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# Blocking the B7-H4 pathway with novel recombinant antibodies enhances T cell-mediated antitumor responses

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**B7-H4 inhibits T-cell activation and is widely expressed by solid neoplasms. We have recently demonstrated that the expression of B7-H4 on the surface of malignant cells in vivo is inducible, and that novel anti-B7-H4 recombinant antibodies can reverse the inhibition of tumor-specific T cells. Thus, antibodies targeting the B7-H4 pathways may extend the survival of cancer patients by restoring T cell-mediated antitumor responses.**

**Keywords:** ovarian cancer, B7-H4, recombinant antibodies, cell surface expression, targeting, immunotherapy

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Ovarian neoplasms stimulate endogenous immune responses that can result in tumor rejection or progression, depending on which subset of immune cells becomes prevalent within the tumor microenvironment. Indeed, while tumor-infiltrating $CD8^+$ effector T cells (Teffs) are associated with favorable disease outcome,[1] other cell subsets such as regulatory T cells (Tregs) and tumor-associated macrophages (TAMs) promote disease progression via multiple mechanisms, including the secretion of immunosuppressive molecules such as interleukin-10 (IL-10) and indoleamine 2,3-deoxygenase (IDO), as well as the expression of inhibitory molecules such as cytotoxic T lymphocyte-associated protein 4 (CTLA4), programmed cell death 1 (PDCD1, best known as PD-1), and V-set domain containing T cell activation inhibitor 1 (VTCN1, best known as B7-H4).[2] The tumor microenvironment can therefore support immune escape by orchestrating a complex network of immunosuppressive cells and mediators that interfere with the generation and clonal expansion of antitumor Teffs.

The clinical relevance of strategies for the inhibition of B7-H4 in patients affected by malignant conditions or autoimmune diseases has previously been established. The overexpression of B7-H4 correlates indeed with advanced disease stage and poor prognosis in cancer patients,[3,4] as well as with increased tumorigenicity and invasiveness in cancer cells.[5] B7-H4 exists in a soluble variant and as a transmembrane protein expressed on the surface of antigen-presenting cells (APCs). Upon interaction with a hitherto unknown ligand (which we refer to as B7-H4L*),[6] B7-H4 inhibits the activation and proliferation of antigen-specific Teffs (**Fig. 1**). These properties confer a profound translational value to B7-H4 in the context of anticancer immunotherapy. However, until recently B7-H4 was thought to be localized mainly in the cytoplasm of ovarian cancer cells,[6] which would be incompatible with the use of B7-H4-specific monoclonal antibodies.

We investigated the expression of B7-H4 on the surface of established ovarian cancer cell lines and primary ovarian carcinoma cells obtained from patient ascites and solid neoplastic lesions.[7] As expected, B7-H4 was poorly expressed on the surface of ovarian cancer cell lines. Conversely, B7-H4 was detected in significant amounts on the surface of primary malignant cells from ovarian cancer patients. To further explore the expression pattern of B7-H4 in vivo, we inoculated mice with an ovarian cancer cell line that does not express B7-H4 on its surface, but contains cytoplasmic levels of B7-H4 that are detectable by immunoblotting.

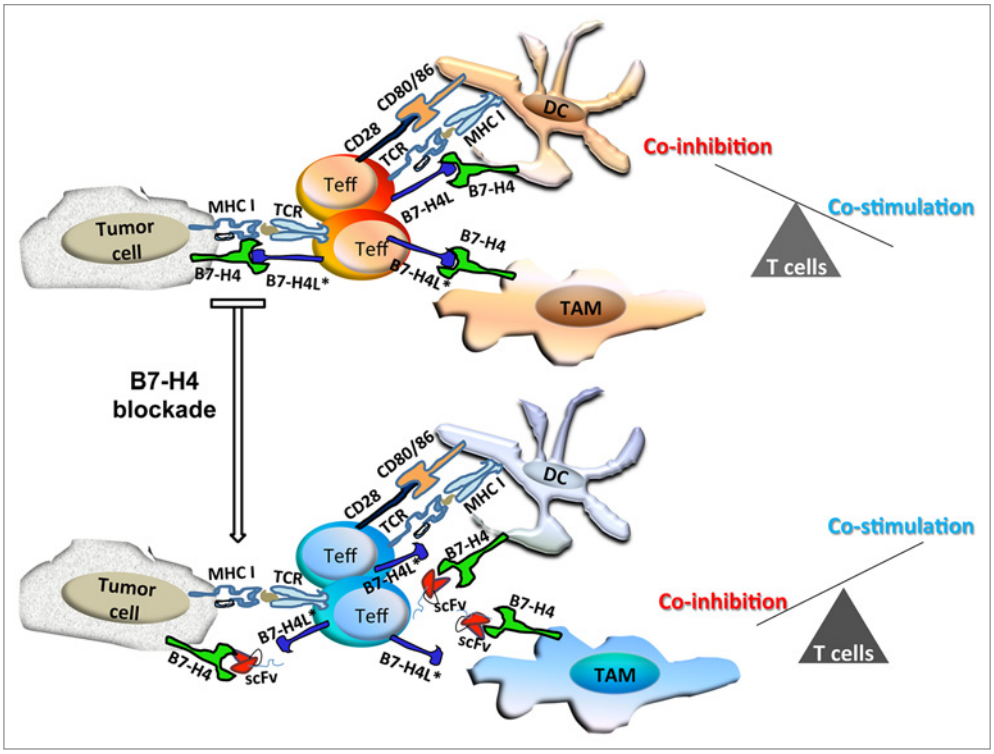


**Figure 1.** Blocking B7-H4 in the tumor microenvironment with specific antibodies potentiates antitumor immune responses. T cells recognize antigens complexed with MHC class I (MHCI) molecules on the surface of antigen-presenting cells (APCs) and some cancer cells, through the T-cell receptor (TCR). The binding of B7-H4 expressed by malignant cells, APCs and tumor-associated macrophages (TAMs) to a putative ligand (B7-H4L*) on the surface of T cells significantly impairs the activation of the latter within the tumor microenvironment. The simultaneous blockade of B7-H4 on various cellular components of the tumor mass, as obtained with specific monoclonal antibodies, can revert T-cell inhibition and hence favor the elicitation of T cell-mediated antitumor responses.

The expression of B7-H4 on the surface of these cells increased upon an in vivo passage, but was rapidly downregulated when harvested cells were placed in culture. These data are consistent with previous findings by Chen and colleagues, demonstrating that TAM-derived factors can promote the expression of B7-H4 on the surface of Lewis Lung carcinoma (LLC) cells in vivo.[8] Zhang and colleagues also demonstrated that the levels of B7-H4 at the plasma membrane, but not within the nucleus or the cytoplasm, inversely correlate with the amount of lymphocytes infiltrating renal cell cancer (RCC) lesions, providing a direct link between the expression of B7-H4 on the surface of malignant cells and Teff inhibition.[9] Altogether, these results indicate that the amounts of B7-H4 on the surface of cancer cells increase in response to microenvironmental cues and may promote immune evasion. The expression of B7-H4 at the surface of both malignant and tumor-infiltrating immunosuppressive cells establishes a rationale for the development of therapeutic approaches based on the targeting of B7-H4.

Recombinant single-chain variable fragments (scFvs) allow for the targeting of selected cell populations both as naked molecules and upon conjugation to endotoxins, nanoparticles, radioisotopes, or protein domains. Moreover, scFvs can be fused to T cell-relevant signaling domains to generate so-called chimeric antigen receptors (CARs), molecules that provide T cells with the ability to respond to antigens in the absence of MHC presentation. Combining protein- and cell-based screening approaches, we isolated scFvs specific for human B7-H4 from a novel yeast display scFv library that had been created from B cells found in the ascites and peripheral blood mononuclear cells (PBMCs) of ovarian cancer patients. In particular, scFvs were selected based on their affinity for soluble and cell-surface B7-H4, as well as on their ability to reverse T-cell inhibition. We generated in vitro model systems in which B7-H4 molecules expressed either in cis on APCs or cancer cells, or in trans on tumor-polarized macrophages (third party inhibition), could inhibit the activation of tumor-specific T cells. Using these systems, we demonstrated that an anti-B7-H4 scFv (called 3#68) could fully reverse T-cell inhibition as mediated by recombinant B7-H4, by peptide-pulsed B7-H4$^{+}$ APCs, by tumor-polarized B7-H4$^{+}$ macrophages admixed with B7-H4$^{-}$ cancer cells, as well as by B7-H4-transduced malignant cells. Another anti-B7-H4 scFv, namely 3#54, exerted a quantitatively reduced but qualitatively similar activity. Consistently, 3#68 was more efficient than 3#54 in delaying tumor growth in a humanized mouse model of ovarian cancer. These results not only confirm that B7-H4 exerts potent immunosuppressive effects as it inhibits T cell-mediated antitumor responses, but also support the use of 3#68 for blocking B7-H4 within the tumor microenvironment and hence

favoring the (re)activation of tumor-specific T cells (**Fig. 1**).

A major obstacle against the development of effective anticancer immunotherapy stems from the inadequate stimulation of tumor-specific Teffs, at least in part reflecting the lack of T-cell co-stimulation within the tumor microenvironment. Consistent with this view, the simultaneous targeting of multiple immunological checkpoints such as those mediated by CTLA4 and PD-1 has recently yielded impressive clinical results among melanoma patients.[10] Monitoring the inhibition of B7-H4 signaling in vivo together with that of other immunosuppressive pathways might improve our understanding of the mechanisms that underlie tumor-dependent T-cell inhibition. We propose that combining the targeting of B7-H4 with other immunotherapeutic strategies, including adoptive T-cell transfer, could functionally modulate diverse cellular components of the tumor microenvironment and hence potentiate T cell-mediated antitumor immune responses.

### Disclosure of Potential Conflicts of Interest

The authors have no potential conflicts of interest to disclose.

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