Humanized monoclonal antibodies specific for CA IX protein are able to suppress hypoxia

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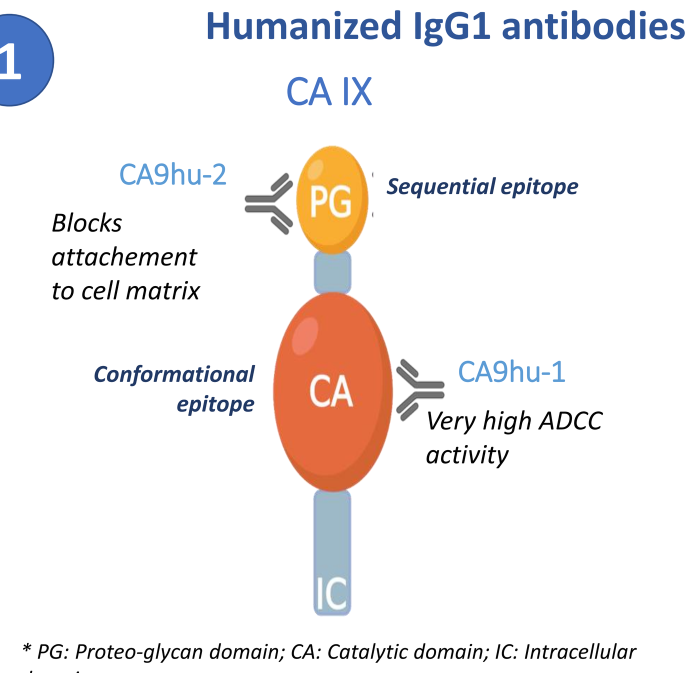
Carbonic anhydrase IX (CA IX), a highly active transmembrane metalloenzyme, represents one of the best markers of tumor hypoxia. The presence of CA IX in hypoxic tumors is associated with the aggressive tumor phenotype, metastatic tendency and poor response to conventional therapy. Expression of CA IX is principally driven by hypoxia through the HIF1 pathway, the extracellular part of CA IX can be cleaved and the soluble form of CA IX is detected in body fluids. Almost exclusive expression of CA IX in a wide spectrum of solid tumors and its transmembrane localization with extracellularly exposed catalytic region predestine CA IX as a highly promising target for therapeutic management of cancer patients.

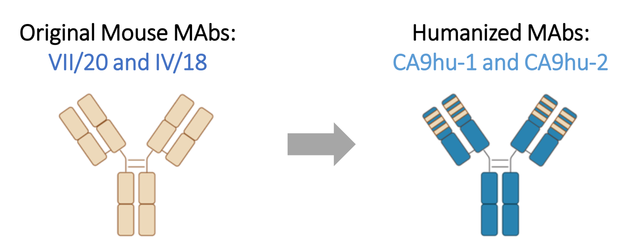
Mabpro developed unique new humanized antibodies specific to the CA IX protein, which could represent a new approach in the treatment of aggressive hypoxic tumors, where CA IX is highly expressed. Both humanized antibodies are of IgG1 isotype. CA9hu-1 antibody recognizes the catalytic domain of CA IX, and has the ability to internalize. The second antibody CA9hu-2 binds to the proteoglycan domain of CA IX and can affect the metastatic potential of cancer cells. Both antibodies inhibit the enzymatic activity of CA IX and the acidification of the extracellular environment, disturb the pH balance in the tumor, as a result of which tumor cells are unable to adapt to hypoxic stress. A unique feature of CA9hu-1 is the ability to activate antibody dependent cellular cytotoxicity (ADCC). In vivo experiments on nude mice showed uptake of humanized CA IX-specific antibodies into xenografts. These antibodies were detected in the xenograft even after 7 days from i.v. applications. CA IX-specific humanized antibodies recognize CA IX protein in hypoxic regions of spheroids with a naturally occurring gradient of oxygen and pH. Preclinical research on organoids derived from tumor tissue showed the activation of apoptotic and necrotic signalling after treatment of organoids with CA9hu-1 in the presence of autologous immune cells and activation of ADCC.

A characteristic manifestation of the action of our CA IX-specific humanized antibodies is also a decrease of oxygen consumption in CA IX-expressing cells treated with CA9hu-1. Furthermore, humanized antibodies are able to affect the hypoxic machinery and suppress hypoxia. Our preclinical results show that CA IX-specific humanized antibodies have a high therapeutic potential and could represent an effective therapeutic approach in monotherapy or in combination with other therapeutics in the treatment of hypoxic aggressive tumors.

1 Humanized IgG1 antibodies CA9hu-1 and CA9hu-2 specific for CA IX protein

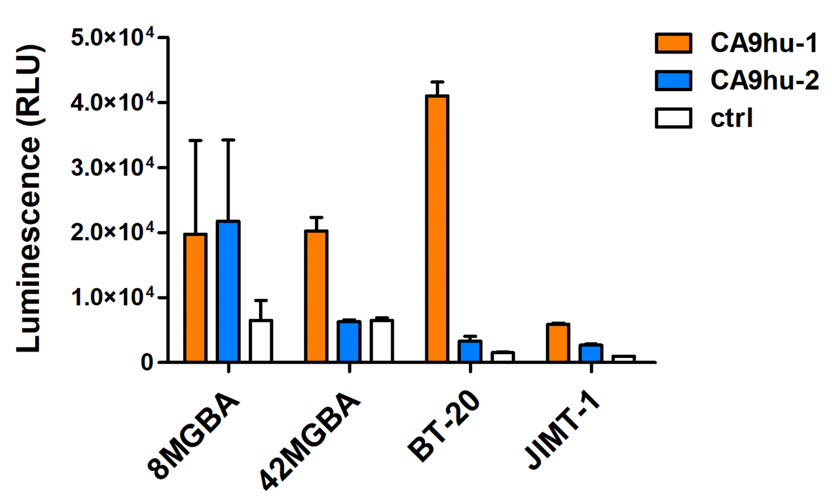


* PG: Proteo-glycan domain; CA: Catalytic domain; IC: Intracellular domain

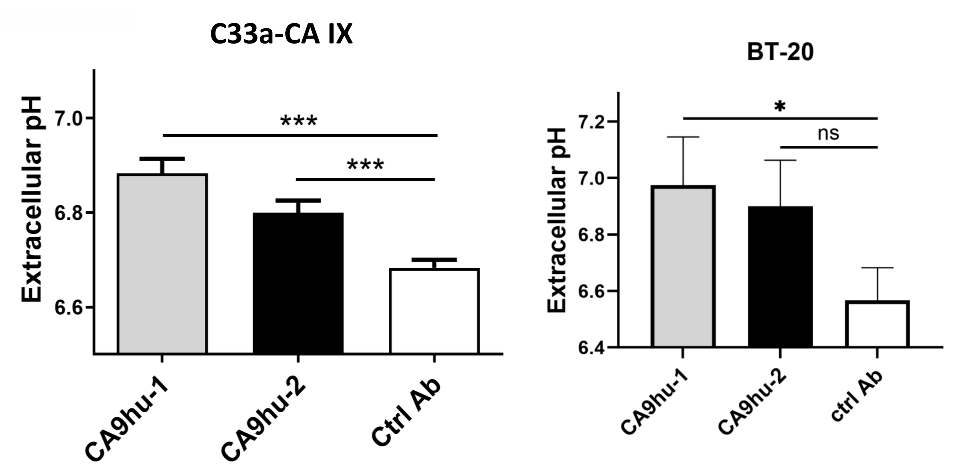


- Mouse monoclonal antibodies IV/18 and VII/20 specific to different CA IX domains were used as a platform for successful development of humanized anti-CA IX therapeutic antibodies
- Based on mouse MAb IV/18 and VII/20 sequences a panel of twenty-five full length humanized antibodies was designed and constructed, using human frameworks – The Pharma Package
- The variants were screened for MHC Class II binding epitopes, Fv glycosylation motifs and deamidation motifs
- Each of the VH domains was synthesized in-frame with a human IgG1 isotype constant domain sequence

2 Humanized antibodies CA9hu-1 and CA9hu-2 retain the ability to activate ADCC pathway and inhibit acidification of the extracellular microenvironment

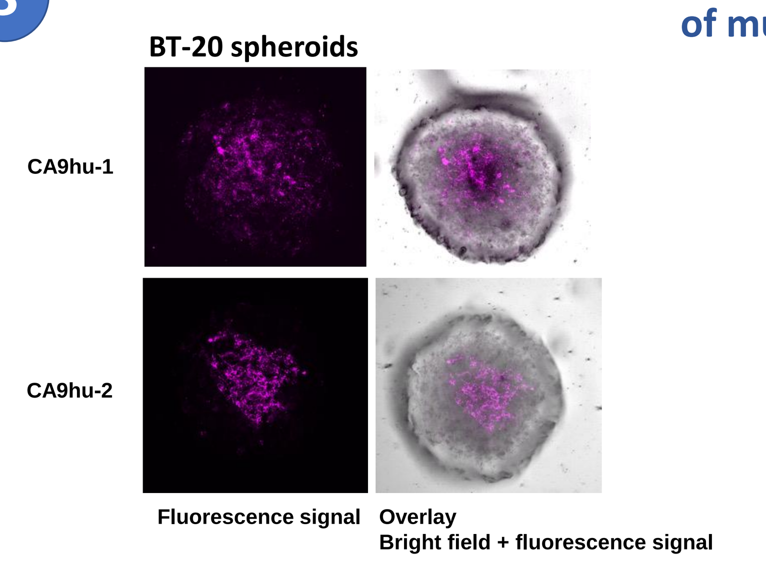


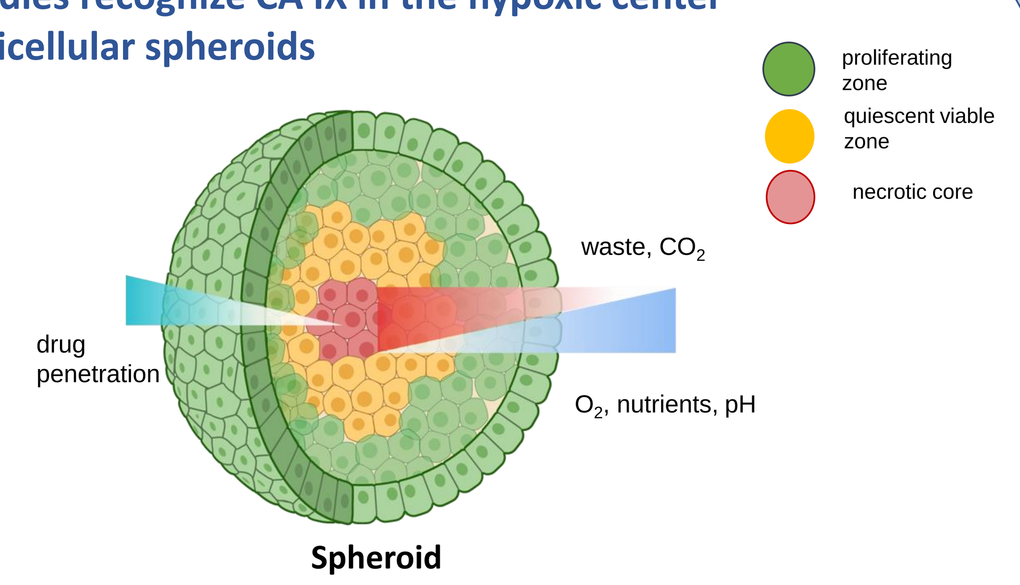
To prove antibody-dependent cell-mediated cytotoxicity (ADCC) of humanized antibodies on cancer cells naturally expressing CA IX, we analysed breast cancer cell lines BT-20 and JIMT-1 as well as glioblastoma cell lines 8-MG-BA and 42-MG-BA.



Humanized antibodies inhibit acidification of the extracellular environment (culture medium) under hypoxia in cells overexpressing CA IX protein (C33a-CA IX) or in cells with hypoxia-induced expression of CA IX (BT-20).

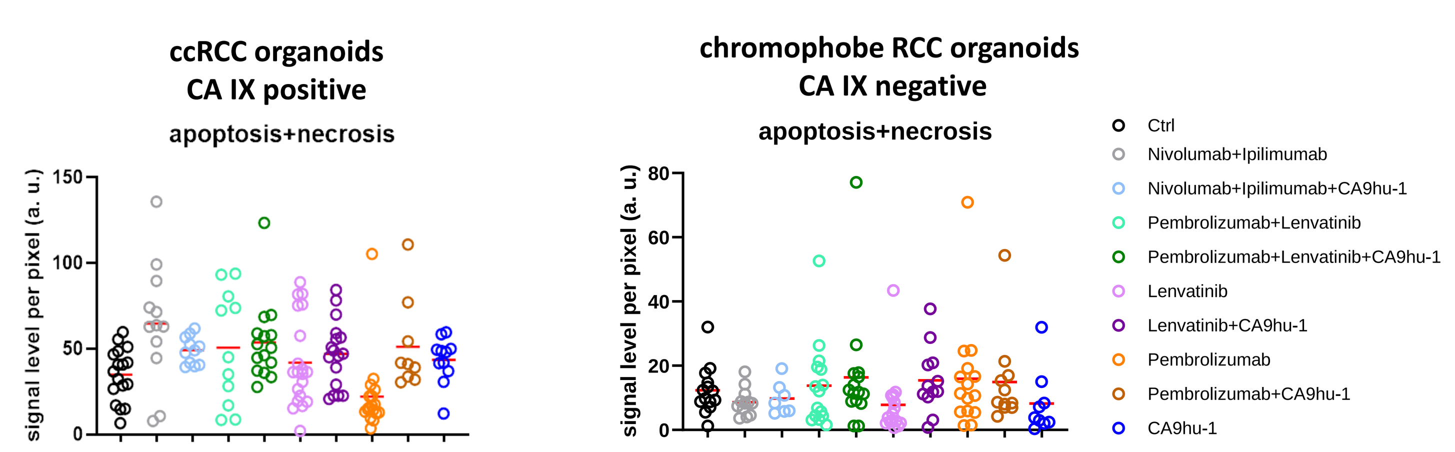
3 Humanized monoclonal antibodies recognize CA IX in the hypoxic center of multicellular spheroids





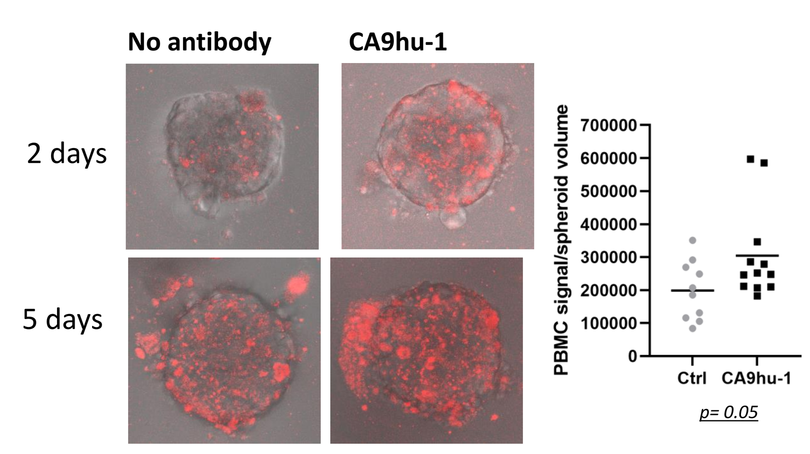
Uptake of humanized antibodies into live BT-20 spheroids with naturally formed gradient of O_2 and pH

4 CA9hu-1 antibody activates cell cytotoxicity in CA IX-positive RCC organoids

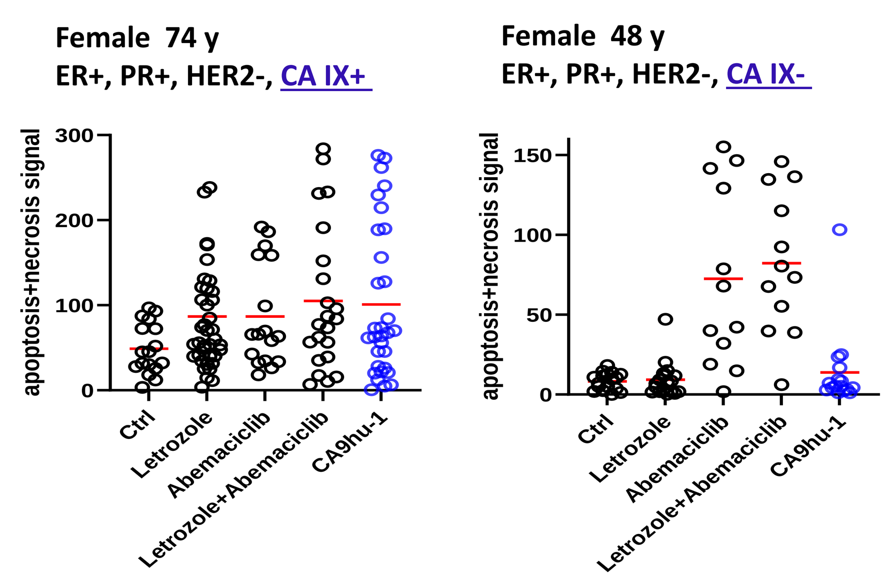


Comparison of the effect of humanized antibody CA9hu-1 with standard of care therapeutics in CA IX-positive and CA IX-negative RCC organoids. RCC organoids cocultured with autologous PBMCs were treated with CA9hu-1 and other therapeutics used in the treatment of RCC. In ccRCC organoids with strong CA IX expression, CA9hu-1 antibody activated apoptosis and necrosis comparable to other therapeutics.

5 CA9hu-1 antibody increases penetration of autologous PBMC into tumor organoids and activates cell cytotoxicity in CA IX-positive tumor organoids

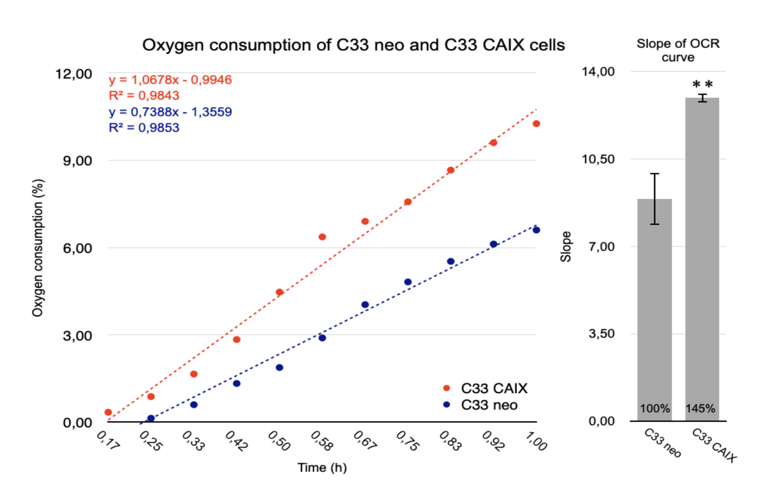


CA IX-positive breast cancer organoids cocultured with autologous PBMCs (red) were treated with CA9hu-1 antibody. CA9hu-1 increases penetration of PBMC into tumor organoids.

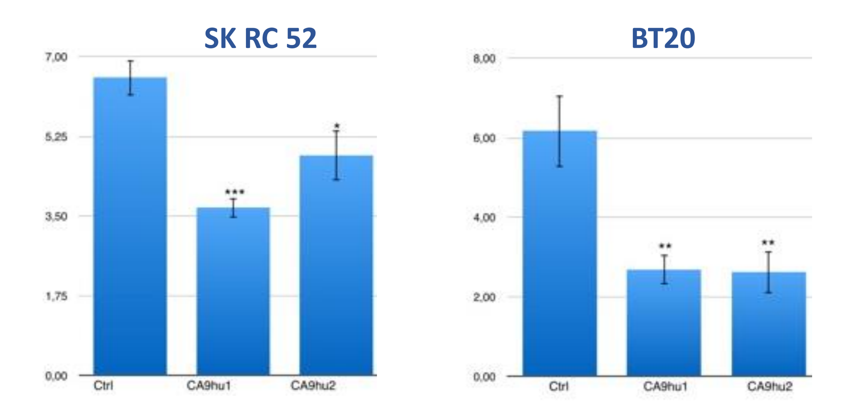


Comparison of cytotoxic effect of CA9hu-1 antibody in CA IX positive vs CA IX negative breast cancer organoids. Breast cancer organoids recapitulate the molecular characteristics of patients' tumor tissue.

6 Humanized antibodies CA9hu-1 and CA9hu-2 reduce oxygen consumption in cells expressing CA IX

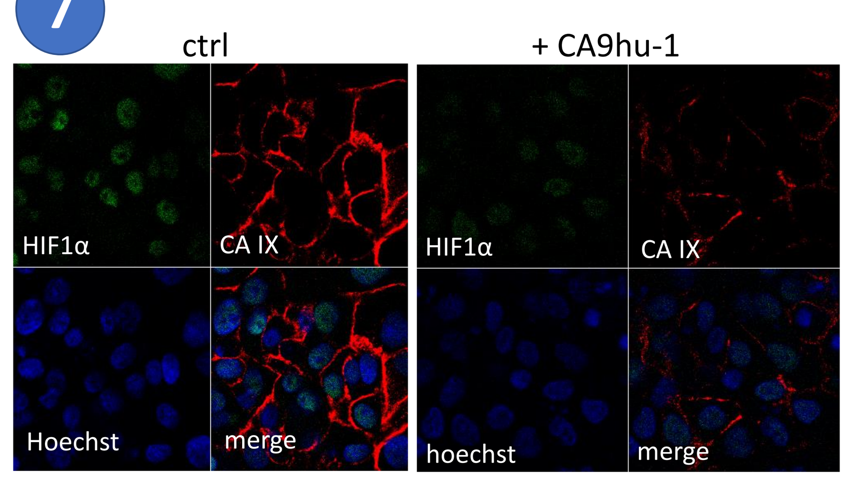


Oxygen consumption is higher in CA IX overexpressing cells compared to CA IX negative C33a cells.

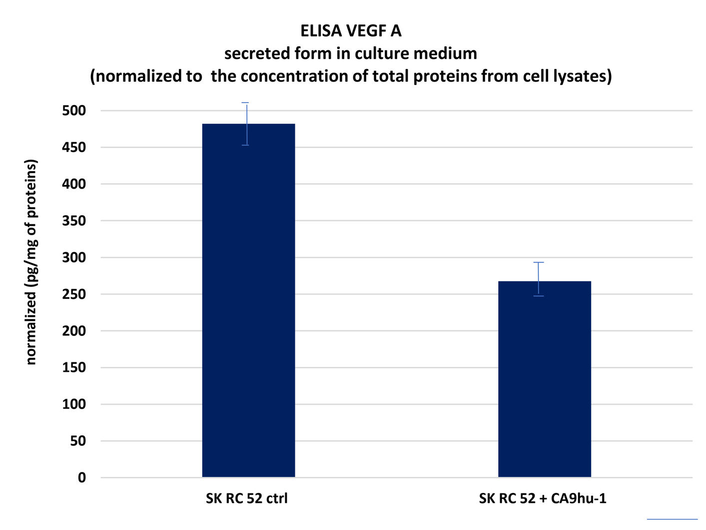


In cells (SK RC 52, BT20) naturally expressing CA IX, oxygen consumption decreases due to the action of humanized antibodies CA9hu-1 and CA9hu-2, which may have a direct impact on the effectiveness of radiotherapy

7 Humanized antibody CA9hu-1 supresses hypoxic signaling



Immunofluorescence of HIF1α and CA IX after treatment of SK RC 52 cells with CA9hu-1 for 48 h in hypoxia (1%O_2)



CA9hu-1 antibody suppresses hypoxia by reducing the level of HIF1α and HIF1-target proteins CA IX, VEGF

Conclusions

CA IX specific humanized antibodies

- recognize hypoxia-induced protein CA IX on the surface of tumor cells
- reduce the adaptation of tumor cells to microenvironmental stress by inhibiting the acidification of the extracellular environment
- reduce oxygen consumption in CA IX-positive cells
- increase penetration of autologous PBMC into tumor organoids
- activate cell cytotoxicity in CA IX-positive tumor organoids
- suppress hypoxia by reducing the level of HIF1α and HIF1 target proteins CA IX, VEGF