BiXAb® MAIT Engagers: solving the problems of classical T-cell engagers in the treatment of solid tumors.

Abstract No. 1005

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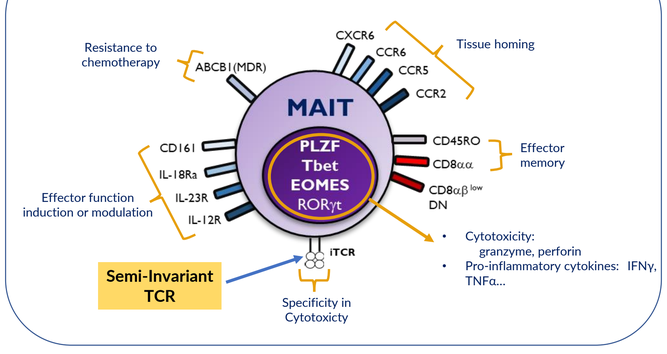
Introduction

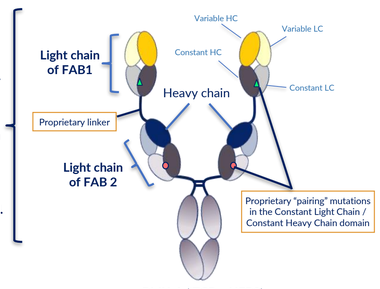
Mucosal-Associated Invariant T cells (MAITs) are an abundant subset of non-conventional T-cells with potent cytotoxic capacity **(up to 20% of circulating T-cells)** that are **naturally resident in many tissues and solid tumors**. They can be activated by a TCR-dependent and independent manner and exhibit a rapid, innate-like response to bacterial and viral infections. MAITs express a semi-invariant TCR and respond to microbial metabolites presented in the context of the MHC-like protein, MR1. They have **potent cytotoxic potential** and readily infiltrate inflamed tissues where their cytotoxic activity can be induced by TCR engagement or by IL-12/IL-18.

Mucosal Associated Invariant T cells.

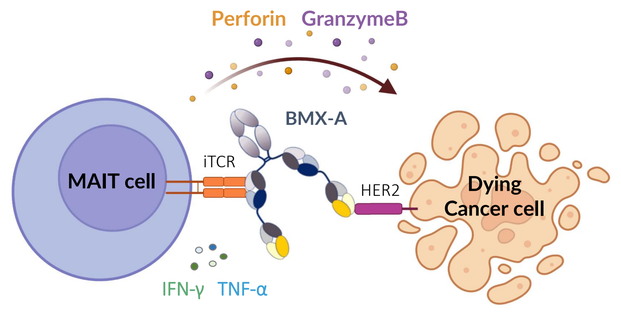


Features of the BiXAb® platform.

- Plug and Play format.
- Swap any 2 FABs.
- Bispecific
- Bivalent (for each target).
- Fc effector function competent or null.
- IgG1 or other.



MAIT cell redirection

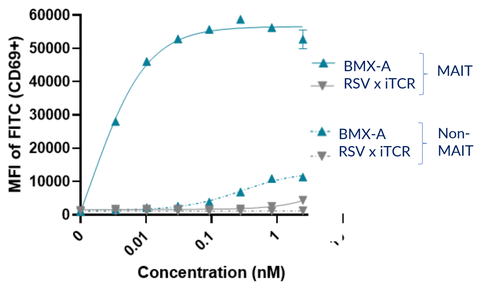


- The BiXAb® binds "in trans" to form an immunological synapse
- Activated/bridged MAIT cells directly kill the cancer cell.
- Local release of cytokines to induce secondary immune cell recruitment.

T-cell redirection is a clinically validated approach to treating haematological cancers but has had limited success so far in solid tumors. Classical T-cell engagers (TCE) bind the epsilon chain of the TCR leading to activation of all T-cells (Cytotoxic CD8s and all CD4 subsets including Tregs) which can lead to Cytokine Release Syndrome (CRS) and associated dose limiting toxicities. Activation of the Treg population in the tumor microenvironment by classical TCEs may also contribute to the reduced activity of this modality in solid tumors. Biomunex Pharmaceuticals, using their proprietary BiXAb® technology, has developed a bispecific antibody to **uniquely engage MAIT cells** and redirect them to kill cancer cells by binding the invariant TCR (iTCR) expressed on MAIT cells and a tumor associated antigen (Human Epidermal Growth Factor Receptor 2; HER2). MAIT cell redirection is expected to **significantly increase efficacy in solid tumors** where there will be **no activation of tumor resident Tregs** with increased immunosuppression and **no overt activation of all T-cell subsets leading to CRS**.

Upon engagement with HER2-expressing cells, the MAIT engager binds and activates MAIT cells

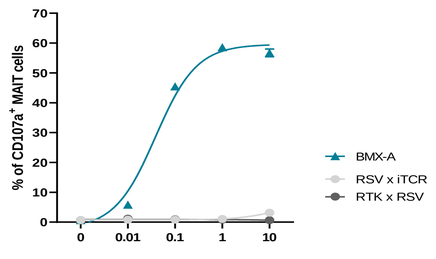
MAIT-cell activation



SKOV3 cells plated. Freshly isolated CD8 population co-cultured for 16 hrs. E:T = 2:1. Gated on MAIT population (CD161^{High}) or non-MAIT population (CD161$^{low/med}$).

The MAIT engager can induce MAIT cell degranulation in co-culture with cancer cells expressing HER2

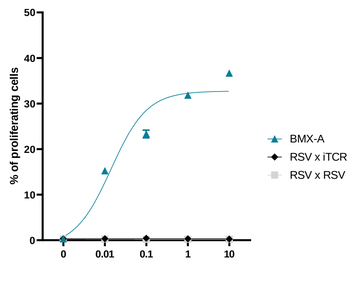
Measurement of degranulation (CD107a)



Freshly prepared CD8 population.HCT-116 cancer cell line co-cultured with CD8s and BiXAb® s for 5h days. E;T ratio = 2:1. FACs read out gated on MAIT (CD161^{High}) or non MAIT (CD161$^{low/med}$) population.

The MAIT engager can induce MAIT cell proliferation in co-culture with HER2+ cancer cells

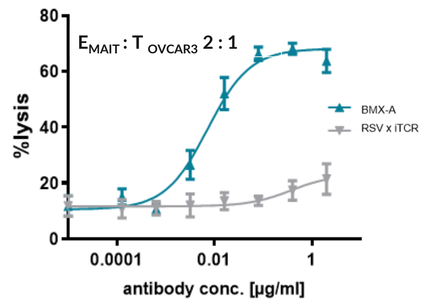
MAIT cell proliferation (FACS)



Freshly prepared CD8 population.SKOV3 cancer cell line co-cultured with CD8s and BiXAb® s for 5 days. E;T ratio = 1:1. FACs read out gaited on MAIT population.

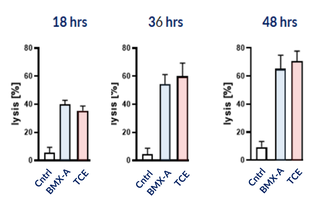
The MAIT engager has potent cytotoxic activity (equivalent to a TCE)

BiXAb® -mediated Cytotoxicity



Freshly prepared CD8 population. OVCAR3 cancer cell line co-cultured with CD8s and BiXAb® s for 18 . Cell lysis measured by Chromium release assay (data in triplicate)..

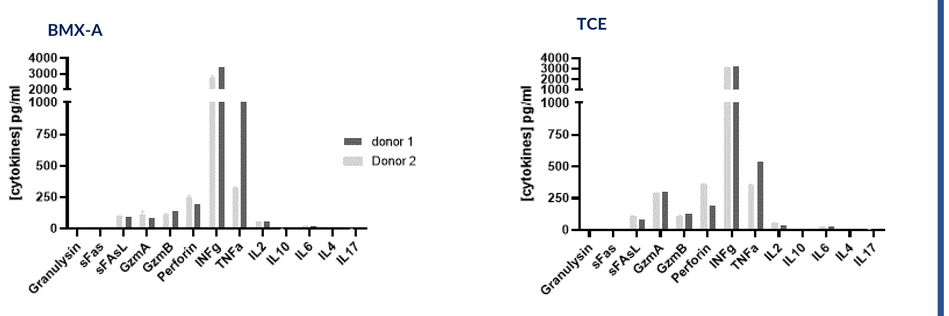
BiXAb® MAIT engager and TCE are equipotent



Freshly prepared CD8 population. OVCAR3 cancer cell line co-cultured with CD8s and BiXAb® / TCE for 18-48 hrs at E:T ratio of 2:1 . Cell lysis measured by Chromium release assay (data in triplicate). BMX-A and TCE a 1 µg/ml.

Upon engagement similar T-cell cytokine profile between MAIT engager and TCE

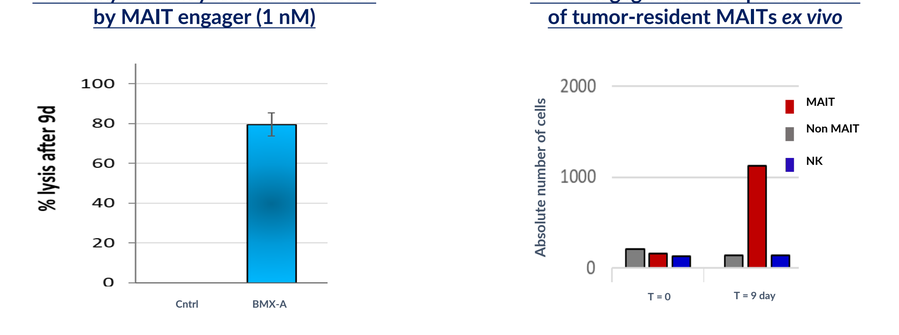
Quantitation of cytokines released by MAIT/CD8 redirection



Freshly prepared CD8 population. SKBR3 cancer cell line co-cultured with effector cells and MAIT engager or TCE at E:T ratio of 2:1 for 20 hrs. Data for 2 donors at 0.1 nM (data in triplicate). Cytokines measured by LegendPlex.

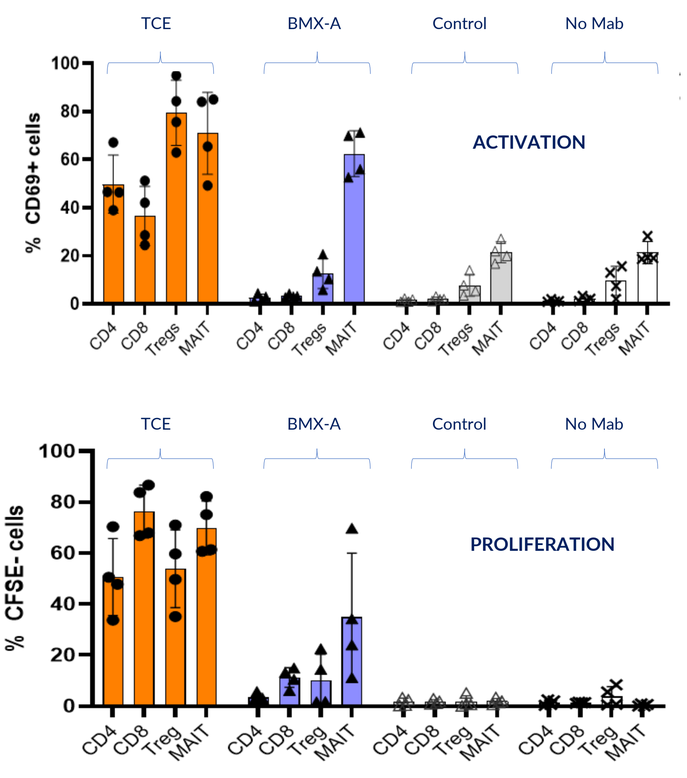
Minimal inflammatory cytokines are released from PBMCs by a MAIT engager compared to a classical TCE.

Quantitation of cytokines released by PBMCs redirection



Freshly prepared PBMCs population. OVCAR3 cancer cell line co-cultured with effector cells and BiXAb® s (1 nM) at E:T ratio of 5:1 for 43 hrs. Cytokines measured by LegendPlex Th kit (data in duplicate). Two donors shown. TCE= CD3 x HER2 drug

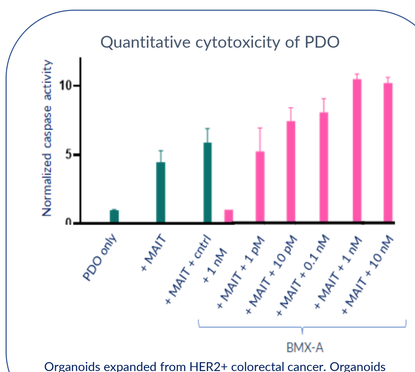
MAIT engager only activates MAITs, whereas, TCE activates all subsets



PBMCs redirected by T-cell engager at increased concentration, coculture with OVCAR3 cells at. E:T = 10:1, Activation (CD69 frequency at 24 hr) and Proliferative cells (CFSE- at 4 days) of conventional CD8 (CD8+CD161low), CD4+, Tregs (CD4+CD25+CD127-FoxP3+) and MAIT cells (CD8+CD161Hi) were analysed by flow cytometry,

MAIT engagers are active on 3D patient derived organoids (PDO)

HUB ORGANOIDS



Organoids expanded from HER2+ colorectal cancer. Organoids incubated with PBMCs and BMX-A for 5 days.

Top image: organoids
Bottom Image: Dying organoids

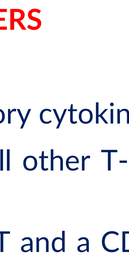
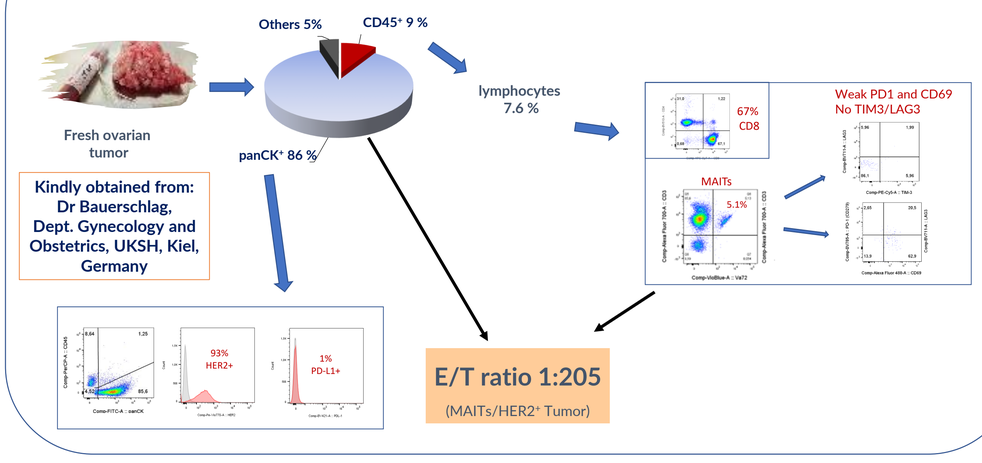


Image taken at 5X of sample incubated with 1 nM BMX-A

Tumor–resident MAITs in fresh human Ovarian cancer samples can be redirected to kill ovarian cancer cells by the MAIT engager *ex vivo*.

Ex vivo analysis on fresh ovarian cancer samples



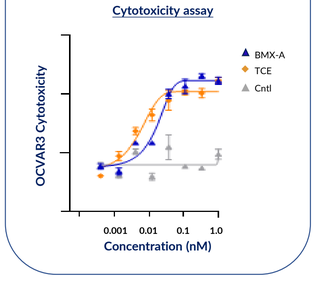
Ex vivo cytotoxicity of Ovarian cancer by MAIT engager (1 nM)

MAIT engager-induced proliferation of tumor-resident MAITs *ex vivo*

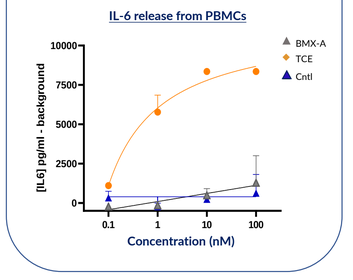
Fresh ovarian tumors were incubated with BiXAb MAIT engager for 9 days. Tumor cell cytotoxicity and immune cell proliferation was performed at the end of the experiment (data in triplicate).

Substantial therapeutic window available with a MAIT engager (whereas it is very narrow for a TCE)

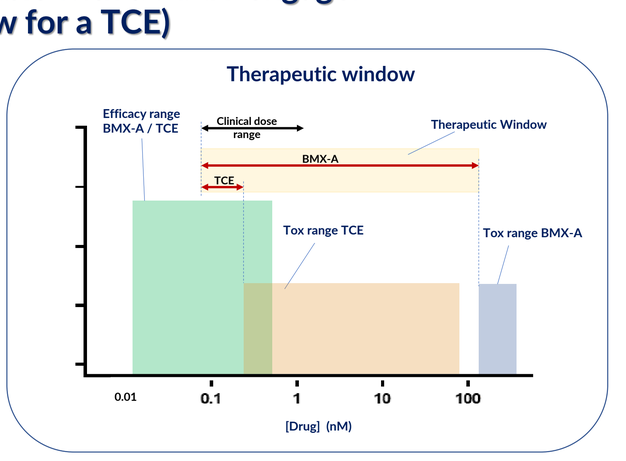
Identical potency



Huge difference in cytokine release



Therapeutic window



Summary

Biomunex Pharmaceuticals has developed a bispecific antibody platform (BiXAb®) that can effectively redirect MAIT cells to specifically kill cancer cells: The BiXAb® MAIT engager.

- MAIT cells are an abundant, potent, cytotoxic T-cell subset: **THE RIGHT NUMBERS**
- MAIT engagers are as potent as classical CD3 T-cell engagers: **THE POTENCY**
- MAIT engagers lead to efficient cancer **CYTOTOXICITY** and local proinflammatory cytokine release
- MAIT engagers ONLY activate MAIT cells; CD3 engager activate T-regs and all other T-cell subsets (Immune suppression in TME): **NO SUPPRESSION AND FREE TO KILL**
- Substantial difference in cytokine release from PBMC mixture between a MAIT and a CD3 engager: **THE SAFETY**
- MAIT engagers can redirect human tumor resident MAITs to eliminate cognate tumor in ex vivo setting: **THE EFFICACY**
- MAIT engagers permit the generation of an **EFFECTIVE THERAPEUTIC WINDOW** which will allow the successful treatment of solid tumors with an off-the-shelf engager approach.