

# IMO-2125, a TLR9 Agonist that Induces High Interferon-alpha Production, as a Candidate for Hepatitis C Therapy

T. Sullivan, E. Kandimalla, L. Bhagat, A. Trombino, D. Cabral, and S. Agrawal  
 Idera Pharmaceuticals, Inc. Cambridge, MA

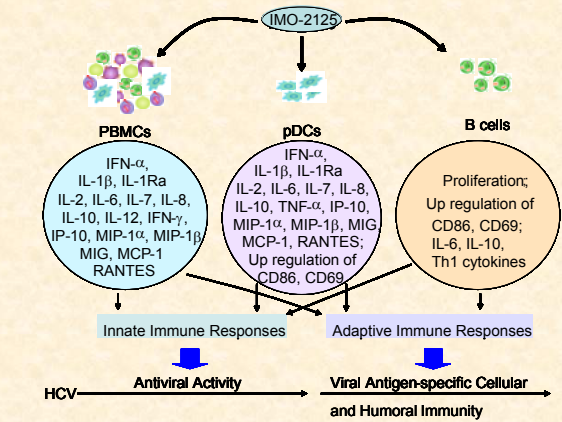
### INTRODUCTION

Oligonucleotide-based compounds have been shown to induce Th1-type immune responses, including IFN- $\alpha$ , through Toll-like Receptor 9 (TLR9). IMO-2125 is a novel DNA-based synthetic agonist of TLR9 designed to stimulate high levels of IFN- $\alpha$  and other cytokines. IMO-2125 is being evaluated as a candidate for treatment of chronic hepatitis C (CHC) infection in humans.

TLRs are specific immune cell receptors that recognize biochemical patterns that may be associated with pathogens. TLR9 in humans is expressed on endosomal membranes of plasmacytoid dendritic cells (pDCs) and B cells. Agonists of TLR9 induce an innate immune response that includes production of IFN- $\alpha$  and other cytokines that have been shown to be useful in developing adaptive immune responses.

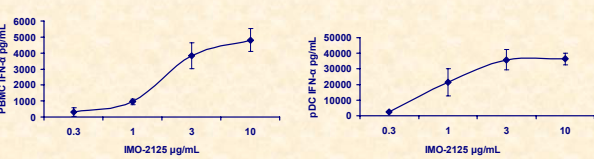
Pegylated recombinant interferon is the standard of care for CHC, combined with ribavirin for increased efficacy. Sustained Viral Response is achieved in 40 – 60% of the general population (Strader *et al.*, Hepatology 39:1147-1171; Dienstag and McHutchison, Gastroenterology 130:231-264). Patients who are non-responders, partial responders, or relapse after standard therapy need therapeutic alternatives. Our hypothesis is that IMO-2125 induction of endogenous IFN- $\alpha$  and other cytokines leading to both innate and adaptive immune responses may provide a therapeutic alternative to pegylated recombinant interferon.

### Proposed Mechanism of Action of IMO-2125



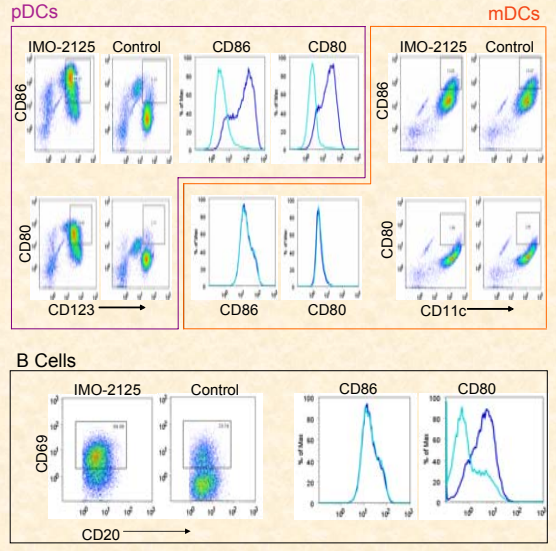
Our hypothesis is that IMO-2125 induction of endogenous IFN- $\alpha$  and other cytokines will lead to both innate and adaptive immune responses to generate antiviral activity.

### IMO-2125 Induces IFN- $\alpha$ in Human PBMCs and pDCs



PBMCs ( $10^6$  cells/well) or pDCs ( $2 \times 10^5$  cells/well) from healthy human donors were incubated for 24 h in the presence of varying concentrations of IMO-2125. Data are means  $\pm$  SEM of 4 donors. These data show that IMO-2125 causes potent induction of IFN- $\alpha$  in human PBMCs, mostly attributable to pDCs.

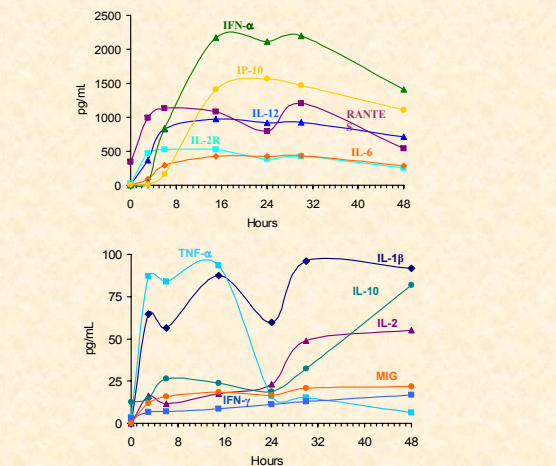
### IMO-2125 Induction of Surface Activation Markers on Human Cells *In Vitro*



Fresh isolated cells were stimulated with 1  $\mu$ g/mL of IMO-2125 or control for 24 h, washed and stained. Samples were analyzed on Beckman-Coulter FC500 MPL Flow Cytometer. Gates were created to include only cells expressing CD123 (pDCs), CD11c (mDCs) or CD20 (B cells).

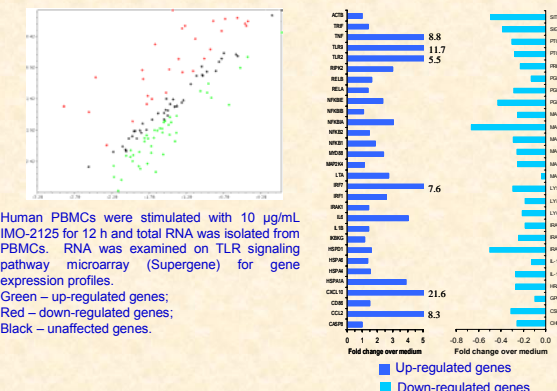
These data show that IMO-2125 activates pDCs and B cells but not mDCs.

### IMO-2125 Induces Cytokines and Chemokines in Human PBMC Cultures

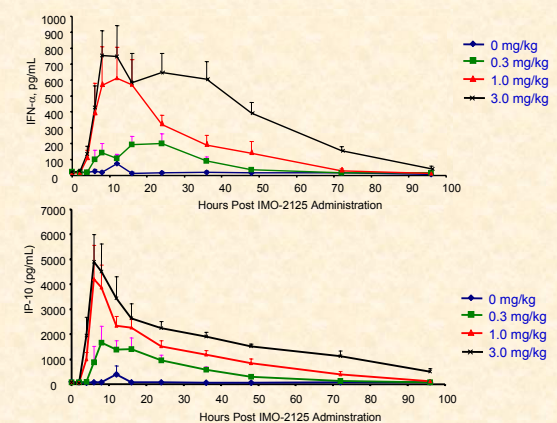


Healthy human PBMC cultures incubated with 10  $\mu$ g/mL of IMO-2125. These data show that IMO-2125 induces a broad array of cytokines and chemokines, many of which have been shown to have direct or indirect antiviral activity.

### Profile of Gene Expression by IMO-2125

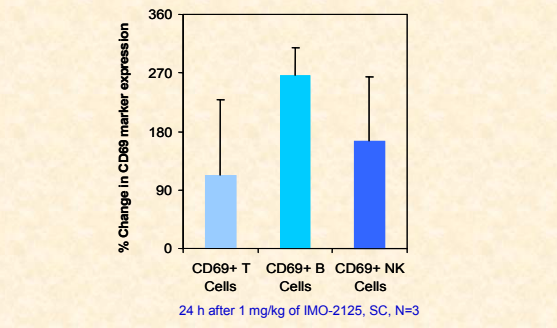


### IMO-2125 Induces Immune Responses in Nonhuman Primates *in vivo*

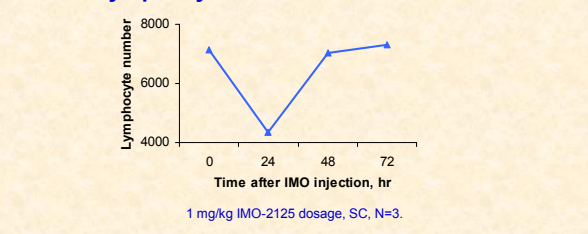


These data show that SC administration of IMO-2125 to nonhuman primates induces prolonged, dose-dependent elevations in plasma IFN- $\alpha$  and IP-10.

### IMO-2125 Induces Surface Activation Markers on Lymphocytes and NK cells in Nonhuman Primates

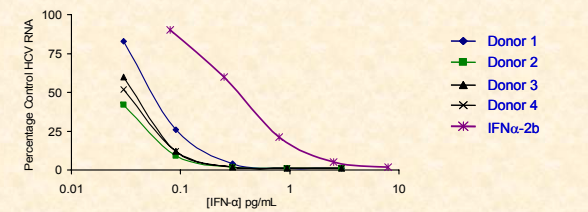


### IMO-2125 Induces Transient Margination of Circulating Lymphocytes in Nonhuman Primates

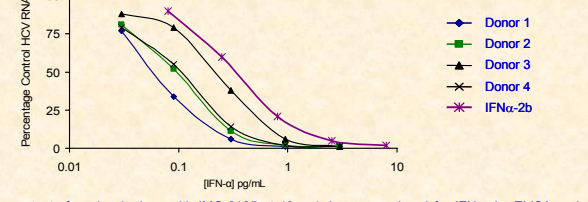


### ANTIVIRAL ACTIVITY OF IMO-2125-INDUCED CYTOKINES/CHEMOKINES IN HCV REPLICON ASSAY

#### Antiviral Activity of Supernatants from Healthy Human PBMCs Treated with IMO-2125

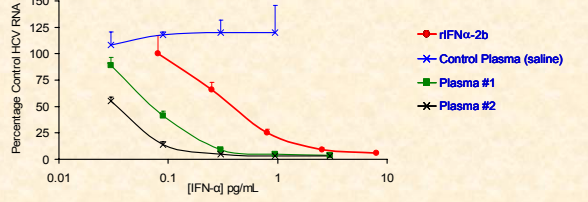


#### Antiviral Activity of Supernatants from Healthy Human pDCs Treated with IMO-2125



Supernatants from incubations with IMO-2125 at 10  $\mu$ g/mL were analyzed for IFN- $\alpha$  by ELISA and diluted to the indicated range. Data are means of N=4. The data show that supernatants from human PBMCs or pDCs incubated with IMO-2125 have greater potency in the HCV replicon assay than does rIFN- $\alpha$ -2b.

### Antiviral Activity of Plasma from Nonhuman Primates Administered 1 mg/kg SC of IMO-2125



Plasma from 48h after dosing was analyzed for IFN- $\alpha$  by ELISA and diluted to the indicated range. Control plasma (administered saline) was diluted equivalently. Data are means from N=4. The data show that plasma from nonhuman primates treated with IMO-2125 has greater potency in the HCV replicon assay than does rIFN- $\alpha$ -2b. All reported Huh7 ET HCV replicon assays were performed at SRI, Frederick MD.

### IMO-2125 DEVELOPMENT STATUS

IND was filed 2Q 2007  
 Phase 1 clinical trial expected to be initiated in 3Q 2007

### Potential for IMO-2125 Combinations with rIFN $\alpha$ -2b and Protease Inhibitor – HCV Replicon Model

Treatment	EC <sub>50</sub>
rIFN $\alpha$ -2b	0.24 pg/mL
rIFN $\alpha$ -2b + RBV	0.11 pg/mL
rIFN $\alpha$ -2b + RBV + NHP plasma*	0.01 pg/mL
NHP plasma	0.03 pg/mL
NHP plasma + RBV	0.02 pg/mL
Protease Inhibitor	0.18 $\mu$ M
Protease Inhibitor + rIFN $\alpha$ -2b (0.1 pg/mL)	0.08 $\mu$ M
Protease Inhibitor + NHP plasma*	0.02 $\mu$ M

Plasma from nonhuman primates (NHP) administered 1 mg/kg sc of IMO-2125 was analyzed for IFN- $\alpha$  by ELISA. An amount equivalent to 0.03 pg IFN- $\alpha$  was added to each well of replicon culture in the combination treatments, as indicated by asterisk. Ribavirin (RBV) was added at 5  $\mu$ g per well. Data are calculated from 6-point concentration curves with N=4 per concentration. The data show that cytokines/chemokines induced by IMO-2125 *in vivo* contribute additional antiviral activity in the HCV replicon assay in combination with rIFN- $\alpha$ -2b or protease inhibitor.

### CONCLUSIONS

IMO-2125 is a novel agonist of TLR9. IMO-2125 produces potent dose-dependent induction of immune response in human PBMCs and pDCs. IMO-2125 increases expression of surface activation markers on B cells, NK cells, and pDCs. IMO-2125 induces dose-dependent induction of IFN- $\alpha$  and other cytokines and chemokines in nonhuman primates. Supernatants and plasma from cell cultures and nonhuman primates, respectively, treated with IMO-2125 have potent activity in the HCV replicon assay. An IND has been filed and a Phase 1 trial in chronic hepatitis C patients is expected to be initiated in 3Q 2007. Antiviral activity in the HCV replicon assay was demonstrated with IMO-2125-induced cytokines/chemokines in combinations with rIFN- $\alpha$ -2b  $\pm$  ribavirin and with a protease inhibitor.

### BACKGROUND REFERENCES

- Yu *et al.*, Nucl Acids Res 30:4460-4469 (2002).
- Yu *et al.*, J Med Chem 45:4540-4548 (2002).
- Kandimalla *et al.*, Nucl Acids Res 31:2393-2400 (2003).
- Kandimalla *et al.*, PNAS (USA) 102:6925-6930 (2005).