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1 **Venetoclax does not impair activated T cell proliferation**

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9 **Short title:** Venetoclax and T cells

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16

17 Allogeneic hematopoietic cell transplantation (allo-HCT) is the standard consolidation therapy for
18 patients with intermediate or high-risk acute myeloid leukemia (AML). However, while patients'
19 outcomes after allo-HCT have significantly improved over the last decade, relapse of the
20 underlying malignancy remains the leading cause of morbidity and mortality. Therefore,
21 development of safe and effective therapeutic strategies to prevent or treat AML relapse is
22 essential¹. Donor lymphocyte infusion (DLI), a well-established treatment of relapsed AML,
23 seems to be an effective prophylactic treatment alone or in combination with the hypomethylating
24 agent azacytidine². Furthermore, maintenance therapy with FLT3 inhibitors seems to be effective
25 in reducing the risk of relapse of FLT3-ITD mutated AML³.

26 The orally available Bcl-2 inhibitor venetoclax, combined with a hypomethylating agent has
27 shown efficacy in newly diagnosed and relapsed or refractory AML⁴. Furthermore, Byrne et al.
28 recently reported the use of venetoclax combined with a hypomethylating agent or low-dose
29 aracytine in 21 patients with relapsed AML after allo-HCT⁵. Results were promising with an
30 overall response rate of 42%, including five complete remissions and three complete remissions
31 with incomplete count recovery, among 19 evaluable patients. Therefore, maintenance after allo-
32 HCT with venetoclax + azacytidine is currently being evaluated in large phase 2 and phase 3
33 studies (NCT04128501, NCT04161885).

34 Nevertheless, since Bcl-2 is important for effector and memory T cell responses⁶ and venetoclax
35 can cause reductions in T cell number⁷, it raises the question of the impact of venetoclax on the T
36 cell mediated graft-versus-leukemia (GVL) effect after allo-HCT and after DLI. Thus, the aim of
37 this study was to evaluate the *in-vitro* effect of venetoclax on alloreactive T cells in the graft-
38 versus-host disease (GVHD)/GVL balance after allo-HCT.

39

40 We first evaluated the effect of venetoclax on activated T cells. T cell activation was achieved by
41 incubating peripheral blood mononuclear cells (PBMCs) with anti-CD3 and anti-CD28 antibodies
42 (Abs) in the presence of different clinically relevant concentrations of venetoclax or vehicle control
43 for 24 hours (online supplemental methods, Figure 1). The viability of the T cell subpopulations
44 (CD3+CD4+, CD3+CD8+) was evaluated by flow cytometry. No difference in viability was
45 observed when the cells were treated with venetoclax or vehicle control for either CD4+ or CD8+
46 T cells (Figure 1A, B). Furthermore, as a control we evaluated the effect of venetoclax on activated

47 B cells. PBMCs were activated by CpG-B and exposed to the same concentrations of venetoclax
48 or vehicle control for 24 hours. A significant decrease in viability of CD19+ B cells was observed
49 in the presence of the highest concentrations of 1 mM and 3 mM of venetoclax (Figure 1C), in
50 agreement with previously published data on B cells⁸. Overall, our findings indicate, that in
51 contrast to activated B cells, venetoclax does not appear to have an effect on activated T cells.
52 Furthermore, we did not find any effect of venetoclax, even at the highest concentration used
53 compared to vehicle control on the proliferation of T cells activated by anti CD3/CD28 evaluated
54 by CFSE assay (Figure 1D).

55 In addition, we evaluated the effect of venetoclax on the induction of apoptosis in T cells. For this
56 purpose, cells were treated after activation for 24 hours with different concentrations of venetoclax.
57 Apoptosis was evaluated by labelling with annexin V and a viability marker. Venetoclax did not
58 induce T cell apoptosis compared to the vehicle control, even at the highest concentrations (Figure
59 E). As a control, we also evaluated induction of apoptosis on B cells and found that the highest
60 concentrations of venetoclax (1 and 3 mM) significantly increased B cell apoptosis as compared
61 to the vehicle control (Figure 1E). Given the established role of Bcl-2 in T cell homeostasis⁶ and
62 the reduced T cell number in venetoclax treated patients⁷, we compared Bcl-2 expression in
63 activated T and B cells to account for the absence of effect of the Bcl-2 inhibitor venetoclax on T
64 cells compared to B cells. Thus, we performed Bcl-2 intracellular labeling and found that Bcl-2
65 expression was significantly higher in activated B cells compared to T cells, (Figure 1F),
66 explaining the differential effect of the Bcl-2 inhibitor on activated T and B cells.

67 Overall, our findings suggest that use of venetoclax to prevent or treat AML relapse after allo-
68 HCT has no detrimental effect on the T cell mediated GVL effect. Furthermore, DLI is an
69 important strategy in the armamentarium of post allo-HCT AML management¹. DLI consisted of
70 the administration of a selected dose of CD3+ T cells to expand the T cell repertoire and enhance
71 the GVL effect. Therefore, based on our results, venetoclax will not impair DLI efficacy and they
72 can be safely combined.

73 Regarding acute GVHD, we do not expect venetoclax to be protective and we cannot exclude that
74 the enhanced GVL effect will come at the cost of an increased risk of acute GVHD. Nevertheless,
75 as already known, we find that venetoclax induce activated B cell depletion. Given the important
76 role of B cells in chronic GVHD pathophysiology⁹, this suggest that venetoclax may contribute to

77 chronic GVHD prevention. We acknowledge that we did not evaluate the impact of venetoclax on
78 other important immune cell subsets involved in the GVHD/GVL balance, in particular regulatory
79 T cells and NK cells. Further studies will be necessary, including *in-vivo* studies, to decipher the
80 exact effect of venetoclax on the different immune subsets involved in GVHD/GVL balance.

81 Overall, use of venetoclax to prevent or to treat AML relapse after allo-HCT seems to be a
82 promising therapeutic strategy that does not impair the GVL effect and may enhance the allogeneic
83 effect. This paves the way for future clinical and immunological studies in these patients and will
84 be important in understanding the possible immunomodulatory effect of venetoclax in the post
85 allo-HCT setting.

86

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90 **Conflicts of Interest**

91 Mohamad Mohty reports grants and/or lecture honoraria from Janssen, Sanofi, MaaT Pharma,
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95 Pharmaceuticals, and Astellas, all outside the submitted work.

96 **Authorship contributions**

97 All authors listed on the manuscript have contributed substantially to this work. LS, BG, MM and
98 FM designed the study, LS, BG, LR, NS and YY contributed the flow cytometry data, and LS and
99 FM performed the statistical analysis. LS, BG and FM prepared the manuscript and figures for
100 publication. All authors

101

102 **Reference**

- 103 1. Dholaria B, Savani BN, Hamilton BK, Oran B, Liu HD, Tallman MS *et al.*
104 Hematopoietic Cell Transplantation in the Treatment of Newly Diagnosed Adult Acute
105 Myeloid Leukemia: An Evidence-Based Review from the American Society of
106 Transplantation and Cellular Therapy. *Biology of blood and marrow transplantation :
107 journal of the American Society for Blood and Marrow Transplantation* 2020.
108
- 109 2. Guillaume T, Malard F, Magro L, Labopin M, Tabrizi R, Borel C *et al.* Prospective phase
110 II study of prophylactic low-dose azacitidine and donor lymphocyte infusions following
111 allogeneic hematopoietic stem cell transplantation for high-risk acute myeloid leukemia
112 and myelodysplastic syndrome. *Bone marrow transplantation* 2019; **54**: 1815-1826.
113
- 114 3. Bazarbachi AH, Al Hamed R, Malard F, Mohty M, Bazarbachi A. Allogeneic transplant
115 for FLT3-ITD mutated AML: a focus on FLT3 inhibitors before, during, and after
116 transplant. *Ther Adv Hematol* 2019; **10**: 2040620719882666.
117
- 118 4. DiNardo CD, Maiti A, Rausch CR, Pemmaraju N, Naqvi K, Daver NG *et al.* 10-day
119 decitabine with venetoclax for newly diagnosed intensive chemotherapy ineligible, and
120 relapsed or refractory acute myeloid leukaemia: a single-centre, phase 2 trial. *The Lancet.
121 Haematology* 2020.
122
- 123 5. Byrne M, Danielson N, Sengsayadeth S, Rasche A, Culos K, Gatwood K *et al.* The use of
124 venetoclax-based salvage therapy for post-hematopoietic cell transplantation relapse of
125 acute myeloid leukemia. *American journal of hematology* 2020; **95**: 1006-1014.
126
- 127 6. Wojciechowski S, Tripathi P, Bourdeau T, Acero L, Grimes HL, Katz JD *et al.* Bim/Bcl-
128 2 balance is critical for maintaining naive and memory T cell homeostasis. *The Journal of
129 experimental medicine* 2007; **204**: 1665-1675.
130
- 131 7. Lu P, Fleischmann R, Curtis C, Ignatenko S, Clarke SH, Desai M *et al.* Safety and
132 pharmacodynamics of venetoclax (ABT-199) in a randomized single and multiple
133 ascending dose study in women with systemic lupus erythematosus. *Lupus* 2018; **27**:
134 290-302.
135
- 136 8. Khaw SL, Mérimo D, Anderson MA, Glaser SP, Bouillet P, Roberts AW *et al.* Both
137 leukaemic and normal peripheral B lymphoid cells are highly sensitive to the selective
138 pharmacological inhibition of prosurvival Bcl-2 with ABT-199. *Leukemia* 2014; **28**:
139 1207-1215.
140
- 141 9. Malard F, Labopin M, Yakoub-Agha I, Chantepie S, Guillaume T, Blaise D *et al.*
142 Rituximab-based first-line treatment of cGVHD after allogeneic SCT: results of a phase 2
143 study. *Blood* 2017; **130**: 2186-2195.
144
145

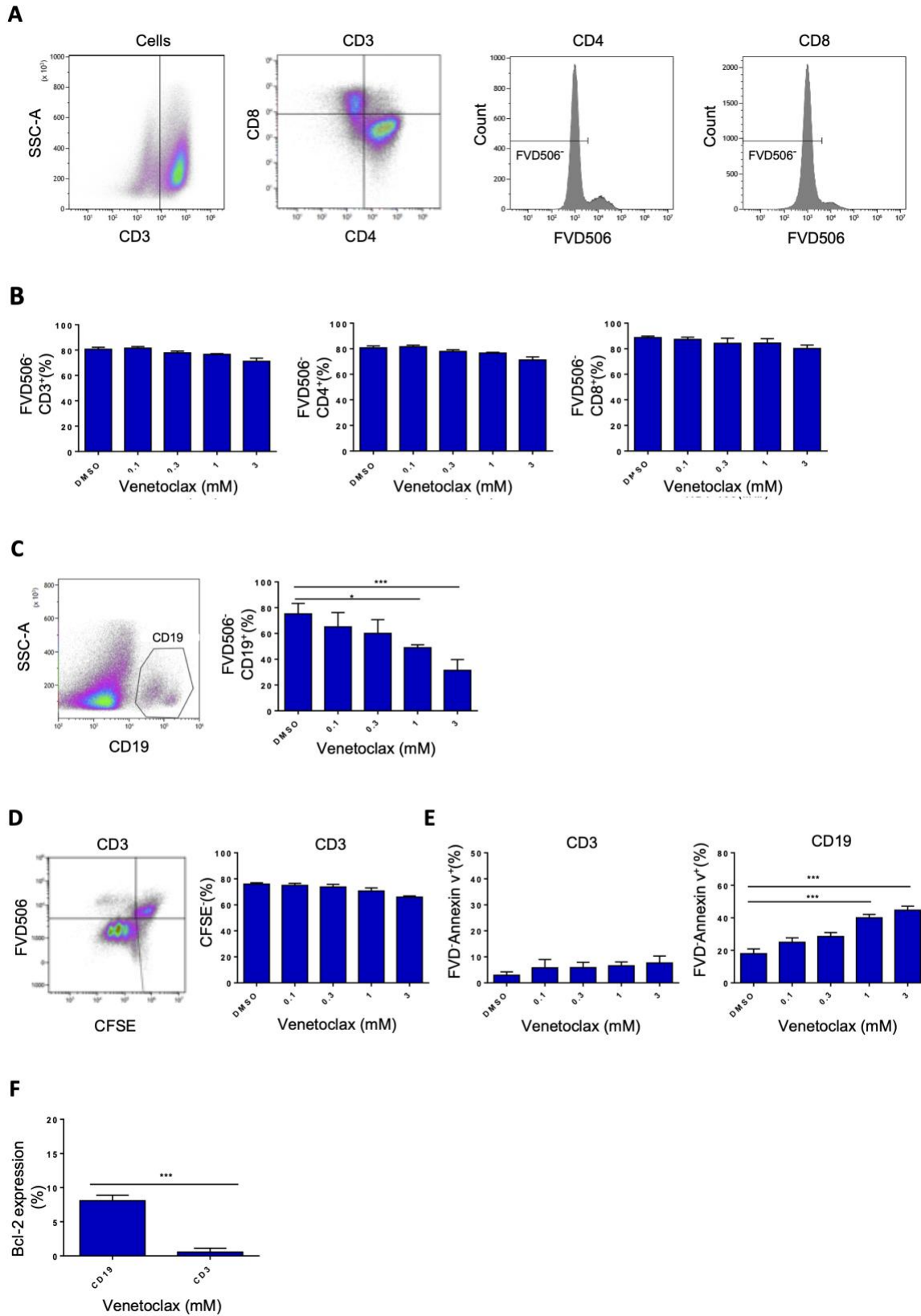
147 **Figure legend**

148 **Figure 1: Effect of venetoclax on T cells and B cells**

149 **(A):** Representative example of gating strategy for viability marker FVD506 analysis on CD4+
150 and CD8+ T cells. **(B)** The graphs represent the mean +/- SD of the % FVD506⁻ cells among the
151 CD3 (left panel), CD4 (middle panel) or CD8 T cells (right panel) treated with the indicated doses
152 of venetoclax for 3 independent experiments.

153 **(C)** The graphs represent the mean +/- SD of the % FVD506⁻ cells among CD19⁺ B cells after
154 treatment with the indicated doses of venetoclax for 3 independent experiments, the % of viable
155 FVD506⁻ cells was obtained in the CD19⁺ B cells gate (left panel). **(D):** Effect of venetoclax on
156 the proliferation of activated T cells. A cytometry plot showing CFSE dilution and FVD506
157 expression on CD3 T cells. The graph represents the mean +/- SD for 3 independent experiments
158 of the % FVD506⁻ CFSE⁻ cells among the CD3⁺ T cells treated with the indicated doses of
159 venetoclax (right panel). **(E): Effect of venetoclax on apoptosis of T and B cells.** The graphs
160 represents the mean +/- SD for 3 independent experiments of the % apoptotic cells FVD-Annexin
161 V+ cells among CD3⁺ T cells (left panel) and CD19⁺ B cells (right panel) after treatment with the
162 indicated doses of venetoclax. **(F):** The graph represents the Bcl-2 expression in activated T and B
163 cells (* $p \leq 0.05$, *** $p \leq 0.001$).

164



167 **Venetoclax does not impair activated T cell proliferation**

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169 **Supplemental data**

170

171 **Material and methods**

172

173 **Venetoclax Preparation**

174 The different concentrations of venetoclax (ABT-199) (Euromedex) were prepared between 0.8
175 and 3 mM. The therapeutic concentration ranged from 0.05 mM to 2 mM¹. Venetoclax was
176 dissolved in DMSO (10mM) which had a concentration of 0.1% in all experiments.

177

178 **Activation of T cells in vitro**

179 Peripheral blood mononuclear cells (PBMCs) were obtained from healthy donors from
180 “Etablissement Français du Sang”, Paris Saint-Antoine-Crozatier. PBMCs were isolated with a
181 standard gradient centrifugation procedure on a lymphocyte separation medium (Lymphosep
182 separation media, Dutscher, Issy-les-Moulineaux, France).

183 PBMCs were cultured at 500,000 cells/well in 96-well round bottom microplate) in RPMI medium
184 (Eurobio) supplemented with 10% Fetal Bovine Serum (FBS), 2mM L-glutamine, 100 U/mL
185 penicillin, 100 µg/ml streptomycin, 1mM sodium pyruvate (Eurobio) and non-essential amino
186 acids (MEM, Life Technologies). The wells were previously coated with anti-CD3 at 10 µg/ml
187 (LEAF Purified anti-human CD3, clone OKT3, Biolegend). The PBMCs were activated in the
188 presence of anti-CD28 (5µg/mL, Anti-Human CD28 Functional Grade Purified, clone CD28.2,
189 eBioscience). After 3 days of culture, the cells were treated with the indicated concentrations of
190 venetoclax ranging from 0.1 to 3 mM for 24 h and then analyzed.

191

192 **Activation of B cells in vitro**

193 PBMCs from healthy donors were cultured at 500,000 cells/well (96-well round bottom
194 microplate, Sarstedt) in RPMI medium supplemented with 10% FBS, 2mM L-glutamine, 100
195 U/mL penicillin, 100 µg/ml streptomycin, 1mM sodium pyruvate (Eurobio) and non-essential
196 amino acids (MEM, Life Technologies). PBMCs were activated in the presence of 1µg/mL of
197 CpG-B ODN 2006 (Miltenyi Biotec, Paris, France) for 24 hours, and treated with different doses
198 of ventoclax and then analyzed by flow cytometry.

199

200 **Proliferation analysis**

201 PBMCs were incubated for 10 min with 1µM of CFSE (Invitrogen) in a light-free environment at
202 room temperature. To stop the reaction, complete cold medium is added and the tube is placed on
203 ice. Then the cells are washed three times with the complete medium and placed in the wells for
204 culture. T cell proliferation was assessed at day-4 of culture by flow cytometry.

205

206 **Analysis of apoptosis and viability**

207 The viability of cells was analyzed by labelling with Fixable Viability Dye eFluor™ 506 (FVD)
208 (Invitrogen) according to the manufacturer's recommendations for 15 min at 4°C. After 2 washes
209 in PBS, the cells were incubated with 1µl of AnnexinV FITC (Biolegend) in AnnexinV Binding
210 Buffer (Biolegend). After 15 min incubation at room temperature and protected from light, the
211 cells were washed with PBS and analyzed with a flowcytometer (Cytoflex, Beckman Coulter).
212 FVD- cells were regarded as viable, FVD+ cells as dead, and FVD-Annexin V+ cells as apoptotic.

213

214 **Flow cytometry analysis**

215 After culture, cells were harvested and washed in PBS (Dulbecco Saline Phosphate Buffer,
216 Eurobio, France), then incubated with the different fluorochrome-conjugated monoclonal
217 antibodies (mAb) in Staining Buffer (BD Biosciences) for 20 minutes, then the cells were washed
218 with PBS and analysed with a flow cytometer (Cytoflex, Beckman Coulter, Villepinte, France).
219 The used mAbs were as follow: CD4-PE, CD3-APC-Alexa 750, CD19-APC Alexa 750 (Beckman
220 Coulter) and CD8-APC (BD Biosciences, Le Pont de Claix, France).

221 For Bcl-2 expression, we performed surface labeling with CD4-Pacific Blue), CD3-APC-Alexa
222 750, (both from Beckman Coulter) and CD8-APC (BD Biosciences) for T cells and CD19-APC
223 Alexa 750 (Beckman Coulter) for B cells. Afterwards cells were stained with PE-Bcl-2 (BD
224 Biosciences, Le Pont de Claix, France) or the corresponding isotype controls, using the
225 Foxp3/Transcription Factor Staining Buffer Set (Thermo Fisher Scientific).

226

227

228 Compensation beads were used for compensation parameters (VersaComp, Beckman Coulter).
229 Data were analyzed with Kaluza Analysis v1.5a software (Beckman Coulter).

230

231 **Statistical analysis**

232 Statistical analyses were performed using GraphPad Prism 6 software (GraphPad Software).
233 Comparisons between groups were performed with the non-parametric test for the cell populations
234 of interest. Observed differences were considered statistical for a p value < 0.05.

235

236 **Reference**

237

- 238 1. Ko TK, Chuah CT, Huang JW, Ng KP, Ong ST. The BCL2 inhibitor ABT-199
239 significantly enhances imatinib-induced cell death in chronic myeloid leukemia
240 progenitors. *Oncotarget* 2014; **5**: 9033-9038.

241

242

243

244