



Clinical, Pathological and Prognostic Implications of RHOAG17V mutations in Peripheral T-cell Lymphomas

Surabhi Jain¹, Ansh Goswami¹, Trisha Mandal¹, Moien Rasheed Lone¹, Prashant Ramteke¹, Mehar Chand Sharma¹, Ajay Gogia³, Atul Sharma³, Mukul Aggarwal², Ranjit Sahoo³, Sameer Bakhshi³, Rishi Dhawan², **Saumyaranjan Mallick**¹

Departments of ¹Pathology, ²Hematology and ³Medical Oncology, All India Institute of Medical Sciences, New Delhi, India
surabhi@sukride.com; drsmallick.aiims@gmail.com



Introduction

- Peripheral T cell lymphoma (PTCL) comprises of a heterogeneous group of lymphoproliferative disorders with varying biological and clinical behavior, which occur beyond the development of T cells in the thymus
- The deregulation of T cell differentiation or maturation may lead to lymphomagenesis
- Various genetic mutations have been identified in the development of these lymphomas

PTCL-NOS	t (5:9) (ITK/SYK), RHOA, FYN
AITL	RHOA, TET2, IDH2, DNMT3A, CD28
ALCL, ALK+	t (2:5) (NPM/ALK)
ALCL, ALK-	t (6:7) (DUSP22/FRA7H), TP63, PRDM1, del TP53
HSTCL	Isochromosome 7q, STAT3, STAT5B
ENKTCL	JAK3, ADAM3A, del PRDM1 and HACE1
EATL type 1	Gains of 9q34, 3q27, 1q, 5q, del16q
EATL type 2	Gains of 9q34, 8q (MYC), del 16q

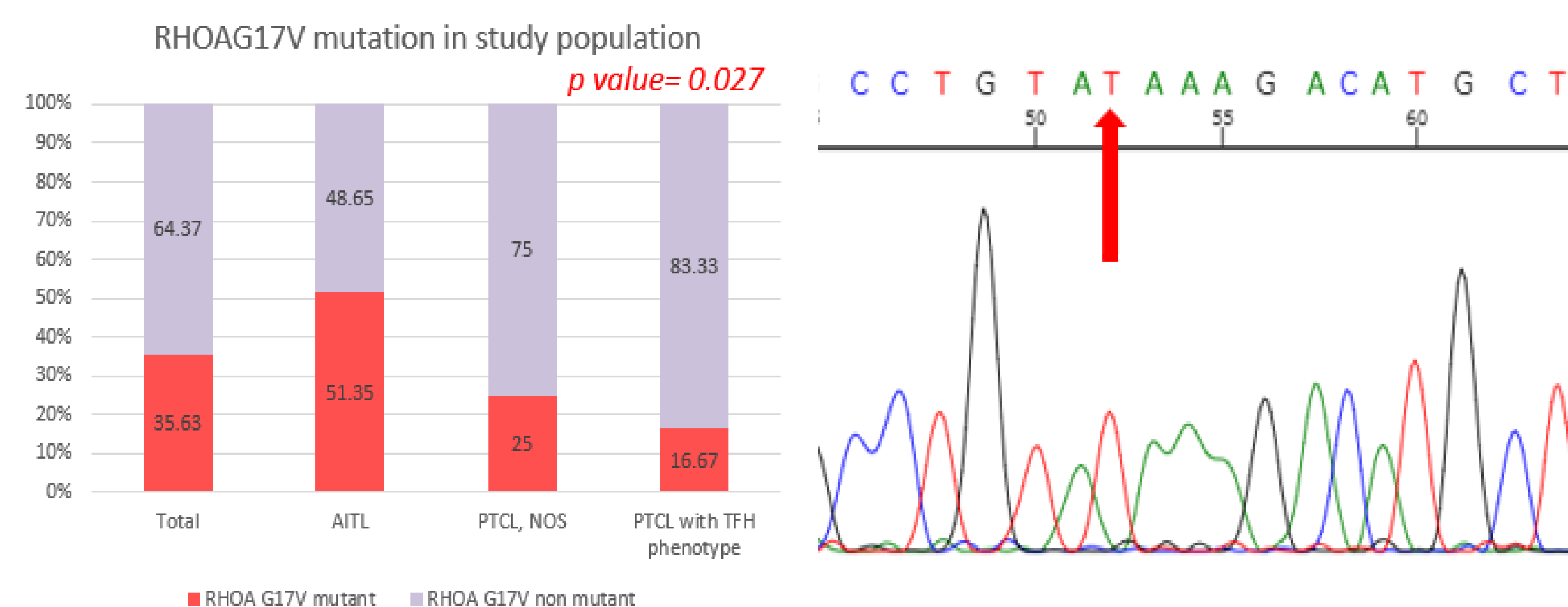
- Mutations in epigenetic modifiers such as IDH2(20-30%), TET2(50-80%), DNMT3A(20-30%) and small GTPase RHOA(60-70%) have been identified by next generation sequencing in Angioimmunoblastic T-cell lymphomas(AITL)
- RAS homolog member A (RHOA) , a member of small GTPases works as a molecular switch in transduction cascades
 - Active GTP bound state plays a significant role in cell functions such as cytoskeletal dynamics, mitogenesis, cell survival, arterial contractility, axogenesis and phagocytosis
 - With mutated RHOA there is compromised binding and this mutant binding inhibits the binding of wild type also. G1 to S phase cell cycle progression is known to reduce with wild type; hence mutated phenotype may increase tumor proliferation
 - Classical missense mutation of G17V (Glycine to valine) is noted in approximately 60% of cases worldwide of AITL and can be noted in PTCL with T-follicular helper (TFH) phenotype
 - The mutation can be tested using molecular methods or commercially available antibody
- RhoA is overexpressed in a wide range of carcinomas of breast, colon, lung, gastric, head and neck, bladder, testicular and PTCLs
- However the association of the dominant negative control with clinicopathological profile of the tumor is yet not clear

Materials and Methods

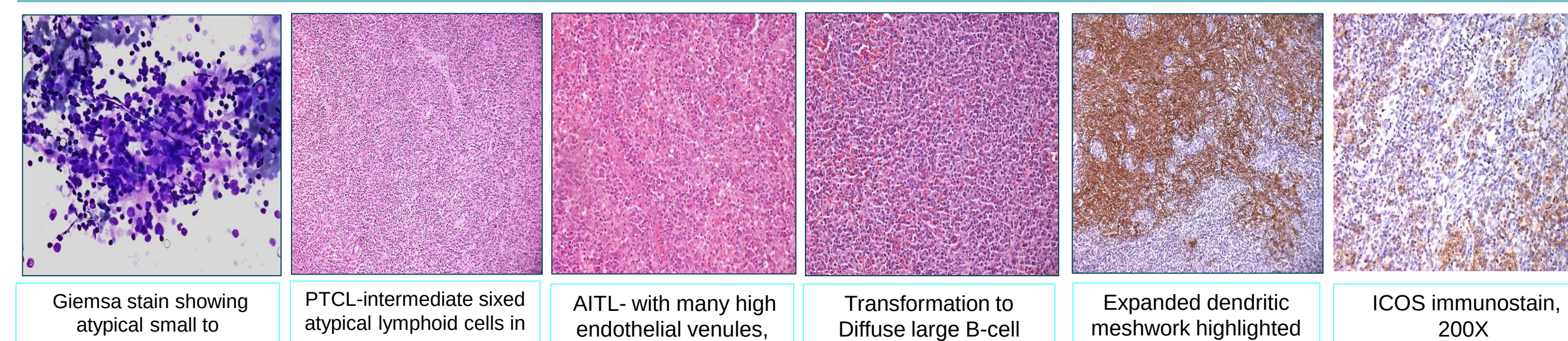
- This was an ambispective study over six years (2015–2020), including 88 patients with diagnoses of PTCL-NOS (44), AITL (37), PTCL-TFH phenotype (P-TFH; 6), and follicular T-cell lymphoma (1) from the archives of Department of Pathology (AIIMS, New Delhi)
- Mutational analysis for RHOAG17V was performed using allele-specific polymerase chain reaction (PCR), validated by the sequencing of cloned products, along with conventional Sanger sequencing
- Detailed clinicopathological correlation was done

Results

- Sanger sequencing of conventional PCR product did not yield any mutation and the results were based on allele specific PCR validated using sequencing of cloned products in E. coli
- RHOAG17V mutation was present in association with diagnosis of AITL (51.35%) in comparison to PTCL-NOS (25%) and P-TFH (16.67%; p-value=0.027)

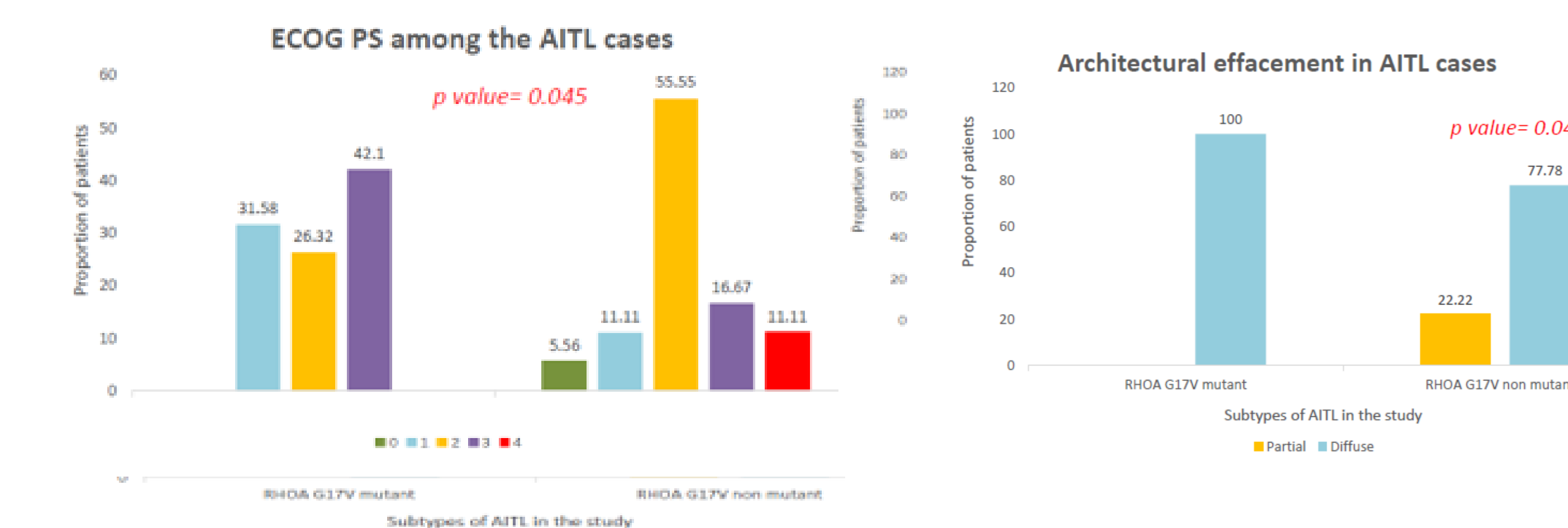


Cytopathology and Histopathology

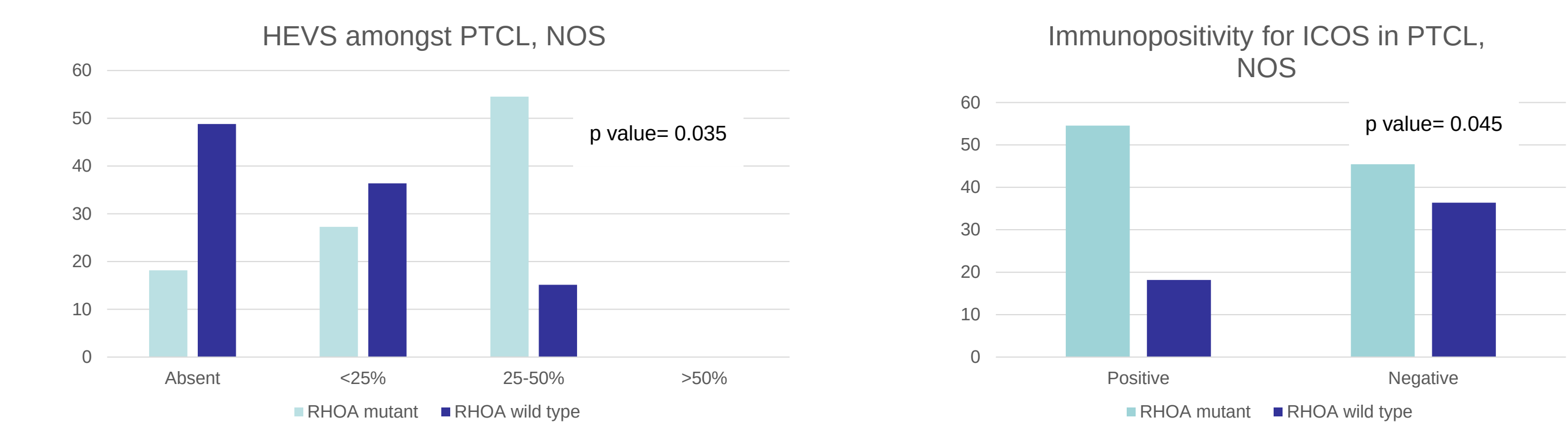


Association of mutational profile with clinicopathological profile

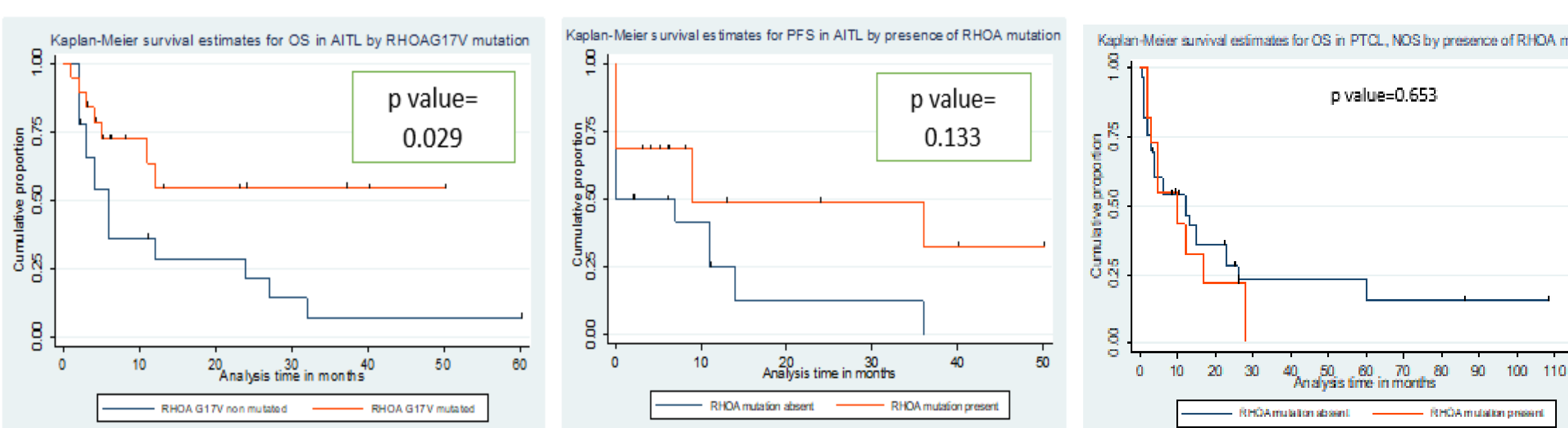
The association between the presence of mutation and diagnosis, clinical factors (demographics, performance status, organomegaly, IPI/PIT score, stage, marrow involvement, histologic and immunophenotypic presentation (pattern, high endothelial venules [HEV], immunopositivity for TFH markers [CD10, ICOS, PD1, BCL6, and others], CD30, and EBER ISH), and survival (overall and progression-free survival) was analyzed.



- Mutant cases of AITL had an overall worse performance status at presentation (p-value=0.045), along with a diffuse pattern of nodal involvement (p-value=0.046)
- Amongst the PTCL-NOS patients, immunopositivity for ICOS (p-value=0.045), along with the presence of increased HEV (p-value=0.035) was noted
- None of these factors showed any association with P-TFH



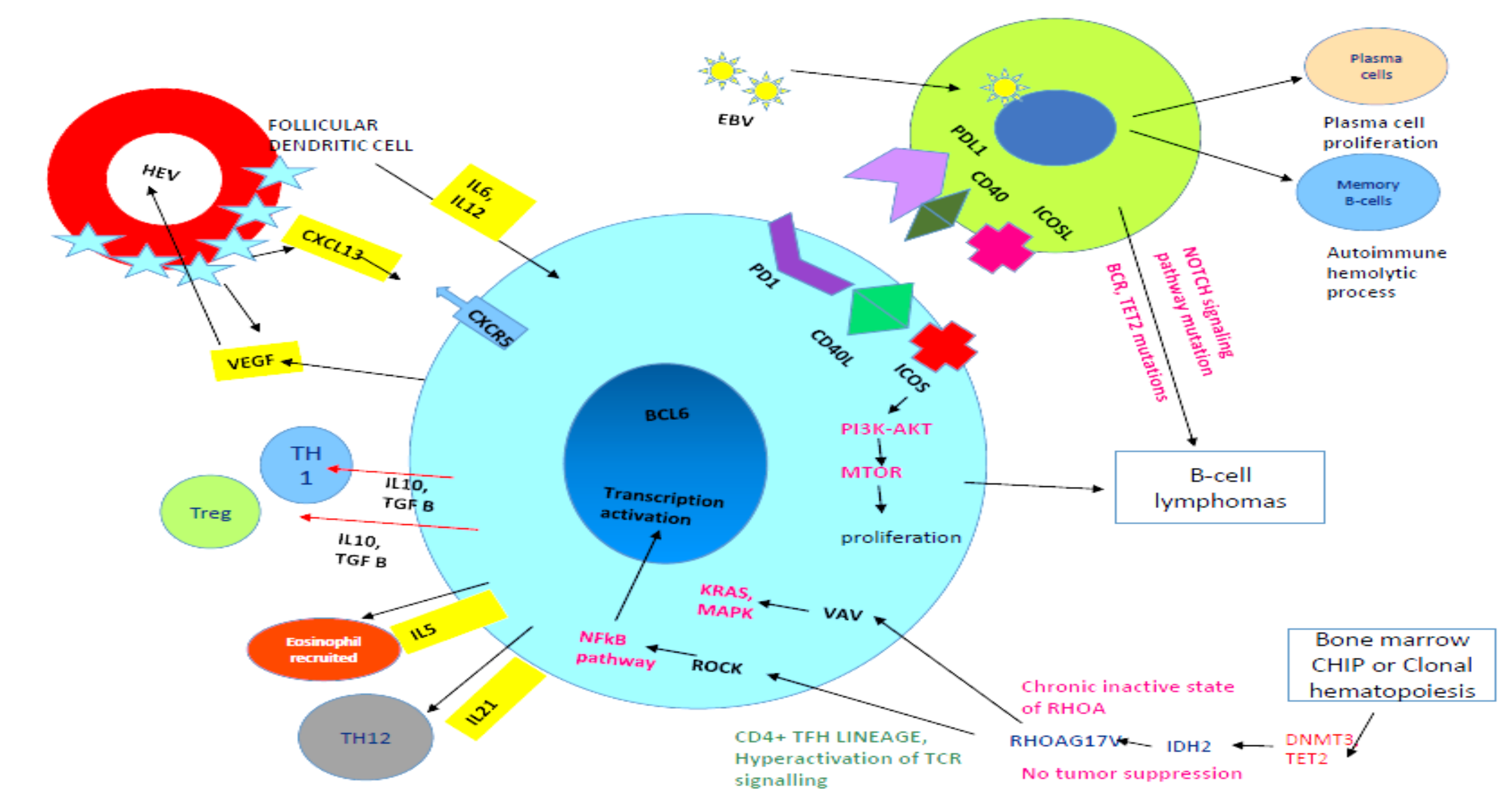
Survival analysis



- In the univariate survival analysis for OS for AITL, wild type status predicted an inferior OS
- On multivariate analysis also to remove the confounding factors, wild type status emerged as a strong negative prognostic marker
- The exact cause of this relationship is not known however RHOA mutation increases ICOS and TCR signaling pathways, which may also lead to proliferation of the immunologically competent T-cell and thereby B-cells which may have an antitumorigenic effect

- Two of the transformed DLBCL cases showed a mutant profile
- No survival advantage was observed in PTCL, NOS or PTCL with TFH subtypes
- Single case of Follicular T-cell lymphoma showed a wild type status

Discussion and Conclusions



- RHOAG17V is a commonly observed mutation in AITL which assigns the TFH lineage to the clonally expanded population. Though seen in PTCL, NOS and PTCL with TFH phenotype; its role in the pathobiology of AITL is of the highest value
- Polymorphous non neoplastic inflammatory infiltrate outnumbers the tumor burden yielding a low variant allele frequency; hence, more sensitive approaches should be designed for mutational analysis in PTCL especially AITL (median variant allele frequency between 0.1 to 49%)
- RHOA G17V Mutant type PTCL, NOS showed an association with ICOS immunopositivity and presence of arborizing vasculature
- RHOA G17V Mutant type AITL had an aggressive presentation, but fared better in overall outcome (p value= 0.029)
- Mutant status of AITL can have prognostic implications and may have translational relevance

Study Group	% Mutant type AITL	Variable showing significant association	Overall Survival prediction
Ondrejka et al. 2016	63% (total n=27)	B symptoms and Hepatosplenomegaly AND Increased number of TFH markers and mean vessel density by ERG IHC in Mutant	No difference in Overall survival
Butzmann et al. 2020	n =10		Overall survival better in mutant
Our study	51.35% (total n=37)	Poor ECOG status and diffuse architectural effacement in mutant type	Overall survival better in mutant

Bibliography

- Ondrejka, Sarah L., Bartosz Grzywacz, Juraj Bodo, Hideki Makishima, Chantana Polprasert, Jonathan W. Said, Bartłomiej Przychodzen, Jaroslav P. Maciejewski, and Eric D. Hsi. 2016. "Angioimmunoblastic T-Cell Lymphomas With the RHOA p.Gly17Val Mutation Have Classic Clinical and Pathologic Features." *American Journal of Surgical Pathology* 40: 335–41.
- Butzmann A, Sridhar K, Jangam D, Kumar J, Sahoo MK, Shahmarvand N, Warnke R, Rangasamy E, Pinsky BA, Ohgami RS. A comprehensive analysis of RHOA mutation positive and negative angioimmunoblastic T-cell lymphomas by targeted deep sequencing, expression profiling and single cell digital image analysis. *Int J Mol Med*. 2020 Oct;46(4):1466-1476. doi: 10.3892/ijmm.2020.4686. Epub 2020 Jul 28. PMID: 32945366; PMCID: PMC7447311.

Thanks to Science Engineering Research Board and AIIMS Research section (SERB-EEQ/2016/402, AIIMS A-653)