1. PUBLISHABLE SUMMARY

Wiskott-Aldrich Syndrome (WAS) is a X-linked rare primary immunodeficiency (MIM no.301000) diagnosed early in the life and many patients with severe WAS do not survive past of 10 years without definitive treatment. WAS is caused by mutations in *WAS* gene that codifies for a cytosolic protein of 502 amino acids. WAS protein (WASp) is expressed exclusively in hematopoietic cells and is present in all types of leukocytes where it has roles in signalling and as a key regulator of actin cytoskeleton reorganization.

The only cure is the allogeneic hematopoietic stem cell transplantation but **compatible HLA-matched-donors are not always available and still have some undesired side effects.** *Ex-vivo* gene transfer of autologous CD34+ cells in patients has emerged as a new therapeutic approach. The **lentiviral vectors (LVs)** are a powerful system of integration and the last generation of self-inactivated-LVs express the transgene under internal promoters instead of the 5´-LTR (*long terminal repeat)*. This characteristic allows the use of physiologic or tissue-specific promoters to express the transgene in corrected cells. The results of the latest clinical trials for GT of WAS are really promising, showing phenotypic correction of most defects. **However, there still room for improvement since the absolute platelet counts have remained low (<50x10^9/l) in the majority of the patients and *was* gene expression was lower in myeloid compared with lymphoid lineages.** Dr. Francisco Martin (GENyO, Granada, Spain) developed an improved second generation LV (AWW) expressing the *WAS* cDNA through a combination of a 500bp fragment of WAS proximal promoter plus 387bp fragment of WAS alternative promoter.

WASHSCGENETHERAPY project (**329284**) consisted on **preclinical studies of this AWW LV for GT of WAS.** We were very interested in its comparison with the actual LV used in clinical trials and also in its improvement. For that, under the supervision of Prof. Adrian Thrasher (UCL, London, UK), several experiments were conducted:

1- Phenotypic rescue of HSCs from WAS patient using the AWW LVs. Human CD34+ cells from bone marrow of a WAS patient were transduced with AWW LV (8.4 copies/cell) and transplanted into sublethally irradiated NSG mice (figure 1). After 2 months, the AWW-transplanted mice showed 35-90% of human hematopoietic cells expressing hWASp (figure 2). We have therefore demonstrated that **AWW can correct human HSCs and express WAS protein in its progeny after transplant into NSG mice.**

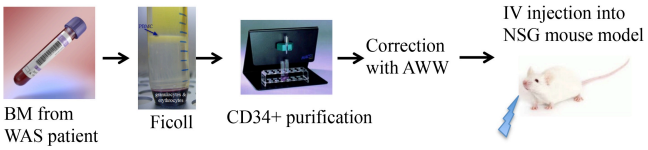

Figure 1: Scheme of the steps of purification, transduction and transplant of hCD34+ cells from a WAS patient into NSG mice.

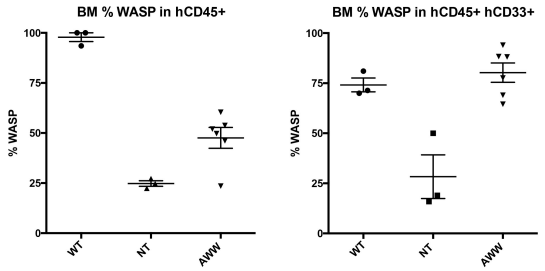

Figure 2: Percentage of human WASp positive cells into human hematopoietic cells (CD45+) and myeloid cells (CD33+) from bone marrow of the NSG transplanted.

2- Comparative analysis of AWW and WW0.5kb LVs in WAS knockout mice (WASKO: *in vivo* (long-term and short-term transplants) and *in vitro* (myeloid differentiation of corrected stem cells).

Our data indicated that AWW restored proliferation and IL2 secretion of mice WASKO-T cells upon CD3 stimulation (figure 3b and 3c). In addition, we observed a tendency of improved restoration levels of hWASp in AWW-granulocytes and myeloid cells (compared to WW0.5kb) obtained after *in vitro* differentiation of transduced lineage negative cells (figure 3d and 3e) and also in myeloid populations obtained from different organs of transplanted mice.

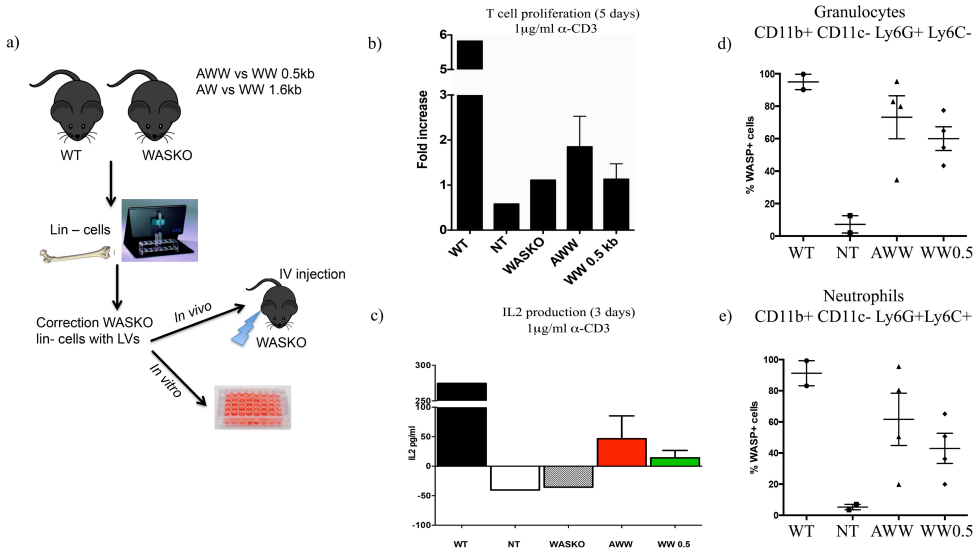

Figure 3: a) Scheme of reconstitution experiments in WASKO mice, b) T cells isolated from spleens were stimulated with anti-CD3 for proliferation analysis and c) IL2 production quantification, d) percentage of hWASp positive cells in granulocytes derived *in vitro* from transduced lin-cells and e) percentage of hWASp positive cells in neutrophils derived in vitro from transduced lin-cells.

3- Generation of an improved AW LV (3rd generation backbone) and its comparison with the vector used in the clinical trials (WW1.6kb). *In vivo* e *in vitro* experiments.

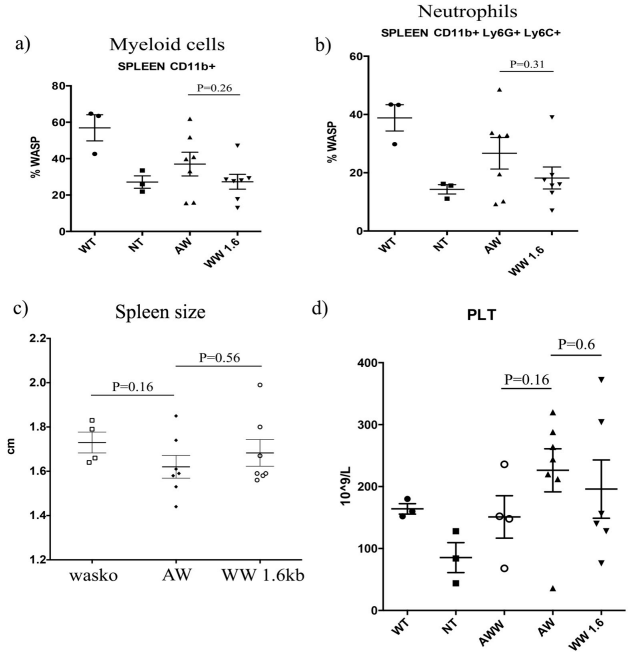

Figure 4: Percentage of hWASp+ cells in a) myeloid cells and b) neutrophils from spleens of transplanted WASKO mice, c) spleen sizes of the AW and WW1.6kb transplanted mice compared with WASKO mice, d) measurement of platelets in blood of transplanted mice after 3 months.

With these preclinical studies we can conclude that the 3rd generation LV incorporating the alternative promoter AW is able to transduce HSCs from WASKO mice improving spleen size and platelets number. When comparing AW with the clinical WW1.6 LV there were some improvements in percentage of hWASP-myeloid expressing cells although they did not reach statistically significance.