Targeting netrin-1/DCC interaction in Diffuse Large-B and Mantle Cell Lymphoma.

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APPENDIX

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Legends for Appendix Figures

Appendix Figure S1. Characterization of lymphoid tumoral hyperproliferations in DCC-D1290N mutant mice.

A. Frequence of lymphoid lesions in DCC mutant (n=29) and control (n=19) mice.

B. Clonal origin of B-cell lymphoma in DCC-mutant mice. Rearranged immunoglobulin VDJ sequences were amplified by PCR as described in “Materials and methods”. The three expected control DJH bands are indicated by arrows on the left, based on the amplification of the spleen DNA of a lymphoma-free DCC+/+ mouse.

C. Histological analysis of lymphoid proliferations in DCC control and mutant mice. High magnifications of sections from control spleen, low-grade FL and high-grade DLBCL stained with hematoxylin-eosin-safran are shown. w: white pulp, r: red pulp, cr: compressed red pulp.

Appendix Figure S2. Netrin-1 acts as a survival factor for ABC-DLBCL and MCL cell lines.

A. Expression of netrin-1 (dark colors) and DCC (bright colors) in lymphoma cell lines. Quantification was performed by Q-RT-PCR in 21 lymphoma cell lines. HPRT housekeeping gene was used as a standardization control. Results are presented relatively to netrin-1/DCC expression levels in Oci-Ly3. Histogram bars corresponding to ABC-DLBCL, MCL and GC-DLBCL are respectively colored in blue, grey and green. Crosshatched bars correspond to other B-cell types of lymphoma.

B-C. Efficiency of netrin-1 (B) and DCC (C) silencing by siRNA in Granta-519 transfected cells. Netrin-1 and DCC expressions are respectively decreased by 65.2% and 78.4% in Granta-519 transfected with specific siRNA as compared to those transfected with scrambled siRNA. Results of Q-RT-PCR are presented as mean+/std of at least 3 independent quantifications, relatively to HPRT housekeeping gene expression levels.
D. Effect of net-1 mAb on netrin-1 expressing Oci-Ly10 cell density. Results are means+/-std indexed to control of four independent experiments. *: p=0.05; two-sided Mann-Whitney U-test.

E. Effect of net-1 mAb on induction of Oci-Ly10 cells apoptosis, detected by TUNEL staining. Left panel: representative images are shown. TUNEL positive cells are labelled in red. Nuclei are counterstained in blue by Hoechst staining. Right panel: quantification of one representative experiment out of three performed is exposed. Results are presented as percentage of TUNEL-positive cells per field, indexed to control mean. **: p=0.006; two-sided Mann-Whitney U-test.

F. Effect of net-1 mAb on netrin-1 negative SUDHL4 cell density. Results are means+/-std indexed to control of three independent experiments. p>0.05; two-sided Mann-Whitney U-test.

G. Caspase-3 activity in Granta-519 (left panel, grey shading) and OCI-Ly3 (right panel, blue shading) cells treated with net-1 mAb antibody or with an unrelated Ig-G1 antibody (Ctl), with or without addition of an excess of netrin-1 to reverse effects of netrin-1-interfering antibody. Results are means+/-std of at least three independent experiments. $^5$: p=0.05; *: p=0.04; $^{35}$: p=0.03; **: p<0.009; two-sided Mann-Whitney U-test.

H. Survival of OCI-Ly3 xenografted mice after treatment with net-1 mAb 20mg/kg (n = 30) or control antibody (n = 30). p<0.0001; Log-rank (Mantel Cox) test.
### Appendix Figure S1

**A**

<table>
<thead>
<tr>
<th>Lymphoid lesions</th>
<th>Percentage of cases</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DCC(^{WT})</td>
</tr>
<tr>
<td>FL</td>
<td>5.2%</td>
</tr>
<tr>
<td>DLBCL</td>
<td>10.5%</td>
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<tr>
<td>Hyperplasia</td>
<td>0</td>
</tr>
</tbody>
</table>

**B**

- DJH1 (1.3 kb)
- DJH2 (1 kb)
- DJH3 (0.7 kb)

**C**

- H&E (x40)
- Control spleen
- FL
- DLBCL
Appendix Figure S2

A

Gene expression (relative to hprt)

B

% of netrin-1 mRNA expression

C

% of DCC mRNA expression

D

Cell density (index to control)

E

Ct

F

Cell density (index to control)

G

Caspase-3 activity (index to control)

H

Survival (%)