Interferon-gamma pathway is activated in a chronically HBV infected chimpanzee that controls HBV following ARC-520 RNAi treatment

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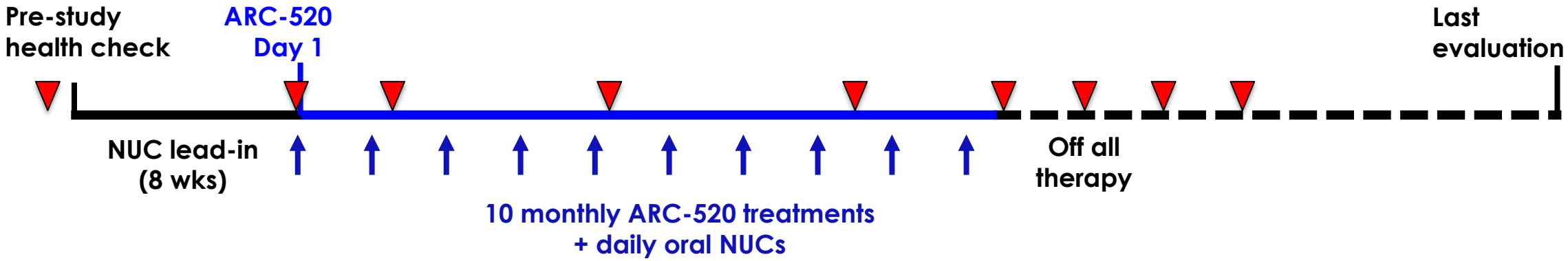
Treatment of chimpanzee with RNAi therapeutic ARC-520



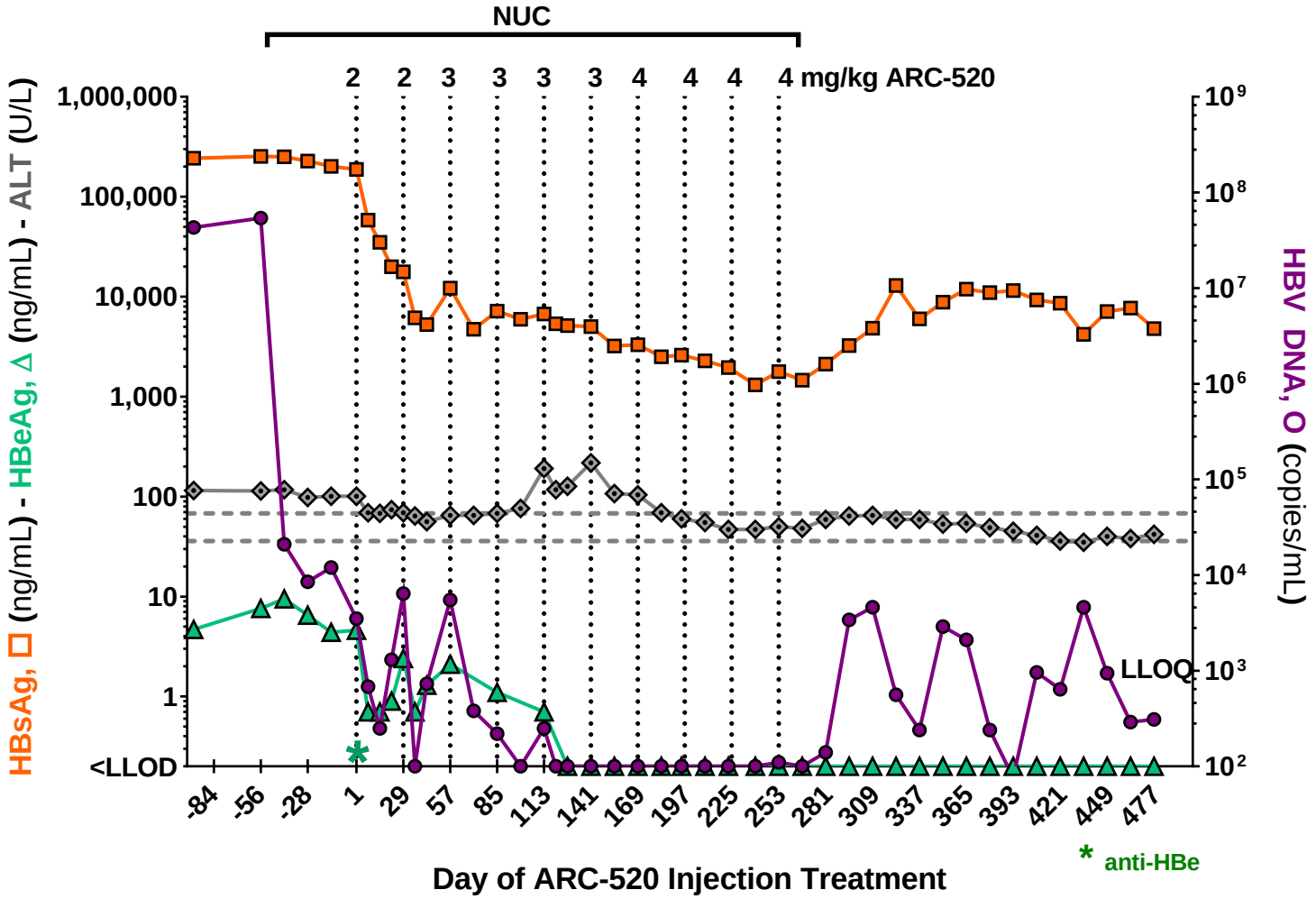
- Chimpanzee A
  - Female
  - HBeAg-positive
  - 7.7 $\log_{10}$ copies/mL serum HBV DNA
  - 2.4 $\log_{10}$ µg/mL HBsAg

- Treatment
  - Daily oral entecavir
  - 2-4 mg/kg ARC-520 dosed monthly
- Monitor safety and efficacy
  - Regular blood collection
  - Periodic liver needle biopsies (▼)

On-treatment response to RNAi + NUC

- Serum HBV DNA undetectable for 17 weeks
- HBeAg negative (after 5th ARC-520 injection), anti-HBe positive
- HBsAg reduced 99.46% (2.3 log_{10})
- Pre-core/pgRNA reduced 99.95%
- Total HBV RNA reduced 99.74%

Following ARC-520 treatments: sustained anti-viral response off all therapy



Sustained response 31 weeks off all therapy

- Serum HBV DNA was 5 $\log_{10}$-fold lower than pre-study
- HBsAg was 1.7 $\log_{10}$-fold lower than pre-study
- HBeAg negative and anti-HBe positive (seroconverted)
- Liver HBV RNA was 99% lower than pre-study

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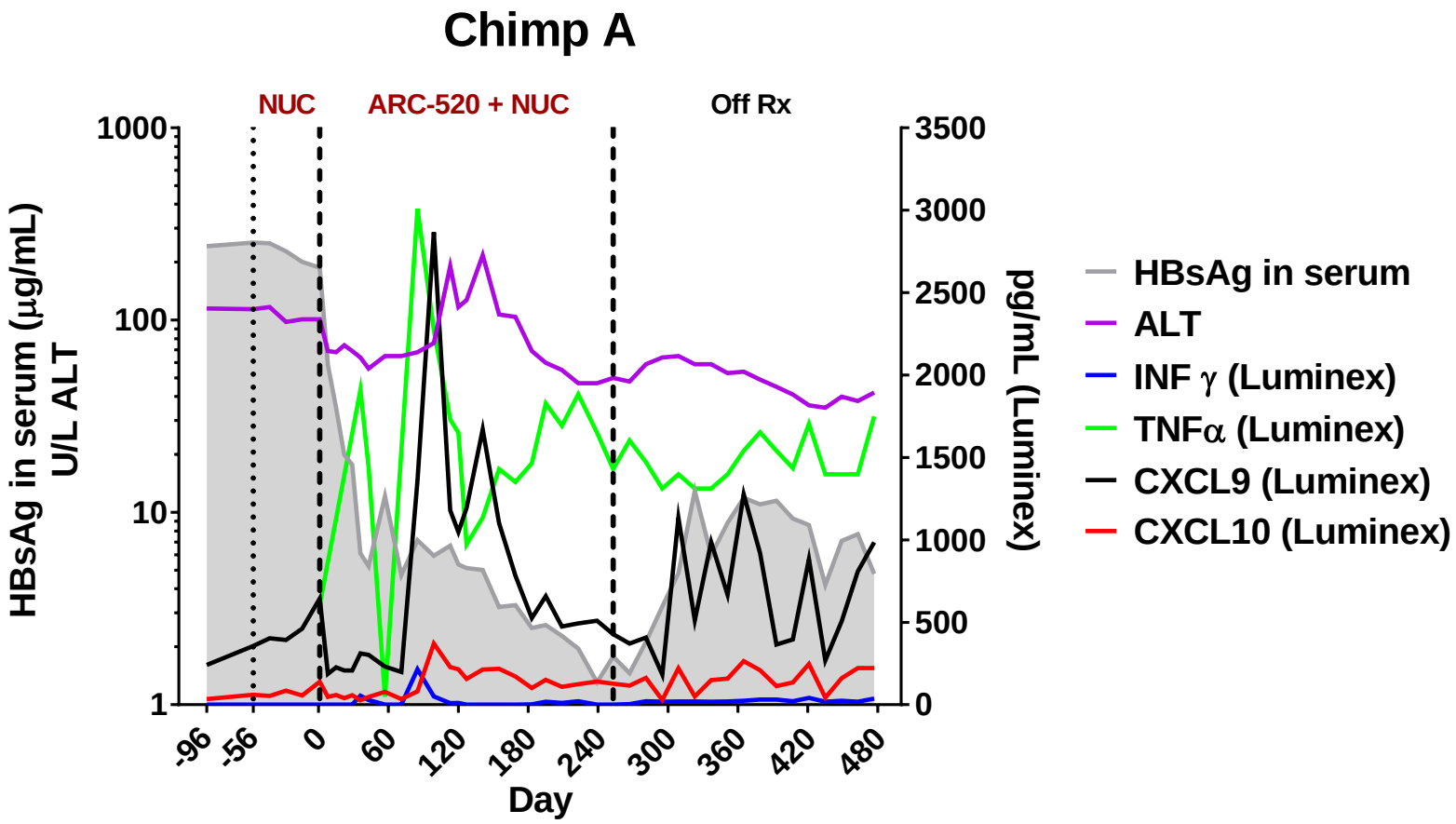
Serum cytokines that increased during ARC-520 treatment



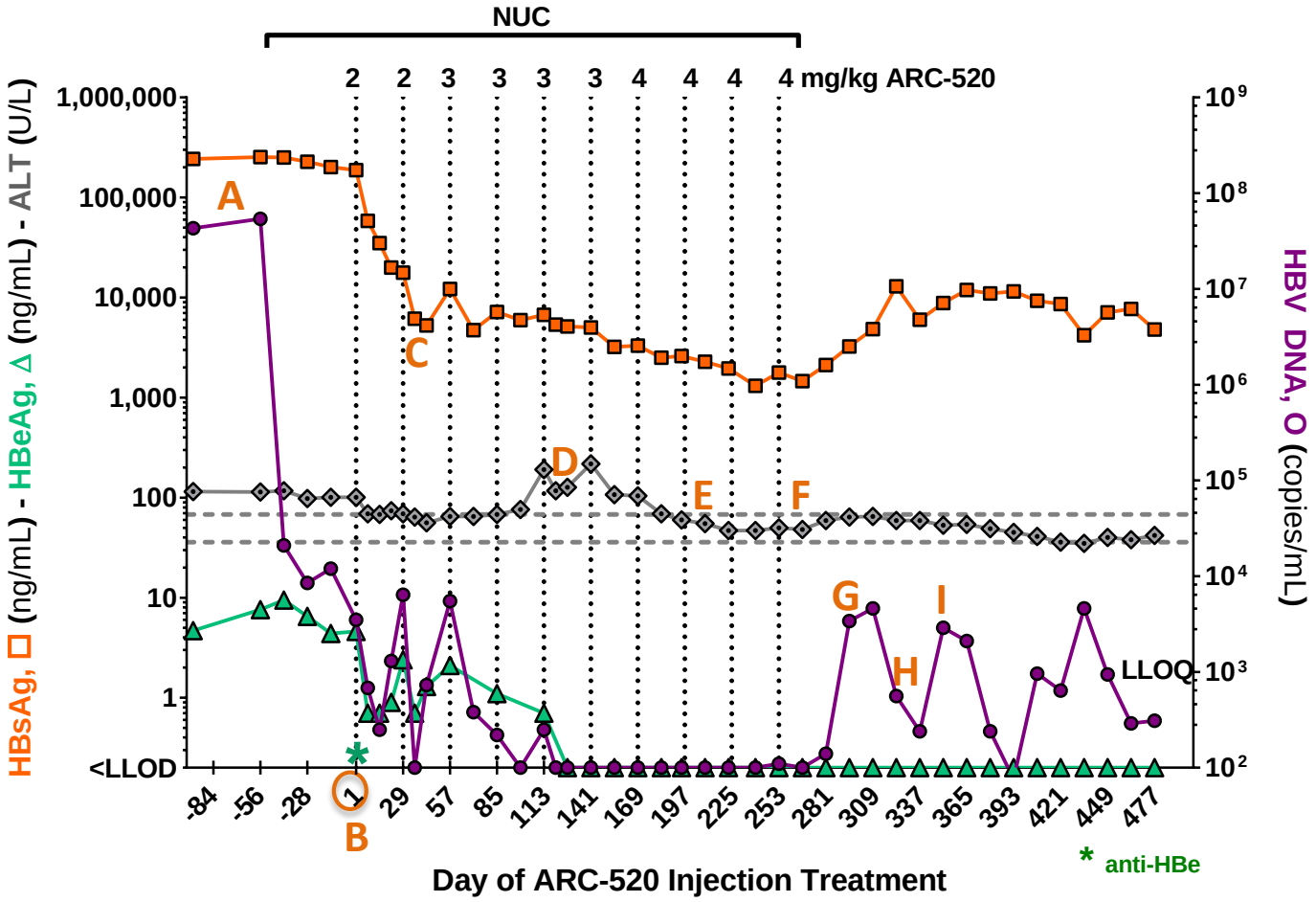
- IFN-γ, CXCL9, TNF-a, and CXCL10 increased when HBsAg was decreased (CXCL9 and CXCL10 are IFN-γ responsive cytokines)
- ALT flare followed elevations of these cytokines
- Off all treatment, elevations of CXCL9, CXCL10 and TNF-a were cyclical

Elevation of IFN-γ responsive cytokines during period of reduced HBsAg, shortly before the ALT flare, and off treatment when HBV was being controlled

Gene Expression Pathway Analysis

- Liver biopsies were collected periodically before, during and after ARC-520 treatment of chimp A
- mRNA-seq generated 40 million reads of each mRNA sample from the liver biopsies
- Expression pathways were assessed by Ingenuity Pathway Analysis (Qiagen)
  - Canonical pathway analysis comparing changes in mRNA-seq reads to published pathways
  - Upstream analysis to identify upstream genes that would result in the observed downstream gene expression pathways

Biopsy Time Points For mRNA-seq Pathway Analysis



Biopsies

A. Day -64: pre-study Health Check (HC)

B. Day 1: after 8 week NUC lead-in, before 1st dose ARC-520

C. Day 36: one week after 2nd dose ARC-520

D. Day 120: during on-treatment ALT flare

E. Day 209: 2 weeks after 8th ARC-520 dose

F. Day 267: 2 weeks after 11th ARC-520 dose

G. Day 295: 5 weeks off all treatment and during serum HBV DNA elevation/ALT increase

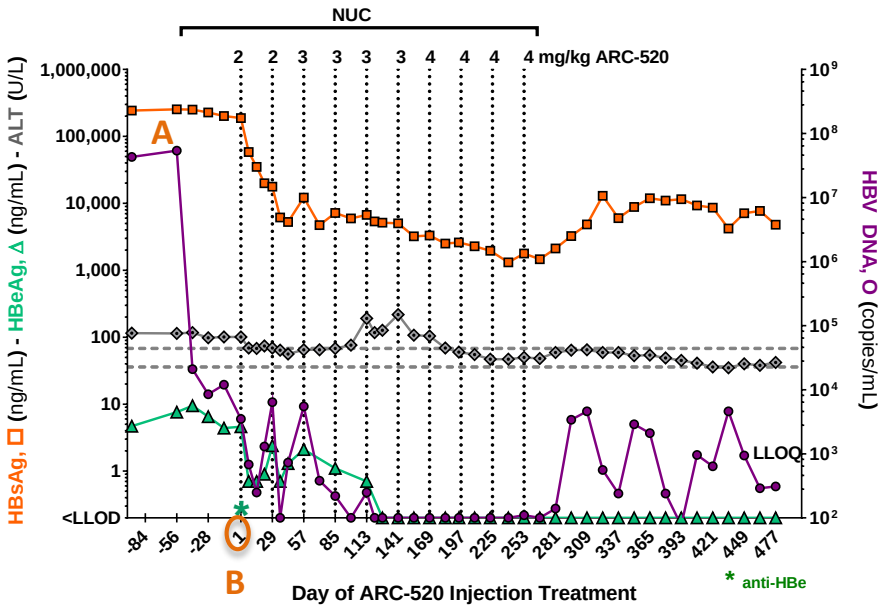
H. Day 323: 7 weeks off all treatment, serum HBV DNA declining

I. Day 351: 11 weeks off all treatment and during second off-treatment serum HBV DNA elevation

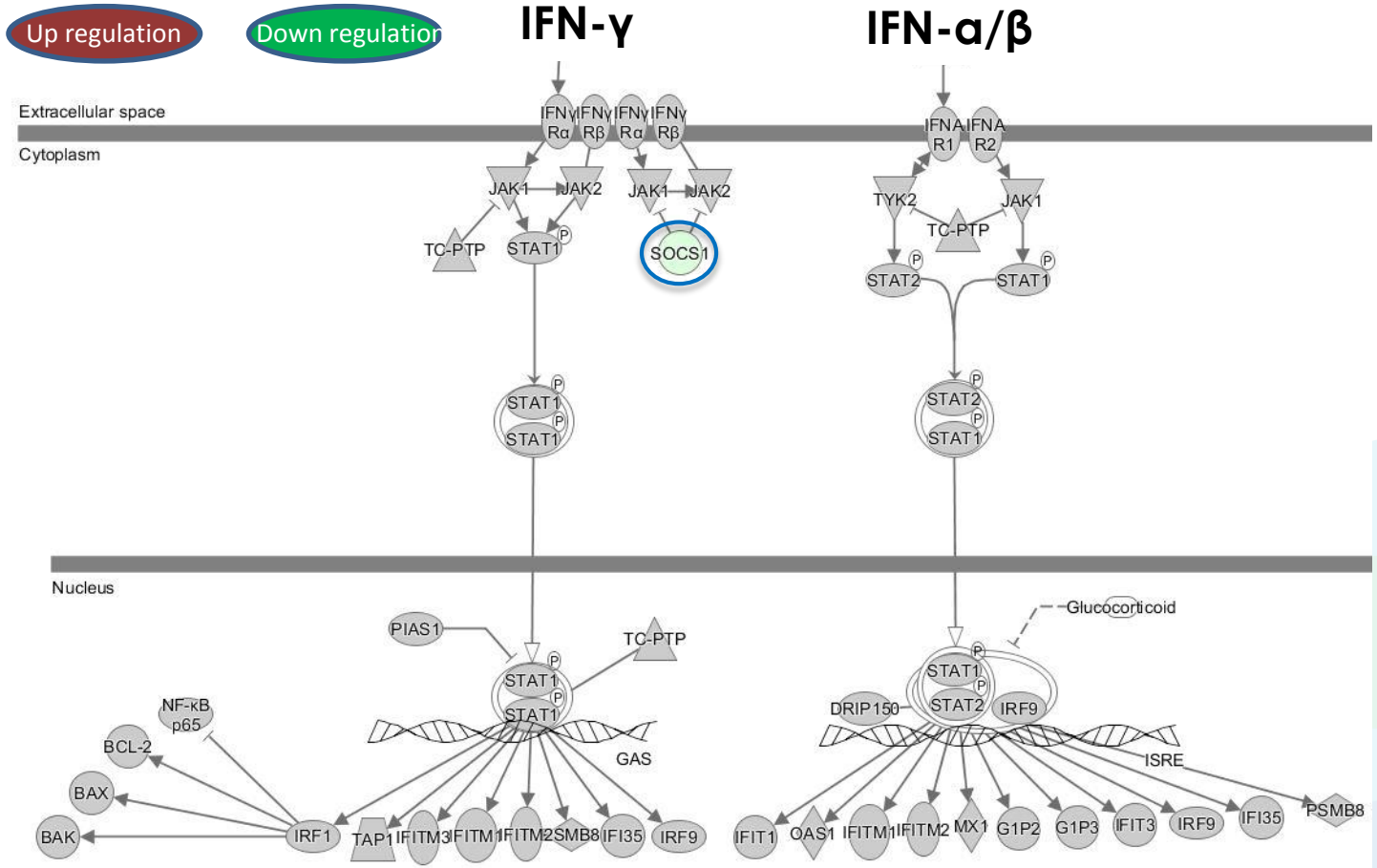
Canonical Gene Expression Pathway Analysis
Ingenuity Pathway Analysis (IPA)

- **IPA software (Qiagen)**
- **All mRNA-seq data normalized to ARC-520 Day 1 after NUC lead-in**
- **Fold change 1.5, FPKM>0.2**

A. Day -64: pre-study Health Check (HC)



(ARC-520 Day 1)



Almost no change in IFN-γ and IFN-α/β pathway gene expression before and after NUC lead-in

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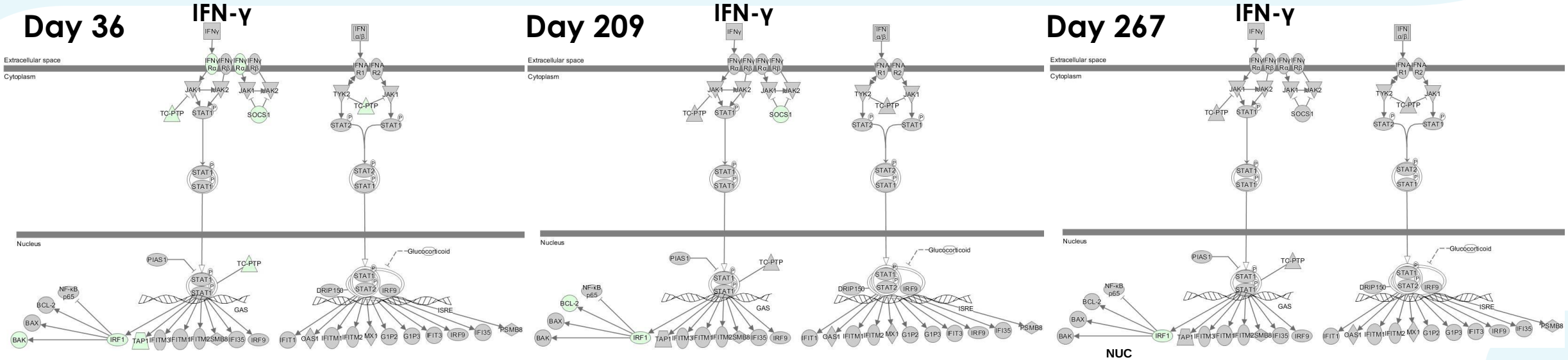
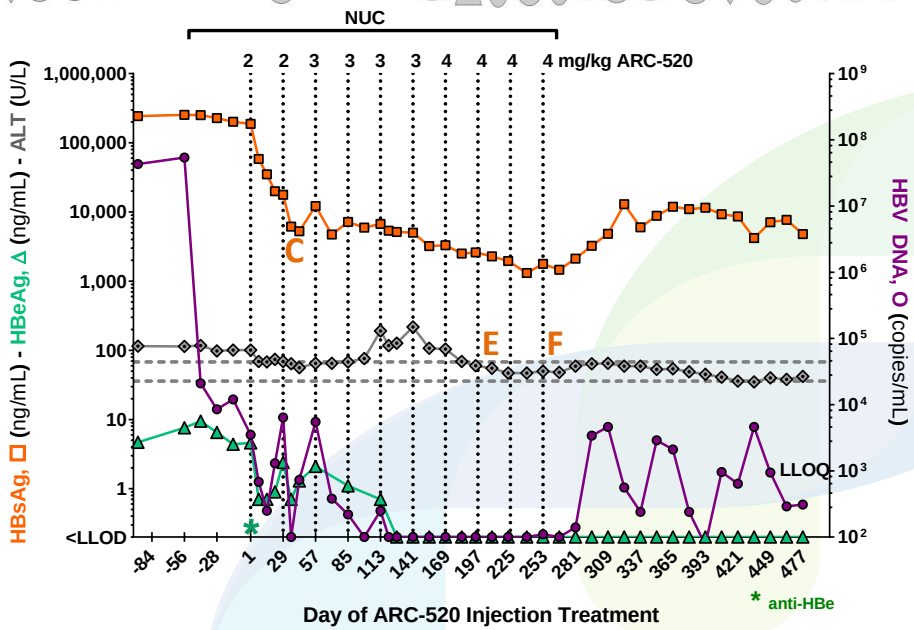
Down-regulation of IFN-γ pathway genes during ARC-520 treatment during periods of improving or quiescent ALTs



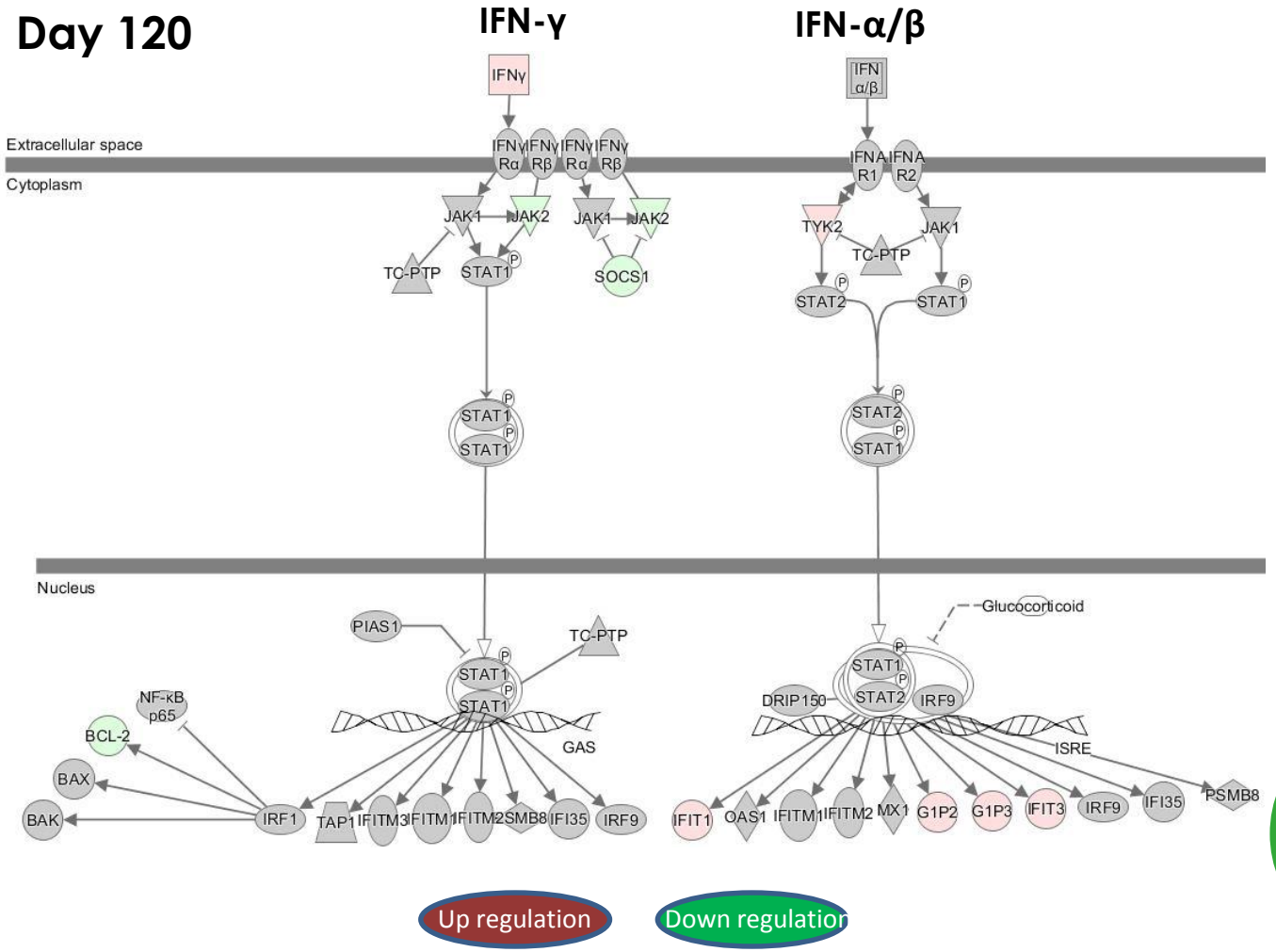
Up regulation

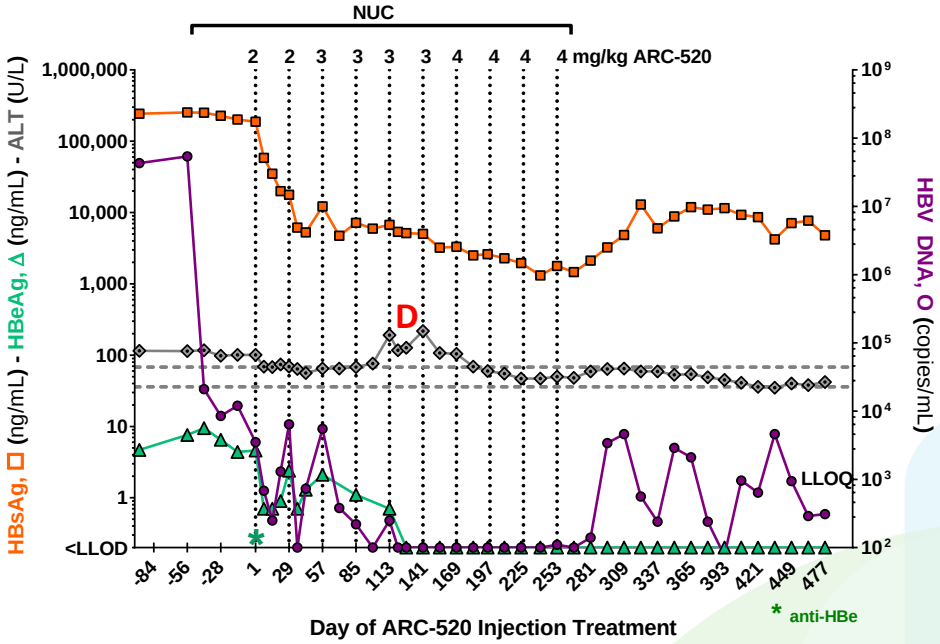
Down regulation

- HBsAg and HBeAg reduced
- Serum HBV DNA undetectable
- ALT normal
- Modest down-regulation of IFN-γ pathway genes



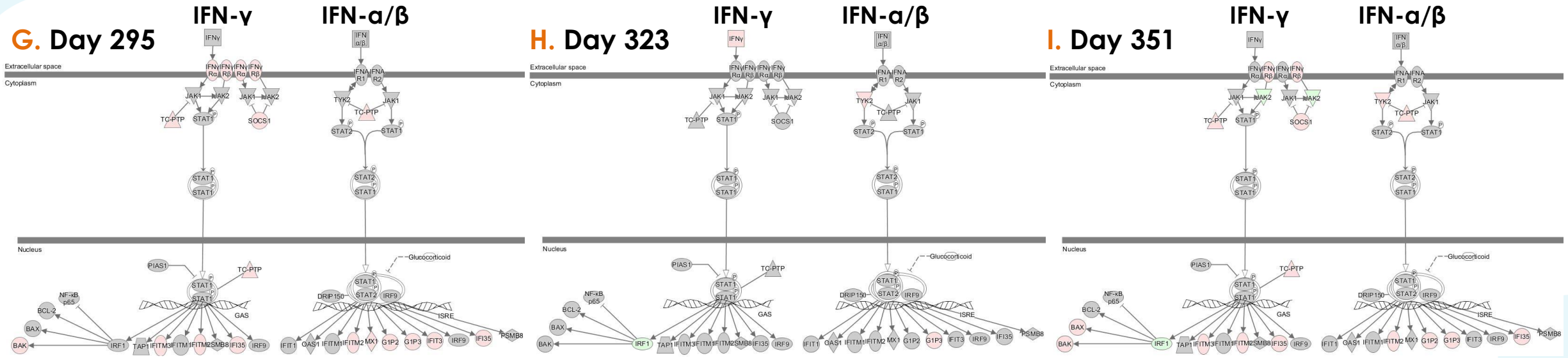
Modest up-regulation of interferon pathways during ALT flare





- ALT flare
- HBsAg reduced
- Serum HBV DNA & HBeAg undetectable
- Modest up-regulation of IFN pathways

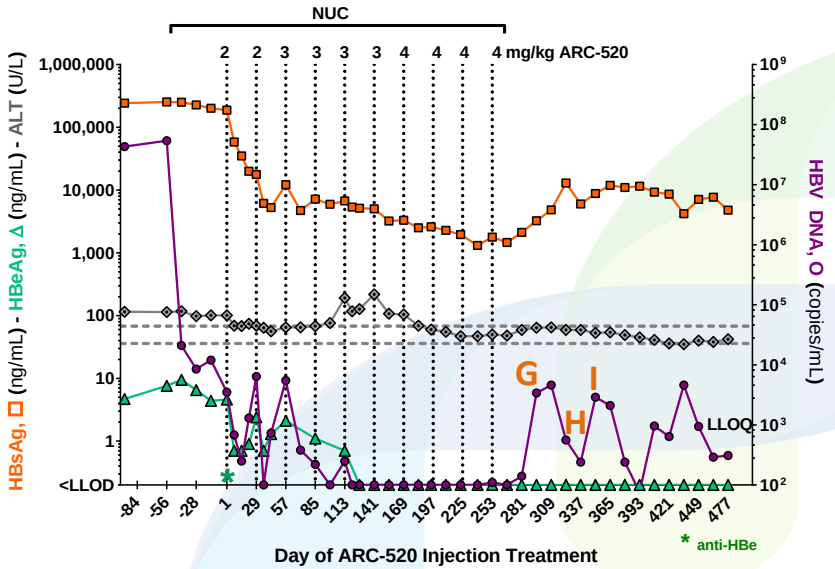
Up-regulation of IFN-α/β,γ pathways off all treatment associated with ALT increase (mild)



Up regulation

Down regulation

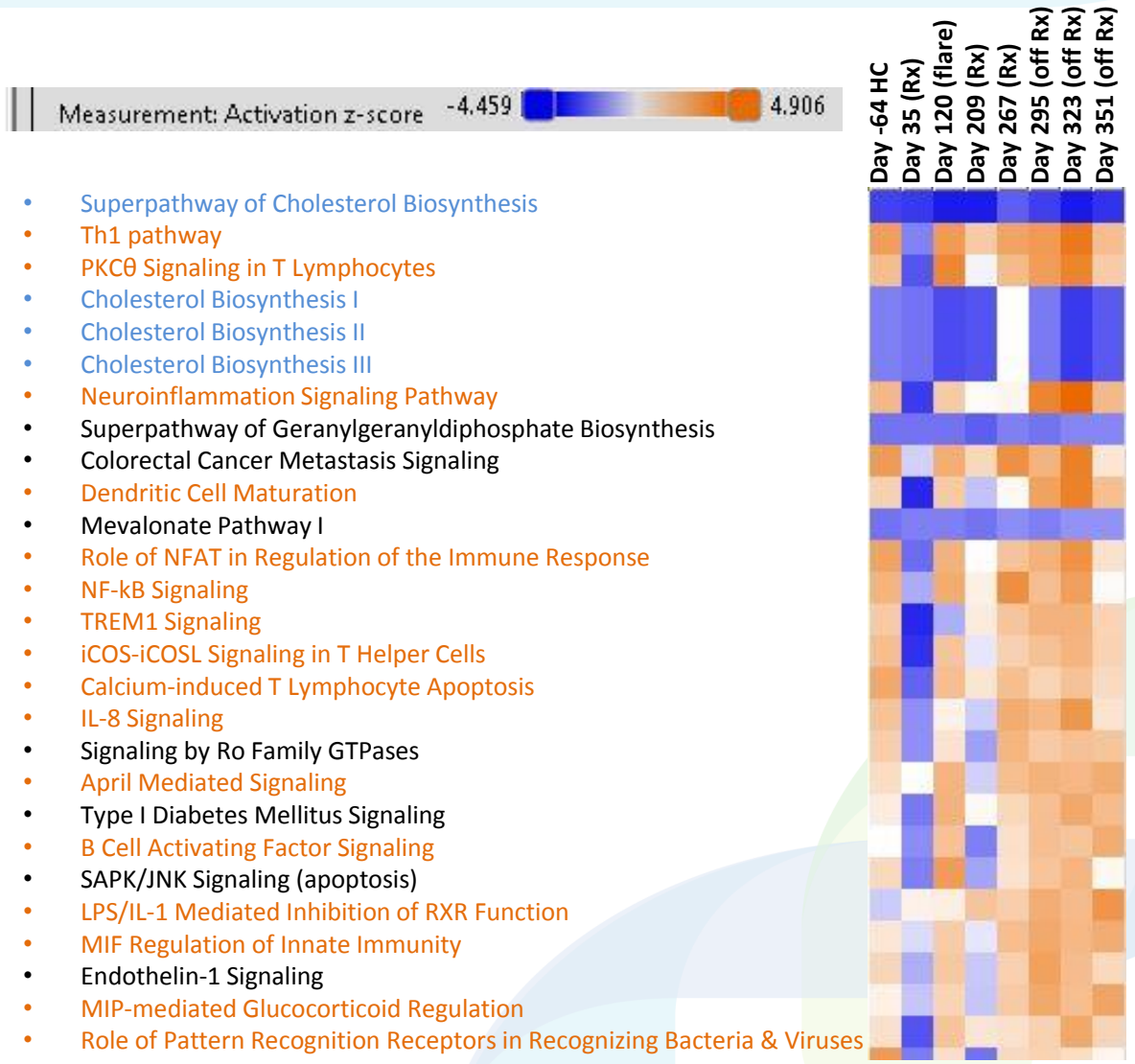
- Cycles of low level elevations and decreases of serum HBV DNA off treatment
- Mild but visible increase in ALT
- Up-regulation of IFN-α/β,γ pathway genes



Canonical pathway analysis shows immune pathways up-regulated

Comparison is to biopsy pre-ARC-520 but after ETV lead-in

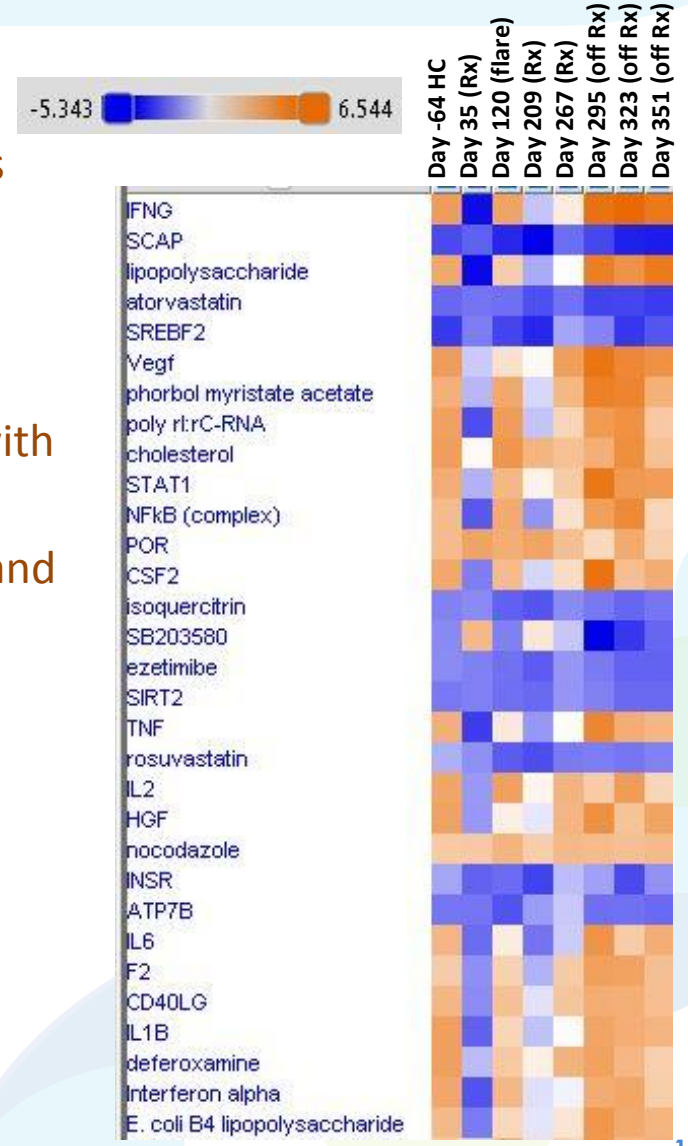
- Th1 pathway (CD4+ T cell role in adaptive immunity)
- PKCθ Signaling in T Lymphocytes
- Neuroinflammation Signaling Pathway (beneficial inflammatory response when controlled)
- Dendritic Cell Maturation
- Role of NFAT (Nuclear Factor of Activated T-cells) in Regulation of Immune Response
- NF-kB Signaling
- TREM1 Signaling (innate & adaptive immune response)
- iCOS-iCOSL Signaling in T Helper Cells
- Calcium-induced T Lymphocyte Apoptosis
- IL-8 Signaling (cellular immune response)
- April Mediated Signaling (promotes B cell proliferation)
- B Cell Activating Factor Signaling
- MIF Regulation of Innate Immunity
- MIF-mediated Glucocorticoid Regulation
- Role of Pattern Recognition Receptors in Recognizing Bacteria and Viruses



Upstream analysis shows INF-γ response pathway as top hit

Comparison is to biopsy pre-ARC-520 but after 57 days of ETV

- **IFNG** – interferon gamma secreted by activated immune cells
- Lipopolysaccharide – communication between innate & adaptive immune cells
- Phorbol myristate acetate – IL12 signaling and production in macrophages
- Poly rI:rC-RNA – inflammatory response
- STAT1 – transcription factor regulated by IFN-alpha, IFN-gamma and IL6
- NFkB – controls transcription, cytokine production, cell survival (relationship with TNFa and LPS)
- CSF2 – cytokine controls production, differentiation, function of granulocytes and macrophages
- TNF – proinflammatory cytokine
- IL2 – proliferation of B cells and T cells
- IL6 – inflammation and maturation of B cells
- CD40LG – expressed on surface of T cells, regulates B cell function
- IL 1B – mediator of inflammatory response
- **Interferon alpha**



Conclusions

- Sustained host control of viremia off all treatment in chimpanzee A
  - HBeAg seroconversion
  - Serum HBV DNA maintained 5 $\log_{10}$ lower than at start of study (near the LLOQ)
  - HBsAg maintained 1.7 $\log_{10}$ lower
  - Off-treatment cycles of serum HBV DNA elevation and decrease coincided with expression of IFNα/β,γ and IFNγ-responsive cytokines that were measured in the serum
- Pathway analysis demonstrated that host control involved innate and adaptive immune responses
  - Upstream expression of IFNγ was most consistent with the activated canonical pathways
  - Interferon alpha also activated
  - T cell and B cell pathways were activated during host control
  - Involvement of dendritic cells, messengers between innate and adaptive immune system
- Host control involved very modest elevations of liver enzymes

Thank you !

Robert Lanford
Deborah Chavez
Kathleen Brasky
Bernadette Guerra

Jason Goetzmann
Dana Hasselschwert

Backup

Immune pathways activated relative to last RNAi treatment

Comparing last time point on treatment to post-treatment

- Interferon-gamma
- Lipopolysaccharide
- STAT1
- TNF
- CSF2
- IL6
- IL 1B
- Poly rI:rC-RNA
- **IFNA2**
- NFkB (complex)
- **IL1RN**
- Interferon alpha
- Phorbol myristate acetate
- **TGFB1**
- **IL27**
- E coli B5 lipopolysaccharide
- **SOCS1** (suppressor of cytokine signaling) modulates IFNG action
- **OSM** regulates IL6
- IL2
- **Interferon**