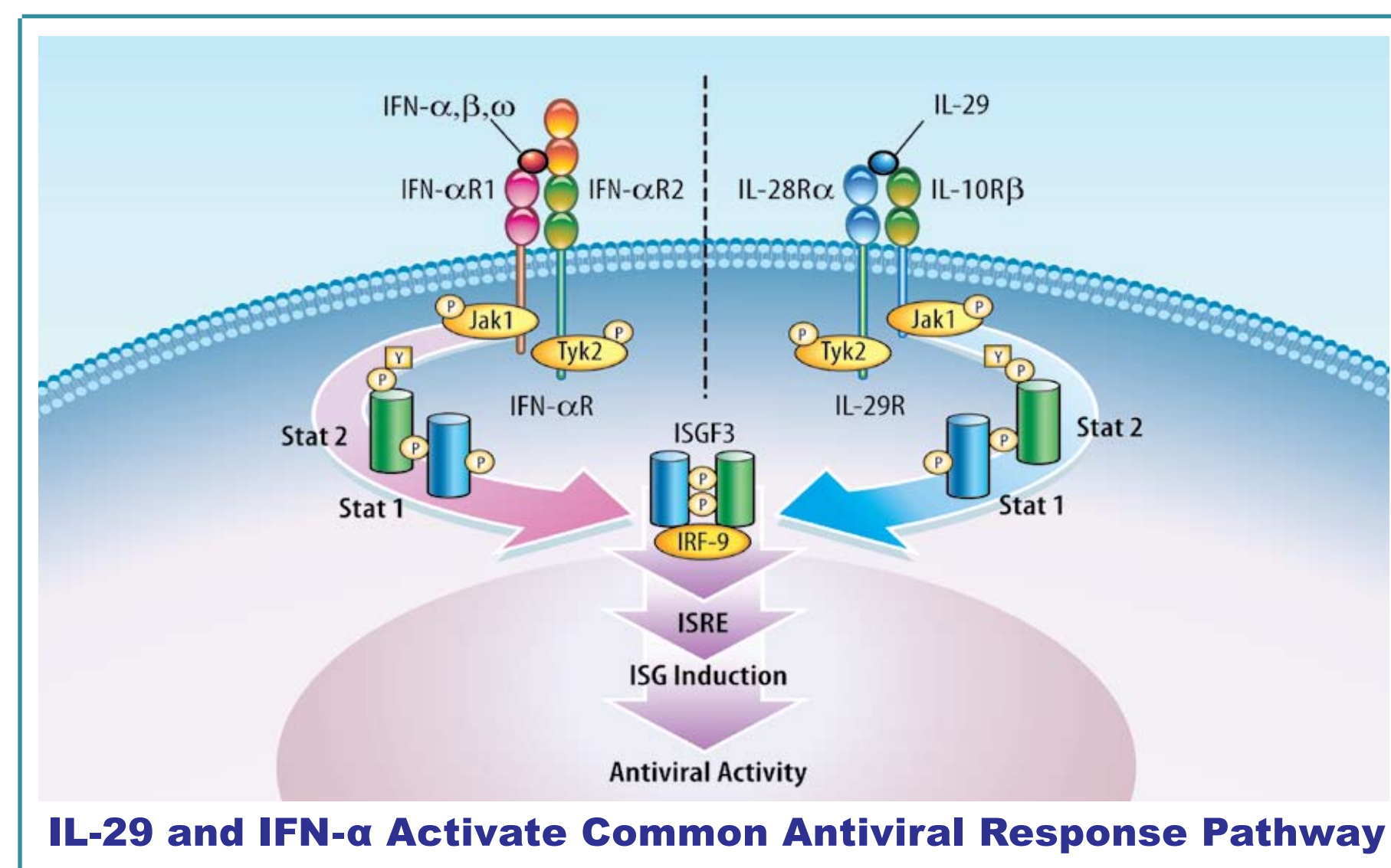


PEG-Interferon Lambda (PEG-rIL-29): Translation of In Vitro Preclinical Data to Clinical Results

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INTRODUCTION

The standard of care for chronic hepatitis C (PEGylated interferon alpha (PEG-IFN- α) + Ribavirin), is associated with significant toxicities. The mechanism of action of PEG-IFN- α is primarily mediated through its direct activity on infected hepatocytes. In contrast, many of the toxicities (e.g., neutropenia and flu-like symptoms) are caused by "off-target" effects. These and other side effects can lead to dose reduction or discontinuation of therapy, contributing to the relatively high rate of treatment failure.



Interleukin-29 (IL-29, also known as IFN- λ) is a Type III interferon that is induced upon viral infection.¹ Interaction between IL-29 and its cognate cell-surface receptor induces an intracellular response similar to that induced by IFN- α including induction of interferon stimulated genes (ISGs). In contrast to the receptor for IFN- α , which is widely expressed, the IL-29 receptor is expressed only on a subset of cell types. Notably, the IL-29 receptor is highly expressed on hepatocytes, the primary site of HCV infection, but is not significantly expressed on immune or bone marrow cells.

Based on the effect of IL-29 on hepatocytes and the expression pattern of its receptor, we have developed PEGylated recombinant IL-29 (PEG-rIL-29) as an alternative to PEG-IFN- α for the treatment of chronic hepatitis C. PEG-rIL-29 was evaluated in preclinical in vitro assays and in a Phase 1a single dose escalation study to test, in part, the following hypothesis:

At pharmacologically active concentrations and dose levels, PEG-rIL-29 does not demonstrate myelosuppressive or systemic immunostimulatory activity.

PRECLINICAL METHODS

In Vitro Bone Marrow Cell Proliferation.

Bone marrow mononuclear cells were isolated from a human bone marrow aspirate. Non-adherent cells were incubated in media supplemented with stem cell factor (SCF), IL-3, IL-6, GM-CSF, and erythropoietin to stimulate proliferation and development. The stimulated cells were incubated with vehicle, PEG-rIL-29, PEG-IFN- α 2a, or PEG-IFN- α 2b. Erythroid colony-forming units and myeloid colony-forming units were enumerated after 7 and 14 days, respectively.

In Vitro Stimulation of Peripheral Blood Mononuclear Cells (PBMCs)

PBMCs were isolated from 10 healthy human donors. Cells were stimulated for 16 hours with vehicle, PEG-rIL-29 or PEG-IFN- α 2a. Culture supernatants were subsequently collected and IL-6 concentration was measured by a Luminex-based immunoassay as an indicator of immune activation and the expression pattern of its receptor.

PRECLINICAL RESULTS

In Vitro Bone Marrow Proliferation

Figure 1a. In Vitro Erythroid Proliferation

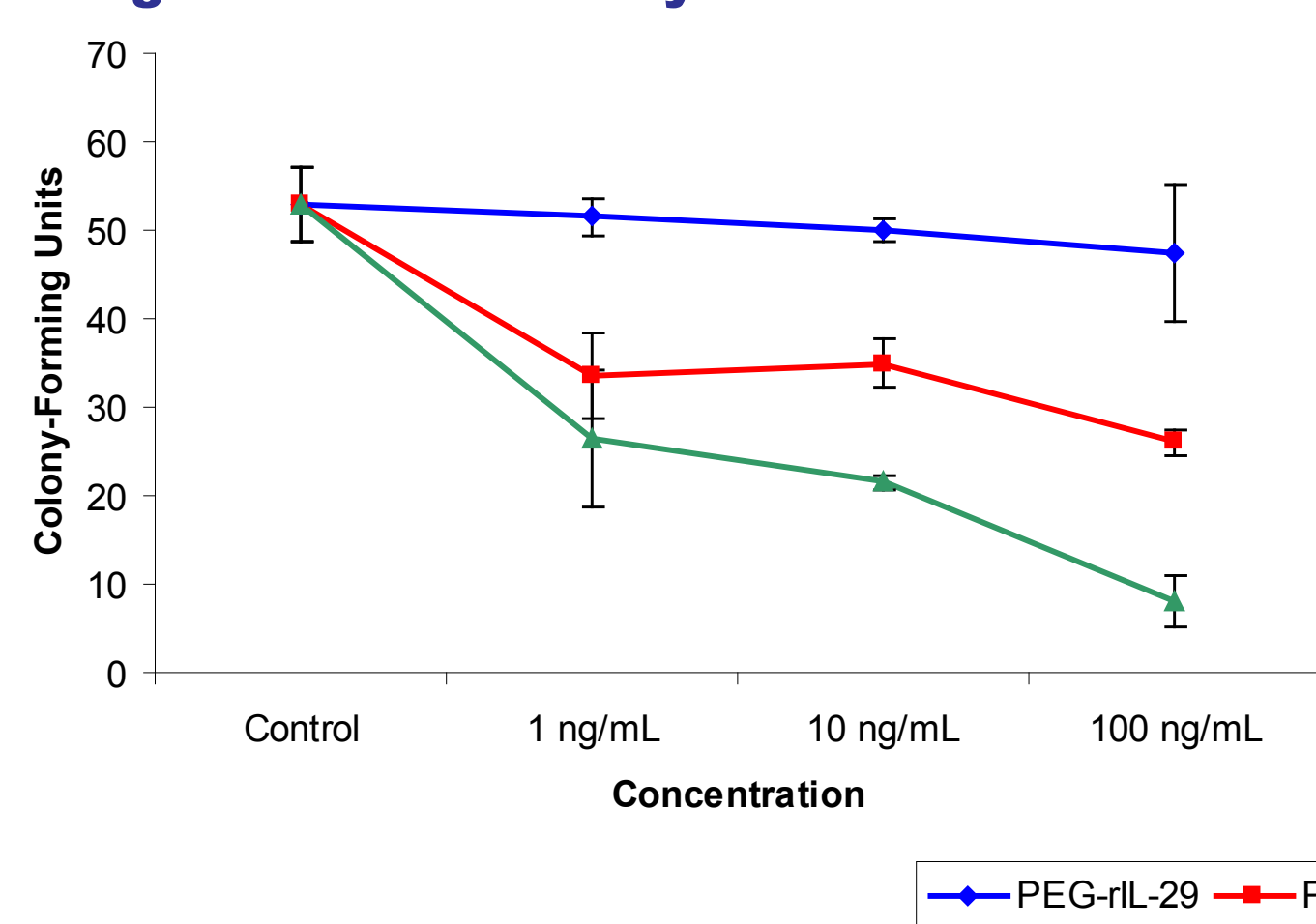
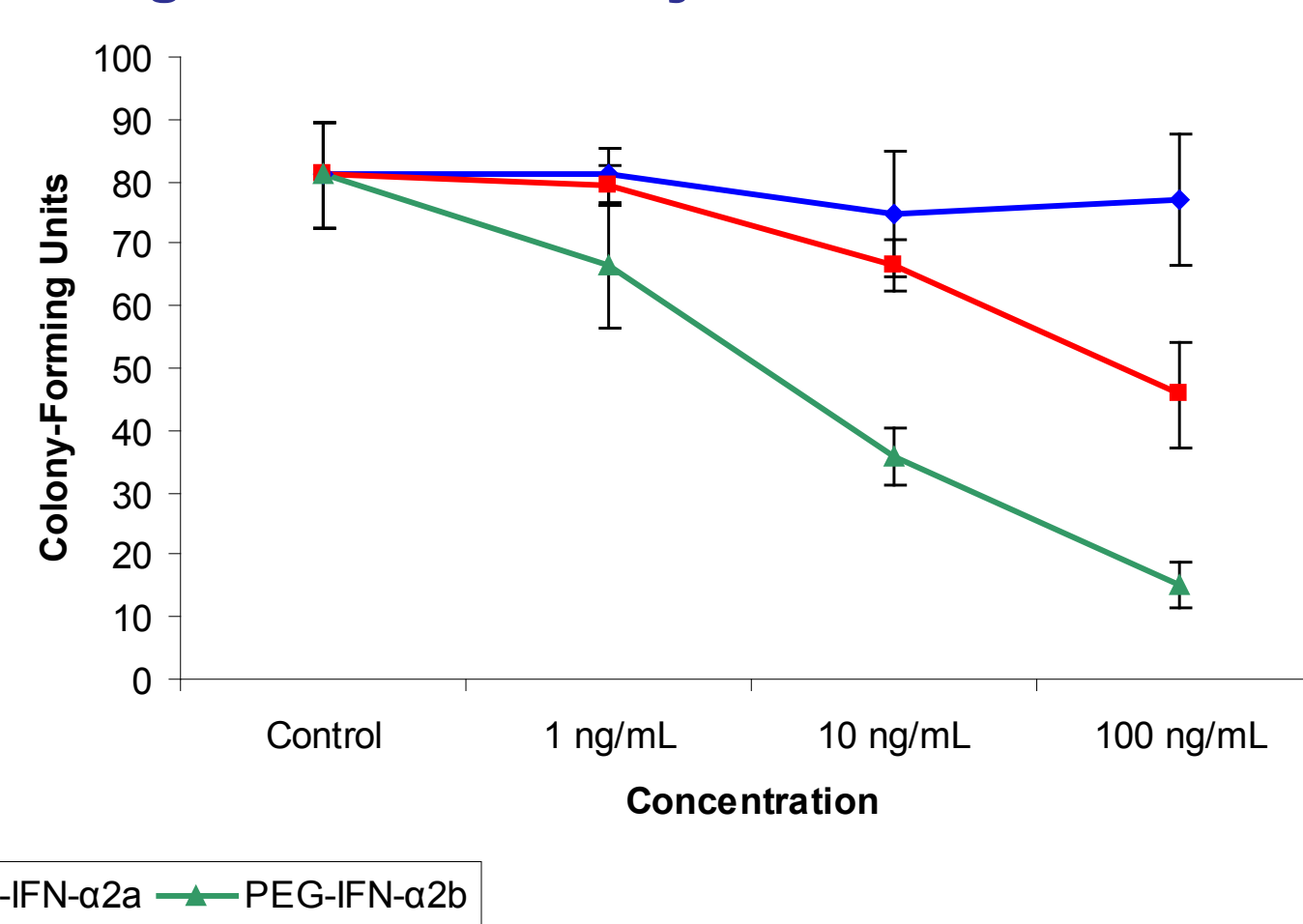


Figure 1b. In Vitro Myeloid Proliferation

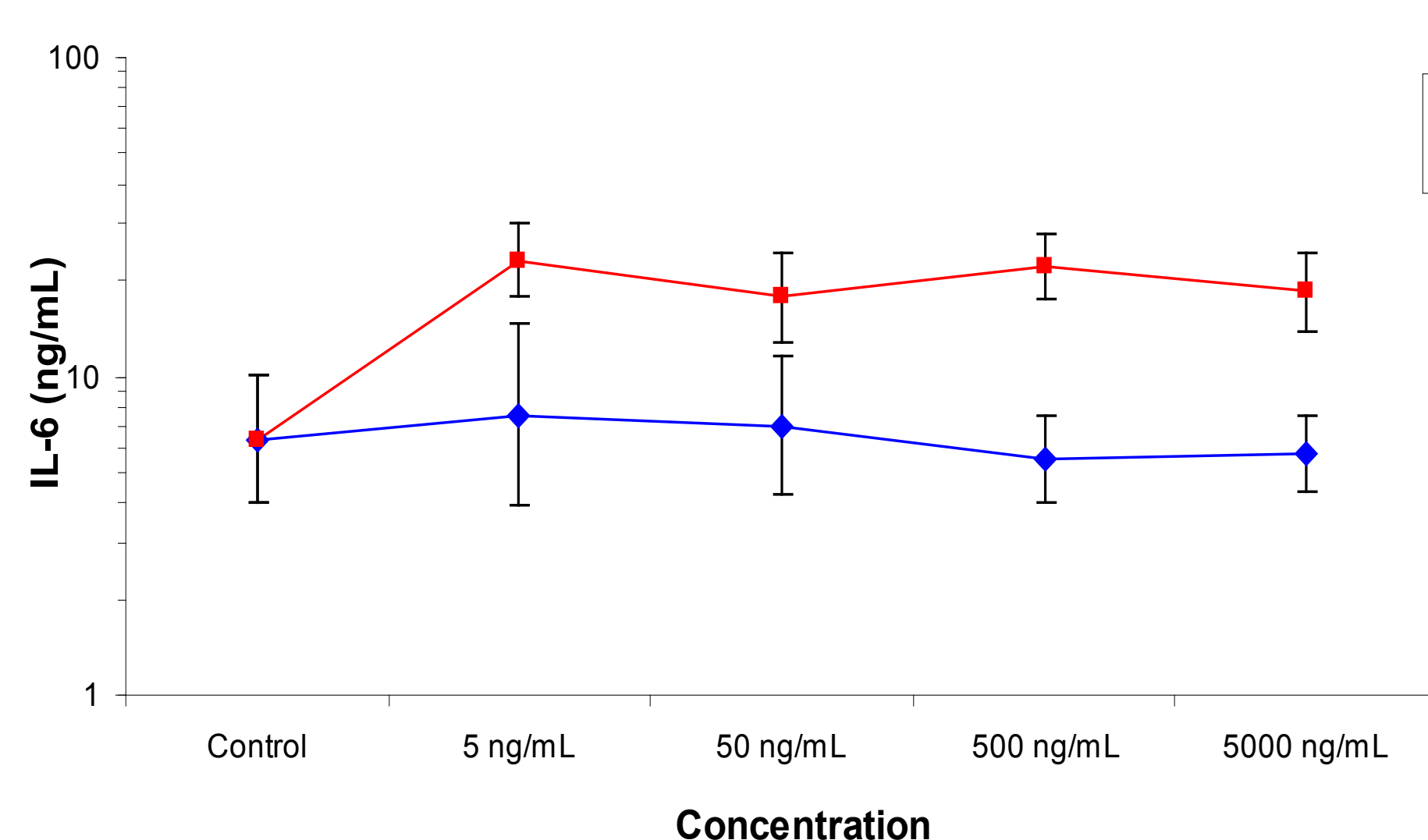


PEG-rIL-29 did not inhibit in vitro proliferation of bone marrow precursor cells.

Bone marrow cells obtained from human bone marrow aspirate were stimulated with PEG-rIL-29, PEG-IFN- α 2a, or PEG-IFN- α 2b. Subsequently, erythroid colonies (Figure 1a) and myeloid colonies (Figure 1b) were enumerated. Points represent average colony counts and error bars represent one standard deviation.

In Vitro Stimulation of PBMCs

Figure 2. In Vitro IL-6 Release



PEG-rIL-29 did not induce the in vitro release of IL-6 by PBMCs. PBMCs were enriched from 10 healthy donors and stimulated with PEG-rIL-29 or PEG-IFN- α 2a. Subsequently, the concentration of IL-6 released into the cell culture medium was quantified by immunoassay. Points represent geometric means and error bars represent one standard error of the mean.

These preclinical in vitro results are consistent with the low level expression of the receptor for IL-29 on bone marrow cells and PBMCs. Notably, at concentrations that induce the in vitro expression of ISGs and inhibit in vitro HCV replication in hepatocytes, PEG-rIL-29 did not inhibit the proliferation of bone marrow cells or stimulate the release of IL-6 by human PBMCs.² These results suggest that pharmacologically active dose levels of PEG-rIL-29 may not induce systemic immune activation or cause hematologic toxicities in clinical studies.

¹ Kotenko, et al., Nature Immunology (2003) 4(1):69-77; Sheppard, et al., Nature Immunology (2003) 4(1):63-68
² Doyle, et al., Hepatology (2006) 44(4):896-906

PHASE 1A STUDY IN HEALTHY VOLUNTEERS

Objectives

- Identify pharmacologically active dose
- Evaluate safety of single dose

Study Design

- Randomized, blinded, placebo-controlled single dose, dose-escalation study
- Healthy subjects, ages 18 to 55
- DLT = any \geq Grade 3 adverse event or lab abnormality related to study drug

Treatments

- 6 subjects per cohort
- Randomized 5:1 to PEG-rIL-29 or placebo administered subcutaneously

Assessments

- Safety, PK, markers of biologic activity
- PD markers: serum beta 2 microglobulin (B2M), OAS
- Immune activation markers: serum IL-6, CRP

CLINICAL RESULTS

20 subjects enrolled and treated with PEG-rIL-29 (n=17) or placebo (n=3)

- 4 dose levels: 0.5, 1.5, 5.0 and 7.5 μ g/kg

Maximally tolerated dose (MTD) = 5 μ g/kg

- DLT in 1 of 2 subjects treated at 7.5 μ g/kg (reversible, asymptomatic, Grade 3 ALT elevation)

Safety Results at All Dose Levels

- No fever, fatigue, insomnia or irritability
- No injection site reactions
- No significant hematologic changes
- No cardiac effects
- No development of antibodies to PEG-rIL-29

Pharmacokinetics

- Dose proportional exposure
- Estimated half-life of 50 to 70 hours
- T_{max} = 8 to 24 hours

Pharmacodynamics

- Dose-dependent increase in B2M starting at 1.5 μ g/kg
- Dose-dependent increase in OAS starting at 5 μ g/kg

Immune Effects

- No detectable increase in IL-6 or CRP

Hematologic Effects

Figure 3a. Median Neutrophil Count

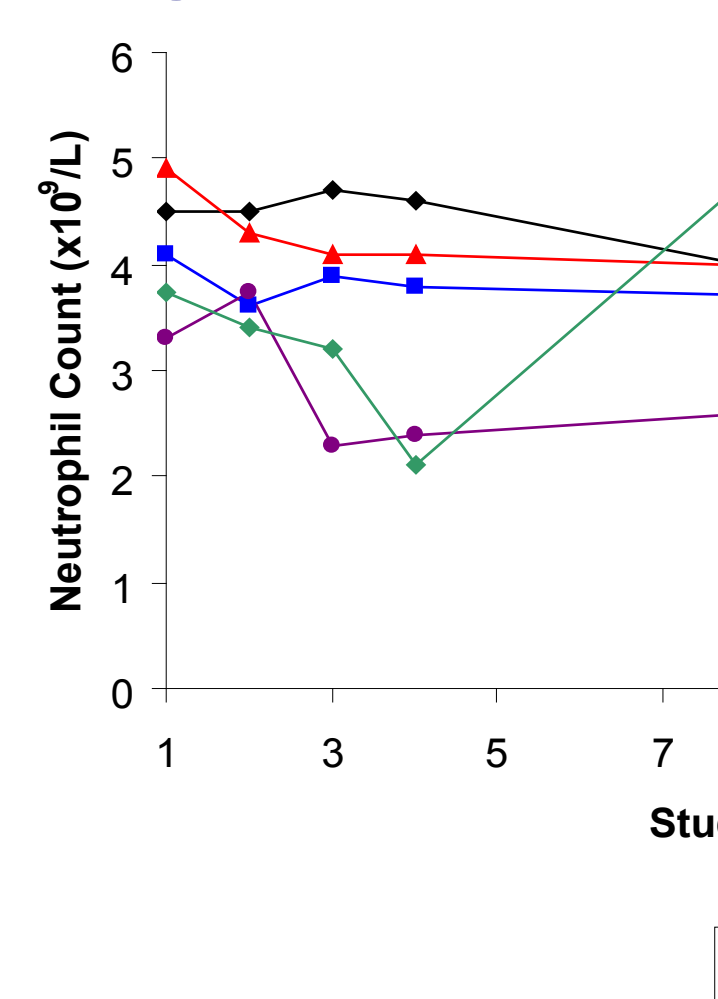
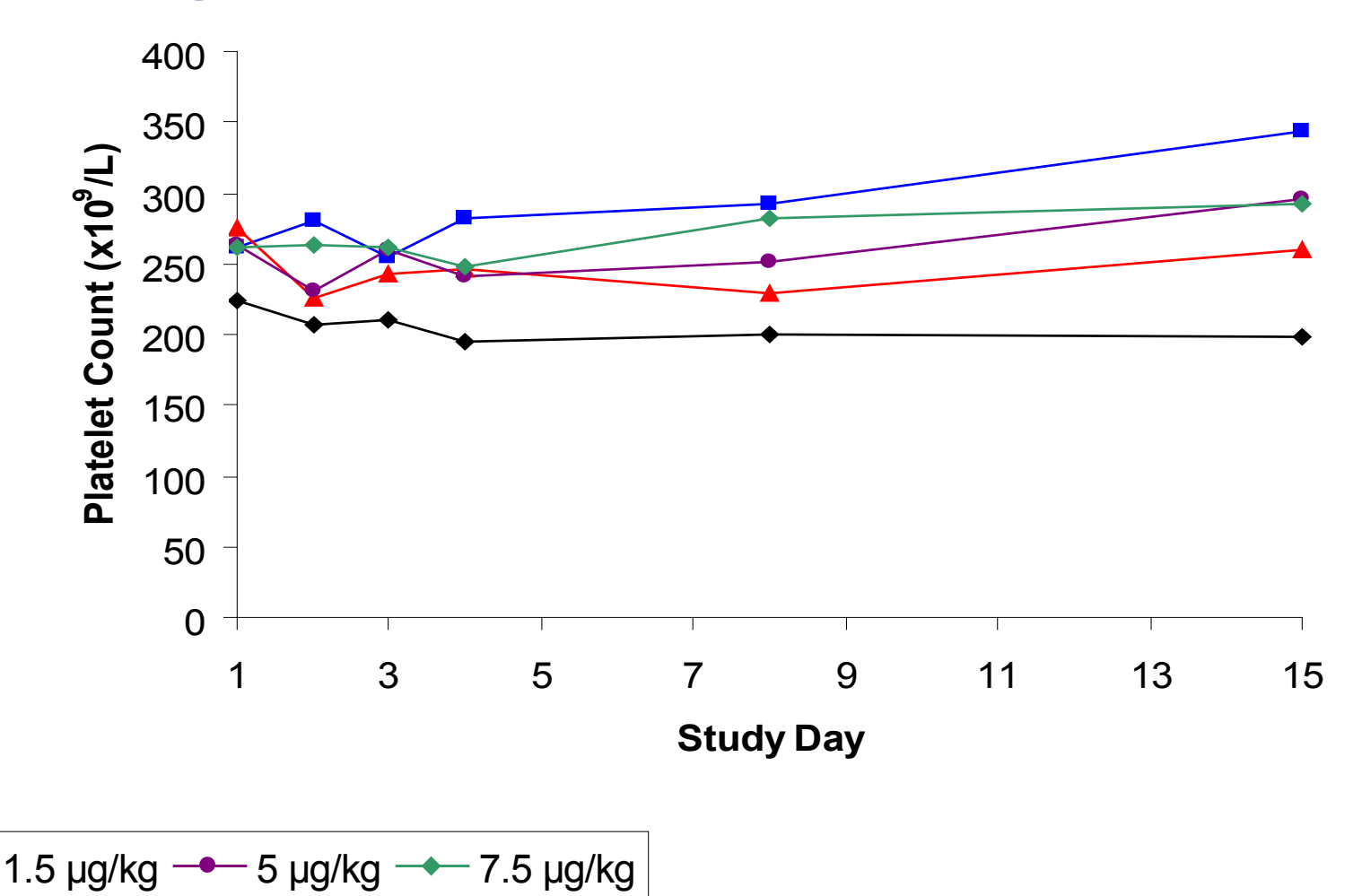


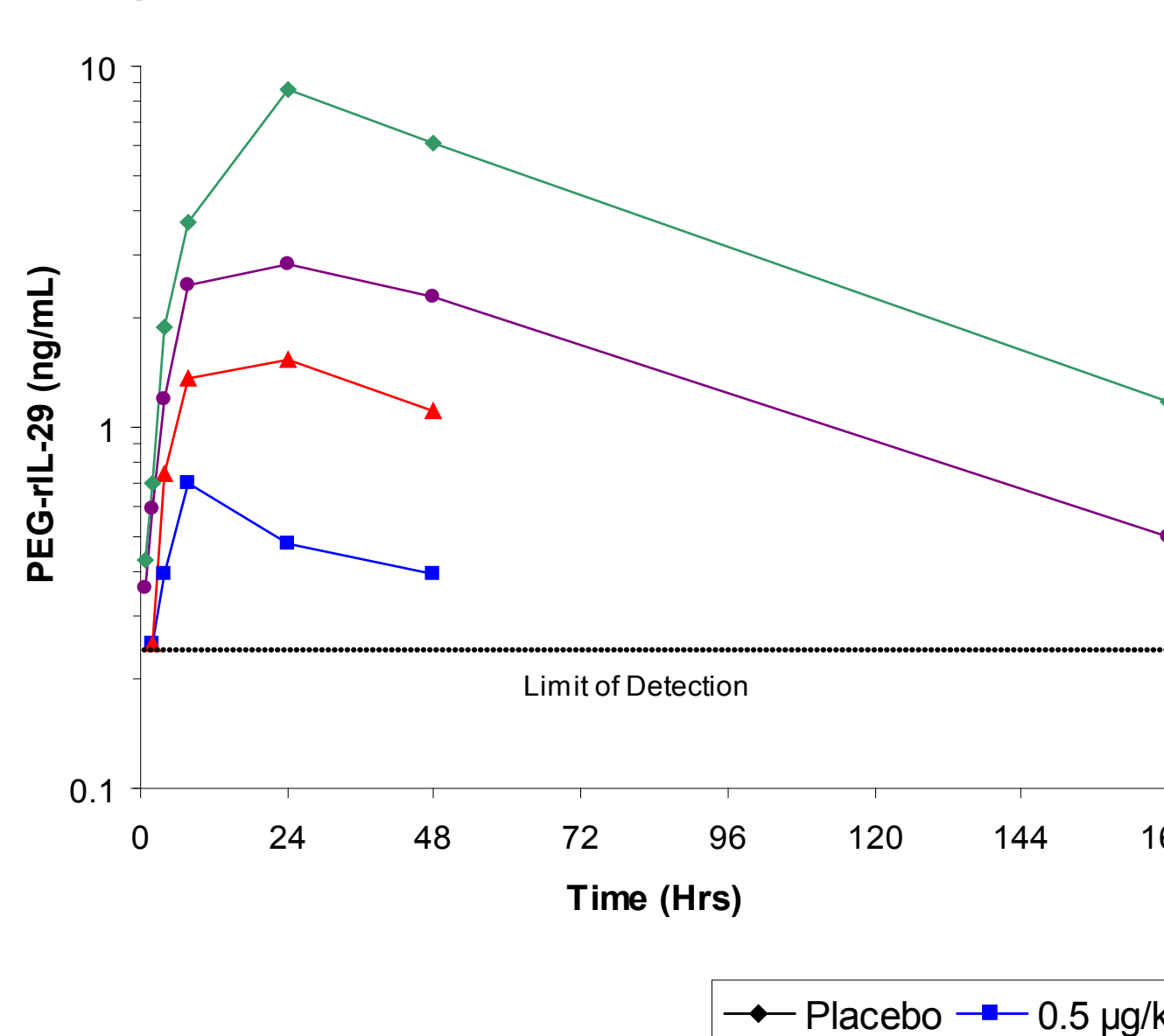
Figure 3b. Median Platelet Count



Neutrophil and platelet counts did not change significantly after a single dose of PEG-rIL-29 at any dose level tested. Neutrophils (Figure 3a) and platelets (Figure 3b) were enumerated by standard hematologic methods at several timepoints following single subcutaneous doses (SC) of PEG-rIL-29. Points represent median cell counts for each dose level tested.

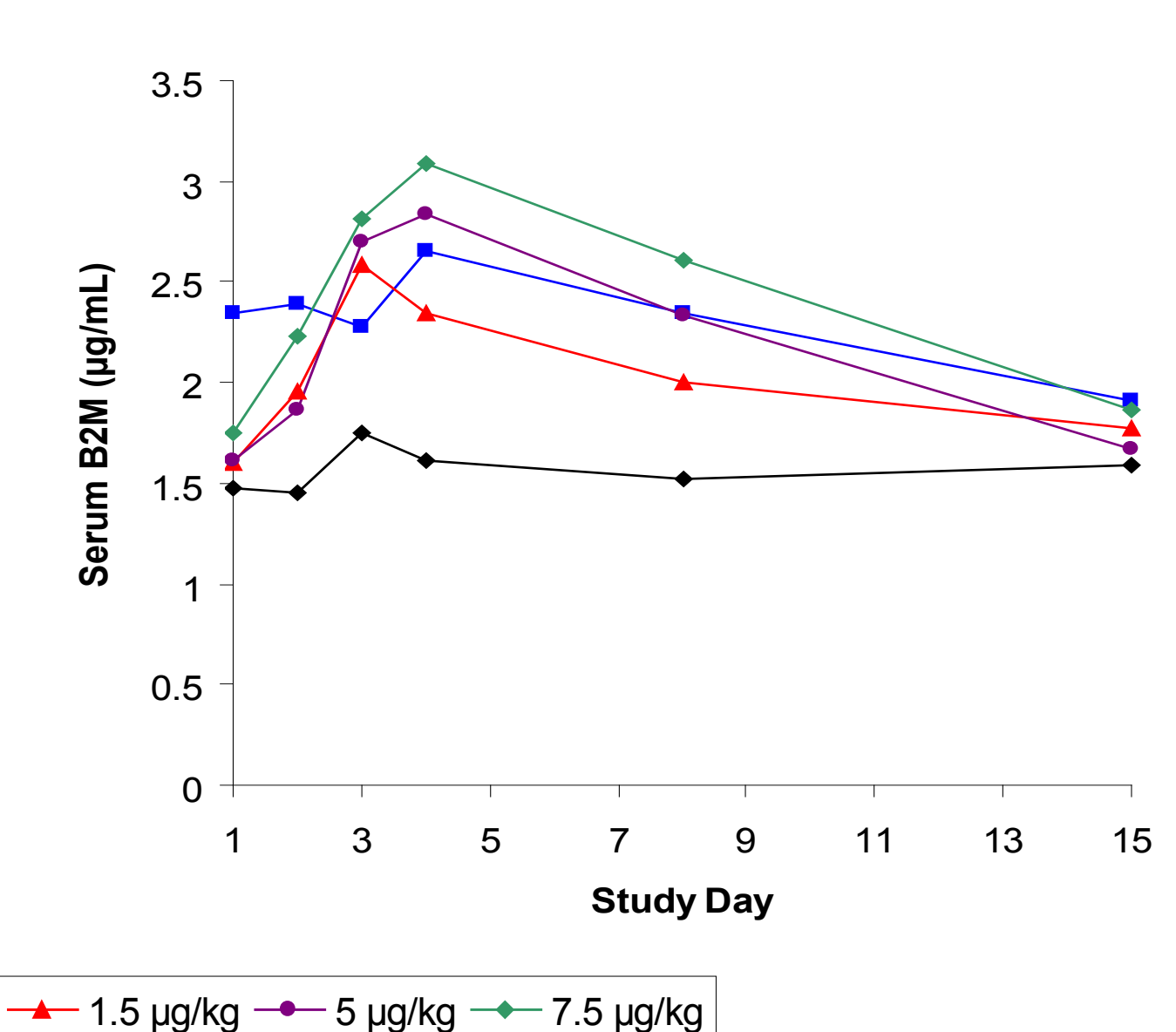
Pharmacokinetics

Figure 4. Median PEG-rIL-29 Concentration



Pharmacodynamics

Figure 5. Median B2M Concentration



Peak PEG-rIL-29 serum concentrations were within concentration levels tested in vitro. Serum concentrations of PEG-rIL-29 were quantified by a validated ELISA at several timepoints following single SC doses of PEG-rIL-29. Points represent median measured PEG-rIL-29 concentrations for each dose level tested. The assay limit of detection was 0.25 ng/mL.

Evaluated PEG-rIL-29 dose levels were pharmacologically active. Serum concentrations of B2M, a biomarker of PEG-rIL-29 pharmacological activity, were measured by a validated ELISA at various timepoints following single SC doses of PEG-rIL-29. Points represent median measured B2M concentrations for each dose level tested.

CONCLUSIONS

- PEG-rIL-29 is well-tolerated at pharmacologically active doses
 - Toxicities associated with single-dose PEG-IFN- α not observed
 - No evidence of myelosuppression
 - No evidence of systemic immune activation
- Dose dependent biological responses observed consistent with the proposed mechanism of action of PEG-rIL-29
- Pharmacokinetics support weekly dosing
- Data support repeat-dose study in hepatitis C patients (ongoing)