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AZACYTIDINE PREVENTS EXPERIMENTAL SCLERODERMIC CHRONIC GRAFT-VERSUS-HOST DISEASE

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Votre Abstract: ***Introduction :*** Graft-versus-host disease (GVHD) has remained one of the major complication of allogeneic hematopoietic stem cell transplantation (HSCT) for the last decades. 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 (Tregs) play a pivotal protective role in the pathogenesis of chronic GVHD by inhibiting alloreactive conventional T cells (Tconvs). Several studies have shown that hypomethylating agents such as azacytidine (Aza) can demethylate the master transcription factor of Treg (Forkhead box protein 3 factor, FoxP3), thus promoting Treg differentiation from Tconvs. This work investigates the impact of Aza in a classical murine model of sclerodermic chronic graft-versus-host disease (B10.D2 - BALB/cJ).

Methods : *In vitro* analyses have been performed to determine the impact of Aza on collagen production. NIH-3T3 fibroblastic cells were plated and stimulated with 50 ng of PDGF or 10 ng of TGF-beta. Cells were then cultured with various concentrations of Azacytidine for 48 hours. After culture, cells were washed and then stained with Sirius Red before quantification of collagen amount by absorbance at 490 nm. Concerning *in vivo* experiments, lethally irradiated (7 Gy) BALB/cJ recipient mice were injected with 10.10^6 bone marrow cells + 70.10^6 splenocytes from B10.D2 donor mice to induce sclerodermic chronic graft-versus-host disease. Following tranplantation, recipient mice were injected with either 0,5 or 2 mg/kg of Aza every 48 hours from day 10 to 30 following transplantation. GVHD severity was scored using a five criteria scale (weight loss, activity, fibrosis, hair loss and mice posture; 0-1-2 points/criteria). Mice were sacrificed at a score of 8/10 (or > 20% weight loss) or at day 52 after transplantation (end of experiment).

Results : Concerning *in vitro* analyses, results suggest a decreased production of collagen at higher concentration of Aza with both stimulations by TGF-beta or PDGF (seen by a gradual diminution of absorbance). For *in vivo* experiments, mice treated with Aza 0.5 mg/kg (n = 14) or 2 mg/kg (n = 25) had significant lower clinical scores of GVHD compared to control ones (n = 23) after the end of the treatment. FACS analysis showed a higher proportion of Treg among CD4+ T cells in the blood of Aza 2 mg/kg mice than in control mice at day 35 following transplantation (P = 0.047), as well as a higher percentage of Tregs expressing the KI67 proliferative marker at the same time point (P = 0.0005). Finally, analyses of the cellular blood components with Cell-dyn demonstrated that Aza 2 mg/kg treated mice were significantly lymphopenic as compared to control mice at day 35 after transplantation (P = 0.05).

Conclusion : Aza prevented sclerodermic GVHD in this classical murine model of chronic GVHD.

Disclosure of Interest: None Declared

Keywords: Azacytidine, chronic GVHD, Fibrosis , Treg