Dynamics of circulating cytokines and chemokines during and after tumor-infiltrating lymphocyte cell therapy with lifileucel in advanced melanoma patients

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Introduction

- Lifileucel is a tumor-derived autologous T cell immunotherapy indicated for the treatment of adult patients with unresectable or metastatic melanoma previously treated with a PD-1 blocking antibody, and if *BRAF* V600 mutation positive, a BRAF inhibitor with or without a MEK inhibitor[1]
- The regimen includes prior nonmyeloablative lymphodepletion (NMA-LD), followed by short course interleukin 2 (IL-2) that contributes to persistence of infused cells[2-4]
  - The safety profile of the regimen is consistent with underlying disease and known safety profiles of NMA-LD and IL-2
- In this study, circulating cytokine levels post-lifileucel infusion were monitored to assess pharmacodynamic effects during and after lifileucel regimen administration
  - In addition, dynamics of cytokines and chemokines were measured to elucidate the mechanism of lymphodepletion in tumor-infiltrating lymphocyte (TIL) cell therapy
  - We also sought to identify potential predictive clinical biomarkers for clinical response to lifileucel

Methods

- Population included 153 patients with unresectable or metastatic melanoma whose disease had progressed on checkpoint inhibitors and, if applicable, BRAF/MEK inhibitors, treated in the C-144-01 (NCT02360579) study[5]
- After tumor resection, patients received NMA-LD (cyclophosphamide on Days −7 to −6 and fludarabine on Days −5 to −1) followed by a single lifileucel infusion (Day 0) and up to 6 doses of IL-2 (Days 0–4)
- Peripheral blood samples were collected at baseline (pre-NMA-LD) and on Days 1, 4, 14, 42, and 84 post-lifileucel infusion
- Serum cytokine and chemokine levels were measured by BioPlex, utilizing the Luminex technology, with a multiplex panel including IL-6, IL-9, IL-10, IL-12, CCL2, CXCL10, IFN-γ, and TNF-α
  - Plasma samples were also tested for IL-15 levels by enzyme-linked immunosorbent assay (ELISA)
  - Serum samples were also tested for IL-7 levels by ELISA

Results

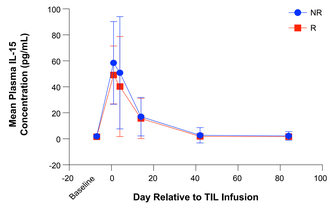
- Samples analyzed across timepoints included both responders (R; CR and PR) and nonresponders (NR; SD, PD, and NE) (**Table 1**)
  - Response evaluated per RECIST v1.1

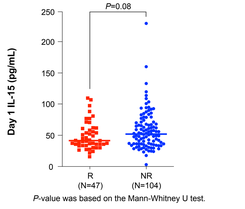
Table 1. Number of Patient Samples Analyzed per Timepoint

Analyte	Baseline	Day 1	Day 4	Day 14	Day 42	Day 84
IL-15	149 R=46, NR=103	151 R=47, NR=104	140 R=45, NR=95	140 R=48, NR=92	135 R=45, NR=90	95 R=38, NR=57
IL-6, IL-7, IL-9, IL-10, IL-12(p40), CCL2, CXCL10, IFN-γ, TNF-α	148 R=46, NR=02	147 R=46, NR=101	135 R=43, NR=92	137 R=46, NR=91	136 R=45, NR=91	101 R=40, NR=61

- The mean levels of IL-15 peaked on Day 1 post-lifileucel infusion, returning to baseline levels by Day 42 (**Figure 1**)
- Kinetics of IL-15 are consistent with an expected increase due to lymphodepletion[6,7]
- IL-15 levels were similar in responders and nonresponders

Figure 1. IL-15 Levels

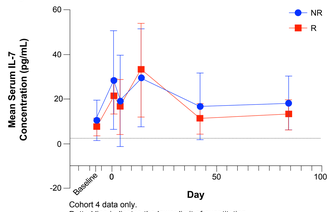




P-value was based on the Mann-Whitney U test.

- Kinetics of IL-7 are consistent with an expected increase due to lymphodepletion[6,7] (**Figure 2**)
- IL-7 levels were similar in responders and nonresponders

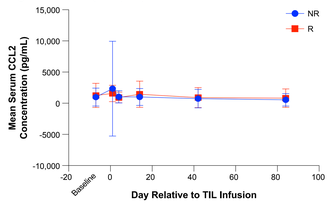
Figure 2. IL-7 Levels



Cohort 4 data only.
Dotted line indicates the lower limit of quantitation.

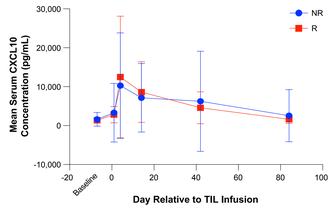
- No increases in mean levels of CCL2 between lymphodepletion and Day 1 were observed (**Figure 3**)
- CCL2 levels were similar in responders and nonresponders

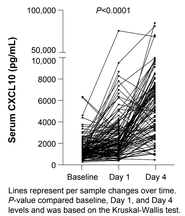
Figure 3. CCL2 (Multiplex) Levels



- The mean levels of CXCL10 were elevated post-lifileucel infusion (**Figure 4**)
- CXCL10 levels further increased at Day 4 post-lifileucel infusion and gradually returned to baseline levels
- CXCL10 levels were similar in responders and nonresponders

Figure 4. CXCL10 (Multiplex) Levels

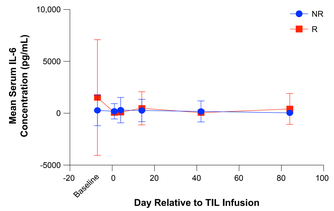




Lines represent per sample changes over time.
P-value compared baseline, Day 1, and Day 4 levels and was based on the Kruskal-Wallis test.

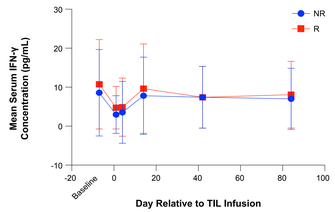
- The mean levels of IL-6 did not increase post-lifileucel infusion (**Figure 5**)
- IL-6, a key mediator of chimeric antibody receptor T cell-associated CRS, remained at low levels following lifileucel treatment[8]
- IL-6 levels were similar in responders and nonresponders

Figure 5. IL-6 (Multiplex) Levels



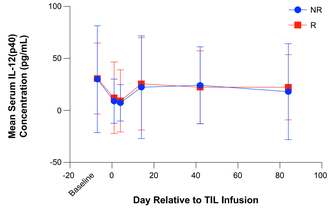
- The mean levels of IFN-γ were below baseline levels on Day 1 (**Figure 6**)
- IFN-γ levels increased by Day 4 and further increased to baseline levels through Day 84
- IFN-γ levels were similar in responders and nonresponders

Figure 6. IFN-γ (Multiplex) Levels



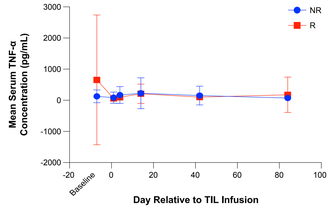
- The mean level of IL-12(p40) was seldom detectable above the lower limit of quantitation (LLOQ) in either responders and nonresponders (**Figure 7**)
- IL-12 levels were similar in responders and nonresponders
- The heterodimeric IL-12(p70) protein was also seldom detectable in our samples (data not shown)

Figure 7. IL-12(p40) (Multiplex) Levels



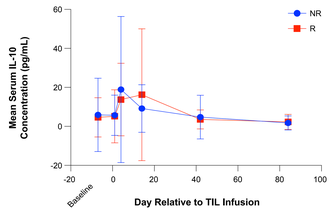
- The mean levels of TNF-α, a measure of immune activation, did not increase post-lifileucel infusion (**Figure 8**)
- TNF-α levels were similar in responders and nonresponders

Figure 8. TNF-α (Multiplex) Levels



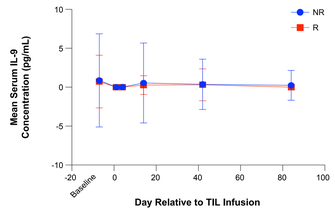
- The mean levels of IL-10 peaked on Day 4 in nonresponders and Day 14 in responders of the lifileucel regimen, returning to baseline levels by Day 42 (**Figure 9**)
- IL-10 levels were similar in responders and nonresponders

Figure 9. IL-10 (Multiplex) Levels



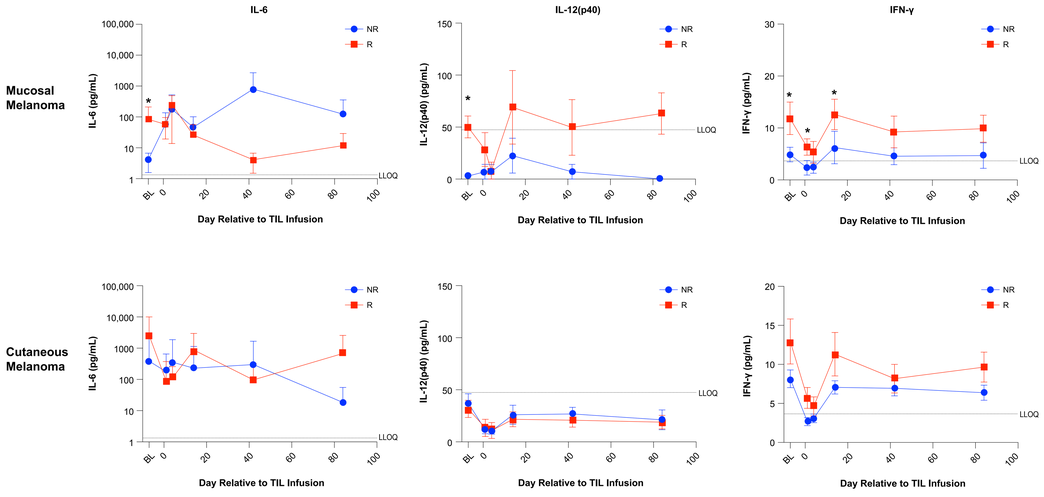
- The mean levels of IL-9 were infrequently detectable above LLOQ in either responders or nonresponders (**Figure 10**)
- Results do not reflect the reported[9] correlation between response and IL-9 baseline levels

Figure 10. IL-9 (Multiplex) Levels



- IL-6, IL-12, and IFN-γ levels in patients with mucosal (n=12, 6 R and 6 NR) and cutaneous melanoma are shown in **Figure 11**

Figure 11. Cytokine Levels By Melanoma Type: Mucosal vs Cutaneous



*Indicates *P*<0.05 vs non-responders based on the Mann-Whitney U test.

Conclusions

- These results support the mechanism of action of lymphodepletion
  - The kinetics of IL-15 are consistent with an expected increase due to lymphodepletion[6,7]
- Circulating CXCL10 levels increased from baseline levels to Day 1 and peaked at Day 4 post-lifileucel infusion and may be a potential pharmacodynamic marker for TIL activity following administration of the lifileucel regimen
- After lifileucel treatment, cytokine levels appeared consistent with a lack of severe systemic inflammation representative of cytokine release syndrome (CRS)
  - IL-6, a key mediator of chimeric antibody receptor T cell-associated CRS, remained at low levels following lifileucel treatment[8]
- All cytokines and chemokines tested showed overlapping distribution of values between responders and nonresponders
  - Further evaluation of cytokine and chemokine levels in the tumor microenvironment may be warranted

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Abbreviations

CCL2, chemokine ligand 2; CCR2, C-C chemokine receptor type 2; CR, complete response; CRS, cytokine release syndrome; CXCL10, C-X-C motif chemokine ligand 10; FDA, US Food and Drug Administration; IFN-γ, interferon-gamma; IL-2, interleukin-2; IL-6, interleukin-6; IL-7, interleukin-7; IL-9, interleukin-9; IL-10, interleukin-10; IL-12, interleukin-12; IL-15, interleukin-15; LLOQ, lower limit of quantitation; NE, nonevaluable; NMA-LD, nonmyeloablative lymphodepletion; NR, nonresponders; PD, progressive disease; PR, partial response; R, responders; RECIST, Response Evaluation Criteria in Solid Tumors; SD, stable disease; TIL, tumor-infiltrating lymphocyte; TNF-α, tumour necrosis factor-alpha.

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