Clinical features of non-infectious pulmonary complications after donor lymphocyte infusion in post-transplant patients: The Nagasaki Transplant Group Experience.

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Introduction

Donor lymphocyte infusion (DLI) is a therapeutic option in immune cell therapies for relapsed hematological malignancies after allogeneic hematopoietic stem cell treatment (allo-HSCT). DLI induces the graft-versus-leukemia (GVL) effect, leading to durable remission. Patients with relapsed chronic myeloid leukemia and adult T-cell leukemia/lymphoma after allo-HSCT responded well to DLI therapy [1-6]. Graft-versus-host disease (GVHD) is an allo-immune reaction-related complication of DLI therapy that has been reported to develop in 40-60% of post-DLI patients, with GVHD-associated mortality of approximately 10% [2-5,7].

Non-infectious pulmonary complications (non-IPC) are an allo-immune reaction-associated complication after allo-HSCT. The disease subtypes of non-IPCs correlate with the intervals from allo-HSCT; peri-engraftment respiratory distress syndrome, idiopathic pneumonia syndrome, and diffuse alveolar hemorrhage are observed in the early post-transplant period, while cryptogenic organizing pneumonia and bronchiolitis obliterans syndrome (BOS) are frequently diagnosed in the late post-transplant period [8-10]. Although previous studies reported non-IPCs after DLI therapy, limited information is currently available on their clinical features, such as the disease subtype of non-IPCs and their outcomes [7,11].

Therefore, we herein retrospectively analyzed 41 patients who received DLI therapy for post-transplant relapsed hematological malignancies. Seven out of 41 patients developed post-DLI non-IPCs, including BOS, nonspecific interstitial pneumonia (NSIP), and acute respiratory distress syndrome (ARDS). We describe the clinical features of non-IPCs after DLI therapy.

Patients and methods

Patient population

The original dataset consisted of 218 patients who underwent first allo-HSCT between January 1, 2009 and December 31, 2018 at 3 institutions in Nagasaki prefecture, Japan. Hematological malignancies relapsed after allo-HSCT in 85 patients. Among these patients, 41 received DLI therapy as a salvage treatment (Supplemental Figure 1). Data on these 41 patients were collected and updated as of August 31, 2019. The present study was approved by the Ethical Committees of each participating institution.

Definition

The present study included patients who did not have any pulmonary complications before DLI therapy, but were newly diagnosed with non-IPCs following DLI therapy.

The definitions of BOS and ARDS were based on the 2014 National Institutes of Health Consensus and the Berlin definition, respectively [12,13]. The diagnosis of NSIP was based on the clinical-radiological-pathological diagnosis; hypersensitivity pneumonitis, infectious complications, collagen vascular disease, and drug-induced pneumonia were excluded from the diagnosis of NSIP [14]. For diagnosis with non-IPCs, bronchoscopy and bronchoalveolar lavage were performed to rule out infectious complications. Bronchoalveolar lavage fluid was evaluated by smear, culture, and real-time quantitative polymerase chain reaction. If bronchoscopy was not performed due to any medical reason, blood and sputum was evaluated.

Acute and chronic GVHD were diagnosed and graded according to established criteria [15,16]. Conditioning regimens were classified as myeloablative or reduced intensity conditioning according to established criteria [17].

Statistical analysis

The cumulative incidence of non-IPCs after the initial DLI was estimated, with (i) death due to a cause other than non-IPCs and (ii) second transplantation being treated as competing events [18]. A graphical presentation was performed using EZR software, version 3.4.3 (Saitama Medical Center, Jichi Medical University) [19].

Results

Non-IPC after DLI therapy

The characteristics of 41 patients who received DLI for relapsed hematological malignancies are shown in Supplemental Table 1. In the present study, 7 out of 41 patients developed non-IPCs after DLI therapy, which were classified into three subtypes: BOS, ARDS, and NSIP. The 6-year cumulative incidence of non-IPC was 18.0% (95% confidence interval, 7.9 to 31.5%) (Figure 1).

Characteristics of patients who developed non-IPCs after DLI therapy

The characteristics at allo-HSCT and transplant procedures of patients with post-DLI non-IPCs are shown in Table 1. Seven patients with post-DLI non-IPCs did not have any pulmonary complications before allo-HSCT. The median age of patients at allo-HSCT was 52 years (range, 24 to 56 years). Graft sources were HLA-matched related peripheral blood stem cells (n=2), HLA-mismatched related bone marrow (n=2), HLA-mismatched related peripheral blood stem cells (n=2), and HLA-matched unrelated bone marrow (n=1). No patient used anti-thymocyte globulin in the conditioning regimens.

The median time between allo-HSCT and the initial DLI was 8.5 months (range, 2.9-24.2). Four patients received 1 cycle of DLI; 1 patient received 2 cycles of DLI without dose escalations; and the other 2 patients received 2 cycles of DLI with sequential T-cell dose escalations. The median initial and total CD3-positive cell doses were 5.5×10^6/kg (range, 1.0-37.0) and 6.0×10^6/kg (range, 1.0-60.0) (Table 2). Before the administration of donor lymphocyte, all 7 patients discontinued immunosuppressive agents as a treatment for relapsed disease. At DLI therapy, 6 of 7 patients had mild chronic GVHD; 5 patients (UPN-01, -02, -05, -06 and -07) had skin lesions of GVHD which was improved by topical agents only, 1 patient (UPN-04) had mucositis and liver disfunction which was stable without systemic immunosuppressive agents. Therefore, each physician decided to perform DLI therapy despite the presence of GVHD.

ARDS after DLI therapy

In the present study, 3 patients (UPN-01, -02, and -03) developed ARDS after DLI therapy. The median interval from the last date of DLI therapy to the development of ARDS was 12 days (range, 12-14) (Figure 2). Three patients had skin rash and/or fever refractory to antimicrobial agents following DLI therapy, and subsequently developed acute and progressive hypoxemia. Thoracic computed tomography (CT) showed diffuse

ground-glass opacities (Figure 3A), leading to the diagnosis of ARDS. For diagnosis with ARDS, bronchoscopy was not performed in 1 patient (UPN-03) due to pancytopenia and respiratory failure.

Regarding the status of GVHD before the diagnosis with ARDS, the progression of acute GVHD following DLI therapy was observed in 2 patients (UPN-01 and -02): grade II acute GVHD in UPN-01 and grade III acute GVHD in UPN-02. The other patient (UPN-03) did not have any symptoms of acute GVHD after DLI therapy.

All 3 patients received a systemic corticosteroid treatment (methylprednisolone (mPSL) 2 mg/kg in UPN-01 and 1000 mg/day in UPN-02; hydrocortisone 200 mg/day in UPN-03) for ARDS, but did not require mechanical ventilation (Table 3). In all patients, the systemic corticosteroid treatment attenuated hypoxemia and resolved diffuse ground-glass opacities.

NSIP after DLI therapy

Two patients (UPN-03 and -04) developed NSIP after DLI therapy; one (UPN-03) developed NSIP following the amelioration of ARDS. The intervals from the last date of DLI therapy to the development of NSIP were 3.5 and 24.7 months in 2 patients (see Figure 2). Respiratory symptoms, such as exertional dyspnea and hypoxemia, were

observed in both patients at the diagnosis of NSIP, and CT revealed the thickening of septal lines and subpleural ground-glass opacities without honeycombing (Figure 3B). One patient (UPN-04) had extensive chronic GVHD at the diagnosis of NSIP, while the other (UPN-03) did not have any symptoms of GVHD. One patient (UPN-04) received bronchoscopy for the diagnose with NSIP. On the other hand, bronchoscopy was not performed in UPN-03 due to pancytopenia and respiratory failure.

Both patients received systemic corticosteroid treatment for NSIP; however, neither responded (Table 3). One patient (UPN-03) died of the underlying disease along with persistent NSIP, while the other (UPN-04) died of the progression of NSIP.

BOS after DLI therapy

Three patients (UPN-05, -06, and -07) developed BOS after DLI therapy. The median interval from the last date of DLI therapy to the diagnosis of BOS was 9.4 months (range, 2.6-61.8) (see Figure 2). Exertional dyspnea and hypoxemia were observed in 3 patients at the diagnosis of BOS. Pulmonary function tests revealed that (i) the forced expiratory volume for 1 second (FEV1) was less than 75% of the predicted value, and (ii) FEV1/forced vital capacity was less than 70% in 3 patients (see Supplemental Table2). CT showed bronchial dilatation, air trapping, and bronchial wall thickening

(Figure 3C and 3D). Bronchoscopy was not performed in all patients with BOS doe to the development of emphysematous changes which could induce pneumothorax.

All 3 patients had symptoms of GVHD at the initial administration of DLI, and showed the progression of GVHD after DLI. In all 3 patients, persistent GVHD was observed at the diagnosis of BOS. Two patients (UPN-05 and -06) received the systemic corticosteroid treatment for BOS and required home oxygen therapy. The other patient (UPN-07) maintained a stable status for BOS with inhaled long-acting β-agonists, and underwent second allo-HSCT for relapsed acute myeloid leukemia. One patient (UPN-05) died of the underlying disease along with persistent BOS; and the other 2 patients (UPN-06 and -07) were alive with persistent BOS.

Discussion

The aim of the present study was to clarify the clinical features of non-IPC following DLI. The results obtained revealed that the 6-year incidence of non-IPCs (ARDS, NSIP, and BOS) after DLI therapy was 18% and 1 out of 7 patients died of the progression of NSIP. Furthermore, we showed that the clinical symptoms of GVHD were present when non-IPCs developed, indicating a close relationship between non-IPCs and GVHD after DLI therapy. This was a relatively large study showing the clinical features of non-IPCs

after DLI therapy; therefore, the results obtained provide important insights into clinical outcomes after DLI.

The present study showed that the non-IPC subtype was associated with the interval from DLI therapy. ARDS developed within 14 days of the last initiation of DLI (i.e., the early post-DLI period), which was consistent with previous findings [11]. A follow-up is needed to monitor the development of ARDS for 2 weeks after the initiation of DLI, and careful attention to not only respiratory symptoms, but also fever and skin rash is required. NSIP and BOS emerged along with chronic GVHD symptoms in the late post-DLI period (≥2 months) following DLI therapy. These results suggest the importance of long-term surveillance to diagnose NSIP and BOS following DLI therapy.

Another important result is that GVHD symptoms were noted prior to the development of early- and late-onset non-IPCs. ARDS emerged along with acute GVHD in 2 out of the 3 patients who used HLA-mismatched donor lymphocytes (see Table 2), indicating that acute GVHD after DLI from these donors affect, at least partly, the development of post-DLI ARDS. In addition, 4 out of 5 patients had chronic GVHD before the development of late-onset non-IPCs (NSIP and BOS). These results were similar to the relationship observed between late-onset non-IPC and chronic GVHD after allo-HSCT

[20-22], which supports the symptoms of chronic GVHD being a significant marker for monitoring late-onset non-IPCs.

In terms of treatment responses, the present study showed that ARDS responded to systemic corticosteroid treatment, while a previous study reported that the response of patients to corticosteroid treatment for ARDS was poor [11]. Since mechanical ventilation was not required in the present cases, who were treated with corticosteroids in the early phase of ARDS, the initiation of systemic corticosteroid treatment needs to be considered as soon as possible when acute and progressive hypoxia with diffuse ground-glass opacities is detected in post-DLI patients, particularly with fever and/or skin rash. In the present study, NSIP in 2 patients and BOS in 2 patients did not respond well to the systemic corticosteroid treatment (see Table 3), indicating that corticosteroid treatment was insufficient for these late-onset non-IPCs. Based on the relationship between late-onset non-IPCs and chronic GVHD, further studies are warranted to clarify whether novel targeting agents, such as a janus kinase 1 and 2 inhibitor (ruxolitinib), Bruton's tyrosine kinase inhibitor (ibrutinib), and selective rho-associated coiled-coil–containing protein kinase 2 inhibitor (belumosudil), are effective against late-onset non-IPCs following DLI therapy [23-26].

There are several limitations that need to be addressed. Firstly, due to the small number

of patients, we were unable to evaluate each factor affecting the development of non-IPCs using a statistical analysis. Furthermore, we did not confirm predictive factors affecting responses to treatments for non-IPCs. Further large-scale studies are needed to reach more definitive conclusions. Secondly, donor lymphocyte could not be determined as the direct cause of ARDS in this study. For the diagnosis with ARDS, any entity was not detected; bacterial, virus and fungal pneumonitis, sepsis, and an aspiration of gastric contents. The previous report suggested that donor lymphocyte release inflammatory cytokine, which may be a first inflammatory hit of ARDS in the patients following allo-HSCT who have active or latent infections [11]. Therefore, the administration of donor lymphocyte was regarded as the trigger of the development of ARDS in this study. However, we could not evaluate not only detail infectious pathogen by next generation sequence but also cytokine following DLI therapy, further studies are needed to confirm the association between DLI therapy and ARDS.

In conclusion, the present study showed the clinical features of non-IPCs following DLI therapy, which supports the importance of the careful management of post-DLI non-IPCs. The early detection of post-DLI non-IPCs based on distinct clinical symptoms would contribute to the initiation of optimal treatment. In the future, novel therapeutic strategies are required to reduce DLI-related toxicity while maximizing the

benefits of GVL effects.

Authorship

Contribution: M.F., H.I., and Y. Miyazaki designed the research, organized the project, and wrote the manuscript; M.F. and H.I. performed analyses and collected data; M.F., H.I, and Y. Moriuchi collected data; and all authors interpreted data and reviewed and approved the final manuscript.

Declaration of competing interests

Y. Miyazaki received honoraria from Novartis Pharma, Nippon-Shinyaku Co., Sumitomo Dainippon Pharma, Astellas Pharma and Celgene Co., Chugai Pharma, AbbiVie GK, and SymBio Pharmaceuticals, and funding from Sumitomo Dainippon Pharma for research and Chugai Pharma for scholarships.

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Figure legends

Figure 1. Cumulative incidence of non-IPCs after DLI therapy

Figure 2. The time of the occurrence and clinical course of non-IPCs following DLI therapy

Black circles and the black bar indicate the diagnosis and on disease of non-IPCs, respectively. Arrows indicate that the patient was alive at the time of the last follow-up.

Figure 3. CT findings at the diagnosis of non-IPCs

A: Diffuse ground-grass opacities and a non-segmental mosaic pattern, indicating ARDS (UPN-03).

B: A thickening of septal lines, subpleural ground-glass opacities, and cord-like shadows without honeycombing, indicating NSIP (UPN-03).

C and D: Inspiratory and expiratory phases, respectively, at the diagnosis of BOS, showing bronchial dilatation, air trapping (red arrow), and bronchial wall thickening (UPN-07).

Supplemental Figure 1. Clinical course of non-infectious pulmonary complications

after DLI therapy for relapsed disease in hematopoietic stem cell transplantation.

In one patient, ARDS overlapped with NSIP.

Abbreviations: allo-HSCT, allogenic hematopoietic stem cell transplantation; DLI, donor lymphocyte infusion; ARDS, acute respiratory distress syndrome; NSIP, nonspecific interstitial pneumonia; BOS bronchiolitis obliterans syndrome.

Abstract

Donor lymphocyte infusion (DLI) is a therapeutic modality for relapsed hematological malignancies after allogeneic hematopoietic stem cell transplantation. We retrospectively analyzed non-infectious pulmonary complications (non-IPCs) following DLI therapy in 41 post-transplant patients with hematological malignancies, and found that 7 developed post-DLI non-IPCs. The 6-year cumulative incidence of non-IPCs was 18.0%. In these patients, non-IPCs were classified into three subtypes: acute respiratory distress syndrome (ARDS), nonspecific interstitial pneumonia (NSIP), and bronchiolitis obliterans syndrome (BOS). The median intervals from the last date of DLI to the development of ARDS and BOS were 12 days (range, 12-14) and 9.4 months (range, 2.6-61.8), respectively; the intervals between DLI and the development of NSIP were 3.5 and 24.7 in 2 patients. Regarding the status of GVHD before the diagnosis with ARDS, 2 out of 3 patients showed the progression of acute GVHD following DLI therapy. One out of 2 patients with NSIP and all 3 patients with BO had chronic GVHD symptoms prior to the development of non-IPCs. In our cohort, 1 patient died of the progression of NSIP. In conclusion, the present study showed the clinical features of non-IPCs following DLI, suggesting the importance of careful follow-ups for non-IPCs in post-DLI patients.

Table 1. Characteristics and transplant procedures

Characteristics	No. of patients
Total	7
Median age at allo-HSCT (range), years	52 (24-56)
Sex	
Male	5
Female	2
Patient match	
Match	3
Mismatch	4
Primary disease	
Acute myeloid leukemia	4
Adult T-cell leukemia/lymphoma	2
Acute lymphoblastic leukemia	1
Type of donor	
HLA-matched related	2
HLA-mismatched related	4
HLA-matched unrelated	1
Stem cell source	
Bone marrow	3
Peripheral blood stem cell	4
Conditioning regimen	
Myeloablative conditioning	4
Flu-i.v. Bu 12.8 mg/kg	1
Flu-i.v. Bu 12.8 mg/kg-TBI (2 Gy)	1
i.v. Bu-Cy	1
VP-16-CA-TBI (12 Gy)	1
Reduced intensity conditioning	3
Flu-Mel	1
Flu-i.v. Bu 6.4 mg/kg	1
Flu-i.v. Bu 6.4 mg/kg-TBI (2 Gy)	1
GVHD prophylaxis	
CyA-based	2
Tac-based	5
Follow-up of survivors, median (range), days	48 (19-76)

Abbreviations: allo-HSCT, allogeneic hematopoietic stem cell transplantation; HLA, human leukocyte antigen; Flu, fludarabine; Bu, busulfan; TBI, total body irradiation; Cy, cyclophosphamide; VP-16, etoposide; Mel, melphalan; GVHD, graft-versus-host disease; CyA, cyclosporine; Tac, tacrolimus.

Table 2. DLI procedures

Abbreviations: DLI, donor lymphocyte infusion; non-IPC, non-infectious pulmonary complication; ARDS, acute respiratory distress syndrome; NSIP, nonspecific interstitial pneumonia; BOS, bronchiolitis obliterans syndrome.

UPN	Type of non-IPC	Age at DLI, y	HLA allele compatibility*	CD3-positive cell dose ($\times 10^6$ cells/kg)		Number of DLI cycles	GVHD at DLI (Grade of GVHD)	Lesions of GVHD	Interval from HSCT to DLI,mo
				Initial	Total				
01	ARDS	30	4 mismatched	10.0	10.0	1	Presence (mild)	Skin	6.4
02	ARDS	44	2 mismatched	10.0	10.0	1	Presence (mild)	Skin	2.9
03	ARDS	55	4 mismatched	1.0	6.0	2	Absence		24.2
	NSIP						Absence		
04	NSIP	56	Matched	5.5	5.5	1	Presence (mild)	Mucous membrane, Liver	9.0
05	BOS	24	Matched	37.0	97.0	2	Presence (mild)	Skin	8.5
06	BOS	53	Matched	1.1	2.2	2	Presence (mild)	Skin	10.1
07	BOS	56	1 mismatched	1.1	1.1	1	Presence (mild)	Skin	4.8

* HLA-A, -B, -C, and -DRB1 were identified by serological and molecular typing.

Table 3. Diagnosis of non-infectious pulmonary complications and treatment

UPN	Type of non-IPC	GVHD at the diagnosis of non-IPCs	IST at the diagnosis of non-IPCs	Treatment	Response of non-IPCs to treatment	Survival after non-IPCs, mo	Cause of death
01	ARDS	Presence	No	Corticosteroid	Improvement	9.0	Underlying disease
02	ARDS	Presence	No	Corticosteroid	Improvement	0.8	Infection
03	ARDS	Absence	No	Corticosteroid	Improvement	4.8	Underlying disease
	NSIP	Absence	No	Corticosteroid	NC	2.8	
04	NSIP	Presence	CyA	Corticosteroid/ CAM	PD	11.3	GVHD with NSIP
05	BOS	Presence	CyA	Corticosteroid	PD	25.7	Underlying disease
06	BOS	Presence	Tac / PSL	Corticosteroid	PD	0.2+	-
07	BOS	Presence	No	Inhaled LABA	NC	10.2+	-

Abbreviations: IST, immunosuppression therapy; PSL, prednisolone; CAM, clarithromycin; LABA, long-acting β-agonists; NC, no change; PD, progressive disease.

Figure 1. Cumulative incidence of non-IPCs after DLI therapy

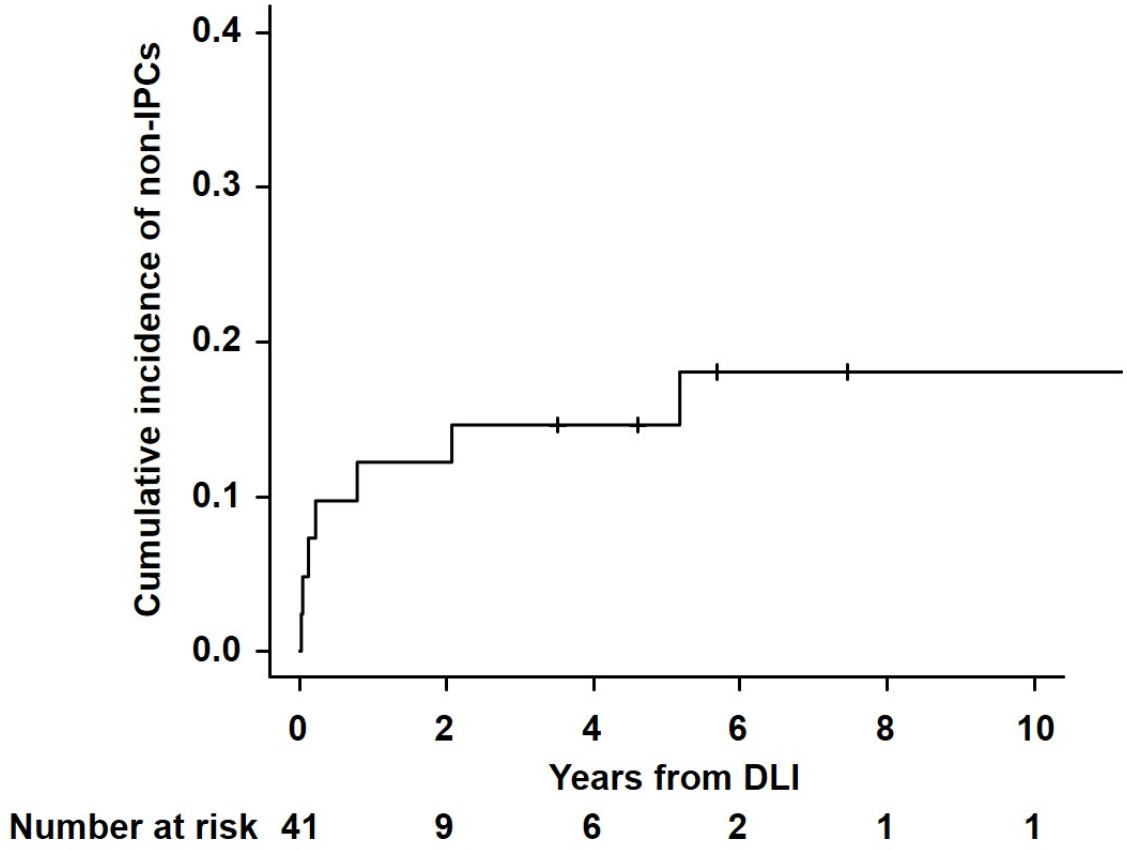


Figure 2. The time of the occurrence and clinical course of non-IPCs following DLI therapy

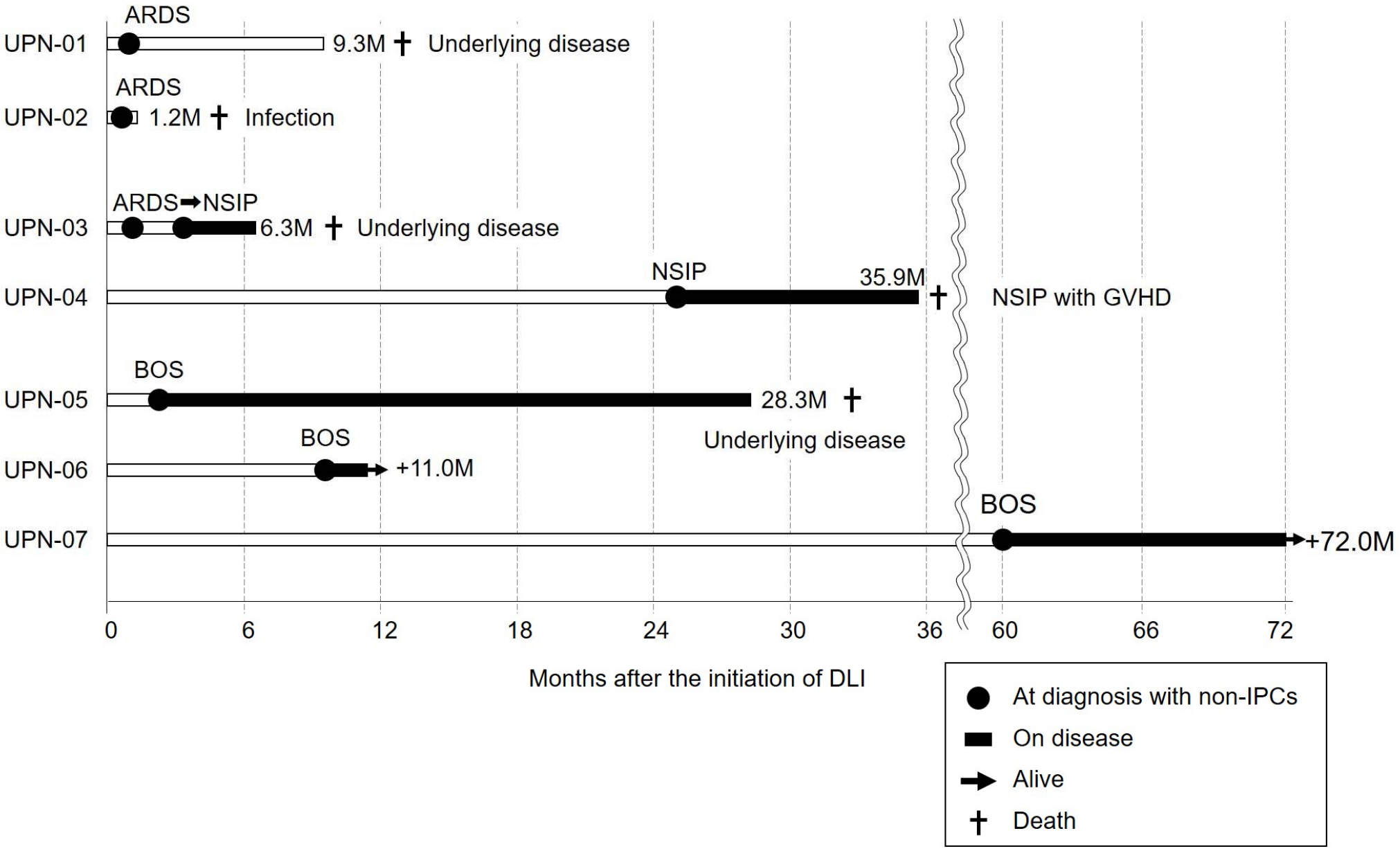
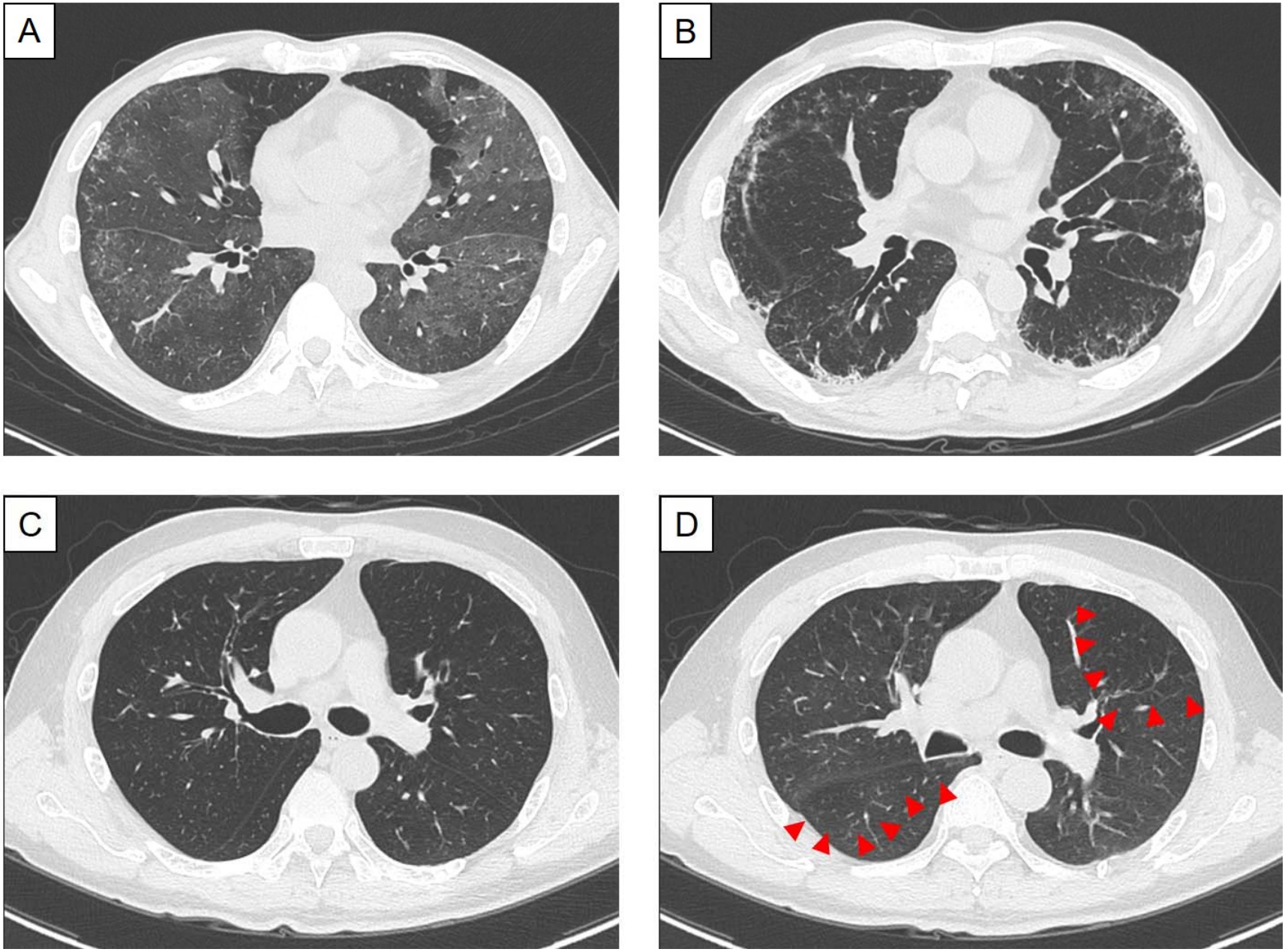


Figure 3. CT findings at the diagnosis of non-IPCs



Supplemental Table 1. Characteristics and transplant procedures of relapsed patients who received DLI therapy

Characteristics	No. of patients receiving DLI
Total	41
Median age at allo-HSCT (range), years	49 (16-62)
Patient Sex	
Male	24
Female	17
Sex match	
Match	22
Mismatch	19
Disease	
Acute myeloid leukemia	17
Adult T-cell leukemia/lymphoma	13
Acute lymphoblastic leukemia	8
Chronic myeloid leukemia	2
Diffuse large B cell lymphoma	1
Type of donor	
HLA-matched related	27
HLA-mismatched related	3
HLA-matched unrelated	10
HLA-mismatched unrelated	1
Stem cell source	
Bone marrow	18
Peripheral blood stem cell	23
Conditioning regimen	
Myeloablative conditioning	26
Reduced intensity conditioning	15
Conditioning regimen including TBI	
No	37
Yes	4
Conditioning regimen including busulfan	
No	19
Yes	22
GVHD prophylaxis	
CsA-based	29
Tac-based	8
Days, allo-HSCT to relapse, median (range)	96 (14-1541)
Days, relapse to DLI, median (range)	44 (7-1008)
Follow-up of survivors, median (range), days	2113
Final status	

Alive	7
Death after relapse (disease-associated death)	26
Death without relapse (treatment-associated death)	8

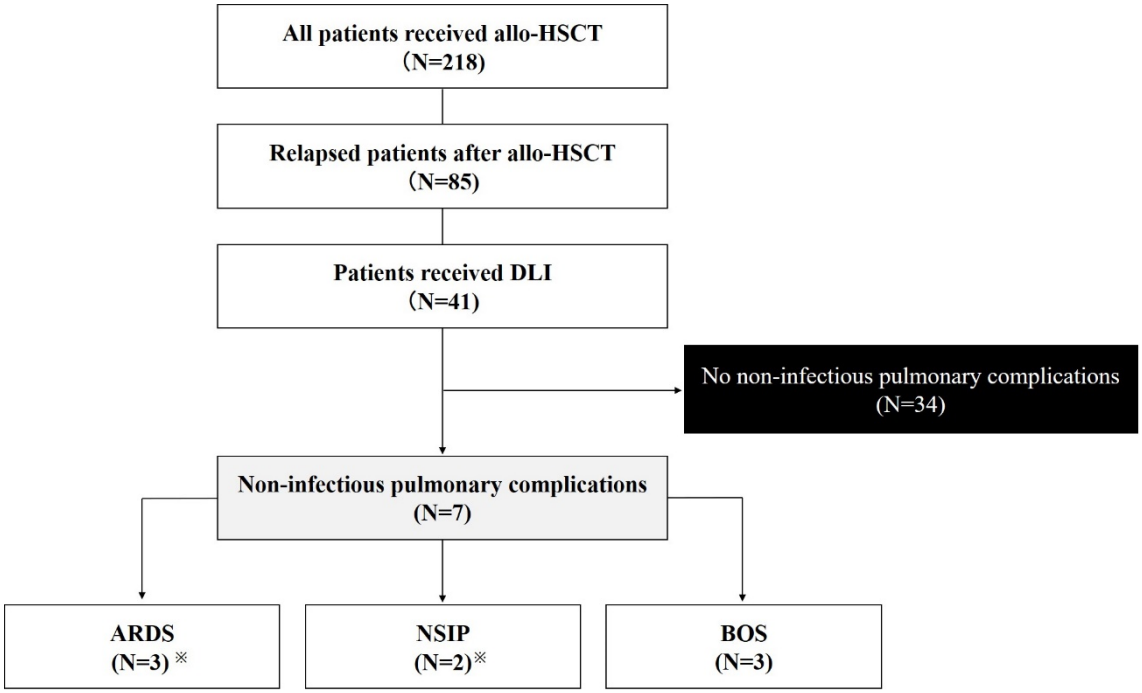
Abbreviations: DLI, donor lymphocyte infusion; allo-HSCT, allogeneic hematopoietic stem cell transplantation; HLA, human leukocyte antigen; TBI, total body irradiation; GVHD, graft-versus-host disease; CyA, cyclosporine; Tac, tacrolimus.

Supplemental Table 2. The results of pulmonary function test at the diagnosis with BOS.

Parameters	UPN-05	UPN-06	UPN-07
VC	2.61	2.49	3.14
%VC	83.2	65.5	97.2
FEV1	0.53	1.14	1.33
FEV1%	20.8	47.9	57.3
FEV1/FVC	0.20	0.48	0.41

Abbreviations: VC, vital capacity; FEV1, forced expiratory volume for 1 second; FVC, forced vital capacity.

Supplemental Figure 1. Clinical course of non-infectious pulmonary complications after DLI therapy for relapsed disease in hematopoietic stem cell transplantation.



In one patient, ARDS overlapped with NSIP.

Abbreviations: allo-HSCT, allogenic hematopoietic stem cell transplantation; DLI, donor lymphocyte infusion; ARDS, acute respiratory distress syndrome; NSIP, nonspecific interstitial pneumonia; BOS bronchiolitis obliterans syndrome.