Introduction
Allogeneic hematopoietic cell transplantation (alloHCT) is a widely accepted treatment option for patients with acquired severe aplastic anemia (SAA), having a suitable donor [1]. Although significant improvement has been made within the last three decades resulting in the increase of the overall survival rates to 56-88%, there is still a proportion of patients who experience primary or secondary graft failure, or who die from other transplant-related complications [2-8]. As previously reported, the risk of graft rejection, which remains the major obstacle for successful transplantation, is associated with long disease duration, preceding transfusions, and low CD34+ cell dose [5, 9]. As the mechanism of graft rejection is based on host-vs.-graft alloreactivity directed against minor histocompatibility antigens of donor origin, this complication is more likely after transplantation from an unrelated donor [1].

For patients with high risk of graft failure several attempts have been made to increase the efficacy of preparative regimen. The use of total body irradiation or limited-field irradiation in combination with cyclophosphamide (CY) +/- antithymocyte globulin (ATG) reduced the risk of graft rejection but resulted in high early and late toxicity [2, 7, 9-11]. More recently, triple agent combinations were introduced including fludarabine or procarbazine in addition to CY and ATG, with promising early results [3, 12-14].

In our center we introduced a novel conditioning regimen incorporating treosulfan, an alkylating agent possessing both myelotoxic and immunosuppressive properties, which, as demonstrated in previous studies on patients with malignant diseases, is associated with low organ toxicity [15-18]. The rationale to introduce the myelotoxic drug was provided by the fact that clonal transformation of SAA to myeloid malignancies, in particular to myelodysplastic syndrome and acute myeloid leukemia is not uncommon [19, 20]. On the other hand, considering the disease specificity, we decided to reduce the total dose of treosulfan from the recommended 30-42 g/m², which is considered myeloablative, to 20 g/m² [16, 17].

In this report we analyze the outcome of the first six SAA patients treated with treosulfan + cyclophosphamide + antithymocyte globulin conditioning in acquired SAA.

Patients and Methods
Patients and donors
From October 2003 to December 2004, six patients with median age of 21 years (range, 14-25) were treated with alloHCT at the Dept. of Hematology and BMT, Silesian Medical University, Katowice, Poland. Median disease duration prior to alloHCT equaled 6 months (range, 3-12). Patients had received a median...
of 22.5 (range, 9-51) red blood cell or platelet transfusions. In one case the transplantation was preceded by immunoablative therapy including the use of ATG, whereas all the remaining patients had been treated with prednisone +/- cyclosporin A. Three patients received alloHCT from an HLA-identical sibling, another three – from an unrelated volunteer selected based on high-resolution DNA typing for both HLA class I and II alleles. In one case the donor was incompatible regarding a single HLA-C locus. Patient characteristics as well as procedure details are listed in Table 1.

Preparative regimen
All patients or their parents signed an informed consent and received conditioning regimen composed of treosulfan 10 g/m²/d iv. on days -7, -6, CY 40 mg/kg/d iv. on days -5, -4, -3, -2, and ATG (thymoglobulin, Genzyme) 2 mg/kg/d on d. -3, -2, -1. Both treosulfan and CY were administered as 2-h infusions and accompanied by adequate hydration. Each, unmanipulated bone marrow and G-CSF stimulated peripheral blood was used as a source of stem cells in three cases and infused on day 0.

Graft-versus-host disease prophylaxis
Graft-versus-host disease (GVHD) prophylaxis consisted of cyclosporin A (CsA) and methotrexate (15 mg/m² on day +1, 10 mg/m² on days +3, +6, +11). CsA was administered at an initial dose of 3 mg/kg iv. from day -1; the dose was further adjusted to the CsA concentration in blood to be maintained between 200-400 ng/ml. From day +21 the route of administration was converted to oral. The CsA administration was continued until day +180 and tapered thereafter.

Supportive care
Patients were hospitalized in HEPA-filtered rooms with positive-pressure air-flow since start of conditioning until engraftment. Infection prophylaxis consisted of acyclovir 5 mg/kg iv. every 8 hours. Daily trimethoprim-sulfametoxazole was discontinued 3 days before alloHCT and restarted after neutrophil recovery. Broad-spectrum antibiotics were introduced in case of neutropenic fever. Cytomegalovirus (CMV) serological status was studied before transplantation in all donor/recipient pairs. After alloHCT, patients were monitored CMV-DNA in peripheral blood leukocytes weekly until day +100 with the use of semi-quantitative PCR method. Patients experiencing reactivation of CMV infection (≥0.8 copies/μL) were treated according to a strategy of pre-emptive therapy with ganciclovir. Hematopoietic growth factors were not used to support neutrophil recovery. All blood products were filtered, irradiated, and tested for IgM anti-cytomegalovirus (CMV) antibodies to be negative. Red blood cell and platelet transfusions were given to maintain hemoglobin of >80 g/L and platelet count >20×10⁹/L.

Assessment of engraftment and response
Myeloid engraftment was defined as the first of 3 consecutive days with neutrophil count >0.5×10⁹/L and platelet engraftment was defined as the first day with platelet count >50×10⁹/L without platelet transfusions. Bone marrow aspirates were examined for morphology and chimerism at 1, 3, 6, and 12 months after alloHCT. Hematopoietic chimerism was evaluated by the analysis of a variable number of tandem repeats.

Results
Engraftment and early complications
All patients engrafted with the median time to neutrophil >0.5×10⁹/L and platelet >50×10⁹/L recovery of 16 days (range, 14-22) and 21.5 days (range, 12-29), respectively. Complete bone marrow donor chimerism was achieved in all patients before day +100. No patient experienced severe complications in the early post-transplant period except of neutropenic fever, which occurred in three cases. Patients required the median of 5.5 (range, 0-6) red blood cell transfusions and 6.5 (range, 3-11) platelet transfusions. One patient experienced reactivation of CMV infection, which was successfully treated with gancyclovir. Oral mucositis was not observed in the study group.

GVHD
None of the patients experienced grade III-IV acute GVHD. In one case grade II acute GVHD occurred and partial response was achieved after the treatment with methylprednisolone at a dose of 2 mg/kg daily. At one year, the cumulative incidence of the overall chronic GVHD was 50%, whereas the incidence of extensive form of this complication equaled 33%.

Survival and performance status
With the median follow-up of 14.5 months (range, 13-27) all patients are alive and disease-free. No severe organ toxicity was observed within the first year after transplantation. In two female patients with child-bearing potential menstruation recurred. Four patients have Karnofsky index of 100%, two patients with extensive chronic GVHD have the performance status of 80%.

Outcome details are listed in Table 1.

Discussion
Graft rejection, GVHD, organ toxicity, and infectious complications are major causes of mortality following alloHCT in SAA [1]. As assessed in registry analyses including large number of subjects the risk of graft rejection equals 5% to 30% and is affected by preceding transfusions [9, 10]. For patients with high risk of this complication various preparative regimens have been developed, however, the optimal one has not been defined so far. In particular, the combination of CY+ATG, which is associated with good outcome after alloHCT from HLA-identical siblings seems insufficient in case of transplantations from unrelated donors [8, 21]. On the other hand, total body irradiation or thoracoabdominal irradiation – containing regimens enable engraftment but result in increased toxicity, including both GVHD and organ complications, which in turn may result in decreased survival and impaired performance status [2, 7, 9-11].
In recent years attempts have been made to improve outcome by combining several immunosuppressive +/- myelotoxic agents with intention to reduce the risk of graft failure while maintaining good tolerance of the regimen. Kim et al. reported outcome of 113 SAA patients treated with CY+ATG (thymoglobulin, 3.75 mg/kg) + procarbazine prior to alloHCT from either HLA-identical sibling or an alternative donor [5]. The probability of graft failure equaled 15% while the overall survival rate at six years was 89.5%. Fludarabine was combined with CY and various preparations of ATG in several studies on alloHCT from alternative donors [3, 9-11]. In the largest cohort (n=38) reported by Bacigalupo et al., with thymoglobulin used at the dose of 7.5 mg/kg, 15% of patients had evidence of graft failure and the actuarial 2-year survival was 73% [3]. Infections, including EBV-related lymphoproliferative disease (EBV-LPD) were the most frequent cause of mortality. Finally, in a study by Dulley et al., 81 patients received conditioning consisting of

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<td>24</td>
<td>15</td>
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ATG, antithymocyte globulin; CyA, cyclosporin A, PDN, prednisone; MP, methylprednisolone; PB, peripheral blood; BM, bone marrow; NC, nucleated cells; WBC, white blood cells, ANC, neutrophils; PLT, platelets; RBC, red blood cells; GVHD, graft-versus-host disease * CMV status defined by serology
busulfan (4 mg/kg) and CY (without ATG) before sibling-alloHCT, with 22% rejection rate and the actuarial survival of 56% at 8 years [4].

In the current study we used treosulfan in combination with CY and ATG with intention reduce the risk of graft rejection. Treosulfan (L-treitol-1,4-bis-methanefulfonate; dihydroxybusulfan) is a prodrug of a bifunctional alkylating cytotoxic agent, which showed a high stem cell toxicity [22]. It has been demonstrated to induce apoptosis in acute myeloid leukemia cells [23]. Addition of treosulfan to a nonmyeloablative conditioning regimen resulted in enhanced chimerism and immunologic tolerance in an experimental alloHCT model [24]. In clinical trials treosulfan was used in patients with hematopoietic malignancies as a part of conditioning regimen at a dose of 3×10 g/m² to 3×14 g/m², in combination with fludarabine or CY [15-18]. In both cases the regimen was characterized by good tolerance and low non-relapse mortality, which could be explained by a predictable pharmacokinetics of treosulfan after its intravenous administration [25].

The above data provided rationale to use reduced-dose treosulfan in a setting of aquired SAA in combination with CY and ATG in order to reduce the risk of graft rejection. In addition, we considered that the dual, immunosuppressive and myelotoxic activity of treosulfan could result in improved outcome, taking into account the possibility of clonal transformation of SAA into myeloid malignancies [19, 20]. Indeed, in some cases, at the time of alloHCT, patients with initial diagnosis of SAA may have already features of MDS, which, however, at that stage cannot be easily diagnosed [26].

All six patients treated with treosulfan + CY + ATG engrafted and remained disease-free with the minimum follow-up of 13 months, despite various risk factors including multiple transfusions and preceding immunosuppression. The tolerance was good with no organ toxicity observed in the early post-transplant period. Neither severe infections nor EBV-LPD were noted despite the use ATG. The dose of thymoglobulin (6 mg) was lower than that previously tested in randomized trials, however, it seemed sufficient to prevent against severe acute GVHD [27]. Chronic GVHD occurred in half of the patients and contributed to deteriorated performance status in two patients, however, in all cases potential risk factors for chronic GVHD were present including peripheral blood as a source of stem cells, HLA incompatibility, unfavorable donor/recipient gender and CMV status combination. Although, because of short follow-up, we were not able to assess late effects of the treosulfan + CY + ATG conditioning, the fact that menstruation reoccurred in both female patients of child-bearing potential suggests that the toxicity, at least with regard to the endocrine system was limited.

Taken together, the addition of treosulfan to CY and ATG does not seem to increase toxicity of the regimen while it may potentate its efficacy by reducing the risk of graft rejection. Our observation provides rationale to test this protocol in a prospective study including larger cohort of patients.

References


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