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Antibodies at the Center of Immunotherapy: Commentary on "Moving a Carbohydrate Mimetic Peptide into the Clinic"

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Commentary

The idea that we can activate the immune system to fight cancer is starting to pay off in clinical practice. Instead of targeting the tumor itself, as in conventional cancer therapies, activating and mobilizing the immune system to attack its "abnormal self" necessitates the training of the immune system to see a collection of antigens expressed on tumor cells. We have recently shown that a novel immunogen can recruit antibodies to breast cancer tumors that can inhibit tumor cell growth [1]. These vaccine-induced antibodies are cross-reactive with Tumor Associated Carbohydrate Antigens (TACA), which are intimately involved in cell survival signaling. The ability of these antibodies to bind to TACA and interfere with signalling events also suggest additive or synergistic modality with chemotherapeutics that are cytotoxic. Most importantly, our studies suggested a potential clinical benefit to the combination of TACA-reactive antibodies as illustrated by the multiple antibodies induced with the P10s vaccine in cancer patients [1]. The sensitization of cancer cell via inhibition of cell survival pathways, show for the first time that signalling pathways interrupted by vaccine induced TACA reactive antibodies causes tumor regression in combination with standard of care chemotherapeutics.

Progress in our ability to manipulate the immune system led to the discovery of monoclonal antibodies (mAbs) that are specific to one epitope of an antigen. MAbs produced *ex-vivo* are fulfilling their original promise as pharmaceuticals in cancer treatment [2-5] and, as investigational tools, by helping investigators fine tune their manipulations of the immune system and design other immune targeted therapies. Our experience with the development of an anti-glycan vaccine allowed us to move the process of producing anti-glycan MAbs from the *ex-vivo* to the *in-vivo* setting and to be able to induce the production of multiple mAbs in patients using the same therapeutic agents, our P10 vaccine.

Many novel mAbs continue to enter the clinic, each designed with modifications to structure aimed at further improving efficacy [6,7]. As we move into an era of precision and personalized medicine, it will become increasingly important to develop closer links between emerging mechanistic insights, mediated by tumor associated antigens (TAA), and the clinical development of mAbs. Glycans or TACA are among TAA targeted by mAbs [8,9]. The ideal target of cancer therapeutics would be a molecule(s) critical for tumor cell survival, expressed at elevated levels on the tumor cell surface and therapeutic benefit should be demonstrable with antibody or T cells to this molecule. By these criteria, TACA stand out as excellent targeted candidates. TACA are intimately related with pathways that mediate cell survival. Tumors expressing a high level of certain types of TACAs exhibit greater metastasis and progression as reflected in decreased patient survival rate than those expressing low level of these TACAs, [10]. mAbs targeting glycans that are either in the clinic or under preclinical development include those for GD2 [7,10-14], and tumor-associated Lewis (Le) glycans [15,16].

TACA, which are elevated in a wide range of solid tumors, mediate apoptosis in tumor cells while sparing normal cells, demonstrating both selectivity and therapeutic activity. When considering this family of antigens as targets, the first challenge is whether mAbs will trigger apoptotic signals because many receptor binding antibodies block, rather than trigger signals. While immunotherapy has focused on approval of mAbs for passive therapy, there are parallel efforts to develop immunogens to induce sustained antibody mediated immunity against TACA [17-20].

Our experience with cancer antigens suggests that TACA are pan-antigens shared across tumor types and are significantly different from the ones expressed on normal cells. TACA are pan-targets because they are intimately involved in cell signalling pathways associated with all cancer cells. The discovery of glycan pan-antigens led to a concept of a pan-approach to immune therapy of cancer where all cancers are targeted by one treatment designed by special manipulation of the immune system. This concept suggests approaches to induce and maintain an immune response against multiple TACA, thus unleashing a powerful multi-prong attack against a tumor. In this context multivalent forms of TACA-based vaccines in particular are meant to induce responses across multiple TACAs by inducing subpopulations of antibodies reactive with the constitutive components of the multivalent vaccine [21- 23].

In contrast to TACA-based vaccines, we have developed potential vaccines based on carbohydrate-mimetic peptides (CMPs) [24]. This approach is similar to using anti-idiotypic antibodies as mimics of TACAs [9,25]. We have shown that CMPs induce anti-tumor-reactive humoral [26-28] and cellular responses [29,30]. We have moved one of these CMPs, with the sequence WRYTAPVHLGDG (referred to as P10s) conjugated to the Pan-T-cell epitope PADRE, into an early-phase clinical trial in Stage IV breast-cancer subjects [1]. This CMP was designed to mimic and induce responses to TACAs that are associated with glycolipid moieties including the ganglioside GD2 and the LeY [28,31].

P10s was computer designed to react with the anti-GD2 antibody ME36.1 and the anti-LeY antibody BR55-2 [28,32]. Therefore, we have extended the notion of mimicry by considering CMPs as pan-immunogens, inducing multiple sets of antibodies reactive with multiple TACAs when immunizing with a single agent [24-29,32]. Conceptually, CMP-induced responses might be a way to manipulate the immune system to generate beneficial low-affinity antibodies that

would sidestep a variety of potential side effects from generating high-affinity antibodies to a particular TACA [28,33] such as GD2 [34].

In keeping with the concept of being a pan-immunogen, P10s was found to react with several anti-GD2 MAbs that include 3F8 [35], 14.G2a [36] and ME36.1 [37] along with the anti-LeY mAb BR55-2 [15,38]. Unlike the other two anti-GD2 MAb 3F8 and 14.G2a, ME36.1 cross-reacts with GD3 [37]. Notably, in a recent project for prioritization of cancer antigens, 4 of the 75 selected antigens were gangliosides (GD2, GD3, fucosyl-GM1, and N-acetyl GM3), and additional targets, like the EGFR and the VEGFR, are known to interact with gangliosides [39,40].

Our observation that P10s immunization enhances reactivity to the ganglioside GD2 and LeY in humans is one example of inducing responses to multiple TACAs with a pan-immunogen, which has therapeutic ramifications such as driving epitope spreading. In our early phase clinical trial we observed some direct clinical benefit in one of our subjects with metastatic lesions as evaluated before and after vaccine treatment [1]. Inducing antibodies to multiple TACA can contribute to overcome immune-escape mechanisms since TACA are always expressed on tumor cells which increases the therapeutic potential of this cancer vaccine.

The evidence in this study further serves as a proof-of-principle for immunization using P10s-PADRE to induce TACA reactive antibodies that are proapoptotic. Monoclonal antibodies, such as anti-GD2 and -LeY antibodies, can mediate signaling pathways extracellularly. This might be accomplished whereby apoptosis signals are transduced via reduction in the phosphorylation levels of focal adhesion kinase (FAK) and the activation of a MAPK family member, p38, upon the antibody binding. Knock down of FAK results in apoptosis and p38 activation. In this context mAbs have defined mechanistic pathways linked to their therapeutic function. Of significance then is that the P10s vaccine can induce antibodies with functionalities similar to mAbs reactive with GD2 and LeY and through these mechanistic pathways can function in association with chemotherapeutics to kill tumor cells or can sensitize tumor cells for more efficient tumor cell killing. This sensitization concept has now provided an opportunity to test the p10s vaccine in a phase 2 study involving HER2 negative, ER positive breast cancer patients in the neoadjuvant setting (NCT02229084, http://www.aymag.com/promising-breast-cancer-vaccine-clinical-trial-offered-arkansas/)

Acknowledgements

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