Depletion of Blood Arginine with Pegzilarginase (AEB1102) in Combination with anti-PD-L1 Increases Tumor Infiltration by Immune Cells and Enhances anti-Tumor Activity

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Poster #869

Introduction

Tumors with an impaired ability to make arginine primarily due to low expression of argininosuccinate synthase (ASS1) have an enhanced sensitivity to extracellular arginine depletion (Fig 1).

Pegzilarginase mechanism of action

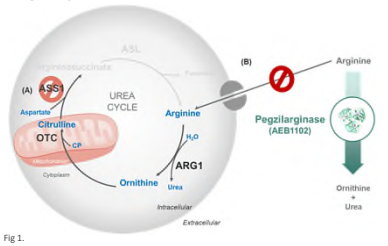

Fig 1.
(A) Impaired arginine biosynthesis due to reduced expression of ASS1 causes reliance on the extracellular pool of arginine for survival and proliferation
(B) Pegzilarginase (AEB1102) restricts the supply of extracellular arginine, thereby starving the tumor cell

1. Native human arginase 1 displays low activity and serum stability
2. Aeglea has developed a bioengineered human arginase 1 (pegzilarginase, AEB1102) with enhanced pharmacological properties through PEGylation and replacement of the natural metal co-factor manganese with cobalt
3. Single-agent pegzilarginase treatment has shown anti-tumor activity in *in vivo* solid tumor models of different histologies with no or low expression of ASS1, including:
   - melanoma
   - small cell lung cancer (SCLC)
   - large cell non-small cell lung cancer (NSCLC)
   - Merkel cell carcinoma

Extracellular depletion of arginine has a number of direct effects on tumors[1], including:
- inhibition of cell proliferation
- induction of apoptosis
- increased autophagy

However, the impact of extracellular arginine depletion on tumor immunogenicity and immune system function has not been clearly established. Given the link between autophagy and MHC antigen presentation to T-cells[2], we hypothesized that treatment with pegzilarginase could modulate immune system function and/or enhance recruitment of immune cells to the tumor microenvironment.

Methods

Balb/c mice bearing palpable (staged) syngeneic subcutaneous CT26 tumors were dosed with pegzilarginase or anti-PD-L1 mAb (10F.9G2) alone, or in combination. At pre-determined time points after treatment initiation, tumors were dissociated and cells were stained with a viability dye to assess the percentage of live cells, or incubated with fluorochrome-labeled antibodies in order to identify relevant cell populations via flow cytometry. Portions of each tumor were also fixed in 10% neutral-buffered formalin and embedded in paraffin for immunohistochemistry (IHC) staining. Serum IFN-γ levels were assessed using a Millipore multiplex assay.

Results

- Tumor growth was inhibited with both pegzilarginase (AEB1102) and anti-PD-L1 antibody monotherapies (Fig 2)
- The combination of AEB1102 and anti-PD-L1 resulted in a greater anti-tumor activity than either single agent (Fig 2)

CT26: AEB1102 + anti-PD-L1 mAb

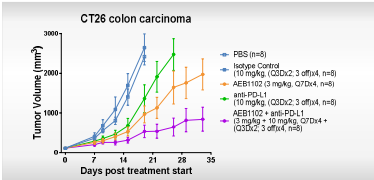

Fig 2. Tumor volumes in a staged subcutaneous CT26 model.

Although no change in cell viability was observed in any group on Day 3 post-treatment (Fig 3A), IHC staining for LC3B indicated increased autophagy in the AEB1102 monotherapy group compared to control (Fig 3 B-D)

CT26: Day 3 tumor cell viability and autophagy

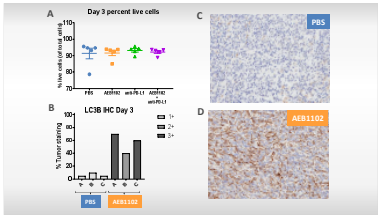

Fig 3. CT26 tumor cell viability (A). Tissues were stained were stained for LC3B and scored as the percentage (and intensity: 1+, 2+, 3+) of tumor cells with positive stain. Brown IHC staining depicts LC3B+ cells in representative sections of tumors from PBS (C) and AEB1102 monotherapy groups (D) (10x).

Day 3 post-therapy tumor immune cell profiling (Fig 4) revealed:
- an increased proportion of CD45+ cells in all treatment groups, consistent with an increased tumor-infiltrating leukocyte (TIL) population
- an increase in macrophages with single-agent or combination pegzilarginase treatment, but not with anti-PD-L1 alone
- an increase in dendritic cells in the combination group

CT26: Day 3 immune cell profiling

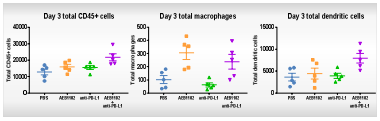

Fig 4. Combination treatment (AEB1102 + anti-PD-L1 mAb) leads to an early increase in CD45+ tumor infiltrating immune cells (TILs). Increases in macrophages were observed in both AEB1102 treatment groups while dendritic cells were increased in the combination group. Cell populations are expressed as counts per 70,000 cells.

Analysis of tumors by immune cell profiling on Day 17 post-treatment (Fig 5) revealed:
- decreased tumor cell viability in the combination group consistent with tumor volume measurements
- increased proportion of CD45+ cells in all treatment groups
- an increase in total T cells in all treatment groups
- a marked increase in CD8+ T cells in the combination group
- an increase in the CD69+ and CD25+ fractions of CD8+ T cells with single-agent or combination pegzilarginase therapy, suggestive of increased CD8+ T cell activation
- an increase in the total natural killer (NK) cells in all treatment groups, with single-agent and combination pegzilarginase therapy showing the highest increase
- a substantial increase in systemic IFN-γ levels with combination therapy.

CT26: Day 17 immune cell profiling

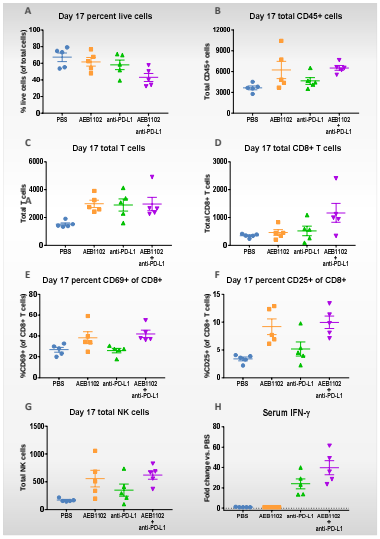

Fig 5. Combination treatment compromises CT26 tumor cell viability (A) and leads to increased CD45+ tumor infiltrating cells (B). This infiltrate is enriched in CD8+ T cells (C and D) and specifically in the fraction of CD8+ T cells that express the activation markers CD69+ (E) and CD25+ (F). The CD45+ infiltrate is also enriched in NK cells (G). Totals are expressed as counts per 70,000 cells. (H) Serum IFN-γ levels are highest in the combination group.

The increase in CD8+ T cells in the combination group on Day 17 post-treatment initiation was also confirmed by IHC staining for CD8 (Fig 6).

CT26: Day 17 anti-CD8 IHC tumor analysis

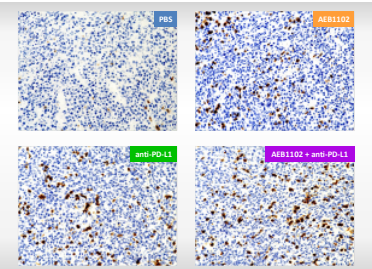

Fig 6. Brown IHC staining depicts CD8+ cells in representative sections of tumors from each treatment group (40x).

Conclusion

- The combination of pegzilarginase and anti-PD-L1 antibody resulted in synergistically greater activity in a staged CT26 model compared to either agent alone
- The enhanced activity in the combination group was accompanied by increased tumor infiltration by CD45+ cells, including macrophages and dendritic cells at early time points; at later time points, an increase in CD8+ T cells, NK cells and serum IFN-γ levels was observed
- These data do not support the hypothesis that arginine depletion is generally immunosuppressive
- These data support a role for arginine depletion in combination with immuno-oncology (IO) agents; and a Phase I/II clinical trial of pegzilarginase in combination with pembrolizumab in advanced small-cell lung cancer is ongoing (NCT03371979)

References
[1]Patil MD et al Oncogene (2016), 1–16
[2]You et al. J. of Hematol. & Onc. (2017) 10:165

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Disclosures All authors are employees of, and have an equity interest in Aeglea BioTherapeutics, Inc.