

HLA-mismatched hematopoietic stem cell transplantation for pediatric solid tumors

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Abstract

Even if the overall survival of children with cancer is significantly improved over these decades, the cure rate of high-risk pediatric solid tumors such as neuroblastoma, Ewing's sarcoma family tumors or rhabdomyosarcoma remain challenging. Autologous hematopoietic stem cell transplantation (HSCT) allows chemotherapy dose intensification beyond marrow tolerance and has become a fundamental tool in the multimodal therapeutical approach of these patients. Anyway this procedure does not allow to these children an event-free survival approaching more than 50% at 5 years. New concepts of allogeneic HSCT and in particular HLA-mismatched HSCT for high risk solid tumors do not rely on escalation of chemotherapy intensity and tumor load reduction but rather on a graft-versus-tumor effect. We here report an experimental study design of HLA-mismatched HSCT for the treatment of pediatric solid tumors and the inherent preliminary results.

Since the 1950s, the overall survival of children with cancer has gone from almost zero to approaching 80%.¹⁻⁵ Although there have been notable successes in treating solid tumors such as Wilms' tumor, some childhood solid tumors have continued to elude effective therapy. In particular, the real challenge remains the cure rate of the high risk pediatric solid tumors such as the listed below: i) neuroblastoma (NB): stage IV disease or other stage with n-myc amplification, resistant or relapsed disease after conventional therapy; ii) Ewing's family tumors (ESFT): stage IV disease or localized tumors with < 80% of necrosis after neo-adjuvant therapy, resistant or relapsed disease after conventional therapy; iii) rhabdomyosarcoma (RMS): metastatic disease or alveolar histology, resistant or relapsed disease after conventional therapy.

With the use of high dose chemotherapy followed by peripheral hematopoietic stem cell (HSC) rescue, dose escalation has been pushed to the edge of dose-limiting toxicities. The examples of NB⁷ and ESFT^{5,8} in this sense,

are paradigmatic. Despite the development of new treatment options, the prognosis of high-risk neuroblastoma patients remains dismal; in more than half of patients the disease returns. High-dose chemotherapy and haematopoietic stem cell rescue, might improve the survival of these patients but the actual 5-years EFS is not yet over 30%. High risk ESFT and RMS as well, have not achieved over these two decades a significant better EFS after a wide use of autologous HSCT, being the 5-year EFS respectively of 51%⁷ and 20%.³ Nowadays, is clear that any further improvements in EFS of these high risk tumors have to be achieved through novel therapeutical approaches. Interest in new therapeutical tools has been renewed at the beginning of this century with the first successful reports of reduced intensity conditioning (RIC) transplants in solid tumors, and with some promising experimental studies that have proved a measurable antitumor immune response against neuroblastoma.^{9,10} In the allogeneic setting, the biological targets of the graft-versus-tumor (GvT) effect are unknown and it has been believed that the GvT effect is questionable in neuroblastoma. Although primary neuroblastoma cells may lack high-level expression of MHC class 1 and class 2 antigens, they should still be good target cells for a cellular immune response given that there is up-regulation of both classes of MHC molecules after conventional therapy and after exposure to proinflammatory cytokines. Minor histocompatibility antigen (mHA) may also be a possible target.¹¹ In 2006, the Accreditation Subcommittee of the EBMT classified HSCT procedures according to prevailing clinical practice in Europe and categorized allo-HSCT for high-risk neuroblastoma as a *clinical option* (with sibling donor) or *developmental* (with well-matched unrelated donor).¹² Stating this background, the AIEOP Study Group for NB together with the HSCT Study Group, started in 2007 the experimental protocol called *Reduced Intensity Conditioning Regimen and Allogeneic Stem Cell Transplantation from Related or Unrelated HLA Identical Donor in High Risk Neuroblastoma*.¹³ Based on this experience, in the following year has been open to the enrollment another protocol named *Reduced Intensity Conditioning Regimen and Allogeneic Stem Cell Transplantation from Related Or Unrelated HLA Identical Donor in Soft Tissue Sarcoma and Ewing Sarcoma*.¹⁴ Both of the studies are still open and enrolling patients. The results of these two novel approaches need a longer follow-up to be completely evaluated but the preliminary observations can be resumed as following: immunotherapeutical approach is feasible, not toxic with an acceptable transplant-related mortality (TRM) and compared with HLA mismatched donors allogeneic HSCT from HLA-mismatched sibling donors has shown no significant evidence of improving outcome following

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allografting. With the goal to obtain a rapidly available donor in any moment of the disease natural history, has been elaborated an experimental design for RIC HSCT in pediatric solid tumors using also full haplotype mismatched donors. The principles of this setting remain the same of the experience with haematological malignancies:¹⁵ the megadose of CD34+ purified stem cell (SC) (>10x10⁶ cells/kg) overcomes the barrier of residual anti-donor CTL-p cytotoxic T cell preventing rejection and allows rapid engraftment with a consequent low TRM and the extensive T-cell depletion (<5x10⁴ CD3+ T-cell) dramatically reduces the incidence of GvHD so that no immunosuppressive therapy is to be administered after HSCT. To investigate the potential role of NK-alloreactivity cell against solid tumors is one of the most intriguing biological objectives of this experimental design. The source of SC is an immunomagnetic-based purification of CD 34+ cell obtained by mobilized peripheral blood mononucleated cells. The donor selection is based on the KIR-KIR-ligand mismatch alloreactivity of NK cells. The T-cell quote contained in the *inoculum* of purified CD34+ has not to overcome the threshold of 5x10⁴/kg of weight of the recipient. The RIC is a fludarabine-based regimen with the use anti-thymocyte immunoglobulin (ATG): melphalan 2x70 mg/sqm, fludarabine 4x40 mg/sqm, thiohepa 2x5 mg/kg, ATG 4x2.5 mg/kg. Since 2009, 4 patients has been enrolled in the study (2NB, 1 ESFT and 1 Wilms' tumor). The state of the disease before HSCT was partial response (<50%) for all the patients. The donors have been mothers in 3/4 cases and father in one case. The median of CD 34+ cells/kg infused has been 19 (15-24) x10⁶. The median of T-cells into the *inoculum* has been 4.51 (1.44-7.1) x10⁴. The median Follow-Up time has been 4 months.³⁻⁷ The median time of take has been 10 days for polymorphonucleated cells⁸⁻¹² and 10 days for platelets respectively.⁹

¹² Complete donor chimerism has been obtained in all of the patients at day +30. The median of absolute number of circulating NK-cells has been as following: at day +15, 0.5 cells/mm³, at day + 30, 8.5 cells/mm³, at day +60, 40.6 cells/mm³, at day +90, 75.1 cells/mm³. No add-back of stimulated freshly NK cells has been infused after the HSCT. No re-activation of CMV or EBV has been reported. About the outcome: 2 patients are alive in complete remission, 1 patient has died for progression of the disease (stage IV n-Myc amplified refractory NB in PR before HSCT) and 1 pts died of lung IFI >100 days after HSCT. From these preliminary results we can resume that: the Fluda-based RIC preparative regimen was associated with a low grade of organ and hematological toxicity (no grade III-IV OMS toxicity was evidenced) in such heavily treated population, extensively T-cell *ex vivo* depletion (average 4.51x10⁶ CD3+ cells infused) allowed no GVHD in our cases, in an half of the patients the HSCT procedure allowed to obtain a complete remission of the disease. To validate these results a longer follow-up time and a larger cohort of patients is certainly needed. In conclusion, early studies of HLA mismatched-HSCT in children with high-risk solid tumors suggest that this is a feasible approach that may improve the outcome in this deadly disease. Although there are a limited number of cases, knowledge of these data provides a basis for decision-making in future studies design. Not only in the subset of patients for whom dose escalation strategies appear to be failing but also for newly diagnosed patients, there is a need to develop newer strategies based on innovative, immunobiological concepts.

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