Abstract: P1017

Title: T-CELL PHENOTYPING SUPPORTS THE USE OF T-CELL ENGAGING ANTIBODIES FOR TREATMENT OF CALRETICULIN MUTATED MYELOPROLIFERATIVE NEOPLASMS

Abstract Type: Poster Presentation

Topic: Myeloproliferative neoplasms - Biology & translational research

Background:

Somatic driver mutations in calreticulin (CALR) are the second most common genetic aberration in Myeloproliferative Neoplasms (MPN), characterized by cytokine-independent activation of Janus kinase/signal transducer and activator of transcription signaling pathway, chronic inflammation and excessive production of mature cells of the myeloid lineage. Current treatment options for CALR-mutated (CALRmut) MPN patients are not curative. Immunotherapies engaging T-cells show promising response rates in hematological malignancies in the clinic. However, T-cell fitness has not been thoroughly investigated in MPN patients for their potential use as effector cells for T-cell engagers (TCE).

Aims:

The objective of this study was to phenotype T cells from CALRmut patients by using mass cytometry (CyTOF) and validate TCE as a potential treatment modality.

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.

Results:

CyTOF-based profiling revealed an increased proportion of effector T cells in CALRmut patients compared to healthy counterparts. In line with these findings, T cells from CALRmut patients had higher basal early T-cell activation. They showed elevated baseline levels of perforin and granzyme B and retained the full capacity to produce intracellular IFNγ and TNFα in the presence of PMA/ionomycin stimulus. The proportion of PD1+ and CTLA4+ CD8+ T cells, as well as of immunosuppressive Tregs in MPN patients was comparable to healthy controls.

In vitro functional assessment of CALRmut patient T cells was done in the presence of CALRmutxCD3 bispecific TCE (JNJ-88549968), which demonstrated CALRmut-selective binding and T-cell mediated cytotoxicity to CALRmut-engineered cells. In an *ex vivo* autologous setting using CD34+ and CD3+ T cells isolated from peripheral blood of CALRmut patients, JNJ-88549968 elicited concentration-dependent cytotoxicity of patient-derived CALRmut CD34+ cells independent of the type of CALR mutation. In addition, T-cell cytotoxicity assays were conducted in a more physiologically relevant setting using CALRmut patient-derived whole blood in presence of CALRmut cancer cells. Whole blood from healthy donors (HDs) was used as a control condition. Similar activity of JNJ-88549968 on T-cell activation and T cell-mediated cytotoxicity to CALRmut cancer cells was observed in CALRmut patient-derived whole-blood and HD whole blood, further confirming that T cells from CALRmut patients can function as effector cells to mediate JNJ-88549968 activity.

Summary/Conclusion:

Phenotyping and functional assessment of T cells from CALRmut patients in the presence of JNJ-88549968 confirmed that CALRmut MPN T cells are functional and can mediate cytotoxicity to CALRmut MPN clone. Taken together, these data validate TCE as a novel therapeutic modality for CALRmut MPN patients. Phase 1

clinical trial of JNJ-88549968, a novel first-in-class CALRmutxCD3 TCE, is ongoing (NCT06150157).

Keywords: Myeloproliferative disorder, Antibody, Bispecific