

Phase I Study of Interleukin-12 in combination with Rituximab in patients with B-cell non-Hodgkin lymphoma.

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Rituximab is a monoclonal antibody that binds specifically to CD20 on B-lymphocytes. While binding of the Fab domain may induce apoptosis, the Fc domain recruits immune effector functions to mediate cell lysis.

Interleukin-12 (IL-12) facilitates cytolytic T-cell responses, enhances the lytic activity of NK cells and induces the secretion of interferon-gamma.

Therefore, we hypothesized that combining IL-12 with Rituximab would augment the immune mediated cell lysis induced by Rituximab.

We conducted a Phase I study of IL-12 in combination with Rituximab in adult patients with B-cell lymphoma to determine the optimal immunological dose of this combination. Rituximab was administered at a dose of 375mg/m² by intravenous infusion weekly for 4 weeks, and IL-12 was given subcutaneously twice weekly. The starting dose of IL-12 was 30ng/kg and this was escalated to 500 ng/kg.

Forty-three patients were treated in this study. Constitutional symptoms and liver enzyme elevations at 500ng/kg of IL-12 were found to be dose limiting. A >20-fold increase from baseline in the serum levels of interferon- γ and a 2.5-5 fold increase in IP-10 levels was seen at IL-12

doses ≥ 100 ng/kg. Objective clinical responses occurred in 29 of the 43 patients (69%), with 8/11 complete responses seen at IL-12 doses ≥ 300 ng/kg. The optimal dose of IL-12 in combination with Rituximab was determined to be 300ng/kg twice weekly starting on day 2.

These data suggest that IL-12 and Rituximab is an active combination and further studies of this combination in B-cell NHL are warranted.

Engineering high affinity scFvs for tumor targeting. K. Dane Wittrup, Div. Bioengineering, MIT.

Mathematical modeling and experiments have previously indicated the potential for substantial limitations in antibody penetration of tumors, a phenomenon termed the „binding site barrier.“ Two technological advancements necessitate re-examination of this process: the widespread availability of antibody fragments one-sixth the molecular weight of an IgG molecule; and the capability to engineer extremely high monovalent antigen-binding affinity by directed evolution. The central question is: at what point does increasing affinity cease to improve integrated tumor exposure, and what rate processes define this limitation? We have implemented a numerical solution of the binding and diffusion equations in prevascular tumor microspheroids, and for physiologically relevant parameter values, the rate of tumor loading is independent of antigen binding affinity. By contrast, antibody retention in the tumor is a strong function of affinity, reaching a limit only when the antibody-antigen dissociation rate is slower than the antigen catabolism rate. This model provides testable predictions regarding the effects of antibody affinity & dosage on tumor penetration and retention. Pursuant to the objective of engineering high affinity antibodies by directed evolution, we have developed a yeast surface display system for combinatorial protein library screening. As a display host, yeast offers the advantages of a eucaryotic secretory pathway and quantitatively precise screening via fluorescent labeling and flow cytometry. Substantial improvements in ligand binding affinity, stability, and soluble expression have been attained; applications of yeast surface display to anti-CEA antibodies will be described.

Anti-CTLA4 in the immunotherapy of malignancies.

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T-cell activation is an important step in the initiation of immunity, and involves complex interactions for co-stimulation of the T cell. CTLA-4 expression on activated T cells competes with co-stimulation through the CD28 molecule and negatively regulates T-cell function, attenuating an immune response. By binding and blocking CTLA-4 with antibodies, it is possible to prevent down regulation of an immune response and increase both humoral and cellular immune responses, particularly when used in combination with antigen specific vaccination. Using preclinical tumor models, it has been shown this approach can induce autoimmunity and significant anti-tumor immunity that can provide protection against both tumor inoculations and spontaneous tumors, and can induce regressions of established disease. A fully human antibody that blocks CTLA4 (MDX-CTLA4) has been generated and is currently in initial human trials. The antibody is well tolerated at saturating doses and shows complex immunologic activity. It is possible that MDX-CTLA4 single agent therapy may activate previous immunity and may release innate anti-tumor immunity. Further trials of MDX-CTLA4 as single agent and in combination with cytotoxics and vaccines are planned.

Combined immunochemotherapy (R-FCM) is superior to chemo-therapy (FCM) alone in recurrent indolent lymphoma - results of a prospective randomized comparison

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The clinical course of indolent lymphoma is characterized by frequent recurrences of the disease and a steadily declining survival curve. However, even in relapse, the disease is sensitive to conventional chemotherapy in a significant subset of patients. In a national multicenter trial, patients with relapsing indolent lymphomas or mantle cell lymphomas were randomly assigned to receive either a anthracyclin-containing chemotherapy (FCM: fludarabine 25 mg/m² d 1-3, cyclophosphamide 200 mg/m² d 1-3, mitoxantrone 8 mg/m² d 1) alone or in combination with an anti-CD20 antibody (375 mg/m²).

Since November 1998, 147 patients were randomized; currently, 70 patients are evaluable for response. In the chemotherapy only arm (FCM), only 18 patients achieved a remission (53% OR: 15% CR, 38% PR) where-as in the combined immuno-chemotherapy cohort (R-FCM), 32 remissions were reported (89% OR: 36% CR, 53% PR). These differences were highly significant for the total group of patients in favor of the R-FCM arm ($p=0.000715$). Similar improvements of remission rates were detected in follicular (95% vs. 68%) as well as mantle cell lymphoma (77% vs. 27). Both treatment options were associated with hematological toxicities of grades II to IV but well tolerated with only 1 and 3 treatment related deaths in the R-FCM and FCM arm, respectively (5.6%). In summary, to the best of our knowledge, this is the first prospectively randomized study which confirms the synergistic benefits of a combined immuno-chemotherapy in relapsed indolent lymphoma.