CD30: a suitable target for immunotherapy?

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The CD30 antigen and its physiological function

CD30 is a 120 kDa transmembrane glycoprotein belonging to the TNF-receptor superfamily and lacking an intracellular death domain. Physiologically, expression of CD30 is found on virus-infected lymphocytes and on a small subset of activated T cells. Furthermore this receptor is involved in the negative selection process of auto-reactive lymphocytes.[1] The CD30 ligand (CD30L) is present on activated T cells, resting B cells, and granulocytes. As shown for most other members of the TNF-receptor family signaling via CD30 is pleiotropic and may result in different responses including apoptosis, differentiation and growth stimulation even in monoclonal cells. This response may depend on the developmental status and environment of the cell.[2] To transduce signaling into the cell CD30 trimerizes after activation via corresponding ligands or in experimental settings with cross linked monoclonal antibodies.[3-5] The physiological function of the receptor is not fully understood. Mouse-knock out experiments imply a role in the formation of the immune system during development, as mice lacking CD30 cannot select and eliminate autoreactive thymocytes and conversely transgenic overexpression of CD30 induces enhanced T cell apoptosis.[6] Signaling via CD30 can inhibit both cell proliferation and activation of T cells. This process is probably involved in the pathogenesis of Hodgkin lymphoma (HL), since the interaction of CD30 expressed on malignant cells and CD30 ligand present on T cells is responsible for suppression of an effective T cell antitumor response.[7] On the other hand, several autoimmune diseases showed an enhanced expression of CD30 and increased serum levels of its soluble form (sCD30), that itself can induce signaling through interaction with membrane bound CD30 ligand.[8] Very recently the CD30/CD30L interaction has been linked to the onset of murine autoimmune diabetes, correlating with the detection of islet-specific cytotoxic T cells which expressed high levels of CD30 and CD30L.[9]

CD30 expression in malignancy

CD30 is expressed on a variety of other neoplasms such as mediastinal B-cell lymphoma, anaplastic large cell lymphoma, peripheral T-cell lymphoma, and embryonal carcinoma.[10-13] Among the different tumors the Hodgkin's lymphoma and the corresponding malignant cells, the Hodgkin-Reed/Sternberg (HRS) cells, seems to be the most promising entity for the clinical development of CD30 based immunotherapy. In HL, CD30 has been first described and is specifically expressed at extremely high levels.[14-17] Based on the rationale of potential clinical use a vast number of murine anti-CD30 monoclonal antibodies (mAbs) have been developed.

Principles of CD30 based immunotherapy of HL

So far, all different approaches available to eradicate tumor cells using antibodies have been tested in HL: so called *naked* antibodies, which either induce target cell death by direct interaction or by induction of antibody dependent cellular or complement dependent cytotoxicity (ADCC or CDC), bispecific constructs, which activate effector cells of the immune system against the target cell, immunotoxins (IT), which deliver a potent toxin linked to an antibody specifically into the target cell, and different radioimmunoconjugates (RIT, see Figure 1). More than a decade ago, Engert et al analyzed the potential use of 40 different MAbs directed against CD25, CD30 and IRac against H-RS cells *in vitro* without evidence of any cytotoxic activity. [16,18] Therefore, the first clinical trials were focused on ITs and RIT, before recent technological developments made it possible to generate bispecific or even human antibodies.

CD30 immunotoxins

Ricin is a ribosome inactivating protein and is extracted from the seeds of Ricinus communis (castor bean). This toxin is most frequently used for the construction of chemically-linked ITs. The most effective

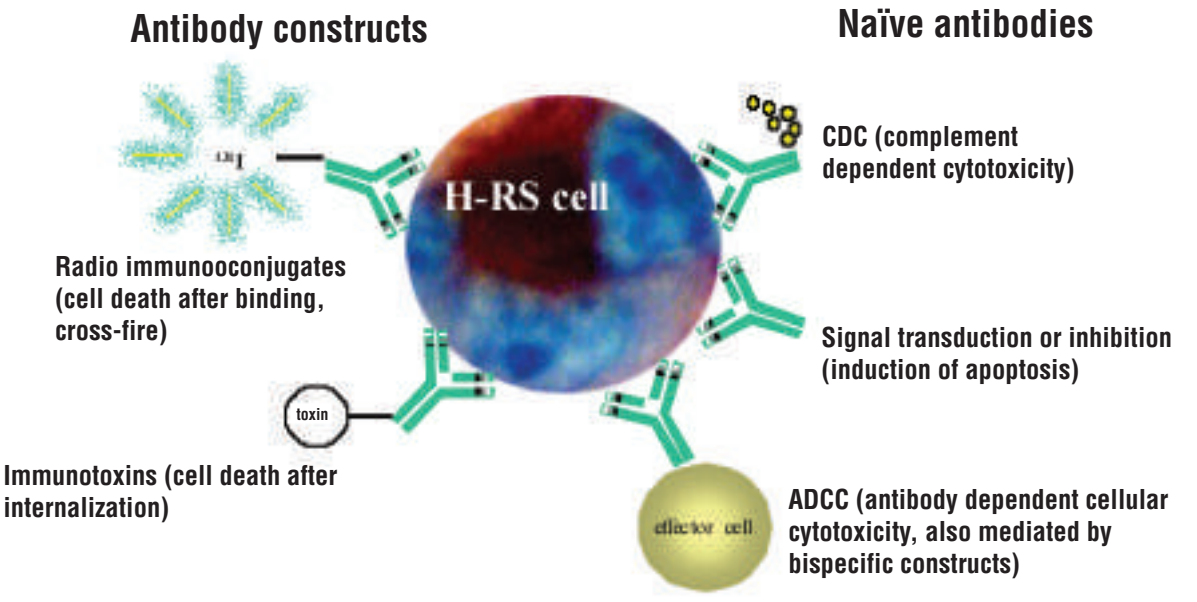

Figure 1. Principles of CD30 antibody-based therapeutics.

anti-CD30 IT, Ki-4.dgA, was selected for a clinical phase-I trial in 17 patients with refractory CD30 positive HL and NHL.[19] The IT was given in four bolus infusions every other day in escalating doses. Side effects were related to the development of vascular leak syndrome and the maximum tolerated dose was already reached at 5 mg/m^2. 40% of patients made human anti ricin antibodies (HARA). Responses included one PR, one MR and two SD. Saporin-S6 is a single-chain type-1 RIP extracted from the seeds of Saponaria officinalis (soapworth). Saporin-S6 ITs for evaluation in HL were constructed by linkage with the CD30 MAb Ber-H2. From the 12 patients treated in two studies overall, four achieved PR and three MR with a median duration of two months20. The MTD of 0.8 mg/kg was established by grade 3 VLS and liver toxicity.

Bispecific Constructs

Bispecific MAbs (Bi-MAbs) or molecules (BSMs) contain two different recognition sites for antigens on tumor cells and immunologic effector cells like macrophages, T-cells or NK-cells.

The murine Bi-MAb HRS-3/A9 specifically binds to the CD30 antigen (HRS-3) and the CD16 surface molecule (A9) triggering specific lysis of the CD30-positive Hodgkin derived cell line L540Cy by NK cells *in vitro*.[21] In a phase-I/II study involving 15 patients with refractory HL, HRS-3/A9 was administered in doses of 1 to 64 mg/m^2 four times every three to four days without reaching the MTD.[22] Fifteen patients were evaluable for response showing one CR, one PR and three MRs. In an additional trial, the effect of concomitant treatment with cytokines (IL-2, GM-CSF) was analyzed.[23] Response included one CR and three PRs.

Another approach targets the high affinity IgG receptor (FcgRI or CD64) that is present on key cytotoxic effector cells. A bispecific molecule was developed by chemically linking Fab-fragments of the murine anti-CD30 MAb Ki-4 and the human anti-CD64 MAb H22.[24] This BSM binds to H-RS cells and CD64 positive immune effector cells (monocytes, macrophages) mediating antibody dependent cellular cytotoxicity. Ten patients with refractory HL were treated i.v. in escalating doses (1 to 20 mg/m^2) on days 1, 3, 5 and 7. Side effects were transient and moderate. The MTD was not reached. Response included one CR, three PR and four SD.

Anti-CD30 based radioimmunoconjugates

Radiolabeled antibodies (RAbs) have been studied for imaging and treatment of lymphomas. The radionuclides used for their construction must have energetic particulate radiation (α- or β-emitters) to focus the radiation dose to defined areas, preserve the binding capacity of the antibody, and should have a stable linkage of the radiometal to the antibody.

In a clinical phase-I trial using the anti-CD30 construct 131I-Ki-4 in relapsed patients with HL, 250-300 MBq 131I labeled Ki-4 was administered at escalating individual total body doses of 0.125 Gy, 0.25 Gy and 0.35 Gy. 22 patients have been treated. Acute toxicity was mild, but 8 patients experienced a grade 4

hematotoxicity 3-5 weeks after treatment, particularly with regard to thrombocytopenia. The main risk factors were the numbers of previous treatments and thrombocytopenia <150,000/μL before radio-immunotherapy, but not the total body dose. Responses included one CR, five PR and three minor or mixed responses.[25] This response rate of 27% with a median duration of 4 months compares to what was reported in the literature. However, ^{90}Y-based constructs might be more promising since ^{90}Y transfers a higher β-energy with a longer pathway compared with ^{131}I. This could be an advantage, especially in patients with larger tumor masses.

Naked antibodies

A vast number of anti-CD30 antibodies have been developed, but only very few of them demonstrated *in vitro* and *in vivo* activity on Hodgkin's-derived tumors. In addition, mAbs containing murine components can generate a human anti-mouse antibody (HAMA) response when administered to patients, thus limiting their utility.[16,26,27] Therefore, human (MDX-060) or humanized (SGN-30) anti-CD30 antibodies have been developed.

SGN-30, a chimeric anti-CD30 mAb, has demonstrated antitumor activity in preclinical models of HL and anaplastic large cell lymphoma (ALCL).[27] In a first phase I single-dose trial this MAb showed minimal toxicity associated with doses of 1-15 mg/kg and antitumor activity was seen in 2/13 patients.[28] In a phase I/II dose-escalation study of six weekly i.v. infusions of SGN-30 at doses of 2, 4, 8 and 12 mg/kg per cohort 24 patients were enrolled (21 HL, 2 ALCL, 1 diffuse large B-cell lymphoma). The MAb was very well tolerated. Preliminary response data (up to the 8 mg/kg cohort) include one CR of a patient with ALCL and five SD in HL. Pharmacokinetics of SGN-30 confirmed a terminal half-life of approximately 3 weeks.

MDX-060 is a fully human IgG1k mAb that recognizes CD30 with nanomolar affinity and mediates killing of HL and ALCL cell lines *in vitro* and in xenograft tumor models.[29] In a Phase I/II open-label, dose-escalation study of MDX-060 in patients with relapsed or refractory HL, ALCL, or other CD30$^+$ lymphomas, MDX-060 was administered intravenously at dose levels of 0.1, 1, 5, or 10 mg/kg weekly for 4 weeks without any DLTs. In the currently ongoing phase II portion, expanded cohorts will receive MDX-060 at 15 mg/kg. To date, 21 patients (HL=16, ALCL=3, Other=2) have been treated without significant infusion-related reactions. While efficacy assessments have not yet been completed in all patients, one patient with ALCL in the 1 mg/kg cohort had a complete response to therapy of 4 months duration and a second remission upon re-treatment with the MAb. This preliminary results indicate MDX-060 to be well tolerated and with clinical activity.[30]

Future strategies

HL is a very complex disease and it took a century from the first description as a specific disease until it could be characterized as a B-cell lymphoma only some years ago. The malignant and CD30 positive HRS cells account for only one in a thousand to one in a hundred cells within the tumor. Most of the tumor volume is comprised of an inflammatory infiltrate that contains mainly reactive T lymphocytes. Thus, it remains questionable whether or not targeting a single cell in huge tumor mass can result in tumor regression, though some responses in relapsed or refractory HL patients have been observed. Combination with conventional chemotherapy might improve the results. *In vitro* studies have shown that there is synergism in between anti-CD antibodies and chemotherapy as it is known from the anti-CD20 antibody rituximab. Also, combination with bortezomib, a proteasome inhibitor, results in a substantial increase in cytotoxicity in HL and ALCL cell lines. Importantly, this synergism was observed also in cell lines being refractory to anti-CD30 antibody treatment alone. Thus, it might be advantageous to test these combinations in clinical trials.

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