

**1078** **AGONISTIC CD40 IGM ANTIBODIES ENHANCE IMMUNE RESPONSES BEYOND THAT OF IGGs**

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**Background** Tumor necrosis factor receptor (TNFR) superfamily member CD40 plays key roles in mediating innate and adaptive immune responses against tumors and requires trimerization to induce agonistic signaling. Anti-CD40 therapeutic IgG antibodies have demonstrated limited anti-tumor activity in the clinic, perhaps due to inefficient multimerization through FcγR engagement in the tumor microenvironment. We generated and evaluated the functional properties of proof-of-concept agonistic anti-CD40 IgM antibodies and have compared their activities to corresponding IgGs.

**Methods** Anti-CD40 IgM and IgG antibodies were generated by inserting the same variable domains into an IgM or IgG heavy chain framework and co-expressing with light chain as well as J chain for IgM. Antibody binding and subsequent activation was evaluated by flow cytometry using human dendritic cells (DCs) and B cells. Mixed lymphocyte reaction (MLR) assay was used to evaluate antibody induced proliferation and cytokine release by human T cells.

**Results** Agonistic anti-CD40 IgM antibodies demonstrated superior binding to primary human cells (DCs and B cells), as well as cell lines (overexpressing human and cyno CD40), as compared to IgG antibodies. Improved binding was due to the enhanced avidity of the multivalent IgMs compared to bivalent IgGs. In NF-κB-luciferase reporter assays, a significant increase in potency was observed with the IgMs in both EC<sub>50</sub> and max activity compared to IgG in the presence or absence of cross-linking. In primary human DC and B cell activation assays, IgMs significantly enhanced the activation of DCs and B cells, as compared to IgGs. In an MLR assay, co-culture of agonistic anti-CD40 activated DCs with T cells, IgM antibodies showed significant T cell activation, as measured by inflammatory cytokine production, whereas IgGs had little to no effect.

**Conclusions** We have discovered that IgM antibodies bind to and signal efficiently through CD40 as compared to the corresponding IgGs, even when IgGs were cross-linked. Efficient multimerization and agonism of CD40 with IgM antibodies may therefore enhance the anti-tumor responses by providing immunostimulatory signals to DCs, thereby promoting anti-tumor responses of T cells.

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