

# Eradication of Colon Cancer Stem Cells by EpCAM/CD3-bispecific BiTE Antibody MT110

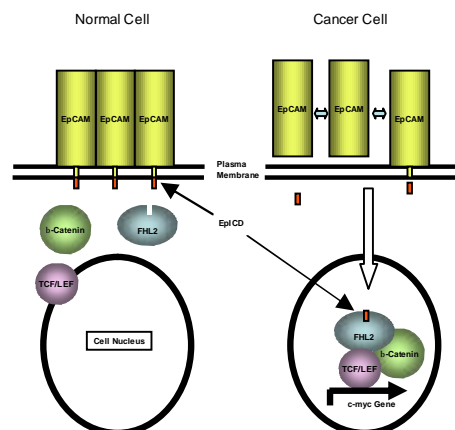
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## Background

Very low numbers of so called cancer-initiating or cancer stem cells (CSCs) are capable to reconstitute large heterogeneous tumors in mice. CSCs are further characterized by expression of certain combinations of surface antigens, and by enhanced expression of detoxifying proteins [1]. In several cancers, treatment with certain chemotherapies has been shown to enrich remaining tumor tissue for CSCs, which may explain the fast relapse of tumors after standard therapy. Here, we sought for a therapeutic means to efficiently eradicate CSC.

A number of recent studies have shown that CSCs derived from colorectal, prostate, breast and pancreas cancers express epithelial cell adhesion molecule (EpCAM, also called ESA). EpCAM is a signalling molecule that activates c-myc and cell proliferation by using the wnt pathway [2]. EpCAM's short intracellular signalling domain is tumorigenic in mice, and in colorectal cancer, expression of both EpCAM and CD44 correlate with a high tumorigenicity of CSC.

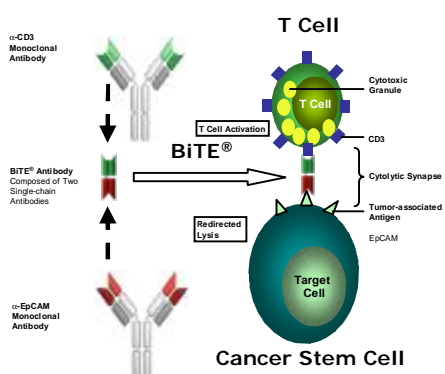
### Nuclear Signalling by EpCAM



For targeting EpCAM on CSCs in colorectal and other cancers, we investigated MT110, a T cell-engaging antibody of the BiTE class with dual specificity for EpCAM and CD3 for the eradication of primary human colon cancer stem cells (pCSC).

MT110, which currently is in a phase 1 clinical trial, has previously been shown to have high anti-tumor activity in SCID mouse models against human colorectal cancer xenografts and against human ovarian cancer tissue derived from patients [3].

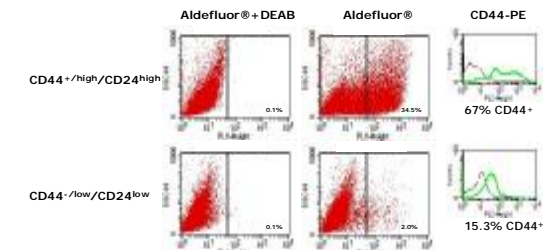
### Generation and Mode of MT110 Action



Here we investigated whether resting T cells redirected by MT110 are capable of completely lysing human colon cancer-derived CSC.

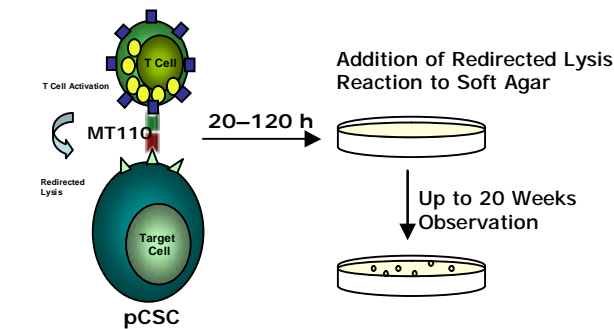
## CSC Cell Line Analysis

### 1. CD44<sup>high</sup>/CD24<sup>high</sup> HT29 Cells Selected *in vivo* Show a Cancer Stem Cell Phenotype

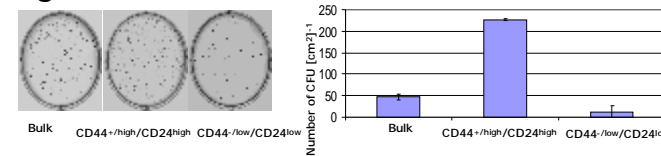


CD44<sup>+</sup>/high/CD24<sup>high</sup> cells are Aldefluor-positive. HT29 xenograft tumor was dissociated and enriched for tumor cells. After CD44 selection both fractions, CD44<sup>+</sup>/high/CD24<sup>high</sup> and CD44<sup>+</sup>/low/CD24<sup>low</sup> were analyzed for aldehyde dehydrogenase ALDH1 using the Aldefluor<sup>®</sup> assay.

### 2. Colony Formation Assay



### 3. CD44<sup>high</sup>/CD24<sup>high</sup> HT29 Cells Have a High Potential to Form Colonies in Soft Agar

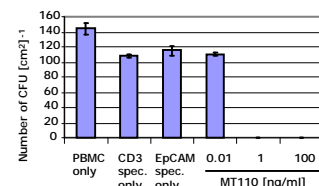


CD44<sup>+</sup>/high/CD24<sup>high</sup> cells display increased colony formation. 20,000 of CD44<sup>+</sup>/high/CD24<sup>high</sup>, CD44<sup>+</sup>/low/CD24<sup>low</sup> and bulk cells were plated onto soft agar and analyzed for colony forming units CFU. Shown are the mean number of CFUs +/- SD per cm<sup>2</sup> of triplicates and the accordant photographs of the complete well.

### 4. CD44<sup>high</sup>/CD24<sup>high</sup> HT29 Cells Are Highly Tumorigenic

NOD/SCID Model				
No. of Injections (CD44 <sup>+</sup> /CD44 <sup>+</sup> )	Cell Number (CD44 <sup>+</sup> /CD44 <sup>+</sup> )	Tumor Formation (CD44 <sup>+</sup> /CD44 <sup>+</sup> )	Average Tumor Size [cm <sup>2</sup> ]	Latency [Days]
2/2	10 <sup>1</sup> /10	-/2	-/0.015	33
2/2	10 <sup>2</sup> /10 <sup>2</sup>	2/2	0.017/0.018	33
2/2	10 <sup>3</sup> /10 <sup>3</sup>	2/2	0.083/0.069	33

### 5. Complete Lysis of CD44<sup>high</sup>/CD24<sup>high</sup> Cells by MT110

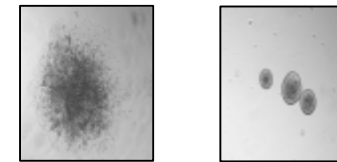


Elimination of CD44<sup>+</sup>/high/CD24<sup>high</sup> cells by MT110. CD44<sup>+</sup>/high/CD24<sup>high</sup> cells were incubated with PBMC effector cells and the respective drug concentration for 20h. All cells were transferred onto soft agar to visualize colony outgrowth. Shown are the mean number of colony forming units CFU +/- SD per cm<sup>2</sup> of triplicates after 14 days of growth in soft agar.

## Patient-Derived CSC Analysis

### 1. Features of Primary Colorectal CSC

- Obtained from 40-year old colorectal cancer patient
- Form spheres:

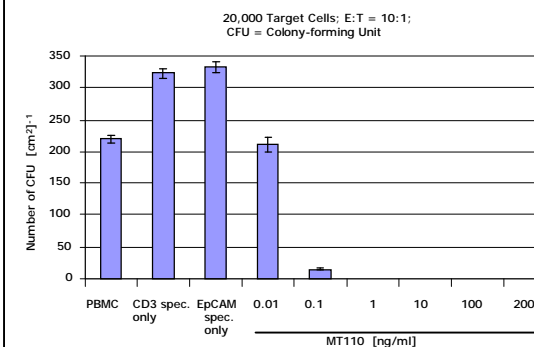
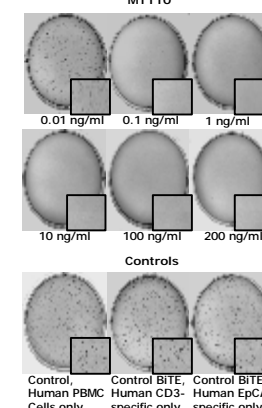


- Positive for following CSC marker: EpCAM, CD44, CD133, CD166, Oct4, Nestin, Telomerase, SSEA 3/4, AP, CA-199

- 100 pCSC cells are tumorigenic in NOD/SCID mice

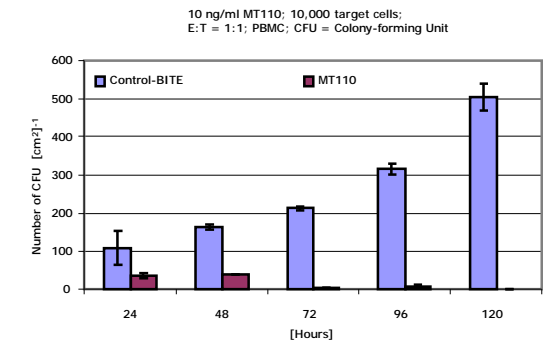
Number of CSC Injected per Site	Number of Injections per Mouse	Numbers of Tumors Formed per Mouse	Tumors per Injection Site [%]	Latency [Days]
10	2	0	0	n.a.
100	4	4	100	42-66
1,000	4	4	100	37-78
10,000	4	4	100	21-30
100,000	4	4	100	10-21
1,000,000	2	2	100	10-21

### 2. Complete Lysis of pCSC by MT110: Dose Dependence



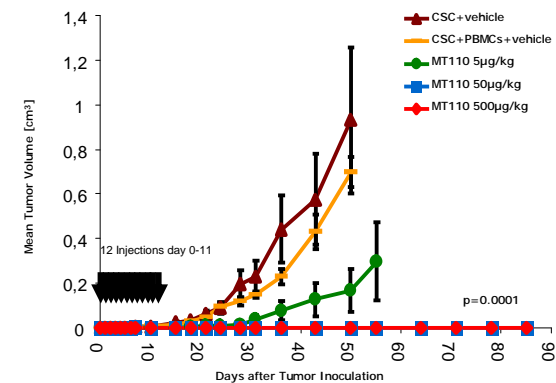
MT110 Dose Response. pCSCs were incubated with PBMC effector cells and the respective drug concentration for 20h. All cells were transferred onto soft agar to visualize colony outgrowth. Shown are representative images and the mean number of colony forming units CFU +/- SD per cm<sup>2</sup> of triplicates after 11 days growth in soft agar.

### 3. Complete Lysis of pCSC by MT110: Kinetics



Complete Lysis of pCSC by MT110 at Low Effector to Target Ratio. pCSCs were incubated with PBMCs or CD8<sup>+</sup> effector cells and 10 ng/ml MT110 or Control BITE Mecoprop for the indicated timepoints. All cells were transferred onto soft agar to visualize colony outgrowth. Shown are the mean number of colony forming units CFU +/- SD per cm<sup>2</sup> of triplicates after 16 days growth in soft agar.

### 4. Tumors Forming from 500,000 pCSC are Completely Prevented by 12 Daily i.v. Injections of 50 µg/kg MT110



Complete Eradication of pCSC by MT110 *in vivo*. 500,000 pCSCs and 10<sup>6</sup> human PBMCs were inoculated into NOD/SCID mice and treated for 11 days with the indicated MT110 concentrations or controls. The mean tumor volume is given to demonstrate the complete elimination of pCSCs at 50 µg/kg and 500 µg/kg MT110. 6/6 mice developed no tumor until day 85.

## Conclusions

- T cells engaged by extremely low doses of MT110 can lyse cancer stem cells from human colorectal cancer patients
  - 1 ng/ml MT110 (18 pM) was sufficient for complete lysis
  - Shown for human pCSC and HT29 colorectal cancer cells
  - A highly sensitive soft agar assay did not detect any colony-forming CSC after several months
  - Activity of MT110 was seen at low E:T ratios
- Tumors grown in SCID mice with a 5000-fold excess of a tumor-forming cell dose are prevented by MT110 treatment
  - Twelve daily doses of 50 µg/kg of short-lived MT110 were sufficient
  - No effect seen with human T cells alone
- Other cancer stem cell targets tested for BiTE antibodies include MCSP (melanoma) and CD33 (AML)