Analysis of Biomarker Responses in Phase I Study of rhIL-18 in Combination with Rituximab in Non-Hodgkin's Lymphoma to Support Phase 2 Dose Selection

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Introduction

- IL-18 is an immunostimulatory cytokine regulating both adaptive and innate immune responses.
- Recombinant human IL-18 (rhIL-18) has demonstrated anti-tumor activity in several preclinical models.
- The current open-label, dose-escalation Phase 1 study evaluates the safety and biological activity of rhIL-18 in combination with rituximab in patients with CD20+ B cell non-Hodgkin's lymphoma.
- IL-18 has the potential to augment the cytotoxic effects of rituximab, (i) by directly enhancing antibody-dependent cell-mediated cytotoxicity (ADCC) through NK cell and monocyte activation and (ii) by inducing a sustained tumor-antigen-specific T cell and antibody response through its stimulatory effects on the adaptive immune response [1]

Objectives

- Elucidate pleiotropic rhIL-18 activity using an extensive panel of plasma based biomarkers (BM)
- Prioritize BMs for future studies
- Support dose selection for a future Phase 2 study in the absence of dose-limiting toxicities and clear efficacy signal due to low patient numbers

Methods

Data

- Subjects received weekly IV infusions of Rituximab (375 mg/m2) on Day 1 of Weeks 1 to 4.
- rhIL-18 was administered as weekly IV infusions on Day 2 of Weeks 1 to 12 in ascending doses from 1-100 µg/kg
- rhIL-18 plasma PK profiles were sampled in Weeks 1, 4, 8 and 12
- Flow cytometry BMs were sampled pre-Rituximab dose, pre-rhIL-18 dose, 4, 48 and 168 hours after rhIL-18 dose in Weeks 1, 4, 8 and 12:
  - Cell counts: total lymphocytes, B cells, CD4/8+ T cells, and CD16+ CD56+ NK cells
  - Cell surface markers: CD69 , CD95L and IL-18R on CD4+ T cells, CD8+ T cells, NK cells and other immune cells
- Soluble mediators were sampled pre-rhIL-18 dose and 4 hrs after rhIL-18 dose in Weeks 1 to12:
  - Cytokines and chemokines: INF-γ, TNF-α, GM-CSF, IL-1, IL-2, IL-6, IL-10, IL-12; MIG, MCP-1, IP-10.

PK/PD analysis

- Biomarker models are based on change from baseline due to large inter-individual and inter-occasion variability of BM measurements.
- Models for BM exposure response relationships were fit using the *nls* and *nlme* functions of S-Plus 7.0.
- Emax model (*Emax* = maximal response, *C* = rhIL-18 concentration, *h*=Hill coefficient, *EC50* = concentration at half maximal response):

$$\%\ \text{change from baseline} = \frac{\text{Emax}\, C^{hc}}{EC50^{hc} + C^{hc}}$$

- Bell-shaped model (µ = concentration at maximal response, *w* = width of response):

$$\text{fold change from baseline} = \text{Emax} \exp\left(-\frac{(C-\mu)^2}{w^2}\right)$$

Results

Pharmacokinetics

- A total of 19 subjects received increasing doses of rhIL-18 in 6 cohorts (Fig. 1)
- Geometric mean of rhIL-18 half-life = 66.2 hrs

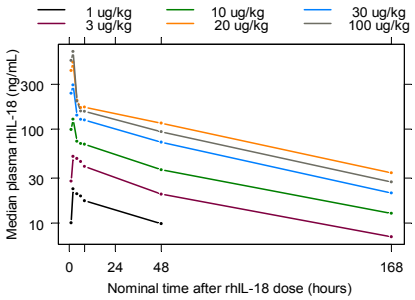

Figure 1: Profile of median rhIL-18 concentrations by dose group

Flow cytometry

- BMs showed a fast (relative to rhIL-18 half-life) and reversible response; time matched rhIL-18 concentrations were used to represent exposure
- No marked, systematic increase or attenuation of responses between cycles
- Among flow cytometry BMs cell counts (total lymphocytes, CD4/8+ T cells, and NK cells) had most robust response pattern: a sharp drop of plasma cell counts at 4 hrs followed by a rebound to baseline levels at 48 hrs (Fig. 2)
- NK cells responded most strongly to rhIL-18 stimulation in terms of their 4 hour response (Emax = -100% , EC90 = 47 ng/mL; see Fig. 3) followed by CD8+ T cells (Emax = -74%, EC90 = 25 ng/mL) and CD4+ T cells (Emax = -71%, EC90 = 190 ng/mL). Due to high variability in BM measurements estimates are associated with relatively high degree of uncertainty.

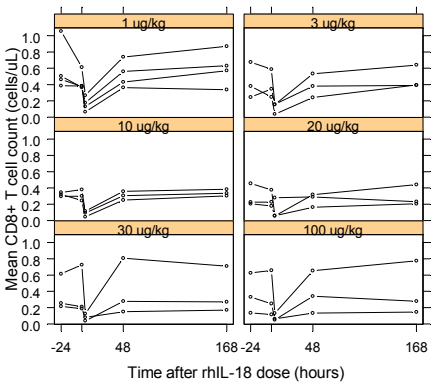

Figure 2: Profile of mean CD8+ T cells response by by patient averaged over rhIL-18 treatment cycles

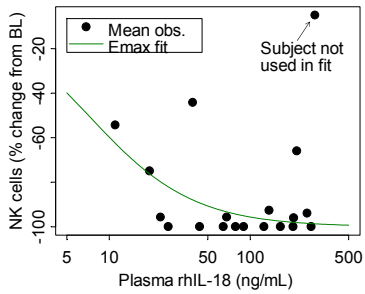

Figure 3: Mean CD16+ CD56+ NK cell exposure response: Cell counts and rhIL-18 concentration at 4 hrs post dose by patient averaged over treatment cycles

- Cell surface markers: CD69+ NK cells showed a pattern of dose dependent increase with maximal response achieved for doses at 20 µg/kg and above (results not shown).

Cytokines and Chemokines

- Most soluble mediators showed potent and repeated stimulation by successive rhIL-18 cycles, with a subset of patients showing especially high peak levels after the first dose of rhIL-18.
- IL-6, IP-10, MCP-1, and MIG had a bell-shaped response pattern with the 100 µg/kg dose cohort typically having sub-maximal response (Fig. 4)
- IL-8 and TNF-α exhibited an approximately linear increase with dose; other soluble markers did not show a clear exposure response pattern

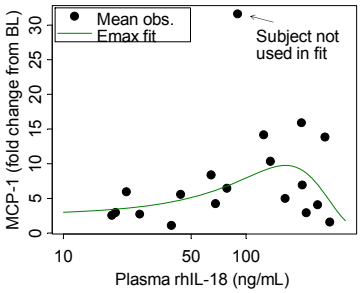

Figure 4: MCP-1 exposure response: MCP-1 concentration versus rhIL-18 concentration at 4 hrs post dose by patient averaged over treatment cycles

Conclusions

- The drop/rebound pattern for cell counts is consistent with rhIL-18 stimulated extravasation of immune cells into peripheral tissues and replenishment through recycling via the lymphatic system and neogenesis. Data from a single biopsy showed increased presence of activated lymphocytes in tumor tissue after rhIL-18 treatment. However, other mechanism of sequestration, such as temporary adhesion to vessel walls, cannot be ruled out without additional data.
- BM analysis confirmed appropriateness of selected rhIL-18 dosing regimen
- Based on their response patterns and dynamics BMs (e.g. CD8+ T cell and NK cell counts) were prioritized for inclusion in the Phase 2 study.
- Doses close to 30 µg/kg were identified as optimal in terms of overall rhIL-18 biological activity taking into account the varied rhIL-18 dose response pattern of the different BM types.
- The BM analysis in this Phase1 study provided crucial support for the design of a future Phase 2 study by confirming the selected dosing regimen. guiding the dose selection and prioritizing biomarkers.

References

[1] Logan, TF and Robertson MJ. Interleukins 18 and 21: Biology, Mechanisms of Action, Toxicity, and Clinical Activity. Current Oncology Reports 2006, 8:114–119.

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