Immune Activation with Plinabulin Enhances Anti-tumor Response Combining Radiation with Immune Checkpoint Blockade

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Abstract

Background

Plinabulin is a first-in-class differentiated tubulin binder that exerts anti-cancer activity primarily by activating GEF-H1-mediated pathways in dendritic cell (DC) maturation, M1 polarization and subsequent T-cell activation (La Sala 2019; Kashyap 2019; Natoli 2021). In a global phase 3 study (Dublin-3, n=559; Han 2024), plinabulin/docetaxel outperformed docetaxel with significant OS/PFS/ORR benefits and 80% reduction in G4 neutropenia (p<0.0001). The doubling of 2- and 3-year survival rates also suggests a durable long-term benefit. When given after radiation in immune checkpoint blockade (ICB)-relapsed/refractory cancers, plinabulin (30 mg/m2) potentiates PD-1 inhibitors with ORR 23% and DCR 54% (Lin 2025). Using scRNAseq, our analysis herein focuses on GEF-H1 immune gene signature at pre/post-treatment (C1D1/C1D4/C3D1) in monocytes/macrophages from PBMCs and non-irradiated tumor biopsies.

Methods

In this phase I study (NCT04902040), 12 patients of mixed cancers (7 PR+SD and 5 PD) were available for examination of GEF-H1 immune activation in PBMCs and 5 of them had pre/post-treatment tumor biopsies (2 NSCLC, 1 fibrolamellar HCC, 1 RCC, 1 SCCHN). For PBMCs, whole transcriptome scRNAseq was performed using Evercode kits (Parse Biosciences) and a DNBSEQ T7 genetic sequencer using Complete Genomics' 100 bp paired end (PE100) high-throughput sequencing kit. For dissociated tumor biopsies, scRNA-seq was performed using 10X genomics and aligned using the hg38 human reference genome. Here, we examined a 47-gene GEF-H1 immune activation signature in peripheral CD14+ and CD16+ monocytes and tumor-infiltrating immune cells (TIICs) focusing on monocyte-derived macrophages (MoMac I-IV) in PR+SD and PD subjects at C1D1/baseline, C1D4 and/or C3D1 timepoints.

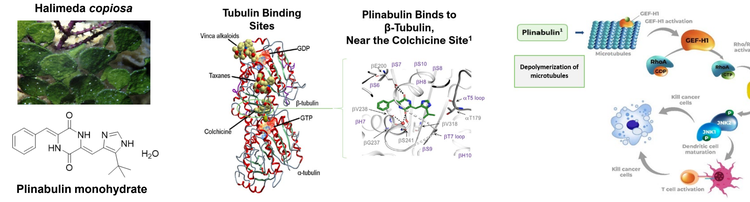
Results

There were significant differences in GEF-H1 immune scores between PR+SD and PD patients in CD16+ and CD14+ blood monocytes at all timepoints. As treatment progressed, a notable increase in %monocyte corresponded to a decrease in %T cells in all patients, although such trends were not significant when examined individually. In patient-matched biopsies, GEF-H1 scores in TIICs and total MoMac were significantly increased in PR+SD group but decreased in PD group (C3D1 vs. C1D1). There was no baseline difference in TIIC-associated GEF-H1 scores between PR+SD and PD groups, whereas total MoMac-associated GEF-H1 score was higher in PD when compared to PR+SD. Further subgroup analysis of MoMac I-IV suggests that AGR1+ MoMac-III represent the dominating pro-tumor M2 phenotype and that plinabulin combined with RT+ICB drives differential monocyte-macrophage responses in responders.

Conclusions

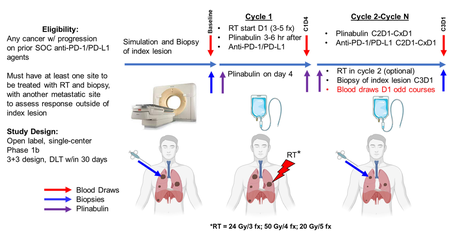
In addition to potent DC maturation for a systemic immune response, plinabulin combined with radiation and ICB promotes proinflammatory monocytes and M1 polarization via a GEF-H1-dependent mechanism with the potential of overcoming acquired ICB resistance from pro-tumor macrophages.

Plinabulin



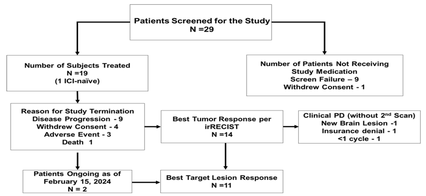
- Plinabulin is derived from the natural marine product Halimide, a fungal metabolite on green algae Halimeda copiosa.
- Screening of anti-cancer effects discovers Plinabulin, a small molecule that had single agent activity against various cancer cell lines.
- Plinabulin binds at a distinct site on beta-tubulin that is distinct from other tubulin agents, like taxanes, vinca alkaloids, colchicine.
- Plinabulin prevents polymerization of microtubules, which leads to GEF-H1 Release. Liberated GEF-H1 is activated upon release Which binds and activates RhoA, leading to Downstream JNK activation.
- Activation of JNK causes dendritic cell maturation In vitro, and in vivo, this boosts antigen induced CD8 T cell activation.
- Plinabulin alters the tumor microenvironment to reduce tumor associated macrophages and enhance M1/M2 ratio.

Phase 1 Trial Schema (NCT04902040)



*RT = 24 Gy/3 fx; 50 Gy/4 fx; 20 Gy/5 fx

Patient Enrollment



Primary Objectives

- To assess the safety and tolerability of plinabulin when administered in combination with a radiation/ immunotherapy regimen in subjects with select advanced solid malignancies after progression on anti-PD-1/PD-L1 mAb
- To assess the objective tumor response rate (ORR) (complete response + partial response)

Secondary Objectives

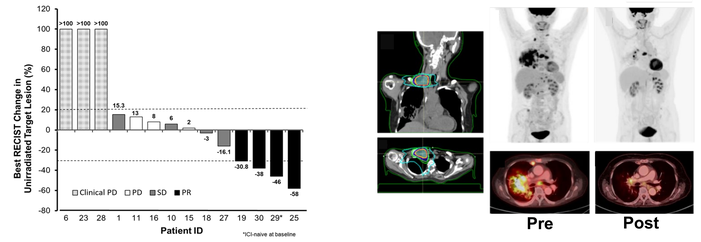
- To assess disease control rate (complete response, partial response [PR] + stable disease [SD])
- To determine progression-free survival (PFS) and overall survival (OS)

Exploratory Objectives

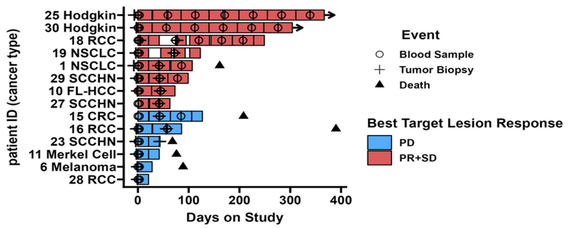
- Dendritic cell activation and phenotypes of immune cells in peripheral blood
- Immune repertoire TCR sequencing change in peripheral blood
- Immune phenotypes in tumor tissue, including DC, T cells, TAMs, pre- and post- treatment
- To explore predictive and response biomarkers for treatment response based on the collected biomarkers

Clinical Responses

(systemic abscopal response comparing baseline and C3D1seen for one patient)



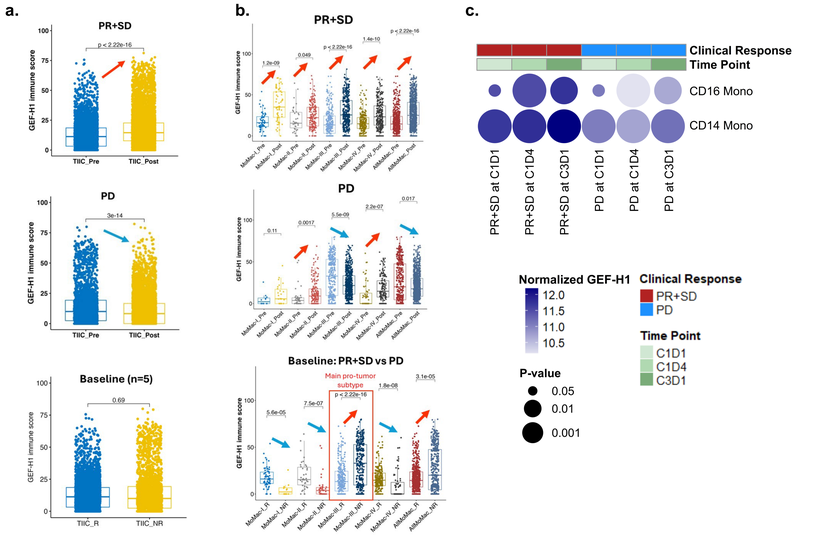
Cancer Types and Treatment Cycles



Results

Single-cell RNA Sequencing Analyses

GEF-H1 Immune Score Computation: The GEF-H1 immune pathway consists of 47 genes that together drives a distinct cell signaling program in DCs (Kashyap 2019). The gene expression counts were first normalized by log 2 Fragments Per Kilobase of transcript per Million mapped reads (FPKM) and then summed up the expression of these genes to establish a normalized.



a. Tumor-infiltrating immune cells (TIIC), **b**. Tumor-associated monocyte-derived macrophages (MoMac), **c**. Peripheral CD14+ and CD16+ monocytes in PBMCs

Conclusions

- This triple combination with the addition of plinabulin after RT initiation (3-6 hours apart) plus anti-PD-1 rechallenge was well-tolerated and provided 54% disease control rate in 13 ICI-refractory patients (3 PR; 4 SD) with 2 heavily pretreated Hodgkin lymphoma patients demonstrating long-lasting anti-tumor responses.
- Despite the mixed cancer types, scRNAseq analysis of tumor biopsies showed that GEF-H1 immune scores in TIICs and total MoMacs were significantly increased in the PR+SD group but decreased in the PD group (C3D1 vs. C1D1). Interestingly, total MoMac-associated GEF-H1 immune score was higher in PD vs PR+SD group at the baseline, which is mostly driving by the MoMacIII subset previously determined as ARG1 (arginase 1) high.
- scRNA-seq of PBMCs shows consistently higher GEF-H1 scores in PR+SD than PD patients for both CD14$^+$ and CD16$^+$ monocytes at the baseline (C1D1) and 2 other timepoints assessed (C1D4, C3D1).
- Plinabulin is a well-suited drug candidate to help invigorate the cancer-immunity cycle by promoting DC maturation and adaptive T-cell activation. The GEF-H1-based mechanistic biomarker approach also warrants future clinical investigation.

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Disclosures

The presenting author has no relevant disclosures.

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