Therapeutic Modalities and Clinical Outcomes in a Large Cohort with LRBA Deficiency and CTLA4 Insufficiency

Nigar Taghizade, MD, Royala Babayeva, MD, Altan Kara, PhD, Ibrahim Serhat Karakus, MD, Mehmet Cihangir Catak, MSc, Alper Bulutoglu, MSc, Zehra Sule Haskologlu, MD, Idil Akay Haci, MD, Ceyda Tunakan Dalgic, MD, Esra Karabiber, MD, Sevgi Bilgic Eltan, MD, Melek Yorgun Altunbas, MD, Asena Pinar Sefer, MD, Ahmet Sezer, MD, Sefika Ilknur Kokcu Karadag, MD, Elif Arik, MD, Zuhal Karali, MD, Aylin Ozhan Kont, MD, Can Tuzer, MD, Sait Karaman, MD, Selver Seda Mersin, MD, Nurhan Kasap, MD, Enes Celik, MD, Dilara Fatma Kocacik Uygun, MD, Sezin Aydemir, MD, Ayca Kiykim, MD, Cigdem Aydogmus, MD, Esra Ozek Yucel, MD, Fatih Celmeli, MD, Emrah Karatay, MD, Emine Bozkurtlar, MD, Semra Demir, MD, Ayse Metin, MD, Neslihan Edeer Karaca, MD, Necil Kutukculer, MD, Guzide Aksu, MD, Sukru Nail Guner, MD, Sevgi Keles, MD, Ismail Reisli, MD, Yasemin Kendir Demirkol, MD, Tugba Arikoglu, MD, Nesrin Gulez, MD, Ferah Genel, MD, Sara Sebnem Kilic, MD, Caner Aytekin, MD, Ozlem Keskin, MD, Alisan Yildiran, MD, Dilek Ozcan, MD, Derya Ufuk Altintas, MD, Fatma Omur Ardeniz, MD, Esin Figen Dogu, MD, Kamile Aydan Ikinciogullari, MD, Elif Karakoc-Aydiner, MD, Ahmet Ozen, MD, Safa Baris, MD

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Therapeutic Modalities and Clinical Outcomes in a Large Cohort with LRBA Deficiency and CTLA4 Insufficiency

Nigar Taghizade, MD[1], Royala Babayeva, MD[2,3,4], Altan Kara, PhD[5], Ibrahim Serhat Karakus, MD[6], Mehmet Cihangir Catak, MSc[2,3,4], Alper Bulutoglu, MSc[2,3,4], Zehra Sule Haskologlu, MD[7], Idil Akay Haci, MD[8], Ceyda Tunakan Dalgic, MD[9], Esra Karabiber, MD[10], Sevgi Bilgic Eltan, MD[2,3,4], Melek Yorgun Altunbas, MD[2,3,4], Asena Pinar Sefer, MD[2,3,4], Ahmet Sezer, MD[11], Sefika Ilknur Kokcu Karadag, MD[12], Elif Arik, MD[13], Zuhal Karali, MD[14], Aylin Ozhan Kont, MD[15], Can Tuzer, MD[16], Sait Karaman, MD[17], Selver Seda Mersin, MD[18], Nurhan Kasap, MD[19], Enes Celik, MD[20], Dilara Fatma Kocacik Uygun, MD[20], Sezin Aydemir, MD[21], Ayca Kiykim, MD[21], Cigdem Aydogmus, MD[22], Esra Ozek Yucel, MD[23], Fatih Celmeli, MD[24], Emrah Karatay, MD[25], Emine Bozkurtlar, MD[26], Semra Demir, MD[27], Ayse Metin, MD[28], Neslihan Edeer Karaca, MD[29], Necil Kutukculer, MD[29], Guzide Aksu, MD[29], Sukru Nail Guner, MD[30], Sevgi Keles, MD[30], Ismail Reisli, MD[30], Yasemin Kendir Demirkol, MD[31], Tugba Arikoglu, MD[15], Nesrin Gulez, MD[8], Ferah Genel, MD[8], Sara Sebnem Kilic, MD[14], Caner Aytekin, MD[32], Ozlem Keskin, MD[13], Alisan Yildiran, MD[12], Dilek Ozcan, MD[11], Derya Ufuk Altintas, MD[11], Fatma Omur Ardeniz, MD[9], Esin Figen Dogu, MD[7], Kamile Aydan Ikinciogullari, MD[7], Elif Karakoc-Aydiner, MD[2,3,4], Ahmet Ozen, MD[2,3,4], Safa Baris, MD[2,3,4]

[1]Department of Pediatrics, School of Medicine, Marmara University, Istanbul, Turkey; [2]Division of Pediatric Allergy and Immunology, School of Medicine, Marmara University, Istanbul, Turkey; [3]Istanbul Jeffrey Modell Diagnostic and Research Center for Primary Immunodeficiencies, Istanbul, Turkey; [4]The Isil Berat Barlan Center for Translational Medicine, Istanbul, Turkey; [5]TUBITAK Marmara Research Center, Gene Engineering and Biotechnology Institute, Gebze, Turkey; [6]School of Medicine, Marmara University, Istanbul, Turkey; [7]Division of Pediatric Allergy and Immunology, Faculty of Medicine, Ankara University, Ankara, Turkey; [8]Division of Pediatric Allergy and Immunology, Dr. Behcet Uz Children's Education and Research Hospital, University of Health Sciences, Izmir, Turkey; [9]Department of Allergy and Immunology, Faculty of Medicine, Ege University, Izmir, Turkey; [10]Department of Allergy and Immunology, Marmara University Training and Research Hospital, Ministry of Health, Istanbul, Turkey; [11]Division of Pediatric Allergy and Immunology, Faculty of Medicine, Çukurova University, Adana, Turkey; [12]Division of Pediatric Allergy and Immunology, Faculty of Medicine, Ondokuz Mayis University, Samsun, Turkey; [13]Division of Pediatric Allergy and Immunology, Faculty of Medicine, Gaziantep University, Gaziantep, Turkey; [14]Division of Pediatric Immunology and Rheumatology, Faculty of Medicine, Uludag University, Bursa, Turkey; [15]Division of Pediatric Allergy and Immunology, Faculty of Medicine, Mersin University, Mersin, Turkey; [16]Department of Allergy and Immunology, Batman Training and Research Hospital, Ministry of Health, Batman, Turkey; [17]Pediatric Allergy and Immunology, Manisa City Hospital, University of Health Sciences, Manisa, Turkey; [18]Department of Allergy and Immunology, Dr. Ersin Arslan Training and Research Hospital, Ministry of Health, Gaziantep, Turkey; [19]Division of Pediatric Allergy and Immunology, Faculty of Medicine, Istanbul Medeniyet University, Istanbul, Turkey; [20]Division of Pediatric Allergy and Immunology, Faculty

of Medicine, Akdeniz University, Antalya, Turkey; [21]Division of Pediatric Allergy and Immunology, Cerrahpasa Faculty of Medicine, Istanbul University-Cerrahpasa, Istanbul, Turkey; [22]Division of Pediatric Allergy and Immunology, Basaksehir Cam and Sakura City Hospital, University of Health Sciences, Istanbul, Turkey; [23]Division of Pediatric Allergy and Immunology, Istanbul Faculty of Medicine, Istanbul University, Istanbul, Turkey; [24]Division of Pediatric Allergy and Immunology, Antalya Training and Research Hospital, Ministry of Health, Antalya, Turkey; [25]Department of Radiology, Faculty of Medicine, Marmara University, Istanbul, Turkey; [26]Department of Pathology, Faculty of Medicine, Marmara University, Istanbul, Turkey; [27]Department of Allergy and Immunology, Istanbul Faculty of Medicine, Istanbul University, Istanbul, Turkey; [28]Division of Pediatric Immunology, Ankara City Hospital, University of Health Sciences, Ankara, Turkey; [29]Division of Pediatric Allergy and Immunology, Faculty of Medicine, Ege University, Izmir, Turkey; [30]Division of Pediatric Allergy and Immunology, Faculty of Medicine, Necmettin Erbakan University, Konya, Turkey; [31]Division of Pediatric Genetics, Umraniye Education and Research Hospital, University of Health Sciences, Istanbul, Turkey; [32]Department of Pediatric Immunology, Dr. Sami Ulus Children Health and Diseases Training and Research Hospital, Ankara, Turkey.

Corresponding Author: Safa Baris, MD, Marmara University, Division of Pediatric Allergy/Immunology, Fevzi Çakmak Mah. No:41, Pendik/Istanbul/Turkey. Phone: +90 216 625 78 11, Email: safabaris@hotmail.com

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Abstract

Background. Lipopolysaccharide-responsive beige-like anchor (LRBA) deficiency (*LRBA*$^{-/-}$) and cytotoxic T-lymphocyte-associated antigen-4 (CTLA4) insufficiency (*CTLA4*$^{+/-}$) are mechanistically overlapped diseases presenting with recurrent infections and autoimmunity. The effectiveness of different treatment regimens remains unknown.

Objective. To determine the comparative efficacy and long-term outcome of therapy with immunosuppressants (ISs), CTLA4-Ig (abatacept), and hematopoietic stem cell transplantation (HSCT) in a single-country, multicenter cohort of 98 patients with a 5-year median follow-up.

Methods. 63 *LRBA*$^{-/-}$ and 35 *CTLA4*$^{+/-}$ patients were followed and evaluated at baseline and every 6 months for clinical manifestations and response to the respective therapies.

Results. *LRBA*$^{-/-}$ patients exhibited a more severe disease course than *CTLA4*$^{+/-}$ patients, requiring more ISs, abatacept, and HSCT to control symptoms. In 58 patients who received abatacept either as a primary or rescue therapy, sustained complete control was achieved in 46 (79.3%) without severe side effects. In contrast, most patients who received ISs as primary therapy (n=61) showed either partial or no disease control (72.1%), necessitating additional ISs, abatacept, or transplantation. Patients with partial or no response to abatacept (n=12) had longer disease activity prior to abatacept therapy with higher organ involvement and poorer disease outcomes than those with a complete response. HSCT was performed in 14 *LRBA*$^{-/-}$ patients; 9 (64.2%) showed complete remission, and 3 (21.3%) continued to receive IS after transplantation. HSCT and abatacept therapy gave rise to similar probabilities of survival.

Conclusions. Abatacept is superior to ISs in controlling disease manifestations long-term, especially when started early, and may provide a safe and effective therapeutic alternative to transplantation.

Clinical implications. This multicenter, observational study on 63 LRBA-deficient and 35 CTLA4-insufficient patients provided comprehensive clinical evaluation and long-term outcomes for different therapeutic options. Early employment of abatacept would achieve better disease control and can replace transplantation without severe side effects. Resistance to targeted therapy should prioritize transplantation.

Capsule summary. This study provided a long-term comparative evaluation of the disease manifestations and treatments in *LRBA*$^{-/-}$ and *CTLA4*$^{+/-}$, favoring abatacept usage in controlling disease symptoms.

Keywords: Inborn errors of immunity, lipopolysaccharide-responsive beige-like anchor, cytotoxic T-lymphocyte-associated antigen-4, immune dysregulation, natural history, abatacept, hematopoietic stem cell transplantation.

Abbreviations. AIHA: Autoimmune hemolytic anemia, **CD:** Chronic diarrhea, **CM:** CTLA4 mutant, **CR:** Complete response, **CTLA4:** Cytotoxic T-lymphocyte-associated antigen-4, **Foxp3:** Forkhead Box P3, **GLILID:** Granulomatous–lymphocytic interstitial lung disease, **GvHD:** Graft versus host disease, **HSCT:** Hematopoietic stem cell transplantation, **ID:** Immune dysregulation, **IEI:** Inborn Errors of Immunity, **ITP:** Immune thrombocytopenia, **LM:** LRBA mutant, **LRBA:** Lipopolysaccharide-responsive beige-like anchor, **PIRD:** Primary immune regulatory disorders, **PR:** Partial response, **NR:** Non-responsive, **Treg:** Regulatory T cells.

Introduction

Inborn Errors of Immunity (IEI) are a group of inherited immune diseases presenting with recurrent infections, autoimmunity, autoinflammation, and malignancies [1, 2]. A subgroup of IEI that impact the development and/or function of regulatory T cells (Treg) present predominantly with immune dysregulation (ID), giving rise to primary immune regulatory disorders (PIRDs) [3-5]. The first described PIRD is immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX), caused by mutations in the Forkhead Box P3 (*FOXP3*) gene [6-8]. Subsequent studies identified additional PIRDs, notably including autosomal recessive lipopolysaccharide-responsive beige-like anchor (LRBA) deficiency (*LRBA*$^{-/-}$) and autosomal dominant cytotoxic T-lymphocyte-associated antigen-4 (CTLA4) insufficiency (*CTLA4*$^{+/-}$) [9-12]. CTLA4 is a potent checkpoint inhibitor critical in maintaining peripheral immune tolerance [13, 14]. CTLA4 is constitutively expressed on FOXP3^{+} Treg cells and transiently expressed after activation in CD4^{+} and CD8^{+} T cells [13]. After recycling to the plasma membrane, CTLA4 enters into a rivalry with CD28 to bind CD80 (B7-1) and CD86 (B7-2) ligands expressed on antigen-presenting cells and removes the CD80/CD86 by transendocytosis ending with T-cell inhibition [14-16]. On the other hand, LRBA protein via its Concanavalin A and Beige and Chediak-Higashi domains is binding to the YVKM motif of the cytosolic tail of CTLA4, controlling vesicular trafficking of CTLA4 by preventing its lysosomal degradation and allowing recycling to the cell surface [17-19]. Recently, an investigational study revealed that CD80 and CD86 initiate divergent CTLA-4 fates after internalization, explaining that the role of LRBA in CTLA4 metabolism is specifically related to the binding of CD86 protein, which provides normal Treg functioning. In contrast, CD80 binding causes CTLA4 degradation via ubiquitination [20].

Human biallelic LRBA deficiency and monoallelic CTLA4 insufficiency are characterized by recurrent respiratory tract infections, lymphoproliferation, and immunodysregulation, manifesting as enteropathy, cytopenias, autoimmune disorders, and malignancies [9-11, 18, 21-24]. In addition, immunological analyses disclosed that the patients had reduced Treg cell numbers with low CTLA4 expression and suppressive capacity, corroborating the phenotypic intertwined between both diseases [11, 12].

Studies regarding clinical manifestations and treatment modalities were reported for both disorders by defining the efficacy of the conventional (immunoglobulin replacement therapy, antimicrobial prophylaxis, and classical immunosuppressants (ISs)), targeted therapies (abatacept-CTLA4-Ig fusion protein, sirolimus-mTOR inhibitor), and hematopoietic stem cell transplantation (HSCT) [25-30]. Abatacept effectively controlled disease activity in most patients, especially for lymphoproliferation, immune cytopenia, and chronic diarrhea in LRBA deficiency [25, 27, 29, 31]. Furthermore, in CTLA4 insufficiency, it also proved effective in controlling symptoms of enteropathy, granulomatous–lymphocytic interstitial lung disease (GLILD), hemolytic anemia (AIHA), immune thrombocytopenia (ITP), lymphoproliferation [27, 32]. Combining abatacept with sirolimus also synergistically mitigated severe patient symptoms [26, 27]. On the other hand, HSCT has been reported in small patient cohorts with end-organ damage, and success rates of symptom control were 17/24 (70.8%) for LRBA and 13/18 (72%) for CTLA4 [26, 27]. Post-HSCT complications have included graft versus host disease (GvHD), graft rejection, multiorgan failure, autoimmunity, and CMV reactivation. Notwithstanding the above treatment options, their comparative efficacy in disease control remains unclear. For example, the benefits and risks of targeted therapy with CTLA4-Ig versus transplantation remain unclear. There is still a debate regarding the long-term use of ISs or conducting early HSCT. By the same token, which disease feature can be controlled with abatacept is not fully established. Finally, factors determining the response rate to treatments are also obscure. Therefore, detailed studies combining *LRBA*$^{-/-}$ and *CTLA4*$^{+/-}$ cohorts are warranted to address the aforementioned raised queries.

In this single-country, multicenter cohort, we evaluate 98 *LRBA*$^{-/-}$ and *CTLA4*$^{+/-}$ patients, and the overarching aim is to reveal the efficacy of different treatment modalities in controlling disease manifestations. In addition, our study showcases where and when abatacept can be more effective. Finally, we also present comparative results regarding outcomes between ISs and transplantation, bestowing better prediction for prognosis.

Methods

Patient and inclusion criteria. The study comprised 98 patients with confirmed *LRBA* and *CTLA4* mutations. The patients were recruited from 24 different immunology centers in Turkey. They were enrolled in the study at different time points starting from November 2016 and followed up prospectively until February 2023. LRBA mutant (LM) 1 to 29, LM35, LM45-50, and CTLA4 mutant (CM) 1 to 12 patients were reported previously [29, 33, 34]. The study protocol was approved by the local ethics committee of Marmara University (IRB number: IRB00009067), and written informed consent according to Good Clinical Practice guidelines was obtained from all parents and adult patients. In patients with young age, assent was also obtained.

Study design. The patients were enrolled in the study prospectively from related centers. A questionnaire, including demographic and clinical data (age at onset of symptoms, age at diagnosis, family history, past infections, autoimmunity, systems involved, and treatments with side effects), was filled out for every patient at baseline and updated every 6 months. We focused on the detailed responses of ISs, abatacept, and HSCT treatments. In addition, we categorized the response rate in terms of used drugs as complete response (CR), partial response (PR), or non-responsive (NR). Blood samples from all the participating patients were sent to the Marmara University Pediatric Allergy and Immunology laboratory for immunological assessments, including serum immunoglobulin levels and extensive lymphocyte subset analysis as described previously [25, 29].

Statistical analysis. The data were presented as mean ± standard deviation (SD) or median with minimum (min)-maximum (max) range. The Kolmogorov-Smirnov distribution test was conducted to determine the normal distribution. The chi-squared test was used for the comparison of categorical values. Comparison between patients and control groups for continuous values was made with a Student unpaired t-test. We performed a propensity score matching (PSM) analysis to reveal the efficacy of abatacept to other immunosuppressants [35]. The analysis was performed in R programming language using the MatchIt package [36]. A 1:1 nearest neighbor matching was performed for the delay in diagnosis, respiratory tract infection, autoimmunity, immune cytopenia, and parenchymal lung disease covariates. Covariate balance using standardized mean differences less than 0.2 after matching was accepted for successful balance [37]. Following successful PSM, chi-squared analysis was conducted on matched data to establish the efficacy of different

treatments on complete response rate using the odds ratio (OR) and the corresponding 95% confidence interval (CI). We also performed a logistic regression analysis examining age as a risk factor for clinical outcomes. Analysis of overall survival (OS) was done using the Kaplan-Meier method (log-rank test). Differences were considered significant at a *p*-value <0.05. Statistical analysis was done using IBM SPSS Statistics version 23.0 and GraphPad Prism 9 (GraphPad Software Inc, San Diego, Calif).

Results

Demographic characteristics, clinical presentations, and immunological features of *LRBA*$^{-/-}$ and *CTLA4*$^{+/-}$. We enrolled 63 *LRBA*$^{-/-}$ (64.3%) and 35 *CTLA4*$^{+/-}$ (35.7%) patients coming from 48 and 21 families, respectively (**Fig.1A**). The cohort had 52 (53.1%) males and 46 (46.9%) females. The patients' demographic characteristics are presented in **Table 1**. The mean current age, age at diagnosis, and age at symptom onset were lower in LRBA deficiency (**Fig.1B**). The median follow-up period for the 98 patients was 5 years (min-max:0.08-25.5). The OS rate was 89.7% (88/98), with higher mortality in LRBA deficiency (**Fig.1C** and **D**).

When the patients were evaluated according to their first clinical presentation (**Fig.1E**), respiratory tract infections (RTIs) were the most common presenting symptoms in both diseases (n=38, 38.8%), followed by chronic diarrhea (CD, n=17, 17.3%), thrombocytopenia (n=10, 10.2%), anemia (n=9, 9.2%), type 1 diabetes (n=7, 7.1%), lymphoproliferation (n=7, 7.1%), arthritis (n=5, 5.1%), and alopecia (n=1, 1.0%). CD was detected higher in *LRBA*$^{-/-}$ (n=15, 23.8%) than *CTLA4*$^{+/-}$ (n=2, 5.7%) (p=0.03). The clinical phenotype of patients was predominantly characterized by common variable immunodeficiency (CVID, n=57, 58.1%); the other phenotypes were ALPS and IPEX (**Fig.1F**). In total, there were 5 asymptomatic *CTLA4*$^{+/-}$ carriers, while full penetrance was observed in all *LRBA*$^{-/-}$ patients (p=0.007).

As we showed previously [29], *LRBA*$^{-/-}$ patients showed a more severe disease course than *CTLA4*$^{+/-}$ ones (**Fig.2A**). After controlling for the age factor, *LRBA*$^{-/-}$ exhibited a higher risk of lower RTI (p=0.01, OR=3.63; 95% CI, 1.35–9.73), CD (p=0.005, OR=4.34; 95% CI, 1.54–12.25), and failure to thrive (p=0.002, OR=6.14; 95% CI, 1.97–11.12) compared to *CTLA4*$^{+/-}$. Gastrointestinal (GI) tract pathologies (available in 40 *LRBA*$^{-/-}$ and 15 *CTLA4*$^{+/-}$ patients) were similar between the respective diseases and revealed diverse upper and lower GI findings; the majority of them were not associated with a specific diagnosis and named as unclassified diarrhea (**Fig.E1A**). In contrast, the radiological evaluations showed higher parenchymal lung diseases in *LRBA*$^{-/-}$ patients (p=0.04; n=40, 63.5% vs. n=15, 42.9%-*CTLA4*$^{+/-}$), especially for parenchymal consolidation (p=0.007, **Fig.E1B**). The ID was mirrored by increased lymphocytic infiltration in different target organs (**Fig.E1C**).

Viral infections, including cytomegalovirus (27% vs. 25.7%), Epstein-Barr virus (12.7% vs. 8.6%), COVID-19 (22.2% vs. 37.1%), and varicella (11.1% vs. 5.7%) were observed equally in both diseases. All the COVID-19-infected patients showed only upper respiratory tract symptoms without hospitalization, except for LM10, who experienced a severe COVID-19 infection that led to her death. *Haemophilus influenzae* was the most commonly isolated bacterial pathogen. Documented bacterial and viral microorganisms are presented in **Table E1** and **Table E2**. Furthermore, malignancies comprising gastric adenocarcinoma (LM5), Hodgkin lymphoma (LM15), non-Hodgkin lymphoma (LM47), gastric adenocarcinoma together with non-Hodgkin lymphoma (LM60), acute lymphocytic leukemia (CM14), Burkitt lymphoma (CM27), and intracranial myofibroblastic tumor (CM26) were observed equally in both diseases' population. The detailed clinical symptoms and treatment modalities, including targeted therapies of patients, are presented in **Table E3** and **Table E4**.

In *LRBA*$^{-/-}$ patients, there were 27 frameshift, 27 nonsense, 8 missense, 2 splice site mutations, and 6 deletions, while in *CTLA4*$^{+/-}$ patients, we observed 15 missense, 14 nonsense, 5 frameshift mutations, and 1 deletion (**Fig.2B** and **C**, **Table E5** and **Table E6**). Consanguinity was prominent (65.3%) in our cohort leading to multiple affected subjects in the same families (n=29). Interestingly, this rate was also high in CTLA4 insufficiency (n=14, 40%). As shown previously [32], among *CTLA4*$^{+/-}$ patients, we did not detect mutation in *CTLA4* exon 4 (cytoplasmic tail). There was no strong genotype-phenotype relationship that governed the manifestations in both diseases. However, in *LRBA*$^{-/-}$ patients, when we compared nonsense vs. missense mutations, lymphoproliferation (n=20 (83%) vs. n=2 (33%), p=0.029) and autoimmunity (n=24 (100%) vs. n=4 (67%), p=0.034) were statistically higher in the nonsense group. Furthermore, we noticed that identical *LRBA* mutation gave rise to divergent clinical outcomes even among siblings but otherwise showed complete penetrance, while among *CTLA4*$^{+/-}$ subjects, there were 4 unaffected mutation carriers, displaying incomplete penetrance, as observed in previous studies [24].

Serum immunoglobulin levels before intravenous immunoglobulin therapy were available for all *LRBA*$^{-/-}$ and *CTLA4*$^{+/-}$ patients and demonstrated proportionally similar distribution in both disorders (**Fig.E2A**). In the whole cohort, there were low IgG in 54

(55.1%), low IgM in 57 (58.1%), and low IgA in 71 (72.4%). Flow cytometric analysis at diagnosis, including T, B, natural killer (NK), T-cell, and B-cell subtypes, was performed in all patients (**Fig.E2B, Table E7,** and **Table E8**). According to the age-matched healthy controls reference values [38], decreased $CD3^+$ T-cell counts were observed in 25 (39.7%) *LRBA*$^{-/-}$ and 19 (54.3%) *CTLA4*$^{+/-}$ patients. At the same time, recent thymic emigrants were low in 52.3% *LRBA*$^{-/-}$ and 62.5% *CTLA4*$^{+/-}$ patients. There were no differences in B- and NK-cell subtypes between the two diseases, and both showed low percentages of memory B cells compared to the age-matched healthy controls.

Therapeutic options and response rates in *LRBA*$^{-/-}$ and *CTLA4*$^{+/-}$. In our cohort, due to recurrent infections and hypogammaglobulinemia, the patients were usually followed up with antimicrobial prophylaxis and immunoglobulin replacement therapy (IgRT, 73 with intravenous and 9 with subcutaneous preparations) (**Fig.3A**, **Table E3**, and **Table E4**). The most commonly used prophylaxis was trimethoprim-sulfamethoxazole (85%) for antibacterial prophylaxis, fluconazole (81.8%) for antifungal, and aciclovir (68.8%) for antiviral prophylaxis. The indication of antifungal and antiviral prophylaxis was not primary and preferred after candidal and herpes infections. *LRBA*$^{-/-}$ patients received mentioned therapies more often than *CTLA4*$^{+/-}$ patients (**Fig.3A)**. Splenectomy was performed in 13 patients (61.5% in *LRBA*$^{-/-}$) due to the intractable chronic cytopenia with splenomegaly. The HSCT was only performed in *LRBA*$^{-/-}$ patients (n=14, 22.2%).

To control immune dysregulatory features observed in the patients, numerous mono or combination therapies with immunosuppressive agents were used (**Fig.3A-C**): Abatacept (n=58, 59.1%), prednisolone (n=54, 55.1%), mycophenolate mofetil (MMF) (n=14, 14.2%), sirolimus (n=9, 9.1%), cyclosporine A (n=9, 9.1%), azathioprine (n=7, 7.1%), rituximab (n=7, 7.1%), hydroxychloroquine (n=3, 3.0%), methotrexate (n=4, .0%), budesonide (n=2, 2.0%), adalimumab (n=2, 2.0%), and sulfasalazine (n=1, 1.0%). Chemotherapy regimens due to malignancies were applicable in 6 patients (6.1%). The management chart of used IS is presented in **Fig.E3**. Abatacept usage was more in *LRBA*$^{-/-}$ (n=42, 66.7%) when compared to *CTLA4*$^{+/-}$ patients (n=16, 42.9%) (p=0.02). In contrast, there was no difference for other ISs (65.1% vs. 57.1%) (**Fig.3B**). Corticosteroids and mycophenolate mofetil were the most applied ISs,

followed by sirolimus and cyclosporine A (**Fig.3C**). The indications to start ISs in 61 patients were AIHA (n=31, 50.8%), ITP (n=29, 47.5%), CD (n=14, 22.9%), lymphoproliferation (n=10, 16.3%), arthritis (n=9, 14.7%), ILD (n=5, 8.1%), demyelinating disease (n=4, 6.5%), malignancies (n=4, 6.5%), hereditary lymphohistiocytosis (n=2, 3.2%), systemic lupus erythematosus (n=1, 1.6%), uveitis (n=1, 1.6%), and alopecia (n=1, 1.6%). The detailed indications of ISs for every patient are presented in **Table E9**. Of note, in patients with autoimmunity, lymphoproliferation, or parenchymal lung disease, the requirement for ISs was more common than in patients without symptoms ($p<0.0001$) (**Fig.E4**). Control of the ID manifestations usually required repeated courses of IS regimens, and 36 patients received $\geq$ 2 ISs before genetic diagnosis or application of abatacept (**Fig.3D**). Furthermore, in general, patients who received ISs showed PR or NR responses, indicating that applicable ISs are not an appropriate way to achieve sustained disease control (**Fig.4A**). Sirolimus was used for enteropathy (LM1, LM17), lymphoproliferation (LM5), AIHA (CM9, CM21), ITP (LM5, CM3, CM9, CM21), parenchymal lung disease (LM10, LM16, LM17). 2 patients (CM3, CM9) showed CR in controlling cytopenia, in the rest of received patients, sirolimus alleviate the symptoms without CR.

In total, 58 patients (59.1%) received abatacept as primary or rescue therapy (**Fig.E3**). Abatacept usage was high in *LRBA*$^{-/-}$ compared with *CTLA4*$^{+/-}$ patients (n=42, 66.7% vs n=16, 42.6%; p=0.02). The detailed indications of abatacept are presented in **Table E10**. The median duration of abatacept therapy was 2.3 years (min-max: 0.8-6.7) with a maintenance intravenous dose of 10-20 mg/kg per 3-4 weeks in 53 (91.3%) and a subcutaneous dose of 125 mg/per week in 5 (8.7%) patients. Apart from other ISs, abatacept displayed a striking control of disease manifestations and demonstrated CR in 74% of *LRBA*$^{-/-}$ and 93% of *CTLA4*$^{+/-}$ patients. The remaining responses were PR or NR (**Fig.4B** and **Table E10**). This response was stable during the follow-up. Besides, when the main clinical phenotypes of the abatacept-received patients were categorized as ID, CD, and lymphoproliferation, each phenotype's response after abatacept therapy was calculated, all patients responded to abatacept therapy but to various degrees (**Fig.4C** and **D**). As we reported previously [25], abatacept therapy revealed better CR in lymphoproliferation (88%) followed by CD (84.4%), and these rates were equal in both disorders. On the

other hand, the ID symptoms showed more heterogenous response rates (CR-62.8%, PR-32.6%, and NR-4.6%) since, in nature, ID presented with many diseases, and it is expected to be associated with different pathophysiological mechanisms [5, 39]. In more detail, CR was observed in the majority of AIHA (100%), ITP (94.1%), and autoimmune hepatitis (100%). Lesser CR was detected in other IDs characterized by arthritis (83.3%), ILD (70.0%), and demyelinating disease (33.3%). All patients with alopecia showed PR to abatacept, whiles type 1 diabetes mellitus was NR. We also analyzed the response rates separately in *LRBA*$^{-/-}$ and *CTLA4*$^{+/-}$ patients; the results showed a similar control pattern, mirroring the shared immune dysregulation in both disorders (**Fig.4D**).

The efficacy of therapeutic approaches between abatacept and other ISs was evaluated using PSM [35, 40]. For this analysis, we accepted patients who received only abatacept (n=25) as the treatment group and patients who used only immunosuppressants (n=20) as the control group. The outcome was to analyze the complete response rate between the groups. After performing a successful PSM matching analysis, we found 18 patients in the treatment group matched with 18 in the control group with SMDs less than 0.2 for analyzed covariates (**Fig.E5A** and **B**). Receiving only abatacept compared to other ISs showed a statistically better CR rate (72.2% vs. 33.3%, p=0.04, OR=5.2; 95% CI, 1.25 – 12.57).

During the follow-up of patients, we stopped abatacept in 18 patients. This proportionally was due to HSCT in 11 (61%), resolved indications in 2 (CM5 showed complete control for AIHA, CD, and lymphoproliferation, CM22 demonstrated complete control for ITP and arthritis), inadequate response of parenchymal lung disease in LM10, adverse effect with EBV reactivation in CM24, purified protein derivative positivity with a negative culture of *Mycobacterium tuberculosis* in LM37 and LM38 at 9th abatacept maintenance doses, in whom abatacept was started again after 1 month of skipping. LM16 had *Aspergillus fumigatus* pneumonia after the first dose of abatacept, leading to treatment discontinuation. However, it was reinitiated after 9 months without side effects (**Fig.4E**). In 14 transplanted patients, there were 11 abatacept-received patients as bridge treatment, revealing good disease control. 9 showed CR (LM2, LM4, LM5, LM9, LM13, LM18, LM29, LM49, and LM57), while 2 demonstrated PR (LM12 and LM21). Generally, the adverse effects of abatacept

were uncommon, usually mild and transient, characterized by mild eczema in LM2, LM3, CM5, and CM8, skin zoster infection in LM29, CM7, and herpes virus in CM25 (**Fig.4F**). At the end of the study, there was no change in IgRT or other prophylaxis rates after abatacept. Notably, with the usage of abatacept, all patients weaned off their other ISs, except for 5 patients (LM10, LM16, LM17, CM26) (**Table E11**).

There were 10 (17.2%) PR and 2 (3.4%) NR patients during abatacept therapy. To determine the factors pertaining to responses, we investigated the distinguishing clinical and immunological features of those patients by comparing them to CR patients (n=46) (**Fig.5A**). Patients with PR/NR showed statistically longer disease course (8.3±4.7 years) than patients with CR (4.5±3.1 years) (p=0.002). Those patients also had more organ involvement characterized by colitis (17% vs. none, p=0.013) and GLILD (25% vs. 2.2%, p=0.025). Further, 2 patients with PR/NR developed cirrhosis during the follow-up (LM19, LM44). The mortality rate in PR/NR was significantly high than in CR patients (n=3 (25%) vs. n=1 (2.2%), p=0.025). They also demonstrated a more dysregulated phenotype characterized by elevated $CD8^+$ T-cell counts and exhausted $CD8^+$ T-cell percentages. Abatacept therapy achieved good disease control in CR patients for various organ involvements (**Fig.5B**). Finally, the probability of survival after receiving abatacept revealed better outcomes in CR than in PR/NR patients (**Fig.5C**). The details of patients with PR/NR to abatacept are presented in **Table E11**.

Fourteen patients (LM2, LM4, LM5, LM6, LM8, LM9, LM12, LM13, LM18, LM21, LM29, LM45, LM49, and LM57) were transplanted because of the advanced disease and poor response to treatment (**Table E12**). The median age of HSCT was 12.1 years (min-max: 7.6-27.3), with a median post-HSCT follow-up of 2.8 years (min-max: 0-8.6). In particular, the indications for the transplantation were persistent hematological findings (LM2, LM5, LM6, LM8, LM9, LM12, LM29, LM49, LM57), severe RTI (LM18), uncontrolled lymphoproliferation and bronchiolitis obliterans (LM6), uncontrolled CD and RTI (LM4, LM6, LM13, LM21, LM45, LM49). A diversity of donors was used, including matched-HLA donors (n=13, 92.3%) and a haploidentical donor (n=1, 7.7%). Conditioning regimens were applied in 12 (85.7%) patients, including 3 myeloablative, 2 reduced toxicity, 7 reduced intensity, and 2 non-conditioning. GvHD was observed in 4 (28.5%). CMV reactivation was detected in 7

(50%) patients. 1 patient (LM12) developed ITP after HSCT requiring ISs (corticosteroid) for 6 months, 1 patient (LM49) required abatacept due to CD and persistent ITP, and he is continuing to use them at the end of the study, LM57 experienced ITP. He is on corticosteroids and MMF at 7 months of HSCT. M13 still receives corticosteroids due to CD at 3 years of HSCT. LM21 developed bronchiolitis obliterans after HSCT. In total, 10 (71.4%) patients could discontinue previous use of IgRT after HSCT, 7 (50%) of them within 6 months, while the other 3 (21.4%) were after 6 months of transplantation. 2 patients had no donor chimerism (LM2, LM6). Both died within one month of transplantation (LM2 due to GvHD, thrombotic microangiopathy, and multiorgan failure; LM6 due to sinusoidal obstruction syndrome and sepsis). LM13 also had no donor chimerism and survived with CD. LM29 showed 29% donor lymphocyte chimerism with complete remission. The rest of the patients demonstrated chimerism levels ranging between 92%-100%. Transplantation from matched sibling donors showed 100% of survival. Overall, the outcome was complete remission in 9 (64.2%), partial remission in 2 (14.2%), no remission in 1 (7.1%), and death in 2 (14.2%).

Survival rates and factors affecting the outcomes. At the end of the study, there was 10 death (10.2%, LM2, LM6, LM10, LM11, LM17, LM35, LM46, LM47, LM56, and CM26). Those were due to severe parenchymal lung disease and COVID-19 (LM10), pneumonia, lymphoproliferation and sepsis (LM11), ILD and sepsis (LM35), intracranial hemorrhage (LM47), and sepsis (LM17, LM46, LM56, CM26). LM2 and LM6 died after HSCT.

The probability of OS was better in *CTLA4*$^{+/-}$ (91.6%) compared with *LRBA*$^{-/-}$ patients (60.5%, p=0.008) (**Fig.6A**). The OS was not different between disease phenotypes classified as CVID, IPEX, ALPS, and unaffected mutation carriers (Data not shown). Although frameshift mutations showed less favorable survival in *LRBA*$^{-/-}$ patients, no statistical differences existed between groups (**Fig.E6A** and **B**). The HSCT was performed only in *LRBA*$^{-/-}$ patients; therefore, we compared the OS between transplanted and non-transplanted *LRBA*$^{-/-}$ patients. As shown in **Fig.6B**, there was no difference between both groups, albeit the probability of OS was better in patients with transplantation. Furthermore, to understand whether the outcome of HSCT was associated with diagnostic delay, we compared patients in terms of diagnostic delay

(<5 years and >5 years). The result revealed similar outcomes between the 2 groups (**Fig.6C**). Additionally, in both disease groups, organ involvements characterized by GI manifestations, lymphoproliferation, and parenchymal lung disease demonstrated significantly less favorable OS than patients without involvements (**Fig.6D-F).**

We evaluated the impact of receiving ISs or abatacept on the OS. Patients who received ISs showed inferior OS (**Fig.7A**). This did not change between CR or PR groups (**Fig.7B**), indirectly mirroring the burden of the disease requiring more medications to control the symptoms. Furthermore, LRBA-deficient patients with transplantation demonstrated significantly better outcomes when compared to ones with ISs other than abatacept (**Fig.7C**, p=0.020). Notably, the time elapsed after receiving abatacept showed a similar probability of survival as the post-HSCT period, delineating that abatacept treatment effectively controls the disease activity and can change the global course of LRBA deficiency (**Fig.7D**). Finally, since patients' ages range from 2 - 67 years, we stratified patients according to age. We observed lower survival for the whole cohort (n=98) in the range of 11-20 years (p=0.002) (**Fig.E7A**). *LRBA*$^{-/-}$ patients also exhibited lower survival for the 11-20 years group (p=0.01) (**Fig.E7B**). The age factor did not show an impact on GI involvement and lymphoproliferation (**Fig.E8A-F**). However, for the 11-20 age group, HSCT exhibited better survival compared to the patients receiving ISs other than abatacept (**Fig.E9A**). A comparison of HSCT vs. receiving abatacept revealed similar outcomes for the 11-20 and ≥ 21 age groups (**Fig.E9B** and **C**).

Discussion

We present detailed clinical outcomes to different treatment modalities of LRBA deficiency and CTLA4 insufficiency in a large multicenter prospectively evaluated cohort. Our study provided for the first time a long-term comparative evaluation of the different types of treatments, including targeted CTLA4-Ig therapy, ISs other than abatacept, and HSCT. We found that *LRBA*$^{-/-}$ patients showed early onset of disease with a more severe phenotype compared with *CTLA4*$^{+/-}$ patients. Therefore, *LRBA*$^{-/-}$ patients required more ISs, abatacept, and HSCT to control the disease activity. Abatacept was able to achieve sustained CR in the majority of used patients. At the same time, other ISs showed transient control that usually provided PR or NR, requiring more therapies like abatacept or transplantation. Our results also addressed the clinical and immunological features that can predict the response to abatacept. Patients with colitis or GLILD showed higher PR to targeted therapy, with a poorer outcome than patients with CR. Importantly, in our study, we observed that conducting HSCT or receiving abatacept showed a similar probability of survival, demonstrating that abatacept therapy can result in sustainable disease control.

LRBA$^{-/-}$ and *CTLA4*$^{+/-}$ diseases are characterized by CVID and ALPS-like phenotypes, affecting many organs and usually present with several autoimmune phenomena, lymphoproliferation, and parenchymal lung disorders, leading to the death of the patients [24, 26, 32, 33, 41]. In presenting patients, early onset of CD, lymphoproliferation, and lung disease should evacuate the diagnosis of diseases [14, 25]. We observed that patients with early diagnosis without end-organ involvements demonstrated better outcomes when compared to patients with lymphoproliferation, GI, and lung involvements. We also showed that disease burden with multiple drug usage inversely affects the outcomes over time. Further, nonsense mutations in *LRBA*$^{-/-}$ patients revealed higher lymphoproliferation and autoimmunity, demonstrating another factor influencing the clinical course. Therefore, the prognosis of the respective diseases depends on early diagnosis and prevention of end-organ damage and, to some extent, the mutation type of the patient.

Previous studies showed that abatacept, sirolimus, and HSCT could provide global disease control in *LRBA*$^{-/-}$ and *CTLA4*$^{+/-}$ patients [26, 27]. There is currently no unanimous

consensus regarding the precise treatment of these disorders. However, according to our current largest applied abatacept treatment results in the literature, initiating abatacept late in the disease course with multiple organ involvements may not be sufficient to induce disease remission or prevent life-threatening immune dysregulation. Therefore, once autoimmune, lymphoproliferative, or GI manifestations occur (after excluding other possible etiological factors like infections and malignancies), abatacept can be considered a first-line targeted drug to achieve better disease control. On the other hand, in our study, the abatacept dose varied between centers, and maintenance with 10-20 mg/kg per month was the more frequently used dose. In addition, the efficacy of abatacept on symptoms was changeable and depended on the type of manifestation (lymphoproliferation>chronic diarrhea>immune dysregulation); therefore, every case should be evaluated separately in terms of response to treatment. Our results also provided long-term good disease control (up to 7 years) without severe side effects showing that abatacept is providing an immune regulatory effect for these disorders rather than making immunosuppression. Data regarding the efficacy of sirolimus in controlling various disease symptoms was available in both disorders [24]. In particular, sirolimus completely controlled cytopenia in 37.5%, while ameliorated enteropathy in 57% of *LRBA*$^{-/-}$ patients [26]. Similarly, 78% of GI manifestations and 62% of GLILID lesions were resolved with sirolimus in *CTLA4*$^{+/-}$ patients [27]. Our result also demonstrated similar results in favoring CR of cytopenia.

Our cohort shares similarities with the reported patients in Jamee et al.'s retrospective review [24], especially regarding gender ratio, current median age, symptom onset age, and age at first autoimmunity and infections presentation. Notably, consanguinity rates were higher in our cohort, particularly among *CTLA4*$^{+/-}$ patients. The low mortality in our patients than reported ones (10.2% vs. 17.3%) could be attributed to several factors; one main reason is the increased knowledge about the diseases, making early diagnosis possible before chronic and severe organ involvements [24]. For this aspect, our study showed a shorter median time of diagnosis and reduced diagnostic delay compared to other published patients [24]. The other reason could be associated with a higher rate of abatacept usage, which was 59.1% in our cohort and 23.8% in previous patients [24]. Other used ISs, including rituximab, sirolimus, MMF, and cyclosporine A, were lower in our cohort than previously reported patients [24].

Overall, our findings indicate a shift in treatment decisions towards the increased utilization of abatacept as a primary therapeutic option for controlling disease manifestations.

Based on our results, the following management approaches can be construed. First, we suggest starting IgRT and antimicrobial prophylaxis in both diseases, especially in patients with the CVID phenotype. Second, regular screening for viral pathogens should be performed, particularly in patients receiving abatacept due to the relatively high number of CMV and EBV infections. Third, once autoimmune manifestations are controlled, in some cases with well-disease control, abatacept can be stopped while closely monitoring these patients by IgRT and antimicrobial prophylaxis. In this line, 2 patients in our cohort (CM5 and CM22) were able to discontinue treatment after revealing control of their manifestations.

In terms of transplantation, due to the variable clinical course of diseases, even with the same mutation among family members, the indication and time of transplantation are still obscure [24, 26-28, 32]. Our results and previous reports show similar successful transplantation results, which were inferior to other IEIs, and in our study, 21.3% of patients continued to receive immunosuppressants after HSCT. The majority of *LRBA*$^{-/-}$ and *CTLA4*$^{+/-}$ transplanted patients in the literature were in the late disease stage with multiple chronic problems, and especially lung involvement was found to be associated with poor outcomes [24, 33]. Likewise, our transplanted patients had multiorgan symptomatology, contributing to high post-transplant complications and less sustained disease control. Therefore, as stated previously, performing HSCT at an early stage can show a better outcome [26, 42], yet further evidence regarding conducting transplantation at an early stage is needed. While the OS did not differ between transplanted and non-transplanted *LRBA*$^{-/-}$ patients, the post-HSCT time elapsed compared with IS treatment showed a better probability of survival, which may favor transplantation. Nevertheless, no comparative study has evaluated the post-HSCT period versus abatacept treatment. In our study, we provided comprehensive data comparing the outcomes of HSCT and abatacept. This study is the first to demonstrate similar outcomes between the two treatment options when evaluated for the elapsed time after each therapy. Our findings highlight the efficacy of abatacept in altering the disease course and suggest its potential as a bridging

therapy for patients who may require transplantation. Hence, our results support HSCT consideration early in patients with no access to abatacept, in case of resistance to conventional ISs and abatacept, or experiencing significant drug-related adverse effects.

The other ongoing debate concerns developing malignancies in patients with prolonged disease courses in *LRBA*$^{-/-}$ and *CTLA4*$^{+/-}$ patients. None of our patients showed malignant transformation under abatacept, suggesting this therapy may re-establish functional immune surveillance to prevent malignant transformation. However, longer-term studies are necessary to investigate this potential benefit [42-44].

Finally, we wanted to mention some limitations in our study. The relatively short follow-up period restricts the ability to ascertain the ultimate clinical course and/or effects of treatment approaches in some patients. Studies with more longitudinal follow-up periods are warranted to answer these questions. The other limitation is related to the various management attempts between centers impeding establishing a decision for standardized management protocols for both diseases. Also, the results may not be fully generalizable to other countries. Lastly, fewer patients with certain disease manifestations hinder concluding the best therapeutic options.

In conclusion, we showed that abatacept therapy could be successfully employed long-term without serious side effects to control and stabilize the diseases. From the therapeutic perspective, an individualized evaluation should be performed for each patient. However, initially considering early employment of abatacept to patients with ID symptoms and close monitoring of effectiveness may be a preferred approach to achieve better disease control. Furthermore, suitable donors should be investigated promptly for possible transplantation during the follow-up.

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Table 1. The demographic features of the study cohort

Patients (n, %)	*LRBA*$^{-/-}$	63 (64.3)
	CTLA4$^{+/-}$	35 (35.7)
Male/Female (n, %)		52/46 (53.1/46.9)
Current age (year); Median (min-max)		17 (2-67)
Age at symptom onset (year); Median (min-max)		4 (0.08-45)
Diagnostic age (year); Median (min-max)		12 (0.4-60)
Diagnostic delay (year); Median (min-max)		5.6 (0.08-40)
Family history (n, %)	Consanguinity rate	65 (66.3)
	Affected relatives	29 (42.0)
HSCT (n, %)		14 (14.3)
Outcome (Alive, n, %)	*LRBA*$^{-/-}$	54 (85.7)
	CTLA4$^{+/-}$	34 (97.1)

HSCT: Hematopoietic stem cell transplantation

Figure and Table Legends

Figure 1. Demographic and clinical features of *LRBA*$^{-/-}$ and *CTLA4*$^{+/-}$ patients. **(A)** The distribution of the patients. **(B)** Comparisons of demographic characteristics between *LRBA*$^{-/-}$ and *CTLA4*$^{+/-}$ patients. **(C** and **D)** The rate of dead/alive patients. **(E)** The main presenting features of *LRBA*$^{-/-}$ and *CTLA4*$^{+/-}$ patients. **(F)** The clinical phenotype of patients. Error bars indicate SEM. Statistical tests: Student unpaired t-test was used in panel **B**. The chi-squared test compared categorical variables **(E** and **F)**. Asterisks indicate the differences.

Figure 2. *LRBA*$^{-/-}$ patients display a more severe phenotype than *CTLA4*$^{+/-}$ patients. (A) The main clinical manifestations in *LRBA*$^{-/-}$ and *CTLA4*$^{+/-}$ patients. The mutations affect different domains of the LRBA **(B)** and CTLA4 **(C)** proteins**.** Most biallelic variants are nonsense and frameshift in *LRBA*$^{-/-}$, while heterozygous missense and nonsense are common in *CTLA4*$^{+/-}$. Red depicts new patients, blue shows old patients, and the square indicates patients with the compound heterozygous mutation. The chi-squared test was performed to compare categorical variables. BEACH: Beige and Chediak-Higashi domain, CC: Coiled-coil, Con A: Concanavalin A-like lectin domain; PH, PH domain associated with beige/BEACH, WD: WD-40 repeat. GLILD: Granulocytic-lymphocytic interstitial lung disease.

Figure 3. *LRBA*$^{-/-}$ patients require more therapies than *CTLA4*$^{+/-}$ to control symptoms. (A) Therapeutic options in *LRBA*$^{-/-}$ and *CTLA4*$^{+/-}$ patients. **(B)** The proportions of used immunosuppressants, abatacept, and transplantation in *LRBA*$^{-/-}$ and *CTLA4*$^{+/-}$ patients. **(C)** The number of various immunosuppressants used to control the symptoms of patients. **(D)** The number of patients who receive 1, 2, 3, or 4 ISs. The chi-squared test was performed to compare categorical variables **(A)**. IS: immunosuppressant, HSCT: Hematopoietic stem cell transplantation.

Figure 4. Abatacept demonstrates better disease control with few side effects compared to other immunosuppressants. The control levels of symptoms after receiving ISs **(A)** or abatacept **(B)**. The impact of abatacept on various disease symptoms in all patients **(C)**, in *LRBA*$^{-/-}$ and *CTLA4*$^{+/-}$ patients **(D)**. **(E)** Reasons for abatacept discontinuation during the study. **(F)** The number of patients who experience side effects during abatacept usage. CR: Complete response, PR: Partial response, NR: Non-responsive.

Figure 5. Patients with end-organ involvement show partial response to abatacept. (A) The comparisons between patients who exhibit CR versus PR/NR to abatacept. **(B)** Radiological improvements after abatacept. CT scan showing bulk mediastinal lymphadenopathies (dashed line circle, **a**), patchy consolidation, and inflammatory nodules (arrows, **b**) in LM61 regressed post-abatacept (**c**, **d**). CT scan of CM16 demonstrating inflammatory nodules with parenchymal tractions (arrows, **e**, **f**) disappeared post-abatacept (**g**, **h**). CT image revealing inflammatory nodules in LM30 before abatacept (arrows, **j**, **k**) and regression after treatment (**ı**, **m**). Enlarged splenomegaly (dashed line circle, **n**) in LM30 before abatacept and decreased size after treatment (**o**). MRI of CM3 showing inflammatory lesions with edema in the right parietal and temporal lobes (dashed line circle, **p**), healed with minimal gliosis after treatment (dashed line circle, **q**). **(C)** Kaplan-Meier curve showing the probability of survival between CR versus PR/NR patients. The chi-squared test was performed to compare categorical variables **(A)**. CR: Complete response, PR: Partial response, NR: Non-responsive, CT: Computed tomography, MRI: Magnetic resonance imaging.

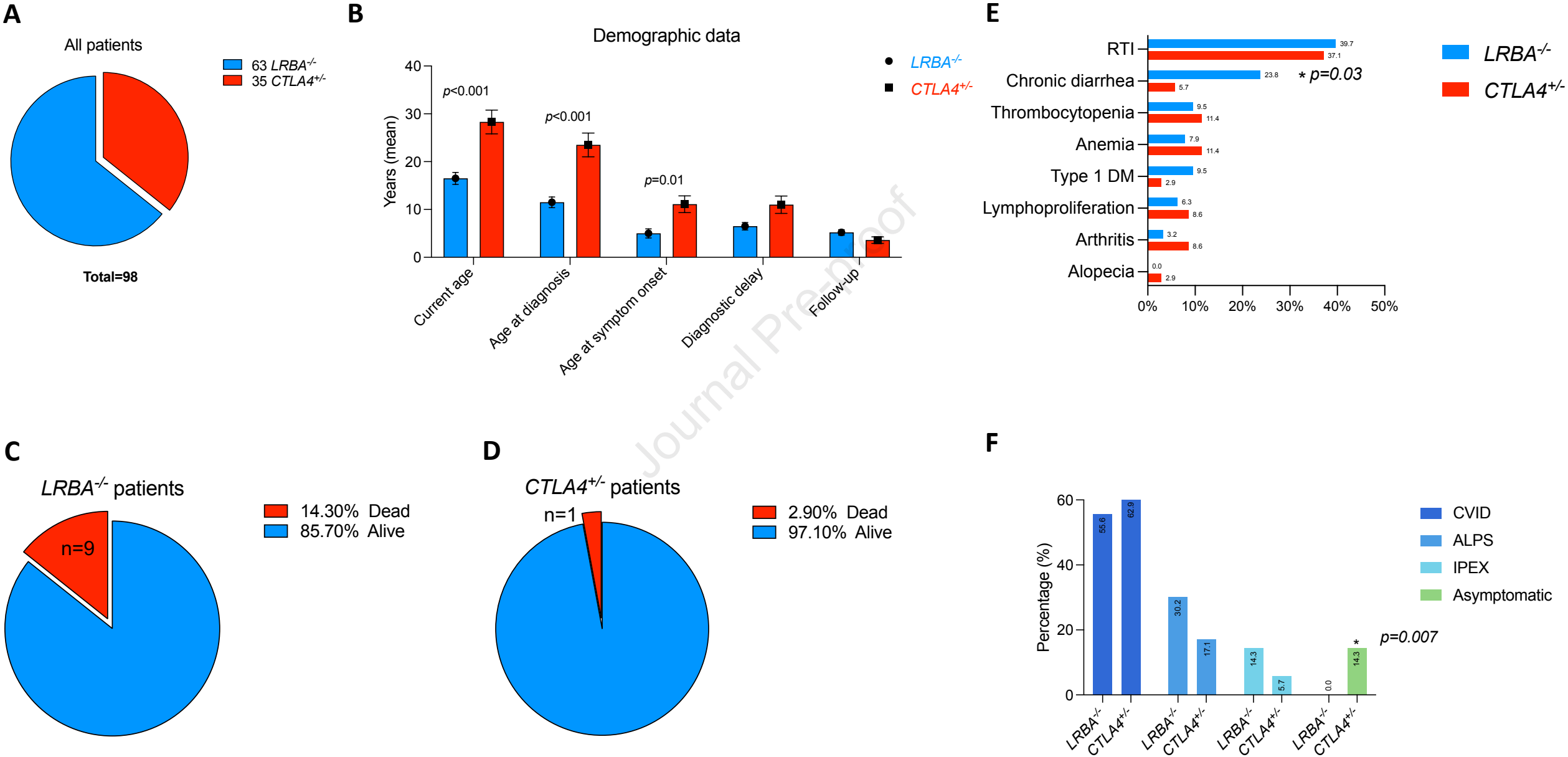
Figure 6. *LRBA*$^{-/-}$ patients demonstrate poorer outcomes than *CTLA4*$^{+/-}$ patients. **(A).** Kaplan-Meier curve indicating the probability of survival between *LRBA*$^{-/-}$ and *CTLA4*$^{+/-}$. **(B)** Overall survival in *LRBA*$^{-/-}$ patients in terms of HSCT. **(C)** The impact of

836 diagnostic delay on survival after HSCT in *LRBA*$^{-/-}$ patients. End-organ involvements,
837 including GI **(D)**, lymphoproliferation **(E)**, and lung involvement **(F)**, and their effect on
838 survival. GI: Gastrointestinal, HSCT: Hematopoietic stem cell transplantation.

839 **Figure 7. Receiving abatacept reveals similar survival to transplantation. (A)**
840 The impact of receiving ISs on survival. **(B)** Comparison of survival between patients
841 who show complete and partial responses to ISs. Probability of survival with HSCT
842 versus ISs **(C)** or abatacept **(D)**. HSCT: Hematopoietic stem cell transplantation.

843 **Table 1.** The demographic features of the study cohort.

Figure 1



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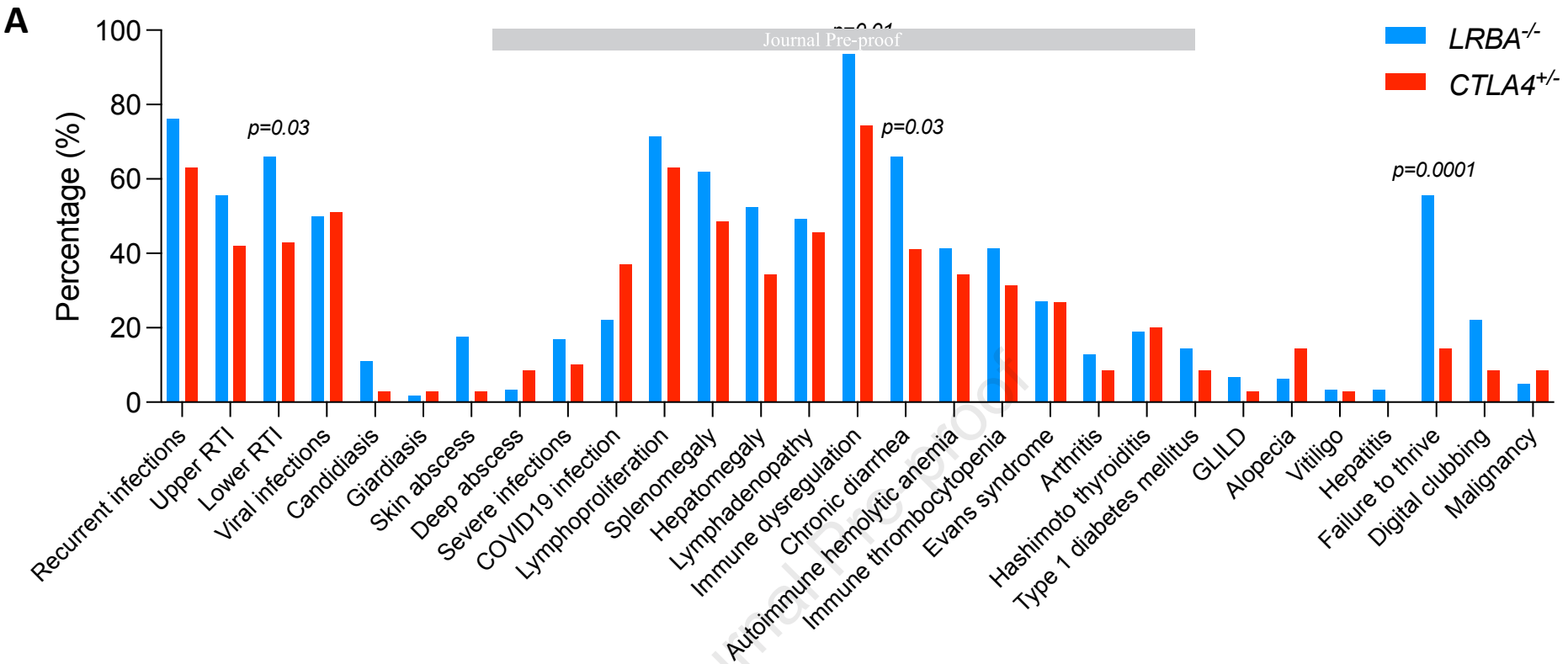

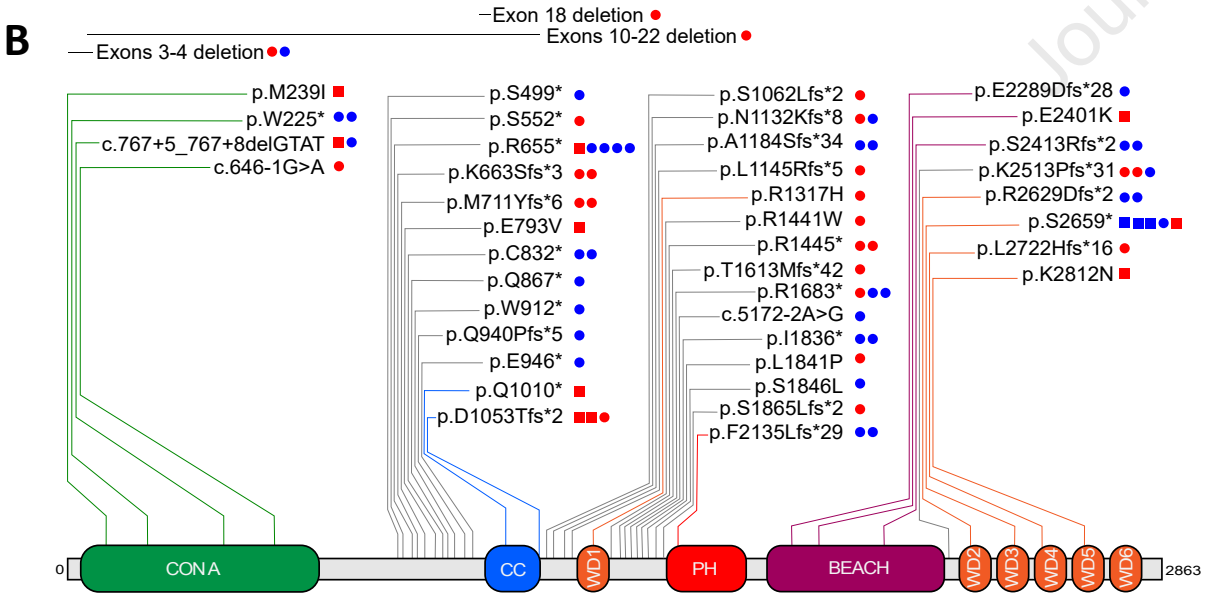

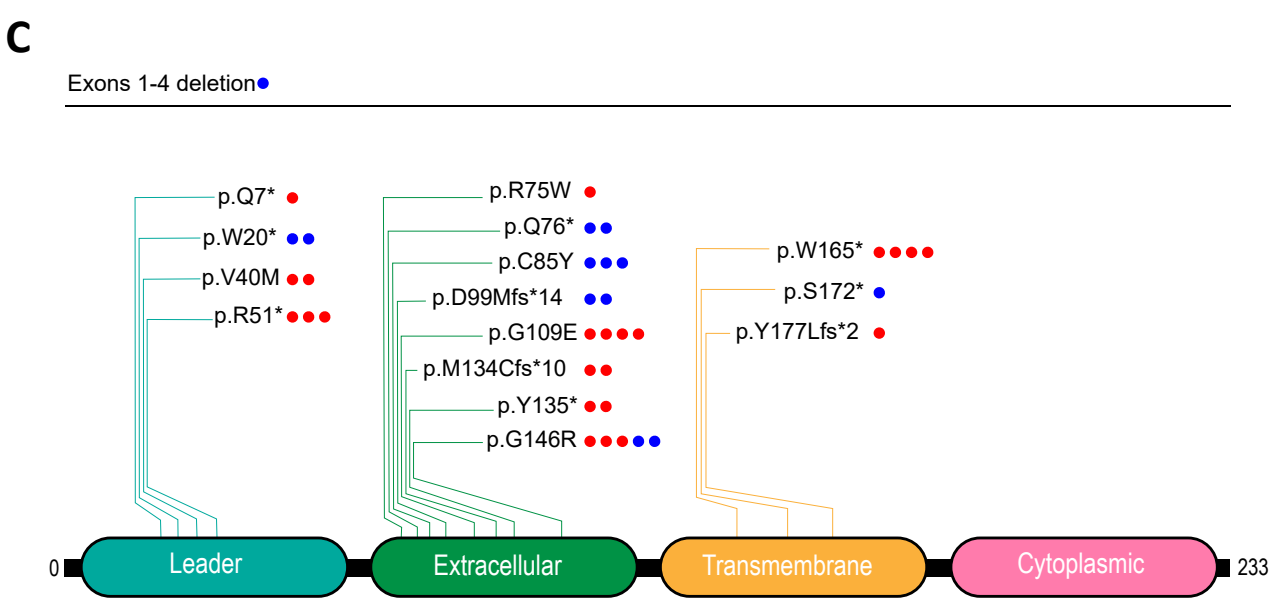

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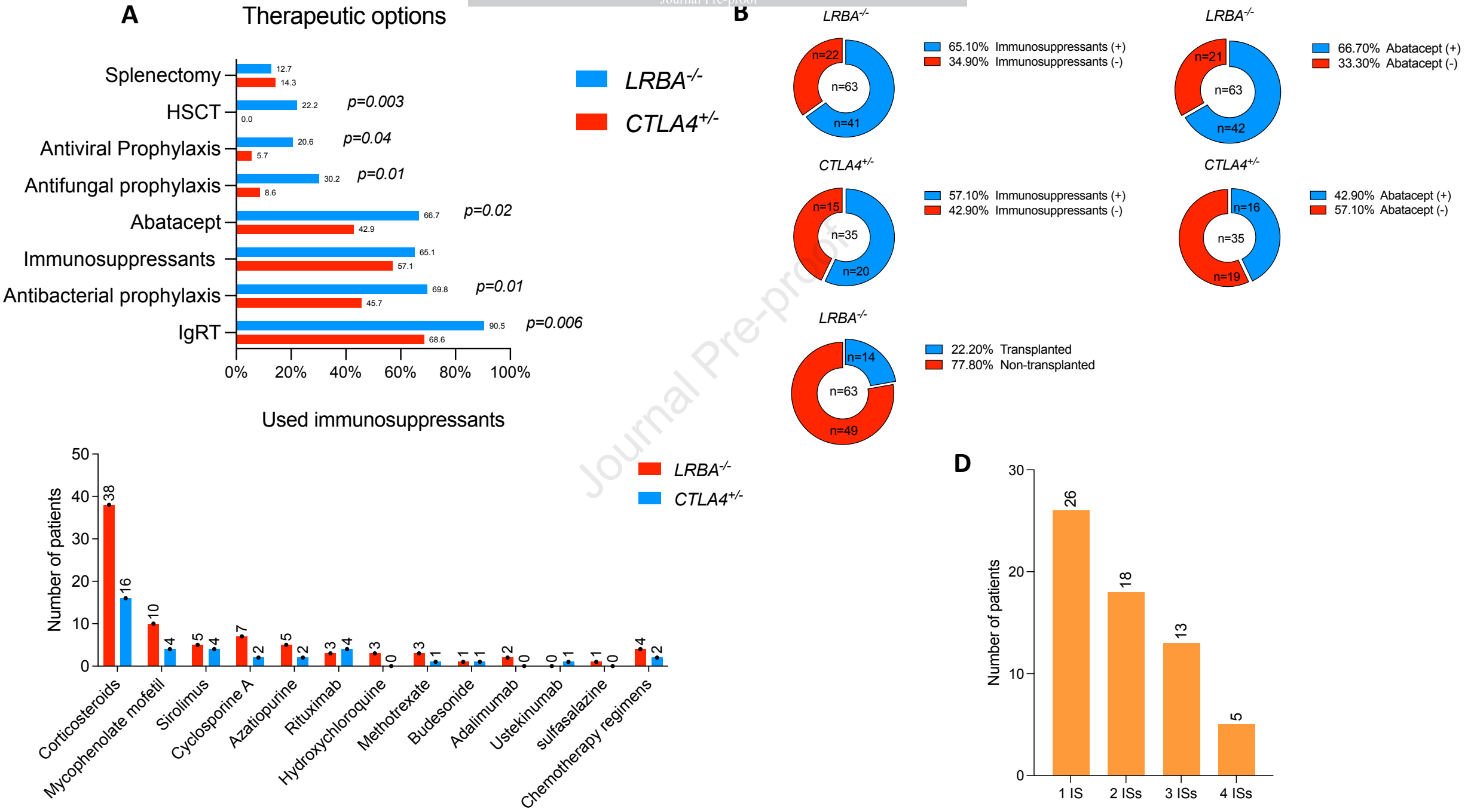
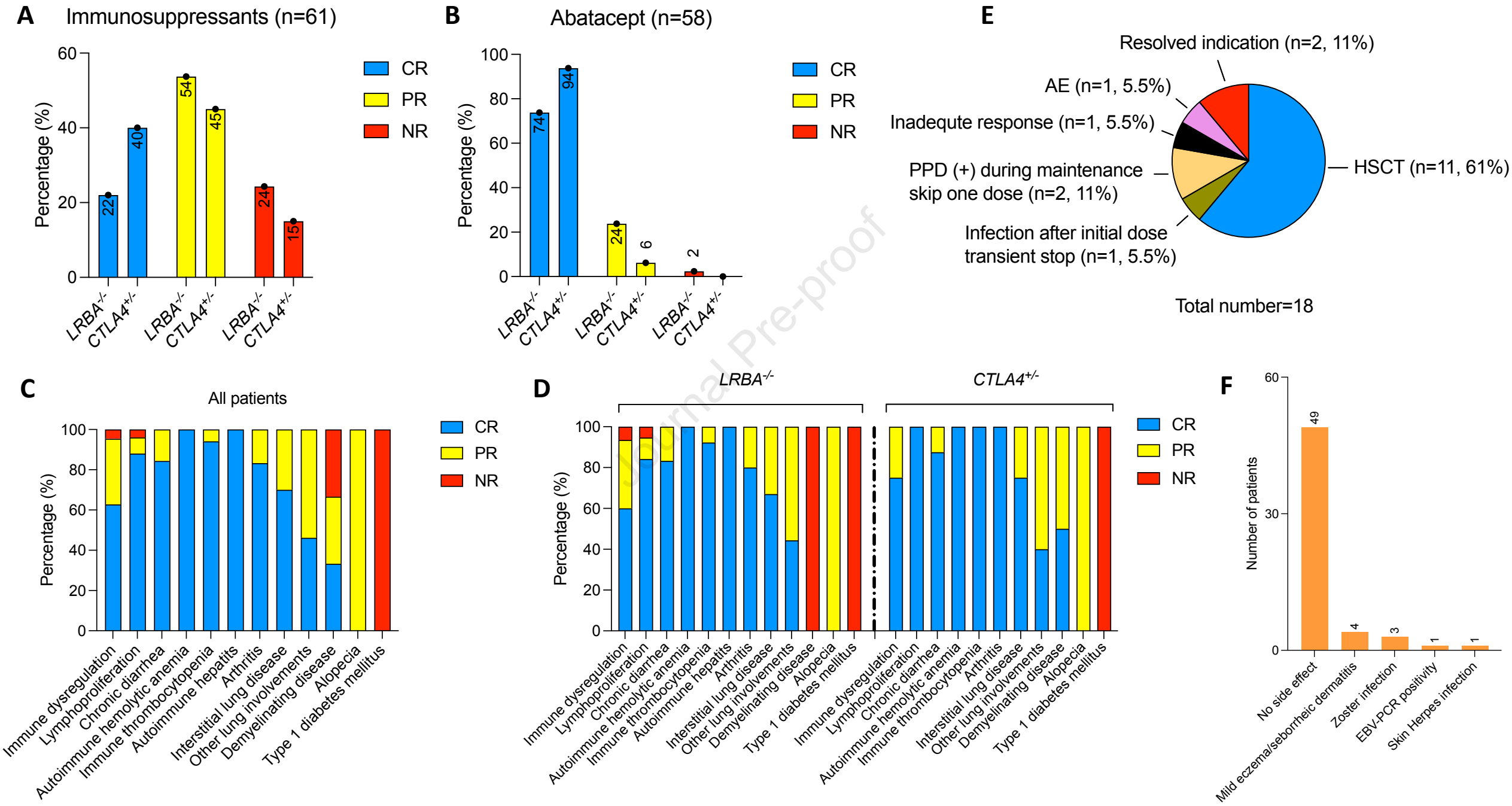


Figure 4



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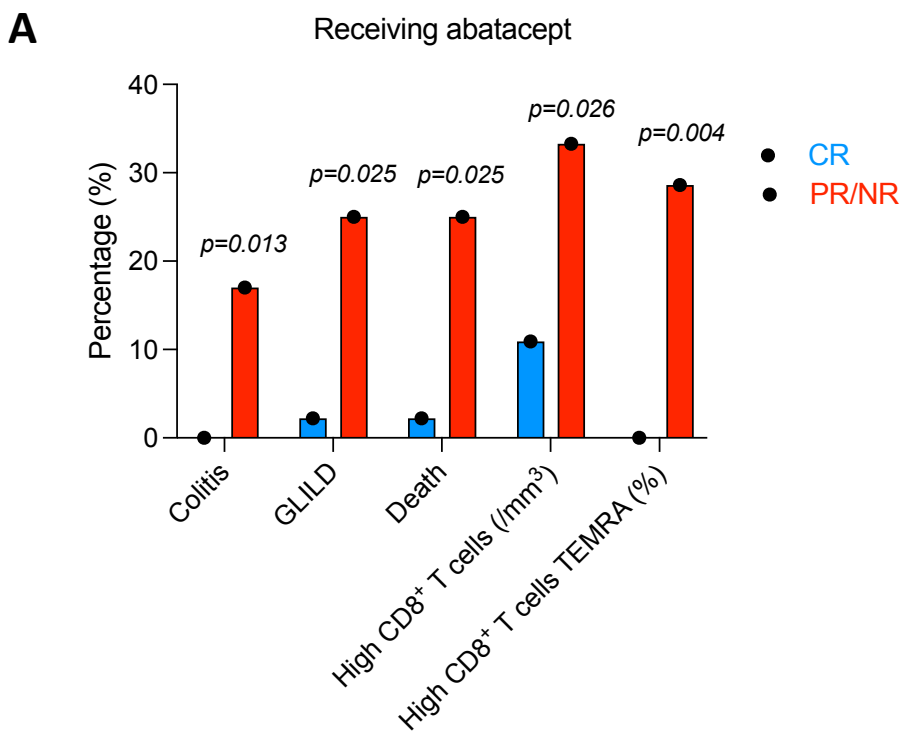

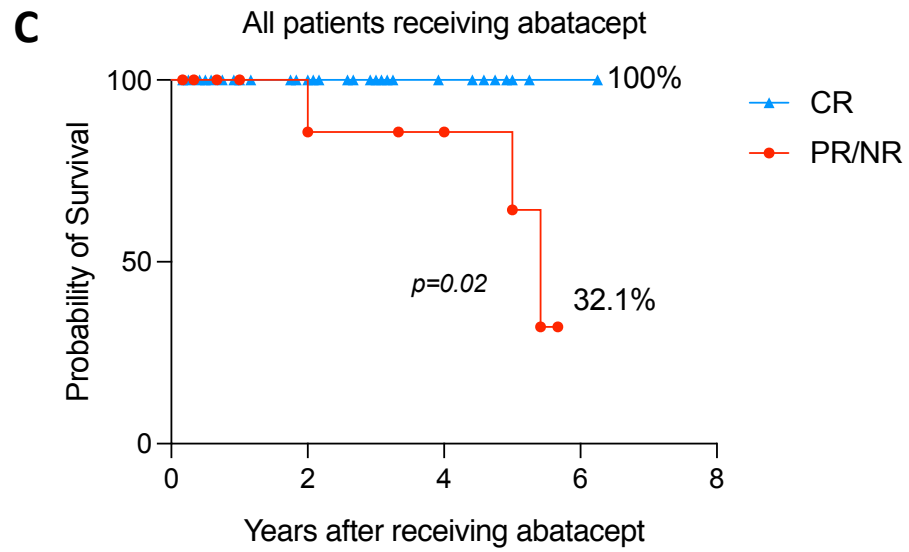

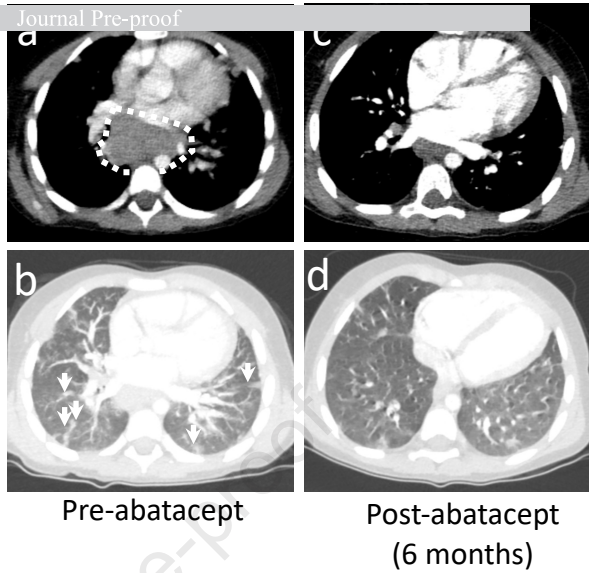

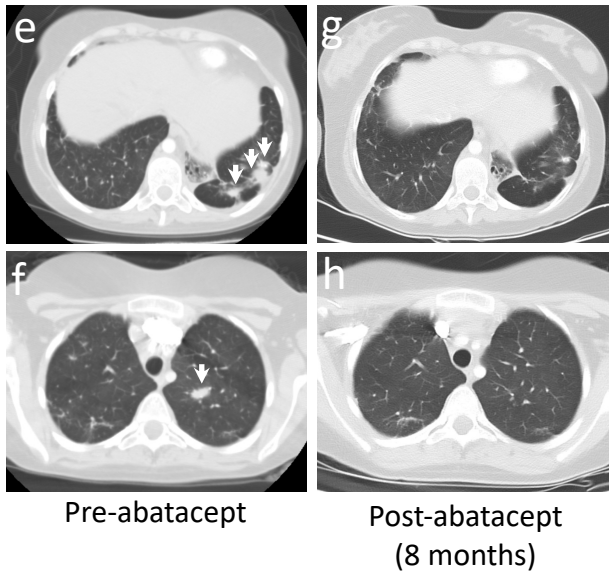

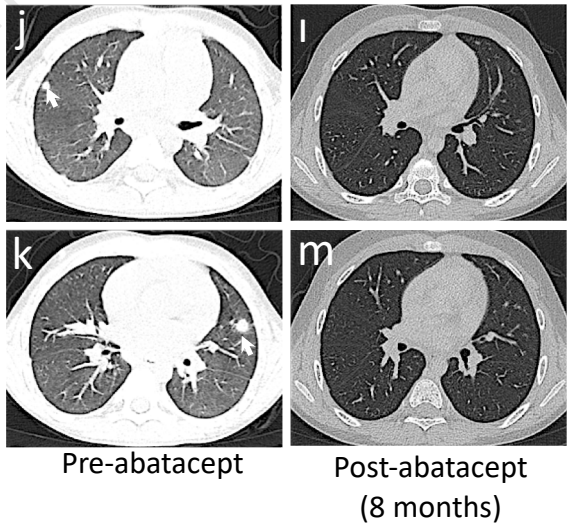

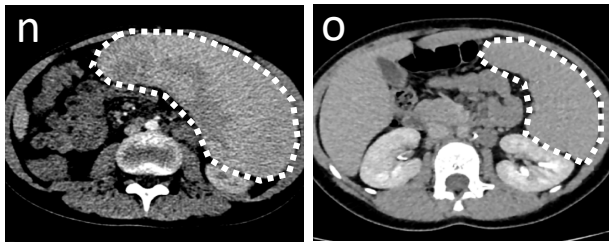

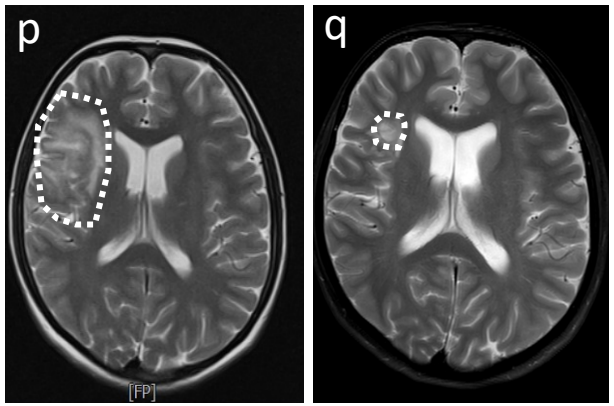

Figure 6

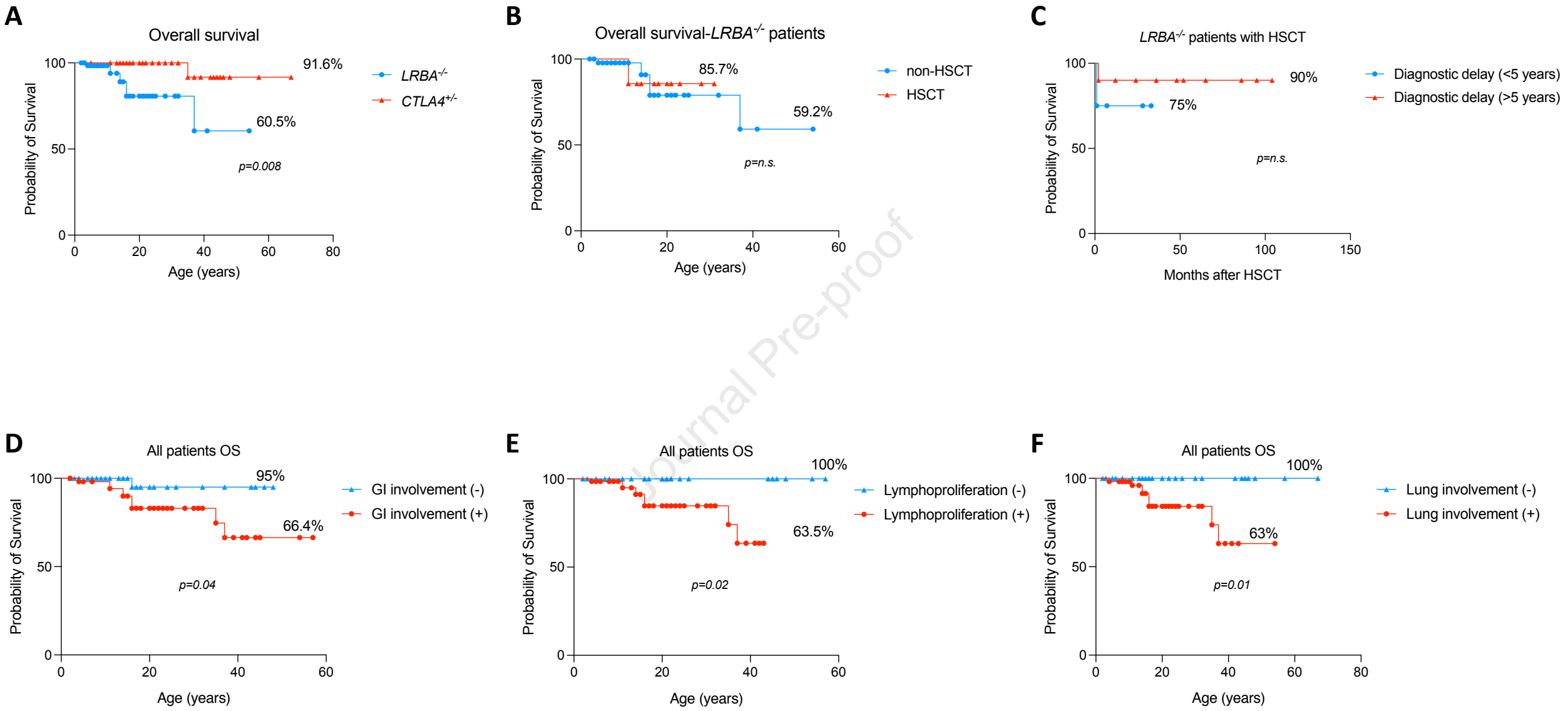

Figure 7