Clinical and radiological resolution of infections during treatment with mbIL-21-expanded FC21-NK cells in patients with relapsed or refractory acute myeloid leukaemia (R/R AML)

L. SILLA[1,2], B. CORREA[1], V. VALIM[2], A. VARGAS[2], C. WEBER[2], S. NICHELE[3], F. SCHERER[2], R. BITTENCOURT[2], A. PAZ[2] and D. LEE[4]

[1]Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil; [2]Hospital de Clínicas de Porto Alegre, Porto Alegre, RS, Brazil;

[3]Hospital Nossa Senhora das Gracas, Curitiba, PR, Brazil; [4]Nationwide Children's Hospital, Columbus, OH, USA

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INTRODUCTION

- High-dose chemotherapy is the cornerstone of treatment for acute myeloid leukaemia (AML)[1,2] but can cause neutropenia associated with treatment-related viral, bacterial and fungal infections,[3] particularly in a population with relapsed/refractory (R/R) disease[4]
- Natural killer (NK) cells have been shown to have both antileukaemic and antimicrobial effects[5] and may be a less toxic treatment option for frail patients with AML
- Double-bright (CD56^{bright}/CD16^{bright}; DB) NK cells are generated from peripheral blood using transfected feeder cells expressing membrane-bound interleukin-21 (mbIL-21) and 4-1BB ligand (FC21)[6]
- FC21-derived NK cells present a unique DB phenotype and express hyperfunctional characteristics with high levels of cytolytic and secretion activity[6]
- In a phase I study (NCT02809092), we investigated multiple doses of DB FC21-NK cells following standard fludarabine, cytarabine and granulocyte-colony stimulating factor (FLAG) induction in patients with R/R AML

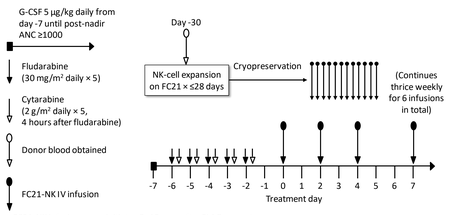
OBJECTIVE

- To investigate preliminary safety and efficacy data in a subgroup of patients with R/R AML and concurrent severe infectious complications from this study

METHODS

- This open-label, phase I study was performed in patients treated at a single centre in Brazil
- Eligible patients were ≥2 years old, with R/R AML; a Karnofsky or Lansky performance status of ≥70; and adequate renal, liver and pulmonary function
- Patients were ineligible if they exhibited uncontrolled infection (infection that had not resolved or shown evidence of resolution after initiating appropriate therapy, excluding chronic asymptomatic viral infections)
- Eligible donors were a human leukocyte antigen-haploidentical relative selected for best NK alloreactivity
- Cytogenetic risk was determined according to European LeukemiaNet (ELN) guidelines[1]
- FC21-NK cells were expanded from donors on FC21 feeder cells as previously described[6]
- Patients were treated with FC21-NK from 10^6 to 10^7 cells/kg/dose; the treatment schema is shown in **Figure 1**

Figure 1: Treatment schema



FC21-NK infusions were initiated 2–15 days after FLAG chemotherapy.
ANC, absolute neutrophil count; G-CSF, granulocyte-colony stimulating factor; IV, intravenous.

- Assessments in this *post hoc* analysis included:
  - Adverse events (AEs); reported from day 0 up to day 56 and graded according to the Common Terminology Criteria for Adverse Events (CTCAE) version 4.0
  - Overall response; assessed at day 28–30 after initiation of protocol using the International Working Group (IWG) response criteria[7]
  - A description of course of infections

RESULTS

Patients

- 16 patients with R/R AML were enrolled; of 13 treated patients, 3 had concomitant infections or high-risk bacterial colonization and were included in this analysis
- Characteristics of these 3 patients are summarized in **Table 1**

Table 1: Patient and disease characteristics

Characteristic	Patient 1	Patient 3	Patient 11*
Sex	Female	Female	Male
Age, years	48	45	2
Severe infections at enrollment	Vancomycin-resistant *Enterococcus*	None	MDR *Escherichia coli (E. coli)*
Bacterial colonization during treatment	*Aspergillus*	*Mycobacterium tuberculosis* (*M. tb*)	None
Cytogenetic risk†	Favorable	Intermediate	Intermediate
Chromosomal aberrations	inv(16)(p13;q22)	None	None
Prior treatments, n	6	6	3
Prior SCT	Autologous	Allogeneic (×2)	Allogeneic (haplo)
Relapse, n or refractory	4	3	Refractory

**Patient treated under compassionate use; †Patients 1 and 3 were wild type for FLT3 internal tandem duplication, not determined for patient 11. Haplo, haploidentical; MRD, multidrug-resistant.*

Treatment exposure and safety

- Treatment exposure is shown in **Table 2**
  - Patients 1 and 3 completed ≥6 IV FC21-NK-cell infusions
  - FC21-NK treatment was stopped after 3 infusions in patient 11 due to worsening jaundice coinciding with rapid *in vivo* expansion of NK cells

Table 2: Treatment exposure

Characteristic	Patient 1*	Patient 3	Patient 11*
Cell dose per infusion	1×10^6/kg & 1.76×10^6/kg	6.94×10^6/kg	9.39×10^6/kg
Number of infusions	6 & 5	6	3

**Patient 1 completed 2 courses of FC21-NK treatment: the first course comprised 6 infusions of FC21-NK only; the second comprised 5 infusions of FC21-NK following FLAG chemotherapy (the 6th infusion was discarded due to contamination of the infusion sample).*

- No patients had dose-limiting treatment-related AEs
- All patients experienced grade 4 febrile neutropenia attributed to haematological toxicity secondary to FLAG
- AEs considered to be related to FC21-NK treatment are shown in **Table 3**; no FC21-NK infusion-related toxicities or cytokine storm syndrome were reported

Treatment outcomes

- Outcomes of patients with severe infectious complications are shown in **Table 4**
  - Patients 1 and 3 demonstrated complete responses (CRs) lasting 151 and 269 days, respectively (**Figure 2**)

Table 3: Adverse events considered to be related to FC21-NK-cell treatment

	Adverse event	Grade	Description
Patient 1*	Worsening of pulmonary symptoms	3	Pre-existing invasive pulmonary aspergillosis, managed with voriconazole
	Probable CNS aspergillosis	2	Resolved without medication
	CNS hypertension	2	Transient, secondary to CNS inflammation
Patient 3	Worsening of symptoms and signs	3	Pre-existing pulmonary tuberculosis

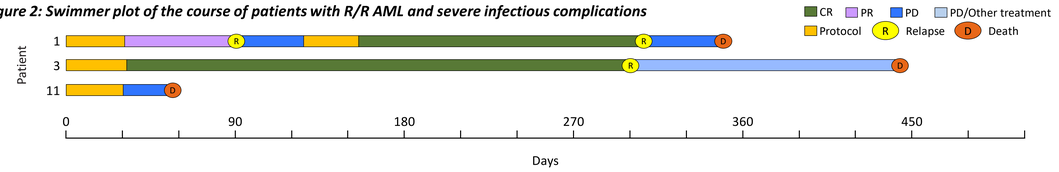
**AEs experienced by patient 1 occurred during the first course of treatment.*

Table 4: Outcome after treatment with FC21-NK following FLAG chemotherapy

Characteristic	Patient 1	Patient 3	Patient 11
Response	CR*	CR	PD
Duration of response,† days	151	301	–
Time to patient death,‡ days	344	440	52

**Response recorded for second course of treatment. Patient achieved PR following first course; †Time from first reporting of response to relapse; ‡Time from start of the protocol (from the first FC21-NK infusion in the case of patient 1 who did not receive FLAG in their first treatment).*
PD, progressive disease; PR, partial response.

Figure 2: Swimmer plot of the course of patients with R/R AML and severe infectious complications



RESULTS (CONT.)

Course of infections during treatment in patients with severe infectious complications

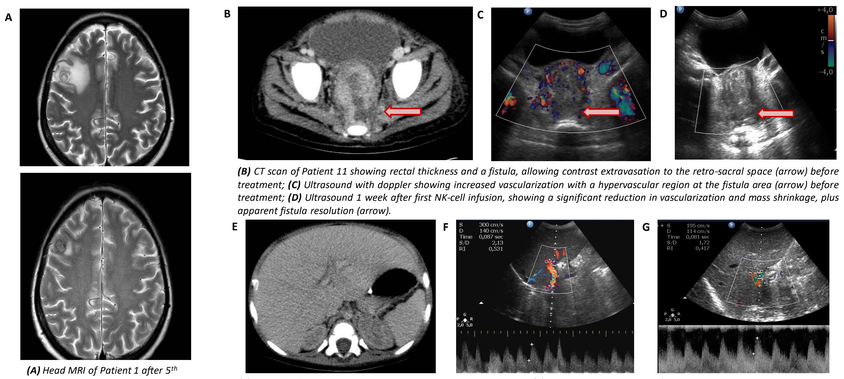
- Course of infections is summarized in **Table 5**
  - Patient 1 was discharged in CR with complete resolution of her mycetoma (**Figure 3A**) and pulmonary symptoms
  - Patient 3 was discharged in CR with resolution of pulmonary infection
  - After discontinuation of FC21-NK treatment, patient 11 experienced significant clinical improvement with almost complete disappearance of cholangitis and rectal mass observed (**Figure 3B–3G)**

Table 5: Course of infections during treatment in patients with R/R AML and severe infectious complications

	Infection	Course of infection/infectious symptoms and lesions
Patient 1	Vancomycin-resistant *Enterococcus* *Aspergillus*	• Pt was enrolled with vancomycin-resistant *Enterococcus* and was receiving prophylactic voriconazole for previous pulmonary aspergillosis • CNS mycetoma and pulmonary aspergillosis were diagnosed* after the 5th FC21-NK infusion. On completion of 6 infusions and following voriconazole dose adjustment, the pt was discharged with AML in PR with infections resolved • 43 days after documentation of PD, a second course of treatment (FLAG + FC21-NK with voriconazole prophylaxis) was given • Pt developed febrile neutropenia, typhlitis and a thigh abscess • Pt was discharged in CR but later died from AML relapse without aspergillosis re-activation
Patient 3	Pulmonary tuberculosis (*M. tb*)	• Pt was receiving prophylactic voriconazole for previous pulmonary aspergillosis. During FLAG treatment, a low-grade fever of unknown aetiology developed; at start of FC21-NK treatment, pulmonary symptoms were reported and tuberculosis was detected† (which was clinically managed with anti-tuberculosis treatment) • Pt was discharged with infection resolved and AML in CR. The pt later died from AML relapse without re-activation of infection
Patient 11	MDR *E. coli*	• Pt had MDR *E. coli* presenting with hepatomegaly, jaundice and ascending cholangitis. He was septic, with a rectal mass causing almost complete obstruction of the GIT and abdominal distension • After stabilizing his condition, FC21-NK was initiated and then discontinued after 3 infusions with *in vivo* expansion of FC21-NK cells. Despite persistent leukaemia, worsening of jaundice and rectal bleeding, significant clinical improvement was achieved with almost complete disappearance of the rectal mass and cholangitis on ultrasound imaging • The pt continued with persistent febrile episodes and died of AML with *E. coli* biliary infection

**Mycetoma was detected by CNS MRI following complaints of intense headache; pulmonary aspergillosis was indicated by micronodules on pulmonary CT and high serum and bronchoalveolar lavage galactomannan levels; †Bilateral micronodular lung infiltration was observed on radiological images and tuberculosis was identified in bronchoalveolar lavage. CT, computed tomography ; GIT, gastrointestinal tract; MRI, magnetic resonance imaging; Pt, patient.*

Figure 3: Resolution of mycetoma, and significant clinical improvement of cholangitis and rectal mass



(A) *Head MRI of Patient 1 after 5th NK-cell infusion, showing parenchymal mycetoma obtained* ***(top)*** *and resolution at day 28 (****bottom****).*

(B) *CT scan of Patient 11 showing rectal thickness and a fistula, allowing contrast extravasation to the retro-sacral space (arrow) before treatment;* ***(C)*** *Ultrasound with doppler showing increased vascularization with a hypervascular region at the fistula area (arrow) before treatment;* ***(D)*** *Ultrasound 1 week after first NK-cell infusion, showing a significant reduction in vascularization and mass shrinkage, plus apparent fistula resolution (arrow).*

(E) *Liver CT of Patient 11 showing an enlarged liver at admission;* ***(F)*** *Ultrasound showing significant increase in spectral velocity at the hepatic artery (PSV = 300 cm/s, EDV = 140 cm/s) before NK-cell infusion;* ***(G)*** *Ultrasound showing significant decrease in spectral velocity at the hepatic artery 1 week after treatment (PSV = 195 cm/s, EDV = 114 cm/s). EDV, end diastolic velocity; PSV, peak systolic velocity.*

CONCLUSIONS

- In this phase I study, multiple IV infusions of FC21-NK administered to patients with serious infectious complications were well tolerated and encouraging signs of antitumor and suspected antimicrobial activity were observed
- FC21-NK-cell infusions might be a possible alternative treatment for frail R/R AML patients
- Elderly, frail AML patients ineligible for SCT could benefit from FC21-NK-cell therapy

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DISCLOSURES

Silla: Nothing to disclose. **Correa:** Nothing to disclose. **Valim:** Nothing to disclose. **Vargas:** Nothing to disclose. **Weber:** Nothing to disclose. **Nichele:** Nothing to disclose. **Scherer:** Nothing to disclose. **Bittencourt:** Nothing to disclose. **Paz:** Nothing to disclose. **Lee:** CytoSen Therapeutics, Kiadis Pharma: stock ownership, consultancy, research funding & patents/royalties; Caribou Biosciences: consultancy; Courier Therapeutics: consultancy.

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CONTACT INFORMATION

For any enquiries, please contact Dr Lucia Silla at dralucia.silla@gmail.com.