Cure of Disseminated Xenografted Human Hodgkin’s Tumors by Bispecific Monoclonal Antibodies and Human T Cells: The Role of Human T-Cell Subsets in a Preclinical Model

By Christoph Renner, Stefan Bauer, Ugur Sahin, Wolfram Jung, Rene van Lier, Georg Jacobs, Gerhard Held, and Michael Pfreundschuh

Cure of a single established human Hodgkin’s tumor growing subcutaneously in severe combined immunodeficient (SCID) mice can be achieved with a complex protocol using two bispecific monoclonal antibodies (Bi-MoAb) directed against the Hodgkin’s associated CD30 antigen and the T-cell triggering molecules CD3 and CD28, respectively, together with human T cells prestimulated in vitro with Bi-MoAbs in the presence of CD30⁺ cells. To adapt this model to the clinical situation, disseminated tumors were established in SCID mice by intravenous injection of 2 × 10⁷ cells of the Hodgkin’s derived cell line L540CY. Treatment of SCID mice bearing disseminated CD30⁺ Hodgkin’s tumors with the combination of CD3/CD30 and CD28/CD30 Bi-MoAbs and naive (ie, not in vitro prestimulated) human T cells resulted in the cure of all appropriately treated animals. T lymphocytes obtained from patients with advanced stage untreated Hodgkin’s disease were as effective as lymphocytes from healthy controls. Treatment was effective even when delayed until 2 weeks after tumor inoculation, and application of Bi-MoAbs into SCID mice with circulating human T cells was as effective as injecting the Bi-MoAbs before the lymphocytes. Treatment results with isolated CD4⁺ and CD8⁺ human T cells suggest that both subsets are necessary for the Bi-MoAb mediated cure of xenografted human tumors in vivo. The efficacy and practicability of this preclinical immunotherapy protocol support and form the basis for the clinical evaluation of this approach in patients with Hodgkin’s disease resistant to standard therapy.

© 1996 by The American Society of Hematology.
Hodgkin’s tumors can be cured even when therapy is started at an advanced stage of disease.

MATERIALS AND METHODS

Animals. Four to six week-old pathogen-free mice with severe combined immunodeficiency (C.B-17 lcr scid/scid) were obtained from the Institut für Versuchstierzucht (Hannover, Germany). Animals were housed and bred in pathogen-free cages and fed with autoclaved standard chow and water. To eradicate residual NK cells, mice were treated on days -3, 0, +3 before and after the application of human lymphocytes with 100 µL anti-asialo GM-1 antibody (Wako, Osaka, Japan) intraperitoneally (IP).

Cell lines and Bi-MoAbs. The CD30+ human Hodgkin’s-derived cell line L540CY and the T-cell activating CD3/CD30 and CD28/CD30 Bi-MoAbs used in this study have been described before.1,2

Establishment of disseminated Hodgkin’s lymphoma in SCID mice. Except for titration experiments, 2 × 10^6 cells of the Hodgkin’s-derived L540CY cell line were injected into the ventral tail vein of SCID mice. To prevent pulmonary embolism caused by the injection of tumor cells, mice were anesthetized by ether inhalation, tumor cells were suspended in 400 µL sterile phosphate-buffered saline (PBS) + 0.1% bovine serum albumin (BSA) and administered intravenously (IV) over a period of 10 seconds. Blood samples were taken every 3 days, and the level of sCD30 was determined by enzyme-linked immunosorbent assay (ELISA).13 For tumor cell titration experiments, animals were divided into groups of ten. All animals that showed signs of advanced tumor disease or that lived longer than 150 days were killed and examined for macroscopic signs of tumor growth. In addition, major organs were resected and subjected to immunohistological and FACScan analysis as described.14 In addition, Northern blot analysis and reverse transcriptase-polymerase chain reaction (RT-PCR) was performed for human CD30 mRNA.

Preparations of T cells. Peripheral blood mononuclear cells (PBMC) were isolated as described.13 After leucine-methion-ester treatment (LME), T cells were negatively enriched by immunomagnetic depletion of CD14, CD16, CD19, and HLA-DR expressing cells using a VarioMACS system (Miltenyi, Bergisch Gladbach, Germany). Briefly, 1 × 10^6 lymphocytes were washed twice in cold MACS buffer (PBS + 1% BSA + 0.05% azide), the cell pellet was resuspended in 100 µL MACS buffer with MoAbs (10 µg) directed against the respective antigens, and the mixture was incubated at 4°C for 15 minutes. After two courses of washing with MACS buffer, goat antimouse immunoglobulin beads (30 µg/mL) were added, and the mixture was incubated at 4°C for 15 minutes. The separation of unlabelled lymphocytes was performed following the manufacturer’s recommendations. The remaining lymphocyte population was >95% CD3+; contaminating cell populations were always <0.5%, and no proliferation after phorbol myristate acetate (PMA) stimulation (10 ng/mL) and phytohemagglutinin (PHA) (1 µg/mL) for 2 to 5 days in culture was observed. For in vivo studies with subsets of human T cells, negative peripheral blood lymphocytes were negatively enriched for T lymphocytes as described above and divided into CD4+ and CD8+ subsets by MACS. Purity of T-cell subset preparations used for IV administration was checked by FACScan analysis and was always >98%.

TREATMENT OF XENOGRAFTED TUMORS IN VIVO

Animals bearing disseminated Hodgkin’s tumors were divided into groups of five and subjected to different treatment protocols. The treatment started with the IV application of bispecific antibody (50 µg/mouse). This was followed by the IV administration of 2 × 10^6 resuspended CD3+ lymphocytes 48 hours later. In one series of experiments, CD8+ and CD4+ T cell subsets were injected instead of CD3+ lymphocytes, and in another series the injection of the lymphocytes preceded the injection of both Bi-MoAbs or controls by 30 minutes, 1 hour, and 3 hours. After treatment, animals were examined clinically and sCD30 levels were assayed every 3 days. Animals were killed after 150 days or when they showed signs of advanced disease. Survival was determined from the day of tumor inoculation until the day the animal was killed. Survival curves were plotted according to the method of Kaplan and Meier, and differences in survival between the treatment groups were determined by log rank test. All animals were examined for macroscopic and microscopic signs of tumor growth. In addition, major organs were resected and subjected to immunohistological and FACScan analysis, as well as Northern blot analysis and RT-PCR for human CD30 mRNA.

Depletion of human T lymphocytes in vivo. To deplete CD8+ or CD4+ T-cell subsets in vivo, each mouse received an IP injection of 100 µg mouse antihuman CD4 and antihuman CD8 antibody (IgG2a; Medac, Hamburg, Germany) or an irrelevant IgG2a control antibody, respectively, 72 hours and 120 hours after the application of CD3+ lymphocytes.

Northern blot analysis. Total RNA was isolated from resected tissues by guanidium isothiocyanate.14 Samples of 20 µg RNA were fractionated on 1% formaldehyde agarose gels, then transferred and cross-linked to nitrocellulose. Blots were hybridized with 32P-labeled probes by using a formamide buffer system (50% formamide, 5 × SSC, 5 × Denhardt’s, 1% SDS, and 200 µg/mL heat denatured salmon sperm) at 42°C for 12 to 16 hours. After hybridization, membranes were washed two times at 42°C for 15 minutes and exposed to X-ray films for 24 to 48 hours. A CD30 antigen specific cDNA fragment of 500 bp length covering a part of the coding region was generated by RT-PCR labeled by random priming (Pharmacia, Freiburg, Germany). A β-actin probe was used as control for equal RNA loading per lane.

RT-PCR. cDNA was generated using poly A-mRNA extracted from Hodgkin cell lines or different tissues resected from sacrificed SCID mice.15 For the specific amplification of the CD30 antigen coding cDNA, a 5′ primer (bp 521-544) and a 3′ primer (bp 1054-1075) amplifying a 533-bp fragment were constructed. PCR was performed at 64°C for 32 cycles and amplified products fractionated on a 2% agarose gel containing ethidium-bromide for DNA staining.

RESULTS

Establishment of disseminated Hodgkin’s-derived tumors in SCID mice. Various numbers of cells of the cell line L540CY ranging from 1 × 10^7 to 2 × 10^7 cells were injected into the ventral tail vein of groups of 10 SCID mice to determine the number of human Hodgkin’s-derived cells necessary to induce disseminated tumors in SCID mice. Animals were killed on day 150 or when they showed signs of advanced tumor disease, which was usually around day 40 after tumor inoculation. Organs were checked for infiltration with human Hodgkin’s-derived cells both macroscopically and immunohistologically, as well as by Northern blot analysis for human CD30 mRNA. The rate of disseminated tumors depended on the number of inoculated L540CY cells. After 1 × 10^7 cells, there was a tumor take rate of only 50%. In contrast, after IV injection of 2 × 10^7 cells, a 100% rate of disseminated Hodgkin’s tumors was observed. Accordingly, this inoculum was used for further experiments. The organs most frequently infiltrated by Hodgkin’s cells after this inoculum were lymph nodes (100%), liver (100%), bones (80%), and lungs (45%).
Soluble CD30 antigen as a parameter for tumor growth. To assure that only xenografted SCID mice with a successful tumor take and established tumors were treated with the immunotherapy protocol, a more prospective parameter than survival or histological examination of involved organs was needed. As shown in Fig 1, serum levels of soluble CD30 derived from the xenografted CD30⁺ Hodgkin’s cells proved to be a reliable marker for tumor growth. After IV injection of 2 × 10⁷ cells, sCD30 levels became detectable 5 days after IV injection of 2 × 10⁷ tumor cells and peaked at day 35. All animals had to be killed on day 40 because of progressive tumor cachexia.

There was a good correlation between sCD30 levels and the degree of tumor infiltration in different organs of tumor-bearing animals as determined by immunohistology, fluorescence-activated cell sorter (FACS) analysis of CD30⁺ cells, and Northern blots for human CD30 mRNA from organs of animals killed at different time points after inoculation of tumor cells.

Prestimulation of human lymphocytes in vitro. In our original protocol, human lymphocytes were prestimulated in vitro for 72 hours with CD30⁺ Hodgkin’s cells in the presence of Bi-MoAb CD3/C30. As this prestimulation in vitro would considerably complicate a clinical protocol, we tested its effect on the efficacy of the Bi-MoAb treatment in vivo. Treatment of tumor-bearing SCID mice with both naïve and appropriately in vitro prestimulated human peripheral blood lymphocytes together with CD3/C30 and CD28/C30 Bi-MoAbs resulted in 100% cure of mice from xenografted Hodgkin’s tumors. This indicates that in vitro prestimulation with CD30⁺ tumor cells in the presence of CD3/C30 Bi-MoAb is not necessary for the induction of effective tumoricidal activity of human lymphocytes in vivo. Therefore, in all following experiments, prestimulation of human lymphocytes in vitro was abandoned.

Treatment of disseminated xenografted Hodgkin’s tumors. Disseminated tumors were established by IV injection of 2 × 10⁷ cells of the Hodgkin’s-derived cell line L540CY into the tail vein of SCID mice. Tumor growth was monitored by determining the sCD30 serum levels of inoculated mice. Only mice with increasing sCD30 levels at day 5 (positive/negative [P/N] >3) were considered to have a tumor take and used for Bi-MoAb treatment. Treatment was started on day 7 after inoculation of the tumor cells. As shown in Fig 2, all appropriately treated animals, i.e., animals that received both the combination of CD3/C30 and CD28/C30 Bi-MoAbs and human lymphocytes, had sCD30 levels below the detection limit by day 20 and survived until day 150 when they were killed. In contrast, all other animals had progressive growth of disseminated tumors and had to be killed by day 40. Experiments using T cells from different donors showed that T cells from five patients with untreated stage III B and IV B Hodgkin’s disease (four with nodular sclerosis and one with mixed cellularity subtype) were as effective in exerting Bi-MoAb mediated cytotoxicity as T cells from five healthy controls.

Macroscopic and microscopic examination of the sacrificed animals showed diffuse tumor growth in the control animals, but no detectable tumor in the appropriately treated animals. That the latter group was indeed cured from their tumors was also suggested by the immunohistological and Northern blot analysis of lymph nodes, spleen, liver, bones, and lungs from these animals on day 150 which showed no detectable remaining CD30⁺ cells or CD30-specific mRNA, respectively (Fig 3). In addition, the more sensitive examination of the same organs by RT-PCR for a human CD30 fragment showed no detectable levels of mRNA in cured mice.

Treatment of advanced disease. To determine the maximal extent of disease that can be influenced by Bi-MoAb-mediated immunotherapy, treatment of SCID mice bearing disseminated xenografted Hodgkin’s tumors was started at different time intervals after the inoculation of the L540CY cells. All animals treated 7 days after tumor inoculation were cured from their tumors. The survival rates obtained with the immunotherapeutic treatment started after 1, 2, 3, and 4 days after immunotherapy were 60% and 20%, respectively. No cure, but significantly (P < .01) prolonged survival compared with control animals was observed when treatment was delayed until 21 days after the inoculation of tumor cells (Fig 4). Immunohistological examination showed that the tumors of animals progressing after immunotherapy were all CD30 positive.

Sequence of application of Bi-MoAbs and human lymphocytes. In the clinical situation Bi-MoAbs would encounter circulating lymphocytes on their way to the tumor site. The binding of the Bi-MoAbs to circulating lymphocytes could interfere with their tumor binding and T-cell activating capacities. To test this possibility, human lymphocytes were injected into tumor-bearing SCID mice 30 minutes, 1, and 3 hours before the application of Bi-MoAbs. As shown in Fig 5, the sequence of the application of the combination of CD3/C30 and CD28/C30 Bi-MoAbs and the human lymphocytes did not influence the efficacy of the treatment: all animals with established disseminated xenografted Hodgkin’s tumors, treated with human lymphocytes and the combination of CD3/C30 and CD28/C30 Bi-MoAbs were cured, no matter whether the antibodies were given 2 days before the human lymphocytes or whether the injection of the lymphocytes preceded the application of the human lym-
phocytes by 30 minutes, 1, or 3 hours, respectively. Again, all control animals showed progressive tumor growth and had to be killed by day 40. Thus, circulating human lymphocytes did not interfere with the efficacy of the Bi-MoAb therapy.

Role of human T-lymphocyte subsets on antitumor effects in vivo. To evaluate the impact of different lymphocyte subsets on tumor cell lysis in vivo, two sets of experiments were performed. When purified resting CD4+ or CD8+ lymphocytes were injected with the combination of CD3/CD30 and CD28/CD30 Bi-MoAbs (Fig 6A), no effect of this treatment was observed when compared with control mice. Only the combination of both CD4+ and CD8+ lymphocytes resulted in the cure of tumor-bearing mice, suggesting that both subsets are crucial for efficient eradication of tumor cells.

To elucidate the question whether the combination of CD4+ and CD8+ human T cells was mandatory for the activation or the effector phase of the tumor cell destruction in vivo, human T-cell subpopulations in the SCID mice were depleted in vivo. To this end, unsorted CD3+ lymphocytes were given to tumor-bearing mice 2 days after injection of the CD3/CD30 + CD28/CD30 Bi-MoAbs, followed 3 and 5 days later by the IP application of anti-CD4 and anti-CD8 MoAb, respectively. As shown in Fig 6B, mice treated with anti-CD4+ MoAb had only a prolonged survival when compared with anti-CD8+—treated and control mice (that received no Bi-MoAbs), while all mice receiving an irrelevant control antibody instead of anti-CD4+ or anti-CD8+ were cured.

DISCUSSION

Our data show that an immunotherapeutic approach using a combination of T-cell activating Bi-MoAbs and human T
Fig 4. Survival of SCID mice bearing disseminated Hodgkin’s tumors according to interval between tumor inoculation and treatment with CD3/CD30 + CD28/CD30 Bi-MoAbs and human lymphocytes. The difference to untreated control animals is significant (log rank test) when treatment is started on day 7 (---, P < .005), day 11 (----, P < .005), day 14 (------, P < .005), and day 21 (-----, P < .01).

Different methods were used to evaluate treatment results in disseminated xenografted L540CY tumors in SCID mice: gross macroscopy, immunohistopathology, serum levels of soluble sCD30, and Northern blot analysis, as well as RT-PCR for human CD30 mRNA. All of these parameters showed a good degree of correlation. All animals with increasing sCD30 levels after therapy died of progressive tumors, while decreasing levels were associated with a successful treatment outcome (Fig 2). Thus, similar to the situation in patients with Hodgkin’s disease, \( sCD30 \) serum levels were simple and reliable monitors for tumor growth and were useful as early predictors for response to treatment. In contrast to observations made after treatment with CD25 (interleukin [IL]-2 receptor \( \beta \) chain) immunotoxins, no selection of CD30 antigens-negative tumors was observed after immunotherapy with CD30 Bi-MoAbs, neither in this T-cell modulating study nor in an earlier study using CD16/CD30 Bi-MoAbs for the specific activation of human NK cells. The reason for this remains unclear, but resembles the clinical situation where loss of expression of CD25, but not of CD30 by Hodgkin and Reed-Sternberg (H&RS) cells, has been observed in end-stage disease (A. Engert, personal communication, July 1995).

We also demonstrated in this study that application of the Bi-MoAbs after the human effector cells, ie, the IV injection of Bi-MoAbs into tumor-bearing SCID mice with circulating human T cells was as effective as the reverse sequence in curing animals from disseminated tumors with injections of the Bi-MoAb 30 minutes, 1 hour, and 3 hours after the lymphocytes, all resulting in a 100% cure rate. While it had been reported that human lymphocytes are removed from the peripheral blood of SCID mice as early as 1 hour after injection using fluorescence in situ hybridization, other studies, including one by our group, have demonstrated the persistence (or recirculation) of a significant proportion of \( ^3H \)-uridine-labeled human lymphocytes in the peripheral blood of the SCID mice as long as 48 hours after IV injection. Irrespective of this controversy, there is no doubt that at least the Bi-MoAbs injected after 30 minutes must have encountered circulating human lymphocytes. This demonstrates that circulating T cells do not interfere with the eventual binding of the Bi-MoAbs to the tumor cells and suggests that the circulating lymphocytes in the peripheral blood of
a patient should not become a crucial obstacle against the successful application of this model to the clinical situation. Finally, we could show that the efficacy of our immunotherapeutic approach does not depend on prestimulation of human effector T cells with antigen-positive tumor cells. This observation has bearing on the design of clinical phase I/II studies, which are considerably facilitated by the omission of an in vitro prestimulation step.

The demonstration of prolonged persistence of the Bi-MoAbs at the tumor site and lymphocyte distribution studies in tumor-bearing SCID mice had already suggested that the combination of CD3 and CD28 binding Bi-MoAbs has the potential for in-situ activation of human T lymphocytes. In addition, we had shown by RT-PCR and Northern blot analysis from tumor tissues taken 6 days after the initiation of treatment that mRNA for activation antigens such as CD25 was upregulated, and proliferation markers (eg, Ki-67) and cytokines (eg, IL-2, tumor necrosis factor [TNF]-β) were strongly expressed by tumor infiltrating human lymphocytes in vivo after application of both CD3 and CD28 Bi-MoAbs.20 Finally, the observation made in this study that prestimulation of human effector lymphocytes in vitro with CD30+ tumor cells can be abandoned without loss of therapeutic efficacy also indicates that both signals that are necessary for efficient activation of cytotoxic T cells can be delivered to the human T cells at the tumor site by cross-linking via the combination of the CD3/CD30 and CD28/CD30 Bi-MoAbs. The fact that the injection of either unstimulated CD4+ and CD8+ human T cells resulted in the loss of therapeutic efficacy points to the necessity of both subpopulations for the activation of cytotoxic T cells in vivo. Finally, the results of our in vivo depletion studies of human T-cell subsets with anti-CD4 and anti-CD8, respectively, support the hypothesis that after the initial activation, which necessitates both CD4+ and CD8+, the cytotoxicity in vivo is exerted by either CD4+ and CD8+, with the CD8+ subset being the more potent effector cells (Fig 6). This is in complete accordance with the situation in vitro, where both CD4+ and CD8+ cells confer Bi-MoAb-mediated cytotoxicity in vitro after appropriate prestimulation.21

Like other experimental approaches to immunotherapy of malignant tumors, the efficacy of our approach using Bi-MoAbs and human T cells depended on the time during the course of the disease at which the immunotherapy protocol was started: while all animals were cured with the treatment started on day 7, there was only a limited effect (significantly prolonged survival, but no cure) when treatment was delayed until day 21. This suggests that the antitumor effects of Bi-MoAb-activated human T cells depend on the tumor load. Whether this is due to immunosuppressive effects exerted by the tumor cells or is caused by pharmacokinetic problems inherent to large tumor masses, such as compromised accessibility for monoclonal antibodies and effector cells, remains to be determined.

The antitumor effects of Bi-MoAbs and human T cells against disseminated human Hodgkin’s-derived tumors are CD30 antigen-specific, but MHC-nonrestricted, as CD30-transfected Chinese hamster ovary (CHO)-cells (that lack human MHC molecules) are as efficiently lysed as CD30+ Hodgkin’s-derived cells, while CHO cells transfected with the vector without insert, are resistant to treatment with Bi-MoAbs and human T cells prestimulated in vitro with CD30+ tumor cells in the presence of CD3/CD30 Bi-MoAbs.21 The non-MHC-restriction might also explain, to some degree, the high efficacy of the Bi-MoAb approach: while the requirement for a specific TCR/MHC interaction limits considerably the number of T cells that can be induced to exert a cytotoxic function, Bi-MoAbs with the indicated properties can confer anti-CD30 antigen-directed specificity to a majority of resting T cells. To another part, the success of this model may also be due to the reciprocal expression of adhesion molecules by the Hodgkin’s-derived tumor cell lines.
(which in this regard closely resemble H&RS cells in vivo) and human T cells. This is suggested by the observation that blocking of the leukocyte function antigen-1 (LFA-1)/intercellular adhesion molecule-1 (ICAM-1) or CD2/LFA-3 adhesion pathway with the respective MoAbs decreases Bi-MoAb-mediated T-cell cytotoxicity in this model. Therefore, the Bi-MoAb-mediated induction of T-cell cytotoxicity against tumors can be expected to be most efficient in neoplastic cells that, in addition to a tumor-associated antigen that serves as a target for a T-cell activating Bi-MoAb, display the respective costimulatory adhesion molecules on their surface. Before all others, this holds true for tumors of hematopoietic origin, such as leukemias, lymphomas, and Hodgkin’s disease. Finally, the demonstration that human T cells derived from patients with untreated Hodgkin’s disease are as effective in this model as T lymphocytes from healthy controls does not only encourage the clinical evaluation of this approach in patients with refractory Hodgkin’s disease, but also suggests that the T-cell deficiency described in Hodgkin’s disease affects mechanisms that are not relevant for the delivery of Bi-MoAb-mediated cytotoxicity or is compensated for by the combined cross-linking of CD3 and CD28 by the Bi-MoAbs via the H&RS cells. This is comparable to our findings of NK cell deficiencies in the same patients: while the NK cells from untreated Hodgkin’s patients had a pronounced defect in classical NK cell activity (ie, cytotoxicity against the K562 cell line) when compared with normal controls, the CD16/CD30 Bi-MoAb-mediated NK cell cytotoxicity against L540CY of these patients was as effective as that of normal controls against H&RS cells in vitro. Of course, one has to be cautious to draw conclusions from our in vivo SCID mouse data to the clinical situation, because normal immunity cannot be restored with human peripheral blood lymphocytes in the SCID mouse; however, that the in vitro findings have bearing on the clinical situation is suggested by early results of our ongoing trial with the NK-cell activating CD16/CD30 Bi-MoAb in patients with refractory Hodgkin’s disease, where we have observed clinical responses at doses as low as 4 mg/m² per patient.

In summary, we have established a preclinical model of a novel immunotherapeutic approach for the treatment of xenografted human tumors using Bi-MoAbs and human T cells. We have demonstrated that this approach is simple, does not depend on expensive and time-consuming in vitro stimulation procedures of human effector cells, and results in a high cure rate of disseminated tumors. We have investigated the role of T-cell subsets and could demonstrate that T cells derived from patients with active Hodgkin’s disease are as effective as lymphocytes from healthy controls in this model. As the parental HRS-3 antibody has been shown to bind specifically to Hodgkin and Reed-Sternberg cell in patients with Hodgkin’s disease, this approach should now be readily applicable to the clinical situation. As the NK cell activating CD16/CD30 Bi-MoAb, which was much less efficient in vitro and in the SCID mouse model than the combined CD3 + CD28 Bi-MoAb model, has shown encouraging activity in our ongoing phase I/II trial in patients refractory to standard treatment (manuscript in preparation), there is reason to expect that the T-cell activating approach with the combination of CD3 and CD28 Bi-MoAb will become a major step forward in the conquest of Hodgkin’s disease.

ACKNOWLEDGMENT
We thank Birgit Bette, Natalie Fadle, and Markus Sühre for expert technical assistance, and Volker Diehl for stimulating discussions.

REFERENCES
8. Staerz UD, Bevan JM: Hybrid hybridomas producing a bispecific monoclonal antibody that can focus effector T-cell activity. Proc Natl Acad Sci USA 83:1453, 1986


Cure of disseminated xenografted human Hodgkin’s tumors by bispecific monoclonal antibodies and human T cells: the role of human T-cell subsets in a preclinical model

C Renner, S Bauer, U Sahin, W Jung, R van Lier, G Jacobs, G Held and M Pfreundschuh