Cancer Therapy: Preclinical

EpCAM/CD3-Bispecific T-cell Engaging Antibody MT110 Eliminates Primary Human Pancreatic Cancer Stem Cells

Michele Cioffi¹, Jorge Dorado¹, Patrick A. Baueule², and Christopher Heeschen¹

Abstract

Purpose: Tumor-initiating cells with stem-like properties, also termed cancer stem cells (CSC), have been shown to sustain tumor growth as well as metastasis and are highly resistant to chemotherapy. Because pancreatic CSCs have been isolated on the basis of EpCAM expression, we investigated whether a targeted immunotherapy to EpCAM using the bispecific T-cell–engaging antibody MT110 is capable of eradicating CSCs.

Experimental Design: We studied in vitro and in vivo the effects of MT110 on CSCs using both established cell lines as well as primary cells of human pancreatic cancer.

Results: Although established cell lines were more responsive to MT110-engaged T cells, also primary cells showed a time- and dose-dependent response to treatment with the bispecific antibody. In addition, the population of highly tumorigenic CSCs was efficiently targeted by the EpCAM/CD3-bispecific antibody MT110 in vitro and in vivo using a mouse model of established primary pancreatic cancer. Pancreatic cancer cells derived from metastases were slightly more resistant to MT110 treatment on the basis of in vivo tumorigenicity studies. This appeared to be related to a higher frequency of an EpCAM-negative subpopulation of CSCs.

Conclusions: Cytotoxic T cells can be effectively redirected against primary human pancreatic cancer cells by T-cell–engaging BiTE antibody MT110 including a subpopulation of highly tumorigenic CSCs.

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Introduction

Pancreatic cancer is the fourth most frequent cause for cancer-related death (1) and is characterized by early metastasis and pronounced resistance to chemotherapy and radiation. Despite extensive research efforts, hardly any substantial progress with regard to improvement in clinical endpoints has occurred over the past decades. The only currently available effective treatment modality for pancreatic cancer requires a very invasive and complex surgical procedure, also known as Whipple procedure. Although these patients show an extended median survival of 20 months, only a minority of patients with local disease are suitable for surgical intervention (~20% of patients; ref. 2). For patients with advanced disease, the introduction of the antimitabolite gemcitabine more than a decade ago improved clinical response by reducing pain and weight loss (3). However, the prognosis of patients with metastatic pancreatic cancer has remained extremely poor with a 5-year survival rate of 3% to 4% and a median survival period of 4 to 6 months (4). Therefore, new approaches for targeting the complex biology of pancreatic cancer are desperately needed to pave the way for the development of more effective treatments for these patients.

Increasing evidence indicates that stem cells may play a decisive role not only in the generation of complex multicellular organisms but also in the development and progression of tumors (5, 6). Cells bearing stem cell properties may have an integral part in the development and perpetuation of different human cancers (7–11). The current consensus definition describes a cancer stem cell (CSC) as a cell within a tumor that is able to self-renew and to produce the heterogeneous lineages of cancer cells that comprise the majority of the tumor mass (6). We and others have recently provided supportive evidence for a hierarchical organization of human pancreatic cancer (12, 13). Importantly, pancreatic CSCs are heterogenic, and a subpopulation of the CD133³ cells identified by additional expression of the chemokine receptor CXCR4 bear exclusive metastatic activity (12). As these tumorigenic CSCs are highly resistant to standard chemotherapy, such cells may be the source of the virtually inevitable relapse of pancreatic
cancer. Indeed, in cases in which bulk disease is eradicated by chemotherapy, only to be followed by a relapse, a plausible explanation is that the CSCs have not been destroyed completely.

Epithelial cell adhesion molecule (EpCAM; CD326) is frequently overexpressed and functionally altered in malignant cells (14, 15), including CSCs (16–18). Certain normal epithelial tissues and embryonic stem cells also express EpCAM (19–21), but emerging evidence indicates that EpCAM on normal epithelial tissues is largely sequestered within intercellular boundaries while becoming accessible on the surface of disintegrated cancer cells (22). Therefore, EpCAM may represent a promising target for immunotherapy of EpCAM-expressing cancer cells including tumorigenic CSCs. As MT110 is a bispecific T-cell–engaging (BiTE) antibody construct, which simultaneously targets EpCAM on tumor cells and the T-cell receptor–CD3 complex on T cells, we hypothesized that MT110 may also target and eradicate EpCAM-expressing pancreatic cancer cells including CSCs by redirecting cytotoxic effector T cells.

MT110 and related EpCAM-specific BiTE antibodies have already shown high antitumor activity in diverse xenograft models (23–26). Importantly, studies in syngeneic mouse models using a BiTE binding to murine EpCAM and murine CD3 showed antitumor activity without affecting normal epithelia, which expressed EpCAM at similar level and distribution as seen in human tissues (27, 28). MT110 is currently tested in a dose-escalating phase I clinical trial enrolling patients with diverse epithelial cancers for safety and initial signs of antitumor activity. Because EpCAM is also expressed in pancreatic CSCs (12, 13), EpCAM-directed immunotherapy may provide a new treatment opportunity for the effective eradication of pancreatic cancer including CSCs as the putative root of pancreatic cancer.

Cytotoxicity assay

Redirected cellular cytotoxicity was assayed using human peripheral blood mononuclear cells (PBMC) as effector cells and various EpCAM-positive human pancreatic cancer cells. PBMC were isolated from healthy donors by Ficoll density gradient centrifugation using standard procedures, washed with PBS, and resuspended in RPMI-1640 complete medium. The cells were seeded in 96-well plates (Nalgene Nunc International) at a concentration of 1 × 10⁴ cells per well in 100 μL of complete medium. The cells were incubated for 24 hours to allow sufficient cell adhesion, and all the microplates were incubated for a total of 72 hours after administration of compounds. The cytotoxic activity was measured by sulforhodamine B (SRB)-based cytotoxicity assay as described previously (30). The protein absorbance of the viable cells at each concentration was expressed as the relative percentage of absorbance compared with the control well without drug exposure. Each experiment was carried out using 3 replicated wells at same drug concentrations, and all the experiments were repeated 3 times.

Western blot analysis

For the analysis of protein expression, human primary pancreatic cancer cells and spheres were homogenized in lysis buffer (40 mmol/L HEPES pH 7.5, 120 mmol/L NaCl, 5 mmol/L MgCl₂, 1 mmol/L EGTA, 0.5 mmol/L EDTA, 1% Triton X-100) containing protease and phosphatase (20 mmol/L a-glycerolphosphate, 2.5 mmol/L...
Na-pyrophosphate) inhibitors. Twenty-five micrograms of cell lysates were analyzed on 12% to 10% SDS-PAGE, and the following primary antibodies were used: anti-Oct4a (2890 Cell Signalling), anti-Nanog (ab21624; Abcam, Inc.), anti-GAPDH (ab8245-100; Abcam, Inc.). Anti-mouse and anti-rabbit immunoglobulin G (IgG) coupled to peroxidase were used as secondary antibodies (Santa Cruz Biotechnology) and the signal was revealed through a chemiluminescent detection kit (ECL Detection Kit; Amersham Biosciences).

RNA preparation and real-time PCR

Total RNAs from human primary pancreatic cancer cells and spheres were extracted with the TRIzol Kit (Life Technologies Inc.) according to the manufacturer’s instructions. A 1 µg total RNA sample was used for cDNA synthesis with SuperScript II reverse transcriptase (Life Technologies Inc.) and random hexamers. Quantitative real-time PCR (qRT-PCR) was carried out using SYBR Green PCR master mix (Qiagen), according to the manufacturer’s instructions. The following primers were used: Nanog: sense-TGAACCTCAGCTGAAACAGGT, antisense-ACTTGACGAGGCACTGAG; Oct3/4: sense-CTTGCTGAGAATGGTGGTGAGGAA, antisense-CTGCAGTGTGGGTTTCGGGCA; Ki67: sense-ACCCACACAGGTGAGAAACC, antisense-ATGTG-GAGGAA, antisense-CTGCAGTGTGGGTTTCGGGCA; Sox2: sense-AGAACCCCAAGATGAGGAGGAA, antisense-CTGCAGTGTGGGTTTCGGGCA; Klf4: sense-TGACCTGAGAATGGTGGTGAGGAA, antisense-CTGCAGTGTGGGTTTCGGGCA; and glyceraldehyde-3-phosphate dehydrogenase (GAPDH): sense-CACACACAGGTGAGAAACC, antisense-ATGTG-GAGGAA, antisense-CTGCAGTGTGGGTTTCGGGCA.

Statistical analysis

Results for continuous variables are expressed as means ± SD. Treatment groups were compared with the independent samples t-test. In case of non-normal distribution, the Mann–Whitney U test was used. Pair-wise multiple comparisons are carried out with the 1-way ANOVA (2-sided) with Bonferroni adjustment. P values < 0.05 were considered statistically significant. All analyses were carried out with SPSS 19.0 (SPSS Inc.).

Results

Redirected lysis of EpCAM expressing primary human pancreatic cancer cells by MT110-engaged T cells

The efficacy of T-cell–mediated redirected lysis by MT110 of EpCAM-expressing cells was investigated for several primary human pancreatic cancer cells. A total of 3 human pancreatic adenocarcinoma xenografts (A6L, 185, and 198), some of which have been described previously (31), as well as 2 established pancreatic cancer cell lines (L3.6pl and MiaPaCa2) were investigated (12). Primary pancreatic cancer tissues were expanded as xenografts.
Isolated cells from these xenografts were cultured at low passage number as adherent cells or spheres for functional enrichment of CSCs (Supplementary Fig. S1). All cells expressed EpCAM, CD133, and CD44, as determined by flow cytometry. Redirected lysis of pancreatic cancer cells by MT110-engaged T cells was monitored by loss of cellular protein using a SRB-based cytotoxicity assay. Unstimulated allogeneic human PBMCs from healthy donors were the source of effector cells that were used at an effector-to-target (E:T) ratio of 5:1, which typically contain between 10% and 20% cytotoxic CD8+ T cells (32). A Control BiTE antibody shared the CD3-binding arm with MT110, but otherwise recognized an herbicide as an irrelevant antigen.

Established pancreatic cell lines L3.6pl and MiaPaCa2 showed lysis at 1 ng/mL MT110, which was slightly increased at 10 and 100 ng/mL MT110 (Fig. 1A and B). Statistically significant redirected lysis of target cells by MT110 was observed only at a concentration of 100 ng/mL MT110 after, minimally, 48 or 72 hours of coculture with comparable responsiveness for the 3 different primary cell lines. MT110-induced lysis of target cells was dose- and time-dependent. Treatment with 1 ng/mL MT110 for 48 hours resulted in 21% cell lysis (P = 0.10) whereas 100 ng/mL MT110 resulted in 41% cell lysis (P = 0.03). After 72 hours at 1 ng/mL MT110, we observed specific lysis in 31% of cells (P = 0.10), and at 100 ng/mL MT110 in 53% of cells (P = 0.01). Representative images show the microscopic changes seen in coculture experiments with primary cell line A6L in the presence and absence of 100 ng/mL each of MT110 and control BiTE (100 ng/mL) and CD3-only binding Control antibody after 72 hours of treatment (Fig. 2C). Clearance of the cell culture plate from adhered cells and compilation of effector and target cells in clumps was evident after treatment with MT110.

### Activation of T cells in cocultures with primary pancreatic cancer cells by MT110

Next, we explored whether MT110 is capable of inducing T-cell activation in the presence of pancreatic cancer cells. CD8+ T cells from 7-day cocultures of PBMCs with primary pancreatic cancer cell lines A6L and 185 were analyzed by flow cytometry for the de novo expression of early T-cell activation marker CD69 and late activation marker CD25. CD8+ T cells in PBMCs were initially resting as they

![Figure 2](Image)

**Figure 2.** Activity of MT110-engaged T cells against human primary pancreatic cancer cells. An MT110 dose response for redirected lysis of primary human pancreatic cancer cells A6L, 185, and 198 is shown using an SRB colorimetric assay measuring the loss of cell protein. Bar graphs are mean values of the percentage of live cells observed after (A) 48 hours and (B) 72 hours of treatment with different concentrations of MT110 at control BiTE. Unstimulated PBMCs were used as effectors at an E:T ratio of 5:1. C, representative images of A6L cells after 72-hour treatment in the presence of PBMCs with MT110 (100 ng/mL) or control BiTE (100 ng/mL) antibodies.
MT110 increased the percentage of DAPI-positive cells compared with the control BiTE, treatment with 100 ng/mL control BiTE or MT110 for 7 days. The expression of the CD8+ T-cell population to 15.9% in A6L and to 21% in 185 cocultures was seen by MT110 treatment vis-à-vis treatment with the control BiTE (Fig. 3B). In addition, treatment with control BiTE significantly reduced cell proliferation as shown by a decrease in the proliferation index of the control BiTE, eventually explaining a reduction in sphere formation of 3- and 4.2-fold for both A6L and 185 cells, respectively, was observed for cells treated with MT110 compared with control BiTE (Fig. 5A and B). In addition, treatment with control BiTE significantly reduced sphere formation by 185 cells, but not by A6L cells. It appeared that larger spheres were formed in the presence of the control BiTE, eventually explaining a reduction in number (Fig. 5A, middle). Consistent with the data from Annexin V staining, the sphere-forming capacity was more strongly reduced in 185 cells compared with A6L cells.

**Induction of apoptosis in primary human pancreatic cancer cells by MT110**

Redirected lysis of A6L and 185 primary pancreatic cancer cells by MT110-engaged T cells was further studied by surface expression of the proapoptotic marker Annexin V using fluorescence-activated cell-sorting (FACS) analysis. Treatment of cells with MT110 at 100 ng/mL for 7 days in the presence of PBMCs resulted in a significant increase of the Annexin V+ cell population (Fig. 4A). Compared with the control BiTE, treatment with 100 ng/mL MT110 increased the percentage of DAPI+ Annexin V+ cells by 10-fold to 29% ± 4.2% in A6L cells and by 3-fold to 51% ± 8.9% in 185 cells. In addition, apoptosis was slightly increased by treatment with 100 ng/mL control BiTE compared with no addition, that is, from 1.5% ± 1% to 3.5% ± 1.9% in A6L cells and from 9.5% ± 2.2% to 17% ± 4.9% in 185 cells. In the Annexin V assay, A6L cells appeared to be more resistant to T-cell–mediated apoptosis by MT110 than 185 cells, which was less apparent in the SRB test that also used shorter assay periods (see Figs. 1 and 2). Consistently, EpCAM+ Annexin V+ cells also increased in MT110-treated cells, with a subsequent decrease in EpCAM+ cells confirming effective targeting of the EpCAM+ cell population (Fig. 4B). Importantly, a significant decrease was also detectable not only for CD133+ cells, in general but also for EpCAM+CD133+ CSC, in particular.

**MT110 treatment reduces capacity of pancreatic cancer cells to form spheres**

We next examined the impact of MT110 treatment on subsequent sphere formation by pancreatic cancer cells as a surrogate assay for the self-renewal capacity of CSCs. Adherent A6L and 185 primary pancreatic cancer cell lines were treated for 7 days with PBMCs and either control BiTE or MT110 at a concentration of 100 ng/mL. The remainder of viable cells was plated under sphere-promoting conditions to assess their remaining sphere-forming capacity. Spheres were quantified after 7 days by a cell counter. A significant decrease in sphere formation of 3- and 4.2-fold for both A6L and 185 cells, respectively, was observed for cells treated with MT110 compared with control BiTE (Fig. 5A and B). In addition, treatment with control BiTE significantly reduced sphere formation by 185 cells, but not by A6L cells. It appeared that larger spheres were formed in the presence of the control BiTE, eventually explaining a reduction in number (Fig. 5A, middle). Consistent with the data from Annexin V staining, the sphere-forming capacity was more strongly reduced in 185 cells compared with A6L cells.
Loss of tumorigenicity of pancreatic CSCs following in vitro treatment with MT110

The most defining feature of CSC is their ability to exclusively form tumors following transplantation into secondary recipients such as immunocompromised mice. We, therefore, evaluated the tumorigenicity of A6L cells that were seeded in equal numbers and treated for 7 days, in the presence of PBMCs, with MT110 or control BiTE. All cells left over in cocultures after treatment with control BiTE or MT110 were implanted into the left or right flank, respectively, of immunocompromised mice without further treatment, and contralateral injection sites were evaluated for tumor formation and size after 60 days. In vitro treatment with MT110 resulted in complete abrogation of tumor formation by A6L cells, indicating that tumor-initiating cells have been efficiently eradicated by MT110-engaged T cells (Fig. 6A and B). This was despite the fact that in vitro MT110 treatment could not completely prevent sphere formation by these cells (see Fig. 5B). All implanted A6L cells treated in vitro with control BiTE, which were injected into a contralateral side to MT110-treated cells, reproducibly gave rise to tumors.

Figure 4. Induction of apoptosis on pancreas cancer cells by treatment with MT110. A, flow cytometric analysis for apoptotic cells used staining for Annexin V and DAPI in A6L and 185 cells after treatment with 100 ng/mL MT110 or control BiTE. Results are from triplicate determinations. B, flow cytometric analysis for CSC markers EpCAM, CD133, SSEA-1 in combination with Annexin V and DAPI after treatment of A6L and 185 with control BiTE or MT110. Data are represented as mean ± SD (n ≥ 3). **, P < 0.01 versus control BiTE.

Figure 5. Effect of MT110 treatment on formation of spheres by pancreatic cancer cells. A, representative images of spheres derived from surviving adherent cells treated for 7 days with either control BiTE or MT110 at a concentration of 100 ng/mL. B, quantification of spheres used a Casy cell counter.
In the next experiment, we further enriched for putatively surviving CSCs by sphere formation during 7 days after a preceding in vitro treatment with MT110–PBMCs or control BiTE–PBMCs for 7 days and before implantation of the cells into immunocompromised mice. After 60 days, tumor take occurred in only one third of the mice in the group receiving spheres obtained after MT110 treatment, whereas all mice developed tumors in the group receiving spheres after treatment in the control BiTE group (Fig. 6C). Consistent with formation of larger but fewer spheres after treatment with control BiTE (see Fig. 5A), tumors induced by such spheres were larger than under control conditions. These data indicate that CSCs enriched within spheres and being resistant to a 1-week treatment with MT110 have a much-reduced tumorigenicity, if any.

To directly compare metastasis-derived A6L cells with primary tumor-derived 185 cells for their susceptibility toward redirected lysis by MT110 a further in vivo experiment used equal numbers of cells (10^4) pretreated with either control BiTE–PBMC or MT110–PBMC for 7 days. Equal cell numbers were implanted into contralateral flanks of immunocompromised mice and tumor volume was determined on day 90 following implantation. The less aggressively growing and in vitro less resistant 185 cells had entirely lost their in vivo tumorigenicity after MT110 pretreatment as compared with control BiTE–treated cells (Fig. 7A and B). In contrast, tumorigenicity and tumor growth by the same number of metastasis-derived A6L cells was significantly reduced, but not abrogated.

Flow cytometric analysis revealed that A6L cells harbored a much more prominent EpCAM-negative subpopulation of 22% of cells compared with 185 cells with only 2% (Supplementary Fig. S5). This subpopulation of A6L cells becomes slightly enriched during sphere formation to approximately 32% of cells, which was also evident in the subpopulation of CD133+ CSCs. In addition, 185 cells enriched EpCAM-negative cells on sphere formation to approximately 15%. EpCAM-low or -negative CSCs are likely to evade redirected lysis by T cells during MT110 treatment in vitro. Nonetheless, they appear to be compromised in their in vivo tumor formation given the significantly reduced tumorigenicity seen after implantation of cells obtained after anti-EpCAM treatment.

Finally, we studied the effects of MT110 in vivo using a model of established primary human pancreatic cancer coimplanted with healthy donor-derived PBMCs. Treatment was initiated on day 7 after cell implantation (Fig. 7C). Control BiTE and MT110, respectively, were administered by intraperitoneal (IP) injection for 14 days with or without coadministration of gemcitabine. Although gemcitabine (in combination with control BiTE) resulted in a modest slowdown of tumor growth, we observed a complete stall in tumor growth for MT110-treated mice (Fig. 7D). As the treatment effects were maintained throughout the follow-up period of several weeks after discontinuation of treatment, these data are consistent with our in vitro findings that the tumors were depleted for tumorigenic/tumor-promoting CSCs. Indeed, on day 40 and, therefore, 19 days after termination of MT110 treatment, investigation of the CSC content in representative tumors using flow cytometric analysis revealed depletion for cells expressing CD133, SSEA-1, and CXCR4 as well as significantly reduced sphere-formation capacity in the MT110 group as compared with control BiTE (Supplementary Fig. S2). The combination of MT110 with gemcitabine did not result in a further reduction in tumor size.
Discussion

Considering the still devastating prognosis of patients with pancreatic cancer (1), the development of novel therapeutic strategies is a prerequisite to eventually achieve better outcomes. Previous studies, including our own for the first time, have shown that pancreatic cancers contain a rare population of undifferentiated cells that are characterized by expression of CD133 or CD44/CD24 (12, 13) that are exclusively tumorigenic and highly resistant to chemotherapy (12, 33). In this study, we show that cytotoxic human T cells engaged by low concentrations of an EpCAM/CD3-bispecific antibody construct have the potential to target highly tumorigenic pancreatic CSCs. Importantly, these cells are sensitive to lysis irrespective of downstream signaling because the targeted surface antigen EpCAM merely serves as an anchor for T-cell engagement. The low amounts of BiTE antibody necessary for redirected lysis (sub-nanomolar) are unlikely to modulate signaling activity of the targeted pathway (Supplementary Fig. S3).

Although prior studies using this technology have employed established cancer cell lines (32), we were curious to investigate whether freshly isolated human pancreatic cancer cells would respond equally well to BiTE. We observed that established human pancreatic cancer cell lines were more sensitive to BiTE treatment than primary
human pancreatic cancer cells. This observation emphasizes the rather limited predictive value of established cancer cell lines for treatment studies. Moreover, the used primary pancreatic cancer cells showed a much more robust in vivo tumorigenicity because usually less than 1,000 isolated cells are capable of tumor formation in immunocompromised mice, whereas up to 10^6 cells derived from established pancreatic cancer cell lines are required for reproducible in vivo tumor formation. Therefore, the model system used in the present study represents the most challenging setting for testing new treatment modalities against pancreatic cancer.

In this respect, our study adds important data to the growing evidence that treatment with EpCAM/CD3-bispecific antibody constructs can target highly tumorigenic CSCs. A recent study has already shown the highly efficient elimination of colorectal cancer–derived tumor-initiating cells using BiTE technology (31). In the present study, we have now extended these findings to pancreatic cancer as one of the most metastatic and highly therapy-resistant cancers (2). The population of tumor-initiating cells was virtually erased from in vivo treated primary human pancreatic cancer cells as the remaining cells could no longer form tumors after transfer into immunocompromised mice. More importantly, in vivo studies in established primary pancreatic cancers revealed disease stabilization in response to MT110 treatment whereas tumors treated with control BiTE or gemcitabine continued to grow and eventually required sacrifice of the animals. The addition of gemcitabine did not lead to a further reduction of tumor size nor did the chemotherapy apparently interfere with the activity of MT110. Harvesting of the tumors revealed that the small remaining tumors were depleted for CSCs and contained mostly differentiated cancer cells as evidenced by flow cytometric and sphere-formation assays. Therefore, the failure of gemcitabine to further reduce the size of these small reminiscent tumors can be rationalized by the lack of response of nonproliferating tumor cells to cytotoxic agents.

For A6L cells, which were derived from a metastatic pancreatic cancer lesion in the liver (29), responsiveness to MT110-engaged T cells was more diverse than for primary tumor-derived 185 cells. If A6L cells were pretreated and all surviving cells implanted, in vivo tumorigenicity was completely abrogated. However, if putatively surviving CSCs were enriched before implantation by sphere formation or the number of injected viable cells was adjusted to the number of viable cells harvested from control cultures and brought to 10^7 cells, we still observed some tumor take for BiTE-treated cells. Importantly, however, the arising tumors grew extremely slowly and were significantly smaller compared with those arising from control BiTE-treated cells. Flow cytometric analysis revealed that A6L cells contained a larger proportion of EpCAM-negative cells than 185 cells, which was also found to increase upon sphere formation (Supplementary Fig. S4). These data are consistent with the notion that sphere-derived EpCAM-negative cells are invasive CSC that have undergone epithelial-to-mesenchymal transition (EMT; ref. 34) and have likely escaped MT110 treatment. Their reduced tumorigenicity, however, indicates that loss of EpCAM is of disadvantage for their CSC properties. Indeed, EpCAM knockdown experiments using siRNA in CSC derived from colorectal cancer tissue support a functional role of EpCAM for tumor initiation, and CSC proliferation and migration (P. Baeuerle, unpublished data). Future studies will have to address the importance of EpCAM signaling for pancreatic CSCs.

An important characteristic of pancreatic cancer tissue is its poor vascularization and strong desmoplastic response (35). Although the in vivo response of BiTE antibody administration on human pancreatic cancer cells embedded in this stromal fortress will still need to be determined, the elimination of circulating cancer (stem) cells by antibody-engaged T cells can already be predicted from the current set of data. Indeed, it is important to note that, based on the systemic nature of pancreatic cancer, elimination of circulating and/or isolated metastatic CSCs may be an important therapeutic approach. Although these cells are still highly resistant to standard therapy, they are more easily accessible to immunotherapy, and their elimination already bears great potential for improving the outcome of patients with pancreatic cancer. Indeed, a recent study in breast cancer pleural effusates showed efficient lysis of these potentially metastatic cancer cells (36).

Disclosure of Potential Conflicts of Interests

C. Heeschen holds a commercial grant from Micromet Inc. P.A. Baeuerle is an employee of Micromet and has equity. No potential conflicts of interest were disclosed by other authors.

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