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T Cell Costimulus-Independent and Very Efficacious Inhibition of Tumor Growth in Mice Bearing Subcutaneous or Leukemic Human B Cell Lymphoma Xenografts by a CD19-/CD3-Bispecific Single-Chain Antibody Construct

Torsten Dreier,‡ Patrick A. Baeuerle,§ Iduna Fichtner,‡ Michael Grün,† Bernd Schlereth,* Grit Lorenzewsiki,* Peter Kufer,* Ralf Lutterbüse,* Gert Rietzmüller,§ Per Gjorstrup,* and Ralf C. Bargou†

We have recently demonstrated that a recombinant single-chain bispecific Ab construct, bscCD19xCD3, in vitro induces rapid B lymphoma-directed cytotoxicity at picomolar concentrations with unstimulated peripheral T cells. In this study, we show that treatment of nonobese diabetic SCID mice with submicrogram doses of bscCD19xCD3 could prevent growth of s.c. human B lymphoma xenografts and essentially cured animals when given at an early tumor stage. The effect was dose dependent, dependent on E:T ratio and the time between tumor inoculation and administration of bscCD19xCD3. No therapeutic effect was seen in the presence of human lymphocytes alone, a vehicle control, or with a bispecific single-chain construct of identical T cell-binding activity but different target specificity. In a leukemic nonobese diabetic SCID mouse model, treatment with bscCD19xCD3 prolonged survival of mice in a dose-dependent fashion. The human lymphocytes used as effector cells in both animal models did not express detectable T cell activation markers at the time of coinoculation with tumor cells. The bispecific Ab therefore showed an in vivo activity comparable to that observed in cell culture with respect to high potency and T cell costimulus independence. These properties make bscCD19xCD3 superior to previously investigated CD19 bispecific Ab-based therapies. The Journal of Immunology, 2003, 170: 4397–4402.

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*Micromet AG, Munich, Germany; †Department of Hematology, Oncology, and Tumor Immunology, Robert-Rösle Cancer Center, Max Delbrück Center for Molecular Medicine, Charité, Humboldt University of Berlin, Berlin, Germany; ‡Experimental Pharmacology, Max Delbrück Center for Molecular Medicine Berlin-Buch, Berlin, Germany; and †Institute for Immunology, Ludwig-Maximilians University, Munich, Germany

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2 T.D. and P.A.B. contributed equally to this study.
3 Address correspondence and reprint requests to Dr. Patrick A. Baeuerle, Micromet AG, Staffelseestrasse 2, 81477 Munich, Germany. E-mail address: patrick.baueuerle@micromet.de
4 Abbreviations used in this paper: NHL, non-Hodgkin’s lymphoma; NOD, nonobese diabetic; ADCC, Ab-dependent cellular cytotoxicity; Ep-CAM, epithelial cell adhesion molecule; VLA-4, very late Ag 4.
need of such high effective concentrations. One is that largely ef-
fective cells with the low-affinity FcγR CD16, such as NK cells,
contribute to ADCC. Second, FcγR binding of a therapeutic Ab
may be effectively competed by the high concentration of endog-
enous IgG. The latter may explain that addition of human serum
can drastically decrease ADCC of an epithelial cell adhesion mol-
ecule (Ep-CAM)-specific IgG1 in vitro (11). The low specific ac-
tivity of IgG1 prompted numerous attempts to increase the cyto-
toxic efficacy of Abs, for instance, by their conjugation with toxins,
prodrugs, or radioisotopes (12). Although such measures
indeed reduced the amount of Ab needed, they typically showed
increased side effects.

One approach to improve the cytotoxic efficacy of Ab-based
treatment are bispecific Abs capable of recruiting T cells (13, 14).
T cells do not usually bear FcγRs and are thus not engaged by
monoclonal IgG1 therapeutics for ADCC. CTLs are among the
most potent cytotoxic effector cells in the organism and are made
responsible for whole organ transplant rejection, graft-vs-tumor
effects in allogeneic blood stem cell transplantation, and sponta-
neous tumor regression. Bispecific T cell recruiting Abs need to
bypass all of the control elements of regular T cell activation,
including the interaction of peptide-loaded MHC class I with a
specific TCR and CD28-mediated costimulation. By binding with
one arm to a T cell trigger molecule, e.g., a component of the CD3
complex, and the other arm to a surface-exposed epitope on the
target cell, bispecific Abs are expected to trigger T cell activation
only when a target cell is tightly bound.

We have selected the CD19 Ag for targeting B lymphoma cells.
CD19 is specifically expressed by normal B cells at most devel-
opment stages (15), is an essential coreceptor for B cell prolifer-
ation, and its expression is highly conserved on various B cell
neoplasias (16). Several anti-CD19 Abs and numerous derivatives
thereof have been tested in preclinical and clinical experiments
(17). However, to date, no CD19 Ab-based therapeutic has pro-
gressed far in clinical development for treatment of B cell malign-
nancies. This is in contrast to Abs targeting CD20, CD22, and
CD52, of which CD20 and CD52 mAbs have reached the routine
therapy (2). The example of cytotoxic anti-CD20 therapeutics
shows that the mature B cell compartment can be ablated along
with the tumor cells without serious consequences for the patient
(3). This may be explained by the repopulation of normal B cells
through target Ag-negative stem cells and the interim supply of Igs
by target Ag-negative plasma cells.

For the past 10 years, several bispecific T cell-recruiting Abs
gainst CD19 have been developed and intensely characterized in
vitro and in the clinic (18–25). Very recently, two approaches have
shown particular promise in preclinical development. One is the
so-called diabody format. It consists of two paired polypeptide
chains, each with variable domains from CD3- and CD19-specific
mAbs. CD19-specific diabodies and, in particular, tandem diabod-
ies, have shown impressive efficacy against human B lymphoma
xenografts in various models (26–28). To be efficacious, this
bispecific format required a cotreatment of animals with anti-
CD28 Abs for additional T cell stimulation, preactivation of hu-
mans T lymphocytes, and total doses in the range of 50–100 μg/
animal (28). Another promising bispecific format is that of a
single-chain bispecific Ab construct called bsCD19xCD3 (29,
30), a variant of which is currently in Phase I clinical trials. In contrast
to the diabody, all four variable domains in bsCD19xCD3 are
aligned on a single polypeptide chain arranging two single-chain Abs
(scFv) in tandem. In vitro studies showed a very high specific activity
of bsCD19xCD3 in the low picogram per milliliter range, activity at
low E:T ratios, and an apparent independence on T cell costimulation
(30). The in vivo therapeutic potential of bsCD19xCD3 has not been
explored to date.

In this study, the in vivo efficacy of bsCD19xCD3 was investi-
gated in nonobese diabetic (NOD)/SCID mice that were s.c. or
i.v. xenografted with a mix of human peripheral blood cells and
human B lymphoma cells of the NALM-6 cell line. The antitumor
effects of bsCD19xCD3 observed in the present study did not require any pre- or costimulation of administered human lympho-
cytes. The independence on T cell costimuli and its high potency
distinguishes bsCD19xCD3 from other T cell-recruiting bispeci-
fic Ab constructs previously tested in SCID mouse models.

Materials and Methods

Cell lines and PBMC preparation

NALM-6 B lymphoma cells were purchased from the Deutsche Sammlung
von Mikroorganismen und Zelllinien (Braunschweig, Germany) and Chi-
nese hamster ovary cells from the American Type Culture Collection (Ma-
nessas, VA). NALM-6 and Chinese hamster ovary cells were cultured as
recommended by the suppliers. PBMC were prepared by Ficoll density
centrifugation from enriched lymphocyte preparations (buffy coats)
obtained from local blood banks. PBMC were prepared on the same day of
buffy coat receipt. Erythrocytes were removed from PBMC by erythrocy-
lysis buffer (55 mM NH4Cl, 10 mM KHCO3, and 100 μM EDTA) and
thrombocytes were removed via the supernatant obtained after centrifuga-
tion of PBMC at 100 × g for 10 min. PBMC were cultured in RPMI 1640
with 1-glutamine (Life Technologies, Grand Island, NY), 10% FCS (Life
Technologies), and 25 mM HEPES (Sigma-Aldrich, St. Louis, MO).

Antibodies

Hexahistidine-tagged bsCD19xCD3 was produced by Micromed (Munich
Germany). Expression and purification was essentially as described previ-
ously (29) with the optimization that multimers were removed by gel fil-
tration. An Ep-CAM-specific single-chain bispecific Ab was used as con-
trol Ab to demonstrate specificity of bsCD19xCD3. This single-chain
bispecific Ab was produced and purified as previously described (31).
FITC-labeled anti-human CD25, CD69, CD2, LFA-1, and PE-labeled anti-
human very late Ag 4 (VLA-4) were purchased from BD PharMingen (San
Diego, CA). FITC-labeled anti-human L-selectin was purchased from R&D
Systems (Minneapolis, MN).

PBMC activation status

Freshly prepared PBMC (2 × 10^5) were incubated in RPMI 1640 with
1-glutamine, 10% FCS, and 25 mM HEPES alone or under stimulating
conditions in the presence of either 4 μg/ml PHA plus 100 IU proleukin
(IL-2; Chiron, Emeryville, CA) or 10 ng/ml bsCD19xCD3. PBMC were cultured for 3 days in the
incubator (37°C, 5% CO2) and the up-regulation of activation markers
(CD25, CD69) and cell adhesion molecules (CD2, L-selectin, LFA-1,
and VLA-4) was investigated by standard FACS analysis. For flow cytometry,
samples were analyzed using a FACS Calibur instrument (BD Biosciences,
Mountain View, CA) equipped with a 488-nm argon laser. Data analysis
was performed using CellQuest software (BD Biosciences). FACS data
were quantitated as histograms by determining the mean fluorescence
intensity as described by Diamond and Demaggio (32).

Animal experiments

All animal experiments were performed in NOD/SCID mice characterized
by T, B, and NK cell deficiency and lack of macrophage function (The
Jackson Laboratory, Bar Harbor, ME). The mice were main-
tained under sterile and standardized environmental conditions (20 ± 1°C room tem-
perature, 50 ± 10% relative humidity, 12-h light-dark rhythm) and re-
ceived autoclaved food and bedding (ssniff, Spezialdiäten, Soest, Ger-
many) and acidified (pH 4.0) drinking water ad libitum. Mice were tested
for leaskness and only animals with IgG levels below 100 ng/ml were used.
All experiments were performed according to the German Animal Protec-
tion Law with permission from the responsible local authorities. In com-
pliance with such regulations, mice had to be euthanized when tumors
reached mean volumes, >10% of body weight. Statistical analysis of tumor
growth was performed with the Mann-Whitney U test.

NALM-6 B lymphoma model

NALM-6 B lymphoma cells were taken from routine cell culture, washed
once, and diluted with PBS. Cells were mixed with pretested PBMC from

healthy donors immediately before s.c. or i.v. injection at the E:T ratios given in the figure legends. In each case, the injection volume of cell suspension was 0.2 ml/mouse. Intravenous treatment with single-chain bispecific Ab or the vehicle (PBS) started 1 h after cell inoculation and was repeated at 3 (i.v. model) or 5 (s.c. model) consecutive days. In one experiment, initiation of single-chain bispecific Ab treatment was delayed to day 4, 8, or 12. In the s.c. model, tumor sizes were measured twice a week with a caliper in two perpendicular dimensions. Tumor volumes were calculated according to (width² x length)/0.5 as a correlate for efficacy. In the i.v. model, mice were investigated once per day for health status. Moribund mice were euthanized, and survival time was taken for the evaluation of therapeutic efficacy. Body weight of mice was determined twice per week as the indicator for tolerability of treatment.

Results
Activation state of transferred human lymphocytes

bscCD19xCD3 requires the presence of human T lymphocytes for biological activity (29, 30). Those can be triggered in vitro by the bispecific Ab to eliminate appropriate target cells without the extra addition of cytokines, mitogenic lectins, or CD28 Abs, as are typically required by other bispecific Ab formats.

To test the activation state of PBMC after 3 days of cell culture before their use as effector cells in our NOD/SCID mouse experiments, a panel of activation markers was screened on cultured cells by FACS. For comparison, an aliquot of PBMC was treated for 3 days with a combination of 4 μg/ml PHA and 100 IU/ml IL-2. Likewise, an aliquot of PBMC was treated with 10 ng/ml bscCD19xCD3, which leads to T cell activation and depletion of endogenous B cells (30).

Under our standard PBMC culture conditions, no significant activation of T cells was detected (Fig. 1). Cell staining by fluorescently labeled Abs against CD25, CD69, VLA-4, CD2, and LFA-1 was very low unless cells were treated with the T cell mitogens PHA and IL-2. Incubation with bscCD19xCD3 had the same effect as PHA/IL-2 in that it led to a robust up-regulation of the activation markers to approximately the same level as that seen with the conventional mitogens. The early T cell activation marker CD69 was not increased, most likely because, as an immediate-early marker, it was already down-regulated after the 3-day stimulation period. Down-regulation of L-selectin in response to both PHA/IL-2 and by bscCD19xCD3 was likewise consistent with a resting stage of the PBMC under our standard cell culture conditions. Our data suggest that the T lymphocytes used in this study as effector cells for bscCD19xCD3 in NOD/SCID mice were not preactivated and did not require an extra stimulus for their full activation by bscCD19xCD3.

Inhibition of s.c. NALM-6 lymphoma growth in SCID mice by bscCD19xCD3

We established a xenotransplant model using the human pre-B lymphoma cell line NALM-6 to investigate the in vivo activity of bscCD19xCD3. NOD/SCID mice were inoculated s.c. with 10⁶ NALM-6 lymphoma cells premixed with 10⁴ unstimulated PBMC isolated from healthy human donors. Previous analyses had shown that within human PBMC the population of CD8/CD45RO double-positive T cells made the major contribution to the short-term cytotoxic activity of bscCD19xCD3, whereas primed CD4-positive T cells contributed to long-term cytotoxic activity (27, 28).

Assuming a frequency of memory CD8 T cells in normal PBMC in the range of 10%, a PBMC:target cell ratio of 1000:1 would correspond to an CD8 E:T cell ratio of 100:1.

bscCD19xCD3 was injected i.v. once a day via the tail vein into cohorts of eight mice on days 0, 1, 2, 3, and 4 after inoculation of tumor cells. Cohorts received doses of the bispecific Ab constructs of either five doses of 1 μg, five doses of 0.1 μg, five doses of 0.01 μg, or five doses of 0.001 μg. As control, one cohort was injected with the vehicle PBS in the absence or presence of human T cells (PBMC).

Subcutaneously injected NALM-6 cells in the absence of PBMC developed a palpable tumor ~30 days after inoculation (Figs. 2-5). Thereafter, the tumor volume rapidly increased to a size requiring euthanization of mice. In the presence of human PBMC, a slightly earlier outgrowth of NALM-6 tumor was noted (see also Figs. 2 and 5), suggesting a stromal support function of human lymphocytes. At cumulative doses of 5 or 0.5 μg bscCD19xCD3, none of the animals developed detectable tumors and survived inoculation of NALM-6 cells for the entire observation period of 76 days. Cumulative doses of bscCD19xCD3 of 0.05 and 0.005 μg were not effective in preventing tumor growth.

In the same experimental setting, we then tested the effect of a 10-fold increased number of tumor cells (Fig. 3). Briefly, 10⁷ PBMC were mixed with 10⁸ NALM-6 cells, giving a presumed effective CD8 E:T cell ratio in the range of 10:1. Under these conditions, a retarded tumor outgrowth was observed; the three highest doses of 5 × 1, 5 × 0.1, and 5 × 0.01 μg bscCD19xCD3 delayed outgrowth of NALM-6 tumors in the three cohorts (n = 5) by 8–20 days compared with controls. In each case, the differences in outgrowth were statistically significant with controls (p < 0.05), showing a dose-response behavior. In the cohort treated with five doses of 1 μg bscCD19xCD3, three of five animals stayed free of tumors for the entire observation period.

FIGURE 1. Expression of activation markers and adhesion molecules on the surface of human PBMC used for lymphoma treatment in NOD/SCID mice. PBMC were cultured for 3 days in the absence or presence of the T cell mitogens PHA plus IL-2 or bscCD19xCD3 at the indicated concentrations. The expression level of indicated cell surface proteins was determined by FACS analysis using FITC-labeled Abs. Mean fluorescence intensity (MFI) is shown on the left.

FIGURE 2. The effect of bscCD19xCD3 on s.c. NALM-6 B lymphoma growth in NOD/SCID mice. Cohorts of eight NOD/SCID mice were inoculated s.c. with 10⁶ NALM-6 cells in the absence (Without PBMC) or presence of 10⁵ human PBMC from healthy donors. The indicated doses of bscCD19xCD3 or a PBS vehicle control were administered via the tail vein on days 0, 1, 2, 3, and 4 following tumor cell/PBMC inoculation. Mean values of tumor growth curves are shown. Numbers indicate the proportion of animals that stayed free of tumors for the entire observation period.
The antitumor effect of bscCD19xCD3 is target specific. bscCD19xCD3 has the potential of binding T cells in the absence of CD19-positive target cells. To test whether this is sufficient for the antitumor activity of bscCD19xCD3, cohorts of eight NOD/SCID mice were treated with a related bispecific construct that shares the C-terminal anti-CD3 scFv with bscCD19xCD3 but has a distinct N-terminal scFv with specificity for the human Ep-CAM. The molecule is referred to as bscEp-CAMxCD3 and has been shown to be active against Ep-CAM-positive but not against CD19-positive cells (30). It is very similar to bscCD19xCD3 with respect to molecular mass and may thus have a similar half-life. No PBMC, PBMC plus vehicle, and a treatment with PBMC plus bscCD19xCD3 were tested in control cohorts. Treatment of NOD/SCID mice with five doses of 1 μg bscEp-CAMxCD3 plus PBMC had no impact on the growth of s.c. tumors and showed a tumor growth behavior very similar to that of the PBMC plus vehicle control (Fig. 5). After treatment with five doses of 1 μg bscCD19xCD3, no tumor growth was seen in any of the eight animals treated and the entire cohort survived the 47-day observation period.

bscCD19xCD3 prolongs survival of NOD/SCID mice with the leukemic NALM-6 B cell lymphoma cell line

The above model established a situation of a localized, extravascular B cell tumor growing in a stroma of human PBMC. The tumor had to be reached by the distantly injected drug and required for drug action the survival of sufficient numbers of human effector cells. In the following, we established a mouse model where 10⁴ NALM-6 cells where injected i.v. into cohorts of eight animals and allowed to developed into a B cell leukemia. As effectors, 10⁷ unstimulated human PBMC were mixed with NALM-6 tumor cells 5 min before injection.

As shown in Fig. 6, 10⁴ NALM-6 cells alone killed all eight animals in the cohort within 45 days. Severe neurological symptoms were observed and mice were euthanized at signs of overt paralysis. Most animals developed severe symptoms between 35 and 45 days after tumor cell inoculation. Coadministration of 10⁷ human PBMC did not show a therapeutic effect but slightly aggravated the disease. bscCD19xCD3 was given on days 0, 1, and 2 using doses of either 1, 5, or 30 μg protein. Later dosing was not tested since the survival of human T cells in mice was expected to be rather short. In all three cohorts treated with bscCD19xCD3, an
increase in survival was observed relative to the two control cohorts (Fig. 6). There was no difference seen between the three doses of 5 and three doses of 30 μg. The 3× 1-μg dose was slightly less efficacious but clearly showed a therapeutic effect. In the cohort receiving the three doses of 5 μg, 50% of the animals survived beyond the 80-day observation period without symptoms. These data show that bscCD19xCD3 is active against both local B cell tumors growing under the skin and disseminated blood-borne tumors.

Discussion

The present study shows that bscCD19xCD3 is effective against human NALM-6 B lymphoma cells in vivo. In NOD/SCID mice with s.c. NALM-6 tumors, cumulative doses of bscCD19xCD3 as low as 0.5 μg (~25 μg/kg) increased survival and effectively suppressed tumor outgrowth in the presence of human PBMC and absence of any T cell costimulatory compounds. In NOD/SCID mice with a leukemic form of NALM-6 tumors, onset of neurological symptoms was delayed in all bscCD19xCD3-treated mice and a certain percentage of mice did not develop symptoms during the entire observation period. In no case, therapeutic effects were observed with the vehicle in the presence of human PBMC. Human PBMC per se did not inhibit but rather promoted NALM-6 tumor growth. Efficacy was lower when the ratio between cocultured PBMC and tumor cells was reduced, showing the dependence of bscCD19xCD3 on effector cells. Bispecificity of bscCD19xCD3 was necessary since a single-chain Ab sharing the CD3-binding property with bscCD19xCD3 but not recognizing CD19 on NALM-6 cells was completely ineffective. This Ep-CAM/CD3 bispecific Ab shows a high in vitro activity against Ep-CAM-positive target cells (30). Altogether these data indicate that bscCD19xCD3 is acting in vivo as a CD19-CD3-bispecific T cell-recruiting Ab construct that can potently activate T cells on its own and direct them against a CD19-positive human B cell lymphoma line. This is in line with the properties of bscCD19xCD3 observed in vitro, i.e., costimulus independence, high potency, and effectiveness at low E:T ratios (29, 30). The observation that bscCD19xCD3 treatment became less efficacious 8 days following tumor inoculation may be explained by a limited lifespan or functionality of human T cells within NOD/SCID mice. bscCD19xCD3 could apparently reach the tumor site when given via the tail vein.

One limitation of the NOD/SCID model is the need to supply human T effector cells. Like the tumor cells, they are available in limited number, and, unlike in previously published experiments (see below), were not prestimulated in any form. The latter circumstance will severely limit the potential of i.v. injected human T cells to adhere to and traverse murine endothelium. Only a small number of i.v. injected human T cells, if any, will therefore be able to reach distant, extravascular tumor sites. We therefore decided to premix NALM-6 tumor cells and human PBMC for s.c. inoculation. This establishes a situation not unlike in natural lymphomas where tumor and T cells occur in close proximity. We expect that the half-life of unstimulated human T cells in NOD/SCID mice is rather limited. This could explain the observed loss of efficacy when bscCD19xCD3 was administered later than 8 days after tumor/PBMC inoculation. In the leukemic model, where NALM-6 and human T cells are i.v. injected together, cells may have a similar chance to distribute in the mouse. The efficacy of bscCD19xCD3 seen under these conditions supports that human T cells and NALM-6 cells stayed in contact within the same compartments. The limited number and reach of human T cells in our NOD/SCID model may underestimate the potential of bscCD19xCD3 in humans where cytotoxic T cells are present in larger numbers and found widespread in the organism. Moreover, potent activation of T cells by bscCD19xCD3 will largely increase their motility, reach, number, and cytotoxic activity.

Pharmacokinetic studies in other nonrelevant species (dogs and primates) have revealed a half-life of bscCD19xCD3 of ~2 h (data not shown). If in NOD/SCID mice bscCD19xCD3 had a comparable half-life, the compound was active for only a few hours following i.v. administrations. The observation that no tumor outgrowth was observed in bscCD19xCD3-treated animals for the entire observation period of 76 days suggests that the initial tumor cell elimination by the short-lived compound was complete or, at least, led to subcritical numbers of tumor cells. It must be assumed that the bispecific Ab did reach the s.c. tumor site after injection via the tail vein and could therefore act sufficiently long at sufficiently high levels with a sufficient number of functional T cells. In humans, the continuous availability of T cells would make a repeated dosing of bscCD19xCD3 much more efficient, raising the opportunity to also treat more advanced tumor stages. The long delay in NALM-6 tumor outgrowth (>3 wk) and the limited lifespan and motility of unstimulated human T cells in mice did not allow investigation of the effect of bscCD19xCD3 on established tumors in the presently established NOD/SCID mouse model. Rather, our current models are investigating a minimal residual disease situation where elimination of a low number of tumor cells reduces the risk of relapse.

Our models allow a limited comparison of the therapeutic potential of bscCD19xCD3 to that of other T cell-recruiting bispecific anti-lymphoma Abs. Some have also been tested in SCID mouse models although different treatment regimens, T cell handling, human B lymphoma lines, and tumor stages were used. A conventional CD19xCD3-bispecific Ab generated by the hybrid-hybridoma technique led to efficient prevention of lymphoma growth in SCID mice but only when human effector T cells were extensively prestimulated and/or costimulated by IL-2, immobilized OKT3 (anti-CD3), and an anti-CD28 mAb (33). Furthermore, a higher Ab dose of 200 μg was injected into each mouse. In another model, a single injection of 50 μg of conventional CD19xCD3 Ab prevented growth of EBV-transformed B cells (34, 35). In this case, EBV-transformed B cells led to strong activation of autologous T cells. In a SCID mouse model using CD19xCD3 diabody and tandem diabody, an antilymphoma effect could be observed with small established tumors, but only upon ex vivo pre- and costimulation of T cells with IL-2, immobilized OKT3 (anti-CD3), and anti-CD28 Ab (36, 37). In these experiments, multiple injections of 50 μg of bispecific Ab were given to the animals. In...
vivo studies using a CD30xCD3 conventional-bispecific Ab in a SCID mouse model for CD30-positive Hodgkin’s lymphoma showed that efficient elimination of established tumors was achieved but, again, only when a second bispecific Ab against CD30 and CD28 was coadministered during T cell targeting (38). Similar observations were made with a bispecific anti-CD3xanti-idiotype Ab for the treatment of B cell non-Hodgkin’s lymphoma (39).

In summary, two features appear to distinguish bscCD19xCD3 from bispecific anti-lymphoma Abs previously tested in mouse models. One is the apparent independence on any T cell costimulatory regimens and the other an almost 2 log higher potency. The molecular and structural basis for these differences, which are also seen in cell culture experiments in vitro (29, 30), is currently under extensive research. It is important to emphasize that the in vitro behavior of bscCD19xCD3 was predictive for its in vivo behavior. We assume that the particular structure of bscCD19xCD3 and its unique CD3-binding moiety are leading to a much more frequent formation of productive immunological synapses between CTL and tumor cells. This may alleviate the need for additional co-stimulatory signals. Ongoing clinical studies will reveal the therapeutic potential and safety profile of a version of bscCD19xCD3 referred to as MT103.

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References

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