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Kikkeri N Naresh, Philippa C May, Alistair G Reid, Alexandra J Marks, Donald Macdonald, et al.. T-cell lymphoblastic leukaemia / lymphoma associated with a microenvironment of thymic asteroid-B-cells in the bone marrow. *Histopathology*, 2010, 57 (4), pp.549. 10.1111/j.1365-2559.2010.03663.x . hal-00580348

HAL Id: hal-00580348

<https://hal.science/hal-00580348>

Submitted on 28 Mar 2011

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Journal:	<i>Histopathology</i>
Manuscript ID:	HISTOP-10-09-0613.R1
Manuscript Type:	Short Report
Date Submitted by the Author:	11-Jan-2010
Complete List of Authors:	Naresh, Kikkeri; Hammersmith Hospital & Imperial College, Histopathology May, Philippa Reid, Alistair Marks, Alexandra Macdonald, Donald; Charing Cross Hospital & Imperial College, Haematology Kanfer, Edward; Hammersmith Hospital & Imperial College, Haematology
Keywords:	Thymus, T-ALL, T-cell lymphoblastic lymphoma, Bone marrow trephine, Asteroid B-cells

T-cell lymphoblastic leukaemia / lymphoma associated with a microenvironment of thymic asteroid-B-cells in the bone marrow

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Abstract:

Aims: Asteroid B-cells are a component of normal thymus. It is currently unclear whether these cells are identifiable in T-cell lymphoblastic leukaemia/lymphoma (T-ALL/LBL) of the thymus. This study aims at identifying asteroid B-cells both in thymic and extra-thymic tissue involved by T-ALL/LBL.

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Methods and results: We revisited thymic, lymph node (LN) and bone marrow trephine biopsy (BMTB) samples from eight patients of T-ALL/LBL. All had been investigated by immunohistochemistry and one by fluorescent in-situ hybridization (FISH). The BMTB

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samples of two of eight T-ALL/LBLs and LN sample in one of them showed presence of asteroid-shaped B-cells with dendritic cytoplasmic processes. These B-cells also expressed CD23 and the features were akin to the unique thymic asteroid B-cells. Both patients had aggressive/resistant disease. Cytogenetic analysis in one showed a complex translocation involving the TCRB gene at 7q35 and a distal region of 9q known to harbour the NOTCH1 gene.

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Conclusion: This is the first report of T-ALL/LBL documenting the presence of asteroid B-cell-rich microenvironment at bone marrow and LN sites. In this small subset, T-ALL/LBL cells are possibly dependent on asteroid-B-cells, and whether targeting of asteroid-B-cells with anti-CD20 monoclonal antibody in such cases will result in clinical benefit remains to be answered.

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Introduction: Though much progress has been made in understanding the immunophenotypic subsets and molecular genetics of T-cell lymphoblastic leukaemia / lymphoma (T-ALL/LBL), relatively little is known about its microenvironment and the role it plays.

The tumour microenvironment has been well studied in many haematological malignancies such as follicular lymphoma and diffuse large B-cell lymphoma.^{1,2} With respect to T-ALL/LBL, in 2001 Winter et al showed the importance of interactions between lymphocyte function-associated antigen-1 (LFA-1) on T-ALL/LBL cell lines and adhesion molecules present on bone marrow stromal cells such as intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1) and E-selectin.³ The bone marrow mesenchymal cell / microenvironment niche may play an important role in growth and survival of leukemic cells, and can contribute to tumour cell proliferation, dormancy, self-renewal or resistance to treatment. Several cytokines secreted by the tumour cells or the microenvironment contribute towards this interdependency.⁴⁻⁶ Among such cytokines, interleukin-8 (IL-8) is highly expressed in T-ALL/LBL cells refractory to chemotherapy. The mechanisms of IL-8 overexpression by T-ALL/LBL cells have been recently explored. It appears that this is dependent on the expression and secretion of the CXCL12 chemokine by bone marrow stromal cells, which then bind to CXCR4 on the leukaemic cells, which in turn upregulates IL-8 production through the nuclear factor-B and Jun-N-terminal kinase (JNK)/activating protein (AP)-1 signalling pathways.⁷ The understanding of the molecular mechanisms underlying the dependence of T-ALL/LBL cells on the microenvironment will help in unravelling the natural history of T-ALL/LBL and in designing alternative therapeutic strategies. Among Philadelphia chromosome-positive (Ph(+))B-LL a subpopulation of leukaemic cells express endothelial markers like VE-cadherin, PECAM-1, and Flk-1 and make them responsive to microenvironment-derived self-renewing and proliferative signals.⁸

The presence of dendritic B-cells with the morphology and immunophenotype of thymic *asteroid-B-cells* was serendipitously observed in the bone marrow stroma / microenvironment of a patient with relapsed T-ALL/LBL. Similar cells were then identified in the lymph node (LN) sample at the time of relapse. [Thymic asteroid B-cells are large B-cells with short dendritic cytoplasmic processes and are normally present in the thymic medulla.](#)⁹ Following this the bone marrow [trephine biopsy \(BMTB\)](#) and LN samples from seven other patients with T-ALL/LBL were re-evaluated, and asteroid-B-cells were found in the marrow stroma of one other patient. In this communication, we describe the findings and discuss the implications.

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Case report: A 47 year old man presented with a four week history of worsening shortness of breath, dry cough, sweats and fevers. Blood tests showed a normal full blood count. Chest x-ray revealed a large mediastinal mass with impending SVC obstruction. He also had a 1cm supraclavicular lymph node on the left side. CT guided Tru-cut biopsy showed T-ALL/LBL. His bone marrow examination showed no evidence of lymphoma or leukaemia. He was HIV negative, and his LDH was 1377u/L. The patient was treated with UKALL XII protocol. Fourteen months after the initial presentation he relapsed with a mediastinal mass infiltrating the sternum and with bone marrow involvement.

Bone marrow trephine biopsy (BMTB) at relapse: There was near-complete replacement of the marrow by sheets of blastic cells. The blasts expressed CD3, TdT, CD99, CD43, and CD10. They are negative for CD20, CD79a, CD34, CD117, CD68, CD68R, myeloperoxidase and HLADR. In addition, there were clusters and singly scattered B-cells, which were identified on the immunostains. These cells were unusual and showed dendritic processes. They were positive for CD20, CD79a, CD23, PAX5, [IgD](#), [CD44](#), OCT2 and BOB.1. The cells were negative for CD21, [CD38](#), [GCET](#), [CD138](#), [CD76](#), [IgM](#), [IgG](#), [IgA](#) and [IgE](#).

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Immunostains CD20, CD79a, CD23 and IgD highlighted the dendritic processes. The cells had features of asteroid-B-cells normally seen only in the thymus (Figure 1).

Cytogenetic analysis on bone marrow at relapse: G-banded chromosome analysis of bone marrow showed complex structural and numerical abnormalities. The karyotype was defined as 47~48,XY, del(2)(p16), der(3)add(3)(p24)add(3)(q26), -5,del(6)(q22), der(7)t(7;?)(q34;?), t(8;11)(p1;q2), add(9)(q34), +16, +19, +20[cp5]/46, XY[7]. Notably, the abnormalities observed included rearrangement of the long arm of one homologue of chromosome 7, which prompted further investigation by interphase fluorescence in situ hybridization (FISH). FISH analysis using a dual colour “break-apart” probe system specific for the TCRB locus at 7q35 (Dako, Denmark) revealed a split hybridisation signal, consistent with rearrangement of TCRB, in 94% of interphase cells examined.

Metaphase analysis using the same probe revealed the presence of the 3'TCRB sequences on the long arm of a chromosome identified by reversed DAPI banding as chromosome 9 (Figure 2), while the 5'TCRB probe was observed on a small F group-sized marker chromosome. Unexpectedly, neither component of the rearranged TCRB was present on the derivative chromosome 7, indicating further complex rearrangement of 7q than was detectable by G-band analysis.

Mediastinal mass biopsy at relapse: Biopsy of the mass infiltrating the sternum at relapse also showed features of T-ALL/LBL. The lymphoid cells were positive for CD2, CD3, CD5, CD7, CD4, CD8 and TdT. The cells were negative for CD20, CD79a, PAX5, CD34 and CD117. Similar to the bone marrow asteroid-B-cells were identified on CD20 and CD79a; but these were fewer in number as compared to the bone marrow sample.

Biopsy of the mediastinal mass at presentation: The biopsy showed essentially similar features and asteroid-B-cells were identified.

Revisit of additional cases: Samples of LN and BMTB from one and BMTB only from 6 other cases of T-ALL/LBL were revisited. All the BMTBs showed extensive involvement by tumour. One of the cases with bone marrow disease showed presence of asteroid B-cells in the bone marrow microenvironment [and this case is discussed in more detail](#).

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Case report of the additional case identified on review: A 38-year old man presented with B symptoms and cough, which had not resolved on antibiotics. A LN biopsy performed elsewhere was diagnosed as non Hodgkin lymphoma and staging CT was carried out showed mediastinal and cervical lymphadenopathy and he was therefore classified as Stage 2B. He was initially treated elsewhere with six cycles of CHOP chemotherapy (cyclophosphamide, doxorubicin, vincristine, and prednisone). With a partial remission to chemotherapy, he developed new left-sided cervical lymphadenopathy and the CT scan and the PET scan confirmed presence of residual active disease in the mediastinum and neck, with new disease in the left cervical region. A bone marrow aspirate and trephine was performed, and it confirmed involvement by T-ALL/LBL. He was then commenced UKALL-12 protocol achieving complete response after the 2nd cycle, then followed by 3 courses of high-dose methotrexate. This was followed by LACE (lomustine (CCNU), cytarabine (Ara-C), cyclophosphamide, etoposide) conditioned autologous stem cell transplant. However, he relapsed within three months in the mediastinum and two months later in the bone marrow.

Bone marrow investigations on the additional case at relapse: The BMTB was hypercellular with a cellularity of nearly 100%, and the marrow showed sheets of blastic cells amounting to over 90% of the marrow cells. They expressed CD3, CD10, TdT and CD99 and were of the cells are negative for CD20, CD43, CD34, myeloperoxidase and CD68R. In addition, there were B-cells with short dendritic processes, and these were positive for CD20 and CD79a. Flow cytometry performed on the bone marrow aspirate showed blasts representing 75% of the bone marrow cells and they expressed cytoplasmic CD3, CD10, TdT, CD5, CD7 and

CD4. They were negative for CD8, CD2, TCR-alpha/beta, TCR-gamma/delta, CD19, CD79a, CD22, cytoplasmic IgM, CD13, myeloperoxidase, CD34, CD15, CD117, CD33 and HLA-DR.

Rest of the cases reviewed: The other six cases included four male and two female adult patients in the age range of 22-32 years. The bone marrow in all the cases and LN in one case showed typical features and immunophenotype of T-ALL/LBL, and the patients were appropriately managed.

Discussion: We report the first case of T-ALL/LBL wherein certain aspects of thymic microenvironment are simulated at the bone marrow and LN sites of disease involvement.

The case also showed a similar microenvironment in the mediastinal site at the time of relapse.

Although the thymus is predominantly a T-cell organ, B-lymphocytes are present in the thymic medulla. A proportion of thymic B-cells are asteroid-shaped larger cells with short dendritic cytoplasmic processes. These asteroid-B-cells express CD20 and CD23, and inconsistently express CD19, CD22, CD37 and IgM. The asteroid-B-cells are located in the cortico-medullary region of the foetal thymus. However, in the adolescent and adult thymus they are randomly distributed in the medulla and are seen in greater numbers close to Hassall's corpuscles. They also form rosettes with T-cells and possibly represent the earliest interaction of maturing T-cells with B-cells.⁹ Their close association with T-cells and medullary epithelial cells suggests a functional role for the asteroid-B-cells in the thymus.¹⁰ However, the exact nature of interaction between the asteroid-B-cells and other cells in the thymus is poorly understood. More recently, the tumour cells of primary mediastinal large B-cell lymphoma of thymic origin were hypothesised to be related to asteroid-B-cells.¹¹

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Asteroid-B-cells are not seen outside of the thymus, though similar large B-cells with a dendritic morphology have been described in the interfollicular region of the LNs.¹²

In the two cases where the asteroid-B-cells were identified in the bone marrow, it is possible that the T-ALL/LBL cells are dependent on the asteroid-B-cells for their survival. This feature of presence of asteroid-B-cells as an important component of the T-ALL/LBL was seen only in two of the 8 cases evaluated, and both had presented with mediastinal involvement. Generally, about one-half of T-ALL/LBL patients present with mediastinal disease.^{13,14} It may be that in a subset of cases that have prominent mediastinal involvement the neoplastic cells may receive survival signals from the asteroid-B-cells.

We could not identify a unique immunophenotype of the neoplastic cells among the two cases of T-ALL/LBL with asteroid B-cells. Though, both the cases of T-ALL/LBL with asteroid B-cells showed co-expression of CD10 in the blasts, this feature was also noted in one of the cases without asteroid B-cells. Furthermore, CD10 expression is reported in close to 20% of T-ALL/LBL and in some studies in over 60%.^{15,16}

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Our index case had an aggressive disease course with disease relapsing within four months after completion of treatment. The second case that was identified on review also had an aggressive course with a short remission after high-dose chemotherapy. The question whether cases of T-ALL/LBL with asteroid-B-cells represent a distinct subset with aggressive behaviour, needs to be addressed. Our index case was associated with rearrangement of the TCRB locus (7q35). This in itself is not unique. A majority of T-ALL/LBL have cytogenetic abnormalities and among them, those involving the four TCR loci are more common. The partner genes involved in the translocations are variable and include HOX11 (10q24), HOX11L2 (5q35), MYC (8q24), TAL1 (1p32), NOTCH1 (9q34), RBTN1 (11p15), RBTN2 (11p13), LYL1 (19p13) and others. These translocations often result in aberrant expression of

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the genes partnering the TCR gene.¹⁷ The observation of TCRB sequences within the distal part of chromosome 9q in case 1 implicates NOTCH1 as a potential partner gene in this case, although this has not been confirmed by molecular analysis.

The presence of asteroid-B-cells closely interacting with neoplastic cells of T-ALL/LBL, apart from possibly defining a [small](#) subset of T-ALL/LBL, raises the question of whether such patients may benefit by targeting the asteroid-B-cells with anti-CD20 monoclonal antibody.

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Figure 1: Bone marrow trephine biopsy of the index case shows diffuse infiltration by blasts (a) positive for CD3 (b) and TdT (c). CD20 immunostain identifies loosely organised asteroid / dendritic B-cells (d & e) that are also positive for CD23 (f).

Figure 2: Fluorescence in situ hybridisation of a representative metaphase cell performed using Dako TCRB split signal probe (Dako, Denmark). This probe system is composed of two contigs, specific for sequences 5' and 3' of the TCRB locus, labelled in green and red, respectively. In normal cells two red-green fusion signals are observed. However, in the index case, an aberrant signal pattern was observed. One intact red-green fusion signal was present on the normal chromosome 7 homologue, marking the un-rearranged TCRB locus. In contrast, the second TCRB hybridisation signal reveals relocation and rearrangement of this region, with part of the green 5' signal hybridised at the distal end of an F group-sized marker, while the remains of the green 5' signal together with the red 3' signal are present at the distal end of the putative chromosome 9 (identified by reversed DAPI analysis). This finding is consistent with rearrangement of TCRB followed by further rearrangement of chromosome 7.