Graft-versus-host disease (GVHD) remains one major complication of allogeneic hematopoietic stem cell transplantation (HSCT). Following unmanipulated peripheral-blood stem cell transplantation, 60% of the patients experience chronic GVHD while approximately 15% of them develop a sclerodermic form of chronic GVHD characterized by multiple organ fibrosis and loss of skin elasticity. Regulatory T cells (Treg) play a pivotal role in the pathology of chronic GVHD by inhibiting alloreactive conventional T cells. Several studies have shown that the hypomethylating agent Azacitidine (Aza) can demethylate the master transcription factor of Treg (Forkhead box protein 3, FoxP3), thus promoting Treg differentiation of conventional T cells. This work investigates the impact of Aza in a classical murine model of sclerodermic chronic GVHD (B10.D2 → BALB/cJ).

**METHODS**

Lethally irradiated BALB/cJ recipient mice were injected intravenously with 10.10^6 bone marrow cells + 70.10^6 splenocytes from B10.D2 donor mice. Recipients were treated with subcutaneous injections of Aza at the dose of 0.5 or 2 mg/kg every two days from day 10 to 30 following transplantation. Mice GVHD severity was evaluated for five criteria (weight loss, activity, fibrosis, hair loss and mouse posture; 0-1.2 points/criteria). Mice were sacrificed at a score of 8/10 (or > 20% weight loss) according to the ethical committee of the University of Liège.

**RESULTS**

MTT assays have been performed on two different cell lines to assess the impact of Aza on metabolic activity of fibroblasts. Aza was added to each wells at different concentration. As shown by Figure A, metabolic activity of KS62 cells (used as control) decrease when Aza concentrations increase (100 mM, p<0.01, 1 µM, p<0.004; 10 µM, p<0.0068). For NIH-3T3 fibroblastic cell lines (Figure B), no effect of Aza on metabolic activity have been observed, except at higher concentration of Aza (10 µM, p<0.0003).

Collagen assays have been performed on NIH-3T3 fibroblastic cell lines. The amount of collagen was quantified by absorbance at 490 nm after staining of collagen fibers by Sirius Red. 500,000 cells/well were plated in triplicate and then put with various concentration of Aza and stimulated by TGF-β or PDGF. As shown by Figure A and B, the amount of collagen slightly decrease when Aza concentration increase even it is not statistically significant except at higher concentration of Aza (Figure A : 100 nM, p<0.005 ; 1 µM, p<0.0005 ; 10 µM, p<0.0001). Figure 8 : 10 µM, p<0.0003)

**CONCLUSION**

In conclusion, Azacitidine seems to be a promising treatment as it prevents cGVHD in the classical murine model of sclerodermic cGVHD. Results suggest that Aza acts mainly through immunomodulation by diminishing the number of alloreactive T cells and promoting phenotypic switch of Tconv into Tregs by demethylation of Tregs master transcription factor FoxP3.