P1017

T-cell phenotyping supports the use of T-cell engaging antibodies for treatment of calreticulin mutated myeloproliferative neoplasms

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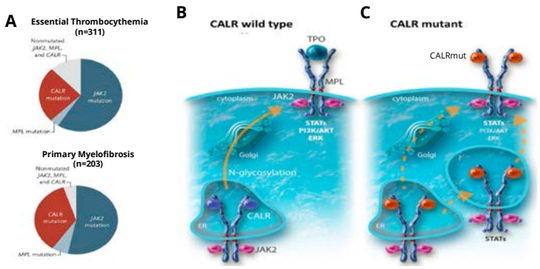
BACKGROUND

- Myeloproliferative Neoplasms (MPNs) are clonal disorders of hematopoiesis characterized by excessive production of mature blood cells of the myeloid lineage. Current treatment options for MPN patients are not curative. Identification of novel therapeutic approaches with a clear disease-modifying effect for the treatment of MPNs is an unmet medical need.
- Mutations in Janus Kinase 2 (JAK2), thrombopoietin receptor (TPOR, also known as MPL), and calreticulin (CALR) are phenotypic drivers in the pathogenesis of MPN. CALR mutations (CALRmut) are the second most frequent in MPN.
- CALRmut are insertions or deletions causing a frameshift in the last exon of the gene, resulting in loss of the endoplasmic reticulum (ER)-retention motif and generation of a 36 amino acid neoantigen. Due to loss of the ER-retention motif, CALRmut is not confined to the ER and is trafficked to the cell surface through interaction with MPL.
- The objective of this study was to phenotype T cells from CALRmut patients by using mass cytometry (CyTOF) and validate T-cell engagers (TCE) as a potential treatment modality



(A) Prevalence of JAK2, CALR, and MPL mutations across MPN in patients diagnosed with Essential Thrombocythemia and Primary Myelofibrosis[1,2]. (B,C) Thrombopoietin (TPO) receptor (MPL) signaling pathway in the presence of CALR wild type (B) or CALRmut (C)[3].

METHODS

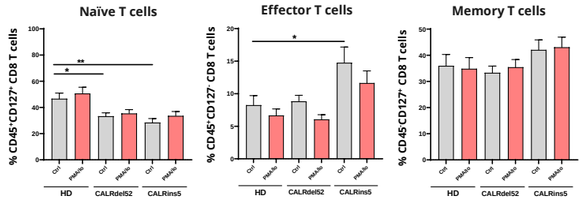
- Peripheral blood mononuclear cells from CALRmut patients and healthy donors were used for CyTOF based immune cell phenotyping. Phenotyping was assessed in the absence of treatment or after stimulation with Phorbol-12-myristate-13-acetate (PMA)/ionomycin.
- Functional assessment of CALRmut patients' T-cell fitness was also explored in vitro in the presence of CALRmutxCD3 bispecific TCE, JNJ-88549968.

Myeloid Malignancies

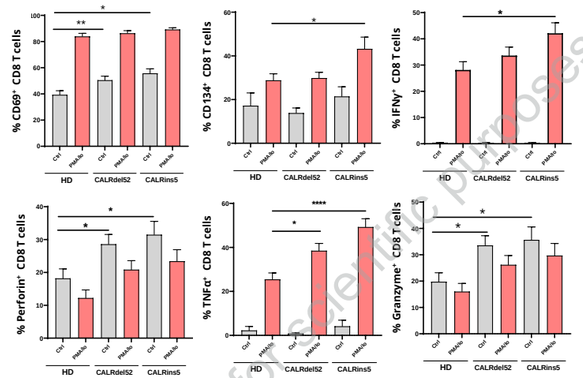
RESULTS

CyTOF-based phenotyping of T cells from CALRmut patients

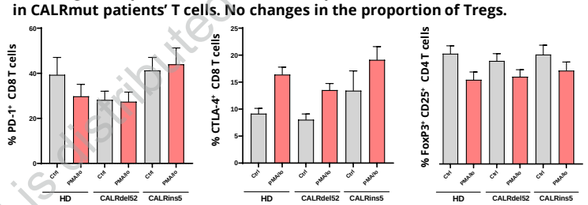
Population of naïve T cells are decreased and effector T cells are increased in CALRmut patients



T cells from CALRmut patients exhibit higher basal early T-cell activation, but retain the capacity to completely respond to PMA/ionomycin stimulus.



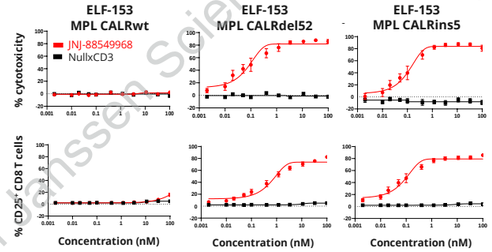
No change in expression of immune checkpoint markers was observed in CALRmut patients' T cells. No changes in the proportion of Tregs.



Phenotypic evaluation of T cells from healthy (n=16), and CALRmut MPN (CALRdel52, n=32, CALRins5 n=21) donors by CyTOF: PBMCs were rested before stimulating the cells with PMA/io (PMA, phorbol 12-myristate 13-acetate; io, ionomycin) for 4 hours, staining with metal-isotype antibodies, and acquisition by CyTOF. The data were averaged and the means ± ± standard error of the mean of the observed data are graphed. Statistical significance is annotated: * p<0.05; ** p<0.01, **** p≤ 0.0001

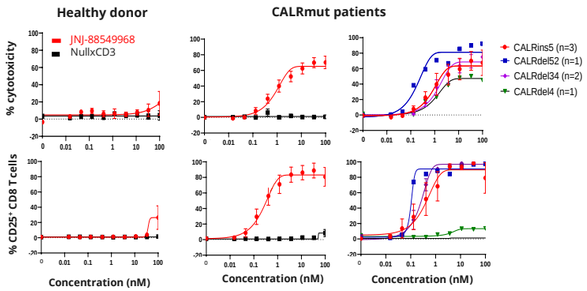
Evaluation of T cells from CALRmut patients for their T-cell mediated efficacy in presence of JNJ-88549968 (CALRmutxCD3)

JNJ-88549968 mediates CALRmut selective T-cell activation and cytotoxicity



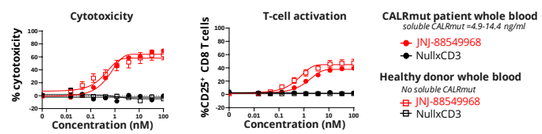
T cells from healthy donors were tested in T-cell cytotoxicity assays with the indicated antibodies and target cell lines. Cytotoxicity of cancer cells and T-cell activation was measured after 72 hours. The assay was conducted at an E:T ratio of 5:1.

JNJ-88549968 induces potent T-cell mediated cytotoxicity to CALRmut patient-derived CD34+ cells in autologous in vitro system, while sparing WT cells



CD34+ and T cells isolated from healthy donors (n=3) or CALRmut MPN patients (n=7) were tested in T-cell cytotoxicity assays with the indicated antibodies. Cytotoxicity of CD34+ cells was measured after 120 hours. The assay was conducted at an E:T ratio of 5:1. 3 out of 7 CALRmut MPN patients were treated with JAK1/2 inhibitor prior cell isolation.

Healthy donor and CALRmut patient derived T cells lead to comparable levels of JNJ-88549968-mediated tumor cell cytotoxicity in whole blood setting



Whole blood from healthy donors (n=6) or CALRmut patients (n=13) was tested in T-cell redirection assays with the indicated antibodies and ELF-153 MPL CALRins5+ target cell lines at an E:T ratio of 1:1. Cytotoxicity of cancer cells and T-cell activation was measured after 72 hours.

REFERENCES:

1. Klampfl T. et al., N Engl J Med (2013); 369:2379-2390; 2. Nangalia J, et al. N Engl J Med (2013);369:2391-405; 3. Vainchenker W and Kralovics R, Blood (2017); 129:667-679

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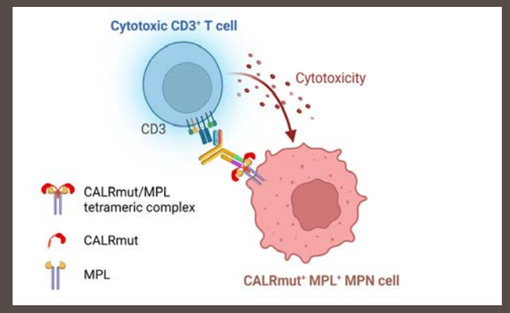
KEY TAKEAWAY

Phenotyping and functional assessment of T cells in the presence of JNJ-88549968 confirmed that T cells from CALRmut MPN patients are functional and can mediate cytotoxicity to CALRmut MPN clones.

These data validate T-cell engagers as a novel therapeutic modality for CALRmut MPN patients.



Schematic explaining mechanism of action: JNJ-88549968 is a T-cell engaging bispecific antibody that recognizes the CD3 antigen on T lymphocytes and CALRmut on an MPN clone. Schematic created with BioRender.

CONCLUSIONS

Immunophenotyping of T cells from CALRmut MPN patients revealed altered T cell populations compared to healthy controls. However, their functional activity was not substantially changed in the presence of stimulation.

Functional assays in the presence of CALRmutxCD3 bispecific T-cell engager, JNJ-88549968, confirmed fitness and functionality of T cells from CALRmut patients.

DISCLOSURES

Anna Kuchnio[1,2], Elsie Samakai[1], Eva Hug[3], Meritxell Balmaña[4], Lut Janssen[1], Ricardo Amorim[1], Ivo Cornelissen[1], Hussein Shehade[1], Yann Abraham[1,2], Andrea Majoros[3], Michaël Broux[1], Isha Taneja[1], Vince Torti[1], Laura Barreyro[1,2], Azra Agic[3], Stefan Moritsch[4], Filippo Benedetti[3], Felix Rosebrock[4], Kathryn Packman[1,2], Janine Arts[1,2], Sonal Patel[1], Ricardo Attar[1,2], Nikki Daskalakis[1,2], Oliver Lomas[1,2], M. Phillip DeYoung[1,2], Oleh Zagrijtschuk[4], Stefan N. Constantinescu[6,7], Robert Kralovics[8], Ulrike Philippar[1,2,8]

1. Current employment at Janssen
2. Current equity holder Janssen
3. Past employment at MyeloPro Diagnostics and Research GmbH
4. Current employment at MyeloPro Diagnostics and Research GmbH
5. Past employment at Janssen
6. Co-founder of MyeloPro Diagnostics and Research GmbH
7. Speakers Bureau of Medscape, SAB GSK JAK2 inhibitor
8. Membership on an entity's Board of Directors or advisory committees and Patent & Royalties

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