ST4-CD40 BISPECIFIC ANTIBODIES ACTIVATE IMMUNE RESPONSES IN A ST4-DEPENDENT MANNER

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Background CD40 is a key costimulatory molecule expressed on professional antigen presenting cells (APCs) and functions to bridge innate and adaptive immunity. CD40 primes dendritic cells (DCs) to upregulate the expression of other costimulatory molecules such as CD80 and CD86 that further activates CD8+T cells. CD40 activation on B cells increases their activation and proliferation that further promotes anti-tumor effects. Agonistic CD40 monoclonal antibodies displayed clinical response, however, the further development was hampered by dose-limiting toxicities possibly due to the systemic activation. To overcome this issue, we have developed a conditional CD40 bispecific antibody which was only activated in ST4-expressing tumor cells. ST4 is a oncofetal protein rarely expressed in normal adult tissues, however, the expression is upregulated in multiple cancers. Therefore, we hypothesized that CD40 crosslinking by engagement of ST4 on the cancer cells could boost the immune response in the tumor microenvironment while minimizing the risk of peripheral toxicity.

Methods To achieve maximal agonistic effect, we have generated a panel of ST4 x CD40 bispecific antibodies in different formats. ST4 x CD40 bispecific antibodies were evaluated in CD40 reporter cells and cocultured with target cells expressing ST4. The potency was further confirmed by measuring IL12 production from primary DCs and CD80 and CD86 expression on the DC and B cells. The anti-tumor efficacy was determined in CD40-humanized C57BL/6 mice bearing MC38-huST4 tumor.

Results ST4 x CD40 bispecific antibodies activated CD40 signaling in a ST4-dependent manner. Similarly, DCs and B cells were activated only when cocultured with ST4-expressing cells. Interestingly, we have identified an optimal format that showing superiority to other formats in inducing more potent CD40 agonism while remained silent in ST4-negative cells. Treatment of ST4 x CD40 displayed a more potent anti-tumor efficacy, compared to an equivalent dose of clinical benchmark CD40 monospecific antibody. Furthermore, tumor-free mice were resistant to tumor rechallenge, indicating an established long-lasting memory response. The ex vivo analysis suggested a focused immune activation in the tumor and no peripheral activation.

Conclusions We have generated bispecific antibodies specific to both CD40 and ST4 that could achieve APC activation only in ST4-expressing tumor microenvironment and demonstrate anti-tumor efficacy without inducing systemic toxicity. The preclinical data warrant further development of ST4 x CD40 bispecific antibody as a potential therapeutic option for solid tumor.