6.47). For PIF/ER patients 6 and 12-month survival rates for PIF/ER (n=30) is 42% (0.237, 0.596) and 20% (0.025, 0.377), respectively. Patients achieving CR/CRh had a median duration of response of 6.9 months (range 1.1-26.4). Four out of 8 patients that attained a complete response underwent hematopoietic cell transplantation, 3 in CR and 1 in CRh (**Fig. 3B**). Median OS was 11.2 months (95%CI: 1.87-not reached), and 6- and 12-month survival rates were 75% (95%CI, 0.450-1) and 50% (95%CI, 0.154-0.846), respectively.

**Biomarkers of response**

To identify potential predictor of response, several biomarkers were captured during the study and retrospectively analyzed. CD123 expression and receptor density on AML blasts was measured by quantitative flow cytometry. CD123 receptor density was higher in patients with PIF/ER compared with LR ($P$ = 0.0283; **Fig. S5A**). However, no association between CD123 expression and response to flotetuzumab was observed (**Fig. S5B, S5C**). Interestingly, *in-silico* (GSE134589)[9] analyses showed that CD123 expression correlated positively with ELN risk category (**Fig. S6A**), and with the expression of inflammatory chemokine genes ($P$ = 0.029) and IFN-$\gamma$ ($P$ = 0.03; data not shown), and that higher CD123 mRNA is associated with PIF and ER in newly diagnosed AML (**Fig. S6B).**

Immune gene signature scores, which were computed as previously published,[9,27] were analyzed in a subgroup of 38 flotetuzumab-treated patients (**Fig. 4A**). As anticipated based on previous findings,[9] patients with PIF/ER showed higher immune infiltration relative to LR patients (**Fig. 4A**). Evidence of complete response from flotetuzumab, defined as achieving either CR, CRh or CRi, was documented in 21%, 44.4% and 60% of patients with low,

intermediate and high immune infiltration, respectively (**Fig. 4A** and **Fig. S7**). Responses were not correlated with the patient's molecular profile, as shown in **Fig. 4B**. Inflammatory chemokine and tumor inflammation signature (TIS) scores were higher in PIF/ER patients compared with LR patients (**Fig. 4C**). Furthermore, unsupervised hierarchical clustering of the expression of 770 immune-related genes in baseline BM samples showed evidence of complete response from flotetuzumab in 26.1%, 33.3% and 66.6% of patients with low, intermediate and high immune infiltration, respectively (**Fig. 5A**). By ranking the genes in the panel, we identified a parsimonious expression signature encompassing the top 10 genes associated with complete response to flotetuzumab (**Fig. 5B**). The expression of the 10-gene classifier, which was computed as the average sum of gene expression across the patient cohort, was higher in patients with heightened levels of BM immune infiltration (**Fig. 5B**; immune clusters defined as detailed in **Fig. 4A** and in previous publications[9]), including neutrophils, macrophages and myeloid cell types, and with inflammatory chemokines and other gene signature scores that reflect a T cell-inflamed, IFN-γ-driven TME, such as the TIS score (**Fig. 5C**). The latter, correlated with the antigen processing machinery and inflammatory chemokine scores ($P < 0.0001$), suggesting the occurrence of antigen presentation and T cell chemoattraction in highly T cell-inflamed samples. The analysis of functional protein association networks revealed that the 10 genes associated with complete response were enriched in ontologies and pathways related to antigen binding and processing, VEGF-activated receptor activity, Notch signaling, micro-RNA regulation in cancer and T helper type 1 (Th1) and Th2 differentiation (**Fig. 5D** and **Table S11**). The ability of the unfavorable ELN risk category (adverse and intermediate) and the novel 10-gene signature score to predict complete response from flotetuzumab, either individually or in combination, is summarized in **Fig. 5E**. Notably, the 10-gene signature score had an AUROC value of 0.854 when considered alone and of 0.904 when in conjunction with the ELN risk category, compared with 0.672 for the ELN risk category alone (**Table S12**).

## DISCUSSION

Since its initial description as a putative marker of leukemic stem cells,[20,21,28] there has been considerable interest in targeting the CD123 antigen in AML. In this report, we present the clinical results of a CD123-targeted immunotherapy for adults with R/R AML. The optimal target dose was determined to be 500 ng/kg/day after 1-week stepwise dose escalation. Similar to other to bispecific antibodies and CAR-T cells, IRR/CRS remain the most frequent and significant AEs observed following treatment with flotetuzumab. The frequency and severity of IRR/CRS may be increased compared to B-cell immunotherapies because of shared target antigen expression on monocytes and macrophages, both of which mediate IL-6 production.[29] In a recent study of autologous T cells transduced with a CD19-directed chimeric antigen receptor (CTL019) lentiviral vector, severe CRS developed in 27% of the patients treated.[30] A subsequent clinical trial of CTL019 has reported a 46% incidence of grade 3-4 CRS in pediatric R/R ALL with a median of 15 organ dysfunction days.[31]

Stepwise dosing combined with pre-treatment dexamethasone, prompt use of tocilizumab and temporary dose reductions/interruptions were successful strategies to prevent severe IRR/CRS while maintaining dose intensity. While the short half-life of flotetuzumab requires administration under continuous intravenous infusion, which can be burdensome for patients and providers, it also affords the ability to manage CRS through fine dosing control before subjects experience severe events. Other approaches to further reduce the severity of IRR/CRS are currently under investigation. Blockade of IL-6 signaling through inhibition of the JAK/STAT pathway with ruxolitinib has been shown to ameliorate CRS in CAR-T murine models[32] and is currently being clinically tested in combination with flotetuzumab.

The findings from our first-in-human study with flotetuzumab indicate that this bispecific molecule provides a novel treatment option for PIF/ER AML. The clinical activity of flotetuzumab was primarily observed in patients with PIF/ER, with CR/CRh rates of 27% and

ORR of 30%. In PIF/ER AML patients who achieved CR or CRh, the median OS was 10.2 months, with 6- and 12-month survival rates of 75% and 50%, respectively.

The observation that benefit with flotetuzumab accrued to patients with PIF/ER is highly encouraging, because these chemotherapy-resistant leukemias have especially poor prognosis and limited treatment options. A retrospective analysis of published literature showed that the response rate to salvage chemotherapy regimens was ~12% for patients with PIF/ER,[33] with subsequent lines of therapy being largely ineffective, and a median OS of only 3 months.[34] The observed response of patients with PIF/ER AML to flotetuzumab is consistent with the observation that an immune-infiltrated, IFN-γ-dominant TME identifies patients less likely to respond to cytotoxic chemotherapy but more likely to respond to immunotherapy.[9]

Further to this observation, the ability of a parsimonious 10-gene signature to predict complete responses from flotetuzumab further underscores the positive relationship between an immune-infiltrated TME and flotetuzumab's mechanism of action. Genes in the signature included *CD8B*, immune checkpoint *ICOS* and *NOTCH2*, all of which reflect a T cell-driven and highly immunosuppressed TME that could be re-invigorated by flotetuzumab. In this respect, increased Notch signaling has been correlated with enhanced CD8 T-cell infiltration in patients with colorectal carcinoma and with inhibited T-cell responses.[35] Furthermore, Notch2, but not Notch1 signaling, is critically required for the generation of cytotoxic T lymphocytes with anti-tumor activity in experimental models of lymphoma and acts as a transcriptional activator of granzyme B.[36,37]

Network analyses also indicated that antigen binding and processing were among the most enriched pathways associated with the 10 genes in our signature, underpinning the potential contribution of enhanced and sustained antigen presentation in the TME to the anti-leukemic activity of flotetuzumab. High antigen presenting machinery scores have been shown to correlate with improved overall response rates to anti-PD-1 or anti-PD-L1 monotherapy in a pooled analysis of 25 distinct solid tumor types.[38] In line with reports of PD-L1 induction by

IFN-γ in solid tumor cell lines and primary tissues, we have previously observed PD-L1 up-regulation of AML blasts incubated with IFN-γ.[39] Notably, primary AML blasts expressing high levels of PD-L1 were less susceptible to flotetuzumab-mediated killing *in vitro.* Furthermore, patients that progressed early (within two-weeks) on flotetuzumab treatment had higher baseline levels of PD-L1 on AML cells. Interestingly, a number of patients with residual disease on flotetuzumab showed higher proportion of PD-L1 positive AML blasts *vs.* basal levels.[39]

In the current study, adverse and intermediate-risk cytogenetics, which would be considered an established negative prognostic factor, acted as a modest predictor of flotetuzumab response. However, combination of high-risk cytogenetics and the 10-gene score improved the ability to predict response to flotetuzumab.

In conclusion, flotetuzumab represents an innovative experimental approach that has demonstrated an acceptable safety and encouraging evidence of activity. Considering that high CD123 expression on AML blasts is also associated with the risk of PIF and with poor prognosis,[17] flotetuzumab targeting of CD123 represents an attractive option for these patients. CD123 expression by leukemic stem cells further contributes to the potential therapeutic value of the target.[20,21,28] The assessment of minimal residual disease eradication, a prognostic factor for relapse and survival in AML, will be of particular interest in future studies of flotetuzumab. The trial is continuing but focused on subjects with PIF/ER AML (ClinicalTrials.gov NCT02152956).

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*Competing interests*

John Muth, Teia Curtis, Erin Fehr, Kuo Guo, Jian Zhao, Ouiam Bakkacha, Kenneth Jacobs, Kathy Tran, Patrick Kaminker, Maya Kostova, Ezio Bonvini, Jan K. Davidson-Moncada: Employees, MacroGenics Inc., Rockville, MD, USA;

Sarah E. Church: Employee, NanoString Technologies Inc., Seattle, WA, USA.

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*Patents*

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**Figure Legends**

**Fig. 1: Lead-in-dose (LID) and use of tocilizumab decrease cytokine release syndrome (CRS) incidence, severity and duration, and lead to increase in total dose intensity. A**) **Summary of dose and dosing schedule for flotetuzumab**: Multi-step lead-in-dose (MS-LID) of 30, 60, 100, 200, 300, 400, 500 ng/kg/day for 24 hours each for Days 1 through 7 given via continuous intravenous (CIV) infusion, followed by 500 ng/kg/day CIV from Days 8 to 28 during Cycle 1, with subsequent additional 28-day cycles dosed at 500 ng/kg/day doses intermittently 4 days on/3 days off per week in 28 day cycles without LID lead-in. **B-D: Lead-in-dose mitigates CRS and consequently leads to improvement in dose intensity**. **B**) CRS grade (mean ± SEM) during each week of cycle 1. **C-D**) Dose intensity (%, mean ± 95% confidence interval [CI]) was calculated as the amount of drug received during the time on study (actual drug delivered) relative to the intended dose during weeks 2-4 following respective LID during week 1 2-step (left) multi-step (right) LID. **E**) Tocilizumab effect on duration of IRR/CRS, irrespective of grade. Only patients for which the drug was not modified as a method of controlling IRR/CRS are included. Mean duration of CRS without tocilizumab 1.8 days (n=42) and with tocilizumab 1.2 days (n=13); $P$ = 0.0375, Student $t$ test. SEM=standard error of the mean.

**Fig. 2**: **Relation between cytokine release syndrome (CRS) and anti-leukemic activity**. **A**) Relation between bone marrow (BM) blast change and number of days patient experienced CRS during cycle 1. **B**) Relation between BM blast change and number of CRS events during cycle 1. **C**) Relation between BM blast change and CRS severity (mean grade for all events per patient) during cycle 1. Simple regression is shown as red line, dotted lines depict 95% CI.

**Fig. 3**: **Best change in bone marrow blasts, duration of remission and overall survival in patients receiving flotetuzumab immunotherapy**. **A**) Fifty patients treated at the recommended phase 2 dose (RP2D) differentiated by AML status at study entry: 45 response evaluable, 40 patients in waterfall plot, 5 progressive disease (PD) on peripheral blood blasts; non-evaluable patients 5 (2 patients withdrew consent, 3 patients withdrawn due to non-treatment related adverse events (TRAE). AML status at study entry and percentage of BM blasts at baseline are indicated. *BM aspirate for this patient was hemodiluted but immunohistochemistry on BM FFPE confirmed the percent blast. **B**) Eight PIF/ER AML patients treated at RP2D who achieved a response on flotetuzumab. Median time to best response represented in red (median 0.8 months, range 0.8-2.1 months) and duration of response in blue (median 9.1 months, range 1.1-26.4 months). Star indicates time at which patients underwent hematopoietic stem cell transplantation (HSCT). Purple depicts overall survival (OS) beyond relapse.

**Fig. 4: The tumor immunological microenvironment (TME) in patients receiving flotetuzumab immunotherapy**. **A**) Unsupervised hierarchical clustering (Euclidean distance, complete linkage) of immune cell type-specific scores and biological activity scores in baseline bone marrow (BM) samples from 38 patients with relapsed/refractory (R/R) AML treated with flotetuzumab immunotherapy (color-coded *per* the legend). ClustVis, an online tool for clustering of multivariate data, was used for data analysis and visualization.[40] The immune landscape from 29 out of the 38 patients in this cohort has been presented in a previous publication.[9] PIF=primary induction failure; ER=early relapse; LR=late relapse. **B**) OncoPrint plot summarizing the molecular profile of patients receiving flotetuzumab immunotherapy. The plot was generated using cBioPortal for Cancer Genomics (https://www.cbioportal.org/). **C**) Inflammatory chemokine score and tumor inflammation signature (TIS) score in baseline BM samples from patients with R/R AML. PIF=primary induction failure (n=25); ER=early relapse;

LR=late relapse AML (n=7); HMA=hypomethylating agents (n=6). Data were compared using the Kruskal-Wallis (KW) test for unpaired determinations. **D**) Correlation between the tumor inflammation signature (TIS) score and antigen processing machinery and inflammatory chemokine scores in baseline BM samples from patients with R/R AML. Spearman rank correlation coefficients and *P* values are shown.

**Fig. 5: The tumor immunological microenvironment (TME) in patients receiving flotetuzumab immunotherapy**. **A**) Unsupervised hierarchical clustering (Euclidean distance, complete linkage) of immune gene expression (n=770 genes in the NanoString's PanCancer IO360 panel) in baseline bone marrow (BM) samples from 38 patients with relapsed/refractory (R/R) AML treated with flotetuzumab immunotherapy (color-coded *per* the legend). Complete responses were defined as either CR, CR with partial hematological recovery (CRh), CR with incomplete hematological recovery (CRi) or morphological leukemia-free state (MLFS) at the end of cycle 1. Partial responses (PR) were defined as > 50% decrease in BM blasts from baseline or decrease to 5-25% BM blasts at the end of cycle 1. ClustVis, an online tool for clustering of multivariate data, was used for data analysis and visualization.[40] **B**) Expression of the top 10 genes associated with complete response to flotetuzumab (CR, CRh, CRi). A ranked gene list ($\chi^2$ values) was generated using Orange3 software package (Version 3.25.0). Unsupervised hierarchical clustering (Euclidean distance, complete linkage). The immune cluster was defined as previously detailed.[9] **C**) Heatmap summarizing the correlation coefficients (color-coded *per* the legend) between our 10-gene signature score and immune cell type-specific and biological activity signature scores in baseline BM samples from patients with R/R AML. **D**) Analysis of functional protein association networks using STRING (https://string-db.org/). Top 20 molecules interacting with the top 10 genes in our signature are shown together with their predicted mode of action (highest confidence interaction scores >0.900). Network nodes (query proteins) represent proteins produced by a single protein-

coding gene locus. White nodes represent second shells of interactors. Empty and filled nodes indicate proteins of unknown or partially known 3-dimensional structure, respectively. Edges represent protein–protein associations. Line shapes denote predicted modes of action. **E**) AUROC curve measuring the predictive ability of the 10-gene signature score for anti-leukemic activity from flotetuzumab. Standard errors and confidence intervals are provided in **Table S11**. AUROC=1.0 would denote perfect prediction and AUROC=0.5 would denote no predictive ability.

**Table 1: Baseline characteristics of AML patients in dose escalation, RP2D and PIF/ER subgroup analyses.**

| **Characteristic** | | **Dose escalation (N=42)** | **RP2D population (N=50)** | **RP2D PIF/ER (N=30) *** |
|---|---|---|---|---|
| Age | Median (range) | 64 (29, 84) | 64 (27, 82) | 59 (27, 74) |
| Gender | Female, n (%) | 18 (42.9) | 19 (38.0%) | 10 (33.3%) |
| AML status at entry | Primary refractory (≥ 2 induction attempts), n (%) | 15 (35.7) | 24 (48.0%) | 24 (80.0%) |
| | Early relapse (CR duration ≤ 6 months), n (%) | 8 (19.0) | 6 (12.0%) | 6 (20.0%) |
| AML risk stratification (ELN 2017) | Adverse, n (%) | 13 (31.0) | 26 (52.0%) | 18 (60.0%) |
| | Intermediate, n (%) | 8 (19.0) | 12 (24.0%) | 7 (23.3%) |
| | Favorable, n (%) | 10 (23.8) | 6 (12.0%) | 5 (16.7%) |
| Secondary AML | n (%) | 10/42 (23.8) | 16/50 (32.0%) | 12/30 (40%) |
| Number of prior lines of therapy | Median (range) | 2 (0, 9) | 3 (1, 9) | 4 (1, 9) |
| Failed induction therapy | Cytarabine based induction chemotherapy, n (%) | N/A | N/A | 21 (70%) |
| | Alternative induction therapy, n (%) | N/A | N/A | 3 (10%) |
| Early relapse (<6 months) | n (%) | 4 (9.5%) | 6 (12%) | 6 (20%) |
| | Median duration of CR (months, range) | N/A | 1.6 (0.8-5.1) | 1.6 (0.8-5.1) |
| BM blasts at time of study enrolment (%) | Median (range) | 40 (2-98) | 46 (5-94) | 41 (5-94) |
| Baseline WBC (10^9/L) | Median (range) | 2 (0.4-29.7) | 1.95 (0.3-67) | 2.35 (0.4-16.2) |

*A subset of the RP2D patient population.

**Legend:** CR=complete remission; RP2D=recommended phase 2 dose; PIF=primary induction failure; ER=early relapse; ELN=European Leukemia-Net; n=number; BM=bone marrow; WBC=white blood cells; N/A=not applicable.

**Table 2: Treatment-related adverse events, all and of grade 3 or higher, occurring in more than 10% of the overall population.**

| Treatment-related adverse events | Dose escalation (n=42) | | Dose expansion RP2D population (n=50) * | | PIF/early relapse population (n=30) ^ | |
|---|---|---|---|---|---|---|
| | **All** n (%) | **Grade ≥ 3** n (%) | **All** n (%) | **Grade ≥ 3** n (%) | **All** n (%) | **Grade ≥ 3** n (%) |
| Infusion-related reaction (IRR)/Cytokine release syndrome (CRS) | 34 (81.0) | 3 (7.1) | 48 (96.0) | 4 (8.0) | 30 (100) | 1 (3.3) |
| Nausea | 11 (26.2) | - | 14 (28.0) | - | 8 (26.7) | - |
| Fatigue | 8 (19.0) | | 6 (12.0) | 1 (2.0) | 3 (10.0) | 1 (3.3) |
| Pyrexia | 8 (19.0) | 2 (4.8) | 11 (22.0) | | 6 (20.0) | |
| Peripheral edema | 6 (14.3) | | 15 (30.0) | 1 (2.0) | 8 (26.7) | |
| Alanine aminotransferase increased | 5 (11.9) | 1 (2.4) | 7 (14.0) | 2 (4.0) | 3 (10.0) | 1 (3.3) |
| Arthralgia | 5 (11.9) | 1 (2.4) | 7 (14.0) | 1 (2.0) | 4 (13.3) | |
| C-reactive protein increased | - | - | 6 (12.0) | 2 (4.0) | - | - |
| Diarrhea | - | - | 11 (22.0) | - | 5 (16.7) | - |
| Hypotension | - | - | 8 (16.0) | - | 4 (13.3) | - |
| Decreased appetite | - | - | 6 (12.0) | 1 (2.0) | 5 (16.7) | 1 (3.3) |
| Tachycardia | - | - | 6 (12.0) | 1 (2.0) | - | - |
| Myalgia | - | - | 8 (16.0) | 2 (4.0) | 4 (13.3) | - |
| Dyspnea | - | - | 9 (18.0) | 3 (6.0) | 4 (13.3) | 2 (6.7) |
| Platelet count decreased | 7 (16.7) | 5 (11.9) | 7 (14.0) | 6 (12.0) | 3 (10.0) | 3 (10.0) |
| Lymphocyte count decreased | 6 (14.3) | 5 (11.9) | 6 (12.0) | 4 (8.0) | | |
| Neutrophil count decreased | 1 (2.4) | 1 (2.4) | 2 (4.0) | 2 (4.0) | 2 (6.7) | 2 (6.7) |
| Treatment-related | 0 | 0 | 1 (2.0) | 1 (2.0) | 1 (3.3) | 1 (3.3) |
| Non treatment-related | 1 (2.4) | 1 (2.4) | 1 (2.0) | 1 (2.0) | 1 (3.3) | 1 (3.3) |

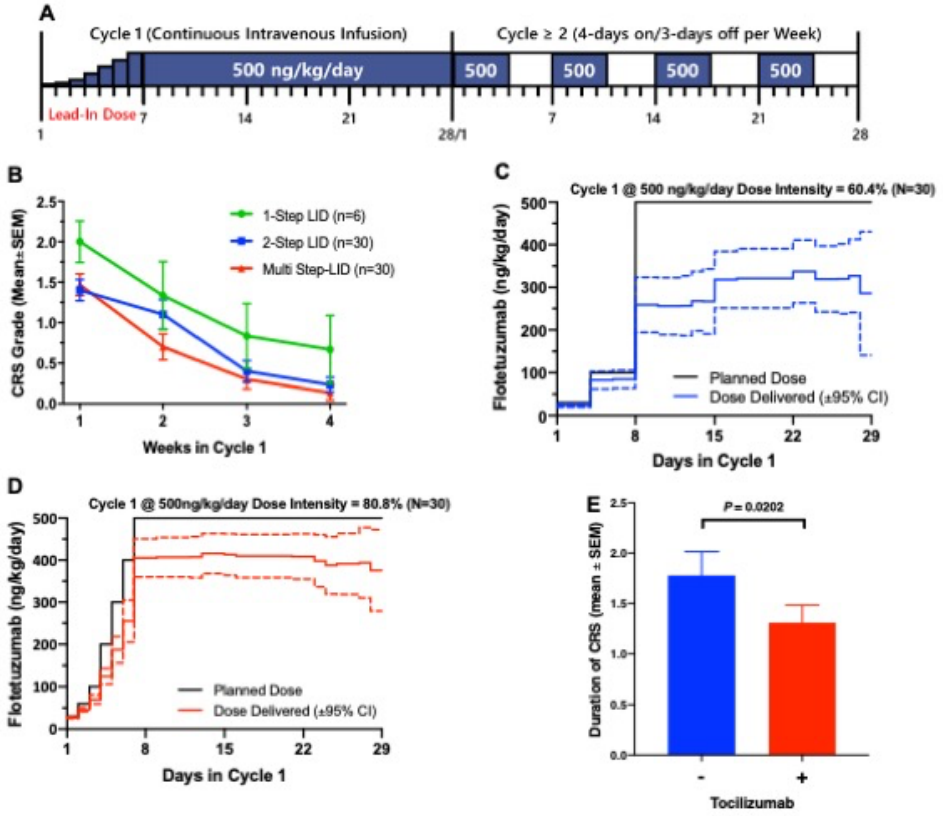
* Includes 46 patients treated in expansion cohort and 4 patients treated in dose escalation.

^A subset of the RP2D patient population.

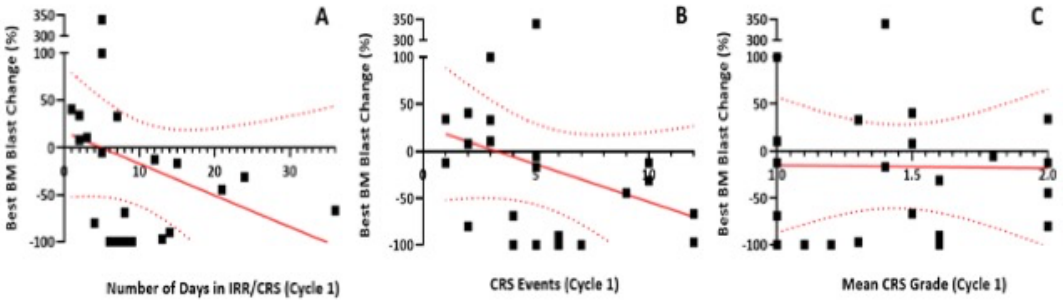
**Table 3: Response Rate in Relapse/Refractory and Primary Induction Failure and Early Relapse AML**

| % (n) | R/R AML (n=50) | PIF/ER AML (n=30) |
|---|---|---|
| **CR** | 12.0% (6) | 16.7% (5) |
| **CR/CRh** | 18.0% (9) | 26.7% (8) |
| **CR/CRh/CRi** | 20.0% (10) | 30.0% (9) |
| **CR/CRh/CRi/MLFS/PR** | 24.0% (12) | 30.0% (9) |

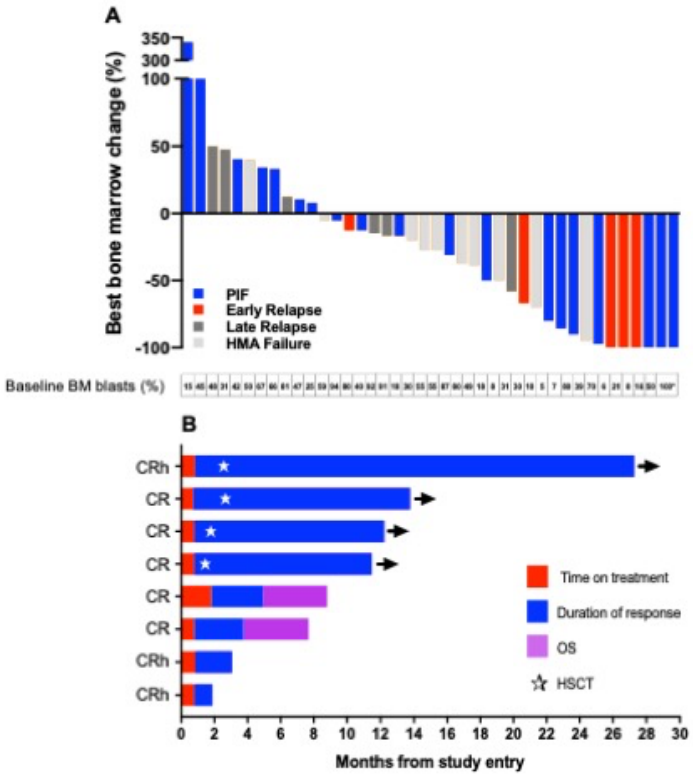
**Legend:** R/R=relapsed/refractory; PIF=primary induction failure; ER=early relapse; CR=complete remission; CRh=complete remission with partial hematopoietic recovery; CRi=complete remission with incomplete hematopoietic recovery; MLFS=morphological leukemia-free state; PR=partial remission.


A
Cycle 1 (Continuous Intravenous Infusion)
Cycle ≥ 2 (4-days on/3-days off per Week)
500 ng/kg/day
500
500
500
500
Lead-In Dose
1
7
14
21
28/1
7
14
21
28
B
CRS Grade (Mean±SEM)
1-Step LID (n=6)
2-Step LID (n=30)
Multi Step-LID (n=30)
Weeks in Cycle 1
C
Cycle 1 @ 500 ng/kg/day Dose Intensity = 60.4% (N=30)
Flotetuzumab (ng/kg/day)
Planned Dose
Dose Delivered (±95% CI)
Days in Cycle 1
D
Cycle 1 @ 500ng/kg/day Dose Intensity = 80.8% (N=30)
Flotetuzumab (ng/kg/day)
Planned Dose
Dose Delivered (±95% CI)
Days in Cycle 1
E
P = 0.0202
Duration of CRS (mean ± SEM)
-
+
Tocilizumab

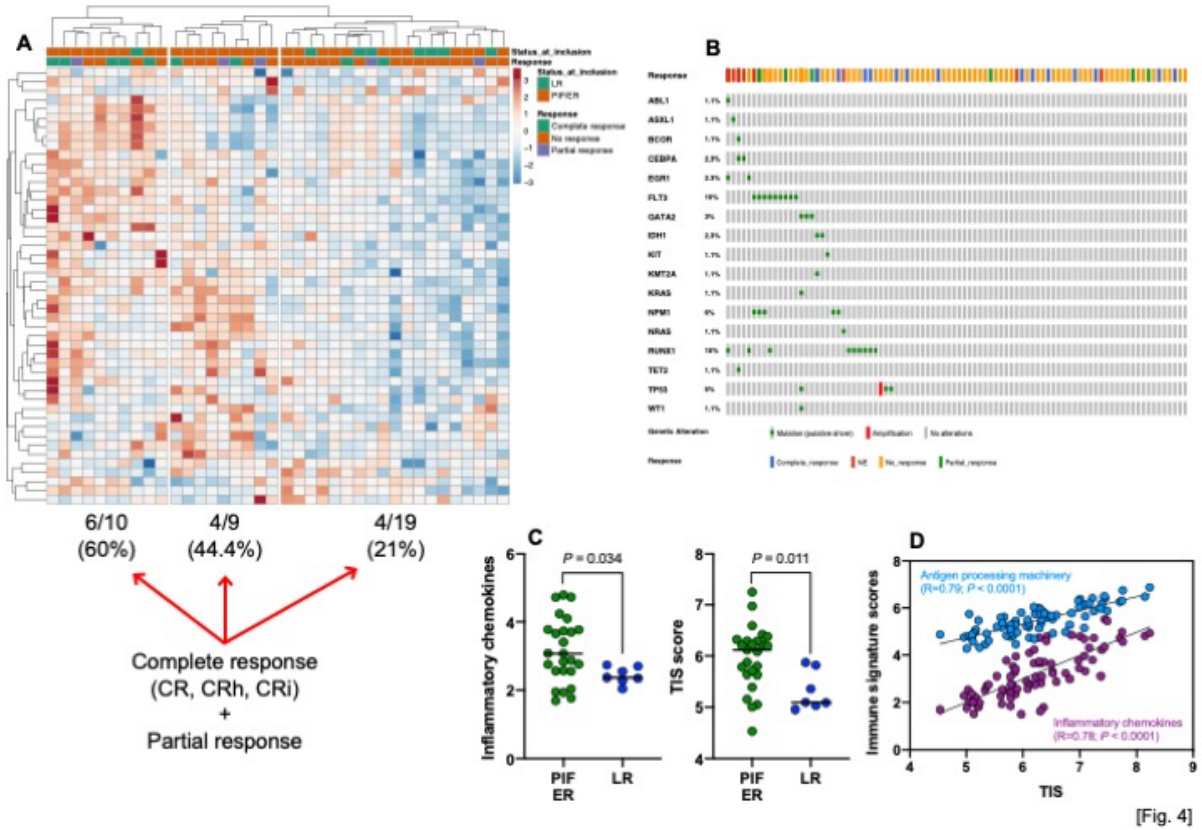
[Fig. 1]


A
Best BM Blast Change (%)
350
300
100
50
0
-50
-100
10
20
30
Number of Days in IRR/CRS (Cycle 1)
B
Best BM Blast Change (%)
CRS Events (Cycle 1)
C
Best BM Blast Change (%)
1.0
1.5
2.0
Mean CRS Grade (Cycle 1)

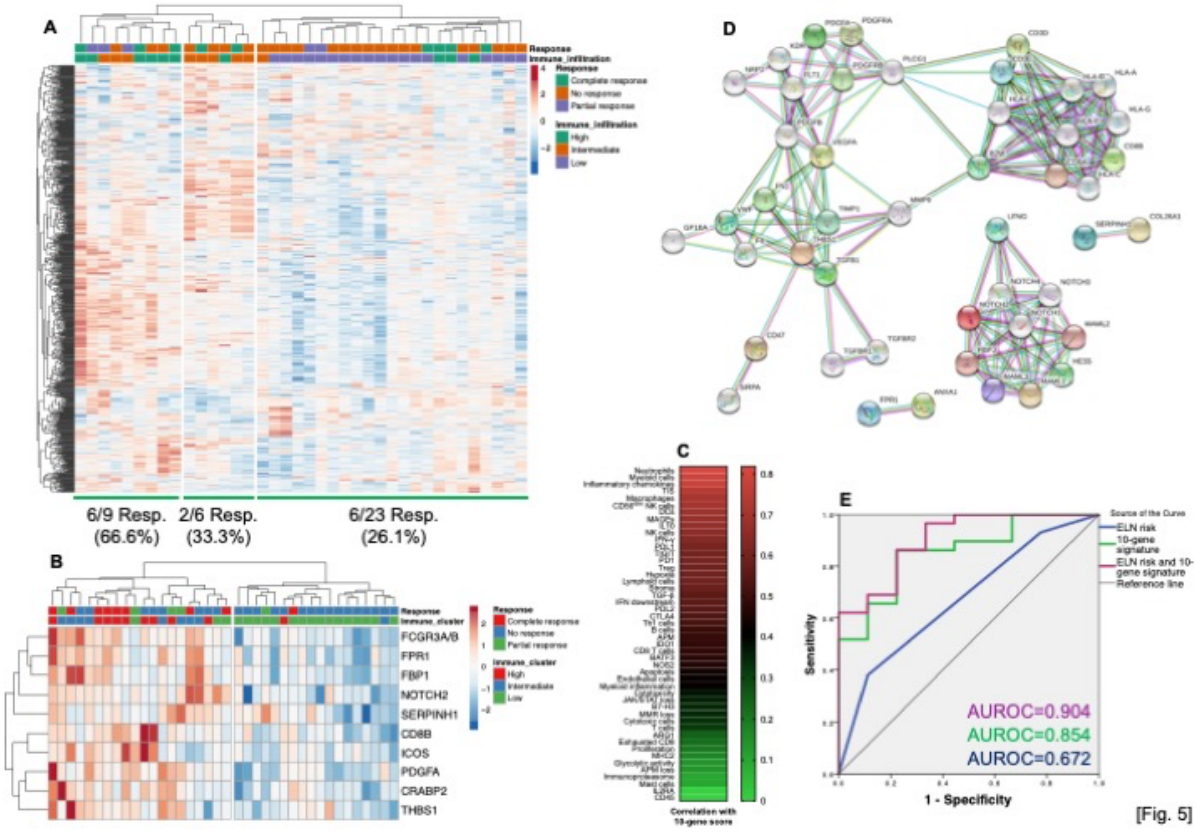
[Fig. 2]


A
Best bone marrow change (%)
350
300
100
50
0
-50
-100
PIF
Early Relapse
Late Relapse
HMA Failure
Baseline BM blasts (%)
B
CRh
CR
CR
CR
CR
CR
CRh
CRh
Time on treatment
Duration of response
OS
HSCT
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30
Months from study entry

[Fig. 3]


A
B
6/10
(60%)
4/9
(44.4%)
4/19
(21%)
Complete response
(CR, CRh, CRi)
+
Partial response
C
P = 0.034
P = 0.011
Inflammatory chemokines
TIS score
PIF
ER
LR
D
Antigen processing machinery
(R=0.79; P < 0.0001)
Inflammatory chemokines
(R=0.78; P < 0.0001)
Immune signature scores
TIS

[Fig. 4]


A
Response
Immune_infiltration
Response
Complete response
No response
Partial response
Immune_infiltration
High
Intermediate
Low
6/9 Resp.
(66.6%)
2/6 Resp.
(33.3%)
6/23 Resp.
(26.1%)
B
Response
Immune_cluster
FCGR3A/B
FPR1
FBP1
NOTCH2
SERPINH1
CD8B
ICOS
PDGFA
CRABP2
THBS1
C
Correlation with
10-gene score
D
E
Source of the Curve
ELN risk
10-gene signature
ELN risk and 10-gene signature
Reference line
Sensitivity
1 - Specificity
AUROC=0.904
AUROC=0.854
AUROC=0.672

[Fig. 5]