CT104

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

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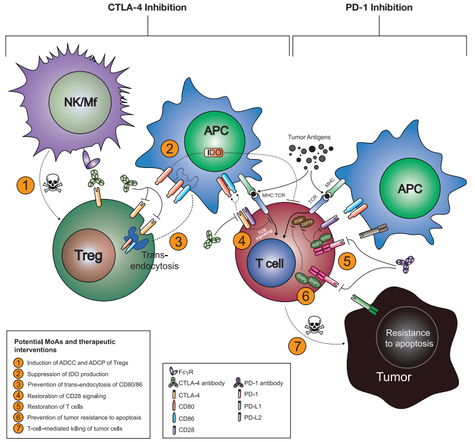
BACKGROUND

- 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**).[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.[2,3,4]

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



- Agenus has developed novel anti-PD-1 and anti-CTLA-4 antibodies, AGEN2034 (human IgG4) and AGEN1884 (human IgG1), respectively, that are currently under evaluation as monotherapy in phase 1/2 studies in subjects with advanced tumors (NCT03104699 and NCT02694822, respectively). A phase 1/2, open-label, multi-arm trial to investigate the safety, tolerability, pharmacokinetics, and biological and clinical activity of AGEN1884 in combination with AGEN2034 in subjects with metastatic or locally advanced solid tumors is currently ongoing (C550-01; ACTRN12618000003279).
- 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 circulating T cells, as measured by expression of the nuclear protein Ki-67, a marker for proliferation that is selectively expressed during active stages of the cell cycle.[5-8] 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.[7] The measurement of Ki-67 during clinical trials may therefore be useful to assess antibody response to therapy and, when measured early, may potentially have future benefit as a biomarker of response.
- 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 combination therapy or commercially available ipilimumab and nivolumab.

OBJECTIVE

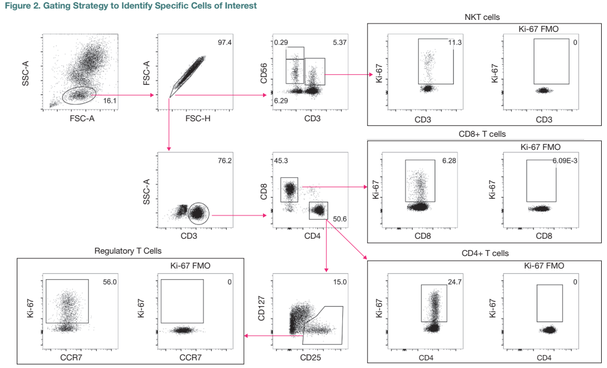
- To assess Ki-67 expression on immune populations after combined blockade of CTLA-4 and PD-1, using the 2-dose regimen of ipilimumab in combination with nivolumab or AGEN1884 in combination with AGEN2034, in patients with advanced or metastatic solid tumors.

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).
- Both nivolumab and ipilimumab were administered intravenously in clinic at the study sites to ensure correct administration and to review for any adverse reaction.
  - When administered in combination, nivolumab was infused first, followed by ipilimumab on the same day.
- Blood samples were collected at different days post-treatment and stained for surface T-cell and natural killer (NK)-cell surface markers and Ki-67 intracellularly.
- A gating strategy was utilized to identify CD4+ T cells, CD8+ T cells, and regulatory T cells, CD56+ NK cells, CD56++ NK cells, and NKT cells (**Figure 2**).
  - Sample flow plots depict staining for Ki-67 and fluorescence minus one negative controls, for which the antibody against Ki-67 was excluded.
  - Numbers depict the frequency of each subpopulation as a percentage of its parent population.

Figure 2. Gating Strategy to Identify Specific Cells of Interest



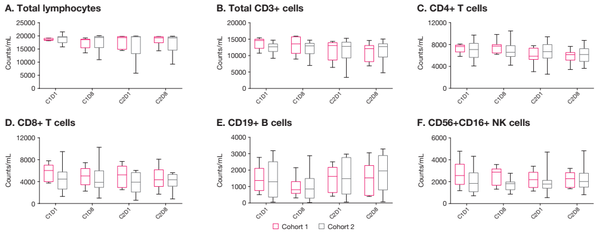
Results

- 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.

Fold Change in Ki-67 Expression

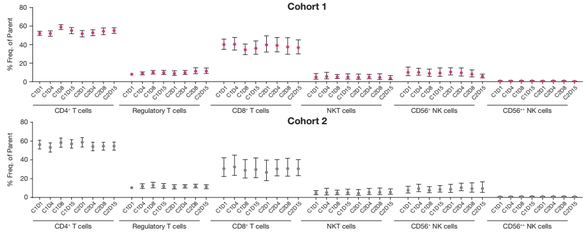
- A highly statistically significant increase in Ki-67 expression was observed in CD4+ T cells in both cohorts (**Figure 5**).
  - Cohort 1: 4.56-fold increase (range, 3.35–6.20) from baseline at cycle 1 day 8 post-treatment ($P<0.001$)
  - Cohort 2: 3.13-fold increase (range, 2.31–4.33) from baseline 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.

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



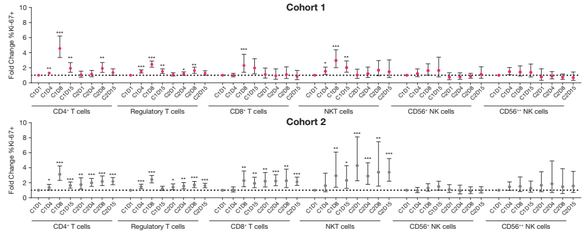
Time points are depicted as C for cycle and D for day after initial treatment. Analysis of total cell counts per mL of blood demonstrated a stable lymphocyte population for both cohorts across different cell types, including total lymphocytes, total CD3+ cells, CD4+ T cells, CD8+ T cells, CD19+ B cells, and CD56+CD16+ NK cells.

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



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.

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



Time points are depicted as C for cycle and D for day after initial treatment. ***$P<0.001$, **$P<0.01$ and *$P<0.05$. Dotted line is baseline. Geometric mean and 95% confidence intervals are shown.

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
- Once safety of Cohort 1 was established, 3 patients were enrolled for the following dose:
  - Cohort 2: AGEN1884 1 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.

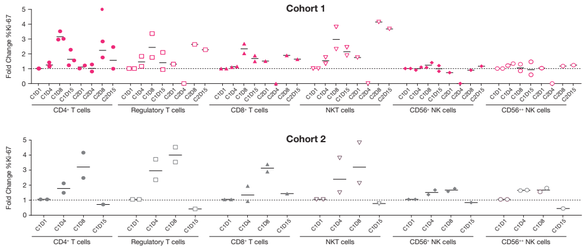
Results

- Cohort 1 and Cohort 2 are still in recruitment as of March 22, 2018.
  - 4 patients are currently enrolled receiving AGEN1884 1 mg/kg q6w / AGEN2034 1 mg/kg q2w.
  - 3 patients are currently enrolled receiving AGEN1884 1 mg/kg q6w / AGEN2034 3 mg/kg q2w.
- 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 1 mg/kg q6w / AGEN2034 3 mg/kg q2w.

Pharmacodynamic Evaluation

- Initial results from this clinical trial demonstrated an increase in Ki-67 expression in CD4+ T cells with the AGEN1884 / AGEN2034 combination, similar to the results seen in the current ipilimumab / nivolumab phase 1 clinical trial (**Figure 6**).
- Analysis of parent population at various clinical study time points identified a consistent frequency of targeted cells in both cohorts over time (data not shown).

Figure 6. Fold Change of Ki-67 Expression per Cell Type Over Treatment Timeline at Various Clinical Time Points for AGEN1884 / AGEN2034 Combination



Time points are depicted as C for cycle and D for day after initial treatment. Geometric mean is shown.

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 finding 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

1. Topalian SL, Drake CG, Pardoll DM. *Cancer Cell*. 2015;27(4):450-61.
2. Hodi FS, Chesney J, Pavlick AC, et al. *Lancet Oncol*. 2016;17(11):1558-1568.
3. Antonia SJ, López-Martin JA, Bendell J, et al. *Lancet Oncol*. 2016;17(7):883-895.
4. Hellmann MD, Gettinger SN, Goldman JW, et al. *J Clin Oncol*. 2016;34(15_suppl):3001-3001.
5. Antonia S, Goldberg SB, Balmanoukian A, et al. *Lancet Oncol*. 2016;17(3):299-308.
6. Das R, Verma R, Sznol M, et al. *J Immunol*. 2015;194(3):950-9.
7. Huang AC, Postow MA, Orlowski RJ, et al. *Nature*. 2017;545(7652):60-65.
8. Bruno S, Darzynkiewicz Z. *Cell Prolif*. 1992;25(1):31-40.

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.