Vγ9Vδ2 T-cells activated by IPH 1201: a possible immunotherapy for chronic HCV infection and its rationale for combination with IFNα

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Background

- Among the cells of the innate immune system, non conventional Vγ9Vδ2 T-cells (γδ) represent 0.5 to 5% of total lymphocytes and are known to exert a broad antiviral activity. They are present only in humans and primates.
- A decrease of γδ T-cells is observed in peripheral blood of chronic hepatitis C patients suggesting a γδ T-cell-specific involvement in the antiviral immune response (Par *et al.*, 2002).
- γδ T-cells can be activated and amplified by non-peptidic antigens such as IPH 1101, currently in clinical trials in oncology and IPH 1201, a second generation activator. Once activated, γδ T-cells release a large amount of Th1 cytokines and chemokines.
- An antiviral activity of activated γδ T-cells, mediated by IFNγ release, was demonstrated in a subgenomic HCV replicon (Agrati *et al.*, 2005).
- Combination of IFNα and IFNγ results in a strong synergistic antiviral activity in replicon model (Larkin *et al.*, 2003).

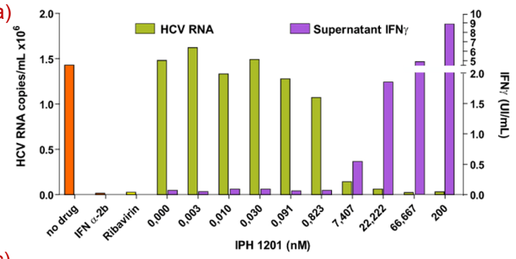
Aims:

- To demonstrate an anti-viral activity of *in vivo* activated γδ T-cells.
- To evaluate a potential combination of IPH 1201 with IFNα *in vitro* and *in vivo*.

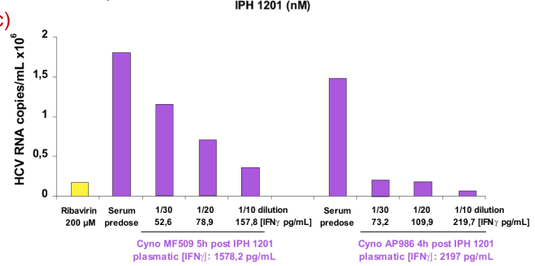
In vitro efficacy

IPH 1201 inhibits HCV replication (Huh7 cell line expressing HCV genotype1b replicon I377/NS3-3')

a)



c)

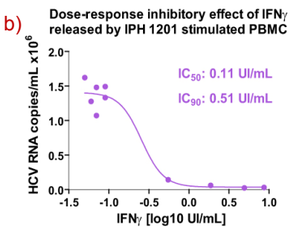


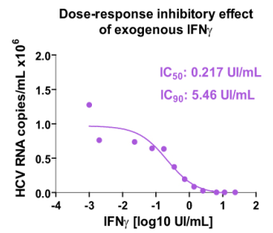
a) Supernatants of hPBMC (containing 0.7% of γδ T-cells) stimulated by increasing doses of IPH 1201 decrease HCV RNA level in a dose-dependent way.
The decrease of HCV replication is inversely correlated with the amount of IFNγ released in the supernatants.

b) The IC_{50} of the IFNγ released is similar to the IC_{50} of exogenous IFNγ but the IC_{90} is ten times lower indicating that, at high doses of IPH1201, IFNγ is probably not the only factor involved in the antiviral activity of IPH 1201.

c) Plasma of cynomolgus withdrawn 4 or 5 hours after IPH 1201 treatment (3 mg/kg SC) induce a strong inhibition of RNA HCV replication in the replicon model.
For an equivalent amount of plasmatic IFNγ, the level of inhibition between animals is different suggesting that other factors released in the plasma by IPH 1201 are involved in the antiviral activity.

b)



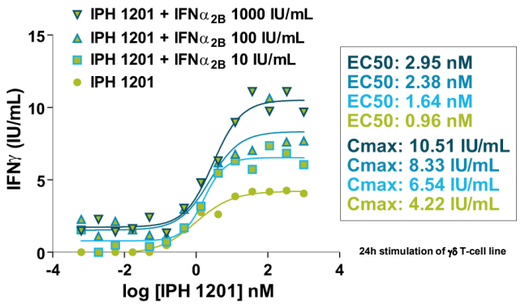


Conclusions and Perspectives

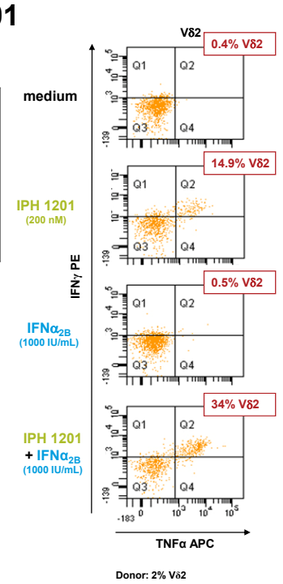
- γδ T-cells, activated *in vitro* and *in vivo* by IPH 1201, inhibit HCV replication in a replicon model: this effect could be partly mediated by IFNγ release.
- IFNα potentiates the IFNγ release induced by IPH 1201 or IPH 1101 selectively on γδ T-cells from healthy donors or HCV infected patients.
- The co-administration of PEG-IFNα to IPH 1201, in the cynomolgus, not only is safe but also induces a long term potentiation of the plasmatic secretion induced by IPH 1201.
- γδ T-cells activation could constitute a new immunotherapy for chronic HCV treatment that can synergize PEG-IFNα antiviral activity.

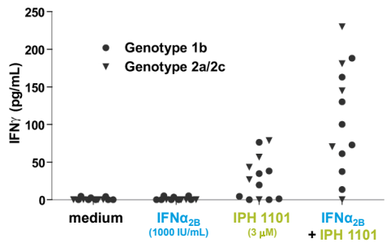
In vitro rationale for IPH 1201 and IFNα combination

IFNα potentiates the effect of IPH 1201



- $IFN\alpha_{2B}$ dose-dependently increases IFNγ release induced by increasing doses of IPH 1201 on isolated γδ T-cells (healthy donor). $IFN\alpha_{2B}$ does not significantly modify EC_{50} whereas Cmax are increased: one hypothesis is that $IFN\alpha_{2B}$ can sensitize γδ T-cells that are weakly responding to IPH 1201.
- Specific intracellular staining of γδ T-cells from PBMC (healthy donor), stimulated by IPH 1201, confirmed that $IFN\alpha_{2B}$ increases the percentage of γδ T-cells positive for IFNγ and TNFα.



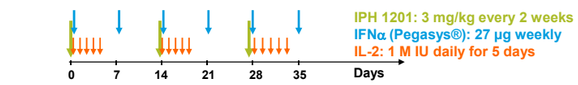


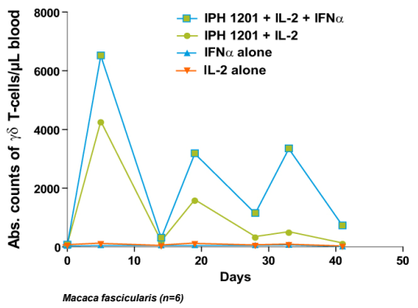
- PBMC from HCV patients can be stimulated by IPH 1101, the first generation of synthetic phospho-antigen.
- The potentiation effect of $IFN\alpha_{2B}$ on IFNγ release is also observed in HCV patients from both genotypes studied.

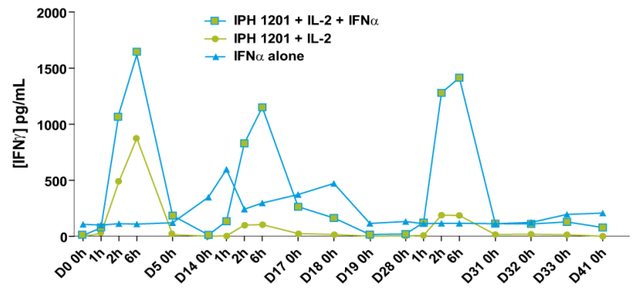
In vivo rationale for IPH 1201 and PEG-IFNα combination

IFNα potentiates *in vivo* the pharmacological effects of IPH 1201

No toxicological finding with the combination IPH 1201 / IL-2 / PEG-IFNα in cynomolgus







- The co-administration of IPH 1201 and IL-2 induces γδ amplification and cytokine release in the cynomolgus. IFNα strongly potentiates the combined effect of IPH 1201 and IL-2 on both parameters, especially at the second and third cycles of treatment.
- No toxicological effect was found with the combination IFNα / IPH 1201 / IL-2 in the cynomolgus, the only toxicological and pharmacological relevant species.