PD1 | ***Validation File***

TARGET PD1
CLONE NAME NAT105C
DESCRIPTION mouse monoclonal
ANTIGEN USED YT cell line
ISOTYPE IgG1
SPECIES REACTIVITY human
LOCALIZATION membrane
POSITIVE CONTROL tonsil
STORAGE BUFFER Tissue culture supernatant: 0.02% sodium azide
Purified antibody: PBS plus 1%BSA and 0.02% sodium azide. MAb concentration: 1mg/ml
STORAGE Aliquot and store at 4C. Do not freeze

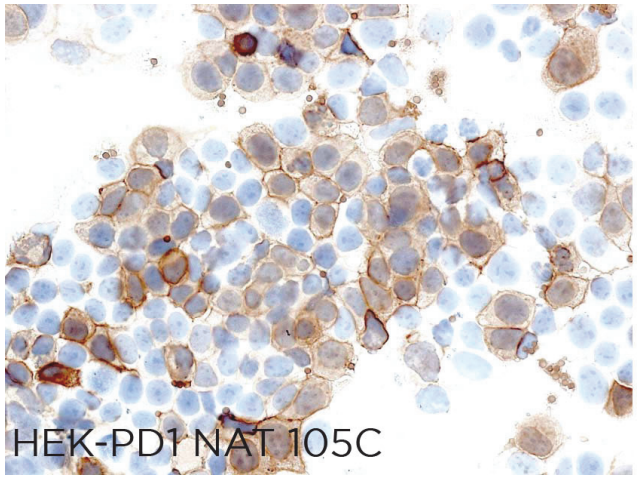
Recommended Inconclusive Not Recommended Not Tested

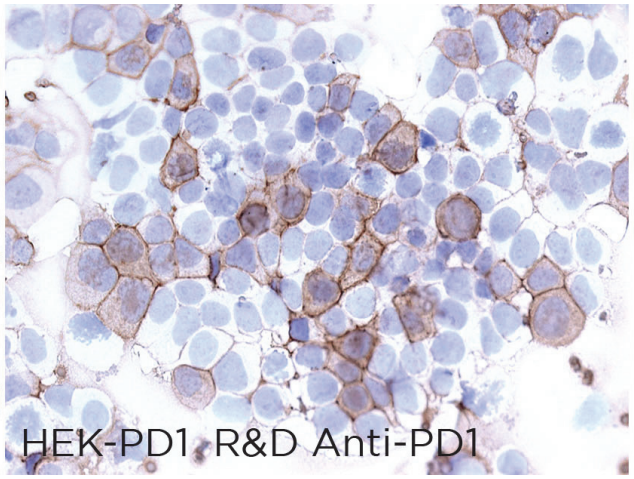
APPLICATIONS

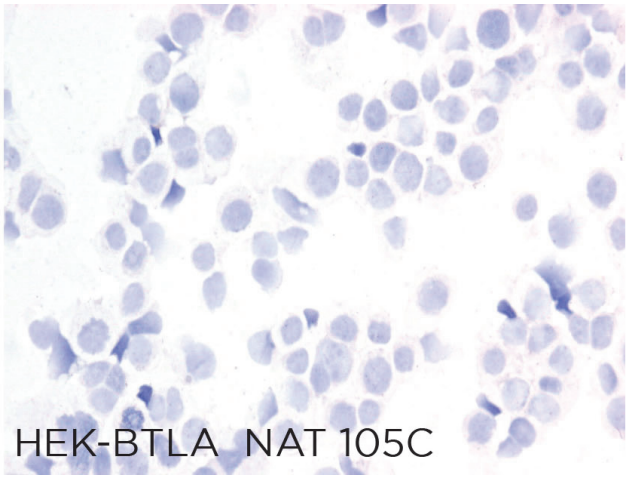
| ICC | ***Immunocytochemistry***

NAT105C mAb is able to detect human PD1 protein in immunocytochemistry.

To confirm that NAT105C mAb recognizes human PD1 protein, immunocytochemistry on frozen cytospins preparations of human PD1 expressed in HEK293 was performed. Goat R&D antibody was used as positive control. Cytospin preparation of human BTLA protein was used as a negative control.


HEK-PD1 NAT 105C

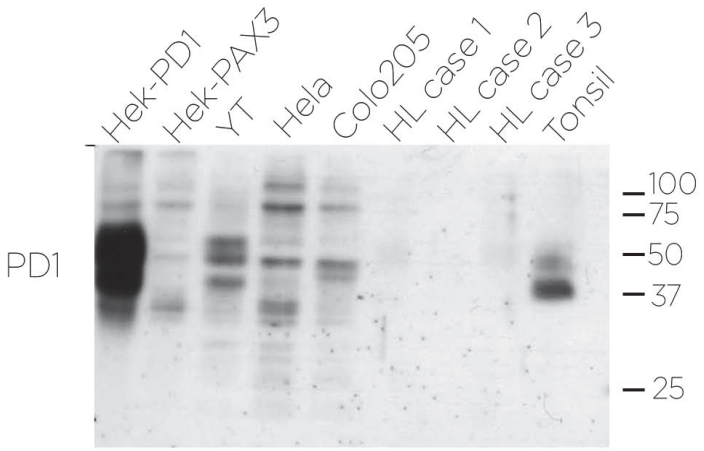

HEK-PD1 R&D Anti-PD1


HEK-BTLA NAT 105C

| WB | ***Western Blotting***

NAT105C mAb is able to detect human PD1 protein by WB.

DILUTION NAT105C no dilution (neat supernatant) or 1:200 purified antibody
R&D antibody was diluted 1:100

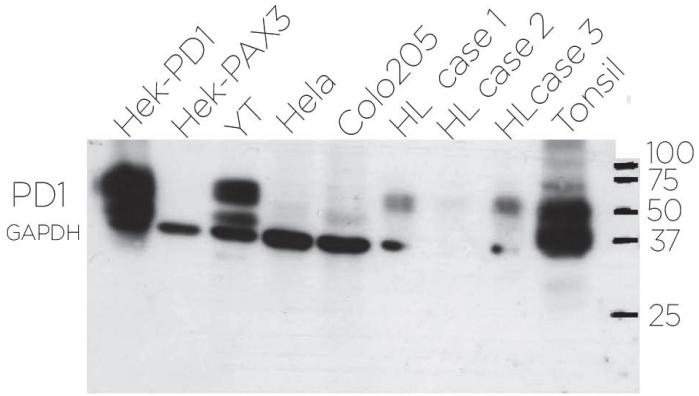


Anti- PD1 R&D goat polyclonal 1:100

Predicted molecular weight: ***32kDa***
Observed molecular weight: ***42kDa***

LANES

Lane 1 Hek-PD1	(20ug) (+)
Lane 2 Hek-PAX3	(20ug) (-)
Lane 3 YT cell line	(100ug) (+)
Lane 4 Hela cell line	(100ug) (-)
Lane 5 Colo205 cell line	(100ug) (-)
Lane 6 Hodgking lymphoma case 1	(100ug) (+)
Lane 7 Hodgking lymphoma case 2	(100ug) (-)
Lane 8 Hodgking lymphoma case 3	(100ug) (+)
Lane 9 Human tonsil	(100ug) (+)

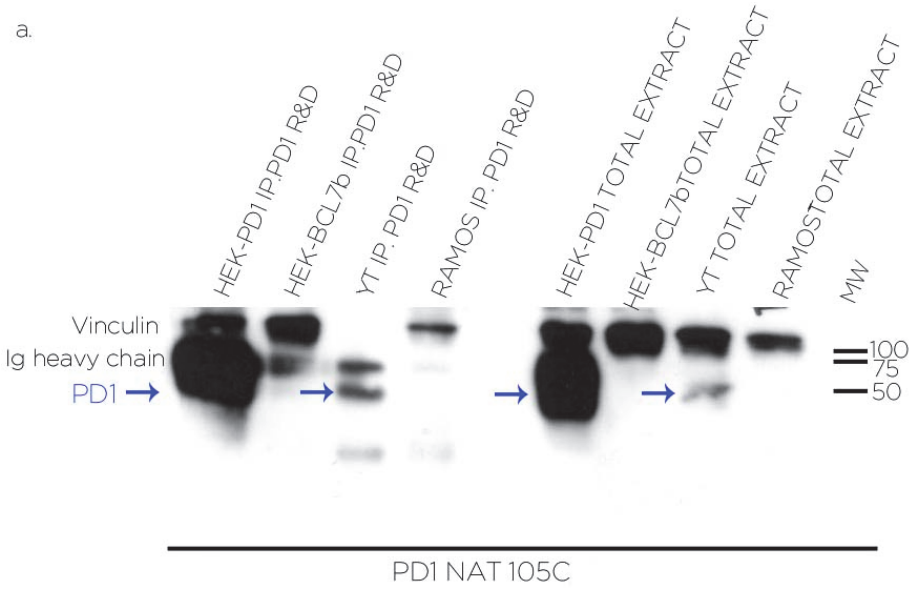


NAT105

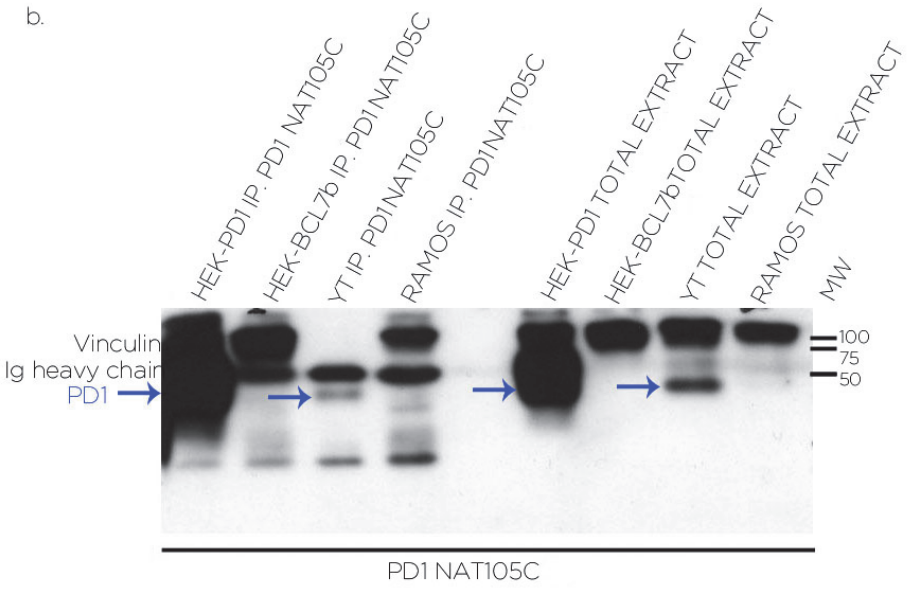
| IP | ***Immunoprecipitation***

NAT105C can be used to immunoprecipitate PD1 protein

DILUTION NAT105C no dilution (neat supernatant)
1:200 purified antibody and R&D antibody was diluted 1:100



a. Immunoprecipitation of protein extracts from HEK-PD1, HEK-Bcl7b, YT and Ramos cell lines with NAT105C mAb (1ul/lane) followed by western blotting with the same antibody (neat supernatant).

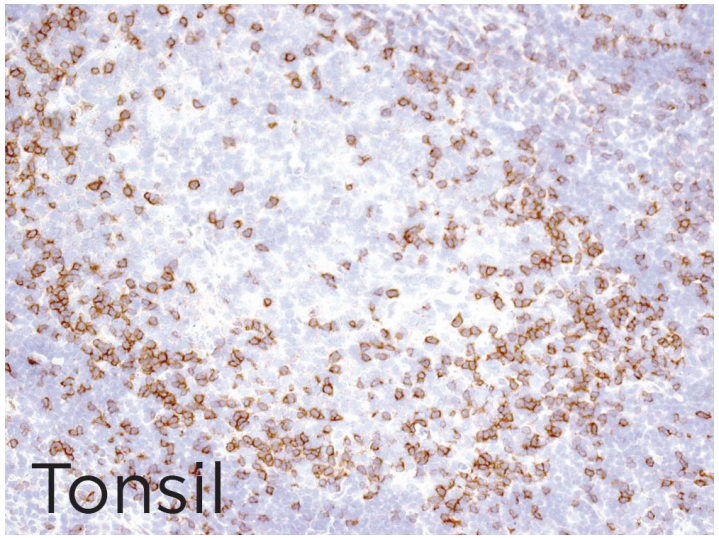


b. Immunoprecipitation of protein extracts from HEK-PD1, HEK-Bcl7b, YT and Ramos cell lines with goat R&D antibody (1ul/lane) followed by western blotting with NAT105C antibody (neat supernatant).

| IHC-F | ***Immunohistochemistry (frozen)***

NAT105C antibody can be used to detect PD1 protein in human frozen tissues.

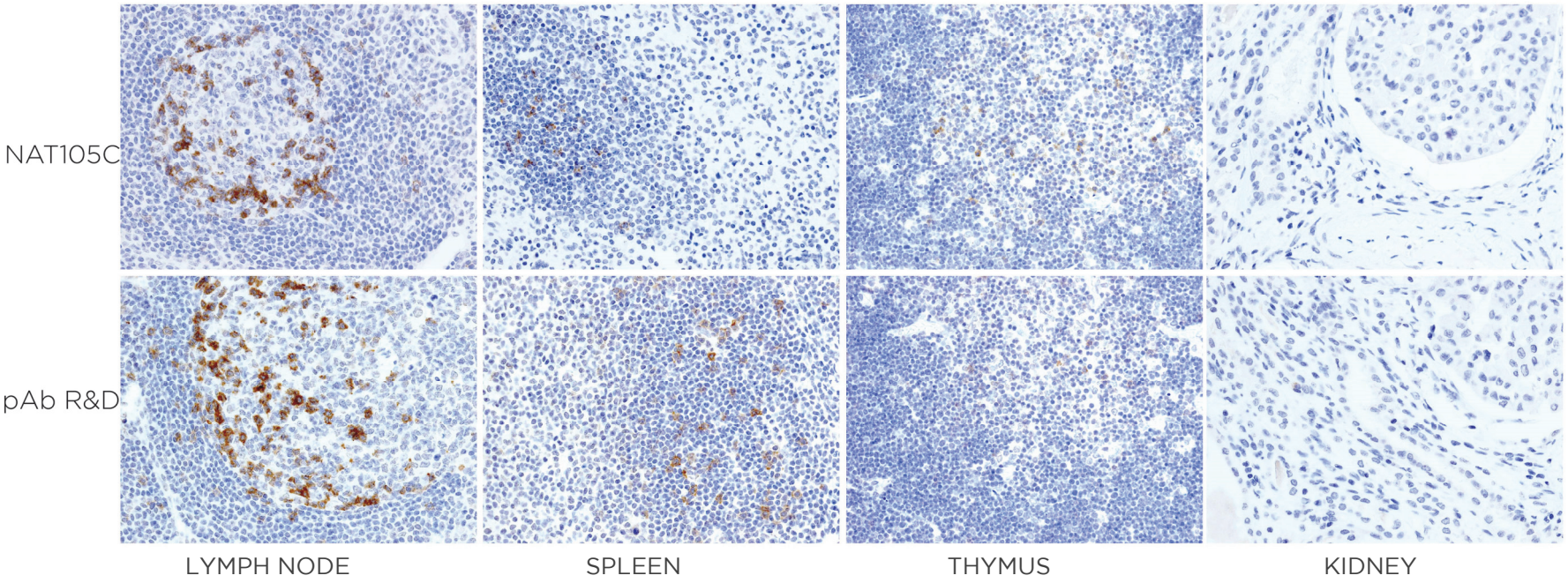
TISSUE SAMPLE Human Tonsil
DILUTION No dilution (Neat Supernatant)



| IHC-P | ***Immunohistochemistry (paraffin)***

NAT105C antibody can be used to detect PD1 protein in human paraffin tissues.

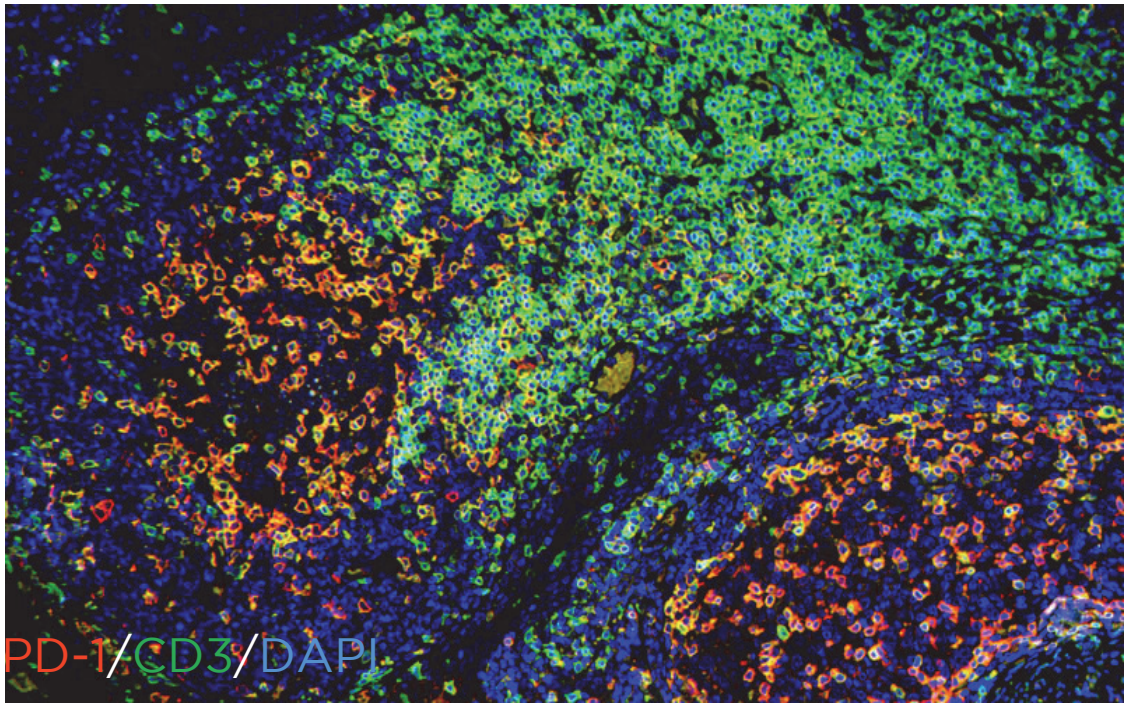
TISSUE SAMPLE Human lymph node, spleen, thymus and kidney.
DILUTION NAT105C no dilution (neat supernatant) or 1:200 purified antibody
R&D antibody was diluted 1:100
ANTIGEN RETRIEVAL 20 minutes ER2 (Tris-EDTA)
DETECTION SYSTEM Novolink kit (BondMax Leica)



| IF | ***Immunofluorescence (paraffin)***

NAT105C antibody can be used to detect PD1 in immunofluorescence.

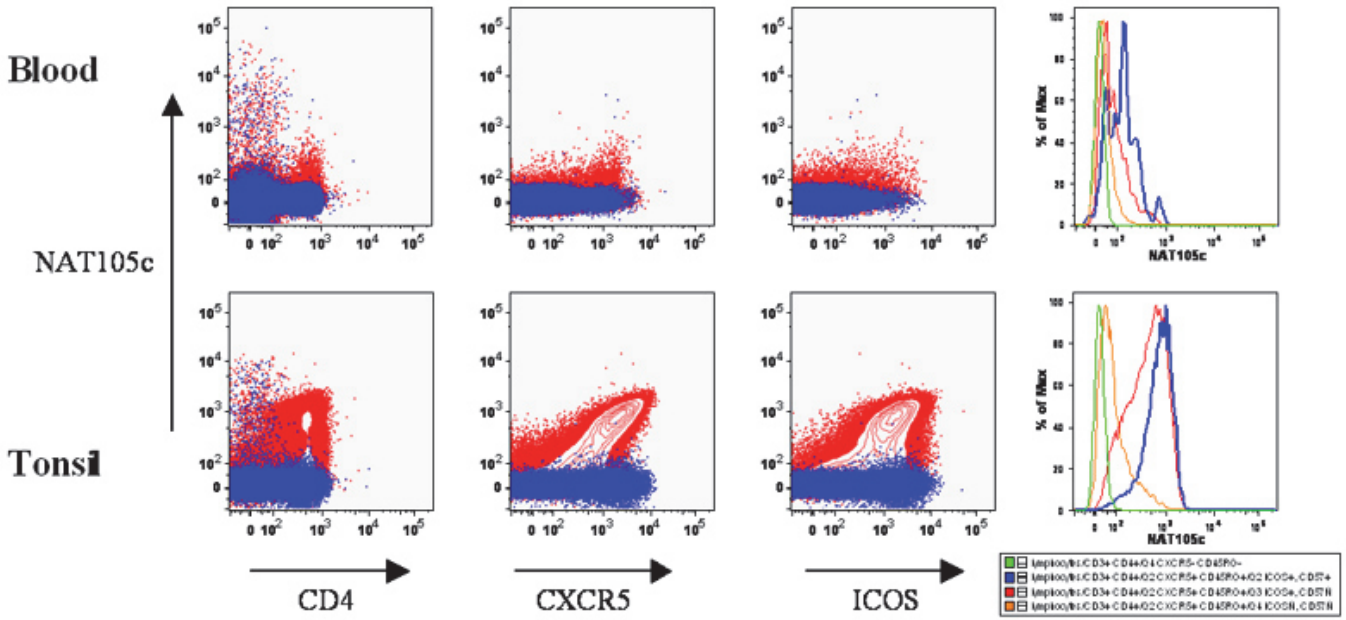
TISSUE SAMPLE Human tonsil
DILUTION NAT105C No Dilution (Neat supernatant) or 1:100 purified antibody
Anti-CD3 antibody (Clone A0452, Dako) was diluted 1:300
ANTIGEN RETRIEVAL 20 minutes ER2 (Tris-EDTA)



| FC | ***Flow Cytometry***

NAT105C antibody can be used to detect PD1 in flow cytometry.

SAMPLE Human blood and tonsillar lymphocytes
Column one. NAT105 (using, as second step, anti-mouse IgG1-PE) and CD4 staining in lymphocytes. Overlaid blue indicates staining in a parallel well with CD4 and anti-mouse iIg1-Pe in the absence of NAT105 (negative control). Column two. NAT105 and CXCR5 staining in CD3+ CD4+ lymphocytes. Column three. NAT105 and ICOS (PerCP) staining in CD3+ CD4+ Lymphocytes. Column four. Overlaid histograms of NAT105 staining in ICOS+CD57+ (blue line), ICOS+ CD57-(red line) and ICOS-CD57- (brown line) CXCR5+ CD45RO+CD4+ lymphocytes, and CXCR5-CD45RO- CD4+ lymphocytes (green line).



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REFERENCES (more than 100 citations)

Rodríguez Pinilla SM, Roncador G, Rodríguez-Peralto JL, Mollejo M, García JF, Montes-Moreno S, Camacho FI, Ortiz P, Limeres-González MA, Torres A, Campo E, Navarro-Conde P, Piris MA. Primary cutaneous CD4+ small/medium-sized pleomorphic T-cell lymphoma expresses follicular T-cell markers. Am J Surg Pathol. 2009 Jan;33(1):81-90.

Rodríguez-Pinilla SM, Atienza L, Murillo C, Pérez-Rodríguez A, Montes-Moreno S, Roncador G, Pérez-Seoane C, Domínguez P, Camacho FI, Piris MA. Peripheral T-cell Lymphoma with Follicular T-cell Markers. Am J Surg Pathol. 2008. Dec;32(12):1787-99.

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Roncador, G., Verdes-Montenegro, J.F.G., Tedoldi, S., Paterson, J.C., Klapper, W., Ballabio, E., Maestre, L., Pileri, S., Hansmann, M.L., Piris, M.A., Mason, D.Y., Marafioti, T. Expression of two markers of germinal center T cells (SAP and PD-1) in angioimmunoblastic T-cell lymphoma. Haematologica. 2007 Aug;92(8):1059-6