The inducible T-cell co-stimulator (ICOS) molecule is expressed on subsets of T cells and is a new marker of lymphomas of T follicular helper cell-derivation

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Supplementary Design and Methods

Cell lines

The cell lines included in this study were obtained from the cell line collection stored in the laboratory of four authors of this paper (JCP, EB, DYM and TM). The investigated series comprised four T-cell lymphoblastic lymphoma cell lines (CCRF-CEM, HUT-78, Jurkat and MOLT-4), the ALK-positive lymphoma line (Karpas 299) and a T/NK-cell leukemia-derived cell line (YT). ICOS was also investigated in some B-cell lymphoma-derived cell lines: Daudi (Burkitt's lymphoma), FL18 (follicular center lymphoma) and Karpas 422, OCI-Ly3, SU-DHL-4, SU-DHL-6 (diffuse large cell lymphoma). Three Hodgkin's lymphoma lines (KMH2, L428, L1236) and a plasma cell lymphoma-derived cell line (Thiel) were also studied. The protocols used to perform cell cultures and prepare cytospins were the same as those described in one of our previous studies.[1]

Antibodies

Three affinity-purified polyclonal antibodies raised against the human co-stimulatory molecule ICOS have been tested on human tissue samples (both cryostat and paraffin-embedded), cell lines and peripheral blood. Of these three reagents, two showed the same staining pattern and one was chosen to carry out this study (details of this antibody are available on request to Dr. T Marafioti, the corresponding author of this manuscript). The selected anti-ICOS antibody demonstrated the same reactivity pattern when applied to either cryostat or paraffin tissue sections. The staining pattern evaluated and established in human tonsil sections was selective and background-free and the antibody worked at a high dilution (guide dilution 1/500) irrespective of whether a manual or automated staining procedure was used and also independently of the detection system (e.g. Dako REAL Envision detection system, Dako, Ely, UK; Bond-polymer refine detection, Leica Microsystems, Newcastle Upon Tyne, UK; and X-Cell-Plus MenaPath, Menarini Daignostics UK, Swindon, UK). For the manual staining anti-

Online Supplementary Table S1. ICOS-positive PTCL, NOS with a T_{FH}-like immunoprofile.

	ICOS	CXCL13	PD-1	SAP	BCL-6	CD10	CD57	CD21 (FDC)
PTCL, NOS								
1	Pos	n.d.	Pos	Pos	Neg	Neg	Neg	n.d.
2	Pos	n.d.	Pos	Pos	Pos	Occasional group of Pos cells	Neg	n.d.
3	Pos	n.d.	Pos	Pos	Pos	Pos	Neg	n.d.
4	(Pos)	n.d.	Pos	n.d.	Pos	Pos	Pos	n.d.
5	Pos	n.d.	Neg	Pos	Pos	Neg	Neg	Neg
6	Pos	n.d.	Neg	Neg	Pos	Neg	Neg	Pos
7	Pos	n.d.	Pos	(Pos)	Pos	Neg	Pos	Pos
8	Pos	n.d.	(Pos)	(Pos) Pos	Some	Neg	Neg	Pos
9	Pos	n.d.	Pos	(Pos)	Some (Pos)	Pos	Neg	Neg
10	Pos	n.d.	Pos	Pos	Pos	Pos	Neg	Pos
11	Pos	n.d.	Pos	Pos	Pos	Neg	Neg	Neg
12	Pos	n.d.	Neg	Pos	Neg	Neg	Neg	Neg
13	Pos	n.d.	Pos	Pos	Some Pos	Pos	Neg	Neg
14	Pos	Pos	Pos	n.d.	Pos	Pos	Neg	Neg
15	Pos	Neg	(Pos)	n.d.	Pos	Neg	Neg	Neg
16	Pos	Pos	Neg	n.d.	Neg	Neg	Neg	(Pos)
17	Pos	Neg	Pos	n.d.	Neg	Neg	Neg	Neg
18	(Pos)	Neg	Pos	n.d.	Neg	Neg	Neg	Neg
19	Pos	Pos	Pos	n.d.	Pos	Pos	Neg	Pos
20	(Pos)	Pos	Pos	n.d.	Pos	Neg	Neg	Pos
21	Pos	Pos	Pos	n.d.	Neg	Pos	Neg	Pos
22	(Pos)	(Pos)	Pos	n.d.	Neg	Pos	Neg	Pos
23	Pos	Neg	Pos	n.d.	Pos	Neg	Pos	Neg
24	Pos	Pos	Pos	n.d.	Neg	Neg	Pos	Neg

Pos: > than 80% of tumor cells positive; (Pos): weak positive; Neg: Negative; n.d.: not determined because exhausted material in the paraffin-block.

Online Supplementary Table S2. Morphological and clinical data of 11 ICOS-positive PTCL, NOS with a T_{RF}-like immunoprofile.

Case Nr.	14	15	16	17	18	19	20	21	22	23	24
Pathological Data											
Pattern	Diffuse	Inter-follicular	Inter-follicular	Partial effacement	Almost complete effacement	Partial effacement	Partial effacement	Diffuse	Partial effacement	Partial effacement	Partial effacement
Infiltrate/Tumor cells	Monomorphic/ Medium	Polymorphic/ Medium	Monomorphic/ Small to Medium	Polymorphic/ Medium to Large	Polymorphic/ Small and clear cytoplasm	Monomorphic/ Medium	Polymorphic/ Small and clear cytoplasm	Monomorphic/ Medium	Monomorphic/ Small	Polymorphic/ Small	Polymorphic/ Medium
FDC	Absent	Absent	Absent	Absent	Absent	Absent	Partially expanded	Expanded	Expanded	Absent	Absent
Vascularity	Neg	Neg	Scarce	Scarce	Mild	Scarce	Scarce	Scarce	Scarce	Mild	Mild
CD20 blasts	Neg	Neg	+++	+++	+++	+	+++	++	+	+++	+
EBER	Neg	Neg	Blasts and small lymphocytes	Neg	n.a.	Small lymphocytes	Neg	Pos	Neg	Neg	Small lymphocytes
Clinical Data											
Age/Sex	79/F	71/M	80/M	70/M	79/F	71/M	64/F	67/M	84/M	58/M	50/F
Stage	III	n.a.	II-A	IV	I	III	III-B	III-A	n.a.	III-A	III-B
Extranodal Involvement	No	n.a.	Yes (Lung)	Yes (Skin, Lung)	No	No	Yes (Lung)	Yes (Lung)	n.a.	No	No
Serous Involvement	No	n.a.	No	Yes (Pleural Effusion)	No	No	Yes (Pleural Effusion)	No	n.a.	No	No
B-symptoms	Yes	n.a.	No	Yes	n.a.	No	Yes	No	n.a.	No	Yes
High LDH	No	n.a.	No	Yes	n.a.	Yes	Yes	Yes	n.a.	No	No
IPI	0-1	n.a.	1	4	0-1	4	4	3	n.a.	1	1
Autoimmune phenomena	No	No	No	No	No	No	No	No	No	No	No
Polyclonal gammopathy	No	n.a.	n.a.	Yes	n.a.	No	No	n.a.	n.a.	n.a.	n.a.
Treatment	CHOP	n.a.	CHOP	CHOP	n.a	CHOP	CHOP	E-SHAP	n.a.	CHOP	CHOP
Alive	Yes	n.a.	No	No	No	Yes	No	No	n.a.	n.a.	No

n.a.: not available; FDC: Follicular Dendritic Cells; Blasts for CD20 were scored as: +: less than 10%, ++: up to 20%, +++: more than 20%; Neg: Negative; Pos: Positive

gen retrieval pre-treatment was carried out using a previously described protocol.[2] Other antibodies used in this study were the monoclonal antibodies CD8 (clone C8/144B), CD20 (clone L26), CD23 (clone MHM6), CD30 (clone Ber-H2), and CD79a (clone JCB117) all from Dako (Ely, UK); BCL-6 (clone LN22), CD4 (clone 4B12), CD10 (clone 56C6), CD21 (clone 2G9), CD25 (clone 4C9), CD57 (clone NK-1) and granzyme B (clone 11F1) all from Leica Microsystems (Newcastle upon Tyne, UK); CXCL13 (clone 53610) from R&D Systems (Abingdon, UK); FOXP3 (clone 236A/E7) from AbCam (Cambridge, UK); PD-1 (clone NAT 105/e3J) (a gift from Dr. G Roncador, CNIO, Madrid, Spain) and PAX5 (clone 24) from BD Biosciences (Oxford, UK). In addition, two rabbit polyclonal antibodies were used, namely c-maf (sc-7866) and SAP (sc-8333), both from Santa Cruz Biotechnology (CA, USA).

Immunostaining and image acquisition

Single and multi-immunoenzymatic labeling was performed as described elsewhere[1,2] on cryostat and paraffin sections of whole tissue biopsies, tissue-arrays, lymphocyte-enriched peripheral blood smears and cytospin preparations of peripheral blood mononuclear cells and lymphoma-derived cell lines. Images were acquired on a Nikon Eclipse E400 microscope equipped with 10x/0.30, 20x/0.50, 40x/0.75 and 60x/0.85 Plan Fluor objective lenses, using a Nikon DS-5Mc digital camera (all from Nikon, Tokyo, Japan) and Adobe Photoshop CS3 Version 10.0.1 image processing/manipulation software (Adobe, San Jose, CA, USA).

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