

# CD3-CD4<sup>+</sup> lymphoid variant of hypereosinophilic syndrome: nodal and extranodal histopathological and immunophenotypic features of a peripheral indolent clonal T-cell lymphoproliferative disorder

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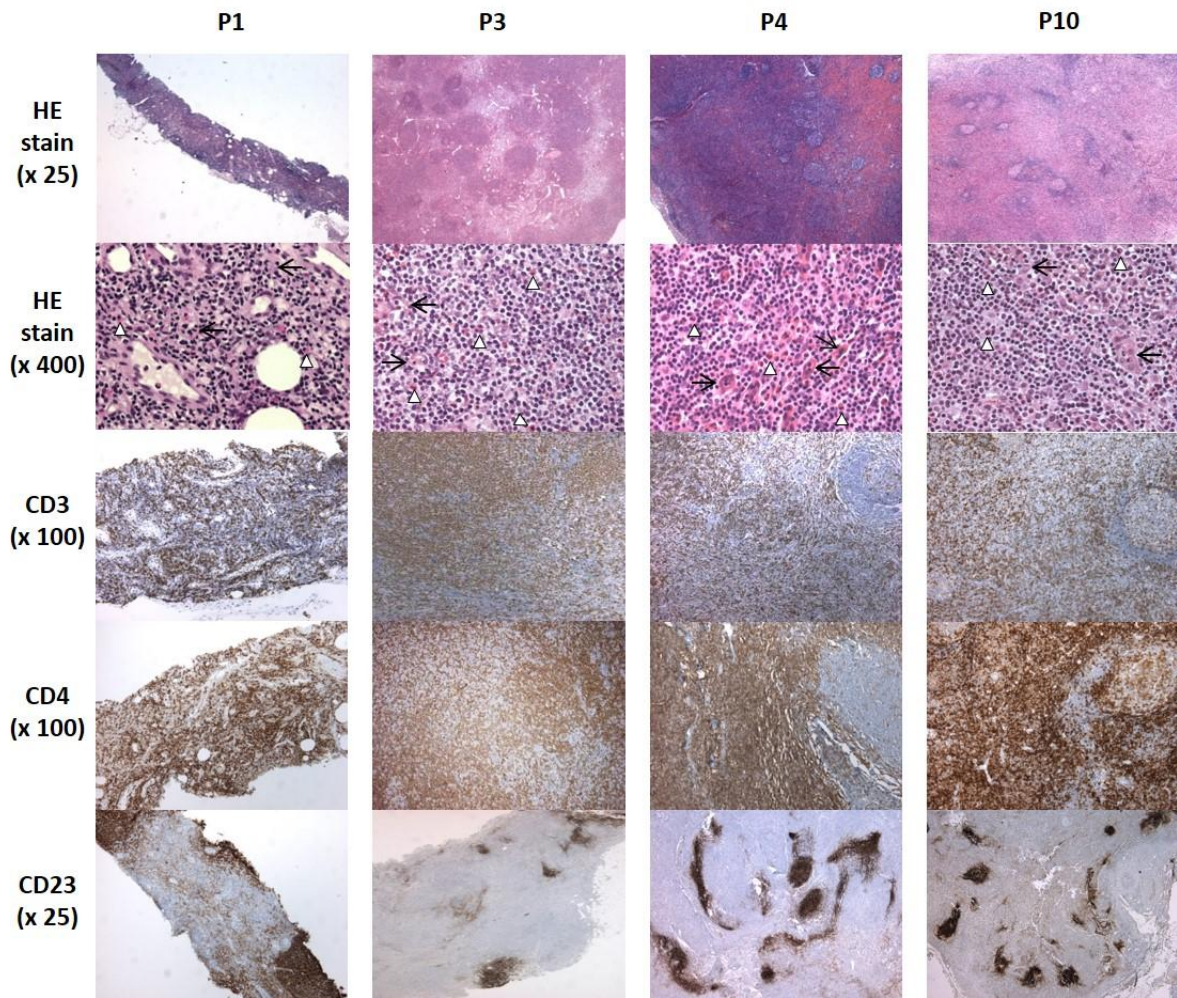
## Supplemental information

### **CD3-CD4+ lymphoid variant of hypereosinophilic syndrome: nodal and extranodal histopathological and immunophenotypic features of a peripheral indolent clonal T-cell lymphoproliferative disorder.**

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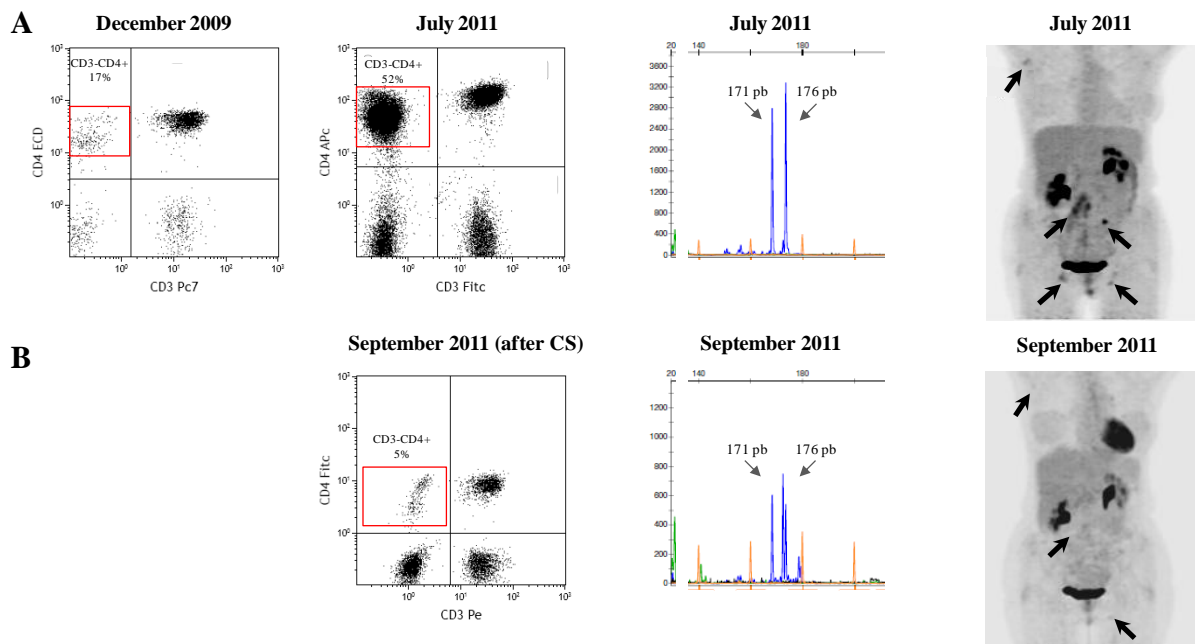
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**Figure S1.** Lymph nodes histopathology in CD3-CD4+ L-HES

Histopathological characteristics of lymph nodes (LN) in 4 CD3-CD4+ L-HES patients. Hematoxylin and Eosin (HE) staining revealed a slight (ex: patient P10) to dense lymphoid infiltration responsible of an effacement of LN architecture (patients P3 and P4). LN architecture is not appreciable on LN needle biopsy of patient P1. In all cases, lymphoid infiltrates spare the peripheral cortical sinuses (not appreciable in patient P1) and cases are composed of CD3+CD4+ T-cells, without any CD10, CXCL13 or PD1 positive T-cells (not shown). The other characteristics are the presence of numerous eosinophils ( $\Delta$ ), rare plasma cells, high endothelial veinules (black arrows), and increased of CD23+ follicular dendritic cells meshwork.

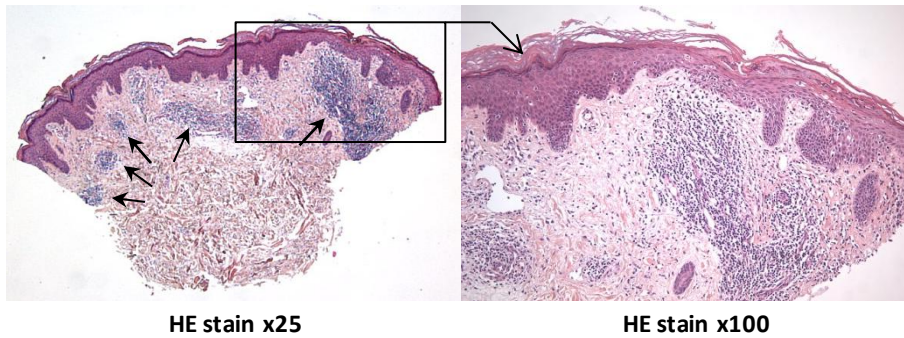


**Figure S2.** Favorable evolution of lymph node involvement in a CD3-CD4+ L-HES patient (P10).

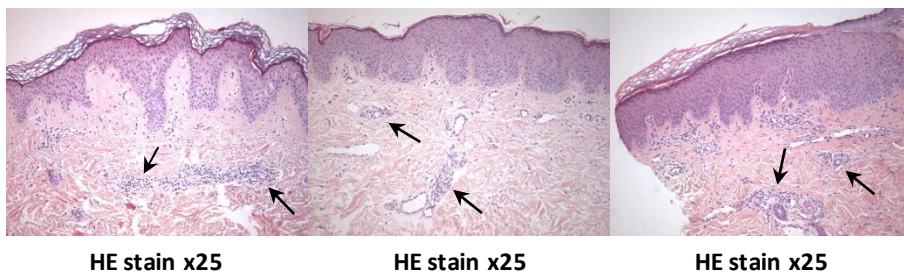
CD3-CD4+ L-HES diagnosis made in 2009 in patient P10 was based on a long-history of blood HE (23 years), an eosinophilic colitis (atypical CD4+ lymphoid infiltrates were observed a posteriori), circulating CD3-CD4+/low T-cells and a clonal TCR $\gamma$  rearrangement. In July 2011, digestive symptoms worsened, eosinophils count increased to 23 G/L and she developed multiple adenopathies. CD3-CD4+ T-cell subsets increased from 17% to 52% of total lymphocytes, TCR $\gamma$  rearrangement study showed the same circulating T-cell clone as in the lymph node (see Fig 2) and 18-Fluoro-deoxyglucose positron emission tomography (18-FDG-PET) showed multiple adenopathies (arrows) (A). Histopathological examination of a coeliomesenteric lymph node did not conclude in a lymphoma (Figure S1) and corticosteroids were started at 0.5 mg/kg/day: 2 months later, the eosinophil count became normal, digestive symptoms disappeared, the circulating CD3-CD4+ T-cell subset decreased, clonal TCR $\gamma$  rearrangement persisted and 18-FDG-PET dramatically improved (B).



**A: 2002**

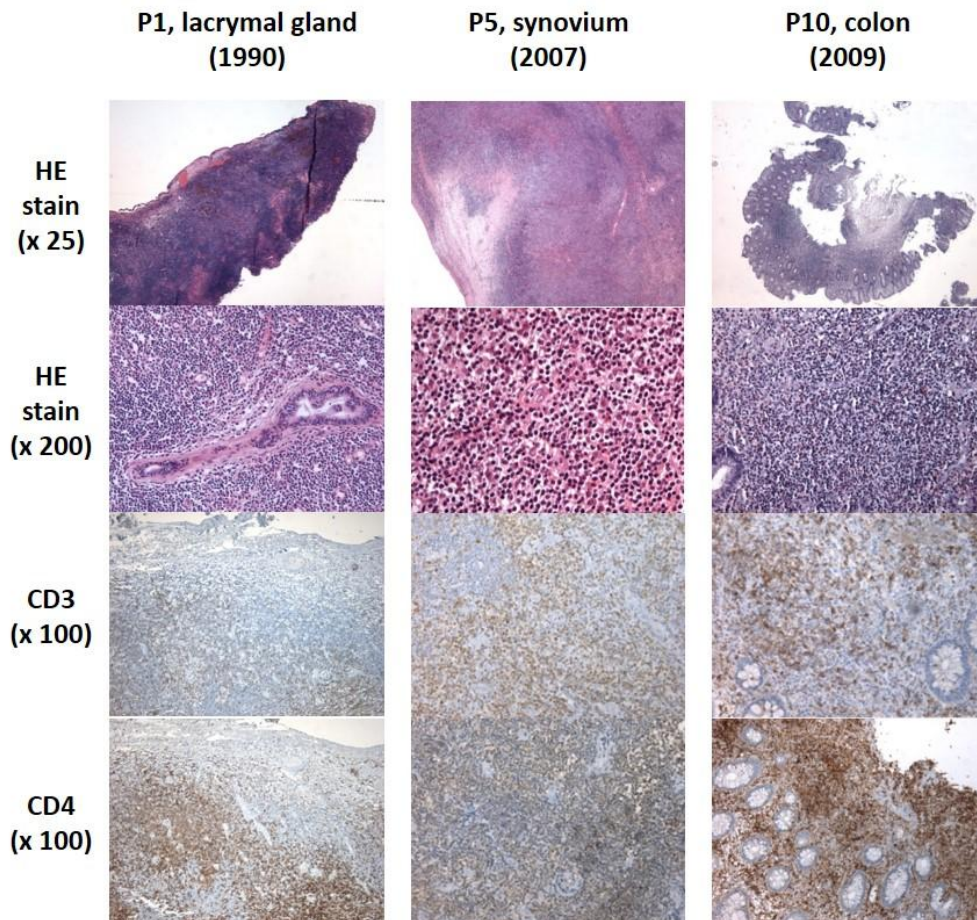


**B: 2005, 2009, 2011**



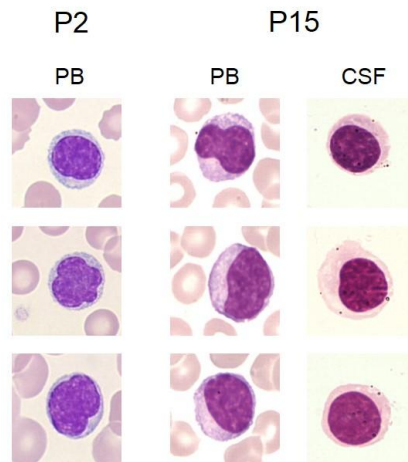
**Figure S3.** Persistence of skin lymphoid infiltrates in a CD3-CD4+ L-HES patient (Ex: patient P8)

At diagnosis in 2002 (A), skin biopsy found dense nodular and perivascular lymphoid infiltrates (arrows) composed of CD2+CD3+CD4+CD5+ T-cell infiltrates (immunohistochemistry not shown), which decreased but persisted over years (B) under corticosteroids alone (since 2002), and then after corticosteroids and mepolizumab (since 2006). Coherently, the first T-cell immunophenotype performed in 2005 showed a CD3-CD4+ circulating subset which decreased from 21% (0,71 G/L) to 6,9% (0,11 G/L) in 2012.



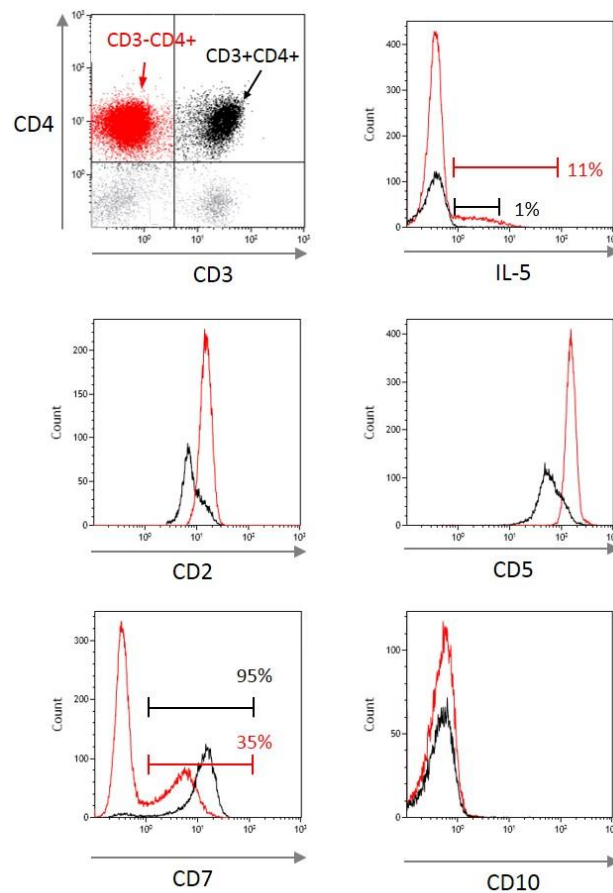
**Figure S4.** Other extra-nodal tissue histopathology in CD3-CD4+ L-HES

Patient P1 had a refractory generalized eczema and “Kimura like” disease with parotid gland enlargement (biopsy demonstrated an infiltration by eosinophils, but was not available for reanalysis), enlarged cervical lymph nodes, subcutaneous swelling, but also lacrymal gland enlargement. The lacrymal glands were highly modified by dense inflammatory infiltrates composed of lymphoid cells and eosinophils. The same eosinophil and lymphoid infiltrates were found in a synovial biopsy of patient P5 who presented a bilateral teno-synovitis of the wrists and intercarpal joints in 2007, and in a colon biopsy of patient P10 who received the diagnosis of eosinophilic colitis in 2009. In all cases, immunohistochemical staining revealed the presence of CD3+CD4+ T-cells without any CD10, CXCL13 or PD1 positive T-cells (not shown). A clonal TCR $\gamma$  rearrangement was detected in all three tissues and still detected in peripheral blood of all three patients in 2013-2014, 23, 7 and 5 years after these biopsies, respectively. All three patients remain in good health status under low-dose corticosteroids (CS) alone (P5 and P10), or under CS and interferon-alpha (P1).



**Figure S5.** Morphology of circulating T-cells of two representative patients, and in cerebrospinal fluid in one patient

As shown here for patients P2 and P15, aberrant circulating T-cells (PB, peripheral blood) were small to medium-sized, with regular nuclei with condensed and sometimes clumped chromatin, or irregular, indented nuclei, with a moderately abundant cytoplasm (such cytological abnormalities was observed in patients with more than 20 % of aberrant T-cells among total lymphocytes, and were confirmed by comparing CD3-CD4<sup>+</sup> versus CD3<sup>+</sup>CD4<sup>+</sup> sorted T-cells in 3 patients). In patient P15 who presented a neuro-meningeal involvement, atypical lymphocytes were found both in blood (left) and cerebrospinal fluid (CSF, right).



**Figure S6.** Typical immunophenotyping characteristics of CD3-CD4<sup>+</sup> circulating T- cells in L-HES (Ex: Patient P14).

In CD3-CD4<sup>+</sup> L-HES, aberrant T-cells produce IL-5 which promotes survival and proliferation of eosinophils (NB: intracellular IL-5 production is detected in permeabilized cells after stimulation by phorbol 12-myristate 13-acetate combined with ionophore, PMA-iono). CD3-CD4<sup>+</sup> T-cells (red), compared to CD3+CD4<sup>+</sup> T-cells (black), share the same surface markers expression with AITL (CD2<sup>+</sup>/hi, CD5<sup>+</sup>/hi, CD7<sup>-</sup>/low). But unlike AITL, CD3-CD4<sup>+</sup> T-cells were always CD10-negative in our L-HES patients.

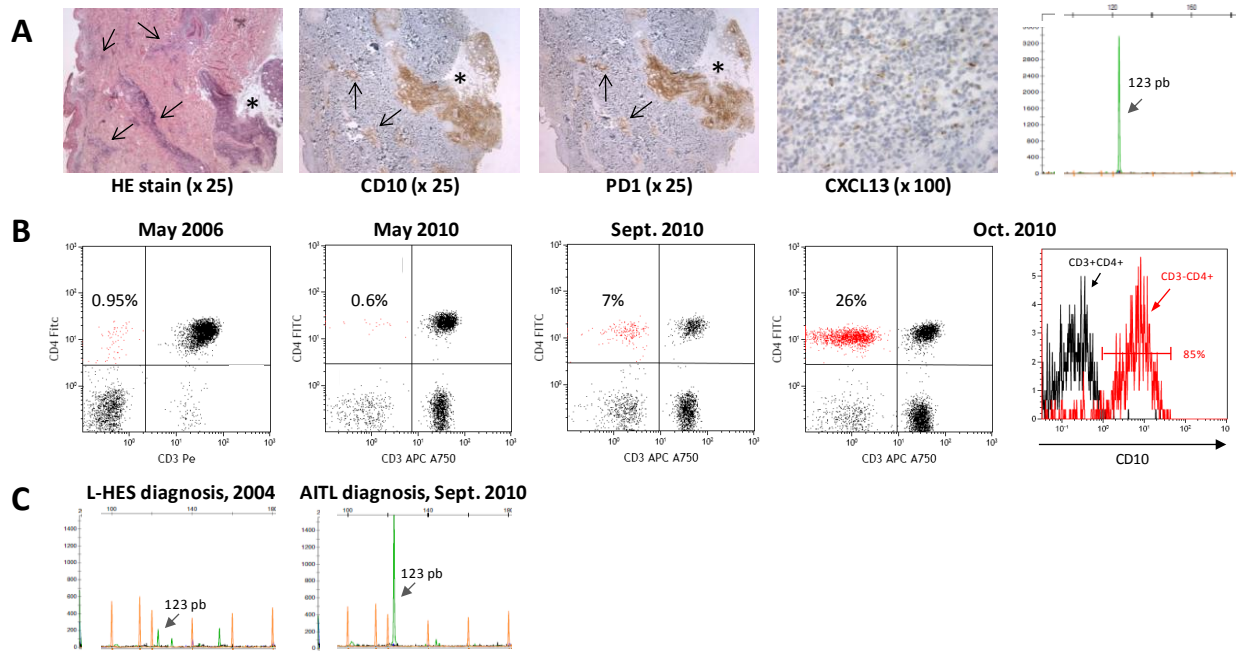


**Supplemental Table S1.** Immunophenotyping characteristics of circulating CD3-CD4+ T-cells in L-HES

Patients	Percentage of CD3-CD4+ T-cells among total lymphocytes during follow-up (min-max)	CD3-CD4+ T-cells characteristics at last sample					
		% of total lymphocytes	CD2	CD5	CD7*	CD10	TCR $\gamma$ rearr
P1	24-78%	46	+	hi	n.a	-	+
P2	39-52%	50	hi	hi	low (16%)	-	+
P3	81-90%	81	+	hi	-	-	+
P4	84-98%	98	+	hi	-	-	+
P5	7-25%	7	hi	hi	low (30%)	-	+
P6	11-17%	11	n.a	n.a	low (6%)	n.a	+
P7	12%-31%	31	hi	hi	-	-	+
P8	7-22%	7	hi	hi	low (28%)	-	+
P9	3.5-7%	3.5	hi	hi	low (10%)	-	+
P10	3-45%	4	+	low	low (45%)	-	+
P11	6-18%	18	+	hi	low (40%)	-	+
P12	2,5-6%	3	hi	hi	low (22%)	-	-
P13	5-7%	6	hi	low	low (16%)	-	+
P14	34-60%	52	hi	hi	low (35%)	-	+
P15	65-79%	75	hi	hi	-	-	+

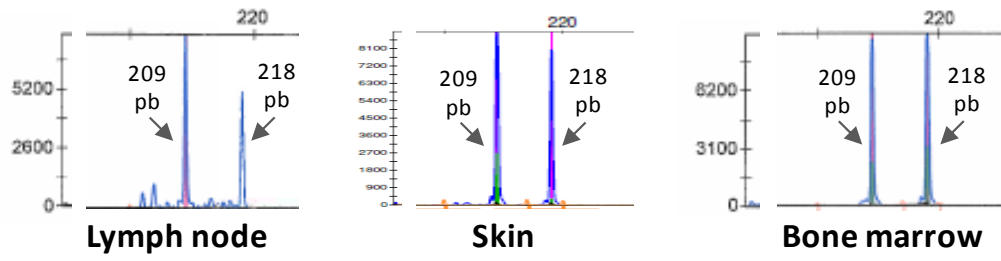
Abbreviations: TCR $\gamma$  rearr, clonal T-Cell Receptor  $\gamma$  rearrangement; +, presence; -, absence; hi, high expression; lo, low expression; n.a, not available.

\*Given percentages are the percentages of CD3-CD4+ T-cells which express CD7



**Figure S7.** Immunophenotyping and histopathological features of AITL in patient P16.

Skin biopsy performed at AITL diagnosis shows large lymphoid infiltrates in all the dermis (black arrows), and in the hypodermis (\*). Lymphoid cells are positive for CD10 and T-follicular helper markers PD1 and CXCL13. A clonal TCR $\gamma$  rearrangement is found in skin lesions (A). Circulating CD3-CD4+ T-cell count increased at AITL diagnosis and 85% of them were CD10-positive (B) (not available in previous samples). The same circulating clonal T-cells were present in 2004 at L-HES diagnosis and in 2010 at AITL diagnosis (C).



**Figure S8.** TCR $\gamma$  rearrangement study in various biopsy at AITL diagnosis in patient P4