Apoptosis in chronic hepatitis C

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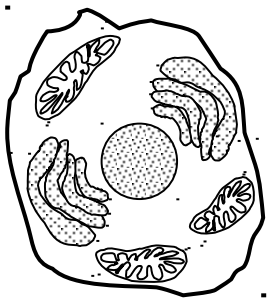
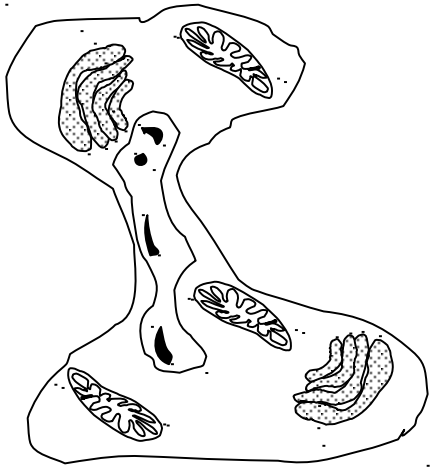
APOPTOSIS

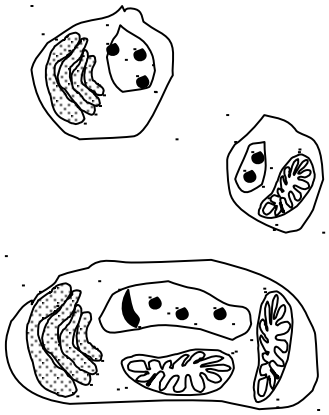
Apoptosis - type I programmed cell death
It is a highly regulated and controlled process during cell lifecycyle that may occur in *Eucaryota* organisms.



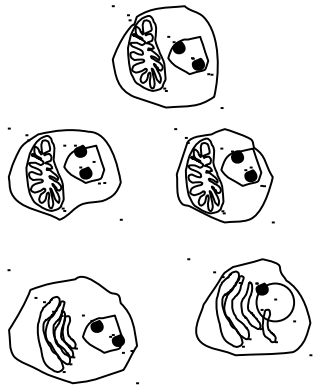
Normal cell



Cell shrinking, blebbing
Chromatin condensation



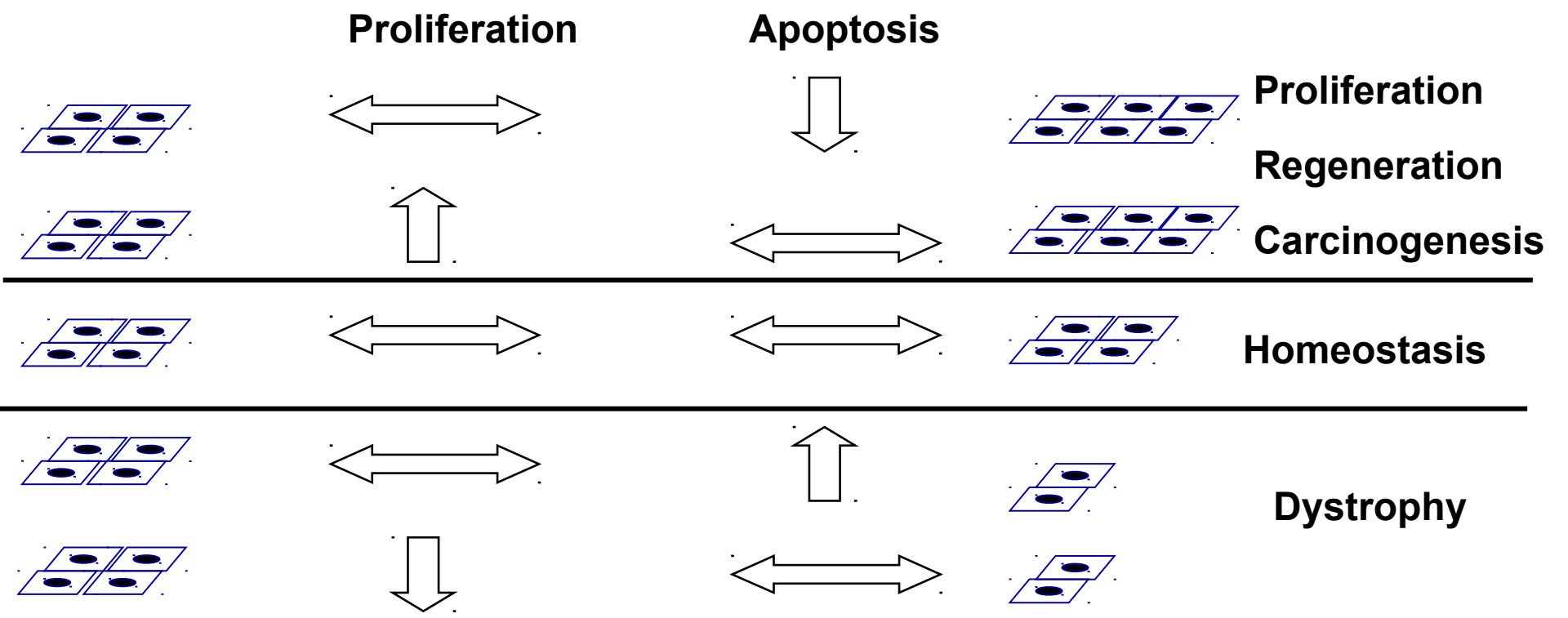
Nuclear fragmentation
Cell fragmentation



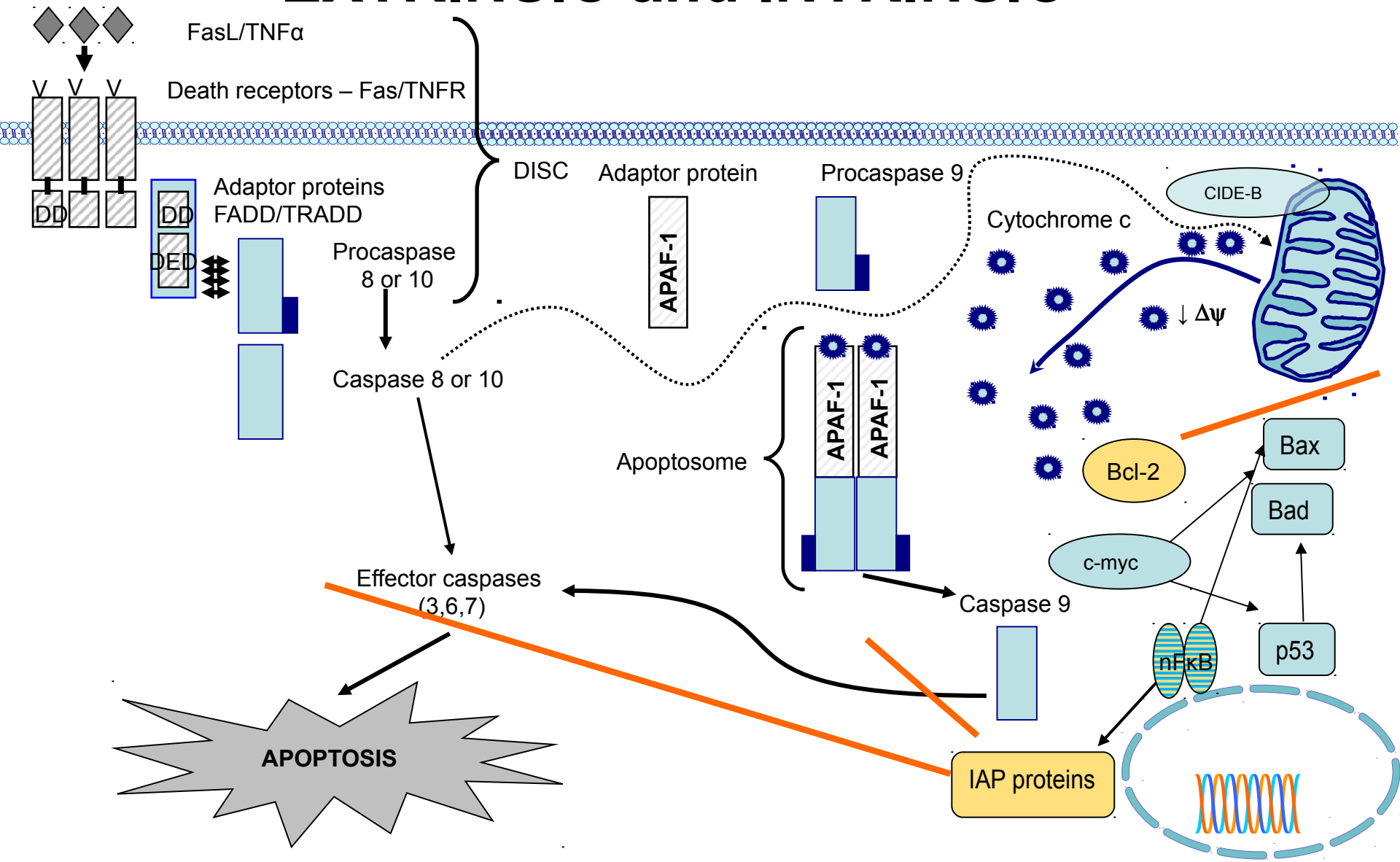
Apoptotic bodies
No inflammation

APOPTOSIS

- plays a role in embryonic development and morphogenesis,
- counteracts proliferation
- controls process of the elimination of defective/damaged/infected cells
- <u>crucial for the maintenance of tissue homeostasis</u>

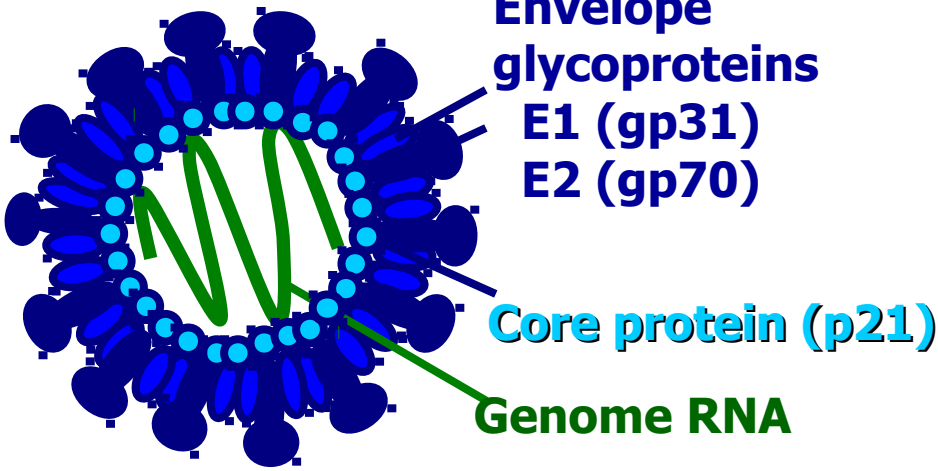


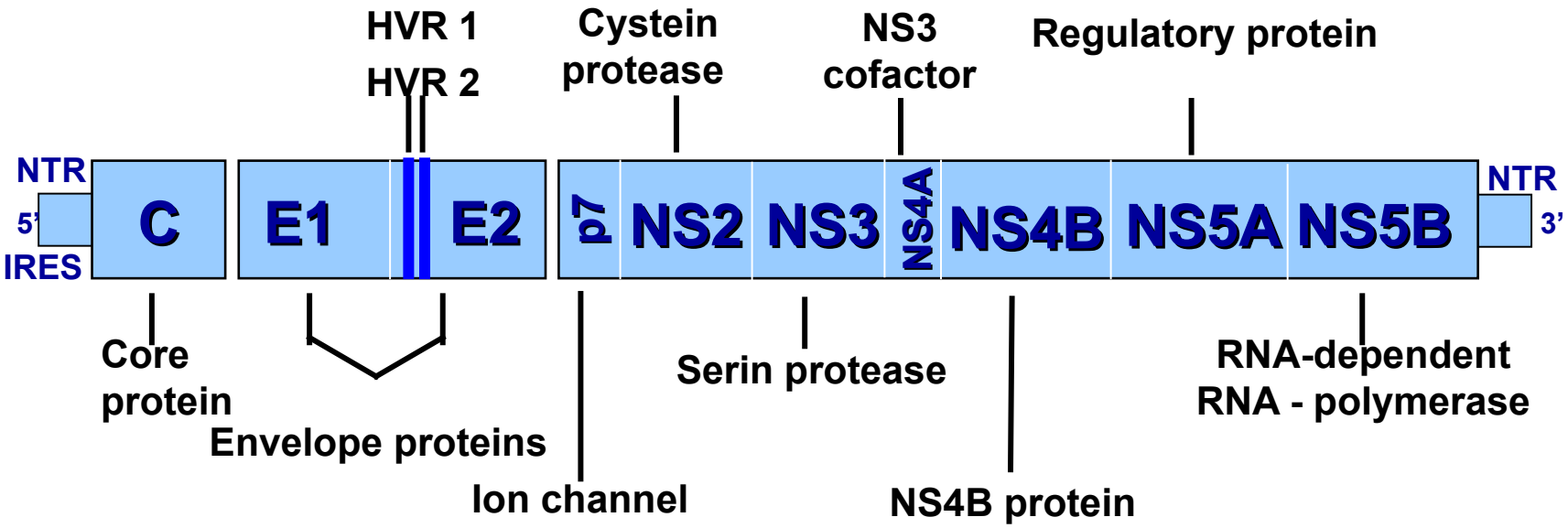
APOPTOTIC PATHWAYS
EXTRINSIC and INTRINSIC



HEPATITIS C VIRUS

- Family *Flaviviridae,* enveloped virus
- Classification D.Baltimore → group IV: ssRNA(+) – positive-sense single stranded RNA
- 7 major genotypes, 67 subtypes, quasispecies





Pathophysiology of CHC

EXPOSURE

Exposure to HCV
Immune response CD4, CD8 T cells, interferon expression

APOPTOSIS

Apoptosis in attempt to clear the virus (15-45% eradication)
Immune evasion-lymphocytes apoptosis among different mechanisms
Establishement of persistent infection

SEQUELAE

Liver damage
Cirrhosis
Hepatocellular carcinoma (HCC)

Hajarizadeh B et al. Nat Rev Gastroenterol Hepatol. 2013 Sep;10(9):553-62

Role of HCV proteins to induce apoptosis

- HCV core protein: activates Fas, activates TRAIL, induces of mitochondrial stress (ROS, Cyt c release),
- NS3/4A complex: induces of mitochondrial stress (ROS, Cyt c release)
- NS5A: binds to protein kinase R leading to the inhibition of anti-apoptotic protein synthesis
- E1, E2: increase FasL expression

Bantel H, Schulze-Osthoff K. Cell Death Differ. 2003;10 Suppl 1:S48-58
Cruise MW et al. J Leukoc Biol. 2005;78(2):412-25
Patel T et al. Hepatology. 1999;30(3):811-5
Korenaga M et al. J Biol Chem. 2005;280(45):37481-8
Nomura-Takigawa Y et al. J Gen Virol. 2006;87(Pt 7):1935-45

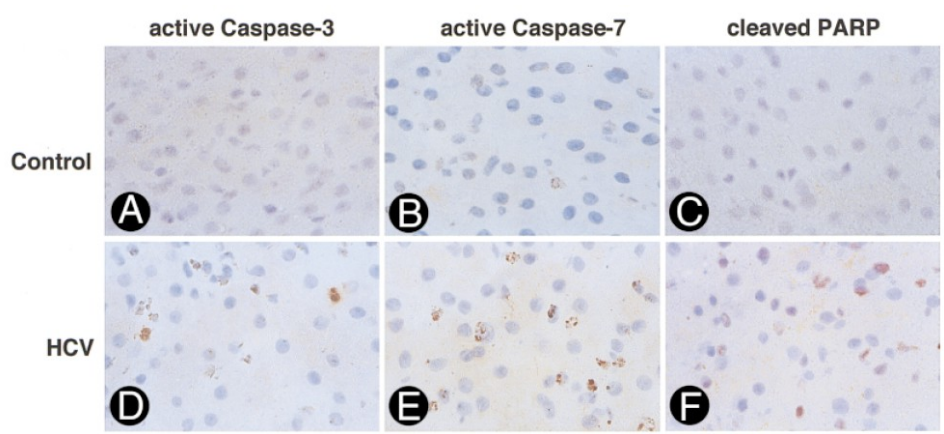
Role of HCV proteins to inhibit apoptosis

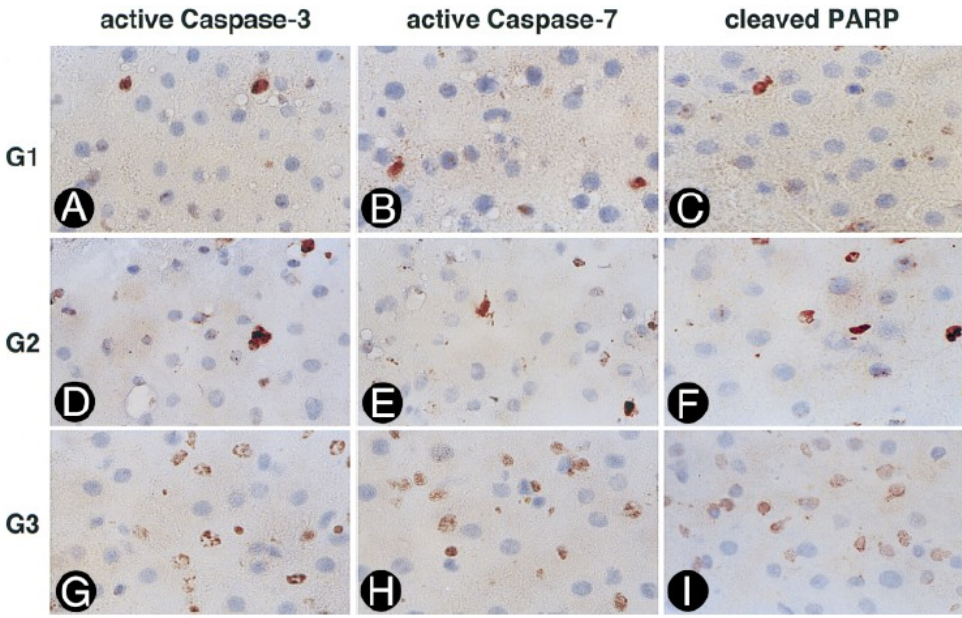
- HCV core protein: binds to c-myc, activates NF-κB, inhibits mitochondrial pathway
- NS3/4A: cleaves MAVS (Cardif) inhibiting intrinsic apoptotic pathway
- NS2: binds to CIDE-B inhibiting intrinsic/mitochondraial pathway
- NS5A:interacts with TRADD blocking caspase activation, activates NF-κB and protooncogen STAT-3 through triggering oxidative stress and ROS production; binds to proapoptotic protein Bax; inhibits proapoptotic protein Bid, activates protooncogen β-catenin, binds to p53 protein
- E1, E2: repress the activation of caspase-8 and the release of cytochrome c from the mitochondria

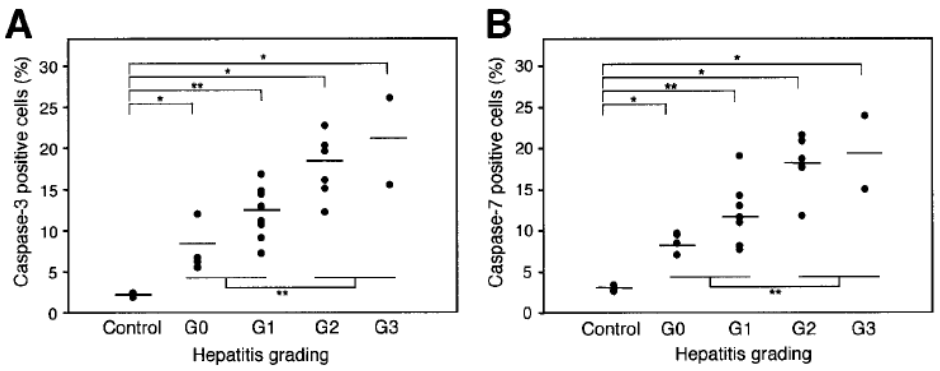
Bantel H, Schulze-Osthoff K. Cell Death Differ. 2003;10 Suppl 1:S48-58
Jiang X et al. PLoS One. 2015;10(7):e0131973
Erdtmann L et al. J Biol Chem. 2003;278(20):18256-64
Lee SH et al. J Immunol. 2005;175(12):8226-35
Kim H, Ray R. Methods Mol Biol. 2014;1155:125-32

Role of apoptosis in pathogenesis of HCV-associated liver inflammation

Apoptosis plays a critical role in HCV-associated liver injury

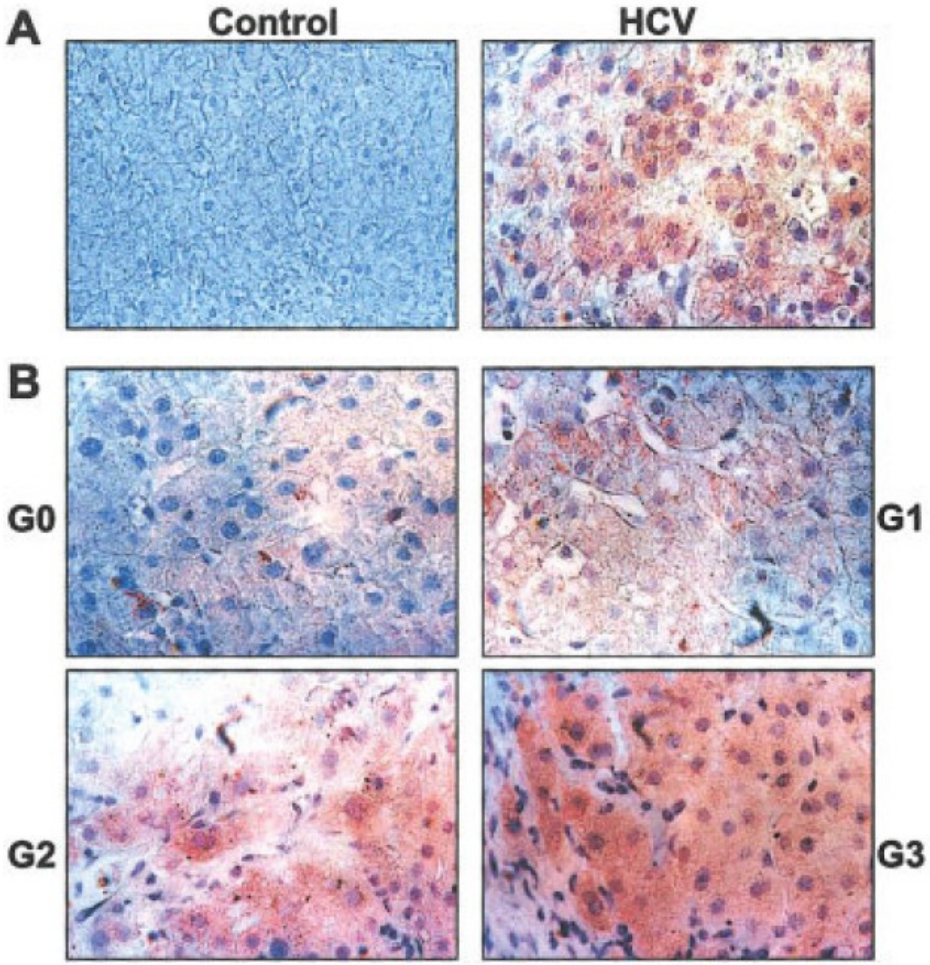




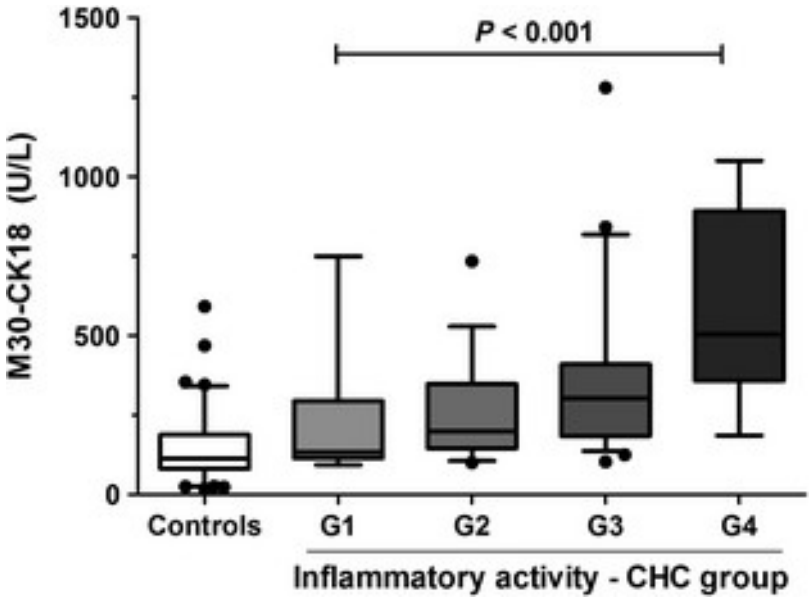


Bantel H. et al. Hepatology. 2001;34:758-67

Role of apoptosis in pathogenesis of HCV-associated liver inflammation



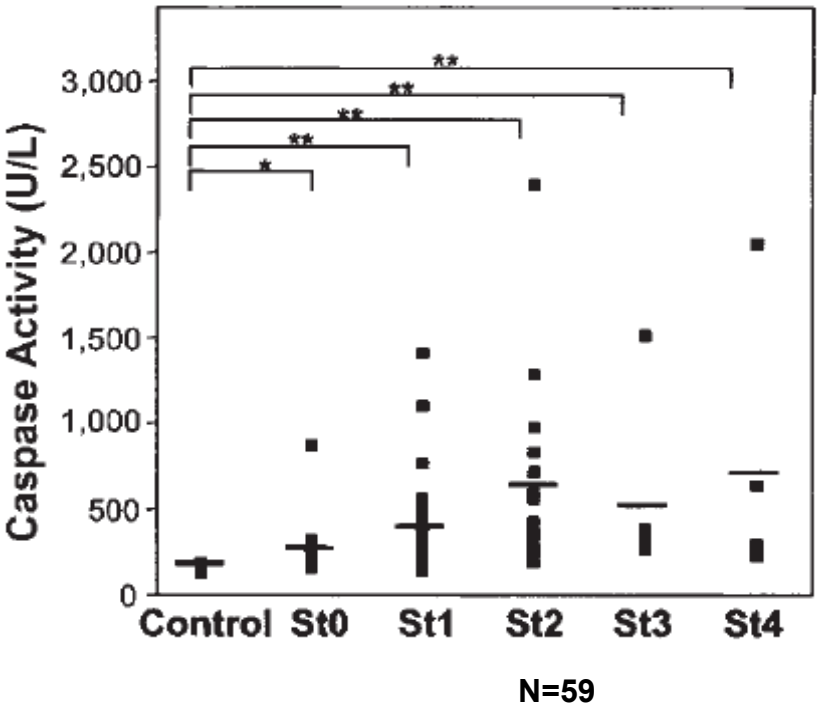
Detection of caspase-mediated CK-18 cleavage in HCV-infected liver biopsies.



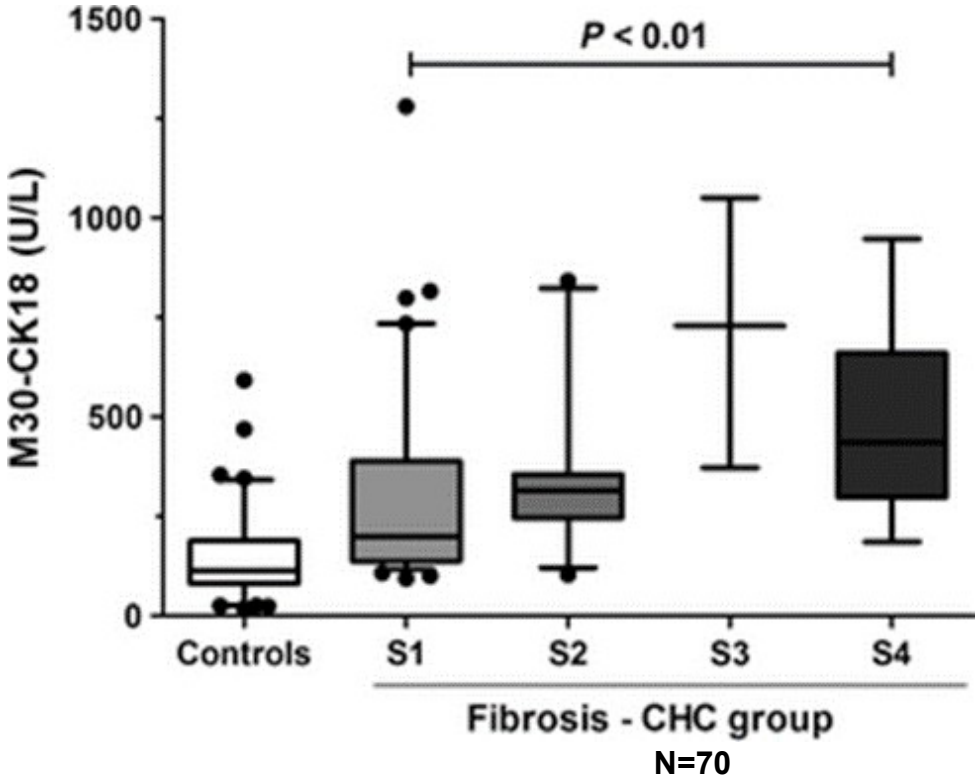
Serum concentration of caspase-cleaved CK-18 epitop M30 and inflammatory activity in liver biopsies in CHC

Bantel H. et al. Hepatology. 2004;40(5):1078-87
Parfieniuk-Kowerda A et al. Liver Int. 2014;34(4):544-50

Role of apoptosis in pathogenesis of HCV-associated hepatic fibrosis



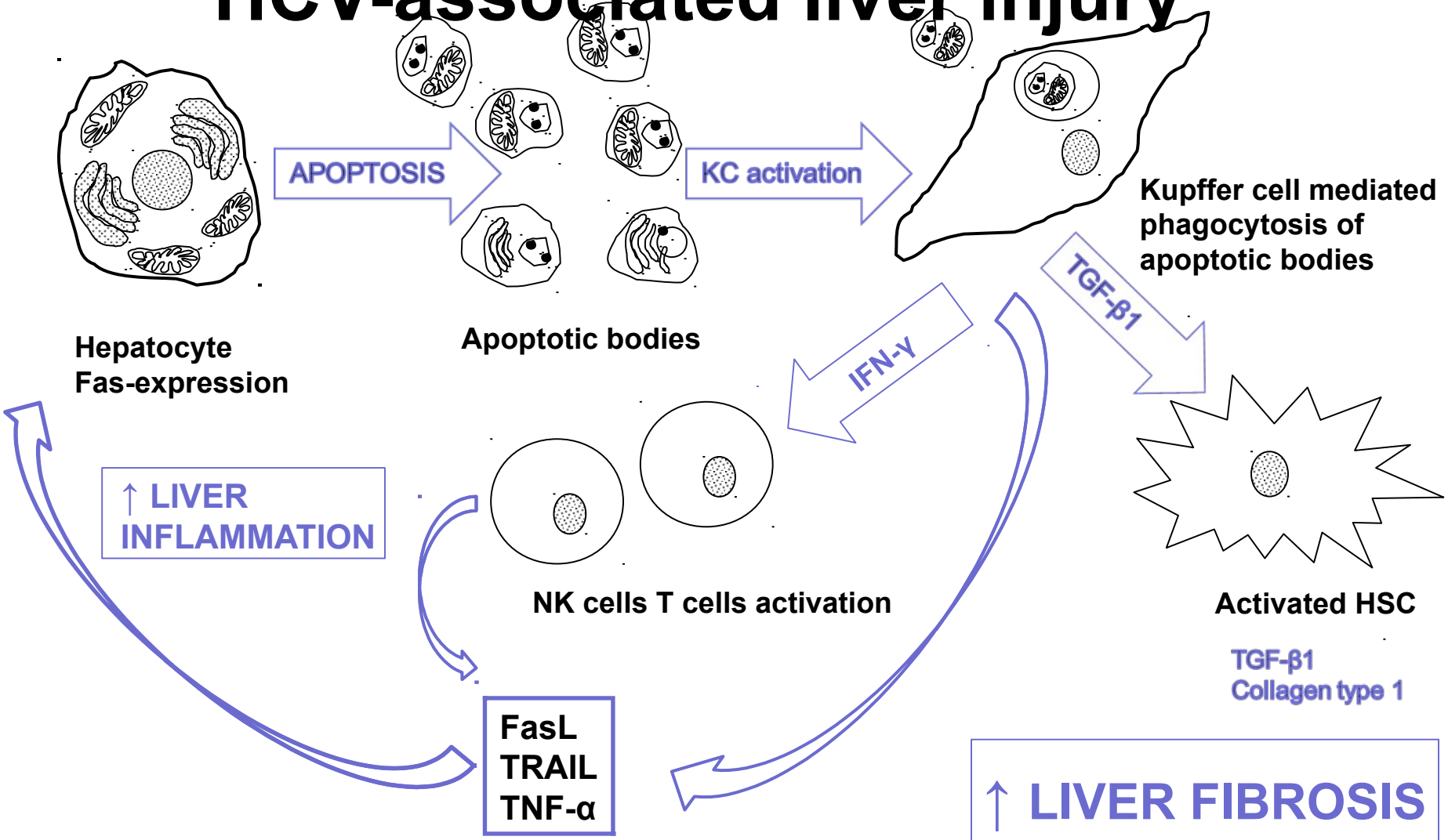
N=59



N=70

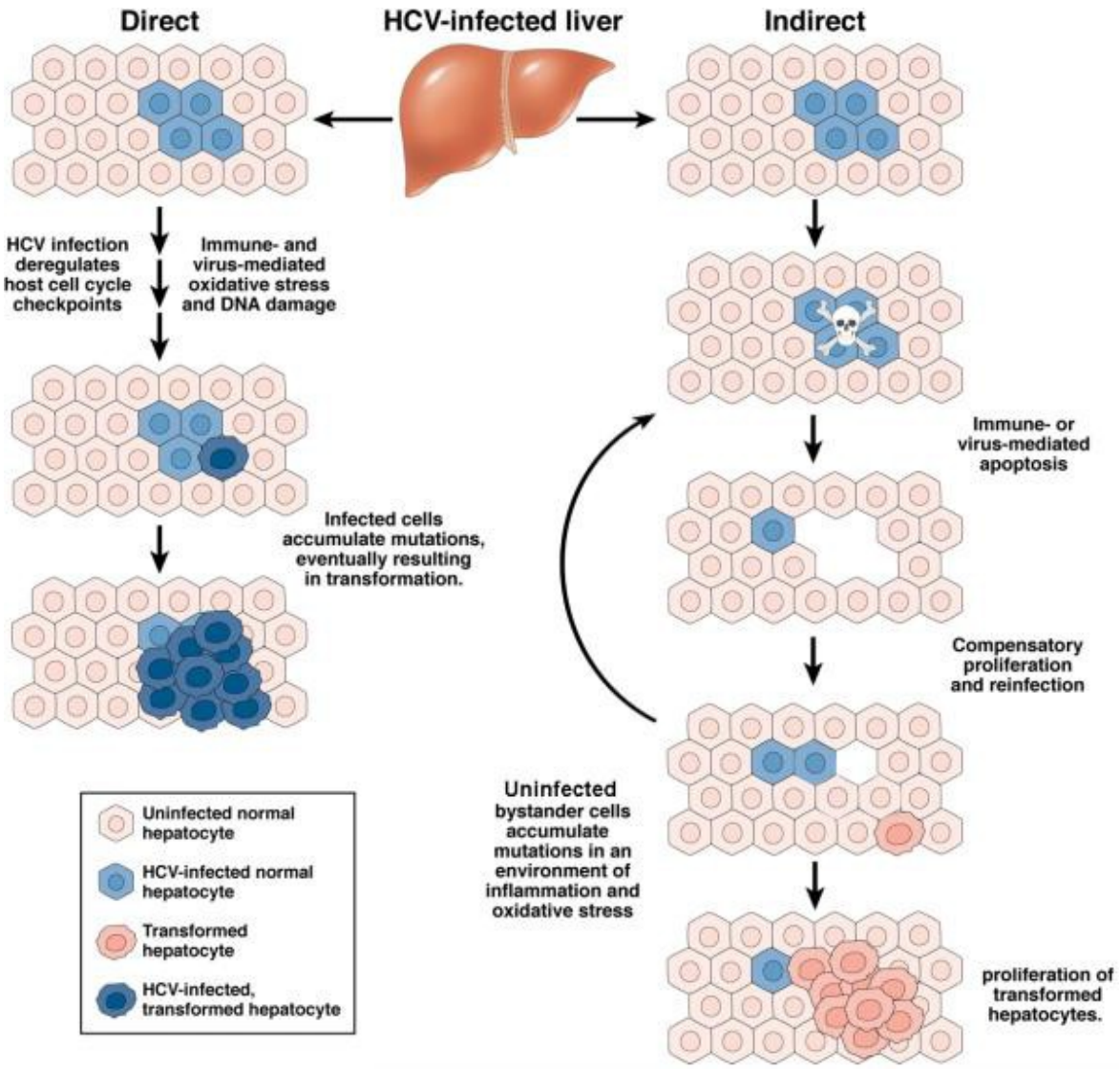
Bantel H. et al. Hepatology. 2004;40(5):1078-87
Parfieniuk-Kowerda A et al. Liver Int. 2014;34(4):544-50

Role of apoptosis in pathogenesis of HCV-associated liver injury



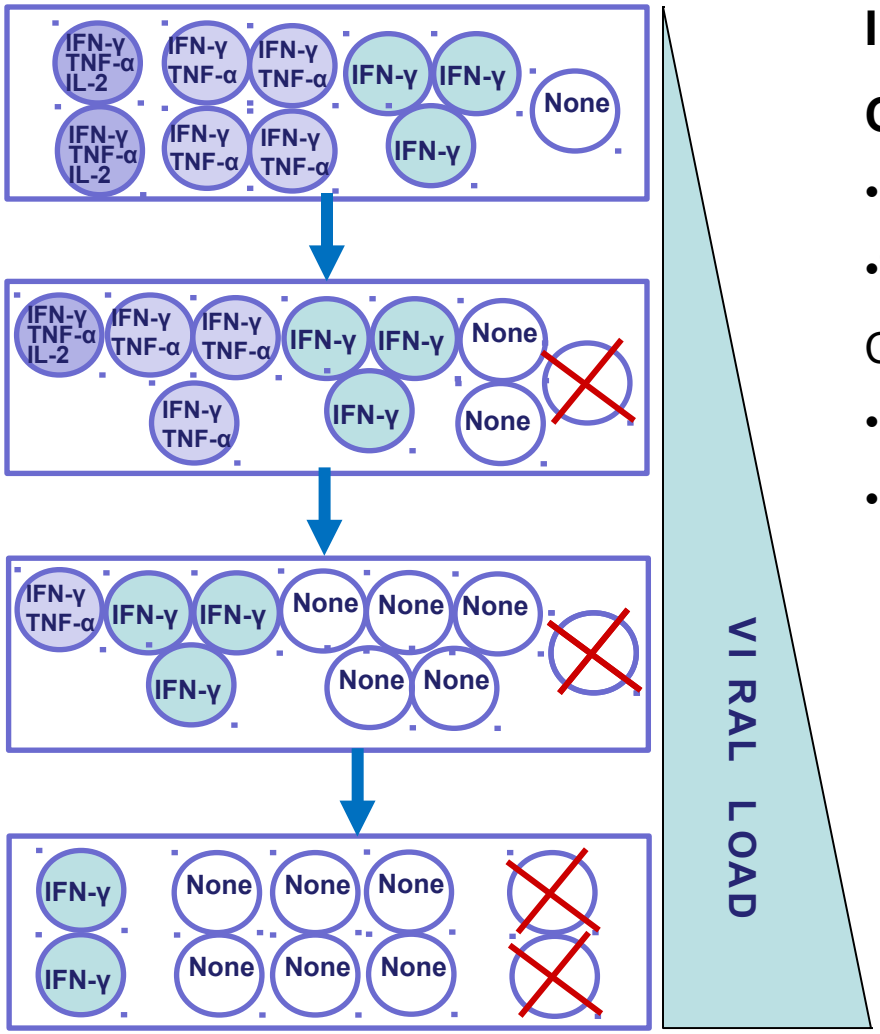
Stegmann KA et al. Gastroenterology. 2010;138(5):1885-97
Malhi H et al. Physiol Rev. 2010; 90(3): 1165–1194

Carcinogenesis in CHC



Lemon SM et al. Gastroenterology. 2012;142(6):1274-8

Apoptosis of lymphocytes in CHC



Increased susceptibility to apoptosis

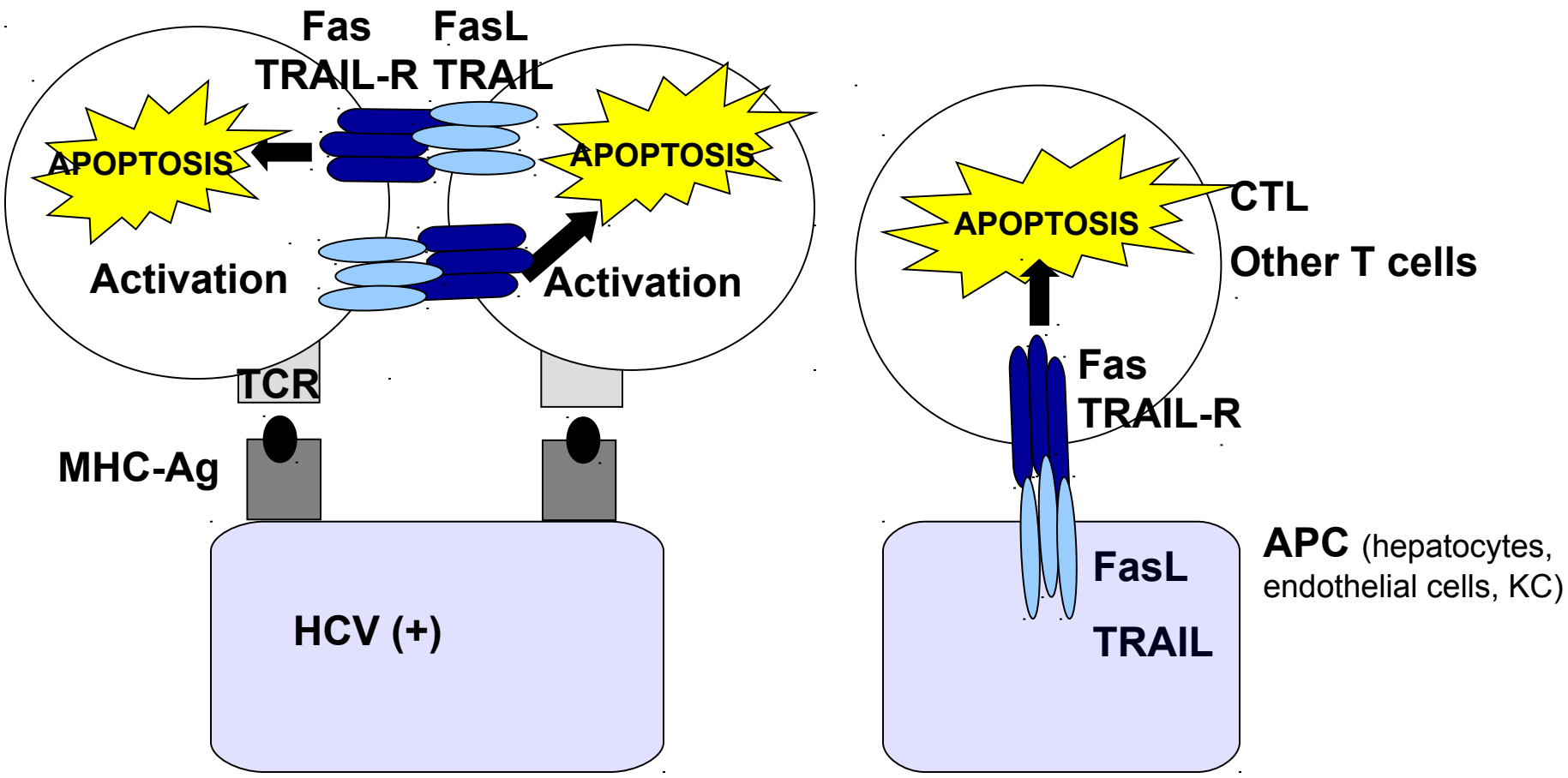
CD4, CD8 T-cells:

- ROS increased in PBMC in CHC
- TRAIL, Fas expression upregulated in NK, CD4, CD8 T-cells
- increased downstream caspase activity
- In HCV-specific CD8+ T-cells:
  1. increased PD-1 expression
  2. increased annexin V expression
  3. increased frequency of caspase 9-mediated T-cell death within the liver
  4. significant functional deficits – phenomenon of T-cells exhaustion

Adapted from:
Yi JS et al. Immunology.2010;129(4): 474–81

Barathan M et al. Apoptosis. 2015;20(4):466-80
Panasiuk et al. Liver Int. 2010;30(3):472-8
Radziewicz H et al. J Virol. 2008;82(20):9808-22
Penna A et al. Hepatology. 2007;45(3):588-601
Stegmann KA et al. Gastroenterology. 2010;138:1885-97

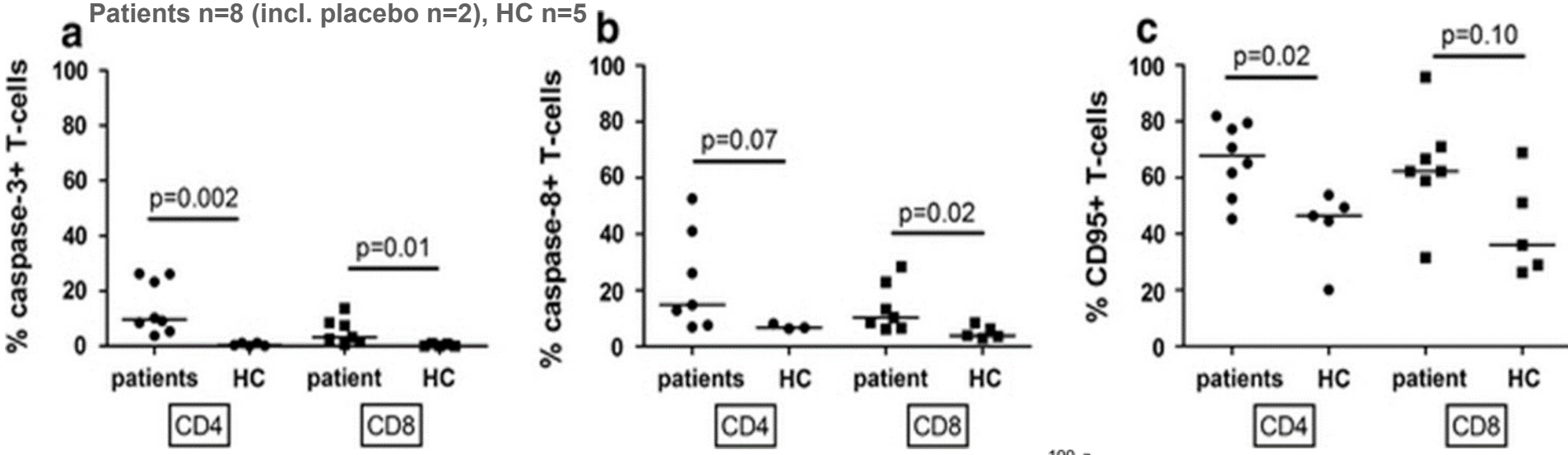
Apoptosis of lymphocytes in CHC



Bantel H, Schulze-Osthoff K. Cell Death Differ. 2003;10 Suppl 1:S48-58

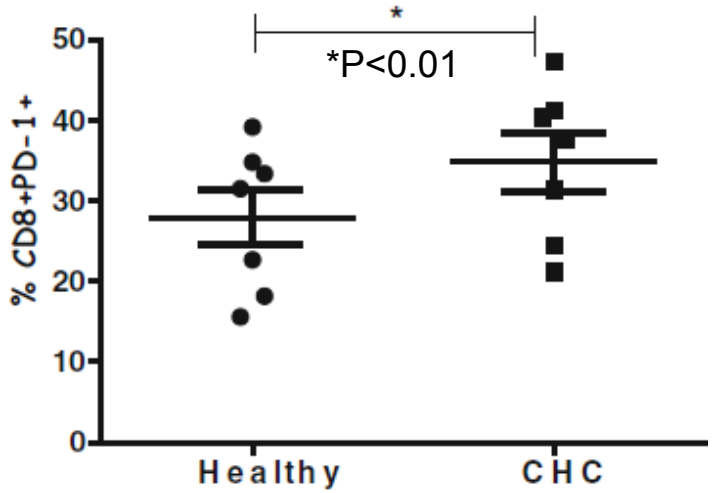
Apoptosis of lymphocytes in CHC

Patients n=8 (incl. placebo n=2), HC n=5



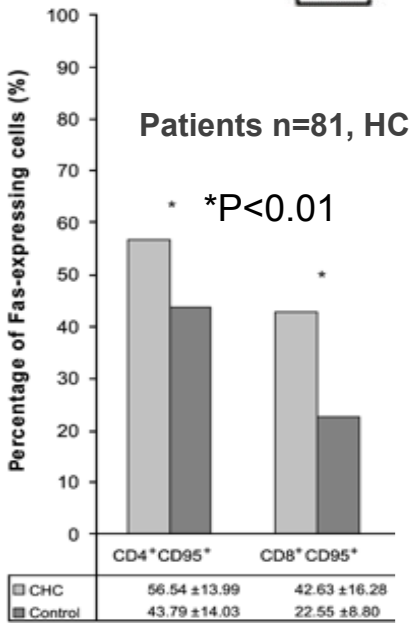
Arends JE et al. Apoptosis. 2011 Sep; 16(9): 959–966

Patients n=7, HC n=7



Barathan M et al. Apoptosis. 2015;20(4):466-80

Patients n=81, HC n=14

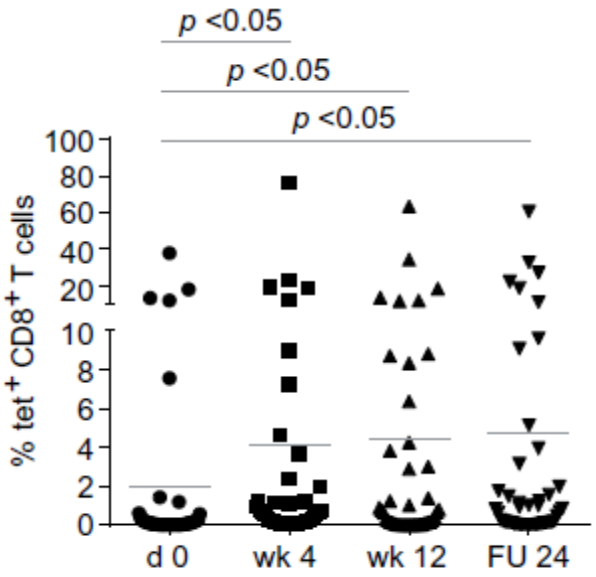


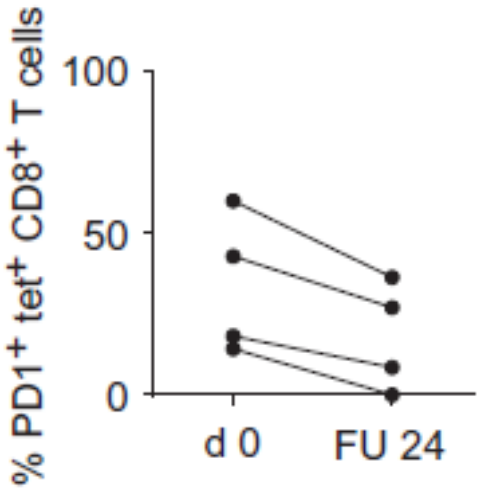
Panasiuk et al. Liver Int. 2010;30(3):472-8

Effects of DAA treatment on T cells population

- 51 previously untreated chronically infected patients undergoing IFN-free therapy with a combination of faldaprevir (NS3/4A protease inhibitor) and deleobuvir (a non-nucleoside NS5B inhibitor) with or without ribavirin
- SVR12 n=28, failure n=13

- prompt restoration of HCV-specific CD8+ T cell proliferation under successful IFN-free therapy
- increase HCV specific responses after *in vitro* expansion per patient

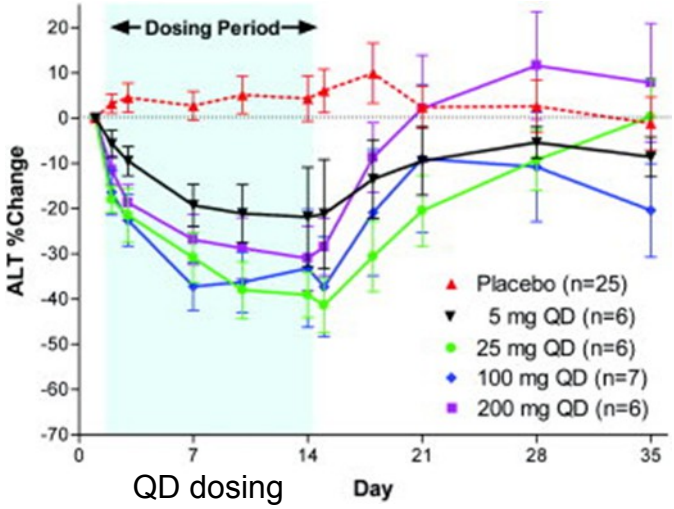




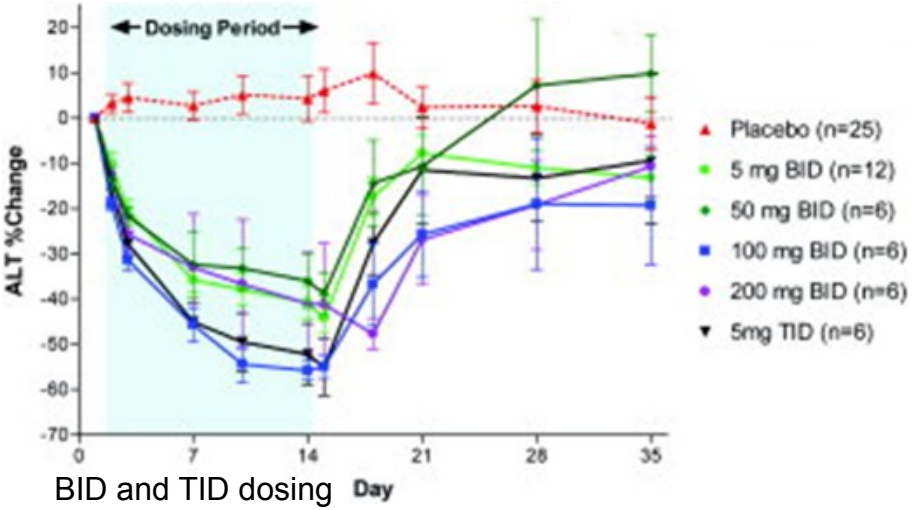
Martin B et al. J Hepatol. 2014 Sep;61(3):538-43

Pancaspase inhibitors in HCV infection Liver inflammation & fibrosis

- Oral emricasan (IDN-6556/PF-03491390), an antiapoptotic irreversible caspase inhibitor, lowers aminotransferase activity in patients with chronic hepatitis C
- CHC n=80,



QD dosing



BID and TID dosing

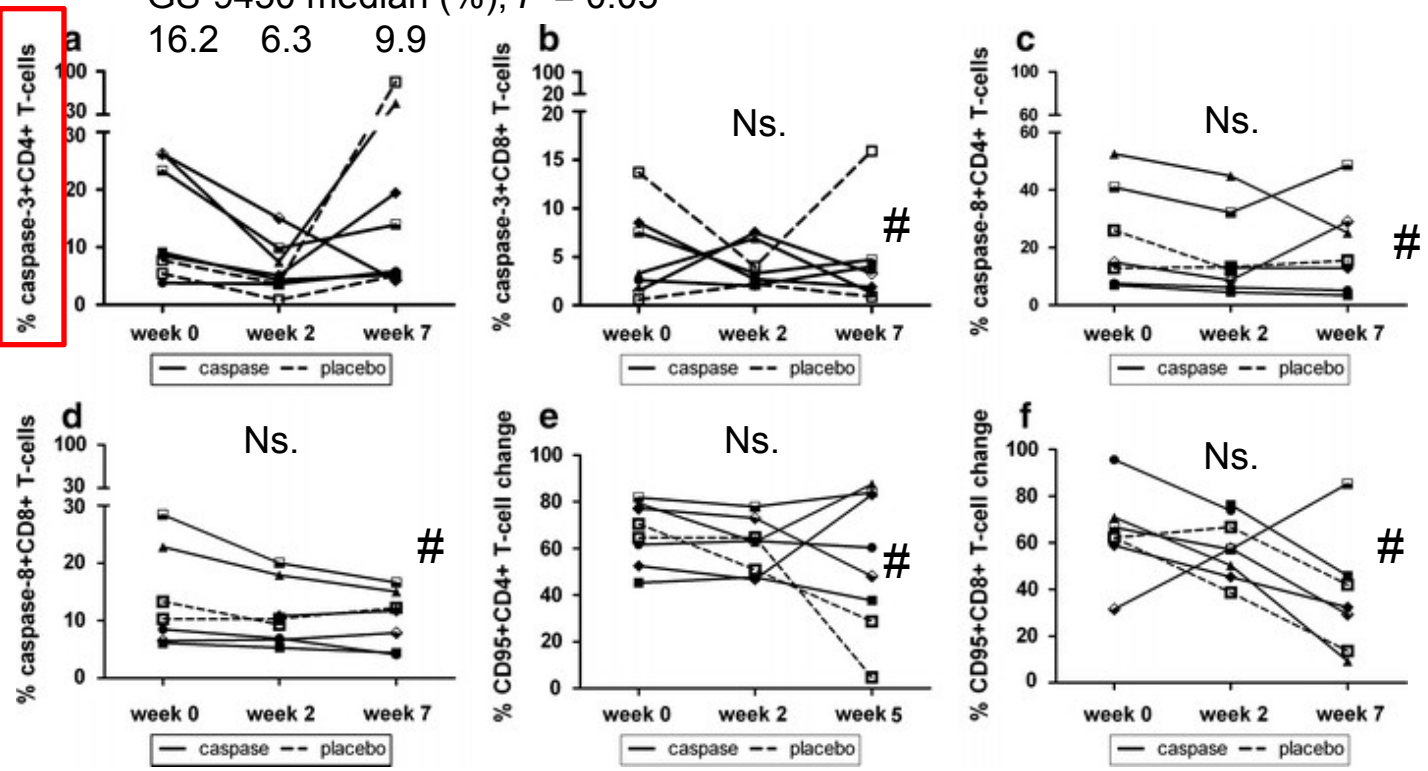
- mean ALT decrease 22-56%
- all HCV treatment groups above 5 mg QD were significantly different from placebo
- HCV-RNA levels remained stable within 1 log unit during the study

Pockros PJ et al. Hepatology. 2007; 46(2):324-9

Longitudinal analysis of caspase-3 and CD95 expression in peripheral T-cells

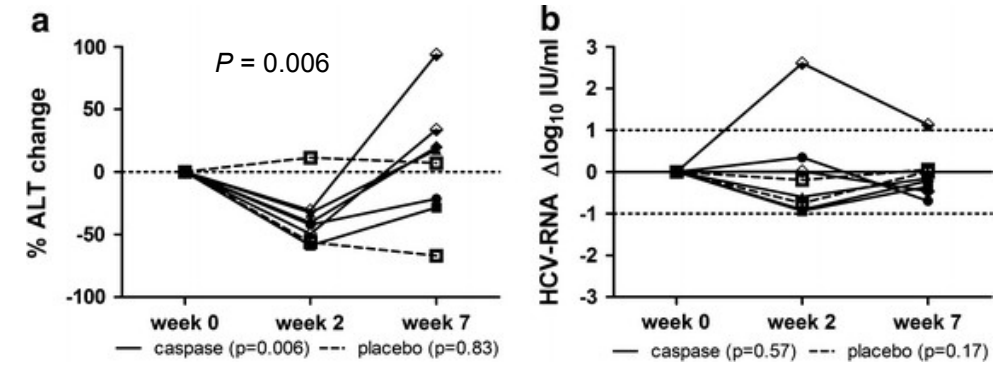
GS-9450 median (%), $P = 0.05$

16.2 6.3 9.9



Patients n=8
(incl. placebo n=2)
HC n=5

ns. GS-9450 vs. placebo



Treatment with low-doses of GS-9450 resulted in lower ALT-values,
but did not affect either the HCV viral load or the peripheral T-cells apoptosis rates (caspase 3, caspase 8, Fas expression)

Arends JE et al. Apoptosis. 2011 Sep; 16(9): 959–966.

Conclusions

The phenomenon of apoptosis in CHC is responsible for liver inflammation and fibrosis, is substantial for cancer formation and faciltate immune evasion

DAA treatment allows to restore HCV-specific CD8 cells population and funcionality of these cells (increased HCV specific responses and reduced expression of the markers of T cell exhaustion, e.g. PD-1) although further confrimation is necessary

Therapeutic interventions in apoptotic signalling are limited by a complex nature of apotosis in CHC and seem to be most applicable in the future in patients with advanced fibrosis / cirrhosis who previously completed successful antiviral treatment