OPTIMIZING TREATMENT FOR DIFFUSE LARGE B-CELL LYMPHOMA

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Treatment of diffuse large B-Cell lymphoma (DLBCL) has changed substantially over recent years. Study groups like the German High-Grade Non-Hodgkin Lymphoma Study Group (DSHNHL) usually treat patients with DLBCL according to age and the International Prognostic Index (IPI). For elderly patients, Coiffier et al. (NEJM 2002;346:235) showed that the addition of Rituximab (R) to standard CHOP chemotherapy administered every 3 weeks (CHOP-21) significantly improved CR, DFS, and OS. The most recent study of the DSHNHL (RICOVER-60) compared 6 or 8 courses of R-CHOP-14 to 6 or 8 courses of CHOP-14. This study which recruited patients (pts.) aged between 61 and 80 years shows a significant improvement of both R-containing regimens over 6 or 8 courses of CHOP-14 without R. The study also demonstrated that 6 courses of chemotherapy are not inferior to 8 courses if 8 courses of R are given. The new standard treatment for elderly patients in our group will thus be 6 x R-CHOP-14. In younger good-risk patients (IPI 0 or I) the MINT study published recently (Lancet Oncology 2006; 7:379) convincingly showed that CHOP or CHOP-like chemotherapy plus rituximab increased event-free survival and overall survival significantly over CHOP alone (3-yr-EFS 79% vs. 59%; OS 93% vs. 84%). Ongoing studies reduce the number of chemotherapy courses to 4 (FLYER-study) in pts. with IPI 0 or compare R-CHOP-21 to R-CHOP-14 in pts. with IPI 1 or bulk. In young high-risk patients (aa IPI 2,3) there is ongoing debate if high-dose therapy (HDT) followed by autologous stem cell transplantation (ASCT) should be integral part of first-line-therapy. The DSHNHL pioneered a large phase II-program combining a HDT program (MegaCHOEP) with and without R and currently is doing a phase III study which compares MegaCHOEP+R to 8 courses of R-CHOEP-14. The first interim analysis is planned for the end of 2006. Future trials in DLBCL will integrate new dosing concepts for R, other monoclonal antibodies, radioimmunotherapy or small molecules to further improve outcome especially in young, high-risk and elderly patients. Patients with relapsed disease are currently treated by HDT/ASCT (CORAL trial) or more experimental approaches like Zevalin®-BEAM followed by autologous transplantation or allogeneic transplantation.

IMPACT OF RADIOIMMUMOTHERAPY ON AGGRESSIVE NHL

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Unconjugated monoclonal antibodies, particularly rituximab, are now an important part of the treatment of B-cell lymphoma. The use of radiolabeled antibodies offers a different way to deliver radiotherapy and take advantage of the well known radiosensibility of lymphoma. Radio-immunotherapy (RIT) is defined as a treatment modality in which cytotoxic radiation is delivered to tumour cells via antibodies binding to tumour-specific or tumour-associated antigen. The antibody serves as transporter for the radioisotope and participates on its own in the tumour killing. The advantage of conjugated radiolabeled antibody over unconjugated antibody is that there is no need to target every to achieve an antitumour effect. Tumour with low or heterogeneous antigen expression, as poorly vascularised and bulky tumour can also be killed by the cross-fire of neighbourhood targeted cells.

Studies of Zevalin in relapsed mantel cell lymphoma

Limited numbers of patients with relapsed mantle cell NHL have been treated with Zevalin. Although the malignant cells in mantle cell lymphoma (MCL) strongly express CD20, the disease often heavily infiltrates the marrow making these patients ineligible for RIT studies. Oki and colleagues reported on 15 patients with relapsed MCL that received treatment with Zevalin. There were 5 objective responses (33%) with all responses being CR/CRu. The median TTP was 4.9 months for all patients and the median DR was 5.7 months. Thus it appears that in relapsed MCL patients, the ORR to single-agent Zevalin is lower than observed for low-grade NHL or large cell NHL. Current trials are using Zevalin after R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine, prednisone) induction for patients with previously untreated MCL. This approach uses the RIT at a time of minimal residual disease. It will be several years before it will be known whether this strategy can improve the otherwise relentless relapses that typically occur in the MCL patient group.

Zevalin in relapsed diffuse large cell lymphoma

Patients with relapsed large cell lymphoma who are in good health, less than 75 years of age, and with chemosensitive disease are usually treated with high-dose therapy with stem cell rescue. However, elderly patients, or those who are not candidates for transplant do not have good therapeutic options and can be considered candidates for trials of RIT. In the phase I trial of Zevalin there were 14 patients with relapsed large cell NHL and 43% responded. A recent trial in Europe treated 104 patients with relapsed or refractory diffuse large cell NHL with a single dose of Zevalin 0.4 mCi/kg (maximum of 32 mCi). They found an ORR of 44% for the entire group with 55% of rituximab-naive patients responding compared to 19% of patients with prior treatment with rituximab-containing regimens. Further follow-up of the patients and full publication of the results are needed before conclusions on the use of Zevalin RIT in this setting can be made. Current trials in the Eastern Cooperative Oncology Group for diffuse large cell NHL are focusing on using Zevalin as adjuvant therapy after completion of R-CHOP induction. The aim is to increase the rate of CR and TTP.

VALIDATION OF THE ANAPLASTIC LARGE CELL LYMPHOMA SIGNATURE

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Anaplastic Large Cell Lymphomas (ALCL) represent a unique subset of lymphomas characterized by the CD30.expression and associated with specific chromosome translocations in which the Anaplastic Lymphoma Kinase (ALK) gene is fused to several partners, most frequently to the NPM gene. Activated ALK chimeras bind multiple adaptor proteins capable of firing several regulatory pathways controlling cell proliferation, survival, and transformation. We have previously demonstrated that the constitutive expression and phosphorylation of ALK chimeric proteins is sufficient for cellular transformation, and its activity is strictly required for the survival of ALCL cells, in vitro and in vivo. To clarify molecular mechanisms and signalling pathways required for NPM-ALK-mediated transformation and tumour maintenance, we analyzed the transcriptomes of ALK positive ALCL cell lines using experimentally controlled approaches, in which ALK signaling was abrogated by a doxycycline-inducible ALKshRNA or by specific ALK kinase inhibitors. Inducible NPM-ALK knockdown or inhibition of ALK enzymatic activity resolved in the modulation of multiple downstream effectors, followed by growth arrest, apoptosis, and tumor regression of xenograft ALCL tumors. The combined analysis of NPM-ALK modulated genes by microarray gene expression profiling identified known and novel downstream targets, grouped in functional clusters of cell cycle and proliferation, adhesion and migration, and in cytokine signalling family molecules. In a functional screen of NPM-ALK regulated genes, we found that the anti-apoptotic protein Bcl2A1 and the transcription factor C/EBPb are strictly regulated by NPM-ALK activity and they sustain the survival and/or growth of ALK positive ALCL cells. Overall, the combination of an experimentally controlled gene expression profiling analysis with a func-

tional RNA interference screening represents a powerful tool to characterize the network mediating tumorigenesis and maintenance of lymphomas. Moreover, this approach will open new avenues to identify suitable targets for specific therapeutic strategies.

NOVEL DRUGS FOR THE TREATMENT OF T-CELL LYMPHOMA

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While never really established in any prospective randomized clinical trial, it is reasonably well accepted that T cell lymphomas are more challenging to treat than B-cell lymphomas. For example, in a study reported by Gisselbrecht et al. (1998), the 5-year survival rate for patients with 1, 2 or 3 risk factors with B- versus T cell lymphoma was 63% versus 60%, 53% versus 36%, and 35% versus 33%, respectively. Similar trends were also observed for the rate of CRs. These data are corroborated to some extent by the categorization of different overall survival rates by histological subtype using the International Lymphoma Study Group (ILSG) classification, which sub-divided the lymphomas into 4 broad groupings. Those histological subtypes with the worse overall prognosis and 5-year survival rates of less than 30% including PTCL, precursor T-lymphoblastic lymphoma, and mantle cell lymphoma. These results have been more recently confirmed by others showing that patients with PTCL have an especially poor outcome with a 5-year overall survival rate of only 26% following treatment with standard doxorubicin containing regimens.[4]

These data underscore the point that new therapies for T-cell lymphoma are urgently needed. The 10 deazaaminopterins are a class of folate analogues that demonstrate greater anti tumor effects than methotrexate (MTX) against murine tumor models and human tumor xenografts in immunocompromised mice.[17-19] The improved activity is due to the more effective internalization by the 1-carbon, reduced folate transporter (RFC-1) and the subsequent accumulation in tumor cells through the formation of polyglutamylated metabolites.[17-20] The reduced folate carrier is a fetal oncoprotein that is almost exclusively expressed on fetal and malignant tissue, and is felt to be the principle means through which pralatrexate, though not necessarily other antifolates, enters the cell. This carrier protein has evolved to efficiently transport reduced natural folates into highly proliferative cells, in order to meet the demands for purine and pyrimidine nucelotides during DNA synthesis. For example, the V_{max}/K_m for pralatrexate is more favorable than for MTX, being incorporated at a rate nearly 14 times greater than that appreciated for MTX. Similarly, the Vmax/Km for the folylpolyglutamyl synthetase (FPGS) mediated glutamylation reactions suggests that pralatrexate is also 10 times more efficiently polyglutamylated compared to MTX. These biochemical features suggest that pralatrexate should be a more potent antineoplastic agent in comparison to methotrexate, an observation corroborated in several preclinical models of lymphoma, where pralatrexate has been shown to be markedly superior to methotrexate.

In early Phase I/2 clinical trials, dramatic activity has been documented in T-cell lymphomas, with little to no activity in patients with B-cell lymphoma. In fact, of over 50 patients treated to date, 8 complete remissions have been documented in 11 T-cell patients evaluable for response, while only one particle remission has been noted in a patient with B-cell lymphoma from over 31 evaluable for response. In select cases, the response appears to defy the natural history of that particular lymphoproliferative malignancy. This early experience is the first to document this unique activity of pralatrexate in T-cell lymphoma, and is beginning to raise new questons about how and why pralatrexate exhibits such striking activity in T-cell malignancies.

PATHOGENESIS OF HODGKIN LYMPHOMA

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In classical Hogkin lymphoma (cHL) the neoplastic Hodgkin and Reed/Sternberg (HRS) cells represent <1% of the lymph node cellularity. Attempts to reveal the largely unknown pathogenesis of cHL through gene expression profiling have so far been restricted to HL cell lines. However, these lines most likely do not retain all important features of primary HRS cells. To generate gene expression profiles from primary HRS cells, ~1000 HRS cells were laser-microdissected from H&E-stained frozen sections of cHL biopsies. After two rounds of in vitro transcription, RNA was hybridized to Affymetrix HG-U133 Plus 2.0 chips. Expression profiles were also generated from similar cell numbers of HL cell lines, microdissected tumour cells from various other B cell lymphomas and lymphocyte-predominant HL (LPHL), and normal mature B-cell subsets that were MACS/FACS-sorted from tonsil or peripheral blood of healthy donors. Unsupervised hierarchical clustering of the 71 samples so far investigated groups the 25 normal B-cell samples separately from the 46 tumor samples (41 biopsies and 5 HL cell lines), suggesting that the different isolation methods (microdissection vs sorting) did not significantly affect the clustering pattern. The further branching of the dendrogram shows that among tumor samples, cell lines grouped apart from primary cases. The latter further split in two sub-branches: one with primary mediastinal B cell lymphomas, Burkitt lymphomas, follicular lymphomas (each of the three forming its own sub-cluster) and diffuse large B cell lymphomas, and the other branch mainly comprising HLs (with both cHLs and LPHLs tending to form discrete sub-clusters) and T-cell rich B-cell lymphomas. A supervised comparison of primary HRS cells with HL cell lines shows a highly differential expression (≥ 4fold change) of ~1200 genes, including many involved in intercellular signaling, chemotaxis, and immune/inflammatory response. These preliminary results suggest that expression profiles can be reliably generated from small numbers of microdissected cells, and that primary HRS cells and HL cell lines seem to differ in various biological features. In about 40% of cases of HL in the Western world, the HRS cells are infected by Epstein-Barr virus (EBV). In EBV-positive cases of HL, three viral proteins, EBNA1 and the latent membrane proteins LMP1 and LMP2a, are expressed. LMP2a harbours a cytoplasmatic motif that is also found in the coreceptors of the B cell receptor (BCR) and that mediates signalling of cross-linked BCR. Through this motif, LMP2a can mimic a BCR, and it has been speculated that LMP2a may replace the survival signal of the BCR in germinal centre (GC) B cells acquiring destructive Ig V gene mutations. This is of potential importance for cHL, as 25% of cHL cases show obviously destructive BCR mutations. The capability of EBV to rescue BCR-deficient human GC B cells from apoptosis was recently directly tested. GC B cells were infected in vitro with EBV to establish lymphoblastoid cell lines. Several lines were identified that carried destructive mutations rendering originally functional Ig V gene rearrangements non-functional. These destructive mutations normally would have caused rapid cell death. Thus, EBV is capable of rescuing BCR-deficient GC B cells from apoptosis.

The role of EBV in the rescue of crippled GC B cells that lost the capacity to express a BCR is additionally substantiated by a striking correlation between the detection of crippling mutations preventing BCR expression (e.g. nonsense mutations) and the presence of EBV in the HRS cells. All cases of HL with crippling mutations that unequivocally prevent BCR expression are EBV-positive. This finding indicates that a GC B cell that had acquired BCR-destructive mutations can survive and become a precursor of an HRS tumour clone only if it is EBV-infected. Together, these recent findings strongly support an important role of EBV in the pathogenesis of classical HL.