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Review

Role of Next Generation Immune Checkpoint Inhibitor (ICI) Therapy in Philadelphia Negative Classic Myeloproliferative neoplasm (MPN): Review of the Literature

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Abstract: The classic, chronic Philadelphia chromosome-negative (Ph-) myeloproliferative neoplasms (MPN) comprises mainly essential thrombocythemia (ET), polycythemia vera (PV), and myelofibrosis (MF) that represent a group of stem cell disorders associated with clonal proliferation of one or more hematopoietic cell lineages with organomegaly and constitutional symptoms. Several studies have shown that the presence of dysregulated immune system and chronic inflammation play important roles in the pathogenesis of these diseases. Lately, the treatment of cancer including hematological malignancy has progressed on the agents aiming for the immune system, cytokine environment, immunotherapy agents, and targeted immune therapy. Immune checkpoints are the molecules that regulate T cell function in the tumor microenvironment (TME). The first line of primary immune checkpoints are programmed cell death-1 (PD-1)/programmed cell death ligand-1 (PD-L1), and cytotoxic T-lymphocyte antigen-4 (CTLA-4). Immune checkpoint inhibitor therapy (ICIT) exerts its anti-tumor actions by blocking the inhibitory pathways in T cells and has reformed cancer treatment. Despite the impressive clinical success of ICIT, tumor internal resistance poses a challenge for oncologists leading to a low response rate in solid tumors and hematological malignancies. A phase II trial on Nivolumab for patients with post-Essential thrombocythemia myelofibrosis, Primary Myelofibrosis, or post-Polycythemia myelofibrosis was performed (ClinicalTrials.gov Identifier: NCT02421354). This trial tested the efficacy of a PD-1 blockade agent, namely Nivolumab, but was terminated prematurely due to adverse events and lack of efficacy. A multicenter, phase 2, single-arm open-label study was conducted including pembrolizumab in patients with primary thrombocythemia, post-essential thrombocythemia or post-polycythemia vera myelofibrosis that were ineligible for or were previously treated with Ruxolitinib. The study showed that Pembrolizumab treatment did not have many adverse events, but there were no pertinent clinical responses hence it was terminated after the first stage was completed. To avail the benefits from immunotherapy, the paradigm has shifted to new immune checkpoints in the TME such as lymphocyte activation gene-3 (LAG-3), T cell immunoglobulin and mucin domain 3 (TIM-3), T cell immunoglobulin and ITIM domain (TIGIT), V-domain immunoglobulin-containing suppressor of T-cell activation (VISTA), and human endogenous retrovirus-H long terminal repeat-associating protein 2 (HHLA2) forming the basis of next-generation ICIT. Our article aims at emphasizing and discovering the role of next-generation ICIT in MPN involving monoclonal antibodies as targeted immunotherapy, or vaccines against selected MPN epitopes that could further highlight the tumor-related immune responses. Therapeutic approaches based on immunotherapy are expanding and hopefully will expand the treatment regimens in patients with MPN. Our laboratory had reported that MDSC were increased in MPN, now we in preliminary studies, we showed that over-expressed VISTA in MDSC, and in progenitor and immune cells which may form the basis for clinical trials using next-generation ICI in the treatment of MPN.

Keywords: Checkpoint Inhibitor; Philadelphia Chromosome; Myeloproliferative Neoplasm

Introduction

The MPN is defined by the clonal proliferation of one or more hematopoietic cell lineages [1]. As per International Consensus Classification [2] (ICC) and World Health Organization (WHO), 5th edition [3] classic MPN comprises mainly myelofibrosis (MF), essential thrombocythemia (ET), and polycythemia vera (PV) [4]. Lately, the focus of the treatment of MPN is based on the agents targeting the immune system, cytokine environment, and immunotherapy agents. The pathogenesis of MPN is not very clear but studies have shown that TNF- α promotes the growth of JAK2V617F-positive MPN cells as compared to controls contributing to clonal formation of mutant copies during MPN progression [5]. Various driver mutations as studied by genetic sequencing and clonal protein expression showed that tyrosine kinase Janus Kinase 2 -JAK2V617F mutation was found in 95% of patients with PV and 50%- 60% of patients with ET and MF [6,7]. These mutations stimulate Janus Kinase and Signal Transducer and Activator of Transcription proteins (JAK-STAT) signaling path of thrombopoietin receptor and erythropoietin receptor [8]. Concurrently profound immune dysregulation and defective immune surveillance also have an important role in the pathogenesis of MPN [9]. The dysregulated genes related to the immune system and inflammation that are implicated in MPN are interferon-inducible gene [10], regulatory T cells (Tregs characterized as CD4 + CD25+ FOXP3+) [11], human leukocyte antigen (HLA) class I and II molecules, natural killer cells, β 2-microglobulin, HLA I antigens (such as LMP7, LMP2, TAP1/2, tapasin) [10,12] and antioxidative stress genes (ATM, TP53, CYBA, NRF2, PTGS1, SIRT2) [13,14]. In addition, increased recruitment of suppressive cells, such as myeloid-derived suppressor cells (MDSC), leads to the escape of tumor cells from immune surveillance thus playing an important part in the etiopathogenesis of MPN [15]. The key events involved in the development of the neoplastic process are oncogenic transformation and immune escape allowing for uncontrolled proliferation and avoidance of apoptosis. Immune checkpoint inhibitory therapy (ICIT) is based on blocking the T cells inhibitory pathways thus promoting anti-tumor immune responses. Oncogenic JAK2 activation results in high expression of programmed death-ligand 1 (PD-L1) on the surface of megakaryocytes, monocytes, platelets, and MDSC which is mediated via the JAK2-STAT3 and JAK2-STAT5 axes [16]. Myeloid malignancies are found to have overexpressed PD-1 pathways and that has gained immense attention recently as pathbreaking therapeutic targets for immunotherapy. One such trial was: ClinicalTrials.gov Identifier: NCT02421354 where the safety and efficacy of nivolumab (PD-1 inhibitor) was tested in eight adult patients with myelofibrosis [17]. However, the study was discontinued due to failure to meet the efficacy endpoint. In 2020, at the annual meeting of American Society of Hematology (ASH), an open label, phase 2, multi-center, single-arm study of pembrolizumab was presented showing its use in patients with primary, post-essential thrombocythemia or post-polycythemia vera MF (NCT03065400) [18]. Nine cases were included, but none showed a clinical response.

The use of ICI in hematological malignancies brings a daunting challenge with a low response rate thus letting the oncologist/molecular physicians change their attention to focus deeply on the TME for novel therapeutic targets. To benefit more patients from immunotherapy, the paradigm has shifted to target alternative new immune checkpoints in the TME such as LAG-3, TIM-3, TIGIT, VISTA, and HHLA2 forming the basis of next-generation ICIT [19] as shown in Figure 1. Our review article aims at emphasizing and discovering the role of next-generation ICIT in MPN involving monoclonal antibodies as targeted immunotherapy or novel inhibitory checkpoints that would further broaden the horizon of tumor-specific immune responses and treatment.

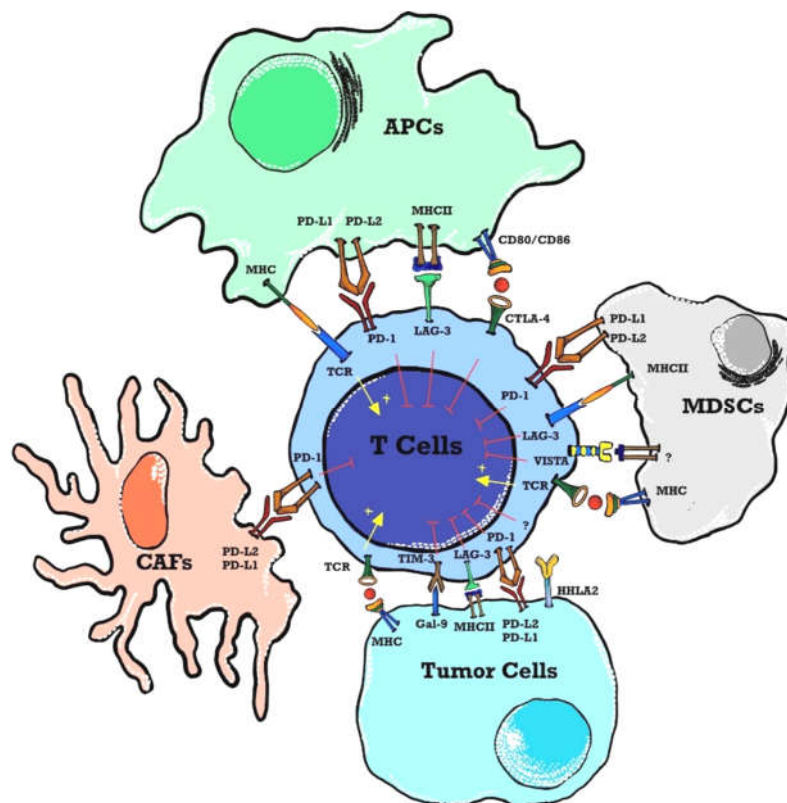


Figure 1. Immune checkpoints in a tumor microenvironment (TME). APCs present tumor antigens to naïve T cells inducing T cell activation. Through the MHC and TCR signaling pathway a first signal for T-cell activation is provided whereas co-inhibitory immune checkpoints suppress T-cell activation in TME. Immune checkpoints are expressed on T-cells, ligands are present on APCs, tumor cells, and stromal cells like CAFs and MDSCs. Abbreviations: APCs - antigen presenting cells; MHC – major histocompatibility complex; TCR – T-cell receptor; TME – tumor microenvironment; MDSCs – myeloid-derived suppressor cells; PD-1 – programmed death 1; PD-L2 – programmed cell death ligand-2; VISTA – V-domain immunoglobulin-containing suppressor T-cell activation; HHLA2 – human endogenous retrovirus-H long terminal repeat-associated protein 2; TIM-3 – T-cell immunoglobulin and mucin domain 3; Gal-9 – Galectin-9; CAFs – cancer associated fibroblasts; LAG-3 – lymphocyte activated gene-3; CTLA-4 – cytotoxic T-lymphocyte antigen-4.

LAG-3 targeted therapy and its role in hematological malignancies

LAG-3 (CD223) is a CD4-associated activation-induced cell surface inhibitory receptor that binds to major histocompatibility complex (MHC) class II molecules and negatively regulates T-cell effector functions [20]. Cells expressing LAG-3 are T cells, a few activated B cells, plasmacytoid dendritic cells (DCs), and neurons [21]. LAG-3 ligands are MHC class-II, galectin-3, and fibrinogen-like protein 1 (FGL1) with MHC-II being the main ligand [22]. LAG-3 attaches to MHC class II with higher affinity than CD4 inducing protein phosphorylation of phospholipase Cγ2 (PLCγ2) and p72syk as well as activation of phosphatidylinositol 3-kinase/Akt, p42/44 extracellular protein kinase, and p38 mitogen-activated protein kinase pathways [23]. Galectin-3 is expressed on activated T cells and tumor cells that are needed for CD8/T-cell and plasmacytoid DC suppression [22]. FGL1 is highly produced by human cancer cells and binding of LAG-3 with FGL1 contributes to resistance /poor response to anti-PD-1/anti PD-L1 immunotherapies [24,25]. This mechanism forms the basis of therapies involving simultaneous blockade of PD-1 and LAG-3 responsible for several T-cell antitumor activities [26–28].

Currently, sixteen LAG-3 targeted immunotherapies are being tested at approximately 97 clinical trials by Bristol-Myers Squibb (BMS-986016), Regeneron Pharmaceuticals (REGN3767 and 89Zr-DFO-REGN3767), Merck (MK-4280), Novartis (LAG525), Tesaro (GSK) (TSR-033), Symphogen

(Sym022), GlaxoSmith (GSK2831781), Incyte Biosciences International Sàrl (INCAGN02385), Prima BioMed/Immutep (IMP321), MacroGenics (MGD013), F-Star (FS118), Hoffmann-La Roche (RO7247669), Shanghai EpimAb Biotherapeutics (EMB-02), Xencor (XmAb841) and Innovent Biologics (IBI323) [29]. LAG-3 targeted therapies are divided into three categories namely monoclonal antibodies, LAG-3 –immunoglobulin fusion proteins, and anti-LAG-3 bispecific drugs [29]. Most trials are phase I/II with two of them reaching phase III including BMS-986016 (NCT05002569) [30] and MK-4280 drugs (NCT05064059) [31]. Table 1 demonstrates the use of LAG-3 agents in hematological malignancies in the current clinical trials.

Table 1. Summarizes all the clinical trials of LAG-3 therapy being used in hematological or related malignancies.

NCT number	Sponsors / collaborators	Title	Drug name	Phase	Tumor / disease	Status	Outcome measures	ICI type	Study Designs
NCT04566978	Memorial Sloan Kettering Cancer Center	89Zr-DFO-REGN3767 in PET Scans in People With Diffuse Large B Cell Lymphoma (DLBCL)	Drug: 89Zr-DFO-REGN3767 Diagnostic Test: PET/CT	Early Phase 1	Large B-cell Lymphoma DLBCL	Recruiting	Biodistribution of 89Zr-DFO-REGN3767 Optimal 89Zr-DFO-REGN3767 mass dose for tumor targeting Optimal time for imaging and tumor uptake post 89Zr-DFO-REGN3767 administration Tumor lesion uptake of 89Zr-DFO-REGN3767 and correlate with LAG-3 expression by IHC	LAG-3	Allocation: Randomized Intervention Model: Sequential Assignment Masking: None (Open Label) Primary Purpose: Diagnostic
NCT03310619	Celgene	A Safety and Efficacy Trial of JCAR017 Combinations in Subjects With Relapsed/Refractory B-cell Malignancies (PLATFORM)	Biological: JCAR017 Drug: Durvalumab Drug: CC-122 Drug: Ibrutinib Drug: CC-220 Drug: Relatlimab Drug: Nivolumab Drug: CC-99282	Phase 1 Phase 2	Lymphoma, Non-Hodgkin Lymphoma, Large B-Cell, Diffuse Lymphoma, Follicular	Completed	Dose-limiting toxicity (DLT) rates Complete Response Rate Adverse Events (AEs) Progression-free survival (PFS) Overall survival (OS) Overall response rate (ORR) Duration of response (DOR) Event-free survival (EFS) Pharmacokinetic (PK)- Cmax Pharmacokinetic (PK)- Tmax Pharmacokinetic (PK)- AUC Health-related quality of life (HRQL) Quality of Life C30 questionnaire (EORTC-QLQ-C30) European Quality of Life-5 Dimensions health state classifier to 5 Levels (EQ-5D-5L)	LAG-3	Allocation: Randomized Intervention Model: Parallel Assignment Masking: None (Open Label) Primary Purpose: Treatment
NCT02061761	Bristol-Myers Squibb	A Phase 1/2a Dose Escalation and Cohort Expansion Study of the Safety, Tolerability, and Efficacy of Anti-LAG-3 Monoclonal Antibody (Relatlimab, BMS-986016) Administered Alone and in Combination With Anti-PD-1 Monoclonal Antibody (Nivolumab, BMS-936558) in Relapsed or Refractory B-Cell Malignancies	Biological: BMS-986016 Biological: BMS-936558	Phase 1 Phase 2	Hematologic Neoplasms	Completed	Safety measured by the rate of Adverse events (AEs), Serious Adverse events (SAEs), death and laboratory abnormalities [Time Frame: Up to approximately 2.3 years]	LAG-3	Allocation: Non-Randomized Intervention Model: Single Group Assignment Masking: None (Open Label) Primary Purpose: Treatment
NCT03598608	Merck Sharp & Dohme LLC	A Phase 1/Phase 2 Clinical Study to Evaluate the Safety and Efficacy of a Combination of MK-4280 and Pembrolizumab (MK-3475) in Participants With Hematologic Malignancies	Biological: pembrolizumab Biological: Favezelimab	Phase 1 Phase 2	Hodgkin Disease Lymphoma, Non-Hodgkin Lymphoma, B-Cell	Recruiting	Percentage of Participants Experiencing a Dose-limiting Toxicity (DLT); Percentage of Participants Experiencing an Adverse Event (AE); Percentage of Participants with Treatment Discontinuations Due to an AE	LAG-3	Allocation: Non-Randomized Intervention Model: Parallel Assignment Masking: None (Open Label) Primary Purpose: Treatment
NCT04913922	Ludwig-Maximilians - University of Munich	An Open-Label Phase II Study of Relatlimab (BMS-986016) With Nivolumab (BMS-936558) in Combination With 5-Azacytidine for the Treatment of Patients With Refractory / Relapsed Acute Myeloid Leukemia and Newly Diagnosed Older Acute Myeloid Leukemia Patients	Drug: Azacitidine Injection Drug: Nivolumab Drug: Relatlimab	Phase 2	Acute Myeloid Leukemia	Recruiting	Maximum tolerated dose (MTD) ; Dose-limiting toxicities (DLTs); Objective response rate (ORR)	LAG-3	Allocation: N/A Intervention Model: Single Group Assignment Masking: None (Open Label) Primary Purpose: Treatment
NCT03865791	Novartis Pharmaceuticals	Modular Phase 2 Study to Link Combination Immune-therapy to Patients With Advanced Solid and Hematologic Malignancies. Module 9: PD001 Plus LAG525 for Patients With Advanced Solid and Hematologic Malignancies.	Biological: PD001; Biological: LAG525	Phase 2	Small cell lung cancer, Gastric/esophageal adenocarcinoma, Castration resistant prostate adenocarcinoma (CRPC), Soft tissue sarcoma, Ovarian adenocarcinoma, Advanced well-differentiated neuroendocrine tumors, Diffuse large B cell lymphoma (DLBCL).	Completed	Clinical Benefit Rate (CBR) at 24 Weeks of PD001+LAG525 by Tumor Type in Multiple Solid Tumors and Lymphoma	LAG-3	Allocation: N/A Intervention Model: Single Group Assignment Masking: None (Open Label) Primary Purpose: Treatment

1. Use of ⁸⁹Zr-DFO-REGN3767 in PET Scans in people with diffuse large B Cell lymphoma (DLBCL) was the pilot study (NCT04566978) [32] undertaken at Memorial Sloan Kettering Hospital in 2022 with the main purpose of the study is looking at the way the body absorbs, distributes, and gets rid of ⁸⁹Zr-DFO-REGN3767 [33]. ⁸⁹Zr-DFO-REGN3767 is comprised of the anti-LAG-3 antibody, REGN3767 labeled with the positron-emitter zirconium-89 (⁸⁹Zr) through the chelator-linker DFO and REGN3767 is an investigational monoclonal antibody that targets LAG-3 receptors. This study is a diagnostic research study determining the optimal time for imaging and tumor uptake post ⁸⁹Zr-DFO-REGN3767 administration. However, it can help evaluate tumor uptake of ⁸⁹Zr-DFO-REGN3767 and correlate with expression of LAG-3 by immunohistochemistry (IHC) in tumors that will be subsequently compared with other biomarkers of TME characterized in biopsies, such as IHC score (LAG-3 and/or other immune cell markers).
2. A safety and efficacy trial of JCAR017 (lisocabtagene maraleucel, also known as liso-cel) (a CD19-targeted chimeric antigen receptor CART-cell therapy) combinations in subjects with relapsed / refractory B-cell malignancies (PLATFORM) (NCT03310619) [34] was done. Relatlimab, BMS-986016 is an anti-LAG-3 fully human monoclonal IgG4-κ antibody that binds human LAG-3 with high affinity and inhibits its binding to MHC-II [35]. This trial was a global, open-label, multi-arm, parallel multi-cohort, multi-center, Phase 1/2 study to determine the safety, tolerability, pharmacokinetics, efficacy, and patient-reported quality of life of JCAR017 in combination with various agents including relatlimab, durvalumab, avadomide, iberdomide, ibrutinib, and nivolumab. The trial was completed, and the studied tumors were diffuse large B-cell lymphoma (DLBCL), non-Hodgkin lymphoma (NHL), and Follicular lymphoma (FL). The objective of the study during Phase 1 was to open different paths to test JCAR017 in combination with other agents in adult patients with R/R aggressive B-cell NHL. Different doses and schedules of JCAR017 were used in several arms and the combination agents were tested in several cohorts per arm. Phase 2 of the study involved the expansion of any dose level and schedule for any arm maintaining safety. All patients from Phase 1 and Phase 2 will then be followed for 24 months for adverse effects, survival, relapse, viral vector safety, and long term toxicity as per guidelines.
3. A similar trial was also designed with relatlimab by Bristol-Myers Squibb, NCT02061761 [36] administered alone or in combination with nivolumab to subjects with relapsed or refractory B-cell malignancies (relapsed or refractory Hodgkin lymphoma (HL) and relapsed or refractory DLBCL and to study its safety, tolerability, dose-limiting toxicities and maximum tolerated dose. The trial completed and studied hematological malignancies including chronic lymphocytic leukemia (CLL), HL, NHL, and Multiple Myeloma (MM). A detailed description of dose-related adverse events was studied and was displayed in the result section of the trial.
4. Favezelimab (MK-4280) is another LAG-3 antibody that is studied in combination with pembrolizumab (MK-3475) in the clinical trial NCT03598608 [37] that was started in July 2018 to study and evaluate the safety and efficacy of these agents in hematologic malignancies.). It included classical HL, DLBCL, and indolent HL. No results have been posted till the writing of this article. This study will also evaluate the safety and efficacy of pembrolizumab or favezelimab administered as monotherapy in participants with classical HL using a 1:1 randomized study design.
5. Relapsed or refractory acute myeloid leukemia (AML) and newly diagnosed older AML are included in the ClinicalTrials.gov Identifier: NCT04913922 [38] to study the combination of relatlimab with nivolumab and 5-azacytidine. No results have been posted yet.

All the above trials included LAG-3 as an ICI agent in the above-mentioned hematological malignancies, however, no trials have been done in the field of MPN. We unfold the mechanism of action of LAG-3 to provide a better understanding of its potential use in the future as depicted in Figure 2.

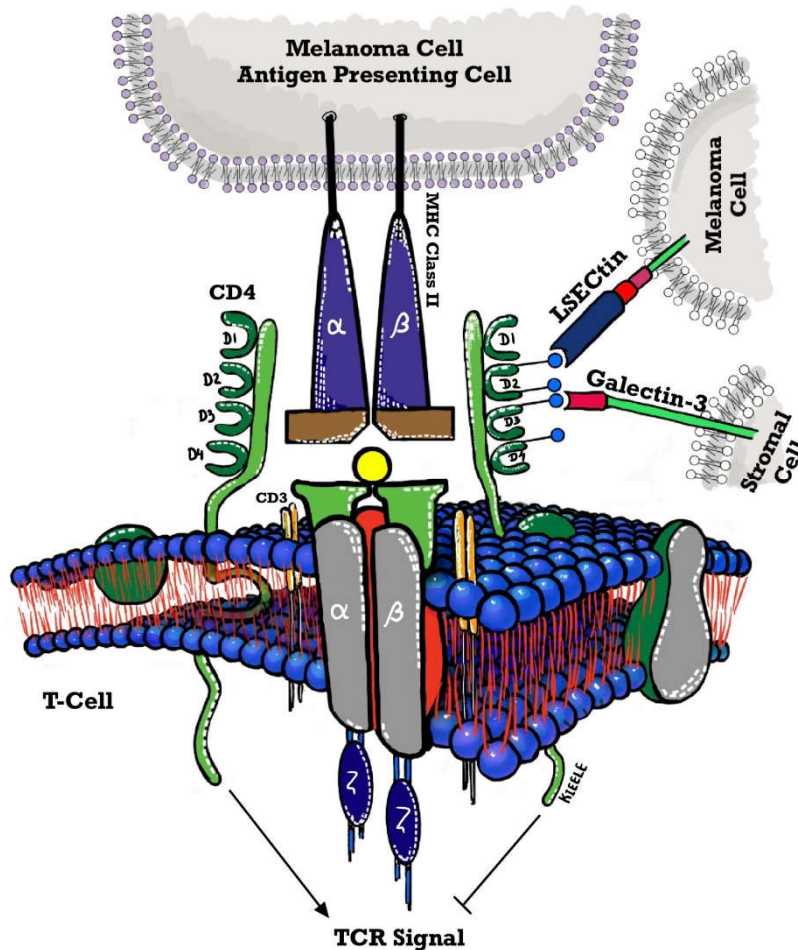


Figure 2. Structural similarities between LAG-3 and CD4. The LAG-3 gene is predicted to be highly structurally homologous to CD4 with four extracellular immunoglobulin superfamily (IgSF)-like domains (D1-D4). LAG-3 binds to MHC class II with high affinity. LAG-3 cytoplasmic domain appears to have three well-defined motifs namely serine-based motif which could act as a PKC substrate, repetitive “EP” motif consisting of a series of glutamic acid-proline dipeptide repeats and relatively unique “KIEELE” motif, highlighted by an essential lysine residue. LAG3 has two additional ligands namely LSEctin expressed on melanoma cells and Galectin-3 expressed on stromal cells and CD8+T cells in TME. Abbreviations: TCR -Toll like recepto.

Mechanism of action of LAG-3

LAG-3 was discovered in 1990, by Triebel and colleagues, as a new 498-amino acid type I transmembrane protein present on activated natural killer (NK) and T cell lines [39]. The LAG-3 gene is found close to CD4 on chromosome 12 in humans (chromosome 6 in mice) displaying structural homology to CD4 with extracellular immunoglobulin superfamily (IgSF)-like domains namely D1–D4 [40]. The structural motifs are conserved between LAG-3 and CD4, translating to the same extracellular folding patterns as a result of which LAG-3 can bind with greater affinity to MHC class II than CD4 [41]. LAG-3 was speculated to be spatially related to the T-cell receptor TCR: CD3 complex present in microdomains of lipid raft promoting clustering of signaling molecules and the development of the immunological synapse however the exact mechanism is still unclear [42]. The cytoplasmic tail for the tyrosine kinase p56^{Lck}, lacks a binding site for LAG-3, which is normally used by CD4 to promote downstream signal transduction of the T cell receptor (TCR) [41]. Conversely, the LAG-3 cytoplasmic domain has three well-defined motifs namely serine-based motif acting as a PKC substrate, repetitive “EP” motif comprising of a series of glutamic acid-proline dipeptide repeats, and

relatively unique “KIEELE” motif, highlighted by an essential lysine residue [43,44]. LAG-3 cytoplasmic tailless mutants neither mediate the inhibitory effects of LAG-3 nor compete with CD4, emphasizing the importance of the function of this domain needed for the transmission of an inhibitory signal [20]. Expression of MHC class II molecules by human melanoma cells is correlated with poor prognosis thus, LAG-3 ligation with MHC-II class, which is seen on melanoma-infiltrating T cells, may facilitate their clonal exhaustion [45]. *In vitro*, the demonstration showed that such an interaction may help tumor cells to adopt an escape mechanism giving them protection against apoptosis, with a recent study showing that MHC class II-expressing melanoma cells causes infiltration of tumor-specific CD4⁺ T cells, mediated by interaction with LAG-3, which in turn negatively regulates CD8⁺ T cell responses [46,47.] Galectin-3 is a ligand, that is expressed by several cells within the TME but not the tumor itself, facilitating interaction with LAG-3 (present on tumor-specific CD8⁺ T cells) that may regulate anti-tumor immune activities [48]. Liver sinusoidal endothelial cell lectin (LSECL) is present in the liver as well as identified in melanoma tumor cells where it stimulates growth by inhibiting anti-tumor T-cell dependent responses [49]. The interaction between LSECL in melanoma cells and LAG-3 inhibited IFN γ production, mediated by effector T cells (antigen-specific), altering the TME [49]. Continuous T cell activation in an inflammatory state, specifically in a tumor, results in persistent co-expression of LAG-3 on T cells along with additional inhibitory receptors (IR) such as PD1, TIGIT, TIM3, CD160, 2B4 leading to T cell dysfunction [50]. Several hematopoietic cell types, including CD11c^{low} B220⁺ PDCA-1⁺ plasmacytoid dendritic cells (pDCs) constitutively express LAG-3 [51] however it is not expressed on any myeloid or lymphoid DC subset. *In vitro*, MHC class II-expressing melanoma cells could stimulate LAG-3 positive pDCs to mature and produce IL-6 which was later confirmed *in vivo* as well with LAG-3 positive pDCs showing increased IL-6 production and an activated phenotype similar to melanoma cells [52]. Bo Huang et al showed that increased IL-6 promotes the release of CCL2 by monocytes *in vitro*, which then may recruit MDSCs thus forming the hypothesis that LAG-3 positive pDCs may indirectly mediate MDSC-related immunosuppression by engaging MHC class II⁺ melanoma cells [53]. LAG-3 functions are regulated by cell surface cleavage mainly ADAM10 and ADAM17 disintegrin/metalloproteases, although in mice soluble LAG-3 seems to have no biological function [54].

V-domain immunoglobulin suppressor of T cell activation (VISTA) targeted therapy and its role in hematological malignancies

VISTA (also known as B7-H5, PD-1H, DD1 α , c10orf54, VSIR, SISP1, Gi24, and Dies1) is primarily expressed in myeloid cells mainly microglia, and neutrophils followed by macrophages, monocytes, and dendritic cells [55,56]. Additionally, it is highly expressed on new CD4⁺ and Foxp3⁺ regulatory T cells [57]. VISTA is a type I transmembrane protein consisting of a single N-terminal immunoglobulin V-domain that has the greatest homology with PD-L1 [58]. The exact function and role of VISTA in regulating the immune system are still complex and not very clear. It works both as a ligand expressed on antigen-presenting cells and as a receptor on T cells [59]. To date, various studies have described the inhibitory effect of VISTA on the immune system and the ability of VISTA-deficiency or anti-VISTA treatment to upregulate immune responses [60]. Due to its predominant expression on macrophages, VISTA is implicated as a potential immunotherapeutic target in melanoma [61]. Studies claim that melanoma survival correlates with PD-L1/VISTA expressions [62,63]. Furthermore, tumor cell expression of VISTA, which is regulated by factor forkhead box D3 (FOXD3), encourages tumorigenesis and promotes PD-L1 expression on tumor-infiltrating macrophages *in vivo* along with increased intra-tumoral T regulatory cells [62]. VISTA is expressed on MDSCs in the peripheral circulation, with a strong positive association between MDSC expression of VISTA and T cell expression of PD-1 in acute myeloid leukemia (AML) patients, although there is no evidence of direct regulation [64,65]. MDSCs are myeloid cells that are defined into subsets namely monocytic MDSCs (CD15⁻) and granulocytic MDSCs (CD15⁺) [66]. Patients with AML displayed increased expression of VISTA on MDSCs highlighting the role of VISTA in MDSC-mediated CD8 T cell response [64]. There is conflicting evidence with some studies supporting that VISTA is an immune checkpoint marker expressed on tumor-infiltrating T lymphocytes and myeloid cells,

causing suppression of T cell activation, proliferation, and cytokine production [67,68] whereas other studies have shown that VISTA is overexpressed in tumor cells and may functions as a co-stimulatory molecule [69,70].

Currently, clinical trials of VISTA-targeted cancer immunotherapy are in progress namely ClinicalTrials.gov Identifier: NCT02671955 [71] and ClinicalTrials.gov Identifier: NCT02812875 [72]. JNJ-61610588 (CI-8993) [71] is a human monoclonal antibody against VISTA with negative checkpoint regulatory and antitumor activities that is being studied in advanced cancer patients. No study results have yet been posted. Meanwhile, a study of CA-170 [72], an inhibitory molecule that selectively aims for PD-L1 and VISTA, is still currently being conducted in advanced solid tumors or lymphomas, although the trial is not recruiting any more subjects and the last update was posted on May 6, 2019. There are pre-clinical trials of VISTA mentioned in hematological cancer and solid tumors involving IGN-381 (mAbs by Ingenica Biotherapeutics) and HMBD-002 (mAbs by Hummingbird Bioscience) [73]. HMBD-002 exerted significant inhibitory effects on tumor progression and its combination with anti-PD-L1 was found to be more effective in tumors that showed abundant MDSC infiltration [74]. Table 2 summarizes the potential clinical trials of VISTA in hematological malignancies.

Table 2. Summarizes the potential clinical trials of VISTA in hematological malignancies.

NCT number	Sponsors / collaorators	Title	Drug name	Phase	Tumor / disease	Status	Outcome measures	ICI type	Study Designs
NCT02812875	Curis, Inc.	A Phase 1, Open-Label, Dose Escalation and Dose Expansion Trial Evaluating the Safety, Pharmacokinetics, Pharmacodynamics, and Clinical Effects of Orally Administered CA-170 in Patients With Advanced Tumors and Lymphomas	Drug: CA-170	Phase 1	Advanced Solid Tumors or Lymphomas	Completed	The number of patients with a dose-limiting toxicity (DLT) in the first treatment cycle; Maximum tolerated dose (MTD) of CA-170 ; Recommended Phase 2 Dose (RP2D) of CA-170	VISTA	Allocation: N/A Intervention Model: Single Group Assignment Masking: None (Open Label) Primary Purpose: Treatment
Preclinical stage	IGN-381 (mAb); Igenica Biotherapeutics	Hematological cancer and solid tumors						VISTA	
Preclinical stage	HMBD-002; Hummingbird Bioscience	Hematological cancer and solid tumors						VISTA	

Tumor and Immune System Interaction Database (TISIDB) [69,75] analyzed the potential relevance of VISTA in cancer immunity across 30 different cancer types and the outcomes were: 1) Almost all types of TILs, with tumor-suppressing or tumor-promoting functions across 30 types of cancers, correlated positively with VISTA expression, including activated CD8 T cells, NK cells, MDSC, and T_{regs} cells 2) VISTA expression levels correlated positively with almost all significant immunomodulators including immune inhibitors, immunostimulators, or MHCs, including but not limited to, the critical immune checkpoints such as PD-L1, PD-1, CD80, and CD86. 3) Additionally, VISTA expression correlated positively with almost all well-known chemokines and their receptors, including but not limited to CXCL1, CXCL8, CXCL10, and CXCR3. VISTA can function as a receptor as well as a ligand interacting with distinct partners modulating immune response. VISTA modulator is a promising target, and its mechanism is worthy of further investigation specifically in hematological cancers including MPN. Targeting VISTA may promote releasing suppression by myeloid cells leading to improve T cell-focused therapies like anti-PD1 and anti-CTLA4 especially when monotherapy resistance of other ICIT appears.

Role of T cell immunoglobulin and mucin domain 3 (TIM-3) as next-generation ICI in hematological malignancies

TIM-3 is a type I transmembrane protein that was discovered on CD4⁺ type 1 helper T cells (TH1 cells) and CD8⁺ cytotoxic T cells (CTLs) [76]. Subsequently, other T-cell subtypes also expressed TIM-3 along with other immune cells including DCs, NK cells, macrophages, monocytes, mast cells, and some malignant cells [77–80]. TIM-3 is pertinently expressed on DCs and macrophages in both humans and mice, specifically in humans where it suppresses IL-12 expression [81,82]. TIM-3 inhibits DCs cell activation and maturation by blocking NF- κ B signaling via a Btk-c-Src signaling-dependent mechanism, interfering with the ability of cytoplasmic toll-like receptors (TLRs) to sense immunogenicity and thereby suppressing anti-tumor immunity [83]. There are four known ligands for TIM-3 namely galectin-9 [84] - which induces apoptosis in TH1 cells, High-mobility group protein B1 (HMGB1) [85] – also called “alarmin”, released from damaged cells and activates phagocytes, phosphatidylserine (PtdSer) [86]- “eat-me” signal induction molecule and carcinoembryonic antigen cell adhesion molecule 1 (CEACAM-1) [87] – known for both *cis* and *trans* interactions with TIM-3. Studies claim that interactions between TIM-3 with its ligands (galectin-9 and Ceacam-1) lead to phosphorylation of tyrosine residues namely Y₂₅₆ and Y₂₆₃, stimulating the release of HLA-B associated transcript 3 (Bat3) from the tail, thereby enhancing T cell inhibitory function by allowing binding of SH2 domain-containing Src kinases and subsequent regulation of TCR signaling [88,89]. Studies have reported that a higher expression of TIM-3 poses a higher risk for myelodysplastic syndrome (MDS) transformation to leukemia as increased levels of TIM-3 and Gal-9 are reported on bone marrow cells and MDSCs from MDS patients [90,91]. This highlights the role of the TIM-3/Gal-9 axis in the blast proliferation, induction of immune escape, and T cell exhaustion supporting disease progression [92]. Bruck et al reported TIM-3 overexpression on exhausted CD4⁺ and CD8⁺ T cells in untreated chronic myeloid leukemia (CML) patients and observed a correlation between PD-1 positive TIM-3 CD8⁺ T cells along with a poor response to Tyrosine kinase inhibitors (TKIs) [93]. Dysfunctional immunity plays a major role in malignancy formation, but many more clinical studies are required to investigate the role of TIM-3 in MPN pathogenesis and establish its role in the formation, therapy resistance, relapse, and immune scoring of this malignancy. Several clinical trials involving co-blockade of TIM-3 and PD-1, have demonstrated promising preliminary results against solid tumors namely HBV-related hepatocellular carcinoma [94–96]. TIM-3 is highly expressed in peripheral blood and bone marrow exhausted T cells in various hematological malignancies, including acute lymphoblastic leukemia (ALL), chronic lymphocytic leukemia (CLL), and multiple myeloma (MM) however, few reports have demonstrated its clinical significance as monotherapy with TIM-3 inhibitors alone [97–99].

Further studies are required to evaluate the efficacy of TIM-3 inhibitors in different types and stages of leukemias and MPNs with emphasis on TME. Currently, the TIM-3 inhibitors used in clinical trials include MBG453 (also known as sabatolimab), TSR-022 (Tesar), BMS-986258,

LY3321367, SYM023, BGB-A425, and SHR-1702 [100,101]. Currently, sabatolimab (high-affinity IgG4 mAb) is the only anti-TIM-3 mAb being investigated in MDS and AML with preliminary safety and efficacy data. ClinicalTrials.gov Identifier: NCT03066648 is an active phase I trial of TIM-3 involving the study of PDR001 and/or MBG453 in combination with decitabine or azacitidine in patients with AML or high-risk MDS [102]. It includes AML, MDS, chronic myelomonocytic leukemia, and bone marrow diseases. No result was posted till the writing of this article, but preliminary results reported that the combination of sabatolimab plus HMA (either decitabine or azacitidine) was associated with mostly grade 1 or 2 adverse events and showed preliminary evidence of antitumor activity with a durable response. As per preliminary follow-up, overall response rates (ORRs) in patients with HR-MDS with sabatolimab plus decitabine and sabatolimab plus azacitidine were 61.1% and 57.1%, respectively, with complete response (CR) rates of 33.3% and 7.1% [100]. TIM-3 is relatively higher expressed on leukemic stem cells than non-tumor stem cells, often with other surface antigens such as CD33, CD123, and CLL, thus targeting TIM3 might be a novel approach in cancer treatment in future [103]. Targeting TIM-3 along with other checkpoint inhibitors or combining TIM-3 inhibition with new immunotherapeutic modalities that activate cancer-specific T-cell stimulatory molecules have immense potential for developing therapies with durable clinical benefits.

Role of T cell immunoglobulin and immunoreceptor tyrosine-based inhibitory domain (TIGIT) as a target for next-generation ICI in hematological malignancies

TIGIT belongs to a family of PVR-like proteins, discovered in 2009, composed of one extracellular immunoglobulin variable domain (a type I transmembrane domain) and a short intracellular domain with one immunoreceptor tyrosine-based inhibitory motif (ITIM) and one immunoglobulin tyrosine tail (ITT)-like motif [104,105]. TIGIT (also called Washington University cell adhesion molecule, WUCAM) along with DNAX accessory molecule-1 (DNAM-1) and CD96 are expressed on NK cells and T cells and share CD155 [polio virus receptors (PVR), nectin and nectin-like (NECL) NECL-5] as a ligand [106,107]. The immunoglobulin variable domain is homologous with other members of the PVR-like family, including DNAM-1, CD96, CD111, CD155, CD112, CD113, and PVRL4 [104]. In both humans and mice, CD155 serves as a ligand of TIGIT, and comparatively, it binds with lower affinity with CD112 and CD113 [105,108]. CD155 is mainly expressed on DCs, B cells, T cells, macrophages, kidneys, nervous system, and intestines [109], CD112 has a wide expression in bone marrow, pancreas, kidney, and lung [110], and CD113 is limited to non-hematopoietic tissues, including placenta, kidneys, testis, liver, and lung [111]. The mechanism of action of TIGIT involves an extrinsic pathway, as a ligand for CD155 [104] or a cell-intrinsic pathway by interfering with DNAM-1 co-stimulation [112,113] or by directly delivering inhibitory signals to the effector cell [105]. The interaction of TIGIT with CD155 sends signals to human monocyte-derived DCs leading to increased secretion of IL-10 and decreased secretion of IL-12 thus promoting tolerogenic DCs that down-regulate T cell responses [106]. For the cell-intrinsic mechanism of action, it was postulated that the high affinity of TIGIT for CD155 out-numbers DNAM-1 for the binding of CD155 leading to T-cell inhibition. This was first observed that TIGIT knock-down in CD4⁺ T cells increased their expression of T-bet and IFN- γ , and this could be overcome by DNAM-1 blockade [112,113]. Several malignancies, including melanoma, breast cancer, non-small-cell lung carcinoma (NSCLC), colon adenocarcinoma, gastric cancer, multiple myeloma (MM), and AML have shown increased expression of TIGIT thus paving the path for anti-TIGIT therapies [114–118].

In mouse pre-clinical models and cancer patients, TIGIT expression on tumor-infiltrating CD8⁺ T cells often correlates with increased expression of other inhibitory receptors such as PD-1, LAG-3, TIM-3, and with decreased expression of DNAM-1 [115,119–121]. Similarly, a high TIGIT/DNAM-1 ratio on tumor-infiltrating T_{regs} was shown to correlate with poor clinical outcomes following ICB targeting PD-1 and/or CTLA-4 [122]. In the pre-clinical mice TIGIT negative mice bearing colon cancer (MC38 model), co-blockade of TIGIT and PD-1 was associated with enhanced effector cell functions of both CD4⁺ and CD8⁺ T cells compared to either therapy alone; and TIGIT/PD-1 co-blockade produced a 100% cure rate [123].

As explained earlier tumor cells create a microenvironment by either promoting secretion of immunosuppressive cytokines such as IL-10 and transforming growth factor (TGF)- β , or by recruiting regulatory cells including T_{regs}, MDSCs, and type 2 macrophages or by affecting immune cell metabolism [124,125]. However, most of these pathways comprise receptor-ligand pairs, and their interaction leading to suppression of the effector functions of T cells and NK cells and thereby impairing anti-tumor immunity [126]. However, despite the enormous success and popularity of ICIT, there is still a substantial number of patients who either do not respond to currently available immunotherapies or develop treatment-related toxicities termed 'immune-related adverse events' (irAEs), which sometimes led to fatalities [127,128]. Thus, there is great interest in discovering new immune checkpoints that can be targeted with safety without affecting the anti-tumor efficacy across various malignancies. TIGIT is a negative regulator of cytotoxic T cells and has emerged as a particularly attractive target for cancer therapy with possibly fewer irAEs than anti-PD-1 or anti-CTLA-4 mAbs [129,130].

Presently, six human clinical trials of anti-TIGIT-mAb of IgG1 isotype are undergoing including etigilimab (OMP-313M32), in phase I/II, either as monotherapy or combinations with PD-1/PD-L1 blockade, for the treatment of solid cancers [131–136].

TIGIT is highly expressed on tumor-infiltrating lymphocytes (TIL) in several hematological malignancies including follicular lymphoma, CLL, classic HL, AML, and relapsed MM, helping in tumor progression and poor outcome [137]. Research studies have shown the immense potential of anti-TIGIT therapy as reported by Catakovic's in vitro experiment showing reduce CLL viability by TIGIT blockade [138]. Anti-TIGIT treatment prevented T cell exhaustion and prolonged survival in MM mice [139]. Current clinical trials based on therapeutic strategies targeting TIGIT have encouraging efficacy in hematological malignancies [140–148]. Table 3 shows current clinical trials of anti-TIGIT antibodies in hematological malignancies.

Table 3. Shows current clinical trials of anti-TIGIT antibodies in hematological malignancies.

NCT number	Sponsors / collaborators	Title	Drug name	Phase	Tumor / disease	Status	Outcome measures	ICI type	Study Designs
NCT0515713	Hoffmann-La Roche	A Phase I/II Open-Label, Multicenter Study Evaluating the Safety, Efficacy, and Pharmacokinetics of Mosunetuzumab in Combination With Tiragolumab With or Without Atezolizumab in Patients With Relapsed or Refractory B-Cell Non-Hodgkin Lymphoma	Mosunetuzumab SC; Tiragolumab; Atezolizumab; Tocolic Phase 1 and 2		Relapsed or refractory (R/R) diffuse large B-cell lymphoma (DLBCL) or follicular lymphoma (FL)	Active, not recruiting	Percentage of Participants With Adverse Events; Best Objective Response Rate (BOR)	TIGIT	Allocation: Non-randomized(Intervention) Model: Single Group Assignment(Masking: None (Open Label)/Primary Purpose: Treatment
NCT04045028	Genentech, Inc.	A Phase Ia/Ib Open-Label, Multicenter Study Evaluating the Safety and Pharmacokinetics of Tiragolumab as a Single Agent and in Combination With Atezolizumab and/or Daratumumab in Patients With Relapsed or Refractory Multiple Myeloma, and as a Single Agent and in Combination With Rituximab in Patients With Relapsed or Refractory B-Cell Non-Hodgkin Lymphoma	Tiragolumab; Daratumumab/HuPDQ2; Rituximab; Atezolizumab	Phase 1	Relapsed or Refractory Multiple Myeloma, Relapsed or Refractory B-Cell Non-Hodgkin Lymphoma	Terminated (slow recruitment)	Percentage of Participants With Adverse Events; Objective Response Rate (ORR) for R/R MM, ORR for R/R NHL; Percentage of Participants With Anti-Drug Antibodies (ADAs) to Tiragolumab / Atezolizumab	TIGIT	Allocation: Non-randomized(Intervention) Model: Parallel Assignment(Masking: None (Open Label)/Primary Purpose: Treatment
NCT05267054	BeiGene	A Phase I/II Study Investigating the Safety, Tolerability, Pharmacokinetics, and Preliminary Antitumor Activity of Ocpelimumab (BG4 A12127) in Combination With Tislelizumab (BG4 A317) or Rituximab in Patients With Relapsed or Refractory Diffuse Large B-Cell Lymphoma	Ocpelimumab; Tislelizumab; Rituximab	Phase 1 and 2	Relapsed Diffuse Large B-cell Lymphoma	Recruiting	Number of participants with adverse events (AEs); Recommended Phase 2 dose (RP2D) of ocpelimumab when administered in combination with tislelizumab or rituximab; ORR, DCR, DOR, TTR, PFS, OS, Host Immunogenicity	TIGIT	Allocation: Non-randomized(Intervention) Model: Parallel Assignment(Masking: None (Open Label)/Primary Purpose: Treatment
NCT04529565	Multiple Myeloma Research Consortium	A Phase I/II Assessment of Combination Immuno-Oncology Drugs Elotuzumab, Anti-LAG-3, Anti-LAG-3 + Pomalidomide + Dexamethasone, Anti-TIGIT, Anti-TIGIT + Pomalidomide + Dexamethasone	Elotuzumab, pomalidomide, dexamethasone; Anti-LAG-3; Anti-LAG-3 + Pomalidomide + Dexamethasone; Anti-TIGIT; Anti-TIGIT + Pomalidomide + Dexamethasone	Phase 1 and 2	Relapsed Diffuse Large B-cell Lymphoma; Multiple Myeloma	Recruiting	Overall Response Rate; Frequency, type and grade of Adverse Events and Serious Adverse Events	TIGIT	Allocation: Randomized(Intervention) Model: Sequential Assignment(Masking: None (Open Label)/Primary Purpose: Treatment
NCT05054442	Merck Sharp & Dohme LLC	A Phase 2, Open-label Study to Evaluate the Safety and Efficacy of MK-7684A (MK-7684 (Vibostolimab) With MK-3475 (Pembrolizumab) Combination) in Participants With Relapsed or Refractory Hematological Malignancies	Biological: Pembrolizumab/Vibostolimab combination	Phase 2	Hematological Malignancies	Recruiting	Number of Participants with a Dose-Limiting Toxicity (DLT); Number of Participants Who Experienced an Adverse Event (AE); ORR, DOR, DCR	TIGIT	Allocation: NA(Intervention) Model: Single group Assignment(Masking: None (Open Label)/Primary Purpose: Treatment
NCT0454246	Compugen Ltd	A Phase 1 Study of The Safety and Tolerability of COM902 in Subjects With Advanced Malignancies	COM902 monotherapy; COM902 in combination with COM701 (both at the RP2D); Triple combination of COM902 + COM701 + Pembrolizumab	Phase 1	Advanced cancer; ovarian cancer; lung cancer; plasma cell neoplasia; MM; HNSCC; Microsatellite stable colorectal carcinoma; MSS-CRC	Recruiting	The safety and tolerability of COM902 monotherapy; To identify the maximum tolerated dose (MTD) and/or recommended Phase 2 dose (RP2D) To characterize the pharmacokinetics (PK) profile of COM902 as monotherapy in subjects with advanced malignancies;	TIGIT	Allocation: Non-randomized(Intervention) Model: Sequential Assignment(Masking: None (Open Label)/Primary Purpose: Treatment
NCT04254137	Seagen Inc.	A Phase 1 Study of SEA-TIG (SEA-TIGIT) in Subjects With Advanced Malignancies	SEA-TIG; saianlimab; brentuximab vedotin	Phase 1	NSCLC; Gastric cancer; Gastroesophageal Junction Carcinoma; classic HL; DLBCL; Peripheral T-cell Lymphoma; Cutaneous Melanoma; Head and Neck Squamous Cell Carcinoma; Bladder carcinoma; ovarian cancer; triple negative breast cancer; cervical cancer	Recruiting	Number of participants with adverse events (AEs); Number of participants with laboratory abnormalities by grade; Number of participants with a dose-limiting toxicity (DLT) at each dose level	TIGIT	Allocation: Non-randomized(Intervention) Model: Sequential Assignment(Masking: None (Open Label)/Primary Purpose: Treatment
NCT04772989	Arcus Biosciences, Inc.	A Phase 1/2a Study to Evaluate the Safety and Tolerability of AB308 in Combination With AB122 in Participants With Advanced Malignancies	AB308; Zimberelimab	Phase 1	Advanced Solid Tumor: NSCLC; Melanoma; cervical cancer; MM/ANHL; DLBCL; Gastric cancer; GE junction cancer; esophageal cancer	Recruiting	Percentage of participants with Adverse Events; Percentage of participants who experience a Dose Limiting Toxicity Number of participants with any adverse events (AEs) and serious adverse events (SAEs); Number of participants with dose limiting toxicity (DLT); Recommended Phase 2 dose (RP2D) of ES0884448 alone or in combinations with ibendomide with or without dexamethasone in participants with relapse/refractory multiple myeloma; Number of participants with overall response (partial or better) as determined by IMWG criteria	TIGIT	Allocation: Non-randomized(Intervention) Model: Sequential Assignment(Masking: None (Open Label)/Primary Purpose: Treatment
NCT05284982	ITeoss Belgium SA	Study of ES0884448 Alone, and in Combination With Ibendomide With or Without Dexamethasone, in Participants With Relapsed or Refractory Multiple Myeloma	ES0884448; Ibendomide; Dexamethasone	Phase 1 and 2	MM	Recruiting		TIGIT	Allocation: Non-randomized(Intervention) Model: Sequential Assignment(Masking: None (Open Label)/Primary Purpose: Treatment

The current and future role of ICI in the management of MPN

The management of MPNs is constantly evolving and highly individualized. Optimal management of MPN patients is based on considering specific disease types, complex decision-making, individualized prognosis, age, comorbidities, and the risks and benefits of available treatment. Patients with PV and ET use aspirin for thrombotic risk reduction as well as hydroxyurea (HU) or interferon-based therapy for cytoreduction [149,150]. As per the revised IPSET score, cytoreductive therapy is reserved for patients with high-risk factors including age > 60 years, previous thrombosis, and JAK2 mutation [151]. HU is associated with significant side effects and subsequently, 24% of patients with PV or ET develop resistance to primary therapy necessitating the need for second-line therapy [152]. Interferon is frequently used as a frontline or second-line therapy including a novel, mono-pegylated formulation called Ropeginterferon alfa-2b, the first and only approved treatment for PV independent of previous hydroxyurea exposure [150,153]. With the advances in molecular science, there is the discovery of the JAK2 V617F mutation and its role in JAK-STAT pathway dysregulation, which led to the development of the JAK inhibitor ruxolitinib, which currently represents the cornerstone of medical therapy in MF and hydroxyurea-refractory/intolerant PV [150]. Furthermore, the JAK1/2 inhibitor ruxolitinib is approved in intermediate to high-risk MF, as well as advanced PV after HU intolerance or failure [154]. JAK inhibitors are known to alleviate symptoms, improve performance status and disease-associated cachexia further adding the survival benefit of these drugs [155]. Long-term follow-up studies showed improved bone marrow morphology (up to 50% of patients might achieve some regression in marrow fibrosis after 60 months) [156] however complete molecular remissions are rare (3 and 6 patients in RESPONSE-I and COMFORT-I trials, respectively) [157,158]. The major limitations of the use of these agents are that

they have debatable disease-modifying activities, there is the likelihood of losing response over time, development of treatment resistance, chronic anemia, and thrombocytopenia stemming from JAK2 inhibition frequently limiting their safety profile and dosing [159].

Ongoing research efforts are dedicated to improving the efficacy and safety profile of established treatment modalities as well as discovering novel therapeutic approaches, many of which target the immune system. Lately, the focus of the treatment of MPN is based on the agents targeting the immune system, cytokine environment, and immunomodulatory agents with targeted therapy. At the American Society of Hematology (ASH) annual meeting in 2020 Mascarenhas et al [18] presented an open-label, multi-center, phase 2, single-arm study of pembrolizumab in patients with primary, post-essential thrombocythemia or post-polycythemia vera myelofibrosis (MF) (NCT03065400). Nine case studies were done, but none had a clinical response. Wang et al published an article demonstrating that PD-1 and PD-L1 expressions were increased in MPN disease in immune cells, including CD4, CD8, monocyte, and CD34+ cells [160]. The potential stimulators of PD-L1 expression are interferon-gamma (IFN- γ), IL-10, VEGF, and hypoxia leading to activation of PD-L1 transcription [161,162]. Treg cells can stimulate B7-H1 expression in MDSCs thus enhancing each other's immune suppression functions [163]. The role of MDSCs in the tumor microenvironment is getting defined day by day and they are implicated in inducing therapeutic resistance to ICI therapy [164,165]. Further studies summarized that in patients with advanced melanoma, non-small cell lung cancer, and breast cancer, there is an accumulation of MDSC that led to resistance to immunotherapy proven by the positive correlation between the MDSC percentage and neutrophil/lymphocyte rate (NLR) (a prognostic marker in both ipilimumab and nivolumab therapy) [166–169]. ICI targeting PD-1 stimulated circulating Treg levels but did not change Granulocyte-MDSC (G-MDSC) and Myeloid-MDSC (M-MDSC) levels. However, the partial response group had a higher baseline level of M-MDSCs, which showed a significant decrease after the first cycle of anti-PD-1 treatment [170]. Therefore, MDSC accumulation plays a significant role within the tumor microenvironment and is implicated in the failure of ICI.

There have been limited studies on the use of ICI in the treatment of MPN as described earlier in the article with three NCI-sponsored clinical trials related to combined immune- therapy (NCT03065400, NCT02421354, and NCT02871323) in 2021 [18,19,171].

We have collected preliminary data in our laboratory showing the expressions of VISTA, TIM-3, and LAG-3 on the progenitor, immune, and MDSC cells in MPN patients. We found that VISTA is the predominant next ICI receptor or ligand found in MPN patients. Other next-generation checkpoints including TIM-3, TIGIT, and LAG-3 were not different in expressions between controls and MPN patients as shown in Figures 3–6. We had previously found MDSC over-expressed in cells including CD34⁺, CD14⁺, CD4⁺, and CD8⁺, and now our preliminary data suggest that VISTA (one of the next generation ICI) as compared to others like TIM-3, LAG-3, TIGIT could be the predominant ICI target in MPN.

Future directions and perspectives

There is now immense interest in integrating immunotherapy into the standard of care for various tumor types specifically hematological cancers, in large part due to the considerable progress made in discovering new immune checkpoint targets as part of next-generation ICIT. First, although combination immunotherapy has shown a ray of hope by yielding significant therapeutic improvement, there is substantial debate over the optimal types and dosage of these modalities. Second, IR blockade can produce remarkable tumor shrinkage and remission in only a proportion of patients, and it is critically important to understand why. While several known factors, such as IR ligand expression, the brevity, and the immunogenicity of neoantigen expression, could contribute, there may be many other factors that remain unknown. Determining these factors and identifying biomarkers that can predict responsiveness to each immunological modality will be critical. Third, while the novel immunotherapies tested in clinical trials represent a significant step forward, it remains important to continue the search for new targets that might be critical components of future combinatorial approaches. This is especially important in the future to promote new modalities with

higher efficacy but reduced adverse events. It is also important to continue to identify new potential immunotherapeutic targets and mechanisms that can lay the foundation of new targeted approaches.

Our preliminary results showed that VISTA than others including TIM-3, LAG-3, and TIGIT, were the predominant next-generation ICI expressed on CD3⁺, CD14⁺, and CD34⁺ cells as measured by the percentage of positive 2nd G- ICI cells (Figure 3) and MFI (Figure 4). Also, we demonstrated that VISTA also wares the predominant 2nd G-ICI on both the G-MDSC and M-MDSC as measured by the percentage of positive cells (Figure 5) and MFI (Figure 6) respectively. This would lead to further clinical trials specifically involving VISTA with a possible combination anti-Vista and anti-PD-1 in MPN disease. This may lead to reviving the ICI therapy in MPN which ICI was found to be a negative trial in using anti-PD-1 only in the treatment of MPN.

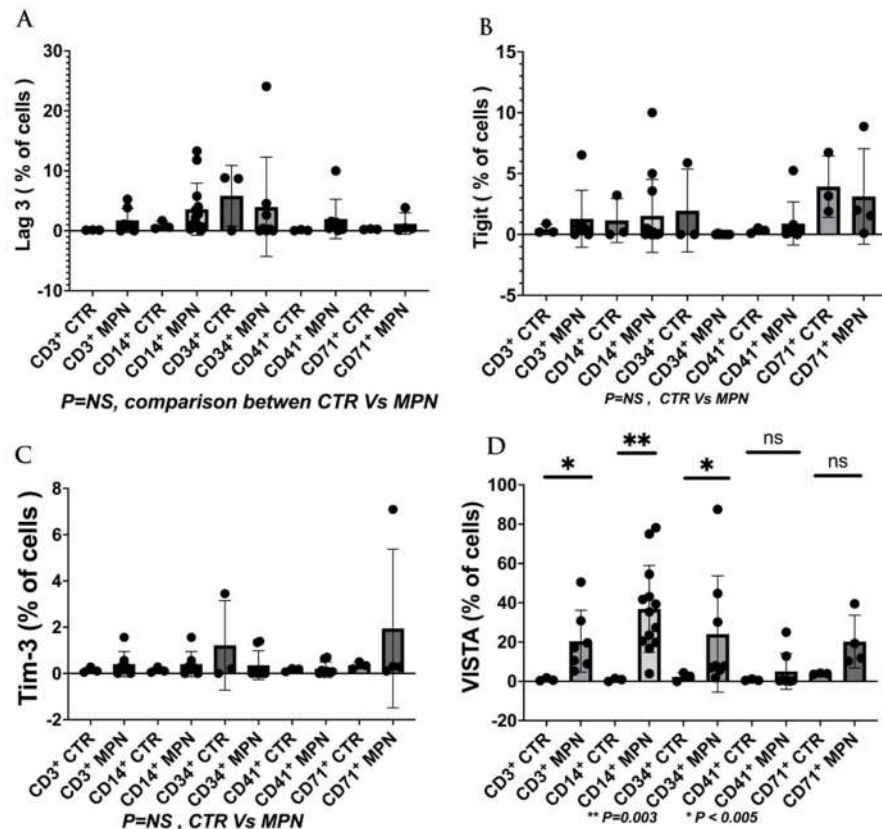


Figure 3. 2nd generation of ICI expressions (% of cells) on the different cell populations were done on MPN patients and controls. The results showed that there were no difference on the LAG-3, TIGIT, and TIM-3 on the different cell population between MPN and controls, but there was a significance of the VISTA expression (between MPN and controls) (mean + SE) on the CD3 (20.4 + 5.94 Vs 0.91 + 0.44 , P<0.05) CD14 (38.86 + 6.12 Vs 0.79 + 0.43, P=0.003) , CD 34 (2.30 + 1.26 Vs 2.30 + 1.28 , P < 0.05), other ICI marker of LAG3 and TIM3 were of no significant difference.

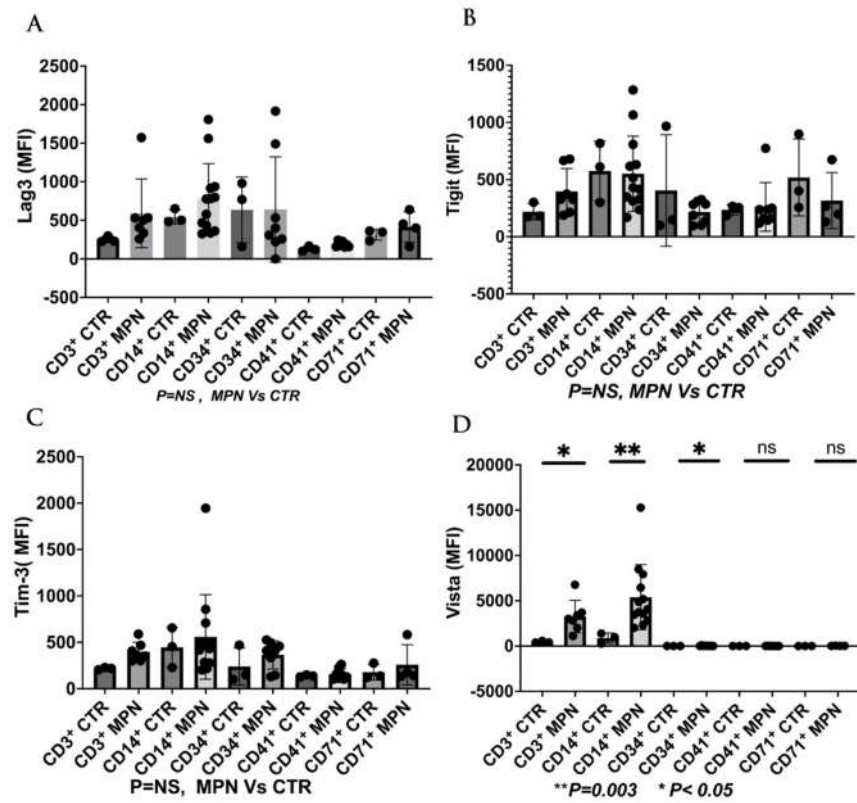


Figure 4. 2nd generation of ICI expression (MFI) on different cell populations on MPN patients and controls. The results showed there were no differences of the LAG-3, TIGIT, and TIM-3 on the different cell population between MPN and controls, but there was a significance of the VISTA expression (between MPN and controls) (mean + SE) on the CD3 (3257 + 673.4 Vs 457,0 + 59.0 , P< 0.05) CD14 (5399 + 994.3 Vs 879.3 + 325.2, P =0.003) , CD 34 (2300 + 1262 Vs 24.0 + 10.4 , P < 0.05)).

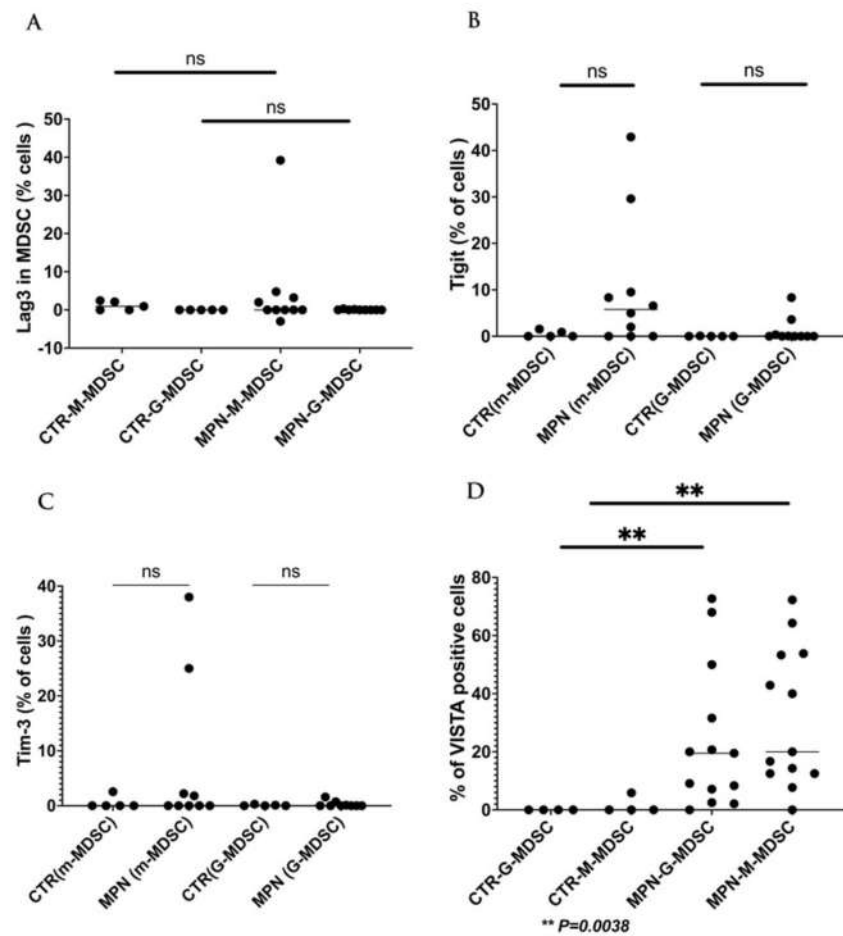


Figure 5. Expression of 2nd generation of ICI (% of positive cells) on the G- MDSC, and M-MDSC in patients with MPN and controls. The results showed there was no difference of the LAG-3, TIGIT, and TIM-3 on G- MDSC or M-MDSC between MPN and controls, but there was a significance of the VISTA expression (between MPN and controls) (mean + SE) on the G-MDSC (23.9 + 6.8 Vs 0.00 + 0.0 , $P<0.003$) , and M-MDSC (31.5 + 6.6 Vs 1.47 + 1.47, $P=0.003$) respectively .

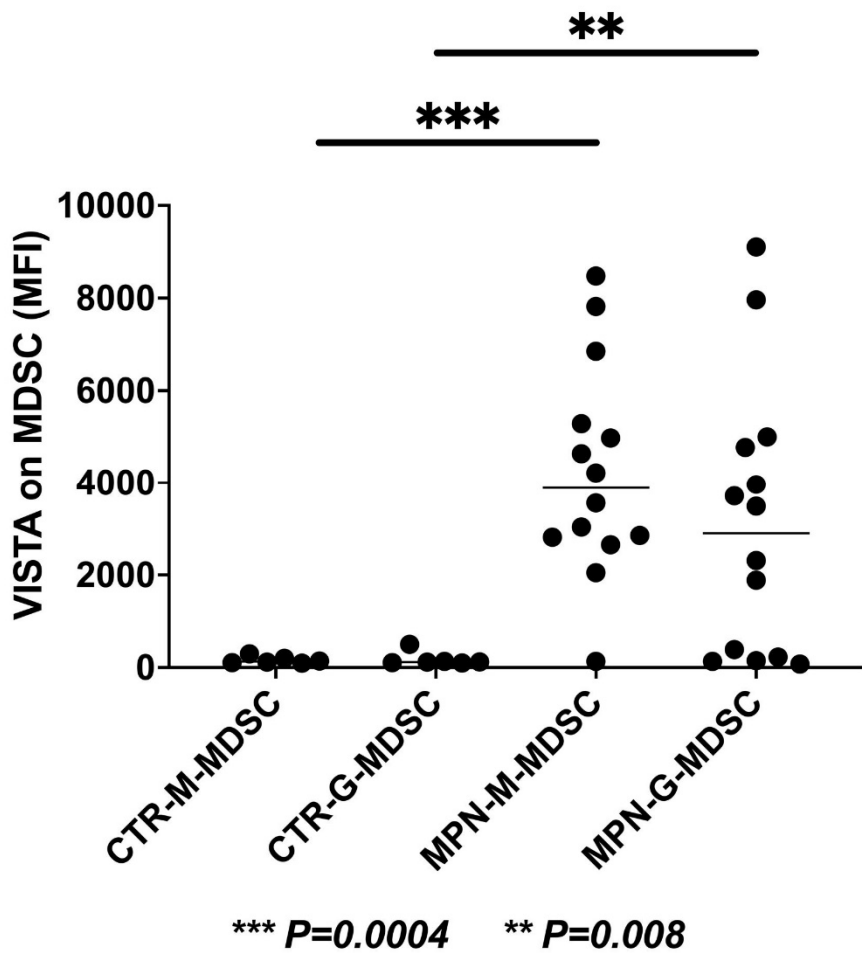


Figure 6. Expression of 2nd generation of ICI (MFI) on the G- MDSC, and M-MDSC in patients with MPN and controls. The results showed there were no difference of the LAG-3, TIGIT, and TIM-3 on GMDSC or M-MDSC between MPN and controls, but there was a significance of the VISTA expression (between MPN and controls) (mean + SE) on the G-MDSC (3085 + 763.6 Vs 179.7 + 64.6 , P= 0.008) , and M-MDSC (4241 + 617.7 Vs 159.7 + 31.29, P =0.0004) respectively.

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