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Short-course IL-15 given as a continuous infusion led to a massive expansion of effective NK cells: Implications for combination therapy with antitumor antibodies

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Supplementary Data:

Supplemental Methods and Materials:

Patients and Methods:

The primary objective of this trial was to define the safety, toxicity profile, and maximum tolerated dose (MTD) for this 5-day (120 hours) continuous intravenous (CIV) IL-15 treatment using a standard 3 + 3 phase 1 dose-escalation design.

Secondary objectives were the characterization of the changes in lymphocyte populations, inflammatory cytokine production and assessment of antitumor response. The patients were age ≥ 18 years, had histologically confirmed metastatic solid tumors, ECOG performance status 0 or 1, DLCO/VA and FEVI > 50% predicted, absolute neutrophil count > 1,500/mcL, platelets > 100,000 mcL, total bilirubin within normal institutional limits, aspartate aminotransferase/alanine aminotransferase AST/ALT ≤ 2.5 upper limit of normal (ULN), serum creatinine ≤ 1.5 x ULN, absence of active central nervous system CNS metastases, no history of clinically significant autoimmune disease or hematopoietic malignancy. No history of severe asthma, did not require use of systemic corticosteroid treatment or inhaled steroids, no evidence of clinically active infection, no history or serology positive for human immunodeficiency virus HIV or hepatitis B or C or human T-cell lymphotropic virus-1 HTLV-1, no clinically significant congestive failure (NYHA Class II or greater), heart disease. Pregnant female patients were excluded. Patients must be more than 4 weeks from their most recent chemotherapy treatment, 6

weeks for nitrosourea mithramycin, 8 weeks from anti-CTLA-4 or anti-programmed death-ligand 1 (anti-PD-L1), more than 2 weeks from radiation therapy, having recovered from previous treatment, not be receiving any other investigational treatment and be able to give informed consent. The patients received the IL-15 as inpatients.

Dose escalation was to proceed unless dose-limiting toxicity (DLT) occurred in ≥ 2 out of 3 or 6 patients treated in one of the dose levels during the first treatment cycle. In the event that ≥ 2 patients in any dosing cohort experience DLTs dose escalation would be halted and the next lower level considered the MTD. The NCI common toxicity criteria version 4 (CTC v4) was used to assess adverse events (AEs).

This was a single institution, open-label, nonrandomized 3 + 3 design, phase 1 dose-escalation study. The patients received IL-15 as inpatients. Intake/output, patient weights, and vital signs involving an R.N. were evaluated in the Clinical Center NIH. Continuous ECG monitoring was not used. Although the study was performed in an inpatient facility, the Clinical Center, NCI, we feel that it would be safe and convenient to be performed as an outpatient. Page 15, line 11: we indicate "In conclusion, the short duration CIV-5 rhIL-15 regimen and its safety profile may make outpatient administration via an ambulatory infusion pump feasible."

This study was performed at the Clinical Center NIH. Groups of 3 to 6 subjects received CIV rhIL-15 at doses of 3, 4, and 5 mcg/kg/day for 5 days provided that the DLT had not been observed. DLT was defined as follows: any grade 3 or 4 toxicity deemed possibly, probably or definitely related to study drug by the NCI principal investigator (PI) during the first cycle of treatment with the following exceptions:

Hematological Exceptions:

- Any asymptomatic grade 3 or 4 lymphocytopenia unless these are deemed signs of significant infection (persistent fevers, labile blood pressure, localized complaints or finding on physical examination of hypoxia or organ dysfunctions).
- Grade 3 granulocytopenia unless there are clinical signs of infections as noted above.
- Grade 3 leukocytosis (WBC > 100,000 mm^3) in the absence of leukostasis.

Nonhematological Exceptions:

- Transient (less than 24 hours) grade 3 hypoalbuminemia, hypokalemia, hypomagnesemia or hypophosphatemia that respond to medical intervention.
- Nonsustained (< 7 days) grade 3 liver function tests (ALT/AST, alkaline phosphatase, total or direct bilirubin) abnormalities in the absence of clinical signs of hepatic dysfunction (lethargy, confusion, anorexia, pruritus or tremors).

Dose escalation proceeded in cohorts of 3 to 6 patients. Patients were not to begin on treatment in the next higher dose level unless all patients treated at the previous level reached 21 days of the protocol, recovered from any clinical or laboratory toxicities and were able to initiate another cycle of treatment unless restaging has demonstrated progression of their disease. Patients receiving the 5-day treatment schedule without evidence of ongoing response after 2 cycles of treatment discontinued rhIL-15. Patients manifesting an ongoing response defined as > 15% decrease in the sum of marker lesions and/or improvement or disappearance of some non-marker lesions received additional cycles. Cycles 1 and 2 were 42 days in length but all subsequent cycles were 28 days in length. Toxicities of only the first cycle were considered in selecting the MTD recommended phase 2 dose (RP2D). Patients could continue rhIL-15 if there was ongoing evidence of clinical activity or for 2 additional cycles beyond radiographic complete response (CR) was observed. Patients with stable disease (SD) after 2 cycles of treatment were followed

for response until disease progression was documented or if they chose to start another treatment. Patients were given nonsteroidal anti-inflammatory drugs (NSAIDs) or other antipyretics, analgesics, antiemetics, antidiarrheal and meperidine for fever, myalgias, nausea, vomiting diarrhea and rigors initially on a prn basis.

Hematology, Clinical Chemistry and Fluorescence Activated Cell Sorting Analysis:

Hematological tests performed after infusion include: WBC, RBC, hemoglobin, hematocrit, mean corpuscular volume, platelet count and percent and absolute numbers of peripheral blood lymphocytes, monocytes, eosinophils, and neutrophils. Bone marrow aspirates were obtained on day 5. The following clinical chemistry tests were also performed: Serum Na, K, Cl, glucose, phosphorus, alkaline phosphatase, ALT, AST, total and direct bilirubin, BUN, creatinine, total protein, albumin, troponin-T, cholesterol, triglycerides, magnesium, amylase, lactic dehydrogenase and uric acid. The following coagulation tests were performed: prothrombin time, partial thromboplastin time and fibrinogen. Assays for soluble IL-2 receptor alpha and 18 concentrations were assessed on these samples using enzyme-linked immunosorbent assay. Plasma, IL-6, interferon gamma, IL-1beta and tumor necrosis factor were assayed by Meso Scale Discovery (MSD) V-PLEX Assay.

Flow Cytometry:

Polychromatic flow-cytometry analysis was performed, as described previously, on heparinized blood obtained preinfusion and at various timepoints after the first infusion, with analysis of multiple leukocyte populations including: CD4, CD8, T cells, B cells, NK cells, T regulatory cells and gamma-delta cells, monocytes and proliferating cells were detected by the analysis of intracellular Ki-67 on cellular subsets [(1, 2)]. Conjugated antibodies were purchased from BD Biosciences (San Jose, CA), Biolegend (San Diego, CA), Ebioscience (San Diego, CA),

Beckman Coulter, Inc. (Brea, CA) or Thermo Fisher Scientific (Waltham, MA). Unconjugated antibodies were purchased from BD Pharmingen and conjugated with either Alexa Flour 594 at 51 µg dye mg antibody or inhouse tandem of cy5PE at 400 µL dye/mg antibody for anti-HLA-DR. Flow cytometry data were compensated and analyzed using FlowJo Software (version 9.9.6 http://www.flowjo.com). Data were analyzed and presented with JMP Software (version 13.0).

ELISA Method for Detection of Host Production of Antibody Directed Toward Infused rhIL-15

Serum samples were analyzed for the *in vivo* production of antibodies to rhIL-15 as modified from[(1)]. This two-arm capture ELISA procedure achieved a limit of quantitation of 156 ng/mL. 156 ng/mL was the limit cutoff for both diluted and undiluted patient samples. The 96-well microliter plate was coated with 100 uL of rhIL-15 at a concentration of 100 ng/mL, sealed, and incubated at 37ºC for 3 hours. The plates were then washed four times with PBST using a plate washer. To eliminate any remaining active plate sites, 300 uL of PBS/3% BSA blocking buffer was added to all wells; incubated at 37ºC for one hour; and washed as described. A commercial affinity purified goat antihuman IL-15 (R&D Catalog No. AF315) was used to form a standard curve for antibody quantitation by serial diluting the antibody from 2,500 to 9.8 ng/mL. Test serum samples were diluted at a ratio of 1:3 and added concomitantly to the appropriate IL-15 coated wells along with positive and negative controls. After an overnight incubation at 4ºC, the plates were washed four times with PBST followed by the addition of 100 uL of biotinylated rhIL-15 at a final concentration determined by prior optimal titrations. The plates were sealed; incubated for two hours at 37ºC and washed four times with PBST. 100 uL of streptavidin alkaline phosphatase diluted with PBS/1% BSA at a concentration of 0.5 ug/mL was added to each well of the plate; incubated for two hours at 37ºC; and washed four times with PBST. 100 uL of the freshly prepared substrate p-nitrophenyl phosphatase freshly dissolved in

prewarmed diethanolamine buffer was added to the wells for color development. The plate was incubated at 37ºC for 40 minutes to one hour and the resultant color was detected at 405 mm absorbance using the Molecular Devices SpectraMax.

References:

1. Conlon KC, Lugli E, Welles HC, Rosenberg SA, Fojo AT, Morris JC, et al. Redistribution, hyperproliferation, activation of natural killer cells and CD8 T cells, and cytokine production during first-in-human clinical trial of recombinant human interleukin-15 in patients with cancer. J Clin Oncol. **2015**; 33(1):74-82.

2. Dubois S, Conlon KC, Müller JR, Hsu-Albert J, Beltran N, Bryant BR, et al. IL-15 infusion of cancer patients expands the subpopulation of cytotoxic CD56^{bright} NK cells and increases NK-cell cytokine release capabilities. Cancer Immunol Res. **2017**; 5:(10)929-938.