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AUTHOR'S VIEW

The *JAK2*V617F and *CALR* exon 9 mutations are shared immunogenic neoantigens in hematological malignancy

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ABSTRACT
Approximately 90% of patients with the hematological malignancies termed the chronic myeloproliferative neoplasms harbor either the *JAK2*V617F-mutation or *CALR* exon 9 mutation. Both of these are recognized by T-cells, which make the mutations ideal targets for cancer immune therapy as they are shared antigens.

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Neoantigens are believed to be of marked importance in the recognition of tumor cells by the immune system due to their immunogenicity. This immunogenicity relies on the emergence of the neoantigens due to somatic mutations, and hence the lack of central tolerance to the mutant antigens. Neoantigens are thus, in theory, optimal targets for cancer immune therapy.[1,2] One problem by targeting neoantigens is the heterogeneity of somatic mutations between different cancer types, and also between patients with the same malignancy. The development of a widely applicable platform for cancer immune therapy based on neoantigen recognition is as such difficult due to this heterogenic mutational landscape.

However certain malignancies have very homogeneic mutations. One such example is the Philadelphia chromosome negative chronic myeloproliferative neoplasms (MPN) which are neoplastic diseases of the haematopoietic stem cells. The MPNs constitute 3 different diseases: Essential thrombocythemia (ET), polycythemia vera (PV) and primary myelofibrosis (PMF). The vast majority of patients with these diseases harbor a driver mutation in either the Janus kinase 2 gene (*JAK2*V617F)[3] or in exon 9 of the calreticulin (*CALR*) gene.[4,5] Hence, 95% of patients with PV and 50% of patients with ET and PMF carry the *JAK2*V617F mutation, and 65% of the *JAK2*wt patients with ET and PMF carry a *CALR*-mutation. The *JAK2*V617F mutation is a point mutation characterized by a single amino acid substitution, whereas the CALR mutations are 1-bp frameshift mutations which generate a completely novel C-terminus in the CALR protein with a 36 amino acid consensus sequence that is shared between all of the more than 50 known *CALR* mutations.[4,5]

We have recently shown that both the *JAK2*V617F and *CALR* exon 9 mutations are recognized by the immune system: We established a $CD8^+$ cytotoxic T-lymphocyte culture specific for *JAK2*V617F, and the T-cells were able to recognize and kill *JAK2*V617F$^+$ cells in a *JAK2*V617F dependent manner.[6] Concurrently, we screened peripheral blood mononuclear cells from *CALR*-mutant MPN patients for spontaneous immune responses against epitopes from the mutant CALR C-terminus and identified significant $CD4^+$ T-cell responses in a significant proportion of the patients.[7] Next, we established a $CD4^+$ T-cell culture specific for one of the mutant CALR epitopes and showed that the T-cells recognize and are activated upon stimulation with both terminally differentiated autologous *CALR*-mutant cells (monocytes) and haematopoietic $CD34^+$ stem cells. Finally, we demonstrated that target cell recognition depends upon the presence of CALR and that the *CALR*-mutant specific T-cells, despite their $CD4^+$ phenotype, were able to kill autologous *CALR*-mutant cells.[8] The occurrence of a CALR-mutation specific cytotoxic $CD4^+$ T-cell responses are of great importance since the occurrence of a tumor specific cytotoxic immune response has a great impact on disease prognosis. As the $CD4^+$ T-cell response has been described as central in activation of the adaptive immune response, we speculate that the anti-CALR-mutation specific $CD4^+$ T-cells may help activate naïve $CD8^+$ T-cells and NK-cells. The $CD4^+$ T-cells may, in theory, also activate B-cells, which ultimately will produce CALR-mutation specific antibodies. The occurrence of such antibodies, however, has not been identified yet. (Fig. 1)

By these results we have shown that the immune system is able to recognize the driver mutation in approximately 90% of patients with MPN. We only detected minor responses in patients against *JAK2*V617F epitopes (unpublished), however numerous strong responses were detected against *CALR*-mutant epitopes. This is probably because the *JAK2*V617F is relatively small and due to the fact, that the mutant JAK2 epitope resembles the wild type JAK2 epitope. In contrast, the mutant CALR C-terminus spans 36 amino acids and the novel

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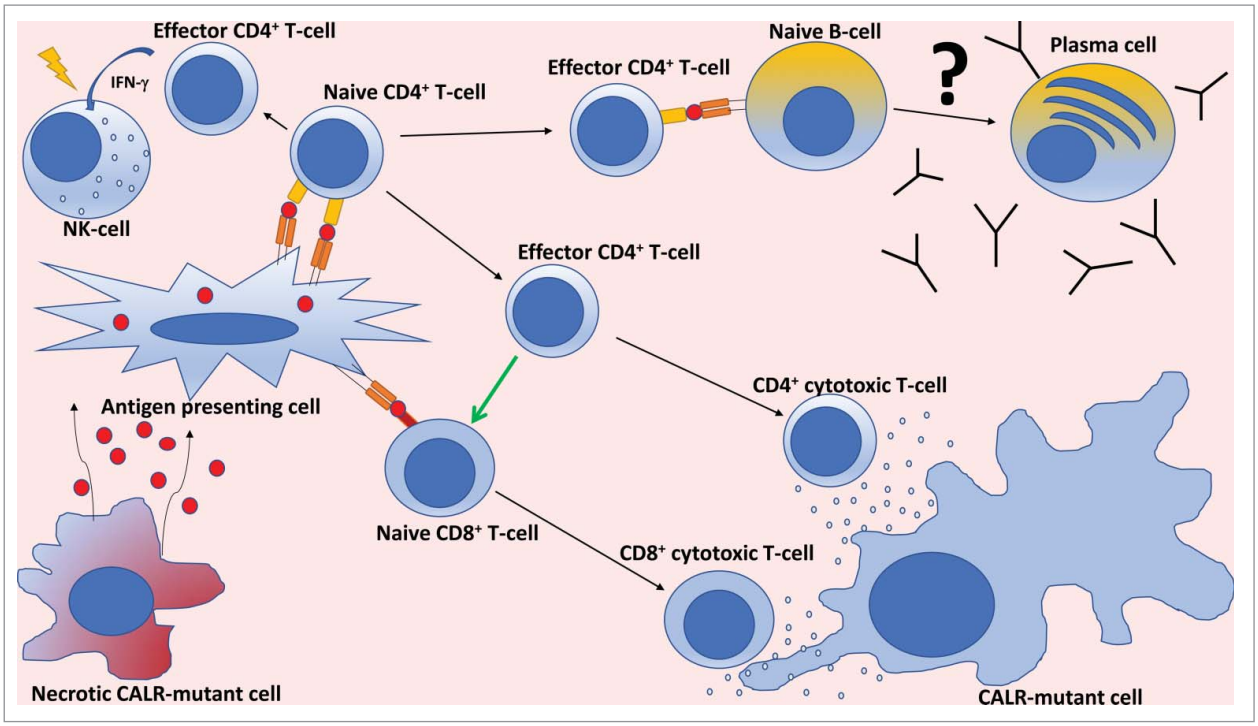

Figure 1. A *CALR*-mutant cell dies shedding its antigens which are taken up by antigen presenting cells (APC). These cells present antigens to naïve $CD4^+$ T-cells and naïve $CD8^+$ T-cells. The naïve $CD4^+$ T-cells are activated and start proliferating and differentiate into effector T-cells. Some of these effector T-cells secrete IFN-γ which activate NK-cells. The $CD4^+$ effector T-cells will, in theory, bind to naïve B-cells which are activated and differentiate to plasma cells that start producing antibodies specific for CALR-mutant antigen. Upon binding to the APC, the $CD4^+$ effector T-cells induce the expression of co-stimulatory molecules which in turn induces maturation of naïve $CD8^+$ T-cells into cytotoxic T-cells. Concurrently some of the $CD4^+$ effector T-cells are also cytotoxic and exert their cytotoxic function on *CALR*-mutant target cells together with the $CD8^+$ cytotoxic T-cells.

sequence has no homology with the wild type sequence or any other known peptide sequences. In light of the immunogenicity of the *CALR*-mutations, we wonder how patients are able to establish *CALR*-mutant disease at all, as the immune system is expected to clear the malignant cells. Firstly, MPN is characterized by elevated amounts of cytokines and is, by some researchers, characterized as a "cytokine disease." This massive deregulation of cytokines, both inflammatory and anti-inflammatory, could potentially render the bone marrow immunosuppressive, possibly by upregulation of immunoregulatory proteins such as PD-L1. Secondly, the *CALR*-mutant specific T-cells could potentially be regulatory T-cells which upon binding to CALR-mutant peptides become activated and start secreting immunosuppressive cytokines. Finally, the lack of rejection could be due to an exhausted anti-*CALR*-mutant immune response due to prolonged exposure to mutant CALR antigens. Future experiments are planned to look further into these possible explanations.

How may the immune recognition of the *JAK2*V617F and *CALR* exon 9 mutations be used for novel therapies, and how can we enhance the anti-tumor immune response? We believe that an effective tumor immune surveillance may be reinstated by peptide vaccination with either JAK2-mutant peptide or CALR-mutant peptide. However, we speculate that the immune response needs to be augmented by either targeting immune check points such as the PD1-PDL1 axis by either monoclonal antibodies or peptide vaccination,[9] or by concurrent treatment with an immunostimulatory cytokine such as interferon-α (IFN-α). Giving further impetus to this notion is the fact, that IFN-α is used as first line treatment of MPN in several countries, and that the mechanism of action of IFN-α is believed to rely on reinstatement of defect tumor immune surveillance.[10] Hence, peptide vaccination with either JAK2-mutant epitopes or CALR-mutant epitopes in combination with immune checkpoint inhibition or IFN-α could prove to be a completely new and potentially curable treatment modality for MPN.

Disclosure of potential conflicts of interest

No authors have conflicts of interest to disclose. However, it should be noted that Morten Orebo Holmström, Hans Carl Hasselbalch, and Mads Hald Andersen have filed a patent regarding the *CALR* exon 9 and *JAK2*V617F mutations as a target for cancer immune therapy. The patent has been transferred to University Hospital Zealand, Zealand Region and Copenhagen University Hospital at Herlev, Capital Region according to Danish Law concerning inventions made at public research institutions.

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