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Ask the Expert

# Rabbit Anti–T Cell Globulin in Allogeneic Hematopoietic Cell Transplantation

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Anti–T cell globulin (ATG) is polyclonal IgG from rabbits immunized with human thymocytes or a human T cell line. Prophylaxis using ATG infused with conditioning for adult marrow or blood stem cell transplantation reduces both acute and chronic graft-versus-host disease (GVHD). However, ATG is not or minimally efficacious in steroid refractory GVHD treatment. Regarding preemptive therapy, ATG is promising; however, further work is needed on establishing adequate biomarkers to be used as triggers for preemptive therapy before it can be used routinely. Relapse is not increased by ATG, except possibly in the setting of reduced-intensity conditioning. Infections are probably increased when using high but not low-dose ATG, except for Epstein-Barr virus–driven post-transplantation lymphoproliferative disorder, which may be increased even with low-dose ATG. Survival is not improved with ATG; however, survival free of immunosuppressive therapy is improved. Pharmacokinetics of ATG are highly variable, resulting in highly variable areas under the time-concentration curves. Optimized dosing of ATG might improve transplantation outcomes. In conclusion, ATG reduces GVHD and, thus, may improve quality of life, without compromising survival.



## INTRODUCTION

Clinically significant graft-versus-host disease (GVHD), ie, grade 2 to 4 acute GVHD (aGVHD) or extensive or moderate/severe chronic GVHD (cGVHD), occurs in 40% to 90% of recipients of T cell–replete allogeneic hematopoietic cell transplantation (HCT) (for cGVHD, the up to 90% pertains to patients surviving 1 year). It leads to morbidity, mortality, and poor quality of life. Unfortunately, prophylaxis of GVHD with small molecule immunosuppressive drugs or with pure ex vivo T cell depletion (without in vivo T cell depletion) has been associated with increased relapse and infections [1-5]. Anti–T cell globulin (ATG) is promising as GVHD prophylaxis that may not result in increased relapse or fatal infections in adults undergoing bone marrow transplantation (BMT) or peripheral blood stem cell transplantation (PBSCT). This is less clear in the setting of pediatric BMT or PBSCT and adult and pediatric cord blood transplantation (CBT). Thus, here we review the use of ATG first in the setting of adult BMT/PBSCT and then in the setting of pediatric BMT/PBSCT and CBT. We also review the impact of ATG and ATG pharmacokinetics (PK) on immune reconstitution and its possible association with susceptibility to infections and relapse.

The name *anti–T cell globulin* is imprecise because ATG contains antibodies expressed not only on T cells but also other cells, and it does not contain total serum globulin but only IgG. A precise name would be ***A**nti–**T** cell and other cell Ig**G***.

## ATG FORMULATIONS

As shown in Table 1, ATG is manufactured by immunizing animals with human thymocytes (ATGAM [Pfizer, New York, NY] and Thymoglobulin [Sanofi, Paris, France]) or Jurkat T lymphoblastoid cells (ATG-F [Neovii Biotech, Waltham, MA]) and subsequently extracting IgG from the sera of the immunized animals. Rabbits are used for the production of Thymoglobulin and ATG-F, whereas horses are used for the production of ATGAM. The rabbit products cause more profound and longer lymphocytopenia than the horse product, despite the horse product being given at a higher dose [6]. Interestingly, horse ATG appears to be more efficacious than rabbit ATG when treating aplastic anemia [6], though not all studies confirm this [7]. For prophylaxis of GVHD, rabbit ATG is efficacious whereas horse ATG is not efficacious [8-10]. In

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**Table 1**
ATG Formulations

| | ATGAM (Pfizer) | Thymoglobulin* (Sanofi) | ATG-F* (Fresenius/Neovii) |
|---|---|---|---|
| Animal immunized | Horse | Rabbit | Rabbit |
| Human cells for the immunization of the animal | Thymocytes | Thymocytes | Jurkat cells (T lymphoblastoid cell line) |
| Lymphodepletion in vivo | ± | + | + |

* The immunized rabbits are pathogen free, the thymocytes (obtained from pediatric donors undergoing cardiac surgery in case of Thymoglobulin) are screened for known viruses, and the IgG from the immunized rabbits is pasteurized, ensuring safety. The rabbit IgG is exposed to human erythrocytes that adsorb antibodies against antigens on their surface. In case of ATG-F, the rabbit IgG is also adsorbed on human placental cells.

the randomized study of horse ATG prophylaxis in patients with aplastic anemia, the incidence of cGVHD was even higher in the horse ATG arm compared with the no ATG arm, though this was not statistically significant [8]. Thus, only rabbit ATG is reviewed further.

Thymoglobulin contains antibodies against multiple antigens, including CD1a, CD2, CD3/T cell receptor, CD4, CD5, CD6, CD7, CD8, CD11a/CD18 (LFA1), CD11b, CD16, CD19, CD20, CD25, CD28, CD29, CD30, CD32, CD38, CD40, CD44, CD45, CD49, CD50 (ICAM3), CD54 (ICAM1), CD56, CD58, CD61, CD81, CD82, CD95, CD98, CD99, CD102 (ICAM2), CD126, CD138, CD147, CD152 (CTLA4), CD184 (CXCR4),CD195 (CCR5), CD197 (CCR7), HLA class I, beta-2-microglobulin, and HLA class II [11,12]. Antigens targeted by ATG-F have not been studied as extensively as for Thymoglobulin; however, it is likely that the number of antigens targeted by ATG-F may be lower than that of Thymoglobulin (eg, CD4 and HLA-DR antibodies are lacking in ATG-F [12]). This may be because (1) Jurkat cells are relatively homogeneous whereas thymocytes are heterogenic (include T cell precursors, T cells, dendritic cells, B cells, plasma cells, macrophages, and stromal/epithelial cells), and (2) because during the production of ATG-F (but not Thymoblobulin), the rabbit IgG is adsorbed on human placental cells. Compared with Thymoglobulin, a higher concentration of ATG-F is needed to achieve the same degree of complement mediated lysis [13-15]. Perhaps this is the reason why a higher dose of ATG-F appears to be needed to achieve a similar degree of GVHD reduction. ATG-F for GVHD prophylaxis has been administered in recent studies at a dose of 15 to 60 mg/kg, whereas Thymoglobulin is administered at 2.5 to 10 mg/kg. The European Blood and Marrow Transplant Group recommends, based on consensus opinion, 30 mg/kg ATG-F or 7.5 mg/kg Thymoglobulin, divided into 3 doses administered on days -3, -2, and -1 (for 8/8 HLA allele-matched unrelated donor transplantation) [16]. Further work is needed to establish the optimal dosing. See the Pharmacokinetics section (below) for our opinion on the dosing.

## PROPHYLAXIS VERSUS THERAPY OF GVHD WITH ATG

Prophylactic ATG is typically administered during conditioning. Because of its relatively long half-life (3 days to 6 weeks), ATG can persist in the HCT recipient for weeks to months, suppressing or killing T cells infused with the graft. This is thought to be the primary mechanism of reduced incidence of GVHD as reviewed below.

In contrast to the efficacy of ATG for GVHD prophylaxis, treatment of established GVHD with ATG has produced disappointing results [17,18]. However, this has been studied only for steroid refractory GVHD.

In the upcoming paragraphs, we will first review ATG use for GVHD prophylaxis (ATG in conditioning) and later ATG use for preemptive therapy (post-transplantation administration of ATG to patients at high risk of developing GVHD per early post-transplantation biomarkers).

### *GVHD Reduction by ATG in Conditioning for Adult BMT/PBSCT*

The impact of ATG on GVHD has been studied in 5 randomized studies, multiple nonrandomized studies, and several studies comparing the GVHD incidence between patients with high versus low ATG serum levels (who were treated with a uniform dose of ATG) (Table 2). In all of the randomized studies and most of the nonrandomized studies, aGVHD and/or cGVHD incidence was reduced. Overall, the impact of ATG appears to be greater on cGVHD than aGVHD (Table 2). This is expected to lead to improved quality of life. This has been so far documented in 2 randomized studies and 1 nonrandomized study [19-21]. The anti-GVHD effect of ATG may be less pronounced in the setting of BMT compared with PBSCT [22].

The mechanism (how ATG reduces GVHD) is probably multifactorial, as ATG is polyclonal. ATG includes IgG specificities against antigens expressed on T cells, B cells, natural killer cells, granulocytes, monocyte/macrophages, dendritic cells, endothelial cells and nonhematolymphatic cells, all of which have been implicated in the pathogenesis of GVHD. Leading hypotheses are that ATG kills alloreactive T cells by inducing their apoptosis or complement lysis, interferes with alloreactive T cell traffic (eg, exit from blood to epithelial tissues) or function (eg, activation due to disruption of T cell antigen-presenting cell synapse, proliferation, cytokine production, cytotoxicity), or stimulates development of regulatory T cells [23-26]. Interestingly, a low ATG concentration may stimulate, whereas a high ATG concentration may inhibit, a mixed lymphocyte reaction [27]. Another hypothesis for the anti-GVHD effect is that ATG kills dendritic cells (that present alloantigens) via apoptosis or complement lysis [15,28,29], interferes with their maturation, or stimulates development of tolerogenic dendritic cells [30]. Among all immune cells, ATG has the highest affinity for naïve T cells [31], which are enriched for alloreactive T cells [32]. As ATG administration results in severe naïve T lymphocytopenia (Figure 1) [33-35], we hypothesize that many naïve T cells infused with the graft, including alloreactive T cells, are killed by ATG.

### *Relapse and ATG in Conditioning for Adult BMT/PBSCT*

The impact of ATG on relapse appears to depend on the intensity of conditioning. In 19 of 19 studies on myeloablative conditioning transplantations or combined myeloablative and reduced-intensity conditioning (RIC) transplantations, including the 5 randomized studies, ATG prophylaxis was not associated with increased relapse. In contrast, in 4 of 6 studies on exclusively RIC transplantations, ATG prophylaxis was associated with increased relapse (Table 2). Use of ATG with very low intensity conditioning (eg, 2 Gy total body irradiation only) has not been reported.

The reason why ATG does not increase relapse (after myeloablative HCT) is not known. At least 2 hypotheses exist: (1) ATG selectively interferes with GVHD but not graft-versus-leukemia, and (2) ATG has a direct antileukemic effect,