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CCR Translations

Clinical Cancer Research

A Tumor-Localized Approach to Bypass Anti-4-1BB Immuno-Toxicity

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4-1BB (CD137) is an important costimulatory molecule upregulated on antigen-experienced T cells, however, clinical development of 4-1BB agonists has stalled because of significant liver immuno-toxicity. Using rational protein engineering, a next-generation anticalin-antibody–based therapy achieved localized 4-1BB activation triggered by tumor-expressed antigen, helping to revitalize this pathway in immuno-oncology.

See related article by Hinner et al., p. 5878

In this issue of *Clinical Cancer Research*, Hinner and colleagues (1) reported an original design of a 4-1BB (CD137)/HER2 bispecific anticalin–antibody fusion protein. The preclinical data showed 4-1BB clustering and receptor activation in a HER2 tumor antigen-dependent manner, as well as a potentially favorable profile without obvious signs of liver toxicity.

Compared with anti-PD-1/PD-L1 antibodies that repair or normalize antitumor immunity, agonistic antibodies that target costimulatory pathways such as 4-1BB aim to boost adaptive immunity and have long been considered as one of the most promising immunotherapies. As a member of the TNF receptor super-family, 4-1BB plays a well-established role in promoting the expansion, effector function, and survival of antigen-experienced T cells, especially cytotoxic CD8 T cells (2). Anti-4-1BB mAb delivered systemically is capable of eradicating both immunogenic and nonimmunogenic tumors, as well as in anti-PD-L1, nonresponsive tumors in animal models. However, despite modest efficacy, the first-in-human clinical trials evaluating an anti-4-1BB antibody, Bristol-Myers Squibb's urelumab, exhibited treatment-related liver toxicity, resulting in swift termination of the trial (2). Subsequent anti-4-1BB trials were conducted using either reduced dosing of urelumab, or less potent agonistic antibodies (e.g., Pfizer's utomilumab), which showed improved safety but less promising activity (2).

It remains a major challenge in anti-4-1BB clinical development to decouple antitumor immunity from liver toxicity. Dissection of these pathways has proven difficult, as the cause of toxicity was shown to be CD8 and IFNγ dependent (3), which also play key roles for the proposed efficacy. Nevertheless, the task is not impossible. Liver toxicity is believed to be primarily mediated by liver macrophages activated by nonspecific hepatic memory CD8 T cells triggered by anti-4-1BB (3). Recent work has identified S100A4 as a soluble factor secreted by liver macrophages and plays critical roles in this process (4). Biologics such as anti-S100A4 neutralizing antibody alleviate anti-4-1BB–induced liver pathology without affecting antitumor efficacy (4). Thus, we remain hopeful that novel strategies could be devised to bypass anti-4-1BB immuno-toxicity while retaining significant antitumor efficacy.

Rational protein engineering has emerged as a powerful and popular approach to address issues associated with immunotherapy. Hinner and colleagues utilized an engineered anticalin protein that specifically binds 4-1BB with nanomolar affinity. Anticalins are engineered lipocalins, a family of human plasma proteins characterized by a compact, highly conserved tertiary structure with antibody-like variable loops. These features empower anticalins with not only the capability to target a specific molecule of interest but also the flexibility to conjugate with many other proteins to make bispecific or multispecific biologics. In this design, 4-1BB anticalin was fused with an Fc-modified anti-HER2 antibody (trastuzumab), an IgG4 S228P variant with F234A/L235A mutations to reduce Fc effector functions. After selection of 4-1BB–specific anticalin via phage display and testing various fusion formats to the N- or C-terminus of anti-HER2 heavy or light chain, Hinner and colleagues picked a lead molecule, PRS-343. In this design, 4-1BB anticalin was fused to the heavy chain C terminus of trastuzumab, immediately after the modified Fc region, which exhibited similar geometry as immunologic synapse and preferable functionality. PRS-343 was shown to be functional based on induction of IL2 secretion as well as NF-B reporter signal through coculture experiments containing tumor cells with activated PBMCs or 4-1BB–expressing reporter cells, respectively. Immune cell activation in the presence of PRS-343 correlated with HER2 density on tumor cells, suggesting appropriate antigen-dependent 4-1BB activation. This reagent showed similar *in vivo* pharmacokinetic properties as regular antibodies, with a half-life >14 days in mice and >4 days in cynomolgus monkeys. Although the 4-1BB–related toxicity cannot be fully assessed in monkeys, as PRS-343 has reduced cross-reactivity to cynomolgus 4-1BB, a 4-week GLP-compliant toxicity study in monkeys demonstrated that PRS-343 was well tolerated at doses of 10 and 120 mg/kg. In a human PBMC

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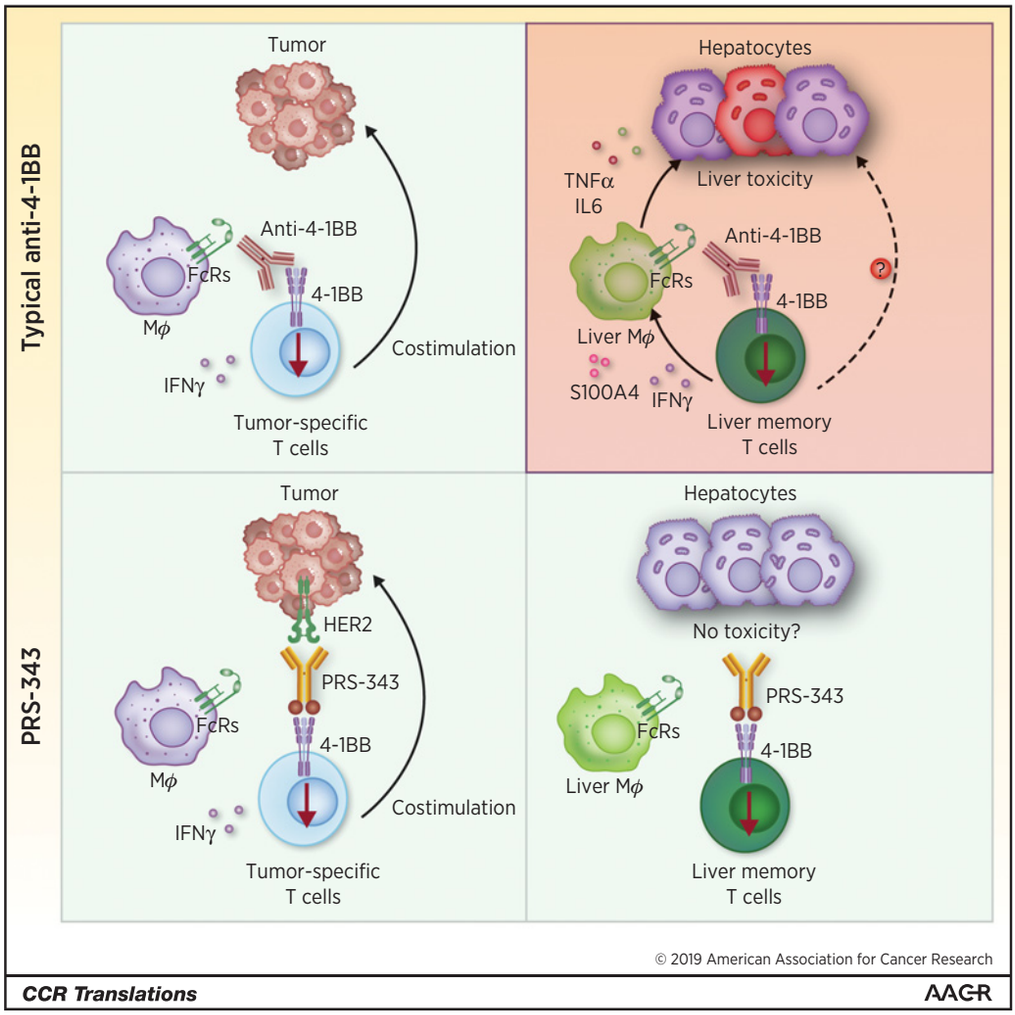

Figure 1.

The mechanisms of antitumor efficacy versus liver toxicity of PRS-343 in comparison with typical anti-4-1BB antibodies. The antitumor efficacy of anti-4-1BB antibodies is mediated by the direct activation (costimulation) of tumor-specific T cells. Liver toxicity is primarily caused by hepatic macrophages activated by nonspecific memory T cells via cytokines such as IFNγ, while direct evidence that liver memory T cells causing hepatotoxicity remain to be uncovered. Both effects are initiated by 4-1BB signaling cross-linked by Fc receptors presented by macrophages etc. PRS-343 theoretically activates 4-1BB signaling only in the presence of abundant HER2 tumor antigen but may not activate bystander hepatic T cells to cause toxicity.

reconstituted HER2$^+$ SK-OV3 tumor cell xenograft in NOG immune-deficient mice, PRS-343 showed antitumor activity in a dose-dependent manner and increased immune infiltrate into the tumor microenvironment without increasing GVHD, as shown by survival. In contrast, an urelumab benchmark antibody 20H4.9, which is functional *in vitro* regardless of HER2 expression and could activate T cells in the peripheral blood, showed little antitumor efficacy but aggravated GVHD. Of note, equimolar dose of trastuzumab-IgG4 control antibody showed similar antitumor activity compared with PRS-343.

As a new player in the 4-1BB therapeutic arena, PRS-343 is currently being assessed in phase I clinical trials sponsored by Pieris Pharmaceuticals. Although a formal analysis of hepatic inflammation and toxicity would be required to establish the safety profile, initial preclinical *in vitro* and *in vivo* data suggest that PRS-343 may exhibit a safer profile than urelumab, perhaps because of prioritized tumor antigen binding (>10-fold higher affinity on HER2 compared with 4-1BB) or different mode/epitope to activate 4-1BB signaling. Given that there was little additive antitumor effect of PRS-343 compared with trastuzumab-IgG4 in their mouse model, it would be interesting to see how the efficacy of PRS-343 compares with the clinically successful and more efficacious single-agent trastuzumab-IgG1 with intact Fc effector functions. Moreover, given the additional recruitment of activated immune cells, it would be important to see whether PRS-343 may offer a new solution for patients who develop resistance to anti-HER2 therapy, and at the same time, whether PRS-343 might augment the side-effects associated with anti-HER2 therapy such as cardiotoxicity.

Overall, this study is noteworthy, as it forms the basis for a phase I clinical trial testing a first-in-class molecule featuring a novel anticalin drug format with interesting mechanisms of

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antitumor efficacy versus toxicity (Fig. 1). Compared with conventional antibody engineering technology, the advantages of anticalin technology include its modularity, increased tissue penetration, high stability, and ease of production and engineering, although these biological benefits remain to be validated in the clinic. Rational engineering strategies are necessary to decouple liver toxicity from efficacy associated with 4-1BB agonist treatment, and this approach may bring us one step closer to feasibly targeting a promising costimulatory molecule more specifically in the tumor microenvironment. Following similar principles, a plethora of next-generation 4-1BB therapies have been designed to effectively leverage this pathway. A recent report had utilized a trimerbody design to guide trimeric 4-1BB agonistic antibody to $EGFR^{+}$ tumors, which induces potent antitumor immunity without systemic toxicity (5). Other potential competitors to PRS-343 include Roche's FAP-4-1BB ligand, with a trimeric split 4-1BBL fused to a monomeric Fab against fibroblast activation protein, a tumor-stromal antigen, CytomX's anti-4-1BB protease cleavable probody, Numab/CStone's PD-L1/4-1BB/HSA trispecific scMATCH3 molecule, as well as the PD-L1/4-1BB bispecific antibody designs from Merus and Inhibrx/Elpiscience etc., the latter of which (INBRX105/ES101) is already entering into phase I clinical trials. Most of these designs, including PRS-343, feature mutations in the Fc that eliminate binding to activating FcRs to avoid potential depletion of 4-1BB-expressing immune cells. Another common theme is the heavy emphasis on tumor microenvironment–dependent 4-1BB activation while reducing systemic auto-activation of T cells. The balance between potential toxicity and efficacy of these various designs still remains to be fully validated, especially given that the tumor specificity of certain antigens may not be ideal. With the rising wave of immunotherapies on the market, now more than ever, enthusiasm for various anti-4-1BB therapies must be tempered with mechanism oriented, rational clinical trial design to maximize clinical utilization while avoiding immuno-toxicity.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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