

Effector memory T cells make a major contribution to redirected target cell lysis by T cell-engaging BiTE antibody MT110

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Background

BiTE antibodies are single-chain bispecific antibody constructs with specificity for CD3 on T cells and a surface antigen on target cells. By transiently bridging T cells and cancer cells, BiTE antibodies are capable to mount a polyclonal T cell response that is no longer limited by T cell receptor specificity, presence of MHC class I, generation and presentation of peptide antigen, or the need for T cell costimulation.

An ongoing clinical phase I trial with blinatumomab, a CD19/CD3-bispecific BiTE antibody, in patients with therapy refractory non-Hodgkin's lymphoma has shown lasting partial and complete tumor responses (Bargou et al., *Science* 321: 974 (2008)). Another BiTE antibody in clinical testing is the EpCAM/CD3-bispecific MT110 for the treatment of patients with EpCAM+ GI tract and lung cancers.

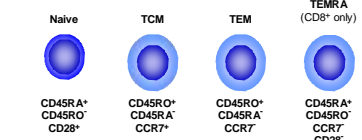
Synopsis

Here, we investigated the contribution of various subpopulations of T cells to redirected target cell lysis mediated by BiTE antibody MT110. Naïve and effector memory $\alpha\beta$ T cells of CD8⁺ and CD4⁺ phenotype as well as NKT and $\gamma\delta$ T cells were freshly isolated from peripheral blood of healthy donors.

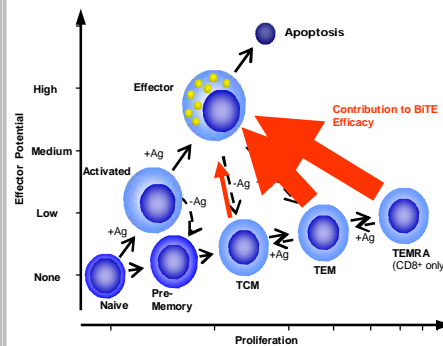
T cell subpopulations were used as an effector to target ratio of 10:1 in cytotoxicity assays analyzing dose response of BiTE antibody MT110 and the percentage of target cell lysis after 18 h as indicators for the efficacy of redirected target cell lysis. As target cells for redirected lysis we used KATO III, a EpCAM expressing human gastric carcinoma cell line. The contribution to redirected lysis of central memory $\alpha\beta$ T cells was indirectly studied. CD8⁺ effector memory T cells of CD45RA⁺ and CD45RO⁺ phenotype proved to be most efficacious in mediating redirected lysis by BiTE antibodies. CD4⁺ effector memory T cells were also highly active but with delayed kinetics, apparently needed to upregulate expression of granzyme B. Naïve CD8⁺ and CD4⁺ T cells showed very low activity. NKT and $\gamma\delta$ T cells were also effective in BiTE-induced redirected lysis, however, were less potent compared to CD8⁺ $\alpha\beta$ T cells.

Our data suggest that, with exception of naïve T cells, all other T cell populations currently analyzed can contribute to redirected lysis by BiTE antibodies.

Markers for T cell subpopulations

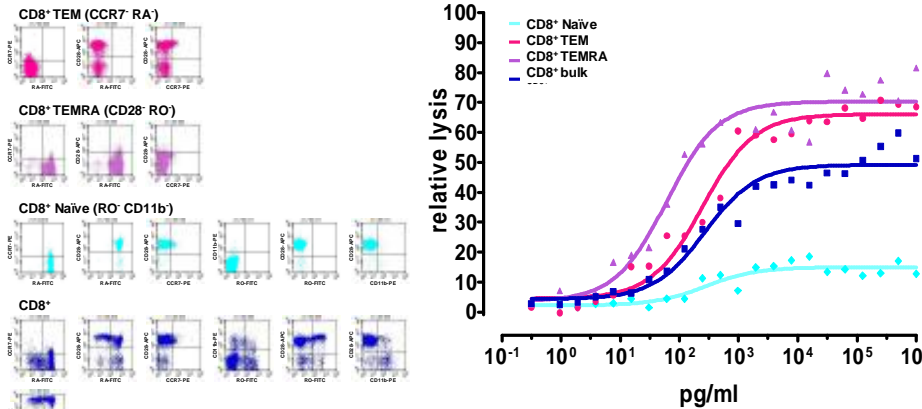


Development of T cell subsets and their effector potential



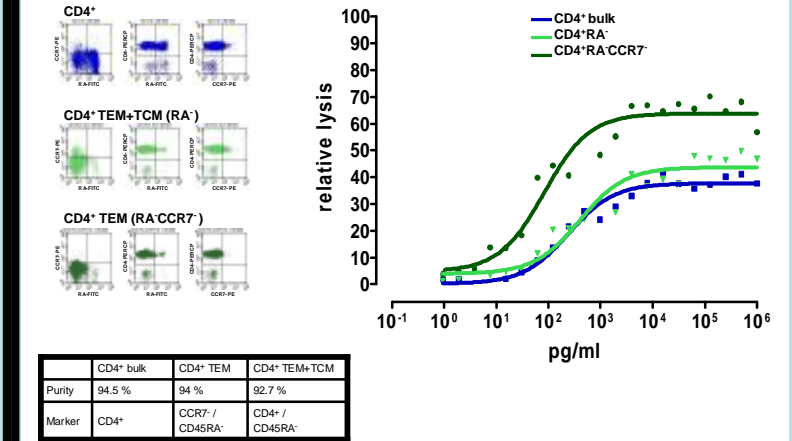
Redirected target cell lysis by CD8⁺ T cell subpopulations

125x 10⁶ CD8⁺ T cells were negatively isolated out of 600x 10⁶ PBMC of one human donor using a Dynabead® kit. Subsequently, 14x 10⁶ naïve CD8⁺ T cells were negatively isolated from 40x 10⁶ CD8⁺ T cells using a mouse anti-human CD45RO antibody, a mouse anti-human CD11b antibody and anti-mouse Dynabeads; 2x 10⁶ CD8⁺ TEM cells were negatively isolated from 40x 10⁶ CD8⁺ T cells using, first, a mouse anti-human CD45RA antibody and anti-mouse Dynabeads and, second, a rat anti-human CCR7 antibody and anti-rat Dynabeads; 3x 10⁶ CD8⁺ TEMRA cells were negatively isolated from 40x 10⁶ CD8⁺ T cells using a mouse anti-human CD28 antibody, a mouse anti-human CD45RO antibody and anti-mouse Dynabeads; Purity of subpopulations was determined by FACS analysis and cytotoxic activity was assessed by an 18 hour chromium release assay using Kato III cells as target (E:T 10:1) and the EpCAM/CD3-bispecific MT110 for recruitment of effector cells.



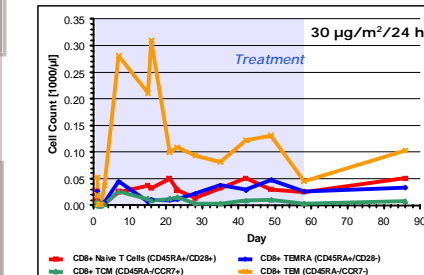
Redirected target cell lysis by CD4⁺ T cell subpopulations

470x 10⁶ CD4⁺ T cells were negatively isolated out of 1,130x 10⁶ PBMC of a single human donor using a Dynabead® kit. Subsequently, 32x 10⁶ CD4⁺ RA⁺ T cells were negatively isolated from 160x 10⁶ CD4⁺ T cells using a mouse anti-human CD45RA antibody and anti-mouse Dynabeads. Subsequently, 11x 10⁶ CD4⁺ TEM cells were negatively isolated from 30x 10⁶ CD4⁺ RA⁺ T cells using a rat anti-human CCR7 antibody and anti-rat Dynabeads. Purity of subpopulations was determined by FACS analysis and cytotoxic activity was assessed by an 18-hour 51-chromium release assay using Kato III cells as target (E:T 10:1) and EpCAM/CD3-bispecific BiTE antibody MT110.



Monitoring of T cell subsets in an ongoing phase I study with CD19/CD3-specific BiTE antibody MT103 (blinatumomab)*

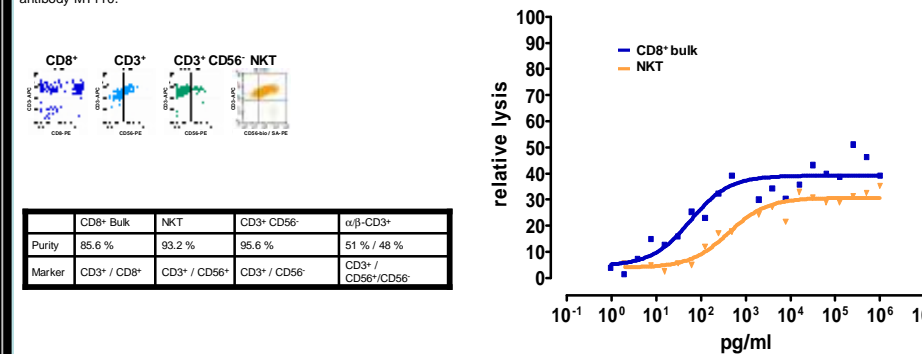
Selective expansion of CD8⁺ effector memory T cells in a NHL patient with a complete response after treatment with MT103 over 8 weeks



*From Bargou R. et al., *Science* 321: 974-977 (2008)

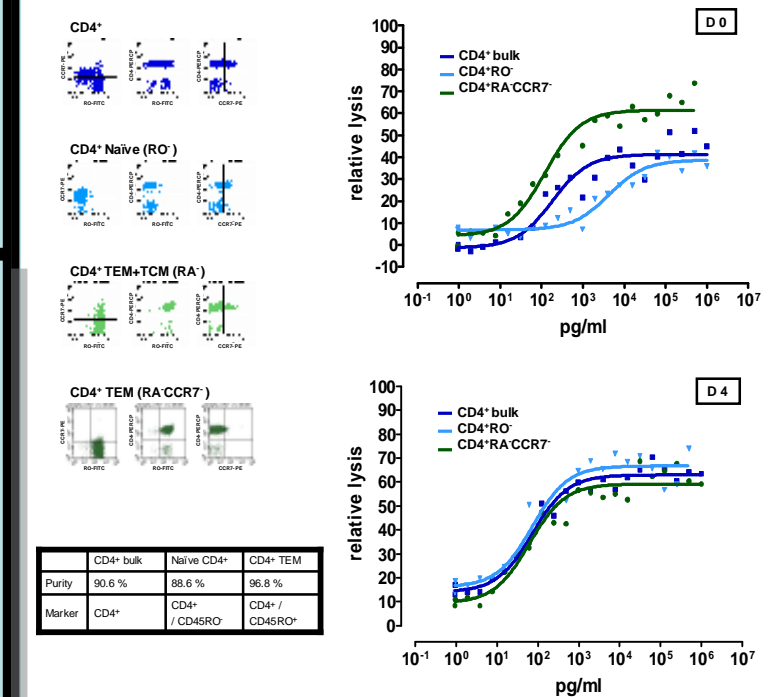
Redirected target cell lysis by NKT cells

242x 10⁶ $\alpha\beta$ T cells were positively isolated from 900x 10⁶ PBMC using a mouse anti-human $\alpha\beta$ antibody and Pan-mouse Dynabeads, and released from beads using dnal releasing buffer. Subsequently, 9.5x 10⁶ NKT cells were positively isolated from 241 x 10⁶ $\alpha\beta$ T cells using a biotinylated mouse anti-human CD56 antibody and Biotin Binder Dynabeads and released from the beads using dnal releasing buffer. As reference, 4.5x 10⁶ CD8⁺ T cells were negatively isolated from 20x 10⁶ PBMC of one human donor using a Dynabead® kit. Purity of subpopulations was determined by FACS analysis and cytotoxic activity was assessed by an 18-hour 51-chromium release assay using Kato III cells as target (E:T 10:1) and the EpCAM/CD3-bispecific BiTE antibody MT110.

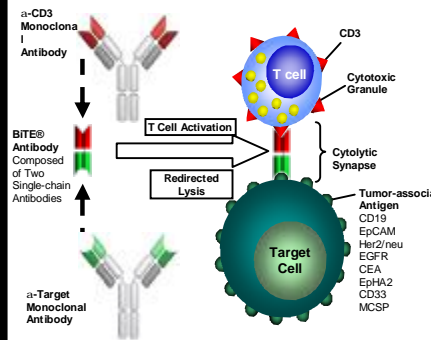


Redirected target cell lysis by freshly isolated vs. aCD3/aCD28 stimulated CD4⁺ T cell subpopulations

214x 10⁶ CD4⁺ T cells were negatively isolated out of 570x 10⁶ PBMC of a single human donor using a Dynabead® kit. Subsequently 13x 10⁶ naïve CD4⁺ T cells were negatively isolated from 100x 10⁶ CD4⁺ T cells using a mouse anti-human CD45RO antibody and anti-mouse Dynabeads; 15x 10⁶ CD4⁺ TEM cells were negatively isolated from 100x 10⁶ CD4⁺ T cells using first a mouse anti-human CD45RA antibody and anti-mouse Dynabeads and second a rat anti-human CCR7 antibody and anti-rat Dynabeads. Purity of subpopulations was determined by FACS analysis. A fraction of the isolated subpopulations was incubated for 4 days in cell culture dishes coated with anti-human CD3 and anti-human CD28 antibodies using medium containing IL-2 (10 IU/ml) before testing of cytotoxic activity. Cytotoxic activity was assessed by an 18 hour chromium release assay using Kato III cells as target (E:T 10:1) and the EpCAM/CD3-bispecific BiTE antibody MT110.



BiTE® antibodies: Generation and mode of action



Results

T Cell Populations Studied	Purity	Marker	Relative Potency of Redirected Lysis
CD8 ⁺ Bulk	91.4 %	CD8 ⁺	+++
CD8 ⁺ Naive	98.3 %	CD28 ⁺ / CD45RA ⁺	-/+
CD8 ⁺ EM	94.8 %	CCR7 ⁺ / CD45RA ⁺	++++
CD8 ⁺ EMRA	91.6 %	CD28 ⁺ / CD45RA ⁺	+++++
NKT	93.2 %	CD3 ⁺ / CD56 ⁺	++
$\gamma\delta$ T cells	93.5 %	CD3 ⁺ / $\alpha\beta$ ⁺	+
CD4 ⁺ Bulk	94.5 %	CD4 ⁺	+++
CD4 ⁺ Naive	88.6 %	CD4 ⁺ / CD45RO ⁺	-/+
CD4 ⁺ EM	94 %	CCR7 ⁺ / CD45RA ⁺	+++
CD4 ⁺ EM+CM	92.7 %	CD4 ⁺ / CD45RA ⁺	++
CD4 ⁺ Naive stimulated	-	-	+++
CD4 ⁺ EM stimulated	-	-	+++

Redirected target cell lysis by $\gamma\delta$ T cells

113x 10⁶ CD8⁺ T cells were negatively isolated from 660 x 10⁶ PBMC of one human donor. Subsequently, 1.6x 10⁶ $\gamma\delta$ T cells were positively isolated from 111x 10⁶ CD8⁺ T cells using a mouse anti-human $\gamma\delta$ antibody and Pan-mouse Dynabeads and released from the beads using dnal releasing buffer. Purity of subpopulations was determined by FACS analysis and cytotoxic activity was assessed by an 18-hour 51-chromium release assay using Kato III cells as target (E:T 10:1) and the EpCAM/CD3-bispecific BiTE antibody MT110.

