Suicide gene therapy (SGT) applied to allogeneic hematopoietic stem cell transplantation (allo-HSCT) has been one of the first clinical applications of gene therapy. In allo-HSCT the antileukemic potential mediated by alloreactive lymphocytes towards patient-specific antigens, such as minor and major histocompatibility antigens, is counterbalanced by the Graft-versus-Host-Disease (GvHD). The risk of GvHD increases with the level of HLA disparity between host and donor, and leads to impaired quality of life and reduced survival, particularly in patients (pts) transplanted from HLA-mismatched donors. In this contest SGT has been applied to modulate alloreactivity, by inserting the HSV-tk gene in donor lymphocytes. This suicide gene/prodrug system requires cell cycle for optimal killing. In the context of allo-HSCT, this characteristic ensures a further level of specificity in GvHD control, by allowing to selectively kill highly proliferating alloreactive cells during GvHD, while sparing resting T cells. We assessed long-term safety in TK cells treated pts.

**Results**

Overall 128 pts have been treated worldwide in 10 phase I-II clinical trials with donor lymphocytes expressing the HSV-tk suicide gene, with the purpose of enforcing the graft-versus-tumor (GvT) effect and/or promoting a functional post-transplant immune reconstitution (IR) while allowing control of GvHD. This approach proved highly feasible, safe and effective in promoting a dynamic and patient-specific modulation of alloreactivity. TK cells engrafted in the majority of pts and a clinical benefit, measured as improvement of hematopoietic chimerism, malignant regression and/or IR, was reported for 65 pts (51%). GvHD grade II-IV was observed in 28 pts (22%) and was always rapidly and completely controlled by the activation of suicide machinery.

A selected population of 57 pts, treated at San Raffaele Institution, was studied in more detail for long-term analysis:
- 23 pts received cells to treat relapse occurring after an HLA-identical allo-HSCT
- Ciceri F et al, Blood. 2007; 109: 4968-470
- 34 pts received cells to improve IR after haploidential HSCT
- Bonini C et al, Blood. 2002; 100: 115a;

No adverse event correlated to the gene transfer procedure was ever reported.

Genetically modified cells engrafted in 90% of treated pts and could be detected in vivo, at low frequencies for up to 14 years (y).

In the HLA-identical setting 11 pts obtained clinical response of the malignant disease and 3/11 are alive with a median follow-up of 15y. Two pts are in complete remission (CR), while one pt affected by chronic myelomonocytic leukemia relapsed 15y post transplant. This pt was subsequently treated with a second transplant from an unrelated match donor and is now in CR 1 year after transplant.

In the haploidential setting, 25 pts/34 reached the target of IR and 9 are alive and in CR with a median follow-up of 7y. Four out of 9 experienced GvHD in the early phase post IR, none presented signs, symptoms, complications related to GvHD and none needed pharmacological treatment in long-term.

According to international guideline for long-term follow-up (Mahjail NS et al, BBMT. 2012; 18: 348-371) all pts underwent regular screening and clinical evaluation.

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**Conclusion**

Long-term assessment confirmed the overall high benefit/risk ratio of the TK-cell approach in allo-HSCT.

A phase III multicentric, randomized clinical trial sponsored by MolMed, is currently undergoing in the context of haploidential HSCT.