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HLA class II as target antigen for lymphoma therapy

Thomas Valerius, MD
Division of Hematology/Oncology, Department of Medicine III
University of Erlangen-Nürnberg, Germany

Antibody therapy has become a new treatment option for lymphoma patients. Antibodies against HLA class II variants - such as 1D10, or Lym-1 - are actively investigated in clinical trials, while „classical" HLA class II antibodies were associated with systemic complement activation and severe toxicity in primate studies. Therefore, we generated novel antibody constructs which should have reduced complement activating capacity, and - at the same time - should improve effector cell recruitment.

For this purpose, variable regions of heavy and light chain genes from classical HLA class hybridoma F3.3, and from the Lym-1 and Lym-2 hybridomas were amplified. Respective genes were cloned into human κ, human cγ1, cα1, or cα2 vectors, which were then stablely transfected into BHK cells. Resulting human IgG1, IgA1 and IgA2 antibody variants were analyzed by ELISA and Western blotting. All constructs demonstrated the expected binding to lymphoma cells. Functional studies with the panel of F3.3 variants revealed that killing by the IgG1 construct was triggered by human NK cells and complement. In contrast, both the IgA1 and IgA2 variants mediated effective lysis by PMN, but did not activate complement or recruit NK cells. Furthermore, bispecific antibodies with specificities for HLA class II, and FcγRI (CD64), FcγRIII (CD16), or FcαRI (CD89), respectively, allowed specific recruitment of selected effector cell populations. In conclusion, novel constructs may improve efficacy and reduce toxicity of HLA class II- directed antibody constructs.

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THERAPY OF CLL WITH HUMANIZED CD52 ANTIBODY (CAMPATH-1H)

Håkan Mellstedt[1], Jeanette Lundin[1], Herman Waldman[2], Geoff Hale[2], Anders Österborg[1]
[1]Centre of Hematology and Department of Oncology, CancerCentreKarolinska, Karolinska Hospital, Stockholm, Sweden. [2]Therapeutic Antibody Centre, Oxford, United Kingdom

CAMPATH-1H, a CDR-grafted, monoclonal antibody, recognizes CD52 on leukemic B cells. The antibody induced a major response (CR+PR) in 25-40% of previously treated CLL patients. A phase II clinical trial was initiated to evaluate the clinical effects of subcutaneous long-term (18 weeks) administration of CAMPATH-1H in previously untreated CLL patients. CAMPATH-1H was given s.c. tiw. At the last follow-up 28 patients have been included. Twenty-seven were evaluated for toxicity and 23 for response. The overall response rate was 87% (4 CR + 16 PR). 96% achieved CR in blood, one PR. In the bone marrow 65% had CR, 18% PR. 21% had CR of the enlarged lymph nodes and 68% PR. The median time to treatment failure has not been reached (16+ months). Most patients had mild or moderate "first dose" flu like symptoms. Three patients had CMV reactivation. Seven patients had transient grade IV neutropenia. Six patients developed transient exanthema/eczema.

The anti-tumor effect of CAMPATH-1H in previously untreated CLL patients is encouraging. Prolonged administration of CAMPATH-1H resulted in a high frequency of CR in the bone marrow. The best effect in CLL is probably noticed during the early phase of the disease when the immune system is not heavily immune-compromised.

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Hu14.18-IL2 immunocytokine in early clinical development: phase I experience and future translational research plans

P.Sondel*, M.Albertini*, S.Gillies#, D. King*, H. Schalch*. J. Gan*, J. Hank*, O.Kashala+, K.Sturmhoefel+
*University of Wisconsin Comprehensive Cancer Center, Madison, WI; #Lexigen Pharmaceuticals, Lexington, MA; +EMD Pharmaceuticals, Durham, NC

Immunocytokines (IC) are recombinant fusion proteins comprised of an antibody directed against a tumor surface antigen and a cytokine linked to the antibody C-terminus. The concept of IC is based on antibody-targeted delivery of cytokines (e.g. IL-2) to the tumor microenvironment. Preclinical tumor models have shown IC to exhibit greater biological activities when compared with the combination of the antibody and cytokine as separate entities. The antibody part in hu14.18-IL2 is directed against GD2, a ganglioside overexpressed by neuroectodermal cancers (e.g. neuroblastoma, melanoma, some sarcomas). A phase 1 safety and tolerability study of hu14.18-IL2 is underway. In this trial, 25 melanoma patients have received various doses (0.8, 1.6, 3.2, 4.8, or 6 mg/m^2/ day) of hu14.18-IL2 given as 4h i.v. infusions on 3 consecutive days per week (one cycle), followed by another cycle 28 days later, in the absence of disease progression. The treatment was generally well tolerated with most patients experiencing grade 1 or 2 adverse events. These events were related to known IL-2 effects. Laboratory parameters were unremarkable, except for 8 patients with asymptomatic and spontaneously resolving grade 3 changes, including: hypophosphatemia, platelets, bilirubin, AST, hypoxia, and hyperglycemia. Thus far, the MTD has not been reached. Nearly all treated patients had a transient drop in lymphocyte counts followed by a rebound lymphocytosis, reminiscent of IL-2, demonstrating biological activity of the IL-2 component of hu14.18-IL2.

We plan to further evaluate hu14.18-IL2 in phase I/II studies in neuroblastoma and melanoma patients. The focus of our studies will be to establish the relevance in humans of some of the key principles of IC, specifically tumor targeting and the induction of tumor-specific immune responses.

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RECOMBINANT IMMUNOTOXINS FOR THE TREATMENT OF PATIENTS WITH CHEMOTHERAPY-RESISTANT CANCER

Robert J. Kreitman, M.D., National Institutes of Health, Bethesda, MD 20892.

Recombinant immunotoxins are fusion proteins containing an Fv fragment of a monoclonal antibody and a truncated toxin. Anti-Tac(Fv)-PE38 (LMB-2) is a single chain recombinant immuntoxin in which the variable heavy (V_H) and light (V_L) domains of the anti-CD25 Mab anti-Tac are fused together via a peptide linker and V_L in turn is fused to a 38 kDa carboxyl terminal fragment of Pseudomonas exotoxin called PE38. LMB-2 was tested in a Phase I trial in 35 patients with CD25+ leukemia, lymphoma and Hodgkin's disease (HD). All 4 patients with chemotherapy-resistant hairy cell leukemia responded with 1 complete remission (CR) and 3 partial responses (PRs), and 4 PRs were also achieved in patients with chronic lymphocytic leukemia (CLL), adult T-cell leukemia (ATL), cutaneous T-cell lymphoma (CTCL) and HD. LMB-2 also is cytotoxic toward activated but not resting T-cells and will be tested for its anti-graft versus host disease activity by selectively depleting alloreative T-cells. More recently engineered recombinant immuntoxins contain a disulfide bond connecting V_L to V_H via cysteine residues engineered into framework residues, instead of a peptide linker. These disulfide stablized recombinant immunotoxins are more stable than their single-chain counterparts and may be produced in higher yield from *E. coli*. Those in clinical testing include RFB4(dsFv)-PE38 (BL22), targeting CD22+ leukemia and lymphomas, SS1(dsFv)-PE38 (SS1P), targeting mesotheliomas, ovarian, lung, head & neck and cervical carcinomas, and B3(dsFv)-PE38 (LMB-9), targeting carcinomas.