CD24 a Potential Immunotherapeutic Target for Mantle-Cell Lymphoma

In the past decade, immune checkpoint inhibitors (ICIs) that re-activate adaptive immunity have transformed the treatment paradigm in various cancer types. More recently, ICIs on innate immune cells have also gained prominence as therapeutic targets, being CD47 the hallmark ICI in the clinic. Lately, CD24 was also described as an innate immune checkpoint with apparent significance in several solid cancer types. In this entry, the role of CD24 as a therapeutic target, with a particular focus on mantle cell lymphoma (MCL) and diffuse-large B cell lymphoma (DLBCL) was discussed.

1. Introduction

CD24 is a small, heavily glycosylated protein attached to the cell membrane by a glycosyl-phosphatidylinositol (GPI) anchor. It has been extensively studied in the context of cancer biology, with it being defined as a cancer stem cell marker in various malignancies, such as breast[1], pancreas[2], and ovarian carcinoma[3]. CD24-based cancer immunotherapy was originally reported decades ago for a subgroup of patients with B-lymphoproliferative disorders [4]. More recently, in a hallmark 2019 report, CD24 was also described as an innate immune checkpoint with apparent significance in several solid cancer types[5]. Specifically, CD24 checkpoint activity relayed anti-phagocytic signals to phagocytes through its interaction with Siglec-10, a lectin expressed on tumor-associated macrophages (TAMs). Accordingly, CD24 blockade using a monoclonal antibody (mAb) induced macrophage-mediated phagocytosis of breast, ovarian, and pancreas cell lines *in vitro* and inhibited tumor growth of xenografted breast cancer cell line in an NSG mouse model [5].

On other hand, CD24 is also reportedly expressed in various hematological malignancies, including non-Hodgkin B-cell lymphomas (NHLs)[6]. Thus, CD24 may be also an immune checkpoint relevant for B cell-NHL. Of note, certain types of NHLs such as mantle cell lymphoma (MCL) or follicular lymphoma (FL), retained CD24 expression in contrast to healthy counterparts [7], with MCL being a more aggressive lymphoma that comprises about 3% to 10% of total NHL cases [8] and not curable with conventional chemoimmunotherapy [9].

2. CD24 as a Potential Immunotherapeutic Target for Mantle Cell Lymphoma

The potential of CD24 mAb treatment and checkpoint targeting was previously preclinically investigated for pancreas, ovarian, and breast carcinoma[5]. In this study, the researchers focused on NHLs where CD24 mRNA expression was similarly high in follicular lymphoma (FL), burkitt lymphoma (BL), MCL, and DLBCL in line with previous studies[6][10]. At protein level, MCL showed the highest CD24 expression, the reason why we decided to focus our work in MCL. Specifically, antibody-mediated targeting of CD24 robustly enhanced the phagocytic uptake of MCL cells yielding over 90% removal of CD24 expressing MCL cancer cells. Phagocytic uptake upon CD24 mAb treatment was significantly greater than upon treatment with the CD47 antibody InhibRx, both in cell lines and primary patient-derived blasts in an autologous setting. In line with this, high expression of CD24, but not CD47, correlated with poor OS in MCL and FL, whereas the opposite was found for DLBCL. The robust increase in phagocytosis upon CD24 mAb treatment was not limited to MCL and was also detected in a panel of carcinoma cell lines expressing CD24. Reversely, only low levels of phagocytosis were observed upon treatment with CD24 mAb in DLBCL, with the CD47 antibody InhibRx having superior effects. Moreover, MCL blasts expressed more CD24 than B cells from healthy donors. Apart from B cells, CD24 is also highly expressed in almost all human tissues (ATLAS database) and other hematological cells [11]. Nevertheless, phagocytosis of healthy PBMCs by autologous macrophages did not increase upon treatment with CD24 mAbs in our experiments. Still, off-target effects of CD24 mAb treatment must be carefully considered in the design of CD24-based immunotherapy. These results highlight the potential of targeting CD24 in MCL, which remains incurable with conventional chemoimmunotherapy [12].

After showing the potential of targeting CD24 for the treatment of MCL, the mechanism behind these mAb-mediated phagocytosis was further investigated. Two different murine antibody clones (SN3 IgG1 and ML5 IgG2a) potently induced phagocytosis of MCL cells, so CD24/Siglec-10 checkpoint activity could exist in MCL similar to previously showed in carcinoma. In the original report on SN3 in carcinoma, its potential checkpoint activity was not evaluated using an F(ab')$_2$ preparation, thus raising the question of whether the observed activity of this antibody is due to checkpoint inhibitor activity or Fc-mediated phagocytosis. The phagocytic activity of both clones was abrogated when an F(ab')$_2$ preparation was used, suggesting that these antibodies triggered antibody-dependent cellular phagocytosis (ADCP) as the main mechanism of action. However, treatment with the complete SN3 mAb in the presence of high concentrations of Fc blocker solution still yielded high levels of phagocytic uptake. Similarly, alternative blocking of the Fc domain of clone SN3, with a goat anti-mouse F(ab')$_2$, also did not negatively impact phagocytic activity. With both approaches, the pro-phagocytic activity of clone ML5 was abrogated. Thus, in these assays, SN3 appeared to have Fc-independent activity. Thus, both proofs to support and disprove checkpoint inhibitor activity of antibody SN3 and/or Siglec-10-CD24 interaction were uncovered, highlighting the need for further investigation into the underlying mechanism (e.g., by constructing a human IgG4 or IgG2 variant).

Highlights of the study

1. CD24 Is Expressed in Several B-Cell Lymphomas, Being Most Highly Expressed in MCL, Where It Correlates with Poor Prognosis in Contrast to Hallmark Immune Checkpoint CD47.

2. CD24 Is a Target for Reactivation of Phagocytosis in MCL, with a Superior Effect Than CD47 Antibody Treatment in both cell lines and

3. CD24 mAb Treatment Increased Phagocytosis of Primary MCL Blasts by Autologous Macrophages and PMNs, but Did Not Induce High Level of Phagocytosis of Healthy Cells.

4. CD24 Antibody-Mediated Phagocytosis Is Superior to CD47 Checkpoint Targeting in MCL and Carcinoma, but Not in DLBCL.

5. Induction of Phagocytosis of CD24 Expressing Cells Is Only in Part an Effect of Breaking CD24-Siglec-10 'Don't Eat Me' Signaling.

3. Conclusions

CD24 mAb treatment in vitro enhanced phagocytic removal of CD24-positive MCL cell lines and primary autologous MCL blasts. This effect of CD24 mAb treatment was more potent than treatment with CD47 InhibRx mAb in MCL, but not in DLBCL. In agreement with this, high CD24 expression correlated with reduced OS in MCL (and FL) but not DLBCL. Thus, CD24 mAb treatment may represent an alternative therapeutic approach for MCL, although the relative importance of the CD24/Siglec-10 interaction as a "don't eat me" signal remains to be elucidated.

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