CD4 as a Target for Immunotherapy

CD4 is a key molecule in the regulation of immune responses and its activity can be modulated using antibodies. In particular, agonistic CD4 antibodies are highly effective in preclinical tumor models either through direct interaction with CD40-expressing lymphomas, or indirectly through the activation of an anti-tumor immune response. To date, limited clinical data have been reported with strong CD40 agonist antibodies; nonetheless it seems likely that targeting this pathway will require a balance between the benefits of potent immune stimulation to drive anti-tumor responses, and the harm that can result from non-specific immune cell activation. We set out to develop novel human anti-CD40 antibodies with different levels of agonist activity to identify a lead candidate for systemic application. Here we describe two novel fully human anti-CD40 mAbs with good agonist activity resulting in immune cell activation and direct anti-lymphoma activity in xenograft models.

Development of novel human anti-CD40 mAbs

Anti-CD40 mAbs (mAbs) were generated by immunization of human IgG transgenic mice (HEL2 strain of Harbour® transgenic mice) with recombinant and cell surface expressed human CD40. Binding to human and NHP CD40 (ELISA and FACS) VL and VH sequences were inserted into human IgG1 and IgG2 vector Transient expression

Summary and next steps

CD40 is a promising and powerful target for immune therapy, but requires an appropriate balance between anti-tumor immune activation and harmful side effects of immune stimulation.

- We have identified 2 novel, fully human anti-CD40 mAbs with good agonist activity when compared to the clinically evaluated mAb B-L1.
- The agonist activity is dependent on expression as an IgG2 isotype and is independent of Fc receptor interactions.
- Next steps are to characterize the biological activity and safety profile of 3C3 and 3G5 mAbs in non-human primates and select a lead candidate for clinical development.

Development and characterization of novel CD40 antibody agonists for cancer immunotherapy

Lauram A. Vitale, Thomas O’Neill, Jennifer Widger, Andrea Crocker, Li-Zhen He, Jeffrey Weidlick, Karuna Sundarapandian, James Storey, Lawrence Thomas, Joel Goldstein, Henry C. Marsh, Jr., Tibor Keler

Cellidx Therapeutics, Inc., Needham, MA and Hampton, NJ

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- **Transient expression**:
  - **Binding to CD40 (ELISA)**
  - **mAb2 Affinities**
  - **CD40 signaling leads to NF-κB activation**.
    - This reporter assay provides a specific readout for signaling via CD40.
    - The IgG1 isotype of 3C3 and 3G5 mAbs bind as well or better than IgG2 isotype.
    - Only the IgG2 isotype have agonist activity measured using the reporter assay.

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