Anaplastic large cell lymphoma in paediatric and young adult patients

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Anaplastic large cell lymphoma in paediatric and young adult patients

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Summary

Anaplastic large cell lymphoma (ALCL) is a heterogeneous disease of debateable origin, which in children is largely anaplastic lymphoma kinase (ALK) positive with aberrant ALK activity induced following the formation of chromosomal translocations. Whilst the survival rates for this disease are relatively high, a large proportion of patients suffer disease relapse, in some cases on multiple occasions and therefore suffer the toxic side-effects of combination chemotherapy. Traditionally, patients are treated with a combination of agents although recent data from relapse patients have suggested that low risk patients might benefit from single agent vinblastine and going forward the addition of ALK inhibitors to the therapeutic regimen may have beneficial consequences. There are also a plethora of other drugs that might be advantageous to patients with ALCL and many of these have been identified through laboratory research although the decision as to which drugs to implement in trials will not be trivial.

Keywords: Anaplastic Large Cell Lymphoma; Anaplastic Lymphoma Kinase; T cell lymphoma; ALK inhibitors

Systemic anaplastic large cell lymphoma (ALCL), first described by Stein et al. in 1985, is a rare, aggressive CD30-positive non-Hodgkin lymphoma (NHL). A significant proportion of ALCLs are associated with the t(2;5)(p23;q35) translocation which was cloned in 1994 by Morris et al., (Morris*, et al* 1994) and following the production of antibodies detecting its gene product - anaplastic lymphoma kinase (ALK) (Pulford*, et al* 1997) our understanding of the mechanisms employed by this oncogene has increased considerably. Indeed, in recent times, ALCL has been sub-divided into two entities based on aberrant ALK expression as a result of chromosomal translocations giving rise to ALCL, ALK+ and ALCL, ALK- sub-classes (Swerdlow*, et al* 2008) although the majority of children are diagnosed with the former. As such, most of the research conducted into mechanisms underlying the generation of ALCL have focussed on the role of aberrant ALK activity mostly in the form of the t(2;5)(p23;q35) product, Nucleophosmin 1 (NPM)-ALK.

Epidemiology and clinical characteristics

ALCL accounts for approximately 10 to 15% of all NHL in children and 1-2% of adult NHL. In children and adolescents, more than 90% of cases are ALK positive (Brugieres*, et al* 2009a) compared to only 40-50% of adult patients, with a median age of around 30 years in ALCL, ALK+ and 55 in ALK negative disease (Sibon*, et al* 2012).

Three major entities have been described: primary systemic ALCL, ALK+, primary systemic ALCL, ALK- and cutaneous ALCL. More recently, a form of ALCL associated with breast implants has been described with these cases being ALK- (Ye*, et al* 2014) as are most cutaneous ALCL, the latter of which are characterised by localised or solitary and often ulcerated skin tumours (Willemze*, et al* 2005). Most patients with systemic ALCL present at advanced stages (stages III-IV) with peripheral intra-abdominal or mediastinal lymph node involvement frequently associated with B symptoms and extra-nodal spread including skin, liver, lung, soft tissue and bone localisation (Brugieres*, et al* 2009a, Sibon*, et al* 2012). Bone marrow involvement is detected in less than 15% of cases on bone marrow biospies and/or smears but, in ALK positive cases, reverse transcription-polymerase chain reaction (RT-PCR) for NPM-ALK can detect minimal disease in up to 50% of cases (Mussolin*, et al* 2005). CNS involvment is rare (Williams*, et al* 2013).

Morphology of ALCL

The definition of ALCL has evolved considerably since the first description of strong CD30-expressing large tumour cells growing within sinuses (Stein*, et al* 1985). They were defined as lymphomas consisting of lymphoid cells that are often large and have abundant cytoplasm and pleomorphic, often horseshoe-shaped nuclei. Besides being $CD30^{+}$, most cases also express cytotoxic granule-associated proteins and epithelial membrane antigen (EMA). Histologically speaking, ALCLs lacking ALK expression are heterogeneous whereas ALCLs expressing ALK were considered relatively homogeneous. However, a study of a large series of ALCL positive for ALK protein provided strong evidence that they show a broad spectrum of morphologic features ranging from small cell neoplasms to an opposite extreme in which very large cells predominate (Benharroch*, et al* 1998) (Figure 1).

Hence, the 2008 World Health Organisation (WHO) classification recognizes five morphological patterns of ALK-positive ALCL sharing the presence of large cells with a highly characteristic morphology (eccentric horseshoe- or kidney-shaped nuclei, often with an eosinophilic region near the nucleus) referred to as 'hallmark' cells (Fig 1): common, small-cell, lymphohistiocytic, Hodgkin-like and composite patterns (Swerdlow*, et al* 2008). An inflammatory background is invariably present but its intensity varies amongst the different morphologic patterns. ALCL, common pattern (60%) is composed predominantly of pleomorphic large cells with the hallmark features described earlier. The tumour characteristically grows within the sinuses or colonizes the paracortex, although complete architectural effacement can also be seen. They often grow in a cohesive manner and thus may resemble a metastatic tumor.

ALCL, lymphohistiocytic pattern (LH-ALCL; 10%) is characterized by tumour cells often clustered around blood vessels, admixed with a large number of histiocytes (Pileri*, et al* 1990). The histiocytes typically have finely granular eosinophilic cytoplasm and small, round uniform nuclei and are associated with varying numbers of plasma cells. They may mask the malignant cells, which are often smaller than in the common pattern, leading to an incorrect diagnosis of a reactive lesion. The key to diagnosis is immunohistochemistry using CD30 and ALK-reactive antibodies; this highlights the malignant cells scattered among the histiocytes and typically concentrated around blood vessels. The 'small cell pattern' (SC-ALCL; 5−10%) shows a predominant population of small cells with irregular nuclei and abundant clear cytoplasm (Kinney*, et al* 1993). Hallmark cells are present but are difficult to detect among small to medium-sized cells. This variant is often misdiagnosed as peripheral T-cell lymphoma not otherwise specified (PTCL, NOS) by conventional examination, but the perivascular distribution of hallmark cells can be helpful for diagnosis. Small tumour cells may be

only weakly positive or even negative for CD30 (Falini*, et al* 1999b) and ALK positivity is usually restricted to the nucleus of tumour cells.

The 'Hodgkin-like pattern' (HL-ALCL; 3%) is characterised by morphological features mimicking nodular sclerosis classical Hodgkin lymphoma, particularly as this latter classification also encompasses cases with a composite pattern in which more than one variant can be seen within a single lymph node biopsy (Vassallo*, et al* 2006). CD15 expression is rarely observed but aberrant expression of PAX5 can represent a diagnostic challenge in HL-ALCL (Feldman*, et al* 2010). Other less frequently encountered patterns include the monomorphic variant, cases rich in multinucleated giant cells or cases with sarcomatoid features. The importance of recognising these rare variants lies in the potential for misdiagnosis with serious clinical consequences. By definition, malignant cells are strongly positive for CD30 in both the membrane and Golgi and the great majority of cases co-express EMA.

Revisions of the 4th Edtion of the WHO classification have established ALK-negative ALCL as a recognised category. Biopsies typically show large pleomorphic cells, sometimes containing prominent nucleoli (Fig. 1). In addition, to a variable degree, "hallmark" cells with eccentric, horseshoe- or kidney-shaped nuclei are seen.

Phenotype and cell of origin

ALCL are considered peripheral T-cell lymphomas as they express one or more T-cell antigens and are often present at peripheral sites although mediastinal involvement is not infrequent (50%) (Lamant*, et al* 2011). However, due to loss of several pan T-cell antigens, some cases may have an apparent 'null cell' phenotype, but show evidence of a T-cell lineage at the genetic level (see below); CD3 is negative in more than 75% of cases, as are proximal T-cell receptor (TCR) signaling proteins (Bonzheim*, et al* 2004). CD2, CD4 and CD5 are positive in a significant proportion of cases (70%) but CD8 is frequently negative. Most cases express cytotoxic associated-antigens TiA1, granzyme B and perforin.

It is therefore considered that peripheral cytotoxic T-cells are the cell of origin of this disease although recent research calls this into question. Identification of the tumour propagating or cancer stem cell for ALCL identified a gene signature characteristic of an early thymic progenitor within this distinct cellular subset (Moti*, et al* 2015). In support of this concept, transcripts for NPM-ALK are detectable in 2/103 samples of newborn cord blood sampled from an otherwise healthy population (Laurent*, et al* 2012). Together these data suggest that the generation of the t(2;5) may be an early

event occuring in primitive haemopoietic cells and perhaps requiring a thymic environment or at least T cell-specific events for transformation although rare ALK+ B cell lymphomas do exist (Laurent*, et al* 2009). In evidence, Malcolm et al. have shown that many ALCLs carry molecular TCR rearrangements that would not normally be selected during normal thymic development in that 2/3rds of tumours examined had major clonal TCRα rearrangements in the absence of a comparable major TCRβ rearrangement (Malcolm*, et al* 2015). Such cells would not normally pass the β-selection checkpoint during thymic development instead undergoing apoptosis. These immunogenetic events are indicative of aberrant events in the thymi of a majority of patients and in mouse models the authors could show that aberrant expression of ALK in the form of the NPM-ALK fusion protein allowed thymocytes to bypass β-selection through up-regulation of Notch1 expression. These data raise the interesting concept of a thymic origin for this disease whereby 'primed' thymocytes aberrantly expressing ALK escape into the periphery to eventually develop into tumours (Fig. 2). Given that the thymus involutes with increasing age, the predominance of this cancer in children also lends support to this theory.

Genetics and Molecular Findings

As previously mentioned, ALCL are subdivided into ALK+ and ALK- sub-classes with the former defined by its genetic features, namely the presence of chromosomal translocations leading to expression of ALK fusion proteins. ALK staining may be cytoplasmic, nuclear, and nucleolar or it may be restricted to either the cytoplasm or, more rarely, the cell membrane, according to the ALK fusion partner. It is important to note that in the small cell pattern and, to a lesser extent, in the lymphohistiocytic pattern, ALK staining may be restricted to scattered large cells and hence is easier to detect without a nuclear counterstain. Tumours associated with the t(2;5) (75 to 80% of ALK+ cases) express NPM-ALK protein and show a characteristic cytoplasmic, nuclear and nucleolar staining pattern. This particular pattern can be explained by the formation of two types of dimers through oligomerisation domains at the N-terminus of NPM which are retained in the fusion protein. The formation of NPM-ALK homodimers mimics ligand binding and leads to activation of the ALK catalytic domain. NPM-ALK can also dimerize with wild-type NPM, a protein involved in nuclear/cytoplasmic trafficking through its nuclear localisation sequence hence accounting for the nuclear localisation of NPM-ALK in tumour cells (Mason*, et al* 1998). However, variant translocations involving ALK and other partner genes on chromosomes 1, 2, 3, 9, 17, 19, and 22 also occur. All result in the aberrant expression of ALK, but the distribution of the staining varies, depending on the translocation partner.

The most frequent variant translocation is the t(1;2)(q25;p23), found in 15% to 18% of ALK+ ALCLs (Lamant*, et al* 1999). In theses cases, which express the TPM3-ALK protein, ALK staining is restricted to the cytoplasm of malignant cells and in virtually all cases there is stronger staining on the cell membrane. Rare cases of ALCL associated with the t(2,17) (p23,q23) show a unique granular cytoplasmic staining pattern. In these cases, the *ALK* gene is fused to the *CLTC* gene, which encodes the clathrin heavy polypeptide (CLTC) (Touriol*, et al* 2000). The implication of the clathrin heavy polypeptide in the hybrid protein accounts for the granular cytoplasmic staining pattern because the CLTC protein is involved in the formation of the clathrin coat on the surface of vesicles. In a single report, the moesin (*MSN*) gene at chromosome Xq11-12 was identified as a new *ALK* fused gene (MSN-ALK fusion protein) in a case of ALCL with a distinct membrane-restricted pattern for ALK (Tort*, et al* 2001). This particular membrane staining pattern for ALK is probably due to the binding properties of the N-terminal domain of moesin to cell membrane–associated proteins. In this case, the *ALK* breakpoint was different from that described in all other translocations and occurred within the exonic sequence coding for the juxtamembrane portion of ALK. In all other translocations, ALK staining is cytoplasmic (TFG-ALK, ATIC-ALK, TPM4-ALK, ALO17-ALK, MYH9-ALK, TRAF1-ALK resulting from the t(2;3)(p23;q11), inv(2)(p23q35), t(2;19)(p23;p13), t(2;17)(p23;q25), t(2;22)(p23;q11.2) and t(2;9)(p23;q33) respectively) (Cools*, et al* 2002, Feldman*, et al* 2013, Lamant*, et al* 2003, Liang*, et al* 2004, Rosenwald*, et al* 1999, Trinei*, et al* 2000). Besides the generation of these defining translocations, ALCL, ALK+ is relatively stable at the level of the genome with few other karyotypic alterations (Salaverria*, et al* 2008, Youssif*, et al* 2009).

In contrast, defining genetic events for ALCL, ALK- have been less forthcoming until recently when rearrangements in the DUSP22-IRF4 locus on 6p25 and TP63 were reported, although these abnormalities are not specific for ALCL, ALK- (Parrilla Castellar*, et al* 2014, Vasmatzis*, et al* 2012). In addition, recurrent activating mutations of JAK1 and/or STAT3 genes have been identified in 20% of cases (Crescenzo*, et al* 2015) and miR155 overexpression has been detected (Merkel*, et al* 2015, Merkel*, et al* 2010). Undoubtedly as techniques develop, more defining (epi)genetic events will be described and other studies for example, have identified alterations to the epigenome in ALCL inclusive of CpG methylation and expression or down-regulation of small non-coding RNA sequences including miRNAs (Ambrogio*, et al* 2009, Hoareau-Aveilla*, et al* 2015, Merkel*, et al* 2015, Merkel*, et al* 2010, Zhang*, et al* 2011).

Overall, whilst potentially providing novel therapeutic targets, these findings align ALCL, ALK+ and ALCL, ALK- as distinct entities. For example, gene expression studies have shown that a 3-gene signature (TNFRSF8, BATF, and TMOD1) can distinguish ALCL, ALK- from both ALCL, ALK+ and

PTCL, NOS (Agnelli, *et al* 2012). Hence, these findings could secure ALCL, ALK- as a distinct entity in the upcoming revision of the WHO classification and as provisionally proposed in 2008 (Swerdlow, *et al* 2008).

Biological mechanisms implicated in the pathogenesis of ALCL

Kinase-induced signal transduction

The large majority of research into the pathogenesis of ALCL has focused on the role of the predominant oncogenic event, the generation of the ALK fusion protein NPM-ALK and its downstream events. It has been shown exhaustively that NPM-ALK can activate a plethora of signal transduction pathways including PI3 Kinase, MAP Kinase and JNK amongst others (Bai, *et al* 1998, Bai, *et al* 2000, Leventaki, *et al* 2007, Marzec, *et al* 2007, Slupianek, *et al* 2001, Turner and Alexander 2006, Turner, *et al* 2007). In addition, STAT3 appears to play a central role in transformation as a consequence of its activation in response to aberrant ALK activity in ALCL, ALK+ (Chiarle, *et al* 2005, Zhang, *et al* 2002) and as a result of mutation events of Jak/STAT proteins in ALCL, ALK- (Crescenzo, *et al* 2015). However, emerging data presented in Varese is unravelling signalling pathways downstream of the TCR, a protein complex that is absent in ALCL. It has been known for some time that ALCL lack expression of TCR proteins whereby there is a striking absence of TCRβ and CD3 expression as well as the proximal TCR cell signalling protein Zap-70 (Bonzheim, *et al* 2004). Until recently it remained unknown as to whether this lack of a functional TCR was oncogene-driven or a consequence of some other activity or indeed if down-regulation of this signalling axis was necessary for cellular transformation. In the interim, epigenetic mechanisms were attributed to silencing by CpG methylation of promoter regions of proximal TCR signalling proteins as well as the IL2R (Ambrogio, *et al* 2009, Zhang, *et al* 2011). The fact that these tumour cells survive and proliferate in the absence of transduction via these signalling pathways is perhaps not surprising given that NPM-ALK can mimic both TCR-induced and IL2R-induced messages (Marzec, *et al* 2013, Turner, *et al* 2007). Whether this is a cause or consequence or indeed if silencing of TCR signalling is essential for cellular transformation remains to be determined but new data generated from a combined approach to human and murine systems provides some insight. In a murine model of ALCL in which all T cells express a TCR specifically recognising ovalbumin peptides as well as NPM-ALK, forced signalling through this TCR (via *in vivo* MHV-ova infection) prevented lymphoid tumour formation suggesting that signalling through the TCR in the presence of NPM-ALK is obstructive for tumour growth (Malcolm, *et al* 2015). However, these data also show that at least transient expression of a

TCR is required for thymic emigration and peripheral tumour development in mice (Malcolm*, et al* 2015). These data suggest that ALCL tumour cells whilst deriving from the T lineage are in some cases unable to functionally act as T cells in the periphery and therefore cannot contribute to an inflammatory response, at least not in an antigen-specific manner via the adaptive immune response. However, innate T cells have been reported whereby response to signalling through the TCR is attenuated allowing cells to act in an innate manner (Wencker*, et al* 2014). Furthermore, some ALCL displayed germline TCR (14%) whilst others resembled γδ T cells at the genetic level (Malcolm*, et al* 2015). Whether such a mechanism applies to ALCL remains to be examined, in particular, if a response to antigen contributed to the lymphomagenic process with subsequent down-regulation prior to or during transformation in the periphery. Reports of ALCL arising in the context of insect bites support this theory to some extent (Lamant*, et al* 2010). Hence, the timing and sequence of events might be of utmost importance to the tumourigenic process (Fig. 2).

Emerging hallmarks and enabling characteristics: Metabolomics

Whilst many of the 'traditional' (hyper)active kinase induced pathways identified in many cancers are likewise functional in ALCL, recent research has begun to focus on other facets of tumour cells and their microenvironment including the so-called enabling characteristics and emerging hallmarks (Chiarle*, et al* 2008b, Hanahan and Weinberg 2011). In this regard, metabolomics has faced a resurgence in cancer research as studies show altered metabolism in tumour cells. Indeed, NPM-ALK was shown to induce a shift towards aerobic glycolysis with increased lactate and biomass production in a PKM2-dependent manner in tumour cells (McDonnell*, et al* 2013). This is very much an emerging area of research in ALCL and with in-depth analysis of proteomics and gene expression datasets will likely provide further insight into the mechanisms of ALCL and even future therapeutic targets.

Emerging hallmarks and enabling characteristics: Immune evasion

Up-regulation of proteins that prevent recognition of tumour cells by the inflammatory system is increasingly recognised in a number of cancers and involves expression by the tumour cells of a number of proteins including PDL1/PD1 and CTLA-4. Likewise in ALCL, NPM-ALK induces expression of PDL1 via STAT3 activity although it remains to be seen whether this has functional consequences to the tumour cells (Marzec*, et al* 2008). More recently, in Varese, the group of Megan Lim presented data showing that ALCL lack expression of CD48, a T cell co-stimulatory

protein and ligand for the natural killer cell receptor 2B4 (Rolland, *et al* 2015). Down-regulation of CD48 has previously been linked with immune evasion in AML (Elias, *et al* 2014) and this was also demonstrated to be the case for ALK+ ALCL. Given the success and enthusiasm for immunotherapies, it is likely that reactivation of the immune system by removing the tumour 'camouflage' will be a viable therapeutic option for ALCL.

However, as presented extensively at the meeting, it has been known for some years that ALK+ ALCL patients display an immune response to the tumour cells; children produce antibodies to ALK and those with a higher titre have a far better prognosis, particularly when combined with an absence of detection of minimal disseminated disease (MDD) (Ait-Tahar, *et al* 2010, Mussolin, *et al* 2013, 2015). A cellular response to tumour cells has also been reported in the form of anti-ALK specific CD8 and CD4 T cells although of course this is insufficient to completely stem the disease (Ait-Tahar, *et al* 2007, Passoni, *et al* 2006, Passoni, *et al* 2002) but efforts continue to be made to understand this activity (Woessmann 2015). It is therefore reasonable to speculate that these children will also respond well to immunotherapies and ALK vaccination has also been proposed as one strategy whereby vaccination of mice with a truncated ALK cDNA has led to CD8 T-cell mediated protection against tumour growth (Chiarle, *et al* 2008a).

Emerging hallmarks and enabling characteristics: Tumour microenvironment

The tumour microenvironment is essential to tumour growth providing a supportive network. It has previously been shown that platelet derived growth factor receptor (PDGFR) and *PDGFB* expression levels are elevated both in transgenic NPM-ALK mouse T cell tumours and primary human ALCL tumours as well as patient plasma (Laimer, *et al* 2012). In NPM-ALK transgenic mice, PDGFRB is a direct target of JUNB and cJUN, and serves as a central mediator of tumour progression and dissemination. Indeed, previously published data clearly show that inhibition of PDGFR with Imatinib induces tumour cell death (in the absence of the other targets of Imatinib activity c-kit and Abl) although the mechanism of this activity remains for the most part undetermined (Laimer, *et al* 2012). A clinical study for ALK+, PDGFR+ ALCL patients combining brentuximab vedotin and imatinib started in Austria in 2015 and is still recruiting. Given that PDGFs and their receptors can drive disease progression through cell autonomous effects in cancer cells via autocrine signalling these data suggest pleiotropic functions of an autocrine/paracrine loop during ALCL tumour progression (Hoch and Soriano 2003). For example, PDGF has been described to have an important impact on the surrounding tumour microenvironment and the PDGF/PDGFR axis promotes invasion and metastasis

(Raica and Ribatti 2010). Cancers characteristically develop embedded in an environment of non-malignant connective tissues and closely associate with the so-called 'tumour stroma'. Reciprocal interactions between tumour cells and the stroma have a profound influence on tumour development and outcome: It is considered a hallmark of cancer (Hanahan and Weinberg 2011). In support of such a role in ALCL, data were presented in Varese showing PDGFR expression on tumour or stromal cells in primary patient tissue to be indicative of prognosis and furthermore that deletion of PDGFR in tumour cells alone (not stroma) delays but does not inhibit tumour growth as does complete ablation of PDGFR (Kenner*, et al* 2015). Hence, accumulating evidence identifies PDGFRB as a central mediator of tumour progression in ALCL cells. While aberrant expression of PDGFRB in stromal cells is intriguing, it raises questions as to whether inhibitors of PDGFR are having an effect on both the tumour cells and its stroma in ALCL; an additional layer of complexity to the role of PDGFR in ALCL which needs to be better explored.

Therapeutic strategies

Front line treatment

One of the peculiarities of ALCL, ALK+ is its extreme chemo-sensitivity in front line and at relapse leading to high response rates with very diverse chemotherapy regimens. Several small series of patients were published in the late nineties with very similar event-free survival (EFS) rates of about 65-75% despite quite diverse first-line chemotherapy regimens involving a number of drugs, with differing cumulative doses and varying durations of treatment (Table 1) (Brugieres*, et al* 1998, Laver*, et al* 2005, Lowe*, et al* 2009, Rosolen*, et al* 2005, Seidemann*, et al* 2001, Williams*, et al* 2002).

Most paediatric groups have now adopted the ALCL99 chemotherapy backbone as the reference chemotherapy; given low cumulative doses of agents that are associated with long-term side effects such as anthracyclines and alkylating agents in this protocol. This regimen, derived from the Berlin-Frankfurt-Munster (BFM)-B NHL protocol, combines a pre-phase and 6 alternating courses over a period of 4 months. Long-term toxicity is expected to be very limited but acute toxicity is quite significant especially hematologic toxicity with grade 4 neutropenia reported in 60%, mucositis in 15% and significant weight gain in 20% of patients (Wrobel*, et al* 2011). Therapeutic results are very similar to those obtained with other regimens: in the ALCL99 trial, the 2 year EFS and overall survival (OS) rates were 74% and 92.5% respectively in a large series of more than 350 patients from Europe and Japan (Brugieres*, et al* 2009a).

Adult patients are generally treated according to protocols designed for diffuse large-cell lymphoma mostly with anthracycline-containing regimens (CHOP, CHOEP (CHOP + etoposide)) or ACVBP (Falini, *et al* 1999a, Gascoyne, *et al* 1999, Savage, *et al* 2008, Schmitz, *et al* 2010, Sibon, *et al* 2012, Suzuki, *et al* 2000). Results are very similar to those obtained in children with a 5-year EFS and OS of around 65-80% and 70-90% respectively in ALK positive ALCL (Table 1). ALCL, ALK- are usually treated with the same protocols as the ALK+ disease even though the EFS for ALK- patients is poor ranging from 15% (Falini, *et al* 1999a) to 46% (Schmitz, *et al* 2010). However, the good prognosis of ALK positive ALCL seems to be largely related to age of incidence since the prognosis of ALK- and ALK+ ALCL is similar in patients less than 40 years (Savage, *et al* 2008, Schmitz, *et al* 2010, Sibon, *et al* 2012).

The prognosis of primary cutaneous ALCL is excellent with a treatment based on surgical excision or local radiotherapy for localized lesions (Kempf, *et al* 2011). However, there is still no consensus for the treatment of multifocal cutaneous ALCL although the efficacy of Brentuximab (Duvic, *et al* 2015) or vinblastine (Laly, *et al* 2015) have been reported recently. Multi-agent chemotherapy is only recommended for extra-nodal spread beyond local to regional lymph nodes.

Prognostic factors

Several factors have been shown to be associated with a higher risk of treatment failure in children, i.e. clinical factors such as presence of mediastinal disease, visceral (defined as lung, liver, or spleen), or cutaneous involvement (Le Deley, *et al* 2008), high risk histologic subtype defined by the presence of a lympho-histiocytic or small cell component (Lamant, *et al* 2011), positive PCR for NPM-ALK in peripheral blood and/or bone marrow at diagnosis (MDD) (Damm-Welk, *et al* 2007, Mussolin, *et al* 2005), low anti-ALK antibody titres at diagnosis (Ait-Tahar, *et al* 2010) and detection of minimal residual disease (MRD) by PCR for NPM-ALK in the blood after the first course of chemotherapy (Damm-Welk, *et al* 2014). These factors can be employed in the design of future treatments in order to stratify patients into risk groups. For example, a combination of MDD and ALK antibody titres detected at the time of diagnosis can be used to stratify patients into high (20% of patients), intermediate (49% of patients), and low risk groups (31% of patients) with a progression free survival (PFS) of 93%, 68% and 28%, respectively as shown in a cohort of 128 patients from the BFM and Italian groups, ($p < 0.0001$) (Mussolin, *et al* 2013).

Treatment of relapse

There is still no gold standard for the treatment of relapse. Several small retrospective studies have demonstrated that patients suffering from relapsed ALCL, ALK+ still have a 50-60% chance of survival. In these studies various therapeutic approaches including a wide variety of chemotherapy regimens and in most publications, autologous or allogeneic hematopoietic stem cell transplantation after complete remission (CR) were used (Brugieres*, et al* 2000, Mori*, et al* 2006, Woessmann*, et al* 2011). Several prognostic factors, such as a short time to relapse and positive immunostaining with an anti-CD3 antibody on tumour specimens at diagnosis, have been shown to be associated with a high risk of failure after first relapse (Brugieres*, et al* 2000, Mori*, et al* 2006, Woessmann*, et al* 2011). In order to evaluate a risk-adapted strategy, stratified according to the time of relapse and immunophenotype, the European Intergroup for Childhood non Hodgkin Lymphoma (EICNHL) launched a prospective trial, the ALCL relapse protocol. The final results of the ALCL relapse protocol confirmed the good efficacy of allogeneic haematopoietic stem cell transplantation in high risk relapse leading to a 3 year EFS after relapse of 64%, whereas the results obtained with autologous stem cell transplantation in intermediate risk relapse were quite disappointing (3 year EFS of only 41%). In a small cohort of 21 patients with low risk relapse (relapse more than one year after diagnosis, CD3 negative), weekly administration of vinblastine proved its efficacy with a 3 year EFS of 85% (Ruf*, et al* 2015). The ability of vinblastine to stimulate a dendritic cell response, suggests that the remarkable efficacy of vinblastine monotherapy in patients with relapsed/refractory ALCL could be related not only to the cytotoxic effect of vinblastine, but also its ability to boost the patient immune response against ALK (Tanaka*, et al* 2009a, Tanaka*, et al* 2009b).

Following the first report demonstrating the efficacy of weekly vinblastine in relapse patients (Brugieres*, et al* 2009b), two different trials, one from the EICHNL group and the second from the Children's Oncology Group (COG), aimed to assess whether adding administration of weekly vinblastine to one year of standard chemotherapy would improve EFS of newly diagnosed ALCL patients (Alexander*, et al* 2014, Le Deley*, et al* 2010). In both trials, this strategy failed to reduce the incidence of relapse. However, in the ALCL99 trial, in which the duration of standard chemotherapy was only 4 months, the addition of an 8 month maintenance stage with weekly vinblastine chemotherapy significantly delayed the occurrence of relapse (13 months versus 6.5 months in patients without vinblastine) (Le Deley*, et al* 2010). In the COG trial (Alexander*, et al* 2014), in which vinblastine was combined with one year of standard chemotherapy, no benefit from the addition of vinblastine was shown. The recent results of the ALCL relapse protocol showing durable remission in patients treated with weekly vinblastine for 2 years (Ruf*, et al* 2015), suggest that the duration of vinblastine treatment might need to be increased to 2 years to reduce the risk of recurrence after discontinuation of treatment. Given the excellent results obtained with

vinblastine in first relapse, the low cost of this drug, its good safety profile compatible with outpatient treatment and the absence of known long term side effects, it seems important to test whether this drug could substitute multi-agent chemotherapy for front line treatment. This is one of the aims of the planned ALCL2 study from the EICNHL group.

New drugs

Several promising novel agents are being investigated for the treatment of ALCL and may lead to profound modifications in therapeutic strategies in the near future. Brentuximab vedotin is an anti-CD30 antibody–drug conjugate that selectively delivers an anti-microtubule agent, monomethyl auristatin E into CD30-expressing cells. Several phase 2 studies have shown high response rates in ALCL. The first reported an overall response rate of 86% and a complete response rate of 57% in 58 adult patients with relapsed/refractory ALCL. For the 16 ALCL, ALK+ patients included in this trial, overall response and complete remission rates were 81% and 69% respectively, in patients who had achieved a complete response; the median duration of response was 13 months with either autologous or allogeneic stem cell transplantation after CR, or prolongation of treatment with brentuximab vedotin for 12 months (Pro*, et al* 2012). On the basis of these results, brentuximab vedotin was approved in the USA and Europe for the treatment of relapsed ALCL in adults following failure of at least one multi-agent chemotherapy protocol. Brentuximab vedotin is given as an outpatient treatment at a dose of 1.8mg/kg every 3 weeks and is generally well tolerated. The most significant side effect is peripheral neuropathy described in 40% of patients that usually resolves within the first few months following the end of treatment. Given this side effect, prolonged treatment with brentuximab vedotin may be difficult to manage. Thus, this drug has mostly been used as a bridge to transplant in relapsed patients. However, it may also be a feasible option when used in combination with multi-agent chemotherapy (Fanale*, et al* 2014) and is currently being tested for front-line treatment in association with cyclophosphamide, doxorubicin, and prednisone (CHP) in adults (NCT01777152) and with ALCL99 in children (NCT01979536). Of note, a recent report demonstrated that retreatment with brentuximab vedotin is possible and induces a response; a complete response rate of 63% was reported in 8 ALCL patients who had previously achieved an objective response with prior brentuximab vedotin treatment (Bartlett*, et al* 2014).

ALK inhibitors are also very promising since ALK tyrosine kinase activity is essential to the survival of ALK+ ALCL. Crizotinib, an orally available dual ALK/MET inhibitor, currently approved for

advanced ALK-positive non-small cell lung cancer (NSCLC) in adults has been shown to induce high response rates: 7/8 ALCL patients achieved CR in a paediatric phase 1 trial (Mosse*, et al* 2013) and all 9 patients in a retrospective report of adults treated with crizotinib for refractory/relapsed ALCL (Gambacorti Passerini*, et al* 2014). Crizotinib is given orally and shows a good tolerability profile; the most frequent treatment-related adverse effects are transient mild visual disorders and elevated amino transferase levels. Unlike in NSCLC in which most patients treated with crizotinib experience resistance after a few months, no progressions have been described for ALCL during crizotinib treatment so far except for 2 patients with early relapses within 2.5 months of treatment initiation (Gambacorti Passerini*, et al* 2014). Even though it induces CR in most cases, Crizotinib has not yet proven curative since it may require life-long treatment; abrupt relapses of ALK+ lymphoma following crizotinib discontinuation have been described (Gambacorti Passerini*, et al* 2015). Most patients so far have been treated with crizotinib to induce CR as a bridge to transplant or as a prolonged treatment (Gambacorti Passerini*, et al* 2014, Mosse*, et al* 2013). Prospective multi-centre clinical trials, with strict molecular monitoring will be required to evaluate whether discontinuing crizotinib in ALCL is safe and to establish the optimal duration of treatment. Several clinical trials testing the efficacy of crizotinib in ALCL patients are on-going including a trial conducted by the COG group in which newly diagnosed children and adolescents with ALCL are randomised at diagnosis to receive either crizotinib or brentuximab vedotin in combination with multi-agent chemotherapy (NCT01979536). The EICNHL group also plans to evaluate whether the addition of crizotinib to multi-agent chemotherapy is able to improve EFS in high and intermediate risk ALCL.

There are at least 10 other ALK inhibitors currently at various stages of investigation (Katayama*, et al* 2015). Given the rarity of ALCL and the good response rate obtained with crizotinib, only a few of them have been tested in ALCL to date. A CR has been reported in several ALCL patients treated with ceritinib, a second generation ALK inhibitor shown to be able to induce CR in crizotinib-resistant xenograft models (Richly*, et al* 2015). Besides ALK inhibitors, several other therapeutic options as described above are plausible and include inhibitors of PDGFR, JAK-STAT, mTOR, PI3K, immune checkpoint inhibitors and vaccination against ALK (Eyre*, et al* 2014). The availability of such a large number of new therapeutic options should allow for improvements in the treatment of ALCL sparing low risk patients from the acute toxicity of multi-agent chemotherapy and reducing the failure rate in high risk patients. Given the rarity of this lymphoma, only prospective international therapeutic trials including both children and adults with ALCL will allow the evaluation of the role of these different therapeutic options in front-line as well as at relapse within a reasonable period of time.

Conclusions

It is obvious that we are not short of therapeutic targets for the treatment of ALCL, in particular ALK+ ALCL, although given the already high cure rates and relatively small number of patients it will be difficult to decide which are the most promising targets to take forward to clinical trial. However, the development of robust prognostic markers will assist in stratifying patients in order that low risk individuals can be assigned to less toxic treatment arms.

Author Contributions

All authors wrote the paper, analysed the literature and edited the document. SDT designed the review and provided figure 2; LB provided the table; LL provided figure 1.

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Table 1: The outcome of various chemotherapy strategies applied to the treatment of ALCL, ALK+ patients.

Reference	Strategy	#of patients	EFS	OS
Children and adolescents				
(Brugieres, *et al* 1998)	B cell strategy (COPADM (cyclophosphamide, vincristine, prednisone, doxorubicin, dexamethasone, methotrexate) + Maintenance)	82	66%	83%
(Seidemann, *et al* 2001)	B cell regimen (BFM-B)	89	76%	ND
(Williams, *et al* 2002)	B Cell regimen (LMB)	72	59%	65%
(Rosolen, *et al* 2005)	T Cell regimen	34	68%	85%
(Laver, *et al* 2005)	APO (doxorubicin, prednisone, vincristine) +randomization of HDMTX and high dose (HD) AraC	86	72%	88%
(Lowe, *et al* 2009)	Compressed T-cell regimen	86	68%	80%
(Brugieres, *et al* 2009a)	B-cell regimen + randomization of vinblastine	352	73%	92%
(Alexander, *et al* 2014)	APO + randomization of vinblastine	125	74%	84%
Adults				
(Falini, *et al* 1999a)	Various doxorubicin containing regimens	53	82%	71%
(Gascoyne, *et al* 1999)	Various doxorubicin containing regimens	31	82%	93%
(Suzuki, *et al* 2000)	Various doxorubicin containing regimens	83	ND	72%
(Savage, *et al* 2008)	Various doxorubicin containing regimens	87	60%	70%
(Schmitz, *et al* 2010)	6 to 8 courses of CHO(E)P (cyclophosphamide, doxorubicin, vincristine, etoposide, prednisone)	78	76%	87%
(Sibon, *et al* 2012)	ACVPB (doxorubicin, cyclophosphamide, vinblastine, prednisone, bleomycin) or CHOP	64	72%	82%

EFS, event free survival; OS, overall survival; ND = not determined.

Figure Legends

Figure 1: Morphological variants of ALCL A. Common type ALCL consists only of large-sized cells (arrow: hallmark cell) (H&E). B-C. Malignant cells are often masked by reactive histiocytes in Lympho-Histiocytic ALCL (H&E) (arrow: hallmark cell) and highlighted by ALK1 antibody staining. D-E. In the Small Cell variant, hallmark cells (arrow) are scattered among a predominant population of small-sized neoplastic cells positive for ALK1 staining. F. In ALK-negative ALCL, tumour cells are often more pleomorphic (H&E). G. ALK-negative ALCL may share a typical perivascular distribution of CD30-positive neoplastic cells as seen with ALK-positive ALCL

Figure 2: A thymic origin for ALCL. In this model, the t(2;5) or variant translocation occurs in haemopoietic stem cells or thymic progenitors whereby NPM-ALK is permissive of cellular survival in the thymus despite aberrant TCR rearrangements. These 'primed' cells may go undetected until a secondary event(s) occurs that leads to clonal expansion and tumour development. This event may be induced as a consequence of an inflammatory response as evidenced by ALCL in the context of insect bites but might also be initiated in an innate manner. ETP = early thymic progenitor, DN = double negative thymocyte, DP = double positive thymocyte, SP = single positive.

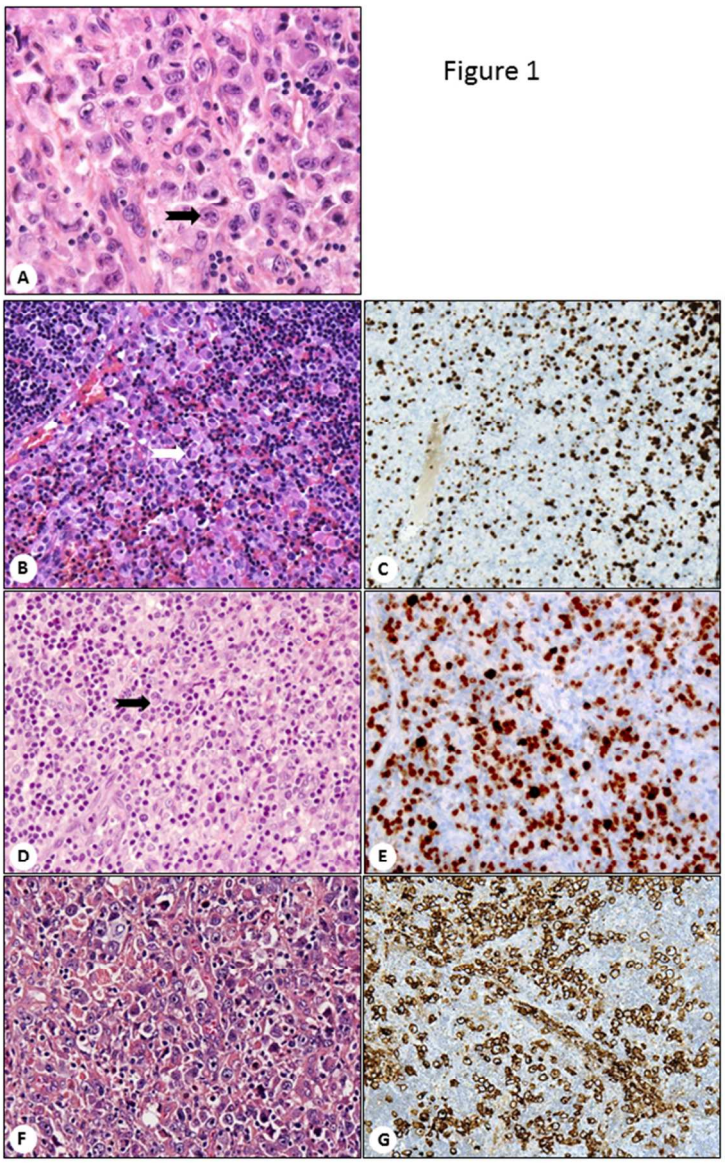
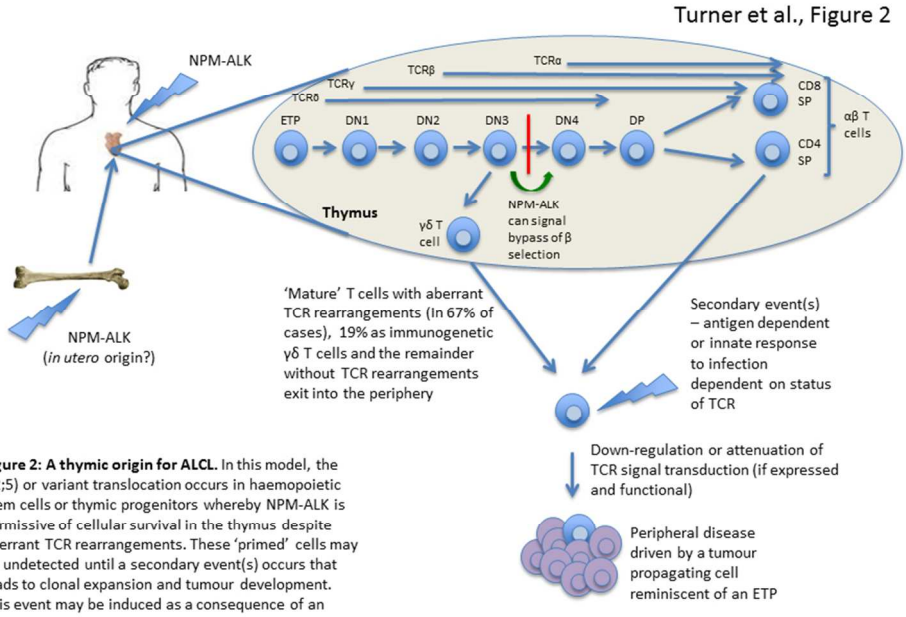


Figure 1

190x254mm (96 x 96 DPI)



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