



# Evidence of immune elimination, immuno-editing and immune escape in patients with hematological cancer

Morten Orebo Holmström<sup>1</sup> · Sabrina Cordua<sup>2</sup> · Vibe Skov<sup>2</sup> · Lasse Kjær<sup>2</sup> · Niels Pallisgaard<sup>3</sup> · Christina Ellervik<sup>4,5,6</sup> · Hans Carl Hasselbalch<sup>2</sup> · Mads Hald Andersen<sup>1,7</sup>

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## Abstract

There is mounting evidence that the immune system can spontaneously clear malignant lesions before they manifest as overt cancer, albeit this activity has been difficult to demonstrate in humans. The calreticulin (*CALR*) exon 9 mutations are driver mutations in patients with chronic myeloproliferative neoplasms (MPN), which are chronic blood cancers. The *CALR* mutations generate a neo-antigen that is recognized by patient T cells, and T cells isolated from a patient with a *CALR*-mutation can recognize and kill autologous *CALR*-mutant cells. Surprisingly, healthy individuals display frequent and strong T cell responses to the *CALR* neo-antigens too. Furthermore, healthy individuals display immune responses to all parts of the mutant *CALR* epitope, and the *CALR* neo-epitope specific responses are memory T cell responses. These data suggest that although healthy individuals might acquire a *CALR* mutation, the mutant cells can be eliminated by the immune system. Additionally, a small fraction of healthy individuals harbor a *CALR* exon 9 mutation. Four healthy individuals carrying *CALR* mutations underwent a full medical examination including a bone marrow biopsy after a median follow up of 6.2 years. None of these patients displayed any signs of *CALR*-mutant MPN. Additionally, all healthy individuals displayed strong *CALR* neo-epitope specific T cell responses suggesting that these healthy individuals retained their *CALR*-mutant cells in the editing stage for several years. Thus, we suggest that *CALR*-mutant MPN could be a disease model of cancer immuno-editing, as we have demonstrated that *CALR*-mutant MPN displays all three stages described in the theory of cancer immuno-editing.

**Keywords** Neo-antigen · *CALR* · Immuno-editing · Myeloproliferative neoplasms · T cell memory · CITIM 2019

## Abbreviations

BCR-ABL1 Breakpoint cluster region-Abelson murine leukemia virus oncogene homolog  
*CALR* Calreticulin  
 $T_{CM}$  Central memory T cell

MPN Chronic myeloproliferative neoplasms  
 $T_{EM}$  Effector memory T cell  
 $T_{EMRA}$  Effector memory T cell expressing CD45RA  
ET Essential thrombocythemia  
JAK2 Janus kinase-2  
MART-1 Melanoma antigen recognized by T-cells  
MYD88 Myeloid differentiation primary response 88  
NPM-1 Nucleophosmine-1  
PMF Primary myelofibrosis

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✉ Morten Orebo Holmström  
holmeren1@yahoo.dk

<sup>1</sup> National Center for Cancer Immune Therapy, Herlev Hospital, Borgmester Ib Juuls Vej 25C, 5. Sal, 2730 Herlev, Denmark

<sup>2</sup> Department of Hematology, Zealand University Hospital, Roskilde, Denmark

<sup>3</sup> Department of Surgical Pathology, Zealand University Hospital, Roskilde, Denmark

<sup>4</sup> Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark

<sup>5</sup> Department of Production, Research, and Innovation, Region Zealand, Sorø, Denmark

<sup>6</sup> Department of Laboratory Medicine, Harvard Medical School, Boston Children's Hospital, Boston, USA

<sup>7</sup> Institute for Immunology and Microbiology, University of Copenhagen, Copenhagen, Denmark

PV Polycythemia vera  
TSA Tumor specific antigen

## Introduction

After decades of scientific discussion, it is now widely accepted that the immune system can spontaneously recognize and kill neoplastic cells, and thereby prevent the occurrence of overt cancer in healthy individuals. Studies performed in murine models with induction of tumors by treatment with carcinogenic compounds such as methylcholanthrene in mice showed that immune deficient mice were more susceptible to develop cancer compared to immune-competent mice [1]. In humans this notion was supported by epidemiological data showing that individuals with immune deficiency, e.g. patients infected with human immunodeficiency virus and patients receiving immunosuppressive therapy after solid organ transplantation were more prone to develop cancer [2, 3]. Even more, several case reports have described how patients with cancer suddenly experience a spontaneous cancer regression [4]. Others have shown that patients with vitiligo have a decreased risk of malignant melanoma [5], a feat that is probably mediated by melanocyte specific T cell responses. Even more it was demonstrated that certain HLA-types confer protection against breakpoint cluster region-Abelson murine leukemia virus oncogene homolog 1 (BCR-ABL1)<sup>+</sup> chronic myeloid leukemia [6] and nucleophosmine (*NPM*)-1 mutant acute myeloid leukemia (AML) [7]. The mechanism of these activities probably involves the ability of protective HLA-types to present highly immunogenic tumor-cell epitopes to specific T cells.

The different stages in the interplay between the immune system and cancer were suggested by Burnet [8], and refined by Schreiber et al. to formulate the theory of cancer immunoeediting [9, 10]. In this review we describe frequently occurring spontaneous immune responses against neo-antigens encoded by the calreticulin (*CALR*) exon 9 mutations. These mutations are driver mutations in a substantial proportion of patients with the blood cancer diseases termed the chronic myeloproliferative neoplasms (MPN). Most importantly, we demonstrate that healthy individuals display strong and frequent T cell responses to *CALR* neo-epitopes as well, and show that these responses are mediated by memory T cells. These data could suggest that healthy individuals might acquire a *CALR*-mutation, but that the mutant cells are cleared followed by the generation of T cell memory. We believe that, based on these data we have provided evidence of elimination – the first of Schreiber’s three E’s in the theory of cancer immune-editing.

## Recognition of tumor antigens on transformed cells lead to tumor rejection

It has often been shown that a strong anti-tumor T cell response is highly important to attain a proper anti-tumor immune response [11]. T cells recognize tumor cells by recognition of tumor antigens presented on HLA-I and II. As such, T cell responses to tumor specific antigens (TSA) in a healthy individual could provide evidence that the immune system in the healthy individual was previously challenged with transformed cells, but the cancer cells were cleared, and memory T cells specific to antigens expressed by the neoplastic cells were generated. Tumor antigens are classified as either tumor associated antigens (TAAs) or TSAs [12]. There are three types of TAAs: antigens involved in differentiation such as melanoma antigens recognized by T-cells (MART)-1; over expressed antigens such as HER2/neu and cancer germline antigens (or cancer testis antigens) such as antigens in the melanoma antigen gene (MAGE) family. TAAs are expressed by both malignant cells and by healthy cells. Detection of an immune response against TAA in healthy individuals does not provide the direct evidence, that the immune system has been challenged with neoplastic cells, as the TAA-specific immune response can also be explained by autoimmune reactions. However, TSAs are only expressed by malignant cells. TSAs include viral antigens such as E7 in human papilloma virus induced cancer and neo-antigens such as antigens encoded by the *BRAFV600E* mutation. Immune responses to viral antigens does not provide evidence, that the individual has been challenged with malignant cells, as the antigen specific immune response can likely result from a viral challenge. In contrast, immune responses to established neo-antigens in healthy individuals most likely indicates that the immune system in the individual has been challenged with transformed cells, but that the immune system has cleared the lesion. As such, several studies have tried to establish if healthy donors display immune responses specific to defined neo-antigens.

## Absence of primary T cell responses to neo-antigen derived epitopes in healthy individuals

The most frequently occurring somatic mutations in human cancer are the *KRAS*, *NRAS* and *HRAS* mutations as these genes harbor several mutational hotspots [13]. Several studies have attempted to establish if healthy donors display T cell responses to RAS derived neo-antigens. Six studies investigated whether primary T cell responses to

RAS neo-epitopes occurred in healthy individuals, and all failed to detect such an immune response [14–19]. Interestingly, one study investigated the occurrence of autoantibodies specific to RAS neo-epitopes in healthy individuals and found that a small fraction of healthy individuals harbored autoantibodies to RAS mutations [20]. Another study on immune responses to the *BRAFV600E* mutation in healthy individuals failed to find any primary response [21]. One study on the immune response to the Philadelphia chromosome translocation, identified immune responses against the BCR-ABL translocation in 3/18 healthy individuals after in vitro stimulation with peptide-pulsed autologous dendritic cells [22]. Another study completely failed to identify any immune responses against the *BCR-ABL* translocation in healthy individuals [23]. Two studies have investigated T-cell responses to the myeloid differentiation primary response 88 (*MYD88*) L265P mutation, which is frequently found in patients with lymphoma [24]. Neither of those studies identified responses to the *MYD88L265P* mutation in primary cultures [25, 26], but in both studies, neo-antigen-specific T-cell cultures were established in T cells from healthy individuals by repeatedly stimulating with antigen.

A subset of patients with AML harbor *NPM-1* mutations, [27] and one study observed frequent T-cell responses against NPM-1 neo-epitopes in T cells from healthy individuals [28]. However, the authors did not investigate the phenotype of the responding cells; therefore, it was not possible to establish whether the neo-antigen-specific T cells were in fact naïve T cells or memory T cells. The MART-1 antigen has received a lot of attention, because it is a highly immunogenic antigen that was used as a vaccine therapy in metastatic melanoma. One study identified spontaneous immune responses to MART-1 antigen in healthy individuals; however, upon analysis of the specific T cells that responded, all displayed a naïve T-cell phenotype [29].

### Neo-epitope-specific T-cell responses in patients with Philadelphia chromosome-negative chronic myeloproliferative neoplasms

Philadelphia chromosome-negative chronic myeloproliferative neoplasms (MPNs) are neoplastic diseases of hematopoietic stem cells. MPNs comprise three diseases—essential thrombocythemia (ET), polycythemia vera (PV), and primary myelofibrosis (PMF). These diseases have overlapping symptoms, and may be difficult to distinguish them from each other [30, 31]. Generally, ET and PV are hyperproliferative MPNs: ET is characterized by elevated platelet levels in peripheral blood, and PV is characterized by elevated red cell mass in peripheral blood. In contrast, PMF

is considered an advanced stage MPN; patients with PMF display bone marrow fibrosis, which leads to cytopenia, and in some patients PMF progresses to AML. The mutational landscape in MPN displays high homogeneity as more than 50% of patients harbor the *JAK2V617F* mutation [32, 33] and 15–20% of patients have a mutation in exon 9 of the *CALR* gene [34, 35]. To date, over 50 types of *CALR* exon 9 mutations have been described, but all share the feature that they generate a mutant C-terminus, with a novel mutant 36 amino-acid peptide sequence. Recently, we showed that patients with *CALR*-mutant MPN display strong, frequent T-cell responses to neo-epitopes derived from the mutant *CALR* C-terminus [36]. The T-cell responses were mainly mediated by CD4<sup>+</sup> T cells, which might be explained by the fact that we mainly investigated responses to long epitopes. In another study, we demonstrated in detail that T cells from patients with *CALR*-mutant MPN recognized several epitopes from the mutant C-terminus, and that T cells isolated and expanded from a patient with *CALR*-mutant MPN could recognize and kill autologous *CALR*-mutant cells [37]. These findings spurred the initiation of a clinical trial that investigated the safety, efficacy, and immunological effects of a mutant *CALR* peptide vaccination in patients with *CALR*-mutant MPN (NCT03566446).

### T cells from healthy individuals display responses to *CALR* neo-epitopes

Given the high frequency of primary responses to *CALR* neo-epitopes in patients, we investigated whether specific immune responses to the *CALR* neo-epitopes occurred in healthy individuals. Interestingly, the two groups that discovered the *CALR* mutations investigated whether the mutations occurred in healthy individuals and in individuals with other types of cancer, but *CALR* mutations were only identified in patients with MPN or myelodysplastic syndrome [34, 35]. Consequently, healthy individuals were not expected display a primary response to *CALR* neo-epitopes, because they had not been challenged with the mutation. Most surprisingly, we recently showed that healthy individuals do display strong and frequent primary responses to *CALR* neo-epitopes [38]. Immune responses to both long and short nonamer epitopes were identified in healthy individuals. Moreover, immune responses against the neo-epitopes were more frequent and stronger in healthy individuals than in patients with cancer. Even though these findings are highly surprising they fit well with earlier studies that demonstrated that patients with advanced cancer could not mount a strong anti-tumor immune response. Indeed, our finding fits even better with the theory of cancer immuno-editing, which states that transformed cells in patients with advanced cancers evade immune-mediated destruction [9]. A recent study

demonstrated that effector T cells from patients with MPN showed significantly higher expression of the exhaustion marker, programmed death receptor-1, compared to T cells from healthy individuals [39]. This finding might explain the different responses to neo-epitopes between healthy individuals and patients. Similar to the responses in patients, the responses in healthy individuals were mainly mediated by CD4<sup>+</sup> T cells. We screened a peptide library that spanned the entire mutant CALR C-terminus, and we identified an immunogenic hotspot. However, immune responses against all parts of the mutant C-terminus were identified. Hence, cross-reactivity seems to be an unlikely explanation to the unanticipated high frequency of responses identified in healthy individuals. Next in that study, we investigated whether the somewhat paradoxical occurrence of *CALR*-mutant MPN could be explained by an age-mediated loss of the immune response to CALR neo-epitopes. We compared CALR neo-antigen-specific immune responses in young healthy individuals (ages 18–21 years) to immune responses in older healthy individuals (ages 50–64 years). No difference was found in neither the frequency nor amplitude of the immune response between the two age-defined cohorts. This finding led to the conclusion that the emergence of *CALR*-mutant MPN cannot be explained by an age-mediated loss of immune protection.

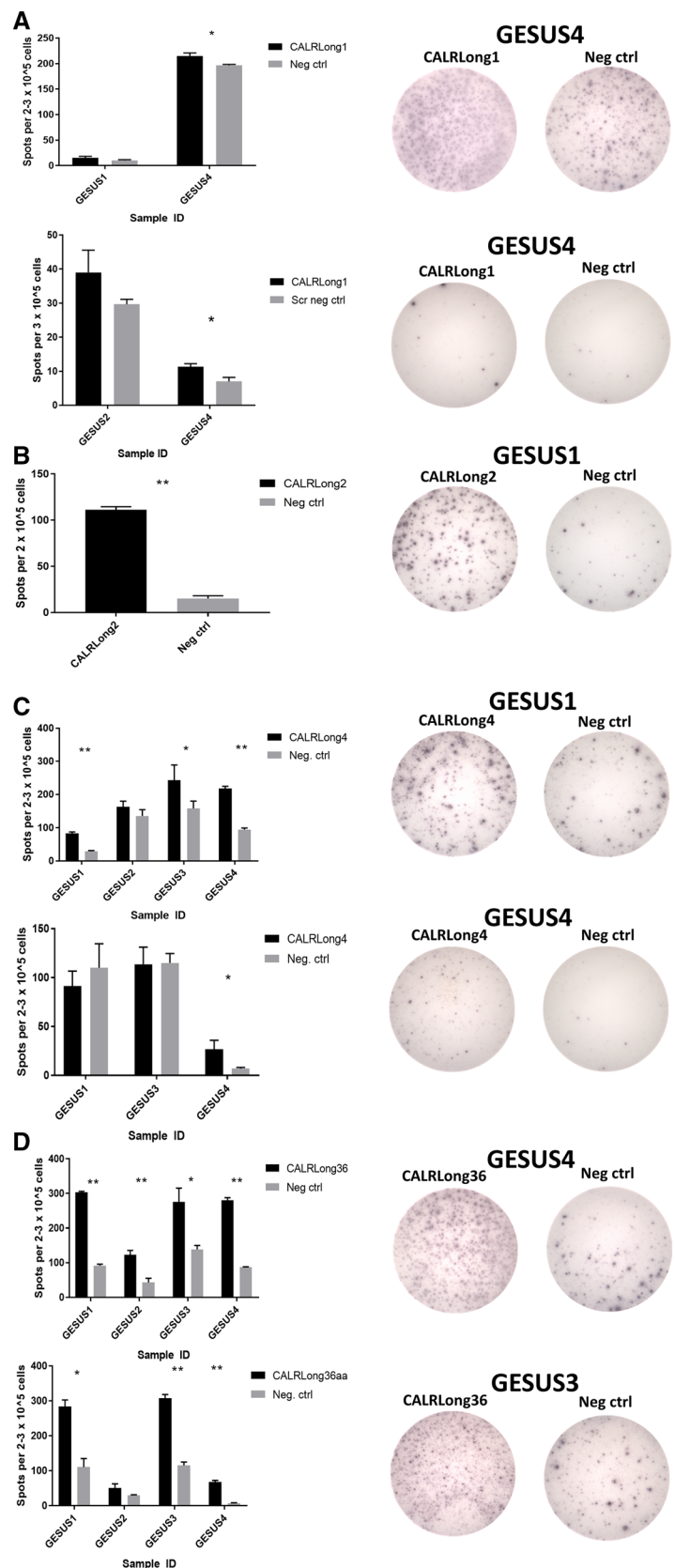
Strong ex vivo responses in healthy individuals against several CALR neo-epitopes indicated that healthy individuals harbor a high amount of freely circulating T cells that specifically recognize the CALR neo-epitopes. These ex vivo responses are noteworthy, because with very few exceptions, it is not possible to detect TAA-specific T-cells, neither with tetramer staining nor with enzyme-linked immunospot (ELISPOT) assays performed on peripheral blood mononuclear cells (PBMC), directly ex vivo without any prior in vitro stimulation [40]. The strong ex vivo responses are most unanticipated, as they suggest that a considerable fraction of T cells in healthy donor peripheral blood are specific to a neo-antigen. As this finding is highly surprising one means to test whether the T cells are indeed specific to *CALR* exon 9 mutation would be to perform isolation of specific cells by tetramers, and test whether these cells may recognize autologous cells with *CALR* exon 9 mutation. Unfortunately, we have not been able to acquire HLA-II tetramers for isolation of specific cells. The frequent and strong ex vivo responses prompted us to investigate the phenotype of CALR neo-antigen-specific T cells, because the strong responses detected both in vitro and ex vivo suggested that these specific T cells might be antigen-experienced memory T cells. Accordingly, we isolated memory T cells with both fluorescence-activated cell sorting (FACS) and magnetically-activated cell sorting. In one of the experiments on FACS sorted cells, the experimental setup allowed us to analyze the amount of effector memory T cells (T<sub>EM</sub>),

central memory T cells (T<sub>CM</sub>) and effector memory T cells expressing CD45RA (T<sub>EMRA</sub>) in the responding Tmemory cell fraction, and showed that in the memory fraction 60% were T<sub>EM</sub>, 22% were T<sub>CM</sub> and 17% were T<sub>EMRA</sub>. In another experiment on FACS sorted cells in which we identified an CALR-mutant specific immune response 45% cells were T<sub>EM</sub> and 55% were T<sub>CM</sub>. Of note, T<sub>EMRA</sub> were not included in the memory cell fraction in this experiment. Our results demonstrated that the CALR neo-antigen-specific T cells were indeed memory T cells. Consequently, we used highly pure CD4<sup>+</sup>CD45RO<sup>+</sup> memory T cells from two healthy individuals to establish bulk cultures of T cells specific for two different CALR neo-epitopes; subsequently, we established clones from these cultures by limiting dilution. The clones displayed cytotoxic phenotypes and could lyse target cells pulsed with the CALR neo-epitope. One final experiment to perform with these cells would be to identify recognition and killing of autologous target cells stably expressing the mutant CALR C-terminus, which would ultimately prove that antigen experienced T cells isolated from a healthy donor are able to recognize and kill autologous cancer cells. Taken together, these data suggest that healthy individuals, from time to time, acquired a mutation in exon 9 of *CALR*. However, given the high immunogenicity of these mutations, the mutant cells were effectively cleared by the immune system, which in turn, generated memory T cells specific to the mutations (Fig. 1).

### A small fraction of healthy individuals harbor *CALR*-mutant cells in peripheral blood

Recently, a Danish population-based study showed that a small fraction of healthy individuals harbor a *CALR* mutation [41]. However, that study also showed that the prevalence of the *JAK2*V617F mutation in healthy individuals is much higher (3.2% of the total population) compared to the prevalence of *CALR* mutations (0.16% of the total population). In addition, the *JAK2* mutation occurred more frequently than *CALR* mutations in patients with MPN. Nevertheless, the frequency of these two mutations varied considerably between patients and healthy individuals. Among patients with MPN, the *JAK2* mutation was approximately 5.6-fold more frequent than *CALR* mutations; however among healthy individuals, the *JAK2* mutation was 19-fold more frequent than *CALR* mutations. This finding implies that individuals with a very low burden of *CALR*-mutant cells may simply clear the mutant cells. In contrast, patients with *JAK2*V617F-mutant cells fail to clear the transformed cells, due to the fact that the *JAK2*V617F mutation is not as immunogenic as the *CALR* mutations.

**Fig. 1** Strong, frequent, specific in vitro and ex vivo immune responses against mutant CALR epitopes in cells from healthy controls with a low CALR-mutant allelic burden. **a** IFN- $\gamma$  ELISPOT results show responses against CALRLong1 peptide epitope in cells from two healthy individuals; (top left) in vitro results; (top right) representative in vitro wells; (bottom left) ex vivo results; (bottom right) representative ex vivo wells. **b** IFN- $\gamma$  ELISPOT results show a response against CALRLong2 peptide epitope in cells from one individual with a low CALR-mutant allelic burden; (left) in vitro results; (right) representative in vitro wells. **c** (Top left) In vitro IFN- $\gamma$  ELISPOT results show responses against CALRLong4 in cells from four healthy individuals; (top right) representative in vitro wells; (bottom left) Ex vivo IFN- $\gamma$  ELISPOT responses against CALRLong4 peptide epitope in cells from three healthy individuals; (bottom right) representative ex vivo wells. **d** IFN- $\gamma$  ELISPOT results show responses against CALRLong36 peptide epitope in cells from four healthy individuals; (top left) in vitro results, (top right) representative in vitro wells. (Bottom, left) Ex vivo results; (bottom, right) representative ex vivo wells. Error bars display standard error of the mean. \* $p \leq 0.05$  according to the distribution free resampling (DFR) rule. \*\* $p \leq 0.05$  according to the DFR2x rule [46]. GESUS1-GESUS4 are donor identifiers



To clarify whether healthy individuals with a *CALR* mutation might harbor a *CALR* neo-antigen-specific immune response, we chose to investigate four healthy individuals with *CALR* mutations that were identified by Cordua et al. [41] to determine the occurrence of *CALR* neo-antigen-specific immune responses. The median allele burden of the healthy individuals at the time of enrollment into the population study was 0.1% (range 0.07–0.37%). Demographic data for these four healthy individuals is shown in Table 1. After a median follow-up time of 6.7 years (range 6.2–7.2 years), measured from the first survey blood draw, we isolated PBMCs from the four individuals. All four underwent a full medical examination to determine whether they had developed overt MPN during the time that had passed since the first blood draw. Surprisingly, none of the patients had developed *CALR*-mutant MPN. Instead, all had maintained a low (<2%) *CALR*-mutant allelic burden and displayed normal hematological parameters. Bone marrow biopsies were performed on all 4 individuals, and they showed no evidence of MPN. Most interestingly, these patients showed strong *CALR* neo-antigen-specific immune responses (Fig. 2) driven by CD4<sup>+</sup> T cells (Fig. 3). These results suggest that, in these healthy individuals, the *CALR*-mutant clone has been kept at bay by the immune system. Moreover, the immune system has maintained the malignant cells in the editing stage. ELISPOT and intracellular cytokine staining assays were performed as previously described [37].

### ***CALR*-mutant MPN: a model disease for cancer immuno-editing**

Given the data provided above, we speculated that *CALR*-mutant MPN could be a model disease for immune-mediated cancer elimination. The memory T-cell responses identified suggest that *CALR* mutations, even though these mutations are identified rarely in healthy individuals, might evolve quite frequently in healthy individuals. Indeed, the *JAK2V617F/CALR*-mutation ratio in healthy individuals

was significantly higher than the ratio in patients. Additionally, *CALR* mutations comprise more than 50 known variants, however only one mutation, a G to T transversion, can generate the *JAK2V617F* mutation [33]. Thus, at least at the stem-cell level, *CALR* mutations are expected to occur more frequently than the *JAK2V617F* mutation. The difference in *JAK2V617F/CALR* mutation ratios between healthy individuals and patients, compared to the identified memory T-cell responses, suggests that healthy individuals might often develop *CALR* mutations, but the mutations are cleared, and the immune system develops memory T cells specific to the mutations. In addition, we identified individuals that maintained a *CALR* variant allele frequency at a low, stable level for over six years, and did not develop any signs of overt MPN. Because all of these individuals displayed strong *CALR* neo-antigen specific immune responses, this finding suggests that the individuals have maintained the *CALR*-mutant cells in the editing stage, which prevented the development of cancer. Nevertheless, a substantial proportion of patients with *CALR*-mutant MPN do display T-cell responses to *CALR* neo-epitopes [36]. However, not all patients display a response, and patients with advanced cancer (PMF) show significantly fewer responses compared to patients with non-advanced cancer (ET) [36]. These data are consistent with previous reports which show that patients with advanced cancer have an exhausted anti-tumor immune response, and thus cannot mount an effective anti-tumor immune response [42]. Consequently, some patients with advanced *CALR*-mutant MPN have completely lost their *CALR* neo-antigen-specific immune response.

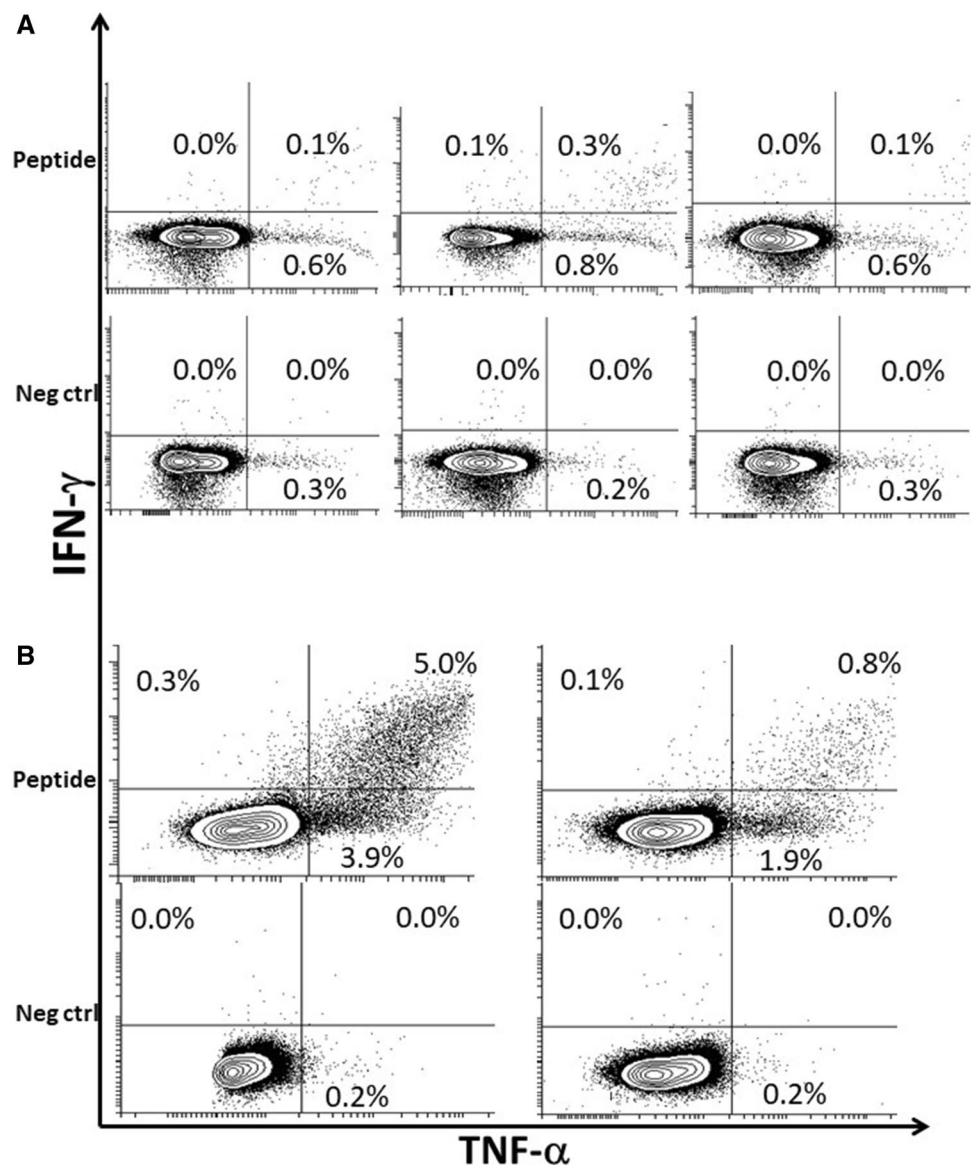
A case study we recently published adds impetus to the hypothesis about immune-mediated control of *CALR* mutations. The case study describes how a patient with *JAK2V617F* PV experiences a shift in disease phenotype. This shift was conferred by a mutational shift from *JAK2V617F* to *CALR* [43]. Over the course of one year, *CALR*-mutant hematopoietic stem cells completely suppressed *JAK2*-mutant hematopoietic stem cells. In our opinion, the expansion of the mutant *CALR* clone was

**Table 1** Demographic data for healthy individuals with *CALR* mutations

Characteristics	The Danish General Suburban Population Study (GESUS) individuals with <i>CALR</i> -mutant allele burdens < 20%
<i>N</i>	4
<i>CALR</i> mutation type (type 1/type 2), <i>N</i>	3/1
Allele burden, %; median (min–max)	0.10 (0.07–0.37)
Age, years; median (min–max)	70 (51–77)
Sex (F/M), <i>N</i>	1/3
Follow up time, years; median (min–max)	6.7 (6.2–7.2)
MPN/non-MPN, <i>N</i>	0/4

*CALR* calreticulin, *MPN* myeloproliferative neoplasms

**Fig. 2** CD4<sup>+</sup> T-cell responses against mutant CALR epitopes in cells from healthy individuals with a low CALR-mutant allelic burden. **a** Cytokine release in CD4<sup>+</sup>-gated T cells from a healthy individual with a low CALR-mutant allelic burden (GESUS1) stimulated with (left) CALRLong1, (middle) CALRLong2, or (right) CALRLong4. **b** Cytokine release in CD4<sup>+</sup>-gated T cells from a healthy individual with a low CALR-mutant allelic burden (GESUS4), stimulated with (left) CALRLong4, or (right) CALRLong36. Percentages indicate the proportion of cytokine-positive cells among all CD3<sup>+</sup>, CD4<sup>+</sup>-gated cells

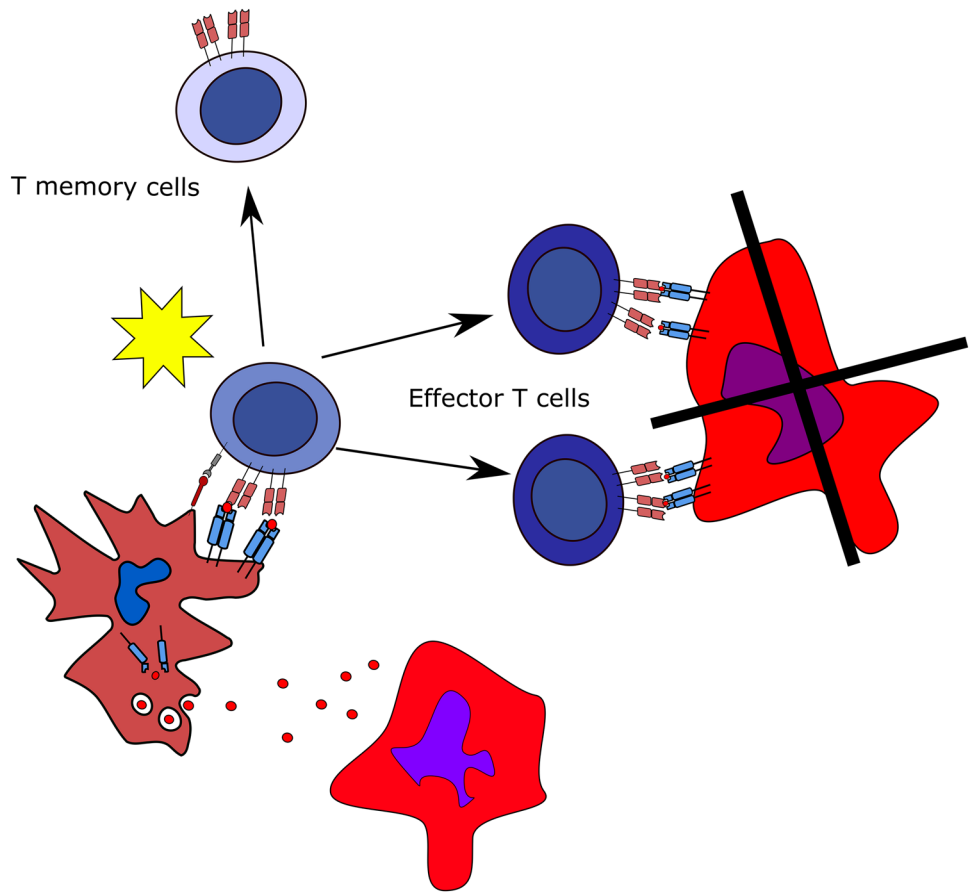


likely due to the loss of immune-mediated control of these transformed cells. Around the time of the mutational shift from *JAK2V617F* to *CALR*, the patient was diagnosed with renal cell carcinoma—another highly immunogenic neoplasm [44], and the *CALR*-mutant clone became measurable for a period of time, when the patient was not treated with the immunostimulatory cytokine interferon-alpha. These findings suggest that the immune system was not able to maintain either cancers in the editing stage, and consequently the two immunogenic neoplasms have evaded the immune system. Accordingly, we believe that immune-mediated tumor control of *CALR*-mutant MPN is of paramount importance in preventing and treating the disease. A comprehensive review of possible immune escape mechanisms in MPN was recently published by Holmström et al. [45].

## Conclusion

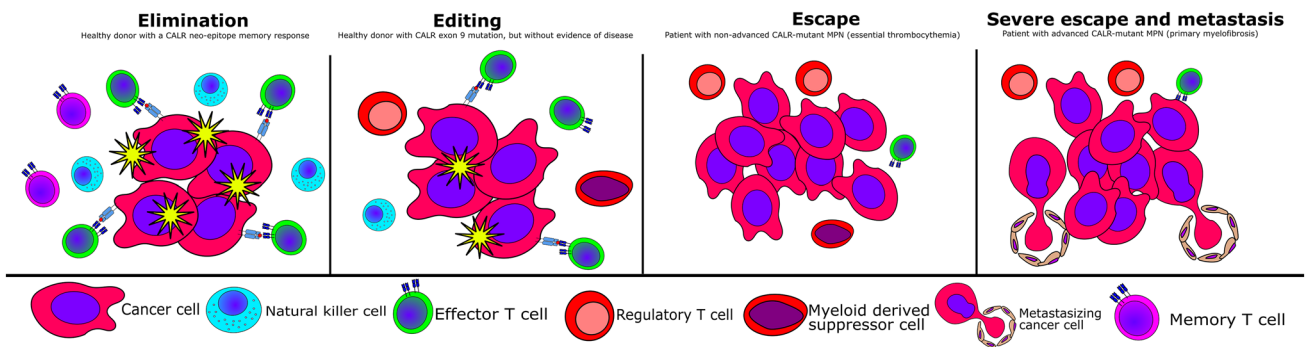
*CALR* exon 9 mutations are highly immunogenic mutations that are only found in patients with MPN and in a small subset of healthy individuals. Stimulation of patient T cells with *CALR* neo-epitopes induces a T-cell response, and T cells isolated from patients that carry a *CALR* mutation can recognize and kill autologous *CALR*-mutant cells. A majority of healthy individuals display strong primary responses to *CALR* neo-epitopes, and the responses in healthy individuals are stronger than in patients. T cells from healthy individuals that are specific to *CALR* neo-epitopes have been

**Fig. 3** Effector T-cell response and the generation of T-cell memory to CALR mutations. (Bottom to top) A *CALR*-mutant cell dies, either by apoptosis or necrosis, and it sheds its mutant *CALR* epitopes to an antigen presenting cell. This cell phagocytoses, processes, and presents mutant epitopes to a naïve T cell, which is then primed. The primed T cell starts to proliferate and differentiate into either effector T cells, which kill the *CALR*-mutant cells, or memory T cells



identified as antigen-experienced memory T cells. Taking these observations together with the low prevalence of *CALR* mutations in healthy individuals, we suggest that healthy individuals can acquire a *CALR* exon 9 mutation from time

to time. However, the mutant cells are cleared, and T cell memory is generated. The small fraction of healthy individuals that carry a *CALR* mutation can suppress the expansion of *CALR*-mutant hematopoietic stem cells and maintain the



**Fig. 4** *CALR* mutations fit into the theory of cancer immuno-editing. (Left) A small quantity of transformed *CALR*-mutant cells are eliminated by the immune system, which results in the generation of memory T cells. (Middle, left) The transformed cells are held at bay by the immune system, which prevents the cancer cells from expanding to give rise to overt cancer. However, the immune system cannot kill all the malignantly transformed cells, and the immune system and the cancer cells now co-exist in a complex interplay, the editing

stage. (Middle, right) The *CALR*-mutant cells are now able to escape immune-mediated destruction, and the transformed cells expand with ensuing *CALR*-mutant MPN. However, the immune system maintains some immune reactivity against the malignant cells. (Right) The immune system has now lost almost all reactivity to the cancer cells. The cancer cells can now expand into the blood stream, which results in the seeding of malignant cells into the spleen and liver (hepatosplenomegaly)



disease in the editing stage. In contrast, patients with overt *CALR*-mutant MPN have lost immune-mediated control of the transformed cells, and this has allowed the mutant cells to expand, resulting in overt cancer. Patients that retain some tumor-specific immune control can maintain the disease in the non-advanced stage (ET); conversely, patients that lose the immune response to *CALR*-mutant cells develop advanced metastatic MPN (PMF) (Fig. 4). Thus, we suggest that *CALR*-mutant MPN may emerge and evolve due to the loss of immune-mediated tumor control, and consequently *CALR*-mutant MPN could serve as a model disease for cancer immuno-editing.

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**Author contributions** MOH: designed the studies, performed the experiments, and wrote the manuscript. SC: designed the studies. VS: designed the studies and performed experiments. LK: designed the studies and performed experiments. NP: designed the studies and performed experiments. CE: designed the studies and performed experiments. HCH: designed the studies and wrote the manuscript. MHA: designed the studies and wrote the manuscript.

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## Compliance with ethical standards

**Conflict of interest** Morten Orebo Holmström, Mads Hald Andersen, and Hans Carl Hasselbalch have filed a patent regarding *CALR* exon 9 mutations as targets for cancer immune therapy. The patent has been transferred to Zealand University Hospital, Zealand Region and to Herlev Hospital, Capital Region, Denmark, according to Danish Law concerning inventions created at public research institutions. The authors declare that there are no other conflicts of interest.

**Ethical standards** All projects described herein were approved by the local Ethics Committee in Zealand Region, Denmark, with the approval numbers: SJ-456, SJ-585, SJ-114, SJ-113, and SJ-452.

**Informed consent** All patients and healthy donors provided both written and oral informed consent on the use of their biological material for research and for publication. The consent was received before the sampling of biological material from the individual.

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