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AUTHOR'S VIEW

Resistance is futile: Targeting the inhibitory FcγRIIB (CD32B) to maximize immunotherapy

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ABSTRACT
Monoclonal antibodies (mAb) are central to the treatment of several types of malignancy. However, these reagents are subject to particular types of resistance. Several resistance mechanisms are regulated by the inhibitory FcγRIIB. We recently developed mAbs to block FcγRIIB and provided *in vivo* proof-of-concept for their ability to overcome FcγRIIB-mediated resistance.

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Introduction

It has long been appreciated that the inhibitory Fc gamma receptor (FcγR) IIB, expressed by numerous cells of the immune system, negatively regulates both innate and adaptive immunity through engagement of immune complexes (IC).[1] Similarly, the knowledge that FcγRIIB negatively regulates mAb-mediated immunotherapy has been known for over a decade. As such, FcγRIIB-deficient mice are able to clear tumors more effectively than WT mice when treated with therapeutic mAbs, indicating that FcγRIIB expression on effector cells (i.e., macrophages and monocytes) leads to suppression of their phagocytic and cytotoxic potential *in vivo*.[2] Moreover, FcγRIIB regulates the antigen-presenting potential of dendritic cells (DC) and FcγRIIB^{-ve} DCs have an improved capacity to activate naive T cells.[3] In addition, it has recently been demonstrated that high expression of FcγRIIB on target tumor cells may also be detrimental to targeted mAb therapy.[4-8] Accordingly, on malignant B cells, such as chronic lymphocytic leukemia (CLL) and mantle cell lymphoma (MCL), the Fc portion of the direct targeting mAb (e.g., rituximab or alemtuzumab), is engaged by FcγRIIB and accelerates mAb internalization from the tumor surface.[4,5,7] As a result of internalization, the Fc-dependent effector-functions of antibody-dependent cellular cytotoxicity (ADCC) and phagocytosis (ADCP) are severely hampered,[4,6,7] reducing therapeutic efficacy in mouse models.[9]

Recently, we tested this hypothesis in humans by performing a retrospective study using tissues from follicular lymphoma (FL) patients treated with rituximab monotherapy and demonstrated that FL patients who expressed medium/high levels of FcγRIIB responded less effectively to rituximab compared to patients with negative/low FcγRIIB levels.[8] These clinical observations add weight to the recent experimental discoveries highlighting the potential therapeutic importance of targeting FcγRIIB in lymphoid malignancies.[4,5,7,8]

To exploit these observations, we used a human phage-display library n-CoDeR®, to identify a panel of highly specific, fully human, FcγRIIB mAbs. Subsequent *in vitro* characterization enabled us to select antagonistic mAbs, capable of blocking IC binding and ligating FcγRIIB without activating it.[10] We tested them in a variety of relevant *in vitro* assays, helping us to identify a lead clinical candidate clone 6G11 (BI-1206). Further *in vitro* and *in vivo* assays alone and in combination with clinically relevant therapeutic mAbs (e.g., rituximab) demonstrated its ability to augment immunotherapy. Of note, we confirmed previous observations that human FcγRIIB does not rapidly internalize from the surface of malignant B cells once ligated, making it a promising target for mAb therapy. Importantly, 6G11 was demonstrated to be safe in human FcγRIIB transgenic (Tg) mice and failed to induce any cytokine storm *in vitro*. Moreover, when combined with rituximab, 6G11 significantly enhanced the depletion of circulatory B cells in a novel human CD20 x FcγRIIB Tg mouse.[10] Furthermore, using a primary CLL patient-derived xenograft (PDX) mouse model, we demonstrated the beneficial effects of combining FcγRIIB mAb with other clinically approved mAbs, including rituximab, obinituzumab and alemtuzumab.[10] Encouragingly, when using CLL samples in the PDX model that were previously defined as refractory to mAb treatment, rituximab alone failed to significantly

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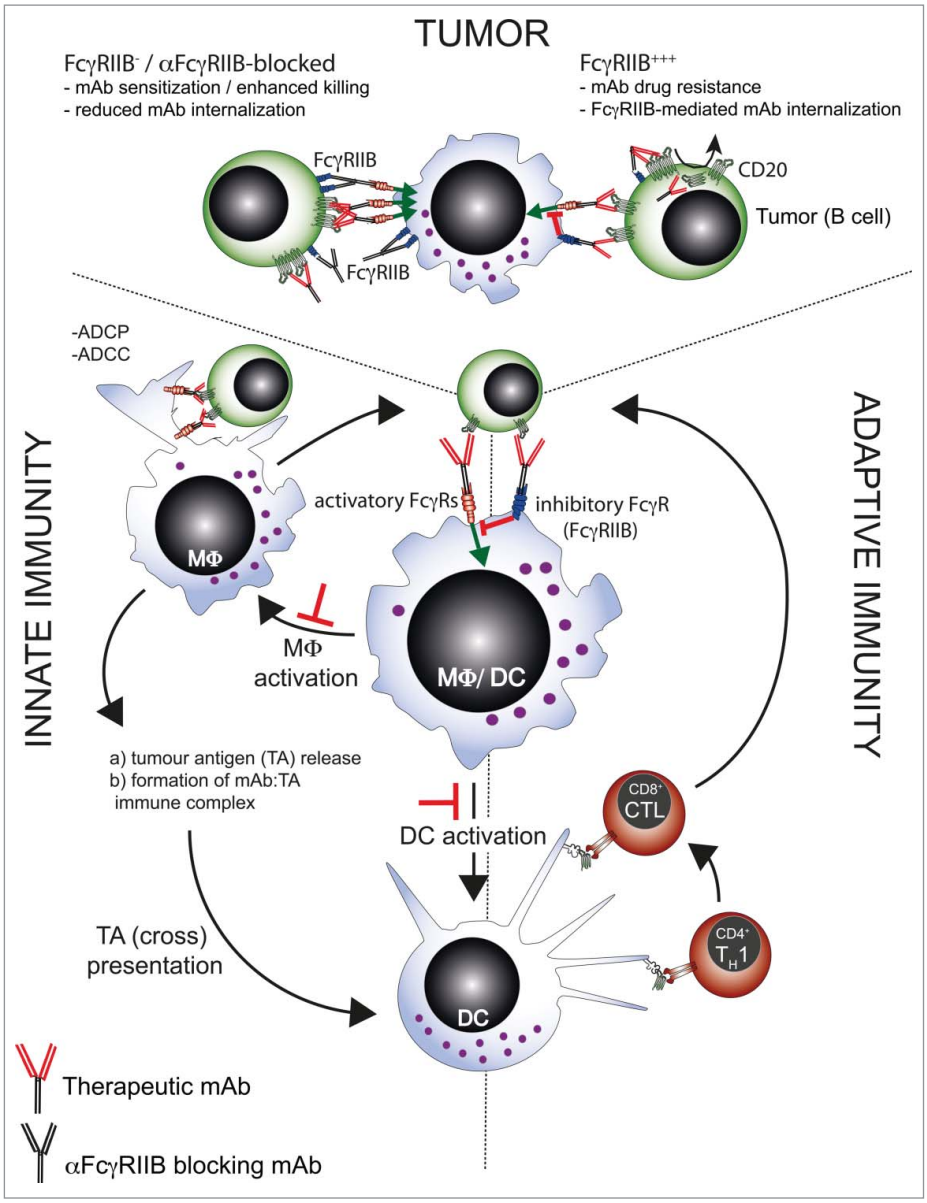

Figure 1. Schematic diagram demonstrating how FcγRIIB can regulate innate and adaptive immunity to influence immunotherapy. FcγRIIB can regulate mAb-mediated immunotherapy at multiple points indicated by the red ⊥ symbol. Within the tumor, FcγRIIB can accelerate the internalization of direct targeting mAbs such as rituximab, leading to drug resistance. FcγRIIB can also transmit negative signals to the innate immune cells such as macrophages and dendritic cells, reducing tumor destruction, antigen release and uptake, presentation and activation of adaptive immunity. Targeting of FcγRIIB using anti-FcγRIIB mAbs may therefore potentially intersect at several points o boost mAb-mediated immunotherapy.

deplete xenografted CLL cells, whereas 6G11 and rituximab combination therapy strongly enhanced their depletion.[10]

In conclusion, our data indicate that human FcγRIIB may offer a safe and promising target for potentiating the activity of therapeutic mAbs currently administered in the clinic (For a schematic of potential mechanism of action see Figure 1). As a result of these pre-clinical data, we aim to test the safety and efficacy of 6G11 in a first-in-human clinical trial in combination with rituximab in non-Hodgkin's lymphoma patients. Additionally, we believe that blocking FcγRIIB on effector cells with an antagonistic mAb will lower their activation threshold, enhancing their cytotoxic potential, as well as potentially augmenting DC activation and cross-priming,[3] Figure 1 perhaps suggesting a broader application for antagonistic FcγRIIB mAbs in the clinic, for example, FcγRIIB^{-ve} malignancies.

Disclosure of potential conflicts of interest

A.R. has received institutional support from BioInvent. M.S.C. acts as a consultant to BioInvent and has received institutional support from BioInvent, Roche and GSK for grants and patents. B.F. is a full-time employee of BioInvent International.

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