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Development of a AXL/PD-1 BiCE as novel immunotherapy agent

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Abstract

Myeloproliferative neoplasms (MPNs) comprise of group of hematopoietic stem cell diseases where acquired mutations lead to excessive formation of myeloid cells, red blood cells, platelets and granulocytes. Essential Thrombocythemia (ET), Polycythemia Vera (PV) and Primary and Secondary myelofibrosis (MF) are the most prevalent MPNs. The phenotypic driving mutations are the acquired unique somatic *JAK2* V617 mutation, *MPL*/*TpoR* (thrombopoietin receptor mutations) and calreticulin (*CALR*) mutations. The latter are +1 frameshift mutations in exon 9 which lead to a new positively charged sequence in the place of the negatively charged C-terminus of wild type CALR, and lacking the ER retention KDEL sequence. Our group established that these mutants are inducing MPNs (15-20% of ET and 27% of MF) via binding in the ER and dimerizing the TpoR, which is then transported in a partially immature state to the cell surface by the rogue chaperone function of mutant CALR. Exposure at the cell-surface is obligatory for oncogenic signal of TpoR in the absence of Tpo ligand. Since the novel sequence is constituted of 36 novel amino-acids that should function as a tumor antigen we examined the possibility of using immunotherapy against this mutant sequence.

In addition to the cell-surface exposed tumor antigen, we report that structure of the N-terminus non-mutated region of CALR slightly differs between wild type and mutant CALR. We used vaccination and showed that naive mice C57-B6 mice respond with antibody and T cell responses to immunization with CALR mutant peptide. In contrast, *Calr* del52 knock-in animals or transplanted animals expressing both wild type Calr and *Calr* del52 knock-in mutant do not respond. We discuss alternate vaccination methods that might be able to break this tolerance. Furthermore, we found that the mutant CALR is also secreted from cells of the clone that do not express TpoR. At levels equivalent to those in patients the circulating mutant CALR acts as a rogue cytokine, forming complexes with endogenous cell-surface mutant CALR and TpoR, but not with TpoR alone, explaining why the mutant CALR only stimulates clonal expansion and not polyclonal thrombocytosis. The circulating mutant CALR will interact with antibodies that recognize the tumor antigen and may exert a sink effect at low antibody doses. Based on our structural studies examining by deuterium exchange mass spectrometry we propose several precise sequences to be targeted by immunotherapy and discuss recent efforts using anti mutant CALR antibodies or bispsecific approaches.

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