**Evaluation of Peripheral T-Cell Subset Proliferation as a Pharmacodynamic Assay to Guide the Development of Anti-CTLA-4 and PD-1 Antibody Combinations in Patients With Solid Tumors**

**OBJECTIVE**

To assess Ki-67 expression on immune populations after combined blockade of CTLA-4 and PD-1, using the 2-dose regimen of ipilimumab in combination with nivolumab. Additionally, a recent study proposed that expression of Ki-67, combined with overall tumor burden, can serve as an early indicator of responsiveness to anti-PD-1 therapy.

**CONCLUSIONS**

These results identify and support Ki-67 expression as a pharmacodynamic biomarker in the context of CTLA-4 (ipilimumab) and PD-1 (nivolumab) blockade, with the absolute increase in Ki-67 expression observed to be numerically dose dependent. A total absolute increase in Ki-67 expression was observed with AGEN2034 (anti-CTLA-4 antibody) in combination with AGEN1884 (anti-PD-1 antibody). This study supports further exploration of the role of Ki-67 expression in the context of combined blockade of pathways involved in the control of tumor growth.

**RESULTS**

- Ki-67 was measured for 3 patients in Cohort 1 who received AGEN1884 1 mg/kg q6w / AGEN2034 1 mg/kg q2w and 2 patients in Cohort 2 who received AGEN1884 3 mg/kg q2w / AGEN2034 1 mg/kg q2w.
- The average fold change in Ki-67 expression across different cell types, including total lymphocytes, total CD3+ cells, CD4+ T cells, CD8+ T cells, CD19+ B cells, and CD56+CD16+ NK cells, was calculated for each cohort.
- Geometric mean and 95% confidence intervals were shown for Ki-67 expression levels.

**Figure 1:** Overview of Pathways Affected by CTLA-4 and PD-1

**Figure 2:** Timing of Administration of AGEN1884 and AGEN2034

**Figure 3:** Total Cell Counts per mL of Blood Shows Stable Lymphocyte Population for Both Cohorts

**Figure 4:** Mean Frequency of Parent Cell Population Over Clinical Study Time Points

**Figure 5:** Fold Change in Ki-67 Expression

**Figure 6:** Fold Change in Ki-67 Expression per Cell Type at Various Clinical Time Points for AGEN1884/AGEN2034 Combination

**Figure 7:** Total Cell Counts per mL of Blood Shows Stable Lymphocyte Population for Both Cohorts

**Figure 8:** Mean Frequency of Parent Cell Population Over Clinical Study Time Points

This analysis was funded by Agenus Inc. (Lexington, MA, USA).

**REFERENCES**

4. This analysis was supported by a grant from the National Cancer Institute of Canada (Funding Grant No. 109868).
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BACKGROUND

• Antigen-specific T-cell activation is regulated by a balance of co-stimulatory and co-inhibitory signals, such as those mediated by inhibitory receptors cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) and programmed cell death protein-1 (PD-1) (Figure 1). Binding of these receptors to their ligands results in impaired T-cell function. For these reasons, antibody blockade of PD-1 and CTLA-4 has been identified as a therapeutic modality to reinvigorate or induce tumor-specific T-cell immunity.1 The combination of inhibition of PD-1 and CTLA-4 pathways by blockade of receptor-ligand interactions has been demonstrated in numerous clinical trials to result in objective clinical response and increased survival in several solid tumor indications, including melanoma and non-small cell lung carcinoma. As a result, ipilimumab (anti-CTLA-4) in combination with nivolumab (anti-PD-1) has been approved as a first line of treatment in patients with metastatic melanoma, and is currently being tested in multiple clinical trials for other indications.2,3

Figure 1. Overview of Pathways Affected by CTLA-4 and PD-1

CONCLUSIONS

• A similar absolute increase in Ki-67 expression was observed with AGEN1884 (anti-CTLA-4 antibody) in combination with AGEN2034 (anti-PD-1 antibody). This was a phase 2, multicenter, open-label clinical study to evaluate the pharmacodynamics of ipilimumab in combination with nivolumab in patients with advanced or metastatic solid tumors (ANZCTR Registration Identifier: ACTRN12617001059358).

Objectives

• To assess Ki-67 expression on immune populations after combined blockade of CTLA-4 and PD-1, using the 2-dose regimen of ipilimumab in combination with nivolumab or AGEN1884 in combination with AGEN2034, in patients with advanced or metastatic solid tumors.

IPILIMUMAB IN COMBINATION WITH NIVOLUMAB

Methods

• This was a phase 2, multicenter, open-label clinical study to evaluate the pharmacodynamics of ipilimumab in combination with nivolumab in patients with advanced or metastatic solid tumors (ANZCTR Registration Identifier: ACTRN12617001059358).

• A total of 21 eligible patients were targeted for enrollment into 2 cohorts, which were enrolled concurrently.

  – Cohort 1: 9 patients; receiving 1 mg/kg of ipilimumab every 6 weeks (q6w) and nivolumab 3 mg/kg every 2 weeks (q2w) until disease progression or discontinuation due to toxicity or a maximum of 12 weeks (2 cycles).

  – Cohort 2: 12 patients; receiving 0.3 mg/kg of ipilimumab q6w and nivolumab 3 mg/kg q2w until disease progression or discontinuation due to toxicity or a maximum of 12 weeks (2 cycles).
Herein, a validated assay was developed to measure Ki-67 expression in various circulating immune cell types in samples from patients treated with AGEN1884 plus nivolumab or AGEN1884 in combination with AGEN2034, in patients with advanced or metastatic solid tumors.

**BACKGROUND**

CT104 ipilimumab (anti-CTLA-4) in combination with nivolumab (anti-PD-1) has been approved as a first line of treatment in patients with metastatic melanoma, and is currently have future benefit as a biomarker of response.

Cohort 1: 3.13-fold increase (range, 2.31–4.33) from baseline at cycle 1 day 8 post-treatment.

**CONCLUSIONS**

Both Cohort 1 and Cohort 2 fully recruited with all patients treated for 12 weeks.

- 9 patients were enrolled to receive ipilimumab 1.0 mg/kg q6w / nivolumab 3 mg/kg q2w.
- 12 patients were enrolled to receive ipilimumab 0.3 mg/kg q6w / nivolumab 3 mg/kg q2w.

**Fold Change in Ki-67 Expression**

- A highly statistically significant increase in Ki-67 expression was observed in CD8+ T cells in both cohorts (Figure 5).
  - Cohort 1: 4.56-fold increase (range, 3.35–6.20) from baseline at cycle 1 day 8 post-treatment (P<0.001)
  - Cohort 2: 3.13-fold increase (range, 2.31–4.33) from baseline at cycle 1 day 8 post-treatment (P<0.001)

- Significant increases in Ki-67 expression were also observed in CD8+ T cells, regulatory T cells, and NKT cells following treatment with combined CTLA-4 / PD-1 inhibition.

**Results**

- Both Cohort 1 and Cohort 2 fully recruited with all patients treated for 12 weeks.
  - 9 patients were enrolled to receive ipilimumab 1.0 mg/kg q6w / nivolumab 3 mg/kg q2w.
  - 12 patients were enrolled to receive ipilimumab 0.3 mg/kg q6w / nivolumab 3 mg/kg q2w.

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  - Cohort 2: 3.13-fold increase (range, 2.31–4.33) from baseline at cycle 1 day 8 post-treatment (P<0.001)

- Significant increases in Ki-67 expression were also observed in CD8+ T cells, regulatory T cells, and NKT cells following treatment with combined CTLA-4 / PD-1 inhibition.

**Figure 2. Gating Strategy to Identify Specific Cells of Interest**

**Figure 3. Total Cell Counts per mL of Blood Shows Stable Lymphocyte Population for Both Cohorts**

A. Total lymphocytes

B. Total CD3+ cells

C. CD4+ T cells

D. CD8+ T cells

E. CD19+ B cells

F. CD56+CD16+ NK cells

Time points are depicted as C for cycle and D for day after initial treatment. Analysis of total cell counts per mL of blood demonstrated a stable lymphocyte population for both cohorts across different cell types, including total lymphocytes, total CD3+ cells, CD4+ T cells, CD8+ T cells, CD19+ B cells, and CD56+CD16+ NK cells.
Herein, a validated assay was developed to measure Ki-67 expression in various circulating immune cell types in samples from patients treated with AGEN1884 plus AGEN2034. Previous studies have suggested that antibody therapy blocking the PD-1/ PD-L1 axis alone or in combination with CTLA-4 mediated increased proliferation in advanced solid tumors. Agenus has developed novel anti-PD-1 and anti-CTLA-4 antibodies, AGEN2034 (human IgG4) and AGEN1884 (human IgG1), respectively, that are currently under clinical investigation.

**BACKGROUND**

CT104 is a clinical trial of AGEN2034 and AGEN1884 in combination with ipilimumab (anti-CTLA-4) and nivolumab (anti-PD-1). It has been approved as a first line of treatment in patients with metastatic melanoma, and is currently being assessed on review of the dose-limiting toxicities during dose escalation in the first 21 days of treatment.

**METHODS**

- **A similar absolute increase in Ki-67 expression was observed with AGEN1884 (anti-CTLA-4 antibody) in combination with nivolumab (anti-PD-1 antibody) blockade, with the absolute increase in Ki-67 expression observed to be numerically dose dependent.**

- **Ki-67 was measured for 3 patients in Cohort 1 who received AGEN1884 1 mg/kg q6w / AGEN2034 3 mg/kg q2w and 2 patients in Cohort 2 who received AGEN1884 0.3 mg/kg of ipilimumab q6w and nivolumab 3 mg/kg q2w until disease progression or discontinuation due to toxicity or a maximum of 1 year.**

- **Time points are depicted as C for cycle and D for day after initial treatment. Analysis of parent population at various clinical study time points identified a consistent frequency of targeted cells in both cohorts over time. Geometric mean and 95% confidence intervals are shown.**

**RESULTS**

- **Both nivolumab and ipilimumab were administered intravenously in clinic at the study sites to ensure correct administration and to review for any adverse reaction.**

- **Figure 4. Mean Frequency of Parent Cell Population Over Clinical Study Time Points**

- **Figure 5. Fold Change of Ki-67 Expression per Cell Type at Various Clinical Time Points for Ipilimumab and Nivolumab Combination**

**AGEN1884 IN COMBINATION WITH AGEN2034**

**METHODS**

- **This is a phase 1, open-label, multi-arm clinical study to evaluate the pharmacodynamics of AGEN1884 in combination with AGEN2034 in patients with locally advanced or metastatic solid tumors (ANZCTR Registration Identifier: ACTRN12618000003279).**

- **A total of 20 eligible patients are targeted for enrollment.**

- **The first 3 patients enrolled received the starting dose below:**
  - Cohort 1: AGEN1884 1 mg/kg q6w / AGEN2034 1 mg/kg q2w
  - Cohort 2: AGEN1884 0.3 mg/kg q6w / AGEN2034 3 mg/kg q2w

- **After 21 days, a safety review was completed with additional ≥7 patients recruited to backfill each cohort.**

- **Both AGEN1884 and AGEN2034 were administered intravenously in clinic at the study sites to ensure correct administration and to review for any adverse reactions.**
  - When administered together AGEN2034 was infused first followed by AGEN1884 on the same day.

- **The primary outcome of the study is to assess the safety and tolerability of AGEN1884 in combination with AGEN2034 in subjects with metastatic and/or locally advanced solid tumors.**
  - This is currently being assessed on review of the dose-limiting toxicities during dose escalation in the first 21 days of treatment.
  - Safety, including physical examinations, vital signs, clinical laboratory tests, electrocardiogram, ECOG performance status, and adverse events, are being evaluated from baseline until end of treatment, defined as end of week 12 or 2 weeks post-final dose of study drug.

- **Secondary outcomes include the change in Ki-67 expression from baseline in CD4+ peripheral blood T cells at cycle 1 day 4 and day 8, and from baseline to the end of the first 6-week cycle.**
CONCLUSIONS

• These results identify and support Ki-67 expression as a pharmacodynamic biomarker in the context of CTLA-4 (ipilimumab) and PD-1 (nivolumab) blockade, with the absolute increase in Ki-67 expression observed to be numerically dose dependent.
• A similar absolute increase in Ki-67 expression was observed with AGEN1884 (anti-CTLA-4 antibody) in combination with AGEN2034 (anti-PD-1 antibody).
• No changes in total immune cell populations were observed after combination therapy in both trials.
• Ongoing work is being conducted to correlate these findings with clinical outcomes to establish a benchmark biomarker assay for anti-CTLA-4 and anti-PD-1 combination treatment of patients with advanced solid tumors.

References

Acknowledgments
Medical writing and editorial support were provided by The Medicine Group, LLC (New Hope, PA, USA), which was funded by Agenus Inc. (Lexington, MA, USA).

Funding
This analysis was funded by Agenus Inc. (Lexington, MA, USA).

Presented at the 2018 Annual Meeting of the American Association of Cancer Research, April 14–18, 2018, in Chicago, IL, USA.