CD30 expression: is it a Rubik cube?

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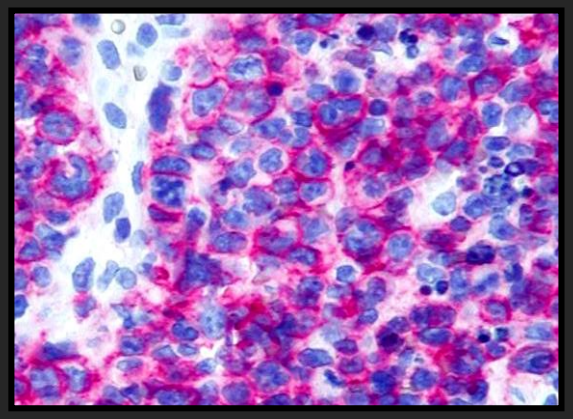

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Citation: Blood Cancer Journal (2017) 7, e603; doi:10.1038/bcj.2017.85

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REVIEW

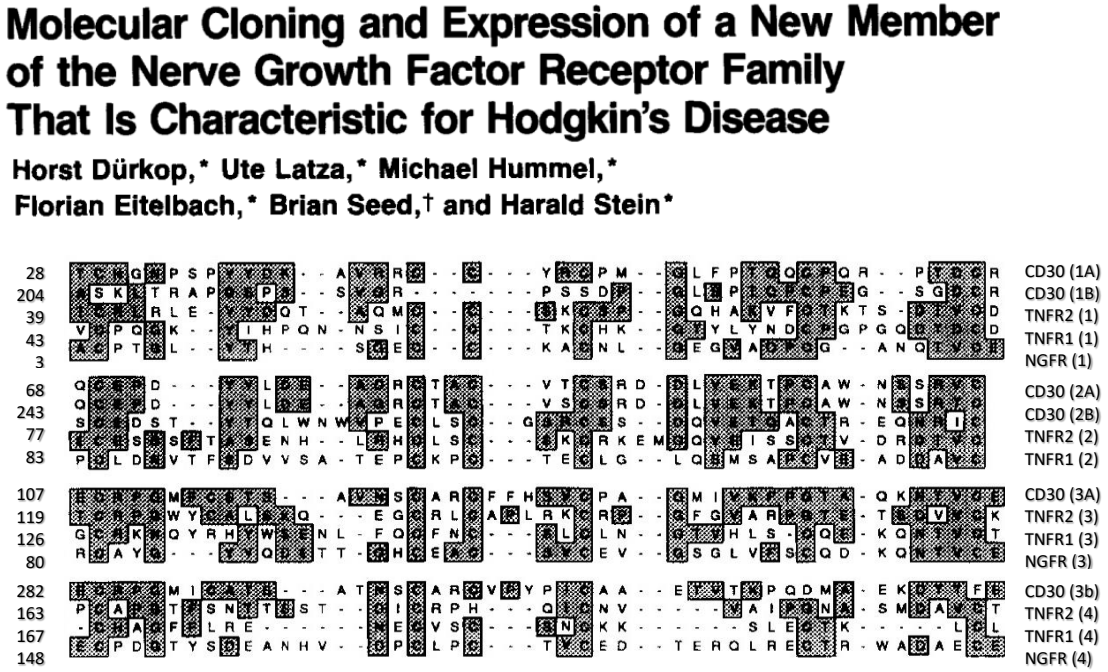
Understanding CD30 biology and therapeutic targeting: a historical perspective providing insight into future directions

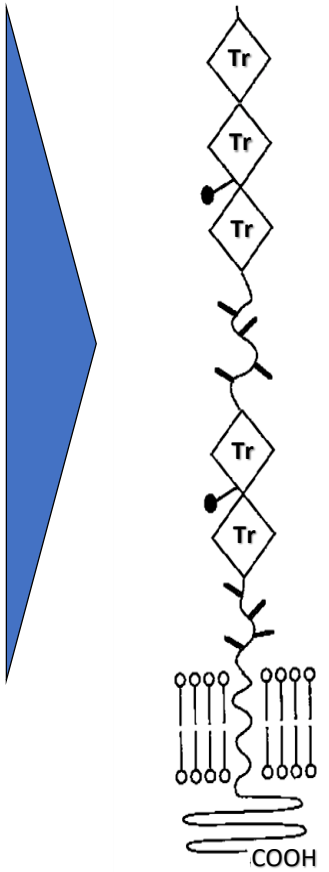
CA van der Weyden[1], SA Pileri[2,3], AL Feldman[4], J Whisstock[5] and HM Prince[1,6,7]

CD30 is a member of the tumor necrosis factor receptor superfamily. It is characteristically expressed in certain hematopoietic malignancies, including anaplastic large cell lymphoma and Hodgkin lymphoma, among others. The variable expression of CD30 on both normal and malignant lymphoid cells has focused research efforts on understanding the pathogenesis of CD30 upregulation, its contribution to lymphomagenesis through anti-apoptotic mechanisms, and its effect on cell survival. Given the restriction of CD30 to certain tumor types, the logical extension of this has been to attempt to exploit it as a therapeutic target. The efficacy of naked anti-CD30 antibodies in practice was, however, modest. Moreover, combinations with bacterial toxins and radioimmunoconjugates have also had limited success. The development of the antibody-drug compound brentuximab vedotin (BV), however, has rejuvenated interest in CD30 as a tumor target. Phase I and II clinical trials in Hodgkin lymphoma, peripheral T-cell lymphoma, cutaneous T cell lymphoma, and even CD30-expressing B-cell lymphomas, have shown the compound is well tolerated, but more importantly, able to deliver meaningful disease control even in patients with multiply relapsed or refractory disease. FDA approval has been granted for its use in relapsed Hodgkin lymphoma and systemic anaplastic large cell lymphoma. A recent phase III trial of BV in cutaneous T-cell lymphoma has confirmed its superiority to standard of care therapies. In this manuscript, we explore the history of CD30 as a tumor marker and as a therapeutic target, both in the laboratory and in the clinic, with a view to understanding future avenues for further study.

Blood Cancer Journal (2017) **7,** e603; doi:10.1038/bcj.2017.85; published online 8 September 2017

CD30 overview: gene located at 1p36





- CD30 (Ki-1, Ki-1 antigen, TNFRSF8, D1S166E)[1,2]
  - inducible, multiform member of the TNFR superfamily[1–5]
  - generally expressed in classical HL and ALCL[5–7]

ALCL, anaplastic large cell lymphoma; HL, Hodgkin lymphoma; TNFR, tumour necrosis factor receptor.

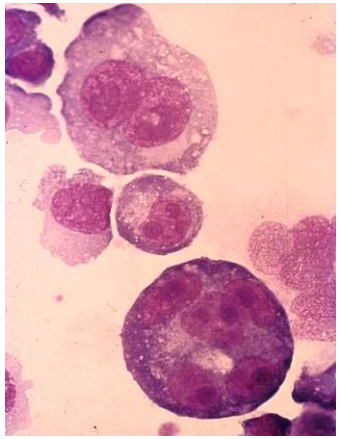
Left image from Dürkop H, et al. Molecular cloning and expression of a new member of the nerve growth factor receptor family that is characteristic for Hodgkin's disease. Cell. 1992;68:421-27. Right image from Barclay AN, et al. editors. Leukocyte antigen factsbook. London: Academic; 1993. 1. Gene symbol report: TNFRSF8. HGNC website. http://www.genenames.org/data/hgnc_data.php?hgnc_id=11923. Accessed 13 November 2013. 2. Josimovic-Alasevic O, et al. Ki-1 (CD30) antigen is released by Ki-1-positive tumor cells in vitro and in vivo. I. Partial characterization of soluble Ki-1 antigen and detection of the antigen in cell culture supernatants and in serum by an enzyme-linked immunosorbent assay. Eur J Immunol. 1989;19:157-62. 3. Dürkop H, et al. Molecular cloning and expression of a new member of the nerve growth factor receptor family that is characteristic for Hodgkin's disease. Cell. 1992;68:421-27. 4. Schwarting R, et al. BER-H2: a new anti-Ki-1 (CD30) monoclonal antibody directed at a formol-resistant epitope. Blood. 1989;74:1678-89. 5. Stein H, et al. The expression of the Hodgkin's disease associated antigen Ki-1 in reactive and neoplastic lymphoid tissue: evidence that Reed-Sternberg cells and histiocytic malignancies are derived from activated lymphoid cells. Blood. 1985;66:848-58. 6. Schwab U, et al. Production of a monoclonal antibody specific for Hodgkin and Sternberg-Reed cells of Hodgkin's disease and a subset of normal lymphoid cells. Nature. 1982;299:65-67. 7. Stein H, et al. Identification of Hodgkin and Sternberg-Reed Cells as a unique cell type derived from a newly-detected small-cell population. Int J Cancer. 1982;30:445-59.

Production of a monoclonal antibody specific for Hodgkin and Sternberg–Reed cells of Hodgkin's disease and a subset of normal lymphoid cells

Ulrich Schwab*, Harald Stein*, Johannes Gerdes*, Hilmar Lemke*, Hartmut Kirchner†, Michael Schaadt† & Volker Diehl†

Nature Vol. 299 2 September 1982 **65**

Ki-1 as the first of 57 clones produced in Kiel



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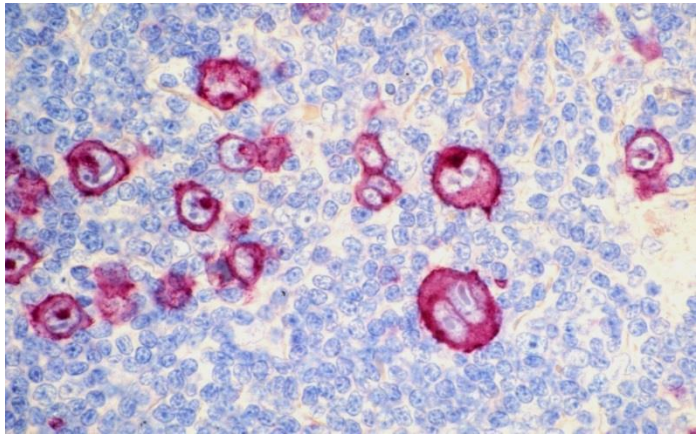
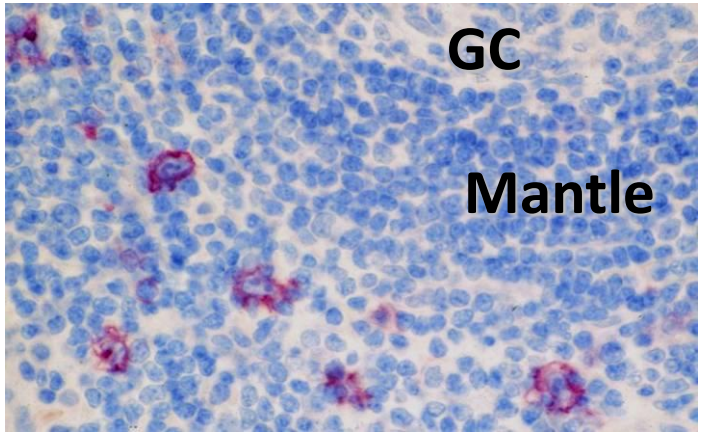


Classical Hodgkin's Disease



Normal Tonsil

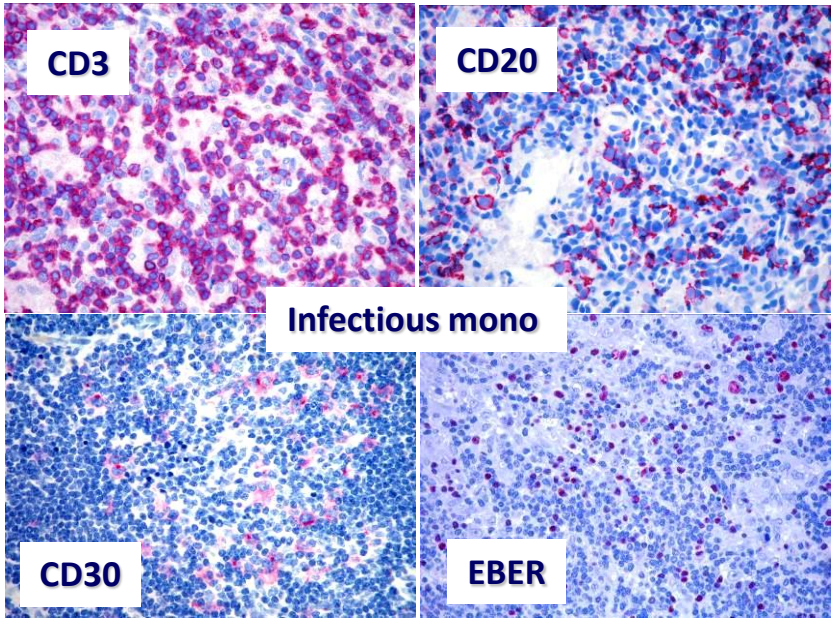
The Expression of the Hodgkin's Disease Associated Antigen Ki-1 in Reactive and Neoplastic Lymphoid Tissue: Evidence That Reed-Sternberg Cells and Histiocytic Malignancies Are Derived From Activated Lymphoid Cells

By H. Stein, D.Y. Mason, J. Gerdes, N. O'Connor, J. Wainscoat, G. Pallesen, K. Gatter, B. Falini, G. Delsol, H. Lemke, R. Schwarting, and K. Lennert

Blood, Vol 66, No 4 (October), 1985: pp 848–858

Table 6. Antigen Profile of Peripheral Blood Lymphocytes After Stimulation or Transformation With PHA, HTLV, EBV, or *S aureus*

Stimulating/ Transforming Agent	Percentage of Positive Cells					
	Ki-1	HLA-DR	T3	Sig	IL 2 R (Tac)	TÜ69
None	0	23	82	12	0	0
PHA	15*	34	93	10	97	98
HTLV II†	95*	99	95	0	96	97
EBV	97*	100	0	100	8‡	9‡
SAC§	91	ND	0	73	86	84



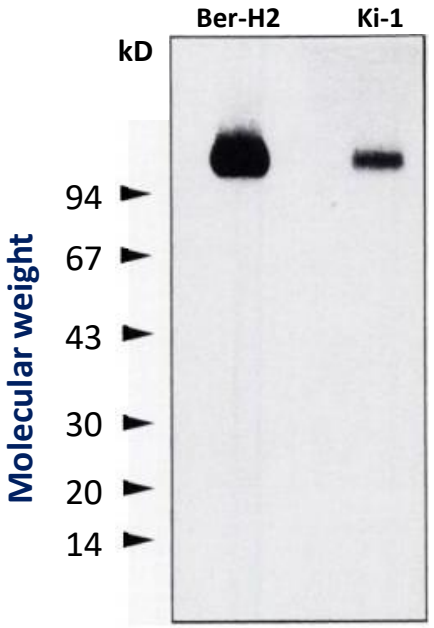
History: Ber-H2 – a new anti-Ki-1 (CD30) mAb directed at a formol-resistant epitope

BER-H2: A New Anti–Ki-1 (CD30) Monoclonal Antibody Directed at a Formol-Resistant Epitope

By Roland Schwarting, Johannes Gerdes, Horst Dürkop, Brunangelo Falini, Stefano Pileri, and Harald Stein

Blood, Vol 74, No 5 (October), 1989: pp 1678-1689

Ki-1 antigen precipitated with Ber-H2 mAb and Ki-1 mAb



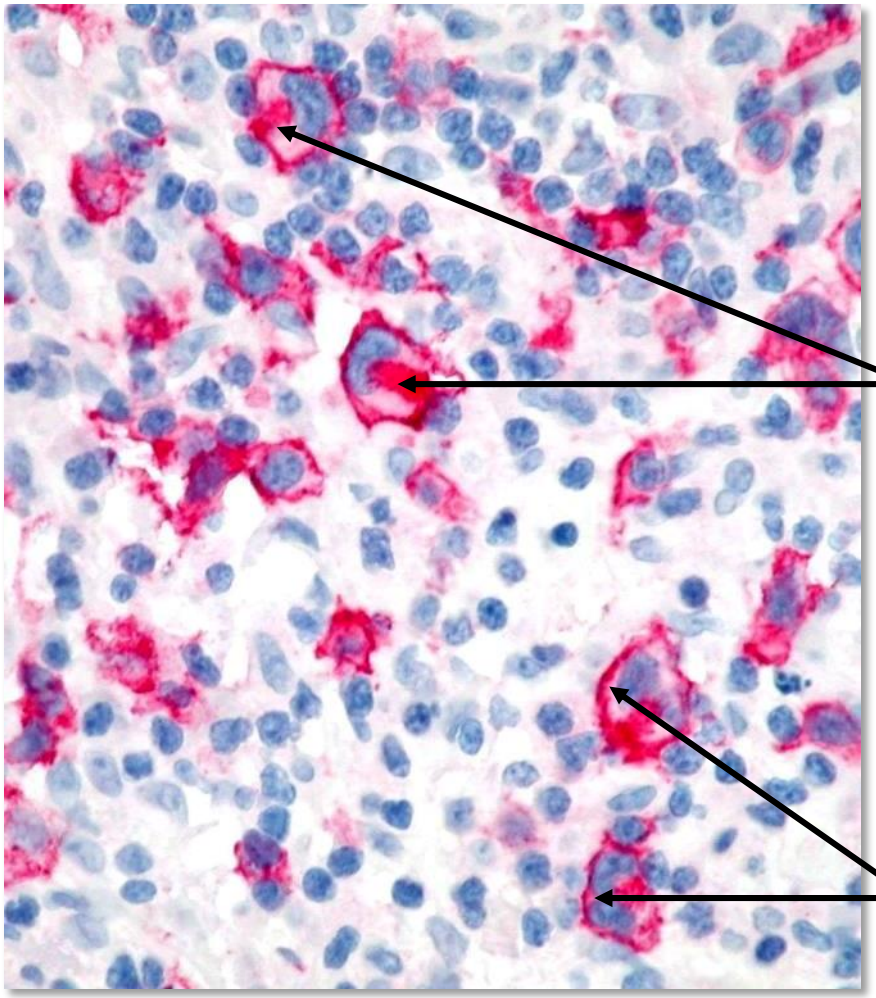
Reactivity of Ber-H2 and Ki-1 mAbs with normal cells of the haematopoietic/lymphopoietic system

	Ki-1	Ber-H2	αIL-2R
Resting peripheral blood cells			
PBMNC	–	–	–
T cells	–	–	–
B cells	–	–	–
Monocytes	–	–	–/+
Granulocytes	–	–	–
Platelets	–	–	–
Activated peripheral blood cells			
PHA blasts (3 days)	+	+	++
SAC blasts (3 days)	+	+	++
γIF monocytes (3 days)[a]	–	–	++
LPS monocytes (3 days)[b]	–	–	++
γIF/LPS monocytes (3 days)[c]	–	–[d]	++

[a] Gamma-interferon-stimulated monocytes.
[b] Lipopolysaccharide-stimulated monocytes.
[c] Gamma interferon- and lipopolysaccharide-stimulated monocytes.
[d] 1–4% large cells with lobulated nuclei positive.
IF, interferon; LPS, lipopolysaccharide; mAb, monoclonal antibody; PHA, phytohaemagglutinin; PBMCN, peripheral blood mononuclear cells; SAC, *Staphylococcus aureus* Cowan.

Schwarting R, et al. Blood. 1989;74:1678-89.

Staining pattern with the Ber-H2 mAb



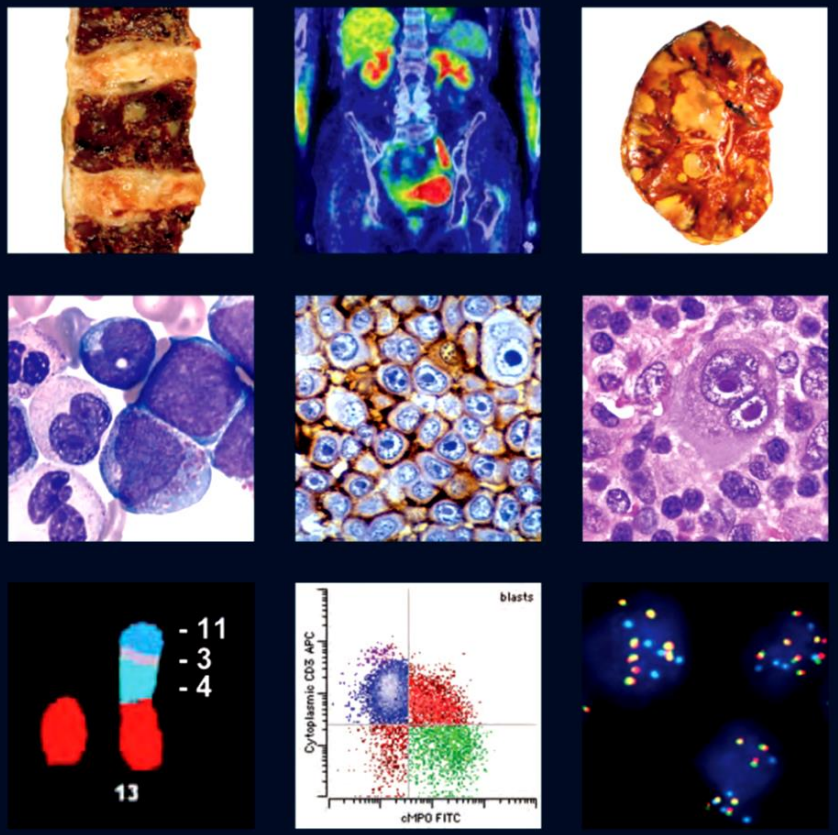
CD30 is synthesized
in the Golgi apparatus
in a precursor form [90 kDa]

Then, it undergoes glyco-
sylation [120 kDa] and
moves to the cytoplasmic
membrane

Image courtesy of S. Pileri.
Rhode M, et al. Am J Pathol. 1992;140:473-82.

WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues

Steven H. Swerdlow, Elias Campo, Nancy Lee Harris, Elaine S. Jaffe, Stefano A. Pileri,
Harald Stein, Jürgen Thiele, Daniel A. Arber, Robert P. Hasserjian,
Michelle M. Le Beau, Attilio Orazi, Reiner Siebert



WHO

2017

CD30 expression in tumours

- Constitutive
  - cHL
  - PMBL
  - DLBCL, anaplastic type
  - ALCL, ALK^+ & ALK^- (including BI-associated ALCL: provisional)
  - Primary cutaneous $CD30^+$ LPD
  - aggressive mastocytosis
  - embryonal carcinoma
- Variable: several types of tumour, mostly lymphoid

cHL, classical Hodgkin lymphoma; DLBCL, diffuse large B-cell lymphoma; PMBL, primary mediastinal B-cell lymphoma.

Falini B, et al. Blood. 1995; 85: 1–14. Pallesen G, Hamilton-Dutoit SJ. Am J Pathol. 1988; 13:446-50. Pileri SA, Am J Pathol. 2003; 162:243-53. Sotlar K, et al. Mod Pathol. 2011; 24:585–595. Swerdlow SH, et al, eds. WHO classification of tumours of haematopoietic and lymphoid tissues. 4th ed. Lyon, France: IARC; 2008.

MATURE T-AND NK-NEOPLASMS

T-cell prolymphocytic leukemia
T-cell large granular lymphocytic leukemia
Chronic lymphoproliferative disorder of NK cells
Aggressive NK cell leukemia
Systemic EBV+ T-cell Lymphoma of childhood*
Hydroa vacciniforme-like lymphoproliferative disorder*
Adult T-cell leukemia/lymphoma
Extranodal NK/T-cell lymphoma, nasal type
Enteropathy-associated T-cell lymphoma
Monomorphic epitheliotropic intestinal T-cell lymphoma*
*Indolent T-cell lymphoproliferative disorder of the GI tract **
Hepatosplenic T-cell lymphoma
Subcutaneous panniculitis- like T-cell lymphoma
Mycosis fungoides
Sézary syndrome
Primary cutaneous CD30 positive T-cell lymphoproliferative disorders
    Lymphomatoid papulosis
    Primary cutaneous anaplastic large cell lymphoma
Primary cutaneous gamma-delta T-cell lymphoma
Primary cutaneous CD8 positive aggressive epidermotropic cytotoxic T-cell lymphoma
*Primary cutaneous acral CD8+ T-cell lymphoma**
*Primary cutaneous CD4 positive small/medium T-cell lymphoproliferative disorder**
Peripheral T-cell lymphoma, NOS
Angioimmunoblastic T-cell lymphoma
*Follicular T-cell lymphoma**
*Nodal peripheral T-cell lymphoma with TFH phenotype**
Anaplastic large cell lymphoma, ALK positive
Anaplastic large cell lymphoma, ALK negative *
*Breast implant-associated anaplastic large cell lymphoma**

CD30 expression in PTCL

by Elena Sabattini, Marco Pizzi, Valentina Tabanelli, Pamela Baldin, Carlo Sagramoso Sacchetti, Claudio Agostinelli, Pier Luigi Zinzani, and Stefano Pileri

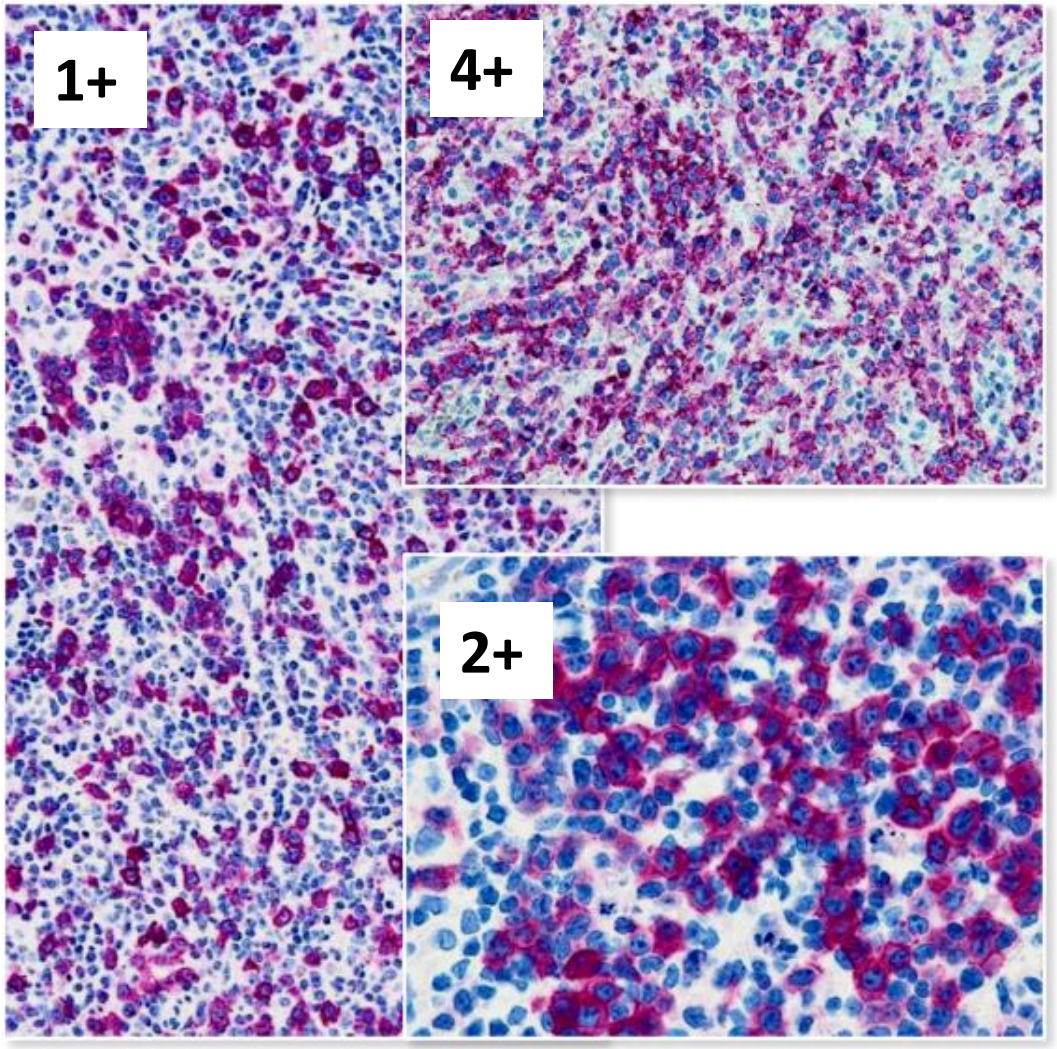
	CD30 IHC score					
	0	1+	2+	3+	4	Score ≥ 2+
PTCL-NOS, n (%) (N = 87)	31 (35.63)	11 (12.64)	18 (20.69)	11 (12.64)	16 (18.39)	45 (51.72)
AITL, n (%) (N = 42)	24 (51.14)	9 (21.42)	5 (11.90)	4 (9.52)	–	9 (21.42)
ENTL, n (%) (N = 10)	2 (20.00)	1 (10.00)	3 (30.00)	1 (10.00)	3 (30.00)	7 (70.00)
MF, n (%) (N = 32)	13 (40.63)[a]	15 (46.88)[b]	2 (6.25)[c]	–	2 (6.25)[d]	4 (12.50)
Transformed MF, n (%) (N = 9)	–	–	3 (33.33)	6 (66.67)	–	9 (100.00)
EATL type 1, n (%) (N = 9)	–	–	2 (22.22)	–	7 (77.78)	9 (100.00)
EATL type 2, n (%) (N = 3)	3 (100)	–	–	–	–	–
All types, n (%) (N = 192)	73 (38.02)	36 (18.75)	33 (17.18)	17 (8.85)	28 (14.58)	83 (43.22)

[a] 2 cases in tumoural phase. [b] 1 case in tumoural phase. [c] Folliculotropic variant. [d] Pagetoid reticulosis sybtype.
AITL, angioimmunoblastic T-cell lymphoma; EATL, enteropathy-associated T-cell lymphoma;
ENTL, extranodal NK/T-cell lymphoma, nasal type; MF, mycosis fungoides.

Sabattini E, et al. Haematologica. 2013;98:e81-2.

Full sections of 192 non-anaplastic PTCLs with the Ber-H2 mAb

Score	
4+	> 75%
3+	50–75%
2+	25–50%
1+	<25%
0	None positive



Sabattini E, et al. Haematologica. 2013;98:e81-2.

Immunohistochemistry as a valuable tool to assess CD30 expression in PTCLs: high correlation with mRNA levels

Céline Bossard,[1] Maria Pamela Dobay,[2] Marie Parrens,[3] Laurence Lamant,[4] Edoardo Missiaglia,[2] Corinne Haioun,[5] Antoine Martin,[6] Bettina Fabiani,[7] Richard Delarue,[8] Olivier Tournilhac,[9] Mauro Delorenzi,[2,10,11] Philippe Gaulard,[12,13,14] and Laurence de Leval[15]

CD30 immunohistochemical expression in PTCLs

% of CD30+ tumour cells	ALCL ALK$^+$ (n = 61)	ALCL ALK$^-$ (n = 19)	PCTL-NOS (n = 141)	AITL (n = 97)	ENTL (n = 28)	EATL (n = 14)	ATLL (n = 9)	HSTL (n = 7)
Score 0, n (%) (< 5%)	0	0	59 (42)	36 (37)	15 (53.5)	7 (50)	4 (44)	7 (100)
Score 1, n (%) (5–24%)	0	0	37 (26)	46 (47)	2 (7)	0	1 (11)	0
Score 2, n (%) (25–49%)	3 (5)	0	13 (9)	10 (10)	3 (11)	0	3 (33)	0
Score 3, n (%) (50–75%)	1 (2)	0	14 (10)	5 (5)	4 (14)	1 (7)	1 (11)	0
Score 4, n (%) (> 75%)	57 (93)	19 (100)	18 (13)	0	4 (14)	6 (43)	0	0
Total positive cases (scores 1–4)	61 (100)	19 (100)	82 (58)	61 (63)	13 (46)	7 (50)	5 (55.5)	0
Strongly positive cases (scores 3–4)	58 (95.1)	19 (100)	32 (23)	5 (5)	8 (28.5)	7 (50)	1 (11)	0

ATLL, adult T-cell leukaemia/lymphoma; HSTL, hepatosplenic T-cell lymphoma.

Bossard C, et al. Blood. 2014;124:2983-6.

CD30: more than a marker in lymphoma

- Differentially promotes cell proliferation and survival via activation of MAPK and NF-kB, increases susceptibility to apoptosis and affects immune response[1–3]
- Variable incidence and intensity of expression in a wide range of malignancies and autoimmune/inflammatory disorders[4,6]
- **Limited expression in healthy tissue makes it an ideal therapeutic target**[6]
- Important factor in the diagnosis and prognosis of several lymphomas[5,7,8]

1. Gruss H-J, et al. Pleiotropic effects of the CD30 ligand on CD30-expressing cells and lymphoma cell lines. Blood. 1994;83:2045-56. 2. Duckett CS, et al. Induction of nuclear factor κB by the CD30 receptor is mediated by TRAF1 and TRAF2. Mol Cell Biol. 1997;17:1535-42. 3. Zheng B, et al. MEK/ERK pathway is aberrantly active in Hodgkin disease: a signaling pathway shared by CD30, CD40, and RANK that regulates cell proliferation and survival. Blood. 2003;102:1019-27. 4. Schwarting R, et al. BER-H2: a new anti-Ki-1 (CD30) monoclonal antibody directed at a formol-resistant epitope. Blood. 1989;74:1678-89. 5. de Leval L, Gaulard P. CD30^{+} lymphoproliferative disorders. Haematologica. 2010;95:1627-30 6. Younes A, Kadin ME. Emerging applications of the tumor necrosis factor family of ligands and receptors in cancer therapy. J Clin Oncol. 2003;21:3526-34. 7. Hu S, et al. CD30 expression defines a novel subgroup of diffuse large B-cell lymphoma with favorable prognosis and distinct gene expression signature: a report from the International DLBCL Rituximab-CHOP Consortium Program Study. Blood. 2013;121:2715-24. 8. Savage KJ, et al. ALK^{-} anaplastic large-cell lymphoma is clinically and immunophenotypically different from both ALK^{+} ALCL and peripheral T-cell lymphoma, not otherwise specified: report from the International Peripheral T-Cell Lymphoma Project. Blood. 2008;111:5496-504.

Anti-CD30 immunotoxin was tested in the treatment of HL

- Preliminary results suggest a role for the anti-CD30 immunotoxin in the treatment of HL

THE LANCET

Volume 339, Issue 8803, 16 May 1992, Pages 1195–1196

Originally published as Volume 1, Issue 8803

SHORT REPORT

Response of refractory Hodgkin's disease to monoclonal anti-CD30 immunotoxin

B Falini, MD [a] (Prof), L Flenghi, MD[a], F Aversa, MD[a], G Barbabietola, MD[a], M.F Martelli, MD[a] (Prof), P Comeli, MD[b], P.L Tazzari, MD[c], M.K Broe, MD[d], H Stein, MD[e] (Prof), H Dürkop, MD[e], G Pizzolo, MD[f] (Prof), A Bolognesi, PhD[g], F Stirpe, MD[g] (Prof), E Sabattini, MD[h], S Pileri, MD[h] (Prof)

[a] Institute of Haematology, University of Perugia, United Kingdom

[b] Institute of Radiology, University of Perugia, United Kingdom

[c] National Institute for Cancer Research, Genova, Italy

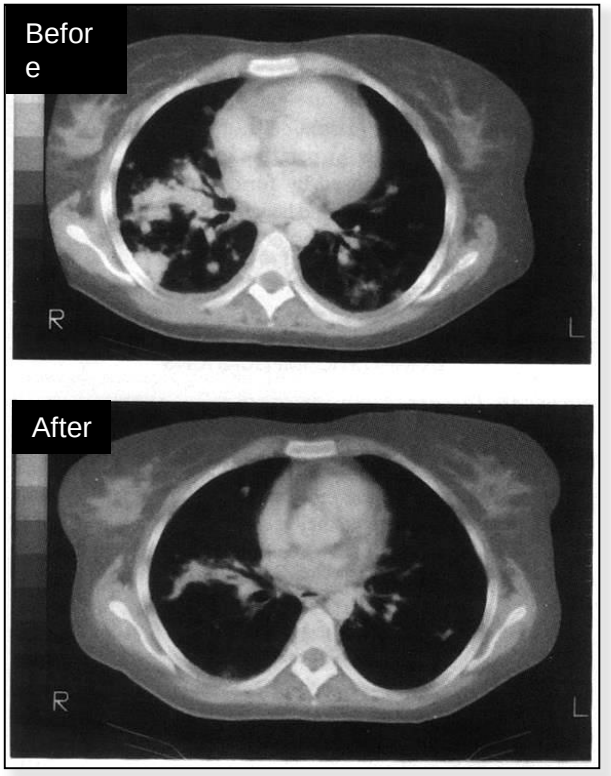
[d] Dako, Glostrup,, Denmark

[e] Institute of Pathology, Free University of Berlin, Germany

[f] Department of Haematology, University of Verona , United Kingdom

[g] Department of Experimental Pathology, University of Bologna, Italy

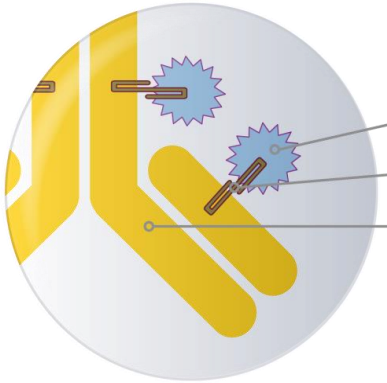
[h] Institute of Haematology, University of Bologna, Italy



Falini B et al. Lancet. 1992;339:1195-6.

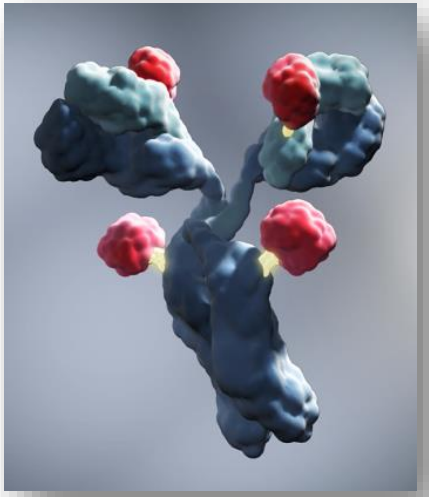
Antibody-drug conjugate: brentuximab vedotin (BV)

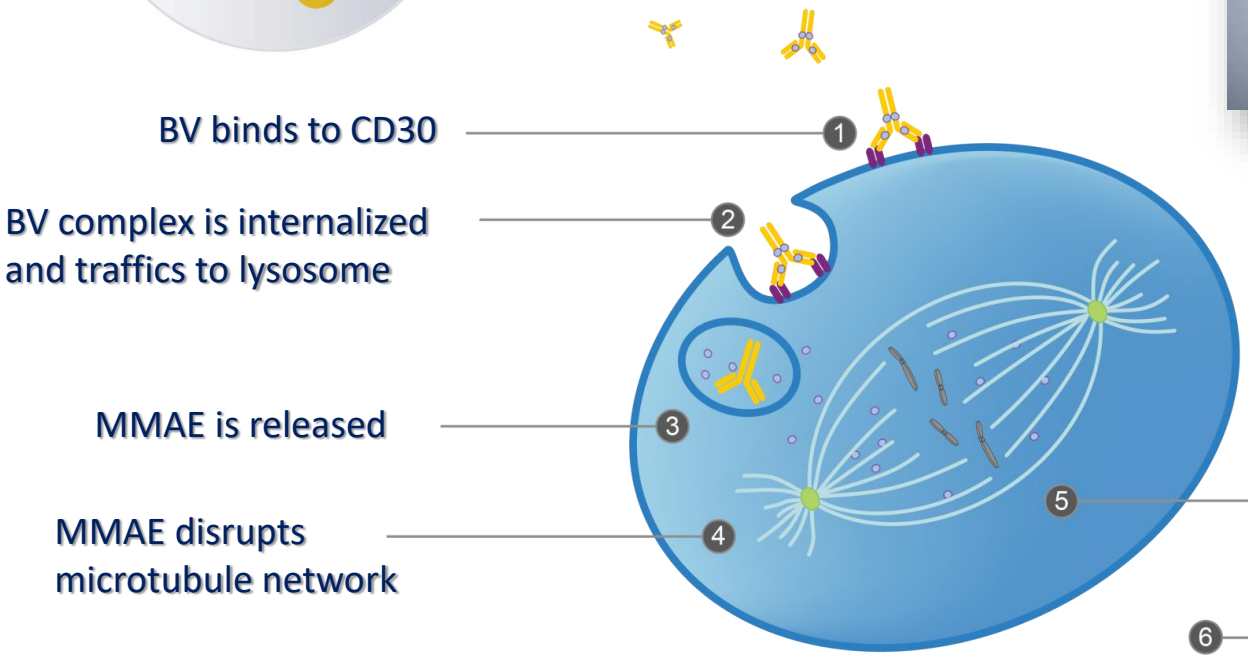
Antibody cAC10 specific for human CD30



BV ADC

- MMAE, microtubule-disrupting agent
- Protease-cleavable linker
- Anti-CD30 monoclonal antibody





MMAE, microtubule-disrupting agent monomethyl auristatin E.

Francisco JA, et al. Blood. 2003;102:1458-65. Van de Donk NWCJ, Dhimolea E. mAbs. 2012;4:458-65.

CD30: more than a marker in lymphoma

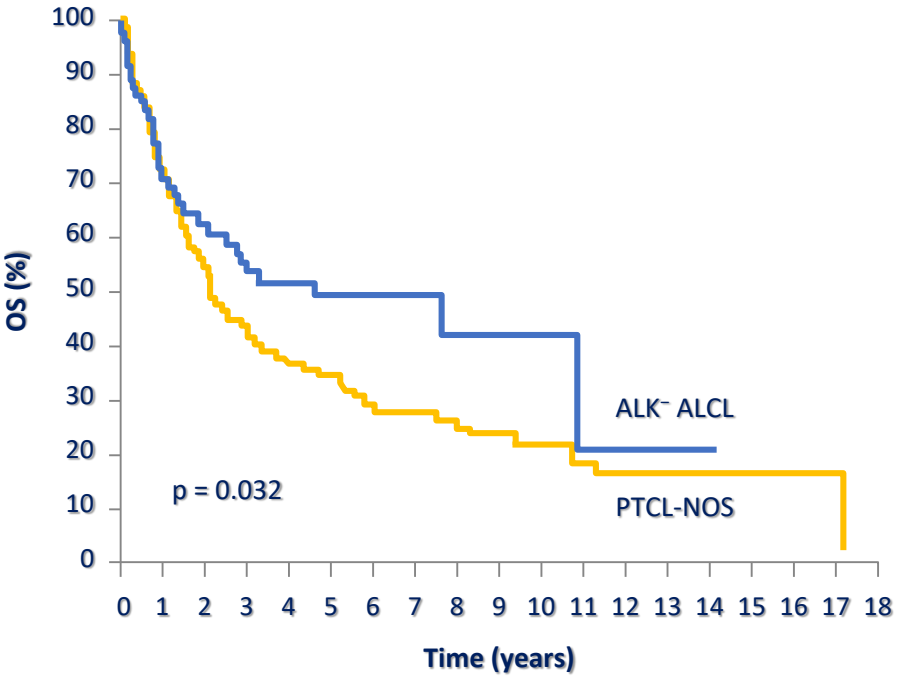
- Differentially promotes cell proliferation and survival via activation of MAPK and NF-kB, increases susceptibility to apoptosis and affects immune response[1–3]
- Variable incidence and intensity of expression in a wide range of malignancies and autoimmune/inflammatory disorders[4,6]
- Limited expression in healthy tissue makes it an ideal therapeutic target[6]
- **Important factor in the diagnosis and prognosis of several lymphomas[5,7,8]**

1. Gruss H-J, et al. Pleiotropic effects of the CD30 ligand on CD30-expressing cells and lymphoma cell lines. Blood. 1994;83:2045-56. 2. Duckett CS, et al. Induction of nuclear factor κB by the CD30 receptor is mediated by TRAF1 and TRAF2. Mol Cell Biol. 1997;17:1535-42. 3. Zheng B, et al. MEK/ERK pathway is aberrantly active in Hodgkin disease: a signaling pathway shared by CD30, CD40, and RANK that regulates cell proliferation and survival. Blood. 2003;102:1019-27. 4. Schwarting R, et al. BER-H2: a new anti-Ki-1 (CD30) monoclonal antibody directed at a formol-resistant epitope. Blood. 1989;74:1678-89. 5. de Leval L, Gaulard P. CD30^{+} lymphoproliferative disorders. Haematologica. 2010;95:1627-30 6. Younes A, Kadin ME. Emerging applications of the tumor necrosis factor family of ligands and receptors in cancer therapy. J Clin Oncol. 2003;21:3526-34. 7. Hu S, et al. CD30 expression defines a novel subgroup of diffuse large B-cell lymphoma with favorable prognosis and distinct gene expression signature: a report from the International DLBCL Rituximab-CHOP Consortium Program Study. Blood. 2013;121:2715-24. 8. Savage KJ, et al. ALK^{-} anaplastic large-cell lymphoma is clinically and immunophenotypically different from both ALK^{+} ALCL and peripheral T-cell lymphoma, not otherwise specified: report from the International Peripheral T-Cell Lymphoma Project. Blood. 2008;111:5496-504.

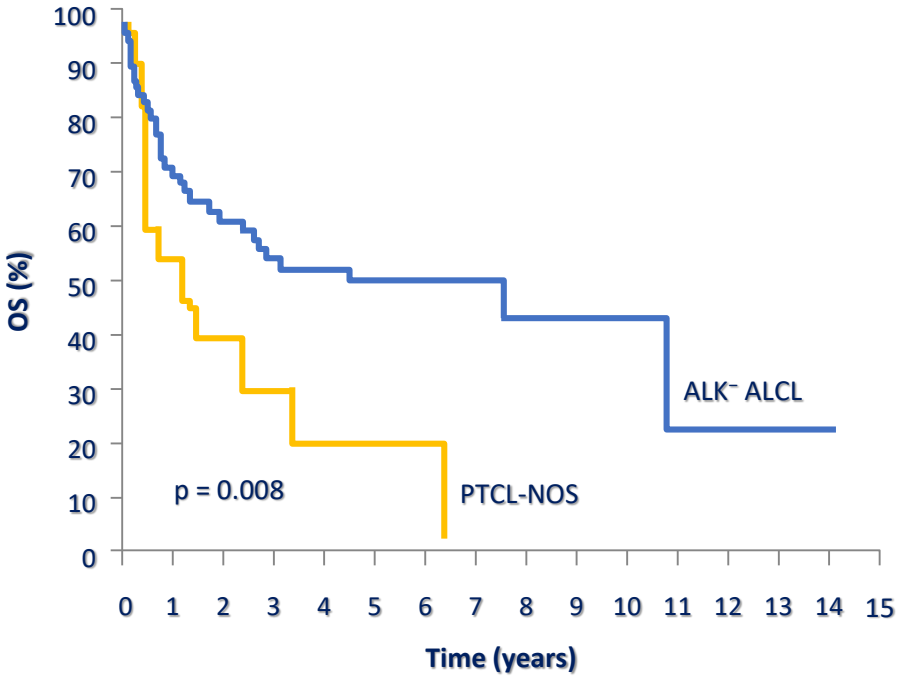
CD30 confers a poor prognosis in PTCL-NOS

- Expression of CD30 on ≥ 80% of cells has been associated with an extremely poor prognosis

Overall survival: ALK⁻ ALCL vs PTCL-NOS



Overall survival: ALK⁻ ALCL vs PTCL-NOS, CD30⁺ ≥ 80%



- High CD30 expression (≥ 80%) was associated with a 40% decrease in 5-year OS (19% vs 32%)

Piccaluga PP, et al. Molecular profiling improves classification and prognostication of nodal peripheral T-cell lymphomas: results of a phase III diagnostic accuracy study. J Clin Oncol. 2013;31:3019–25. Savage KJ, et al. ALK⁻ anaplastic large-cell lymphoma is clinically and immunophenotypically different from both ALK⁺ ALCL and peripheral T-cell lymphoma, not otherwise specified: report from the International Peripheral T-Cell Lymphoma Project. Blood. 2008;111:5496-504.

What are the pathologist perceptions of the role of CD30 staining?

Pathologist perceptions of the role of CD30 immunohistochemistry (IHC) in T-cell lymphoma (TCL)

Robert J. Levak,[1] Randy D. Gascoyne,[2] Graham W. Slack[2]

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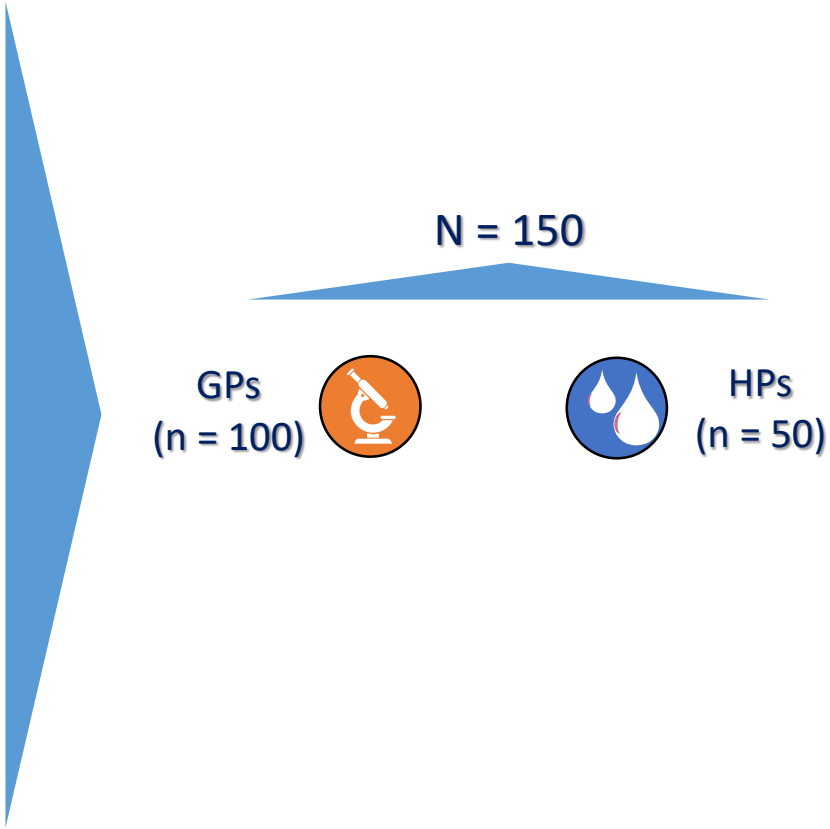
Levak R, Slack GW. Am J Clin Pathol. 2015;144:A196 (abstract LP86).

For which differential diagnosis would your center test for CD30 IHC?

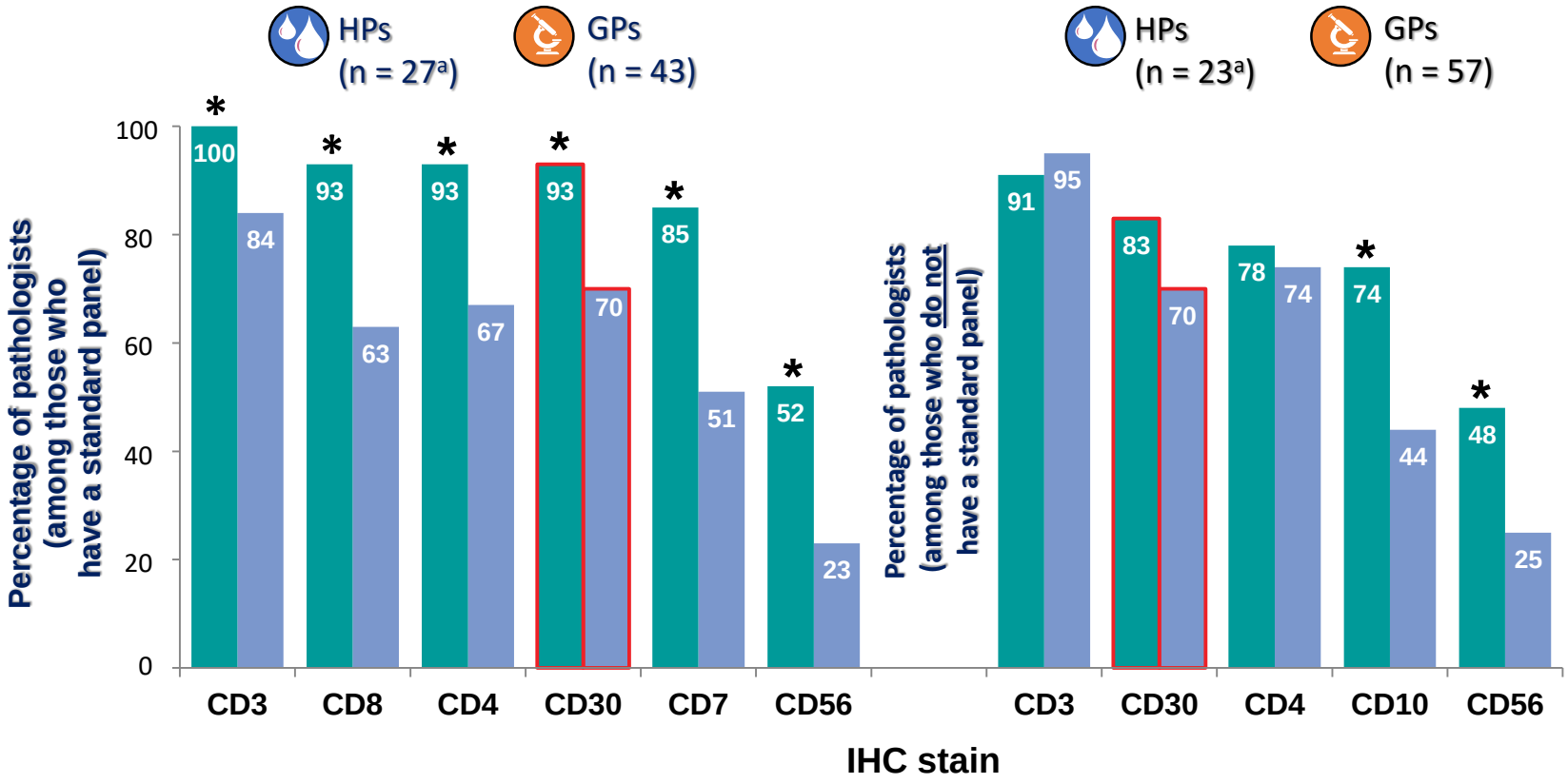
- A 20-minute online survey focusing on CD30 IHC and TCL diagnosis was distributed to 340 pathologists in 2014
  - board-certified or board-eligible
  - in practice for between 3 and 40 years and have
  - made ≥ 1 definitive TCL diagnosis in the past 6 months
- 150 pathologists (44%) completed the survey
- 33% were HPs
  - 48% of the HPs were academics



[a] Statistically significant.
GP, general practitioner; HP, haematopathologist.

Levak R, Slack GW. Am J Clin Pathol. 2015;144:A196 (abstract LP86).

Key IHC stains in standard panel for suspected TCL – an online survey study



Q211. Which of the following IHC stains are in your standard panel for suspected TCL?

Q216. Which IHC stains would you test for when the differential is a TCL?

*Statistically significant.
[a] Small base size.

Levak R, Slack GW. Am J Clin Pathol. 2015;144:A196 (abstract LP86).

What are the results of CD30 staining in different laboratories?

In 2015, 29% of 252 labs failed external QC for CD30 IHC with 4 unstained slides: tonsil, 2 cHL, and EC

- Over a 10-year period, CD30 staining assessment by NordiQC has shown a steady decline in achieving sufficient staining results, decreasing from 92% to 71% in 2015

Proportion of adequate results for CD30 IHC based on 4 NordiQC runs

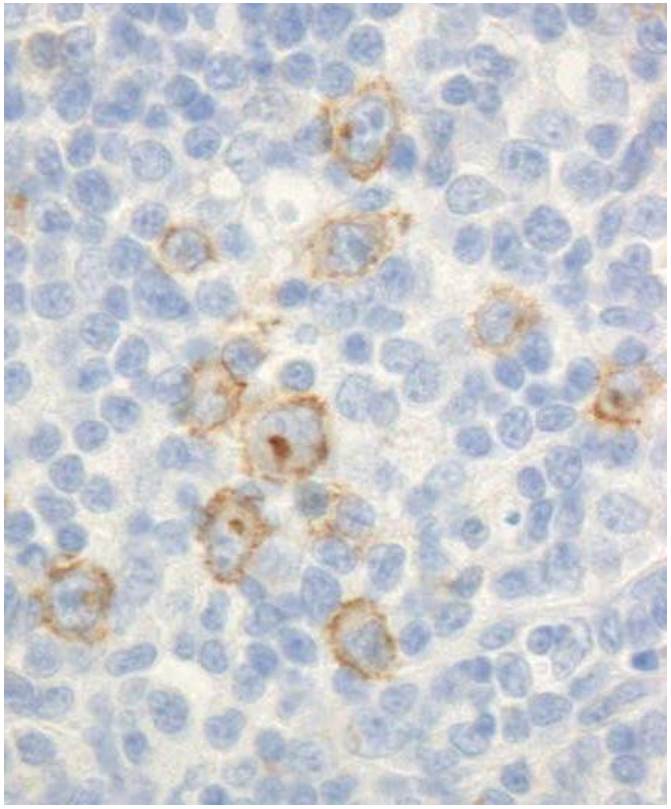
	Run 11 (2004)	Run 25 (2009)	Run 31 (2011)	Run 43 (2015)
Participants	74	126	172	252
Adequate results, %	92	78	77	71

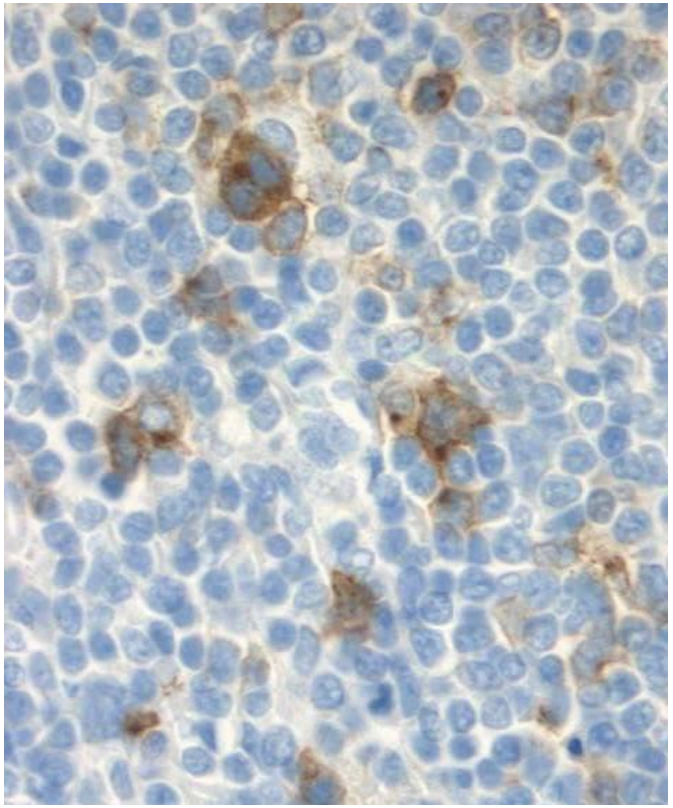
- Insufficient staining was observed in 95% of the laboratories failing QC for the 2015 test run – generally the result of weak staining or complete false-negative results

EC, embryonal carcinoma; NordiQC, Nordic Immunohistochemical Quality Control; QC, quality control.

Nordic Immunohistochemical Quality Control. CD30. Assessment Run 43 2015. Available from: http://www.nordiqc.org/epitope.php?id=5. Accessed July 2016.

Same case stained in two different labs

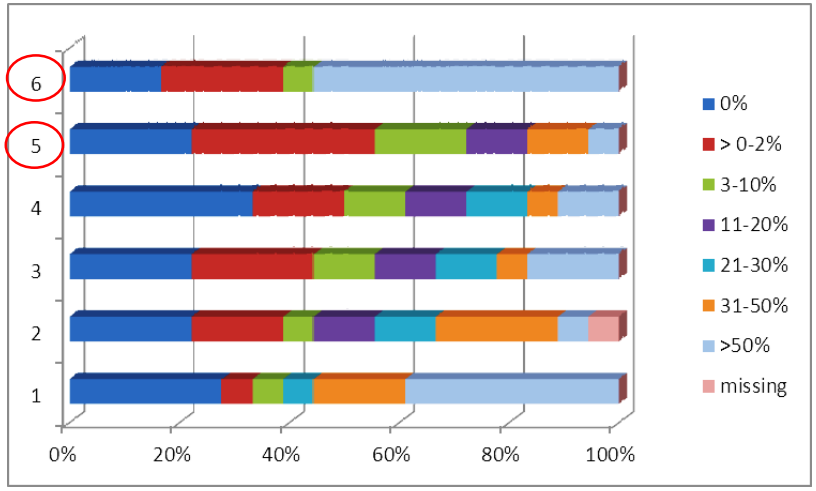


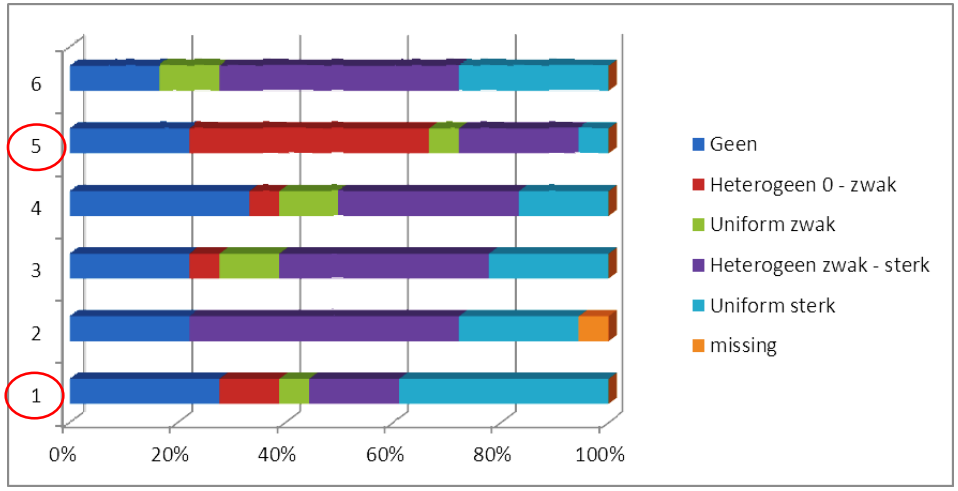


- Same block, same antibody, different technique

CD30 immunohistochemistry validation study by the HOVON Pathology Group

- Technical validation: TMA with 18 cases stained for CD30 in 5 different laboratories (2x DAKO, 3x Ventana)
- Scoring of the TMA slides by the pathologist of that specific laboratory





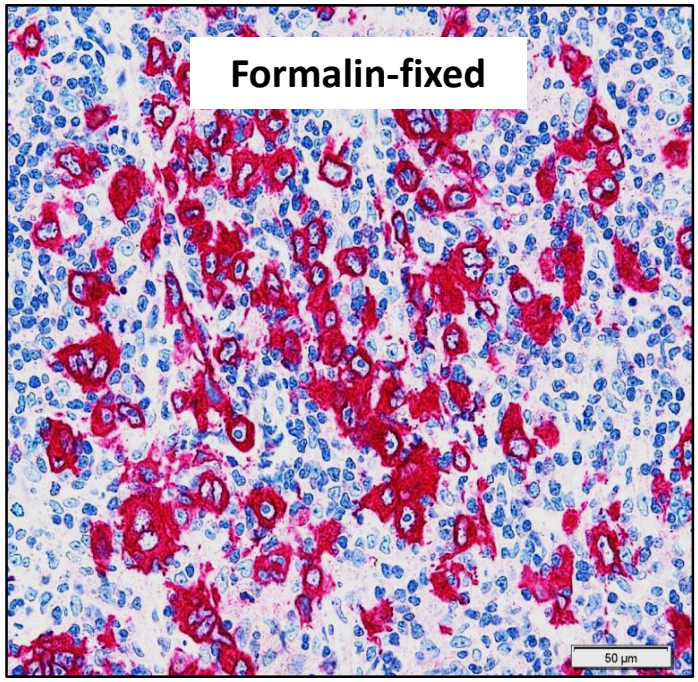
What can cause such situation?

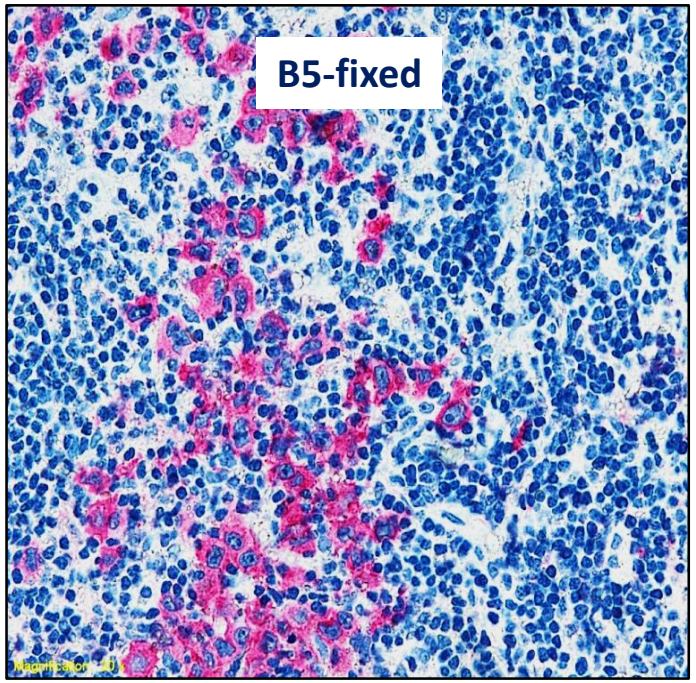
- Fixation
- Antigen retrieval
- Detection method
- Anti-CD30 antibody

- Possible aids:
- Automation
- Digital imaging
- mRNA expression

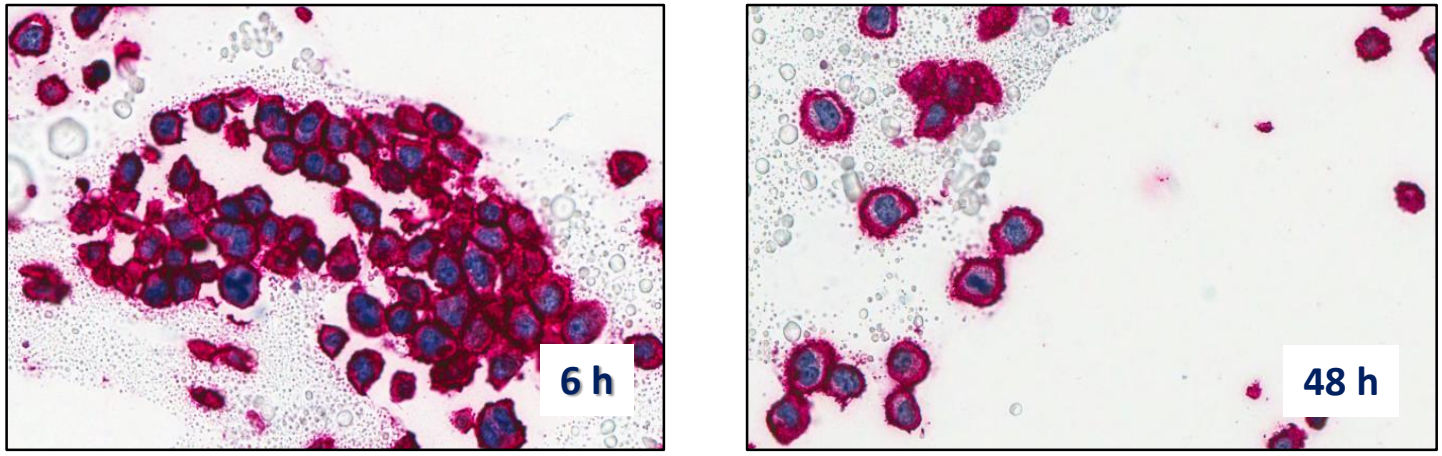
Fixation

- 10% buffered formalin (Lillie's) 24 h: optimal
- Bouin 4–6 h: acceptable
- B5 2–3 h: suboptimal





CD30 detectability is affected by the length of fixation



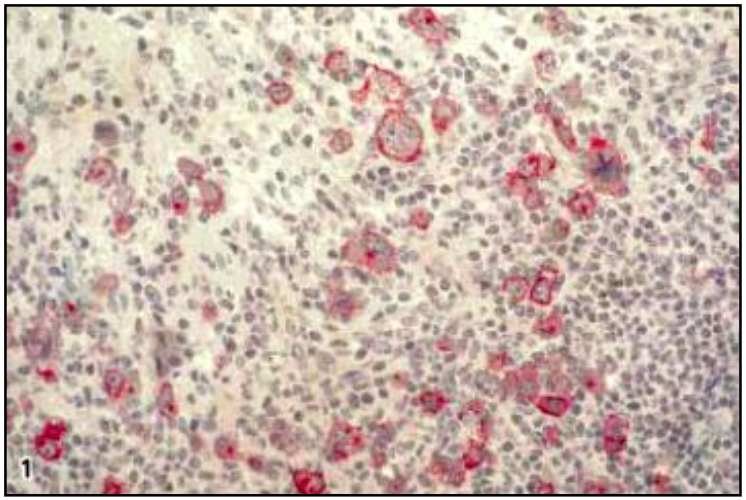
Karpas cell line fixed in 10% buffered (Lillie's) formalin at 6, 12, 18, 24, and 48 h

Antigen retrieval techniques in immunohistochemistry: comparison of different methods

STEFANO A. PILERI[1]*, GIOVANNA RONCADOR[1], CLAUDIO CECCARELLI[1], MILENA PICCIOLI[1], ASPASIA BRISKOMATIS[1], ELENA SABATTINI[1], STEFANO ASCANI[1], DONATELLA SANTINI[1], PIER PAOLO PICCALUGA[1], ORNELLA LEONE[1], STEFANIA DAMIANI[1], CESARINA ERCOLESSI[1], FEDERICA SANDRI[1], FEDERICA PIERI[1], LORENZO LEONCINI[2] AND BRUNANGELO FALINI[3]

Clone	Specificity	Source	Dilution	No AgR	PT	HBAR + citrate	HBAR + Tris-HCl	HBAR + EDTA
Ber-H2	CD30	Professor Stein	1:10	–	–	+ + – –	+ + + –	+ + + +
			1:320	–	–	+ – – –	+ + + –	+ + + +

Following 1 week of formalin fixation!



- MW: 3 cycles, 750 W, 5’ each
- PC: 1–2’ when at pressure

–, completely negative result.
+ – – –, weak positivity in a percentage of cells expected to be positive.
+ + – –, weak positivity in all cells expected to be positive.
+ + + –, moderately strong positivity in all cells expected to be positive.
+ + +, very strong positivity in all cells expected to be positive.

AgR, antigen retrieval; MW, microwave; PC, pressure cooking;
PT, proteolytic treatment; HBAR, heat-based antigen retrieval.

Pileri SA, et al. J Pathol. 1997;183:116-23.

Antigen retrieval

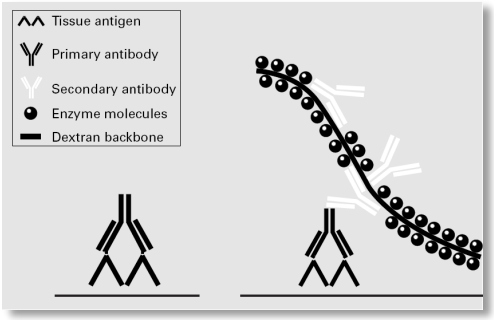
- Automated: PT-link
  EnVision™ flex target
  Retrieval solution (pH high)
  5 min at 92°C

Detection

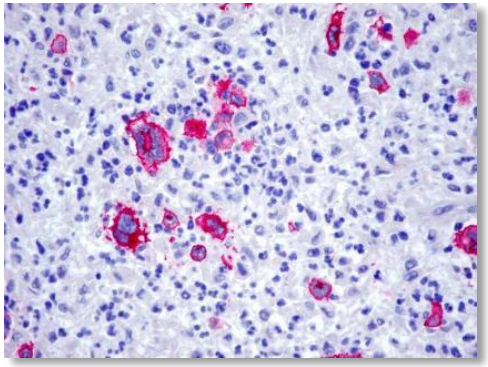
The EnVision™+ system: a new immunohistochemical method for diagnostics and research. Critical comparison with the APAAP, ChemMate™, CSA, LABC, and SABC techniques

E Sabattini, K Bisgaard, S Ascani, S Poggi, M Piccioli, C Ceccarelli, F Pieri, G Fraternali-Orcioni, S A Pileri

Antibody (clone if monoclonal/commercial source)	SABC/LSAB/APAAP	En Vision/CSA
CD1a (O10/Immunotech)	1:2	1:12
CD3 (polyclonal/Dako)	1:300	1:1200
CD5 (CLA229/Medac)	1:5 (inconsistent results)	1:20
CD8 (C8/144B/Dako)	1:5	1:20
CD15 (C3D-1/Dako)	1:5	1:40
CD20 (L26/Dako)	1:200	1:1000
CD21 (IF8/Dako)	1:10	1:40
CD23 (MHM6/Dako)	1:50	1:100
CD30 (Ber-H2/Prof. Stein)	1:10	1:40
CD34 (OBEND-10/Innunotech)	1:20	1:80
CD40 (MAB89/Dako)	1:100	1:400
CD43 (DF-T1/Dako)	1:200	1:800
CD45 (PD7/26+2B11/Dako)	1:500	1:1000
CD45R (UCHL-1/Dako)	1:120	1:480
CD45R (Ki-B3/Prof. Parwaresh)	1:20	1:80
CD57 (Leu7/Becton Dickinson)	1:20	1:40
CD45RA (4KB5/Dako)	1:20	1:80
CD61 (Y2-51/Dako)	1:5	1:5
CD68 (KP1/Dako)	1:640	1:2560
CD68 (PG-M1/Prof. Falini)	1:20	1:80
CD79a (JCB117/ Prof. Mason)	1:10	1:60
Glycophorin A (JC159/Dako)	1:320	1:1280
Neutrophilic elastase (NP57/Dako)	1:10	1:40
FVIIIRAg (F8/86/Dako)	1:6	1:24
Lysozyme (polyclonal/Dako)	1:800	1:3200
TdT (polycolonal/Dako)	1:30	1:80
Cyclin D1 (D1-GM/Novocastra)	Negative	Negative/1:40
κ light chain (polycolonal/Dako)	1:11000	1:22000
λ light chain (polycolonal/Dako)	1:13000	1:26000
MPO (polycolonal/Dako)	1:10000	1:40000
S-100 (polycolonal/Dako)	1:2000	1:8000



Moreover, with the EnVision™+ system the Ber-H2/CD30 – which unpredictably works in over-fixed material – continued to produce vivid staining even in samples soaked in formalin for 1 week



Many CD30 IHC assays are currently available: what are the implications regarding quality, technical failure, and cross-lab data comparison?

Antibodies and assessment marks for CD30, run 43

Concentrated antibodies	n	Vendor
mAb clone Ber-H2	102 9 2 1 1 1 1	Dako Cell Marque Thermo/Neomarkers Biosystems GeneMed Immunologic Zytomed Systems
mAb clone 1G12	9	Leica/Novocastra
mAb clone JCM182	5	Leica/Novocastra
mAb clone CON6D/5	3	Biocare
mAb clone 15B3	2	Leica/Novocastra
mAb clone HRS4	1	Thermo/Neomarkers
mAb EP154	1	Beijing Zhongsan
Ready-to-use antibodies	**n**	**Vendor**
mAb clone Ber-H2 IS/IR602	47	Dako
mAb clone Ber-H2 790-2926	25	Roche/Ventana
mAb clone Ber-H2 790-4858	25	Roche/Ventana

Ready-to-use antibodies	n	Vendor
mAb Ber-H2 MAD-002045QD	2	Master Diagnostica
mAb clone Ber-H2 MAB-0023	1	Maixin
mAb clone Ber-H2 MS-361-R7	1	Thermo/Neomarkers
mAb clone Ber-H2 AM327-5M	1	BioGenex
mAb clone Ber-H2 130M	1	Cell Marque
mAb clone JCM182 PA0790	5	Leica/Novocastra
mAb clone 1G12 PA0153	3	Leica/Novocastra
mAb clone 1G12 CD30-R-7-CE	2	Leica/Novocastra
mAb clone CON6D/5 PM346	1	Biocare
Total	252	

Nordic Immunohistochemical Quality Control. CD30. Assessment Run 43 2015. Available from: http://www.nordiqc.org/epitope.php?id=5. Accessed July 2016.

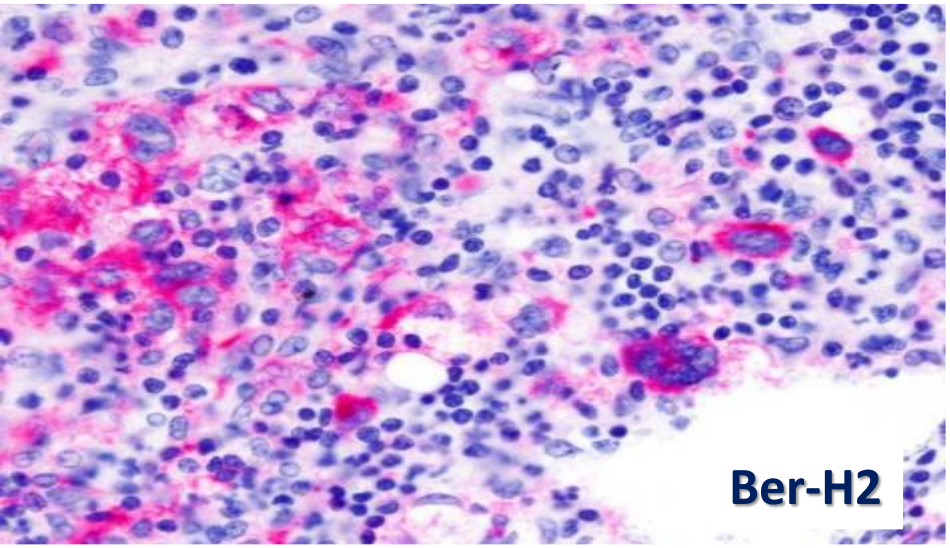
Comparative immunohistochemical studies: Ber-H2

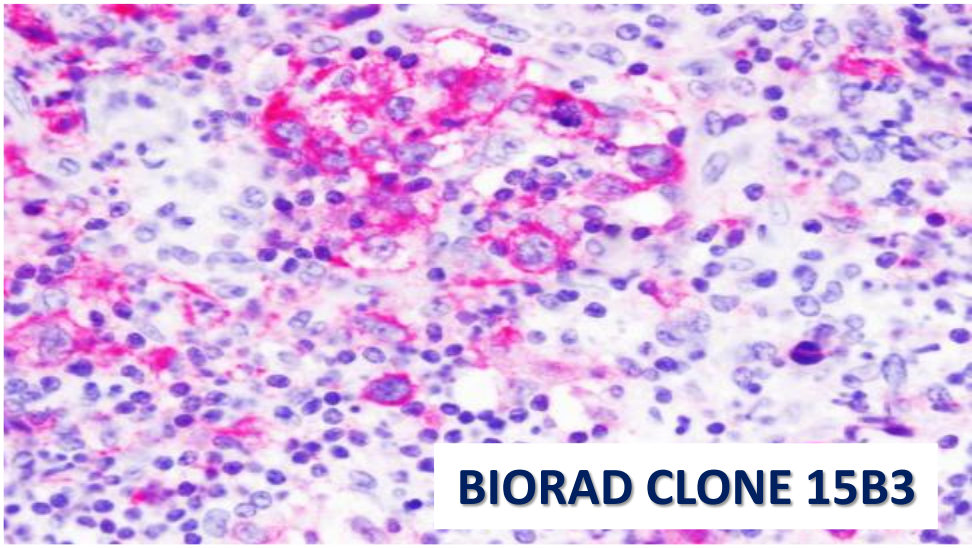
- CD30 BIORAD CLONE 15B3 (diluted 1:40)
- CD30 AbCAM CLONE CD30/412 (diluted 1:200)
- CD30 RAYBIOTECH CLONE MEM268 (diluted 1:200)

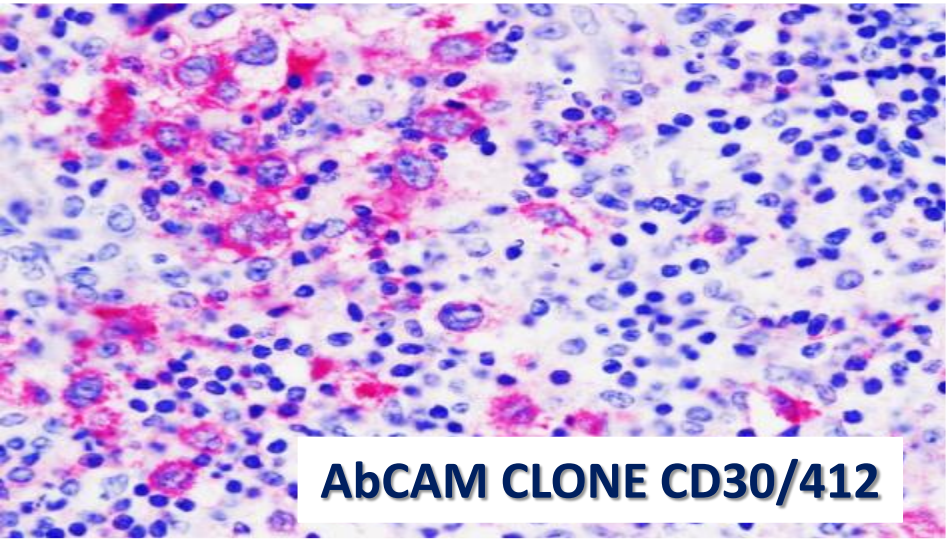
Tested on: DLBCL $CD30^-$
ALK^+ and ALK^- ALCLs
CHL

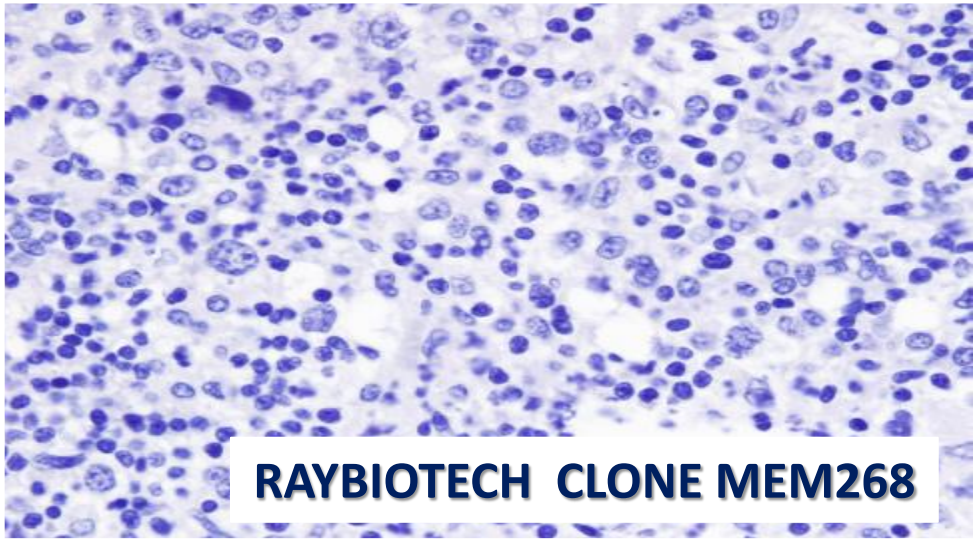
The antibodies produced equivalent results but the clone from RAYBIOTECH gave no staining in all experiments

PT-Link 92°C, alkaline phosphatase


Ber-H2


BIORAD CLONE 15B3

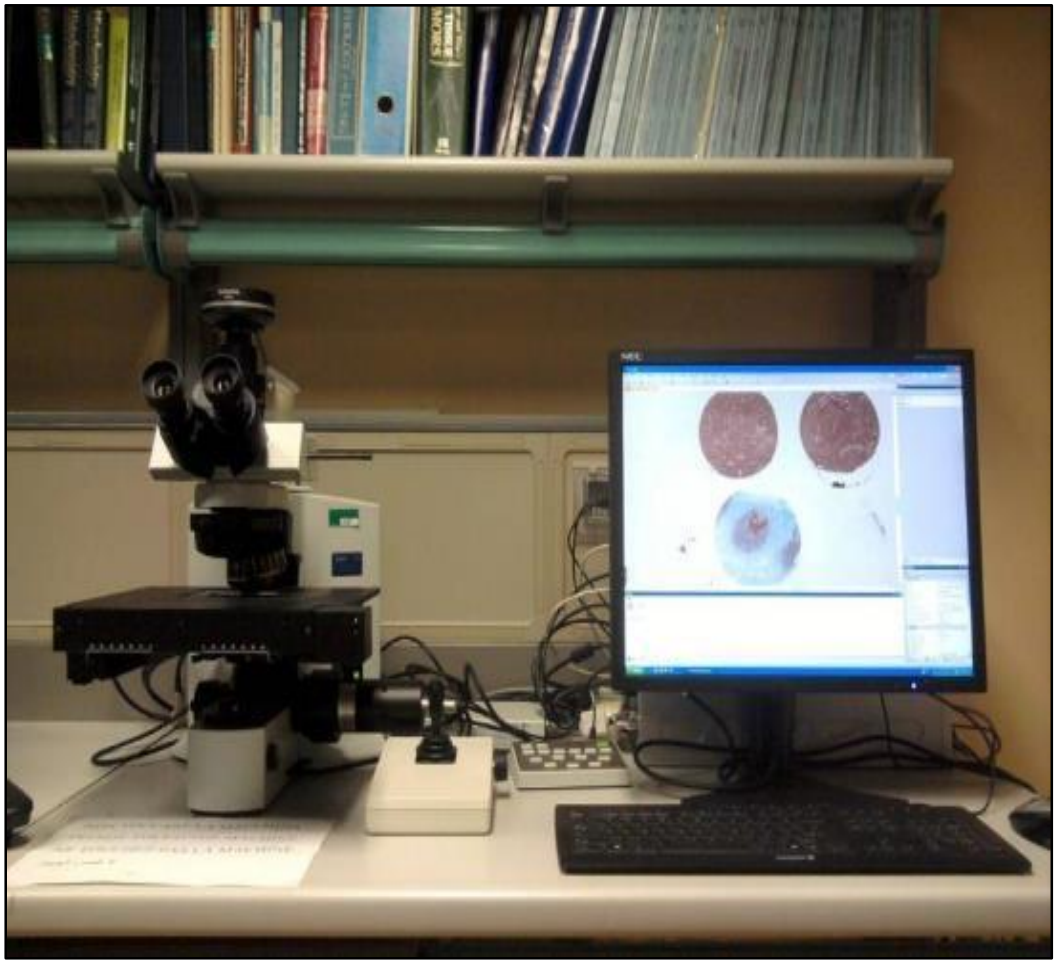

AbCAM CLONE CD30/412

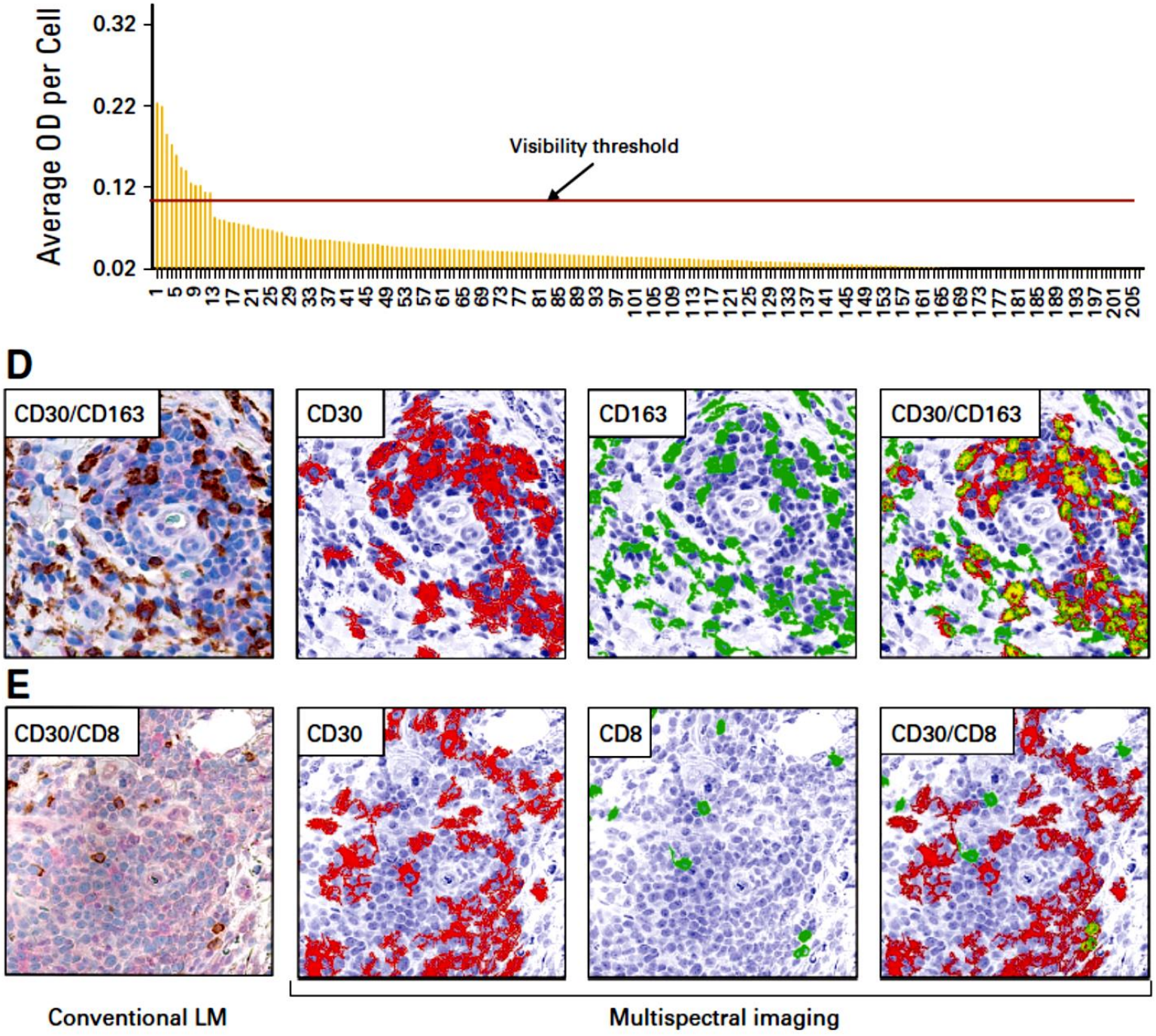

RAYBIOTECH CLONE MEM268

Automation contributes to standardization!

Interpretation by digital image

- Staining intensity
- Location
- P% of (+) cells

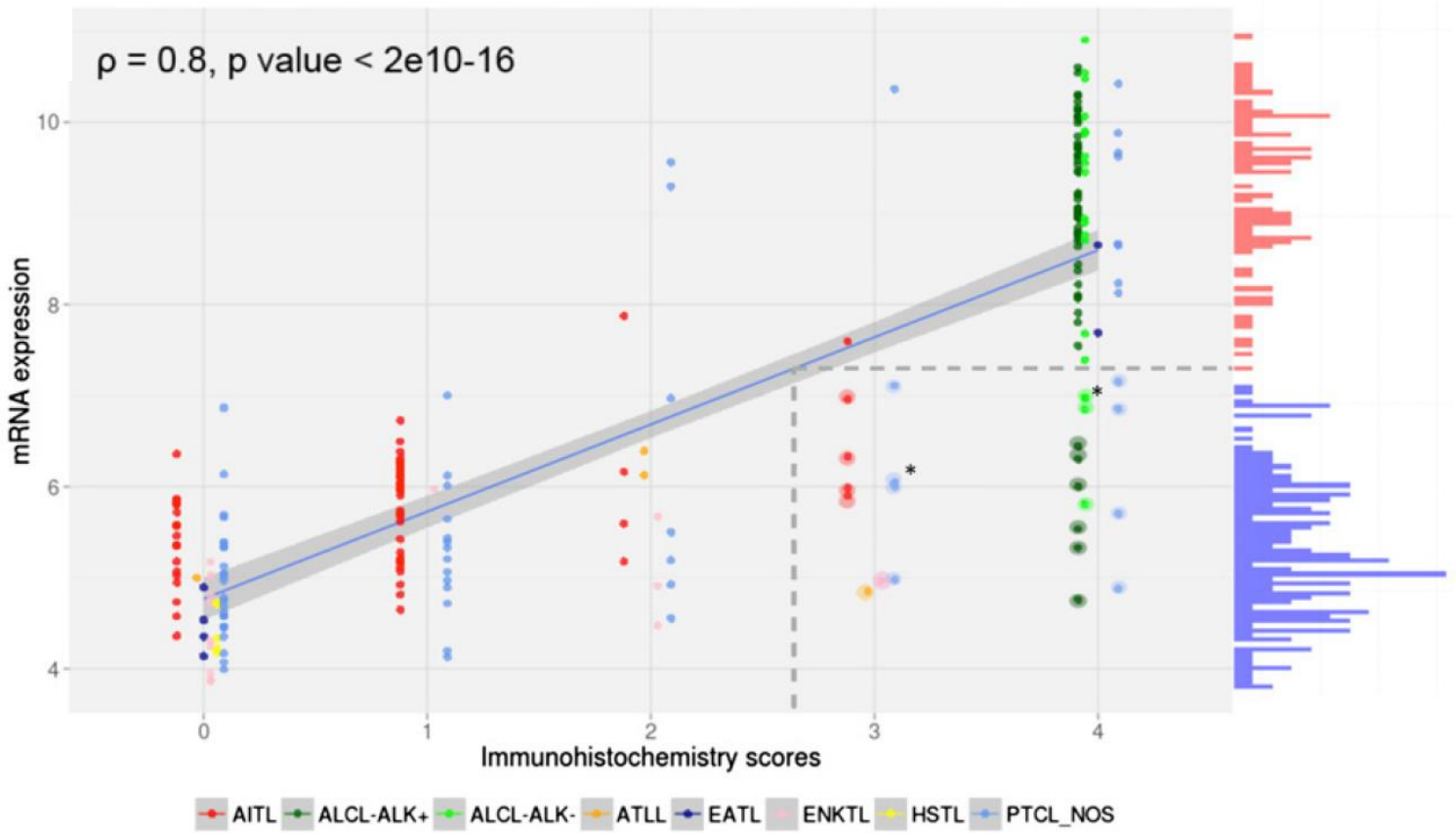



Average OD per Cell
0.32
0.22
0.12
0.02
Visibility threshold
1 5 9 13 17 21 25 29 33 37 41 45 49 53 57 61 65 69 73 77 81 85 89 93 97 101 105 109 113 117 121 125 129 133 137 141 145 149 153 157 161 165 169 173 177 181 185 189 193 197 201 205
D
CD30/CD163
CD30
CD163
CD30/CD163
E
CD30/CD8
CD30
CD8
CD30/CD8
Conventional LM
Multispectral imaging

Correlation between CD30 mRNA and protein expression

Immunohistochemistry as a valuable tool to assess CD30 expression in peripheral T-cell lymphomas: high correlation with mRNA levels

Céline Bossard,[1] Maria Pamela Dobay,[2] Marie Parrens,[3] Laurence Lamant,[4] Edoardo Missiaglia,[2] Corinne Haioun,[5] Antoine Martin,[6] Bettina Fabiani,[7] Richard Delarue,[8] Olivier Tournilhac,[9] Mauro Delorenzi,[2,10,11] Philippe Gaulard,[12,13,14] and Laurence de Leval[15]



Bossard C, et al. Blood. 2014;124:2983-6.


nanoString

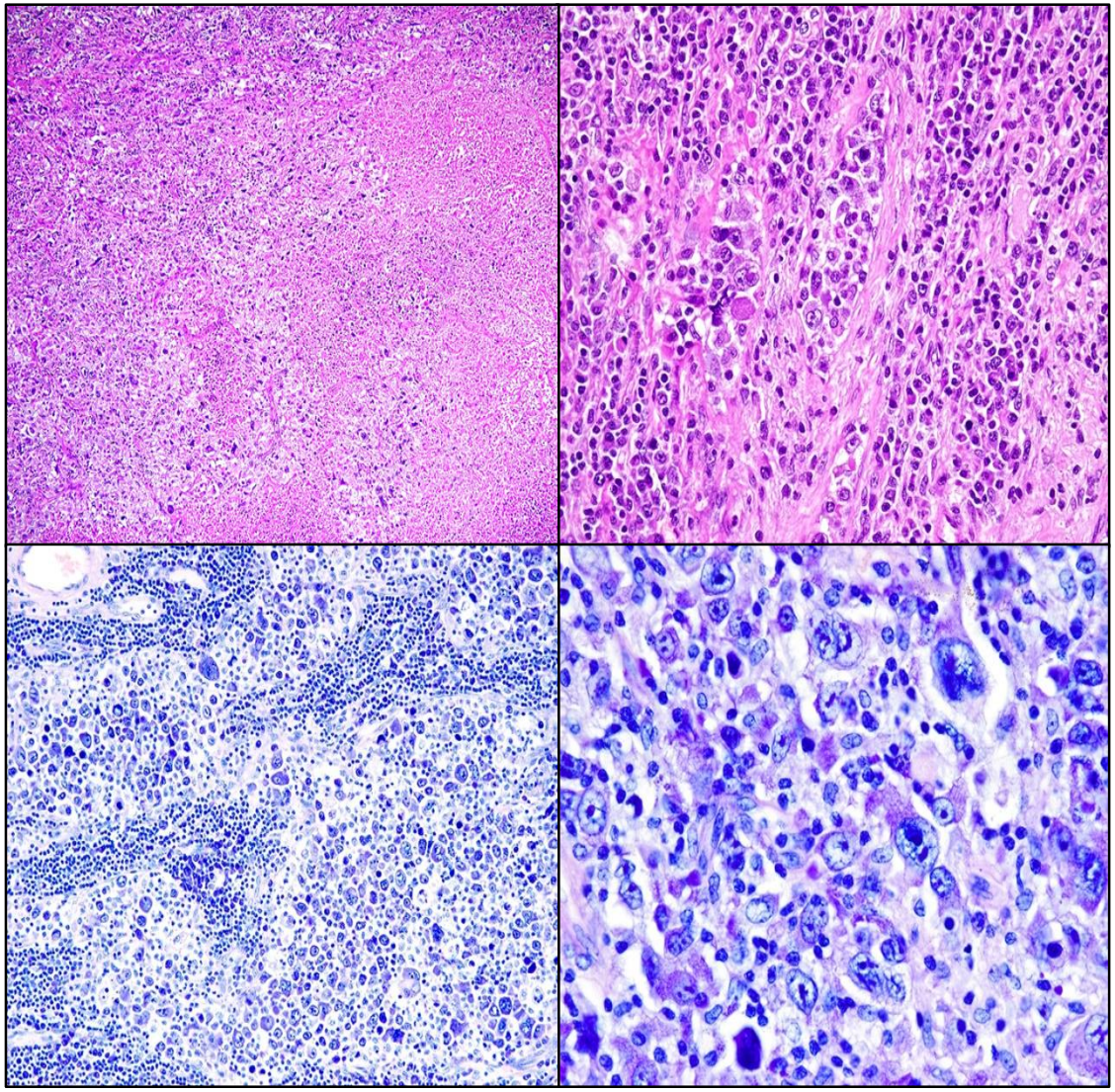
CD30 result interpretation

A complex and puzzling lymphoma case

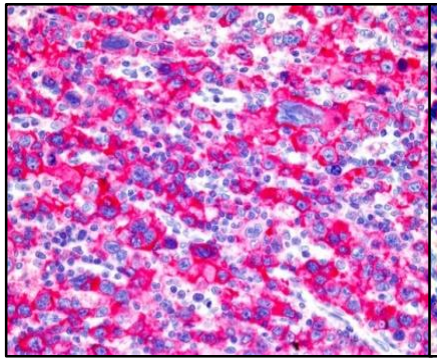
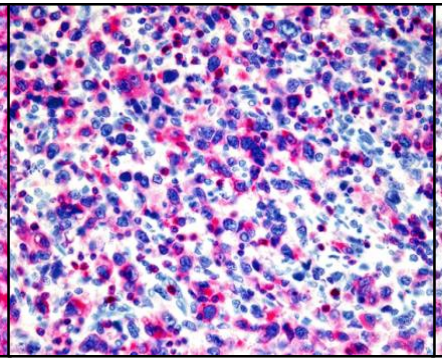
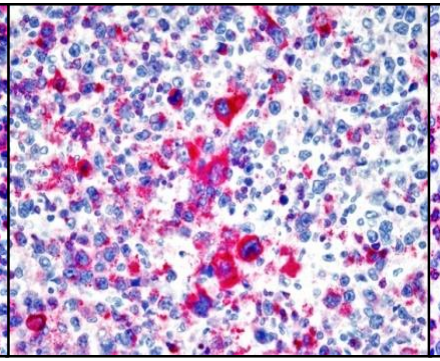
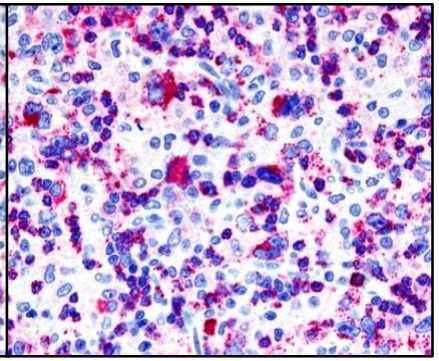
Clinical data

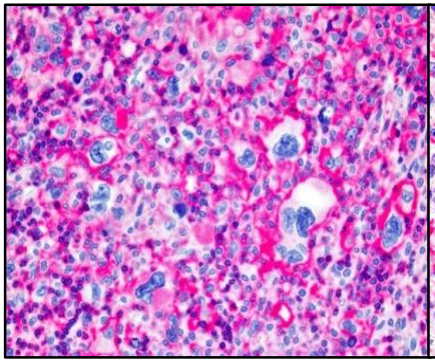
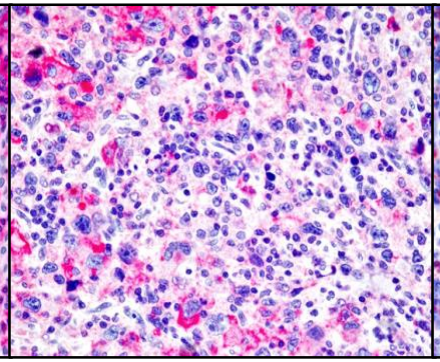
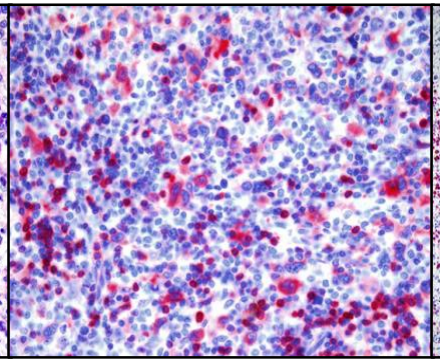
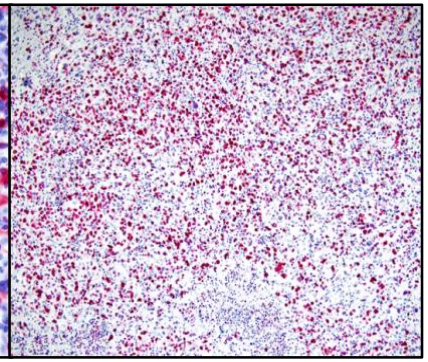
- February 2016
- 66-year-old male
- Latero-cervical lymph node enlargement, mild fever, asthenia, cough, and weight loss (6 kg) during the last 2 months
- Laboratory tests: anaemia (Hb 8.7 g/dL), thrombocytopenia (111 x 10^3/µL), relative lymphopenia (15%), and monocytosis (17%)
- $CD4^+$ lymphocytes (378/2,620/µL)
- PET-CT scan: hypermetabolic lesions in pleura, bones, spleen, and abdominal and cervical lymph nodes

Cervical lymph-node biopsy

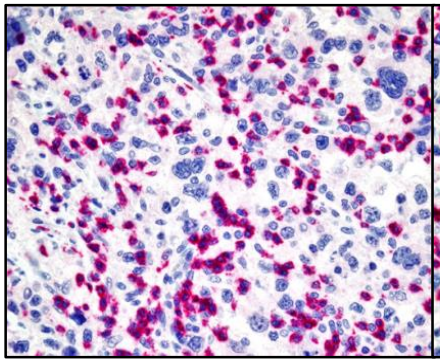
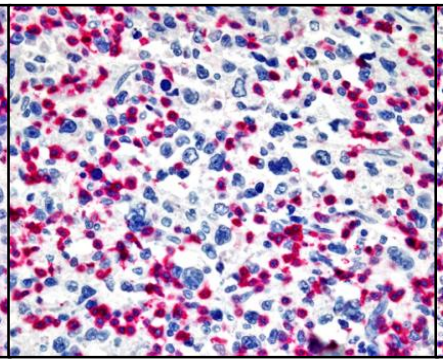
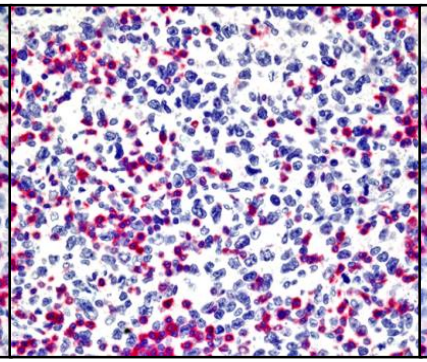
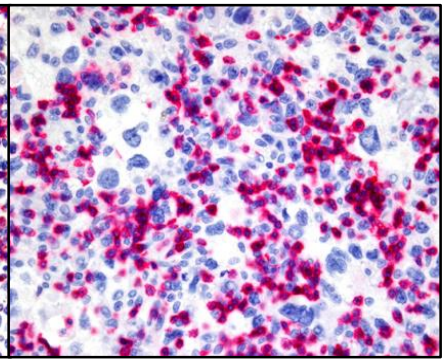


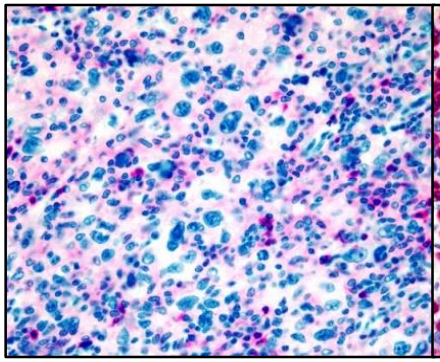
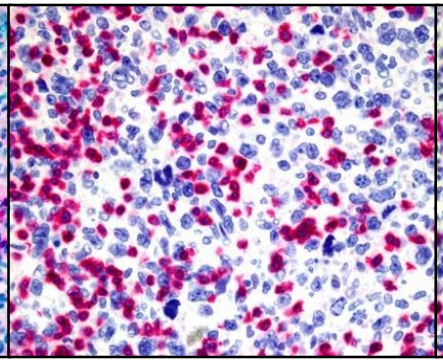
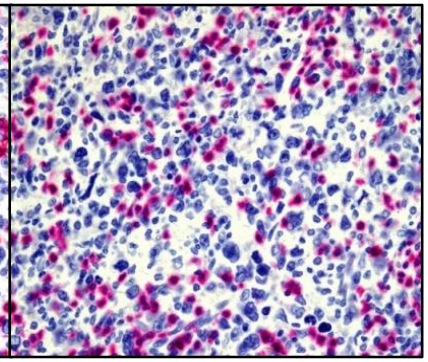
Initial panel

CD30	Perforin	GrB	TIA
			
Strongly positive	Positive	Positive	Positive

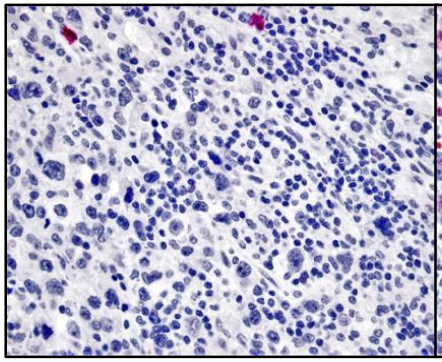
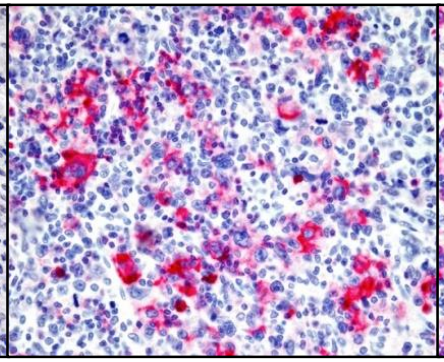
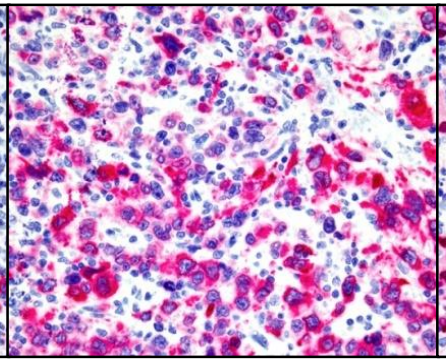
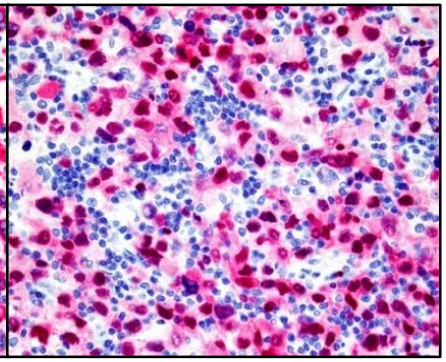
CD45	EMA	BCL-2	MIB1
			
Negative	Weak focally positive	Weak focally positive	90–100% positive

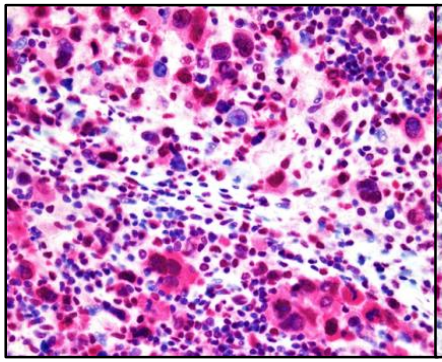
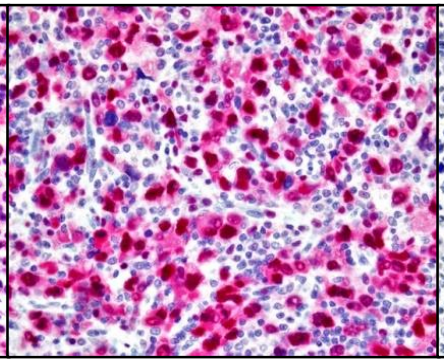
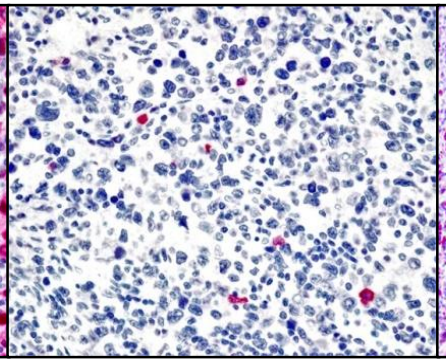
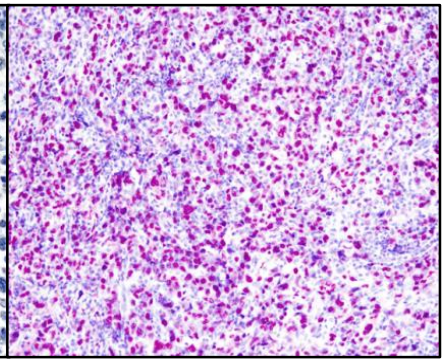
Additional stains

CD3	CD2	CD5	CD43
			
Negative	Negative	Negative	Negative

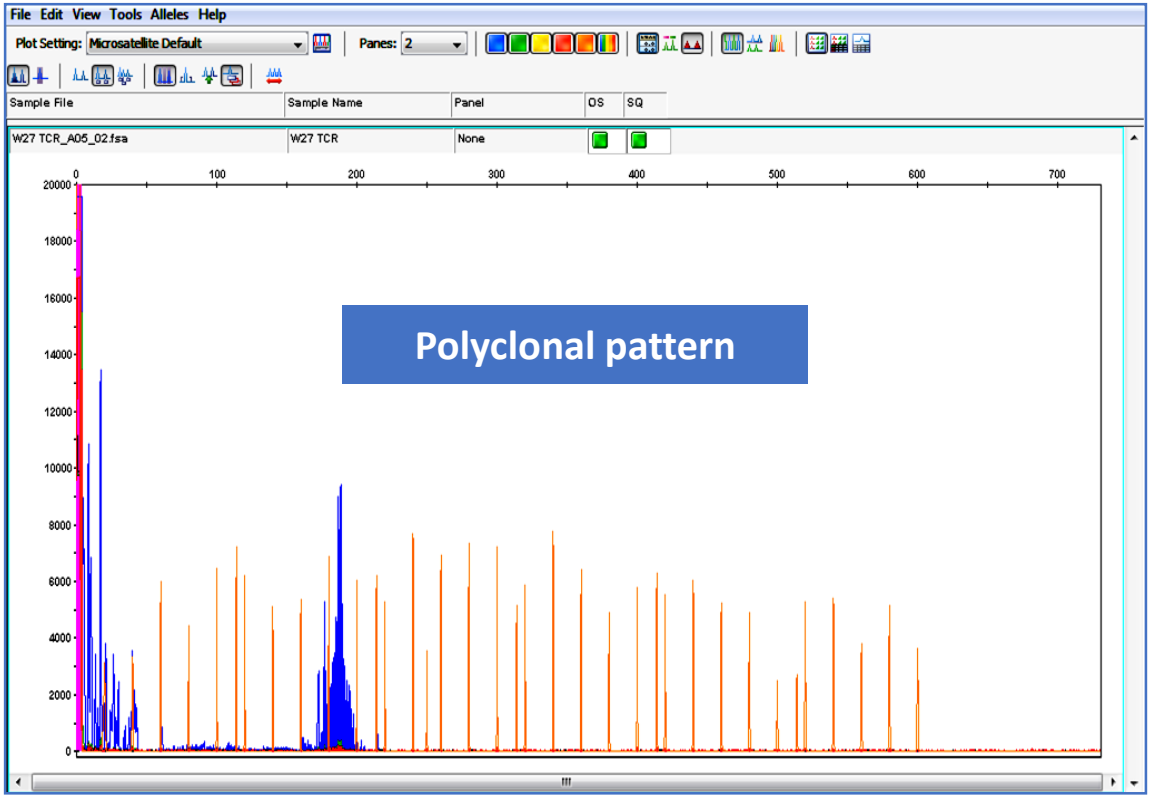
CD4	CD7	CD8
		
Negative	Negative	Negative

Additional stains

CD15	CD20	CD79a	PAX-5
			
Negative	Focally positive	Positive	Positive

BOB.1	Oct.2	CD138	EBER
			
Positive	Positive	Negative	Uniformly positive

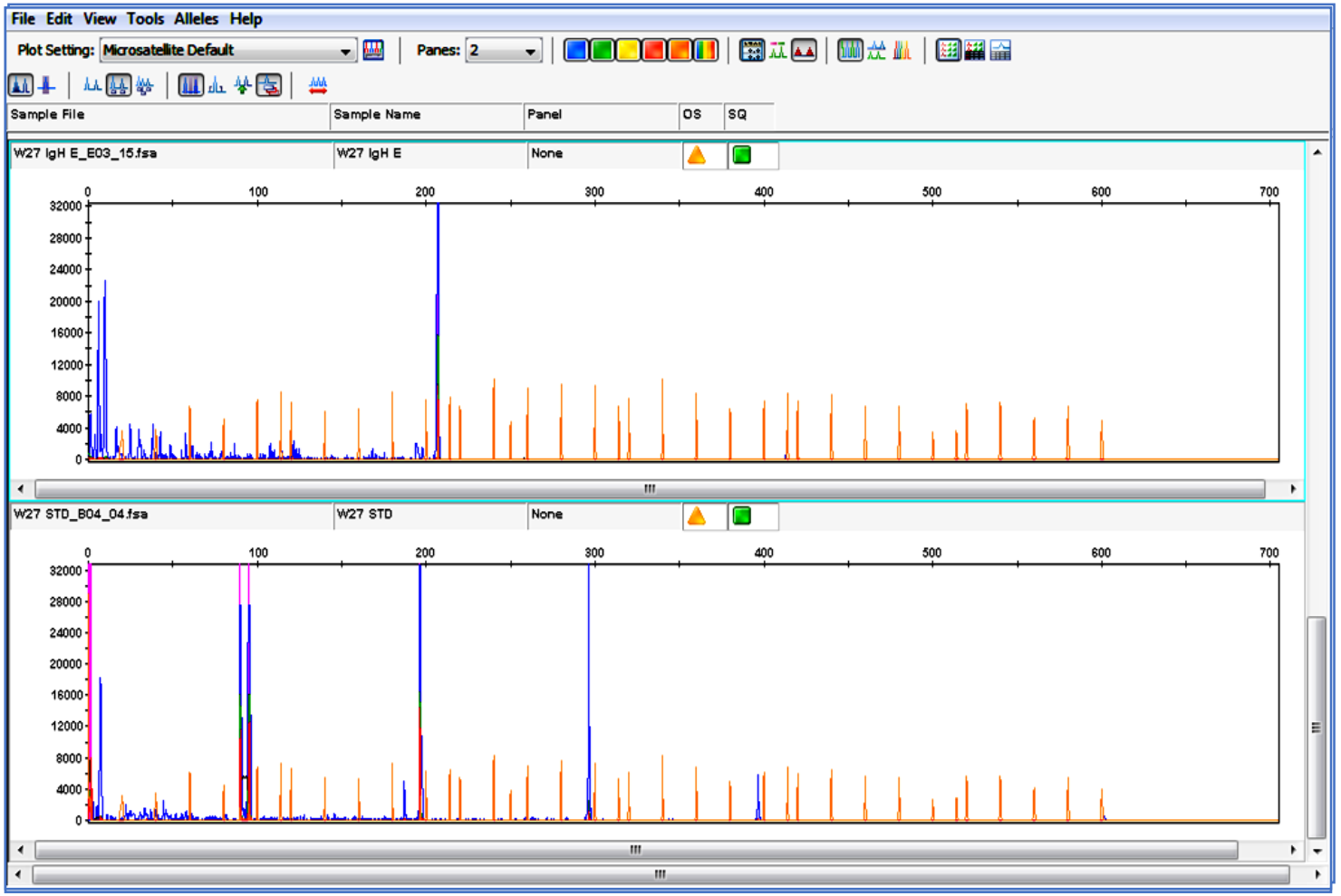
Molecular analysis for TR clonality



Summary table

Peak #	Product size	Peak height	D(x)	% RFU	RPR ratio	Significant
1	176.78	5,286	0.0414	56.3	7.66	No
2	172.69	2,816	0.0337	30.0	18.65	No
3	188.84	9,394	0.0289	100.0	1.81	No
4	187.84	9,314	0.0539	99.1	1.04	No
5	186.85	8,988	0.1014	95.7	1.96	No

Molecular analysis for IGH clonality



Final diagnosis and clinical outcome

- EBV copies: 322,500/mL in plasma and 764/mL in CSF
- HIV RNA levels: 5,732,000 copies in plasma and 300 copies in CSF
- Pre-phase treatment with vincristine + intrathecal prophylaxis with MTX, AraC, and steroids
- CHOEP started but was interrupted soon after because of intestinal perforation; perforation caused the patient's demise

Diagnosis: HIV infection-associated DLBCL, anaplastic variant, possibly stemming from B-reg cytotoxic B lymphocytes

Tabanelli V, Santiago-Pacheco V, Corbellino M, Calleri A, Agostinelli C, Parravicini C, Pileri SA. Histopathology.70:650-6, 2017.

CD30 expression–response enigma

Comment on Jacobsen et al, page 1394

CD30: seeing is not always believing

Kristie A. Blum THE OHIO STATE UNIVERSITY COMPREHENSIVE CANCER CENTER

Key points:

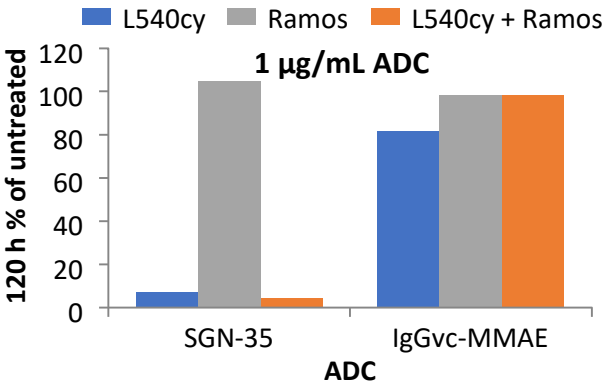
1. CD30 expression by IHC was not correlated to response to a CD30-targeted agent (immune conjugate)
2. Patients with undetectable CD30 by IHC may respond to an immune conjugate and vice versa
3. Discrepancies were observed between CD30 detection by IHC and CD30 detection with more sensitive techniques, thus suggesting possible failure of IHC

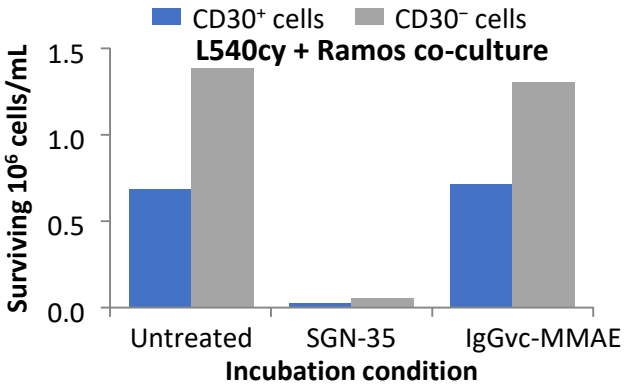
Blum KA. CD30: seeing is not always believing. Blood. 2015;125:1358-9.

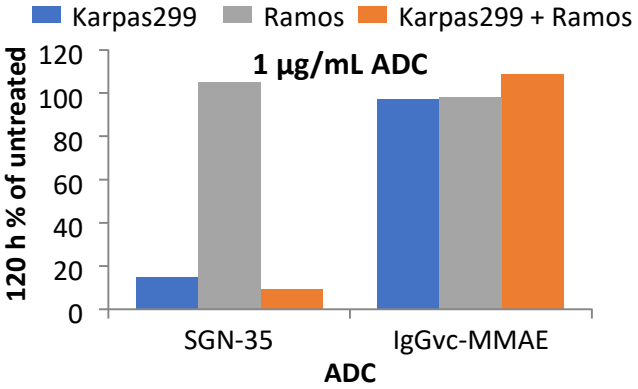
What can cause such a paradoxical situation?

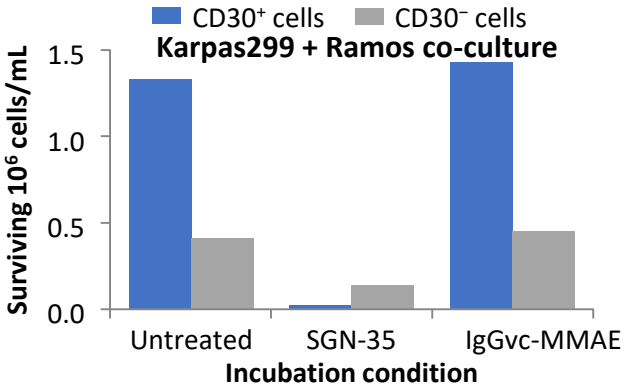
- **Bystander neoplastic cells**
- Microenvironment
- Limitations of IHC

CD30 targeting on mixed cell-line cultures









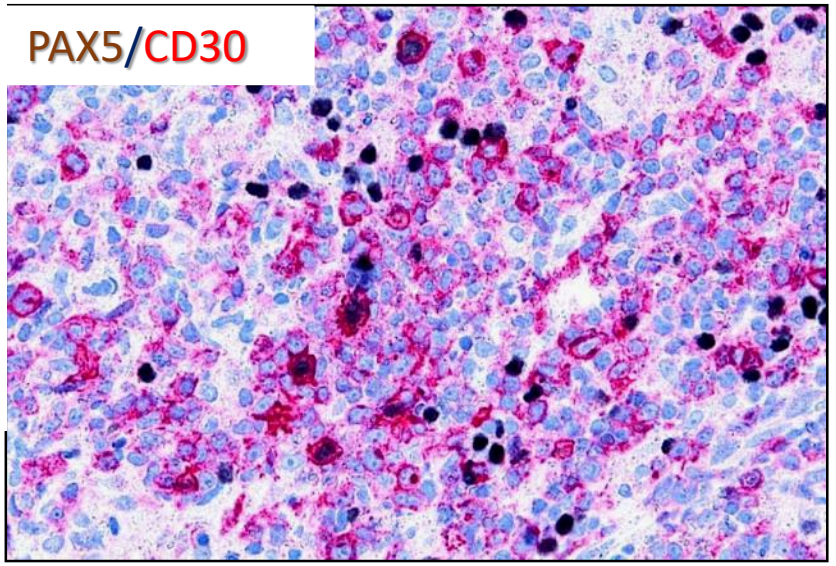
L540cy: HL-derived CD30⁺ cell line
Karpas299: ALCL-derived CD30⁺, CD19⁻ cell line
Ramos: CD30⁻, CD19⁺ cell line
IgGvc-MMAE: nonbinding control

ADC, antibody-drug conjugate.

Okeley NM, et al. Intracellular activation of SGN-35, a potent anti-CD30 antibody-drug conjugate. Clin Cancer Res. 2010;16:888-97.

What can cause such a paradoxical situation?

- Bystander neoplastic cells
- Microenvironment
- **Limitations of IHC**

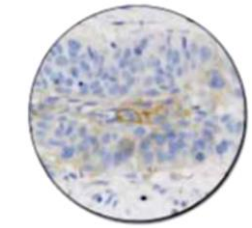


AITL

Do we need guidelines for CD30 IHC testing?

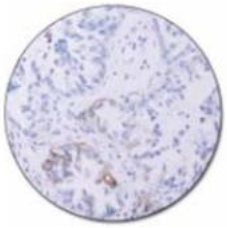
Guidelines for CD30 IHC testing will reduce technical failure and variability

- Pre-analytical parameters are critical to achieving optimal results



PD-L1 IHC 22C3 pharmDx for Autostainer Link 48

PD-L1 IHC 22C3 pharmDx is a qualitative immunohistochemical assay



PD-L1 IHC 28-8 pharmDx for Autostainer Link 48

PD-L1 IHC 28-8 pharmDx is a qualitative immunohistochemical assay

DAKO

VENTANA PD-L1 (SP142) Assay

VENTANA PD-L1 (SP263) Assay

Motor racing is dangerous.
What about pathology?

Misano World Circuit «Marco Simoncelli», Mini Challenge Professional, March 2018