Supplementary material

Supplementary methods

GvHD prophylaxis

Anti-thymocyte globulin (ATG) was added to the conditioning regimen when patients received grafts from a matched unrelated donor (MUD) or when patients with Fanconi or severe aplastic anemia received grafts from a sibling. From 2013 onwards, patients transplanted for AML with cord blood (CB) grafts generally did not receive ATG. ATG was initially dosed at 10 mg/kg. From 2011 onwards, dose adjustments were done in older children (> 40 kg: ATG 7.5 mg/kg; 50% dose reduction was given when preconditioning lymphocyte counts were < 300 * 10^6 cells/L). Timing of ATG moved from start at day -6 to start at day -9 since 2012. From 2016 onwards, ATG was dosed using a Model Based Dosing (MBD) scheme based on absolute lymphocyte counts, cell source and body weight.[27] Cyclosporine A as GvHD prophylaxis was administered for a minimum of 3 months (malignant disease) or 6 months (non-malignant disease) after allo-HCT. Prednisone was given from day +1 in case of CB transplantation and tapered in 2 weeks starting 4 weeks after allo-HCT in benign disorders, and in one week after engraftment in malignant disorders. Methotrexate was given as GvHD prophylaxis on day +1, +3, and +6 in case of a graft from a MUD and, since 2020, in patients receiving a graft from a sibling. Erythrocytes were transfused to maintain a minimal hemoglobin level of 4.3 mmol/L and platelets were administered in patients with evidence or strong suspicion of (mucosal) bleeding. All patients received anti-pneumocystis jirovecii, antiviral and antifungal prophylaxis and antibacterial gut decontamination according to local protocols.

Diagnosis of allo-LS

In case of new onset respiratory signs or unforeseen worsening of respiratory condition with a normal temperature and laboratory parameters unsuspicious for infection, patients underwent the following work up to diagnose allo-LS: pulmonary function tests (PFT; if feasible, and minimum age 6), chest high-resolution computed tomography (HRCT), and NPA or BAL. PFT included spirometry and in some cases CO-diffusion tests and body-box measurements. Chest HRCTs were reviewed by the radiology department, thereby looking for infiltrates, air trapping, pleural effusion, and pneumothorax. NPA and BAL were tested for bacteria, fungi, pneumocystis, mycobacterium, and RV.

IPS was diagnosed according to the American Thoracic Society and defined as evidence of widespread lung injury by clinical symptoms and radiological abnormalities in the absence of a direct infectious cause, cardiac dysfunction, fluid overload and renal failure. BOS was diagnosed according to the National Institutes of Health Consensus Criteria on Chronic GvHD disease 2014 and characterized by forced expiratory volume in 1 second (FEV1)/Forced Vital Capacity < 0.7, FEV1 < 75% and/or evidence of air trapping on PFT or HRCT, in the absence of a direct infectious cause.

Treatment of allo-LS

First line treatment of allo-LS consisted of intravenous methylprednisolone (MP) pulses at a dose of 10 mg/kg/day for 3 consecutive days and 2 mg/kg/day thereafter, tapered after a week by 25% per week to 0.5 mg/kg/day. MP pulses were administered, up to a maximum of 6, repeated monthly, while continuing 0.5 mg/kg prednisone daily. Recovery was defined as normalization of PFT and/or resolved symptoms. IPS patients further received etanercept at a dosage of 0.4 mg/kg twice weekly (8 doses).

Immunosuppressive agents used as GvHD prophylaxis were continued in BOS and often supplemented with FAM. FAM was also given sometimes instead of MP pulses in patients that

would not tolerate high dose steroids. In case of a suboptimal response of BOS to immunosuppressive treatment, the tyrosine kinase inhibitor imatinib was added at a dosage of 100 mg per day to inhibit fibrosis. Supportive care was provided with extra oxygen and mechanical ventilation when necessary. Voriconazole or an alternative antifungal prophylaxis was initiated, if not already given, in case of prolonged immunosuppressive treatment.

Statistical analysis

The association between patient characteristics and the development of allo-LS was studied by univariable and multivariable Fine and Gray competing risk models. Graft failure and death were considered competing events. Considered covariables were age, year of allo-HCT, sex (female vs male), diagnosis (malignant vs non-malignant), serotherapy (yes vs no), aGvHD grade II-IV prior to allo-LS diagnosis (yes vs no), cell source (bone marrow [BM]/peripheral blood [PB] vs CB), donor type (related vs unrelated), human leukocyte antigens (HLA) match grade (partial vs full), conditioning regimen (busulfan-cyclophosphamide with melphalan in myeloid malignancies [BuCy +/- Mel] vs total body irradiation [TBI]), busulfan-fludarabine +/- clofarabine [BuFlu +/- Clo] and non-myeloablative regimens), ATG prophylaxis (ATG with dose adjustment (DA) or model based dosing [MDB] vs no ATG, and ATG without DA or MDB), viral reactivation (>1000 copies/mL yes vs no) prior to allo-LS diagnosis of cytomegalovirus (CMV), Epstein-Barr virus (EBV), adenovirus (AdV), or human herpesvirus-6 (HHV-6), and RV infection prior to allo-HCT (range -28 – -1 days) or early after allo-HCT (range 1 – 100 days) prior to allo-LS diagnosis.5,6 Covariables were entered into multivariable models if $P < .1$. Forward selection was used for selection of independent variables to be used in the final model. Confounder-adjusted cumulative incidence curves were plotted to visualize the variables included in the multivariable model. LOESS regression curves were made to visualize longitudinal markers acquired in follow-up, namely EASIX scores, immunoglobulins, and immune cell counts within the first 100 days after allo-HCT for patients with either BOS or IPS and patients without allo-LS. Data points were included up to the time of follow-up or allo-LS development. Patients without allo-LS who were still at risk of developing allo-LS were excluded from the analysis for EASIX and immune system kinetics if their follow-up time fell within the first three quartiles of the observed diagnosis times for BOS or IPS. Maximally selected rank statistics were used to determine the optimal cut-off points for longitudinal parameters, which were subsequently tested with univariable and/or multivariable Fine and Gray Competing Risk models. Longitudinal markers assigned to a specific time point were from the specific day +/- 7 days and +/- 14 days for day 150. Analyses were performed using R4.03 with packages ggplot2, *survival, survminer, cmprsk, riskRegression, dplyr, forestplot, prodlim, adjustedCurves, pammtools, and lme4.*

Supplementary figures

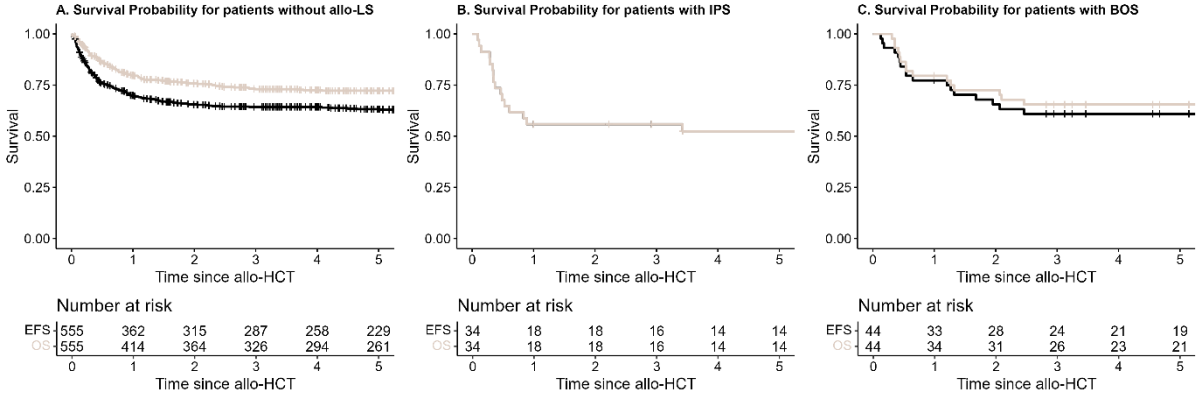

Figure S1. Survival probability. (A-C) Kaplan Meier plots of OS and EFS from the day of allo-HCT for patients without allo-LS (A), patients with IPS (B) and patients with BOS (C).

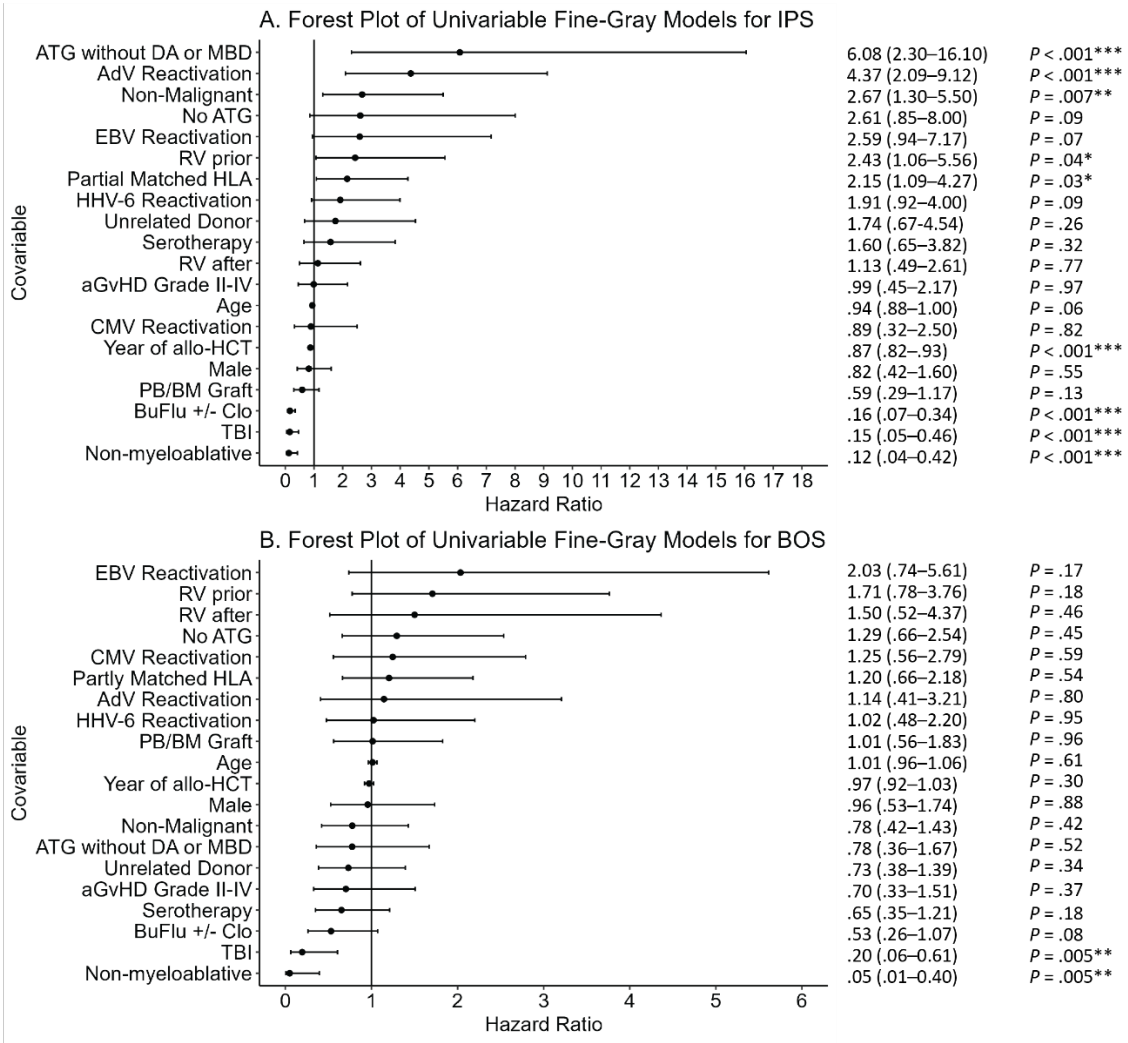

Figure S2. Univariable analysis of risk factors for BOS and IPS. (A-B) Forest plots of univariable Fine and Gray competing risk models for IPS (A) and BOS (B). *P* values < .05 were considered statistically significant. BO: bronchiolitis obliterans; IPS: idiopathic pneumonia syndrome; ATG: anti-thymocyte globulin; DA: dose adjustment; MBD: model based dosing; AdV: adenovirus; CMV: cytomegalovirus; HHV-6: human herpes virus 6; EBV: Epstein-Barr virus; RV: respiratory virus; aGvHD: acute graft-versus-host disease; PB/BM: peripheral blood/ bone marrow; HLA: human leukocyte antigens; TBI: total body irradiation; BuFlu: busulfan-fludarabine; Clo: clofarabine. *: *P* < .05; **: *P* <. 01; ***: *P* < .001.

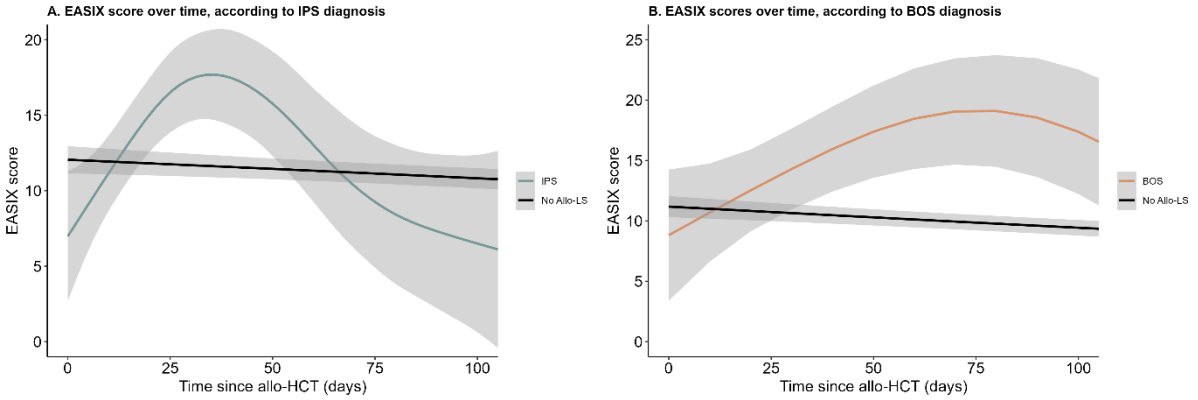

Figure S3 EASIX over time in the first 100 days after allo-HCT. (A-B) LOESS regression curves of EASIX scores in the first 100 days since time of allo-HCT for patients with IPS vs patients without allo-LS (A) and patients with BOS vs patients without allo-LS (B). Data points included were up to either the time of follow-up or the development of BOS or IPS.

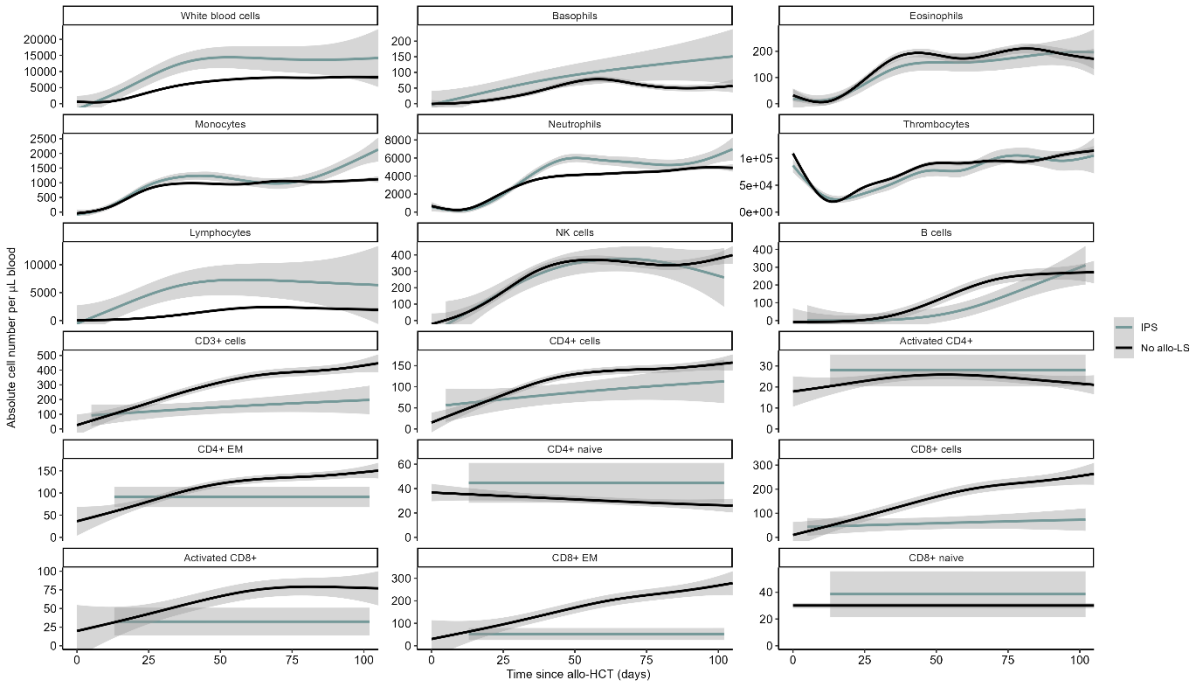

Figure S4A. Cellular kinetics in the first 100 days after allo-HCT. LOESS regression curves of absolute counts of immune cells in the first 100 days since time of allo-HCT for patients with IPS (blue line) vs patients without allo-LS (black line). Data points included were up to either the time of follow-up or the development of allo-LS.

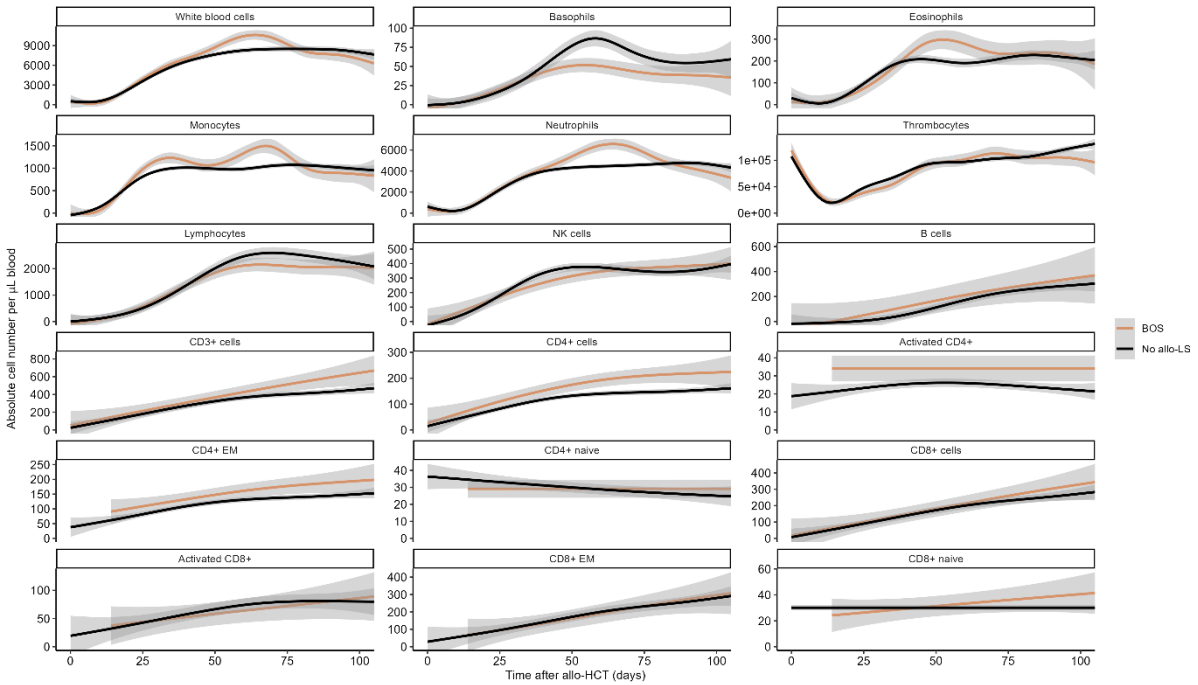

Figure S4B. Cellular kinetics in the first 100 days after allo-HCT. LOESS regression curves of absolute counts of immune cells in the first 100 days since time of allo-HCT for patients with BOS (orange line) vs patients without allo-LS (black line). Data points included were up to either the time of follow-up or the development of allo-LS.

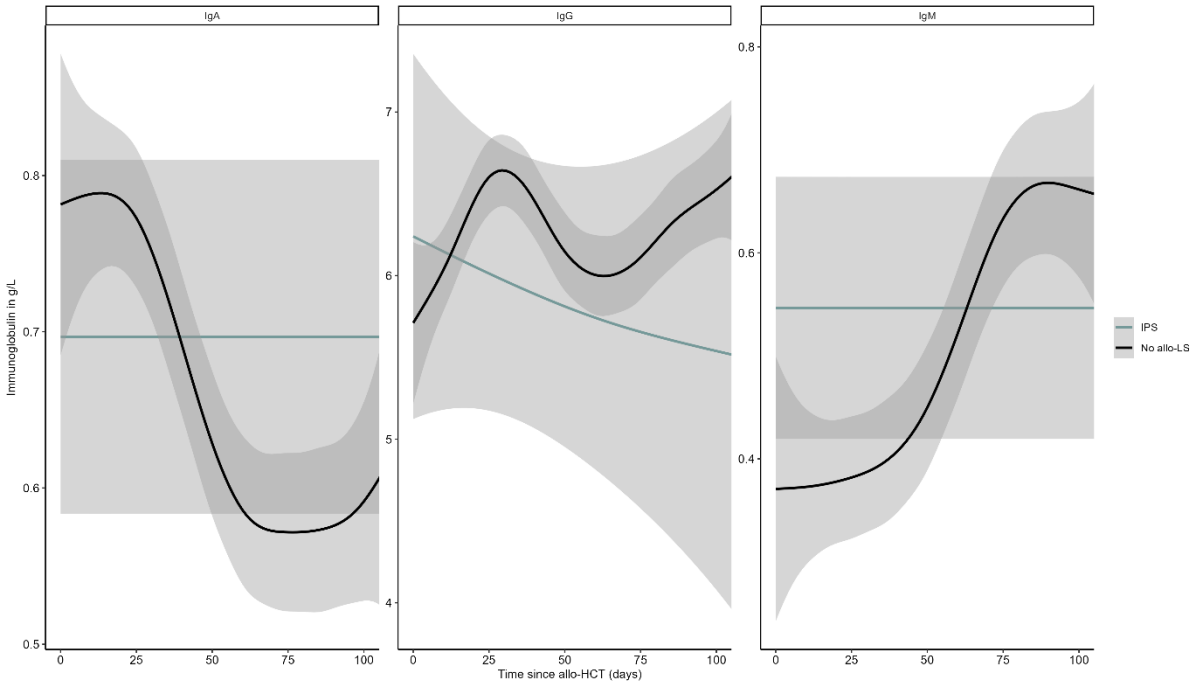

Figure S5A. Immunoglobulin levels in the first 100 days after allo-HCT. LOESS regression curves of immunoglobulin levels in the first 100 days since time of allo-HCT for patients with IPS (blue line) vs patients without allo-LS (black line). Data points included were up to either the time of follow-up or the development of allo-LS.

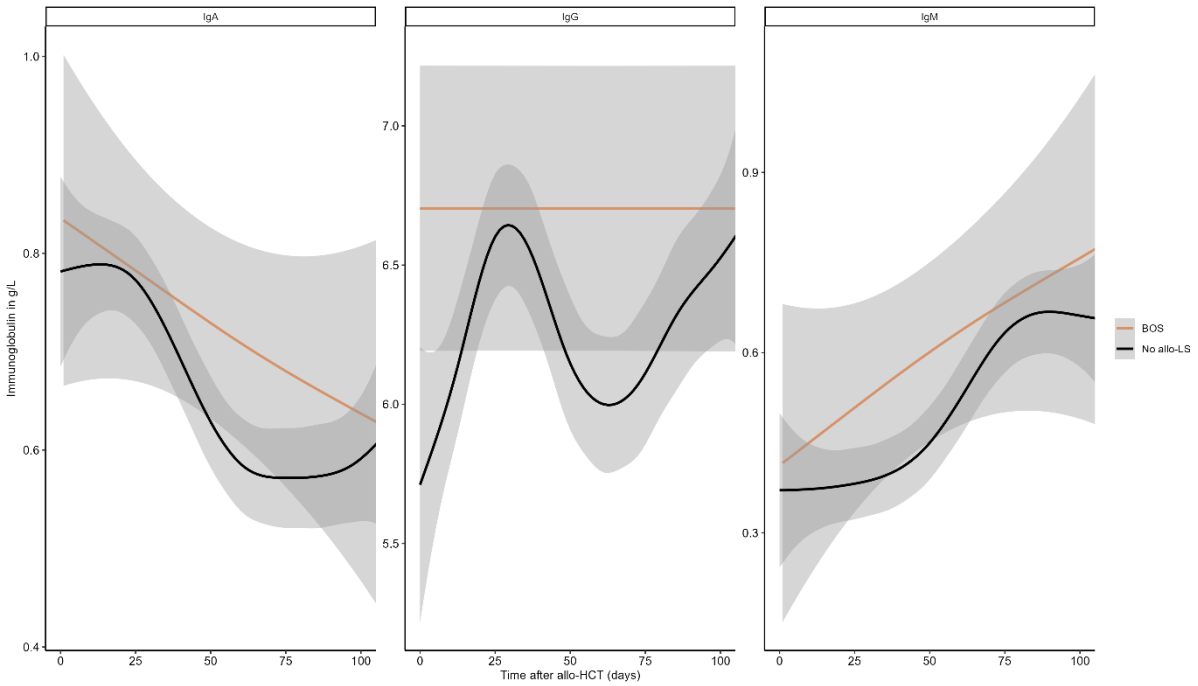

Figure S5B. Immunoglobulin levels in the first 100 days after allo-HCT. LOESS regression curves of immunoglobulin levels in the first 100 days since time of allo-HCT for patients with BOS (orange line) vs patients without allo-LS (black line). Data points included were up to either the time of follow-up or the development of allo-LS.