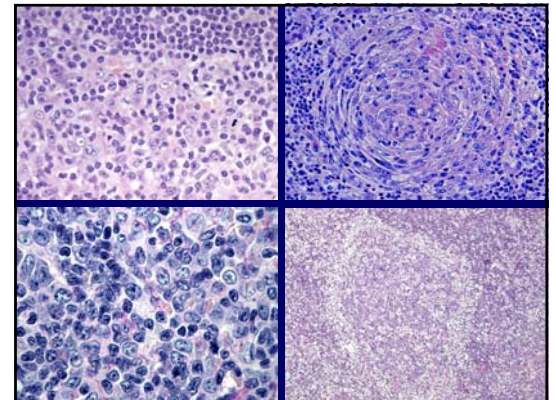


The Biology of Peripheral T Cell Lymphomas



H.K.Müller-Hermelink
Th.Rüdiger
Institute of Pathology, Würzburg

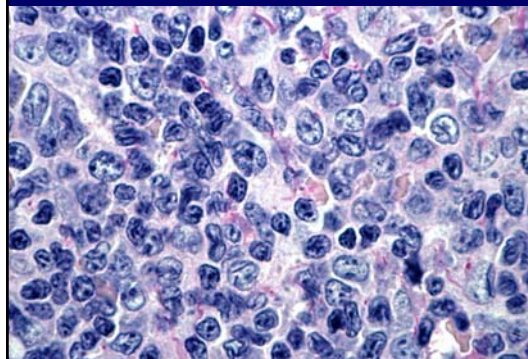
Biomedical Campus Würzburg



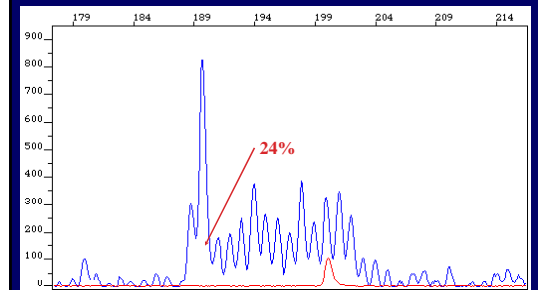
International PTCL classification project

	Europe	North America	East Asia	N
N	288	320	454	1062
PTCL-NOS	20%	33%	22%	266
AITL	30%	16%	18%	216
Adult T-cell leukemia/lymphoma (ATLL)	1%	2%	26%	126
Nasal NK/T-cell lymphoma	3%	6%	14%	90
ALCL, ALK+	9%	13%	4%	86
ALCL, ALK-	11%	7%	2%	67
Enteropathy-type T-cell lymphoma	11%	6%	2%	57
NK/T-cell lymphoma, nasal type	2%	3%	5%	38
Σ	100%	100%	100%	

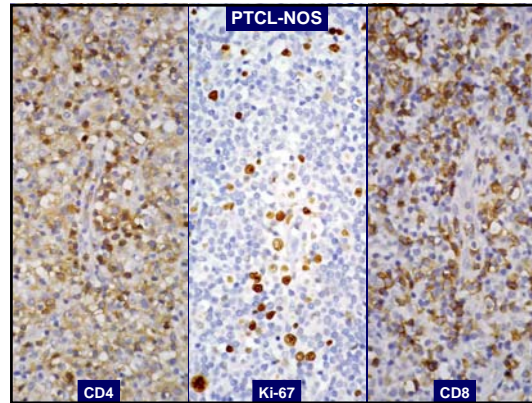
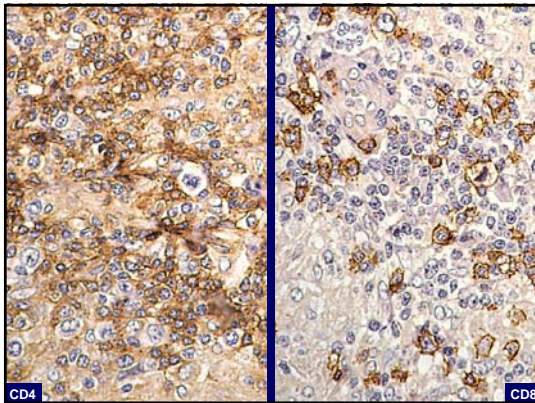
A practical problem in PTCL-NOS



TCR-γ Rearrangement

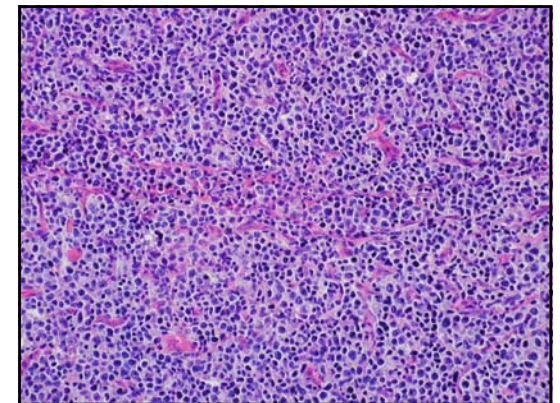
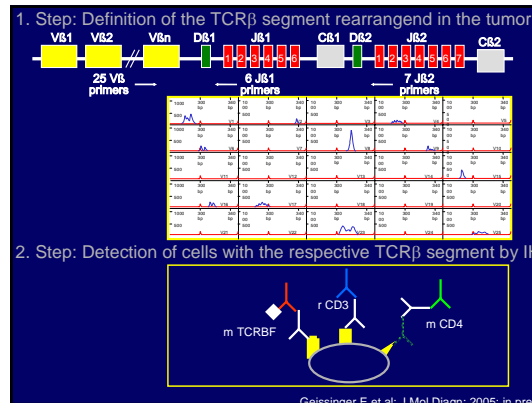
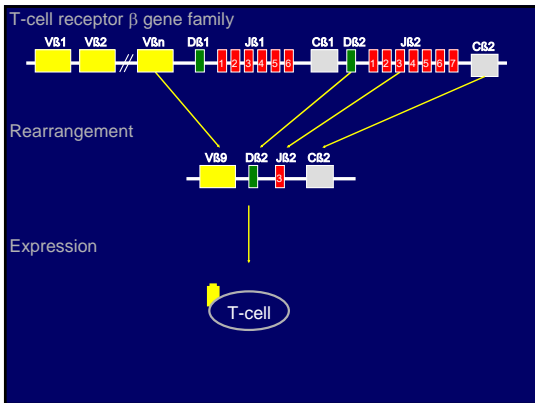


Lee et al.: *Int J Cancer* 103 (2003):12-20.

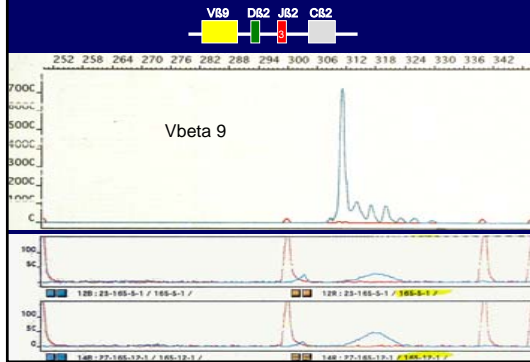


T-cell lymphomas are rich in reactive T-cells
„T-cell rich T-cell lymphoma“

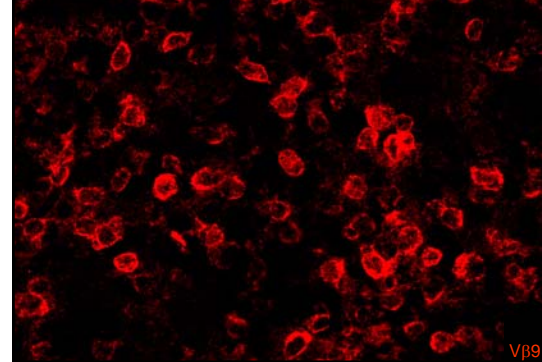
- Identification of the tumor cells in biopsy material is still imprecise for scientific purposes
- Methodological approach:
 - Identification of the tumor cell clone
 - Identification of the functional T-cell population



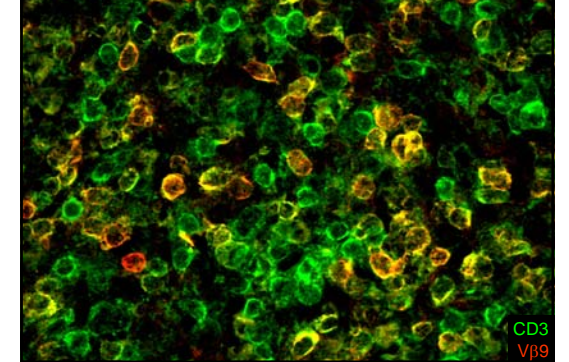
1. Step: Definition of the TCRβ segment rearranged in the tumor



2. Step: Detection of cells with the respective TCRβ segment by IH



2. Step: Detection of cells with the respective TCRβ segment by IH



Material and Methods

- 32 biopsies from 31 patients
 - 21 PTCL-NOS
 - 11 AILT
- Clonal TCRβ rearrangement
 - 18/21 PTCL-NOS (2 oligoclonal)
 - 5/11 AILT
- TCRβ specific antibody available
 - 10/18 clonal PTCL-NOS
 - 3/ 5 clonal AILT

PTCL-NOS

IntNr	Vbeta PCR	CD3	CD4	CD8	5.1	5.2	5.3	7.1	8.1/8.2	9.1	13.6	14.1
5a	V9.1 J1.5	+	+	-	5%	5%	10%	5%	10%	70%	1%	0%
5b	V9.1 J1.5	+	+	-	1%	5%	1%	1%	1%	50%	1%	5%
9	V14.1 J1.6	+	-	-	5%	5%	10%	5%	10%	1%	10%	60%
15	V5.1 J2.2	+	-/-	-	0%	1%	90%	5%	5%	0%	0%	0%
44	V9.1 J2.2	+	-	-	5%	1%	5%	5%	1%	60%	1%	5%
49	V8.1 J1.5	+	+	-	5%	5%	5%	1%	70%	1%	5%	1%
88	V5.1 J2	+	+	-	50%	1%	0%	1%	15%	10%	1%	1%
96	V13.2 J1.1	-	+	-	10%	10%	10%	10%	10%	15%	5%	5%

- PCR-results are family-specific
 - confirmed by independent immunohistochemistry
- On average 65% (40% - 90%) of CD3+ cells were stained with an antibody directed against the TCRVβ-family rearranged in the tumor.
- Antibodies directed against other TCRVβ-families detected <10% of CD3+ cells.

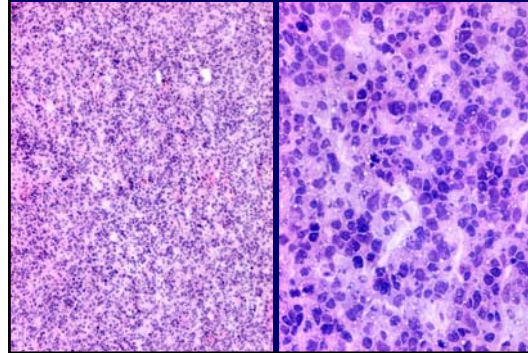
ID	Dx	Single stains					Double Stains						
		CD3	CD5	TLA	GammaTrp B	Perforin	Image	CD3	CD5	TLA	GammaTrp B	Perforin	Image
1	NOS	+	+	-	-	-	CD4	+	+	-	-	-	CD4
3	NOS	+	+	+	+	-	CD8	-	-	-	-	-	DN
4	NOS	+	+	-	-	-	DN	+	+	-	-	-	CD4
6	NOS	+	+	-	-	-	DN	-	-	-	-	-	DN
7	NOS	+	-	-	-	-	CD4	+	+	-	-	-	CD4
9	NOS	+	-	-	-	-	DN	+	+	-	-	-	DN
12	NOS	+	-	-	-	-	CD4	+	+	-	-	-	CD4
16	AILT	+	+	-	-	-	CD4	+	-	-	-	-	CD4
18	AILT	+	+	-	-	-	CD4	-	+	-	-	-	CD4
20	AILT	-	+	-	-	-	CD4	-	-	-	-	-	CD4
21	AILT	+	-	-	+	-	CD4	+	+	-	-	-	CD4
22	AILT	+	+	+	+	-	CD4	+	+	+	+	-	CD4
25	AILT	+	+	-	-	-	CD4	+	+	-	-	-	CD4
26	AILT	+	+	-	+	-	CD4	+	+	-	-	-	CD4

Results of the TCRβ PCR and the immunohistochemical single and double stains in PTCL-NOS and AILT. Differences are indicated in blue.

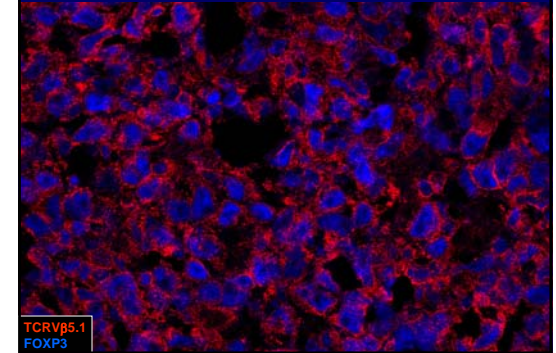
Peripheral T-cell/NK-lymphoma classification: Functional approach

Identification	T _H 1		T _H 2		CTL	NK	T _{reg}	T _{PH}
	TCRβ ⁺ CD4 ⁺ β ⁺	βF1 ⁺ CD4 ⁺ β ⁺	βF1 ⁺ CD4 ⁺ β ⁺	βF1 ⁺ CD4 ⁺ β ⁺	βF1 ⁺ CD4 ⁺ β ⁺	CD2 ⁺ 3 ⁺ 16 ⁺ CD4 ⁺ β ⁺	CD4 ⁺ 25 ⁺ FOXP3 ⁺	CD4 ⁺ 57 ⁺ CXCL13 ⁺
Antigen recognition	frequent antigens with MHC restriction	MHC II restricted extracellular antigens		MHC I restricted intracellular antigen	NKR IgG (ADCC)	TCR MHC	MHCII	
Transcription factor		T-bet	GATA3 MAF				BCL6 ⁺	
Costimulation		IL12	IL4 ⁺	IFN _γ				
Recruitment	Resident in epithelia	CXCR3 CCR5	CCR4			CCR4 CCR5 CD62L	CXCR5	
Effector arm		IFN _γ	IL4, IL5, IL13	Granz.B Perforin FASL	Granz.B Perforin	IL10	IL10 IL21 CD40L	
Functional consequences		Activation of M _φ CTL DTH	Defense against helminths Allergy	Perforin: lysis GranzymeB: apoptosis FASL: apoptosis		Control of autoimmunity	Help for GCb-cells	

PTCL-NOS



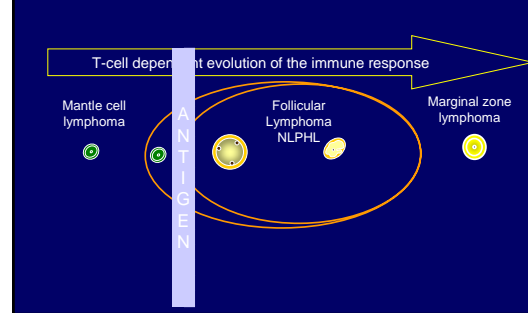
PTCL derived from regulatory T-cells



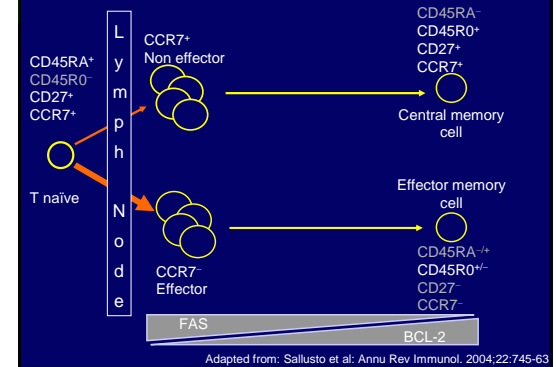
Regulatory T-cells

- Suppression of immune function
 - Autoimmunity
- X-linked immunoproliferative syndrome
- FOXP3 exclusively expressed
 - No expression in naive or activated T-cells
 - Other lymphocytes do not express FOXP3
 - Rare sporadic lymphomas, over 50% of ATLL in Japan

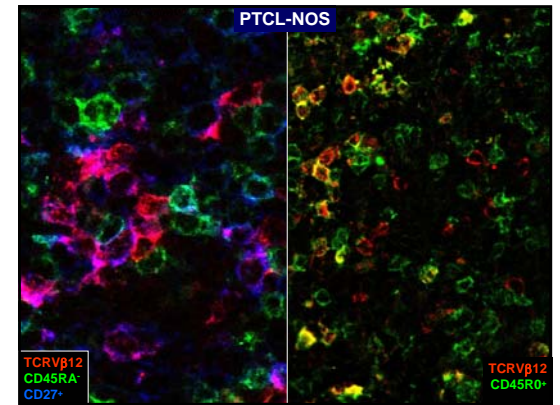
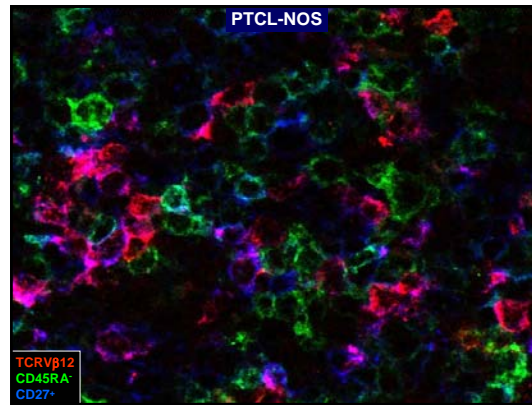
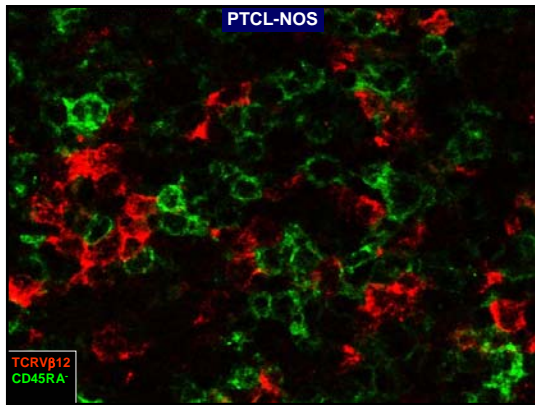
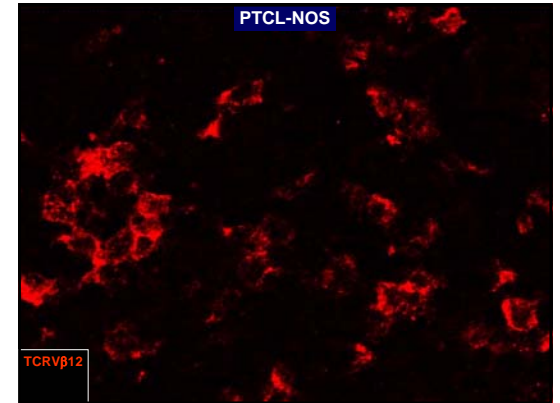
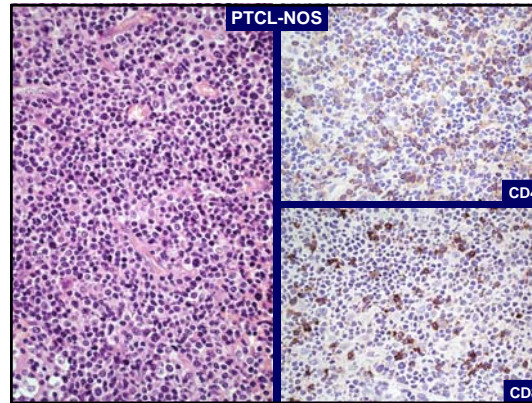
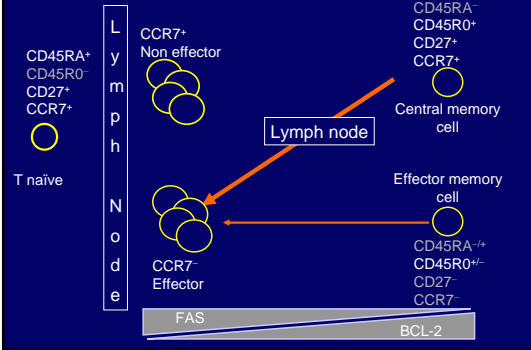
Correlation of B-NHL to sequential steps of B-cell development

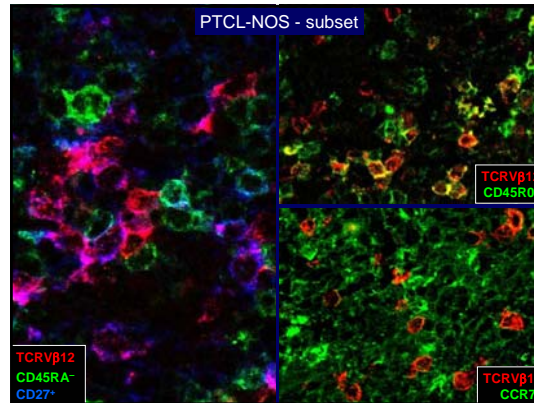
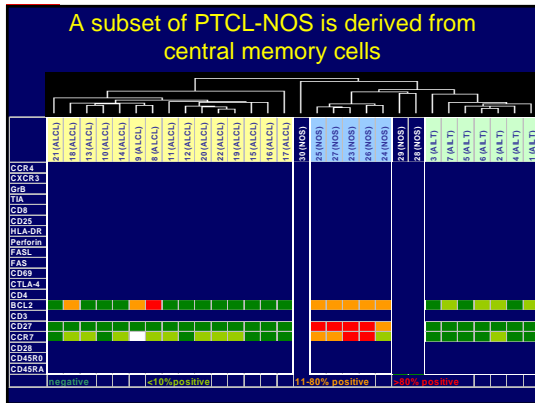


Mature T-cell differentiation



Mature T-cell development: Recall Responses





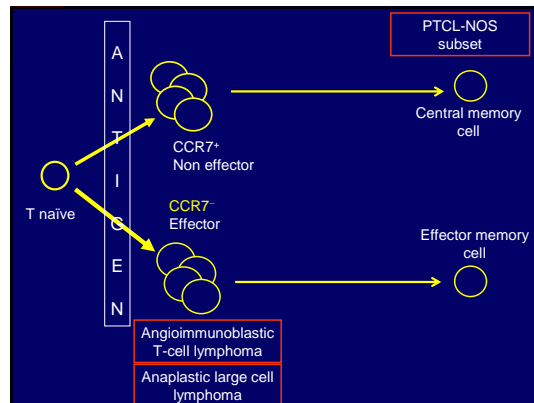
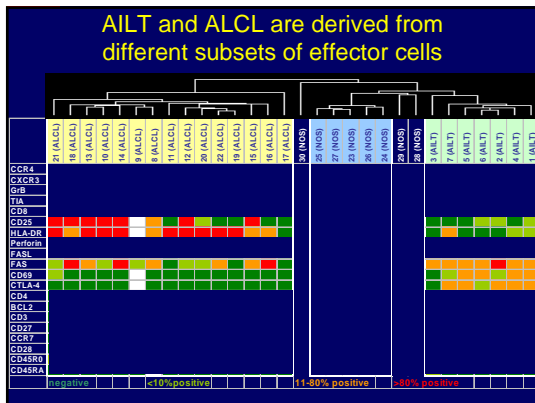
Peripheral T-cell lymphoma, unspecified

Subgroup - Central memory cell

	Explained features	Unexplained features
Phenotype	BCL2 ⁺ , FAS ⁻ , CTLA-4 ⁻ , CD28 ⁺	Proliferation
Homing to T-cell areas of lymph nodes	Preferential lymph node involvement	
Central memory function	Rarer B-symptoms	

PTCL-unspecified with questionable counterpart

- Lennert's lymphoma
- Follicular/ perfollicular PTCL



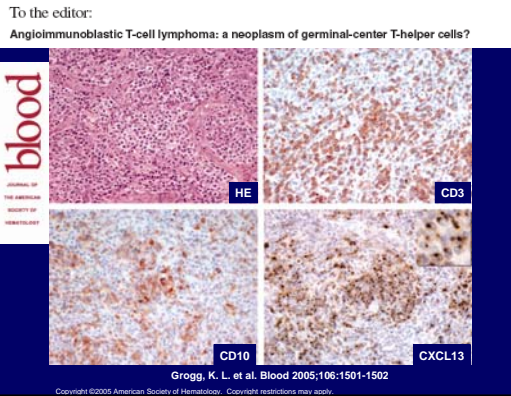
Angioimmunoblastic T-cell lymphoma

Effector or effector memory cell

	Explained features	Unexplained features
Phenotype	BCL2 ⁻ , FAS ⁺ , CTLA-4 ⁺ , CD28 ⁻	
Homing to inflamed peripheral tissues	Extranodal involvement	Lymphadenopathy
T _{H1} effector function	B-symptoms Dysproteinemia	Failure to undergo apoptosis
Stimulated by non-professional APC	Close contact to follicular dendritic cells	

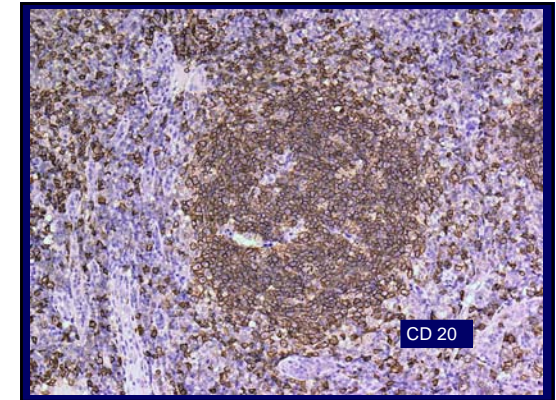
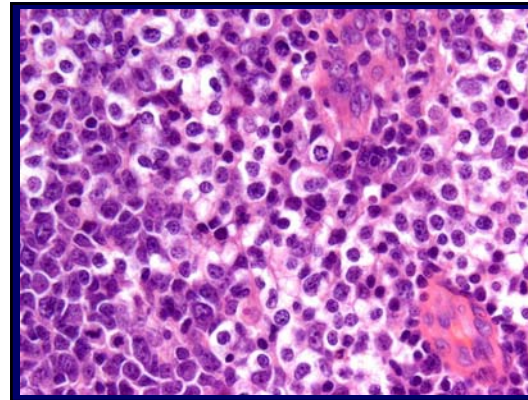
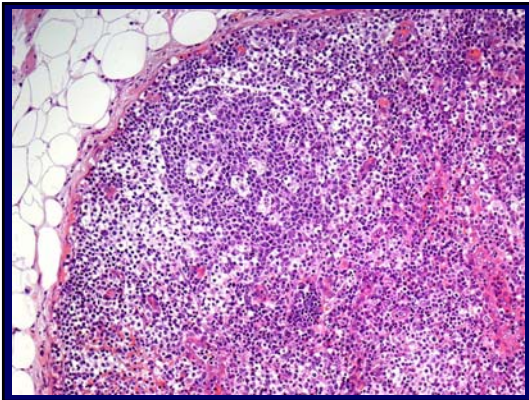
Peripheral T-cell/NK-lymphoma classification: Functional approach

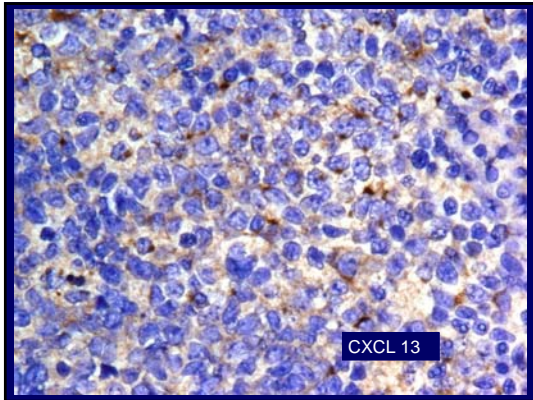
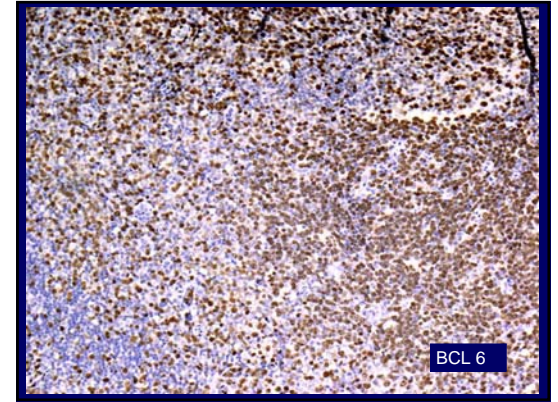
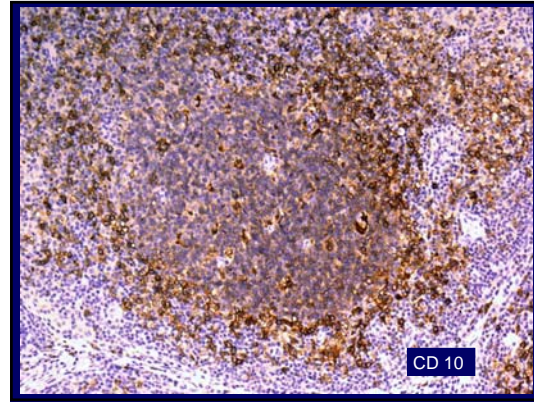
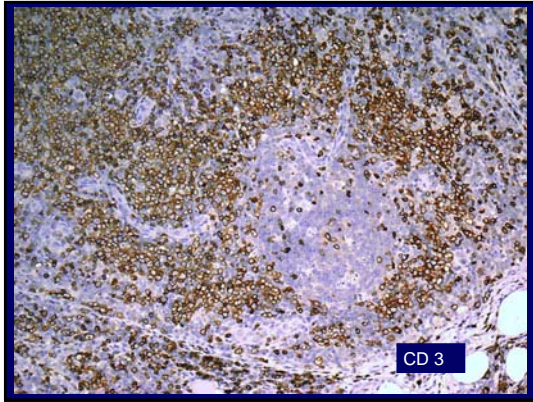
	γ/δ	T _H 1	T _H 2	CTL	NK	T _{reg}	T _{FH}
Identification	TCR δ^+ CD4 $^-$ 8 $^-$	β F1 $^+$ CD4 $^+$ 8 $^-$		β F1 $^+$ CD4 $^+$ 8 $^-$	CD2 $^+$ 3 $^-$ 16 $^+$ CD4 $^+$ 8 $^-$	CD4 $^+$ 25 $^+$ FOXP3 $^+$	CD4 $^+$ 57 $^+$ CXCL13 $^+$
Antigen recognition	frequent antigens with MHC restriction	MHC II restricted extracellular antigens		MHC I restricted intracellular antigen	NKR IgG (ADCC)	TCR MHC	MHCII
Transcription factor		T-bet	GATA3 MAF				BCL6 γ
Costimulation		IL12	IL4 γ	IFN γ			
Recruitment	Resident in epithelia	CXCR3 CCR5	CCR4			CCR4 CCR5 CD62L	CXCR5
Effector arm		IFN γ	IL4, IL5, IL13	Granz.B Perforin FASL	Granz.B Perforin	IL10	IL10 IL21 CD40L
Functional consequences		Activation of M ϕ CTL DTH	Defense against helminths Allergy	Perforin: lysis GranzymeB: apoptosis FASL: apoptosis		Control of auto-immunity	Help for GCb-cells



Teaching case

- 56 yr. old man with cervical lymphadenopathy- lymph node biopsy – local pathologist „peculiar lymphadenitis“.
- Consultant diagnosis : **Follicular/ Perifollicular peripheral T Cell lymphoma**
- Immune histochemistry: CD 3 , CD5 , CD4,CD10, BCL6, weak CD30, CXCL13 positive
- Molecular studies : TCR gamma biconal



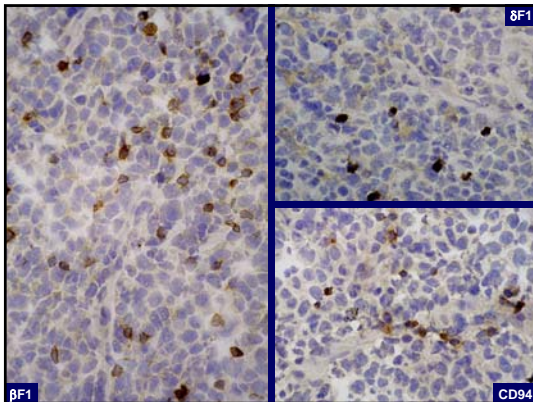
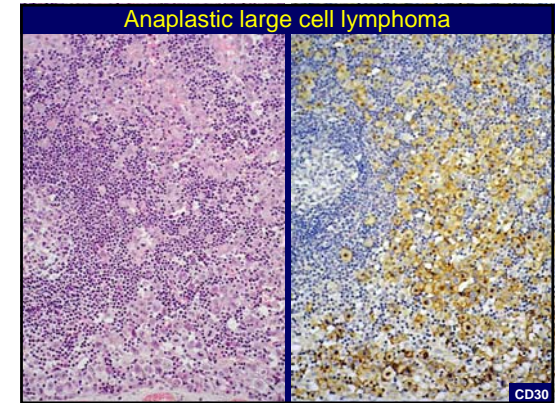
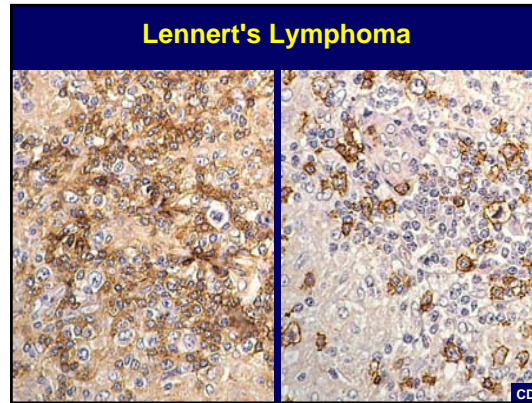
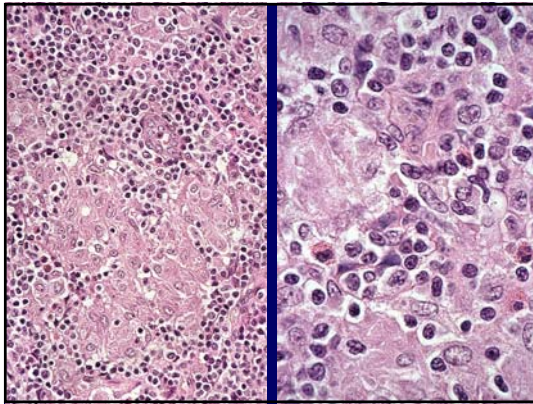


**PTCL-NOS Variant:
Marginal zone-T-cell Lymphoma**

- Morphology
 - Infiltration of the marginal zones of reactive B-cell follicles
 - Atypical clear cell population
- Immunology
 - CD3+, CD5+
 - CD4+
 - CD10+, CXCL13+, CD27+
 - CD8-, TIA1-, GranzymeB-
- Genetics (1 case)
 - 46, XY, del(6)(q21)[3]/46, idem, add(17)(q25)[7]/46, XY[10]

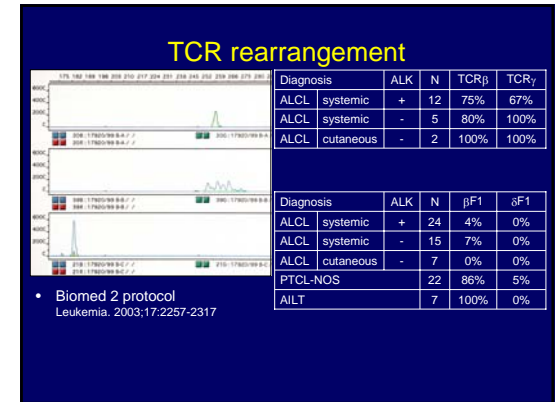
Rüdiger T et. al. : Am J Surg Pathol 24:117, 2000

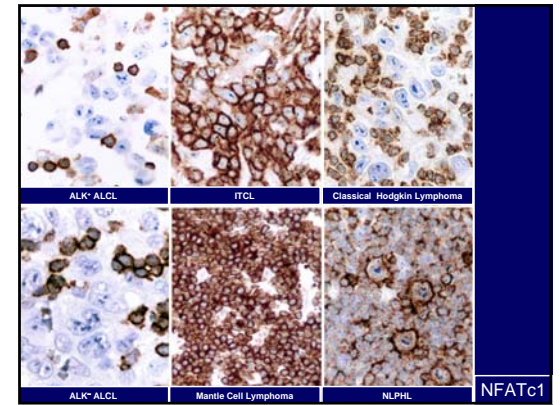
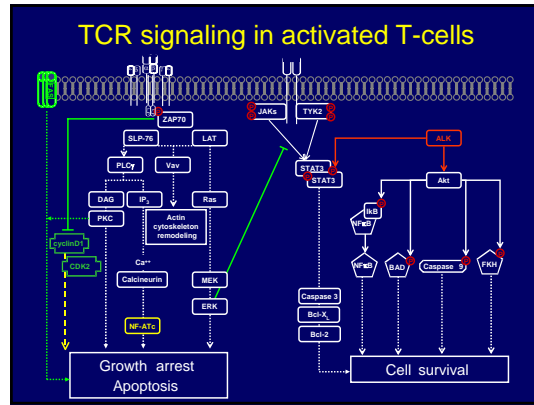
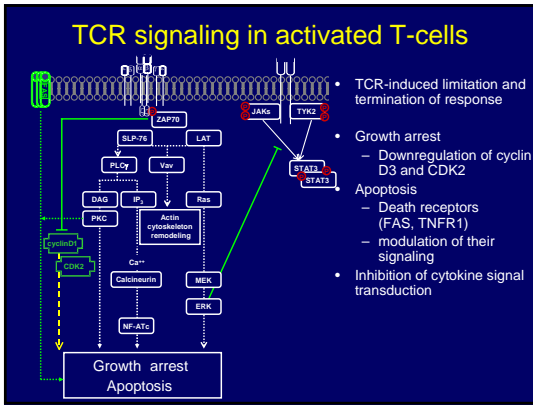
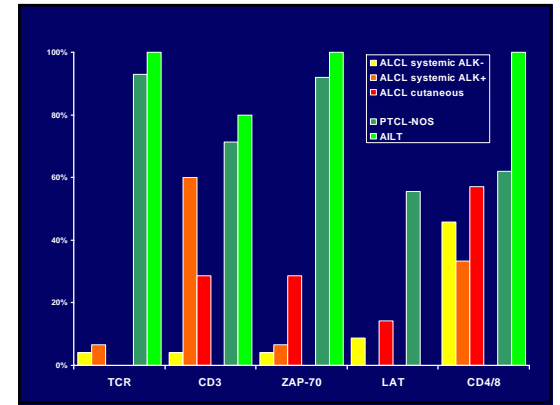
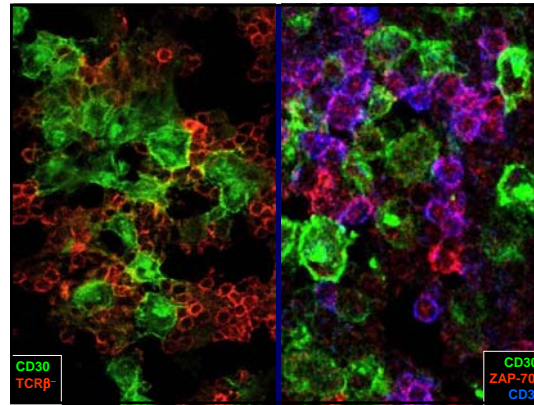
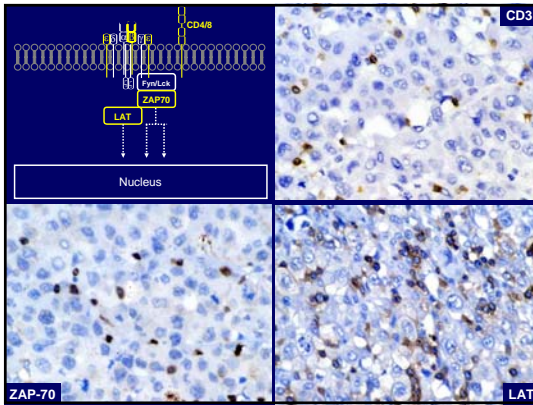
**PTCL-NOS variant
Lymphoepithelioid (Lennert's) Lymphoma**

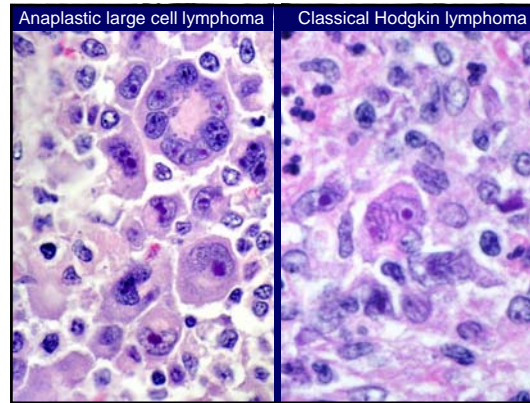
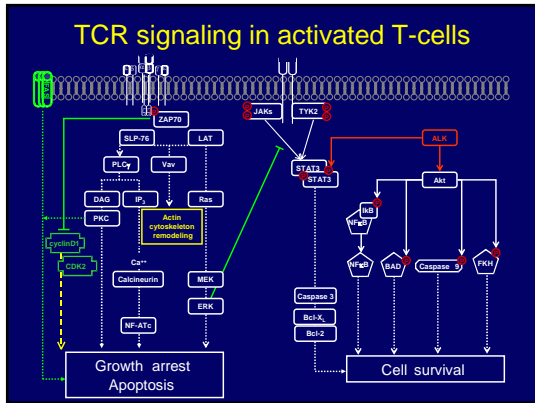


		Frozen	Paraffin
ALCL	systemic		
	ALK+	12	12
	ALK-	5	10
	cutaneous	2	5
PTCL-NOS	CD30+	4	
	CD30-	18	
AILT	CD30-	7	

- T-cell receptor rearrangement
- Immunohistochemistry for
 - TCRβ, CD3, ZAP-70, LAT, CD4, CD8 (Paraffin)
 - TCRβ, TCRδ, CD94 (Frozen)
- Fluorescent stains
 - CD30 - TCRβ
 - CD30 - CD3 - ZAP-70







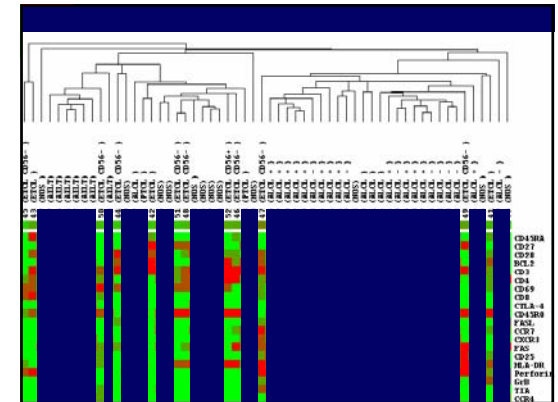
Conclusion

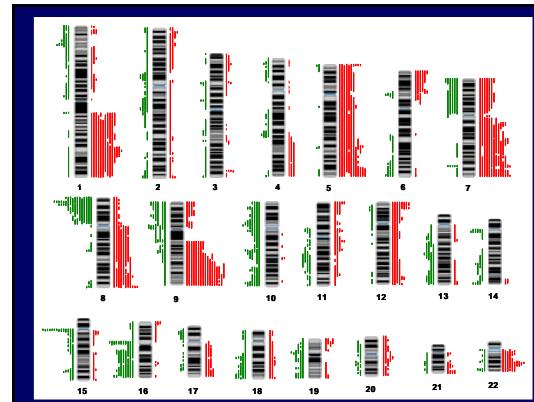
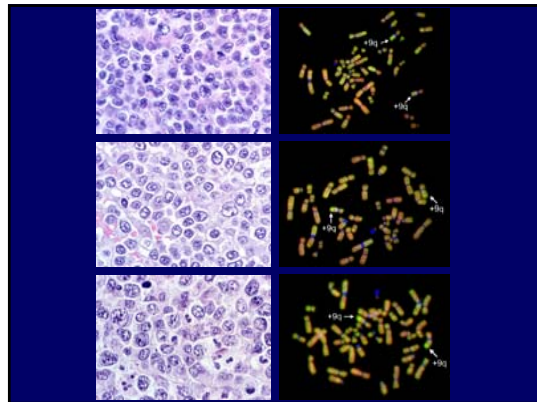
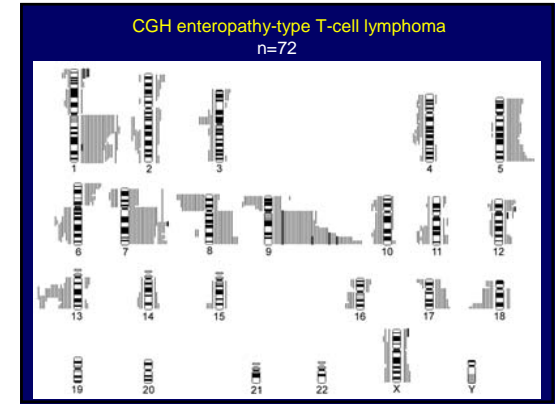
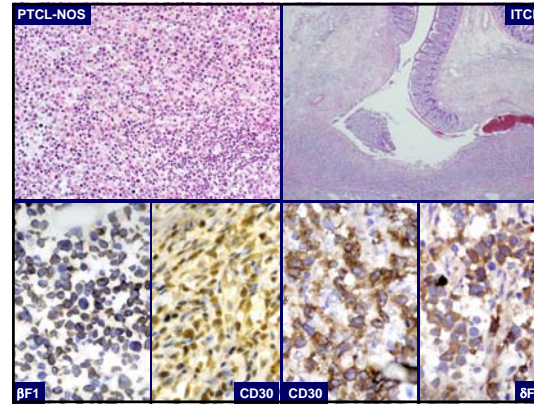
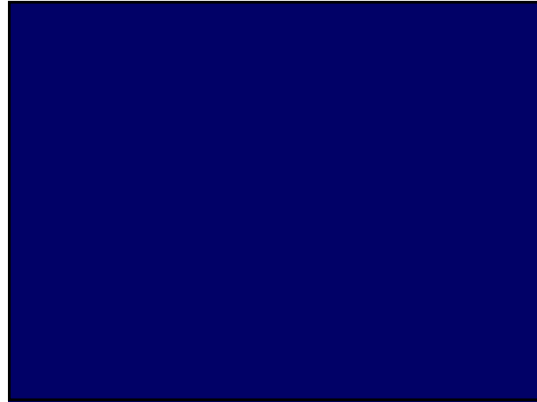
- PTCL can be correlated to normal T-cell differentiation.
 - AILT and ALCL correspond to effector or memory-effector T-cells.
 - A subgroup of PTCL-NOS is derived from central-memory T-cells.
 - ITCL is genetically homogeneous but exhibits a wide range of differentiation.
- ALCL lack T-cell receptors.
 - Contribution to transformation in ALK1⁻ cases.
 - Delineation of ALK1⁻ cases.
- Morphogenesis?
 - Closer cooperation between pathologists and immunologists needed.**



ETL: Two different diseases ?

CD56+ETL	CD56-ETL
Association with celiac disease ??	Celiac disease
Precursor lesion ??	+ 1q, +5q ?
ETL (CD8+, CD56+ monomorphic)	Refractory celiac disease
	+ 9q, -16q
+9q/16q, +8q	ETL (CD8-, CD56-, non-monomorphic)
	+9q/-16q, +1q, +5q

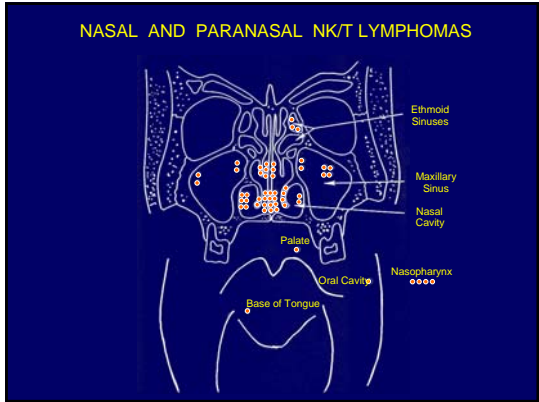
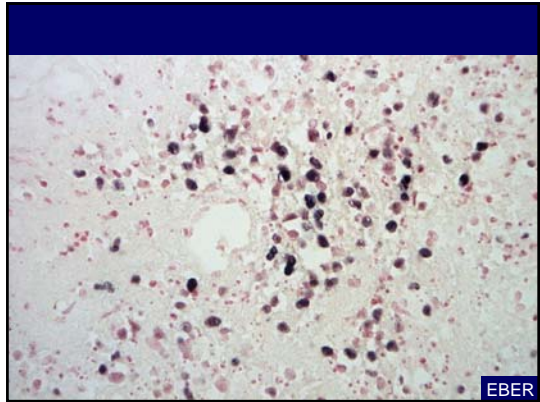
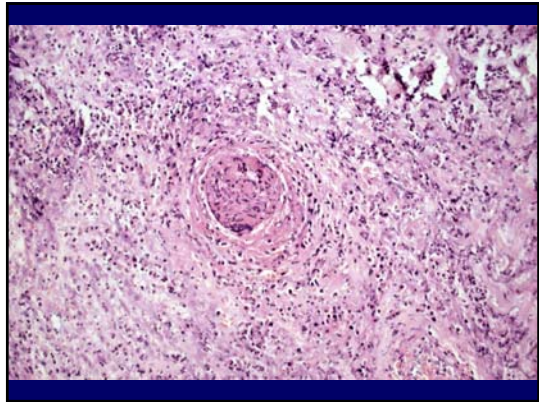


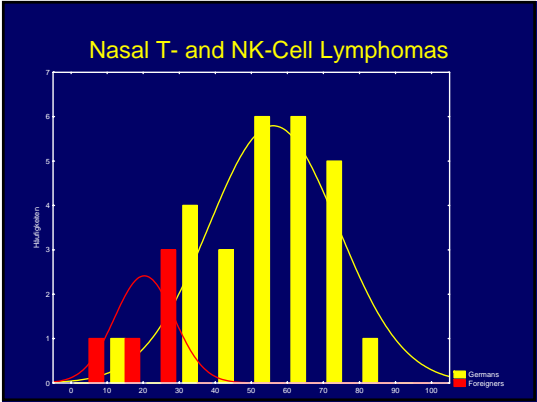


- Array CGH of ETL: preliminary results**
- Gain of 9q is the hallmark genetic alteration in all types of ETL (occurring in 70%)
 - ETL without 9q gains frequently show losses of 16q12.1
 - CD56+ ETL are characterized by frequent gains of 8q
 - CD56- ETL are characterized by frequent gains of 1q and 5q



- ### Similarities and Differences between AILT and ALCL
- Expression of activation molecules
 - CD69 (AILT: 57% vs. ALCL: 0%)
 - HLA-DR (AILT: 16% vs. ALCL: 93%)
 - CD30 (AILT: 0% vs. ALCL: 100%)
 - Expression of CTLA-4
 - AILT: 71% vs. ALCL: 0%
 - Expression of T-cell receptors





Phenotype

	Lineage	CD94	NKG2A	P58.2	P58.1	p140	p70	p50.3
Nasal cavity	NK	+	+	-	-	-	-	-
Nasal cavity	NK	+	+	-	-	+	-	-
Oropharynx	NK	+	+	-	-	-	-	-
Sinus	NK	+	+	-	-	-	-	-
Small bowel	NK	+	+	+	+	+	-	-

