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# Preliminary pharmacodynamic evaluation of AU-007 in a phase 1a dose escalation trial for solid tumors (NCT05267626)

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aulos

**Introduction:**
AU-007 is a human IgG1-LALA antibody that binds to human IL-2 at the CD25 binding site without impinging on the CD122 or CD132 binding motifs on IL-2. This binding leads to an inhibition of IL-2 binding to the CD25-containing trimeric receptor found on regulatory T cells (Tregs), activated effector cells (which upon binding induces reactivation cell death), eosinophils, and vascular endothelium. However, AU-007 binding to IL-2 still allows IL-2 binding to the dimeric receptor expressed on naïve T cells, memory phenotype T cells (a key population to target with cancer immune therapy), NK cells, and NK T cells.

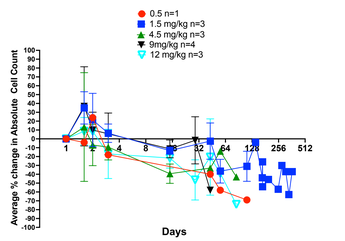
AU-007 is currently in the dose escalation portion (Phase 1) of a dose escalation and expansion (Phase 2) trial in patients with select solid tumors. During dose escalation, AU-007 has been dosed with escalating monotherapy or in combination with either a single escalating low dose of aldesleukin (human IL-2) on Day 1 or dosed concurrently with each AU-007 dose.

During the trial, pharmacodynamic markers in the periphery and tumor biopsies are being collected to investigate the activity of AU-007 or AU-007+IL-2. Here we are reporting updated peripheral blood pharmacodynamic data from the ongoing study. Immunophenotyping of peripheral blood and the inflammatory cytokine interferon-gamma (IFN-γ) as well as peripheral eosinophils were examined, and preliminary results updated here.

**Methods:**
Peripheral blood samples were taken prior to dosing on Day 1 and following dosing at 4 hours, and on Days 2, 3, 15, 29, 43 of cycle 1 and pre-dose/Day 29 on all other cycles. Dosing with AU-007 was done via intravenous dosing by weight-based dosing (mg/kg). Proleukin® (aldesleukin) was dosed subcutaneously by weight-based dose (IU/kg). Whole blood was stained for CD4+ T cells, CD8+ T cells, CD4+ Tregs, NK cells, B cells, and monocytes. Samples were analyzed by flow cytometry using TruCount™ for absolute cell counts. Change from baseline values were determined off absolute counts. In addition, differential hematology counts were taken on Days 1, 15, and 29 of all cycles (or as needed per treating physician's decision) for safety evaluation and to examine eosinophil levels (eosinophils express the IL-2 trimeric receptor). Serum was taken prior to dosing on Day 1 and at 2 hours, 6 hours and Days 2, 3, 15 pre/post and 6 hours, 29 pre/post, 43 pre/post, and subsequent Day 1 of each cycle at pre/post. Samples were analyzed for INF-γ (LOQ 31 fg/ml), IL-2 (LOQ 31 fg/ml), and sCD25 (10 fg/ml) using the ECL Mesoscale Dynamics platform.

## Figure 1: Change in Peripheral Blood Tregs for Cohorts Receiving AU-007 Without and With Proleukin [change in absolute cell numbers (abs)]

**A: Without** Proleukin | **B: With** Proleukin



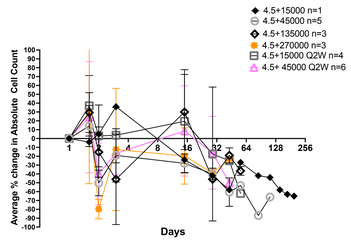
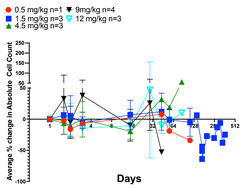
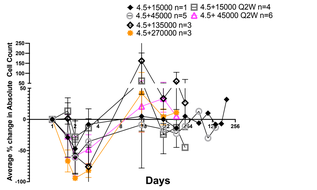




**Figure 1:** Percent change in the absolute number of circulating regulatory T cells. Regulatory T cells were defined as CD3+CD4+CD25+CD127lo of the CD45+ cells. Consistent with the mechanism of action of inhibiting IL-2 from interacting with the trimeric receptor, regulatory T cells decreased in the peripheral circulation. This was observed in both the monotherapy arm and the arms which also included Proleukin and was consistent among patients. **A)** Cohorts receiving only AU-007 **B)** Cohorts with both AU-007 and at least 1 dose of Proleukin

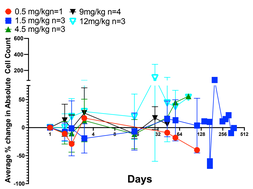
## Figure 2: Change in Peripheral Blood Effector Cells for Cohorts Receiving AU-007 Without and With Proleukin [change in absolute cell numbers (abs)]

**A: CD8+ T cells – Without** Proleukin | **With** Proleukin




**B: NK cells – Without** Proleukin | **With** Proleukin



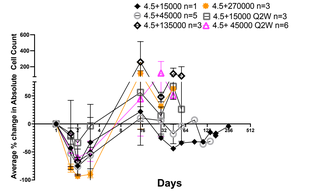
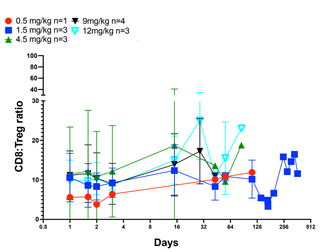




**Figure 2:** Percent change in the absolute number of circulating **A)** CD8+ T cells and **B)** NK cells. Increases in circulating effector cells were observed over time with monotherapy and lowest doses of IL-2 showing lower levels of peripheral effector cell expansion. Expansions were observed the longer the patient stayed on study. This is consistent with the mechanism of action of AU-007 stabilizing low levels of IL-2 and the requirement to build to an activation threshold of IL-2. In the presence of higher levels of IL-2, both CD8+ T cells and NK cells increased higher and earlier. Higher doses of IL-2 are being further explored and results will be reported at a later date.

## Figure 3: Change in CD8+/Treg Ratios for Cohorts Receiving AU-007 Without and With Proleukin

**Without** Proleukin | **With** Proleukin



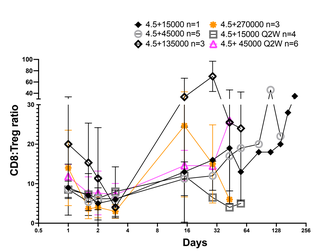
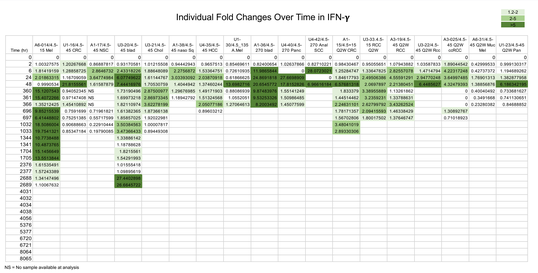




**Figure 3:** Change in the ratio of CD8+ T cells to Tregs observed in the periphery. Consistent with the observations seen in the changes in Tregs and CD8+ T cells, there is an observed trend to an increase in the CD8+/Treg ratio with monotherapy. In the presence of Proleukin, an increase in the CD8+/Treg ratio was observed, particularly at higher doses of Proleukin. Consistent with the mechanism of action, higher doses (of low dose IL-2) and longer exposure trend to higher CD8+/Treg ratios with no observed drug-related toxicity. It is anticipated that increasing doses of Proleukin will further enhance the peripheral response.

## Figure 4A: Fold Change in the Expression of IFN-γ in Patients Dosed With AU-007


NS = No sample available at analysis

## Figure 4B: Fold Change in the Expression of IFN-γ in Patients Dosed With AU-007+Proleukin




NS = No sample available at analysis

**Figure 4 (A) and (B):** A heat map of the change from baseline in the circulating levels of IFN-γ. Light green represents a 0.2- to 2-fold change, mid-green a 2- to 5-fold change and dark green >5-fold change. These preliminary results demonstrate that the longer a patient is on monotherapy, the more likely the patient is to have increases in circulating IFN-γ. This is consistent with the observations in circulating cell populations, particularly Treg and NK cells. The addition of low dose IL-2 in the presence of AU-007 consistently increases IFN-γ in the peripheral circulation.

## Figure 5A: Individual Peripheral Blood Eosinophil Counts in AU-007-Only Cohorts

## Figure 5B: Individual Peripheral Blood Eosinophil Counts in AU-007+Proleukin Cohorts

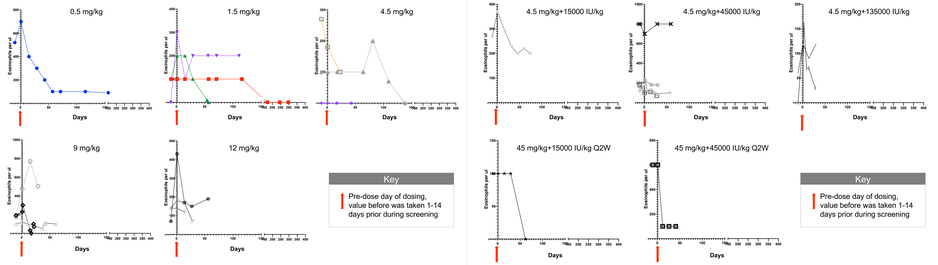



**Figure 5 (A) and (B):** Changes over time in the circulating number of eosinophils. Panel A are the cohorts receiving only AU-007 monotherapy and panel B are cohorts receiving AU-007 with at least 1 dose of Proleukin. All but one patient in the AU-007 monotherapy and AU-007 with Proleukin arms demonstrated a decrease or no change in the circulating levels of eosinophils. A patient in the 9 mg/kg cohort had severe seasonal allergies requiring treatment during time on AU-007 treatment and is consistent with a history of being treated for seasonal allergies. The rise in eosinophils was attributed to the allergy reaction. All patients given AU-007 with Proleukin showed stable or a decrease in circulating eosinophils. This is consistent with the mechanism of action of AU-007 preventing IL-2 from interacting with the IL-2 trimeric receptor on eosinophils.

## Conclusions

The preliminary pharmacodynamic data from the AU-007 Phase 1 trial in multiple solid tumors show treatment with AU-007 as monotherapy or in combination with Proleukin induces changes consistent with the mechanism of action (i.e., redirecting of IL-2 away from regulatory T cells and toward effectors) and consistent with an increase in the overall inflammatory profile of patients. The overall effect increases with time on therapy or with the addition of higher levels of Proleukin. These changes were not associated with any drug-related events (data not shown). AU-007 treatment, with or without Proleukin, led to decreases in eosinophils. Proleukin administered as a single agent is known to raise eosinophil counts, and such increases have been associated with an adverse event profile. No changes were observed in circulating sCD25 levels. IL-2 levels are still being investigated.

## Discussion

The preliminary data presented here support the overall mechanism of action for AU-007. The observed decreases in circulating regulatory T cells and increases in IFN-γ in the presence of the low doses of IL-2 assessed in this trial administered with AU-007 counters what is typically observed if low doses of IL-2 are given in the absence of AU-007. Low dose IL-2 given as a single agent increases Tregs and decreases IFN-γ in circulation and hence is being investigated as a treatment for autoimmune diseases. Proleukin given as a single agent also has a well-characterized adverse event of increasing circulating levels of eosinophils, and lung toxicity can ensue from the eosinophilia. IL-2 interacting with the trimeric receptor on the eosinophils is thought to be the major contributor of this increase. Here, again consistent with the mechanism of action of AU-007 redirecting IL-2 away from the trimeric receptor, AU-007 or AU-007+Proleukin produce decreases in circulating levels of eosinophils, supporting the proposed activity of the antibody. While the overall number of patients per group is small and each cohort has multiple cancer types, the trends observed here with AU-007 and increasing Proleukin doses demonstrate favorable pharmacodynamic effects, including reductions in regulatory T cells, increases in CD8+ T cells and NK cells, increases in CD8+/Treg ratios, and increases in IFN-γ, in a broad range of cancer types. Further investigations with higher doses of Proleukin administered with AU-007 are currently being investigated. Efficacy and safety data for the study are to be reported at a later date.

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