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Hepatitis C and E

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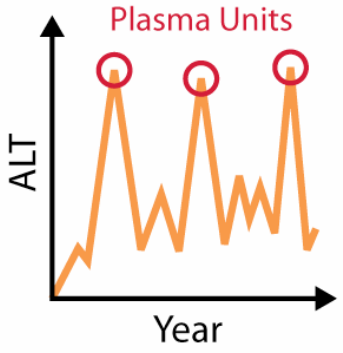
Section A

Background and Overview

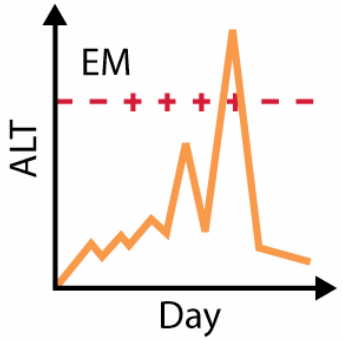
Cloning of HCV, Production of High-Titer Concentrates

Collect units of chronic-phase plasma at ALT peaks (assumes virus is cytopathic)

Chimp → Inoc. FVIII (HCV) →



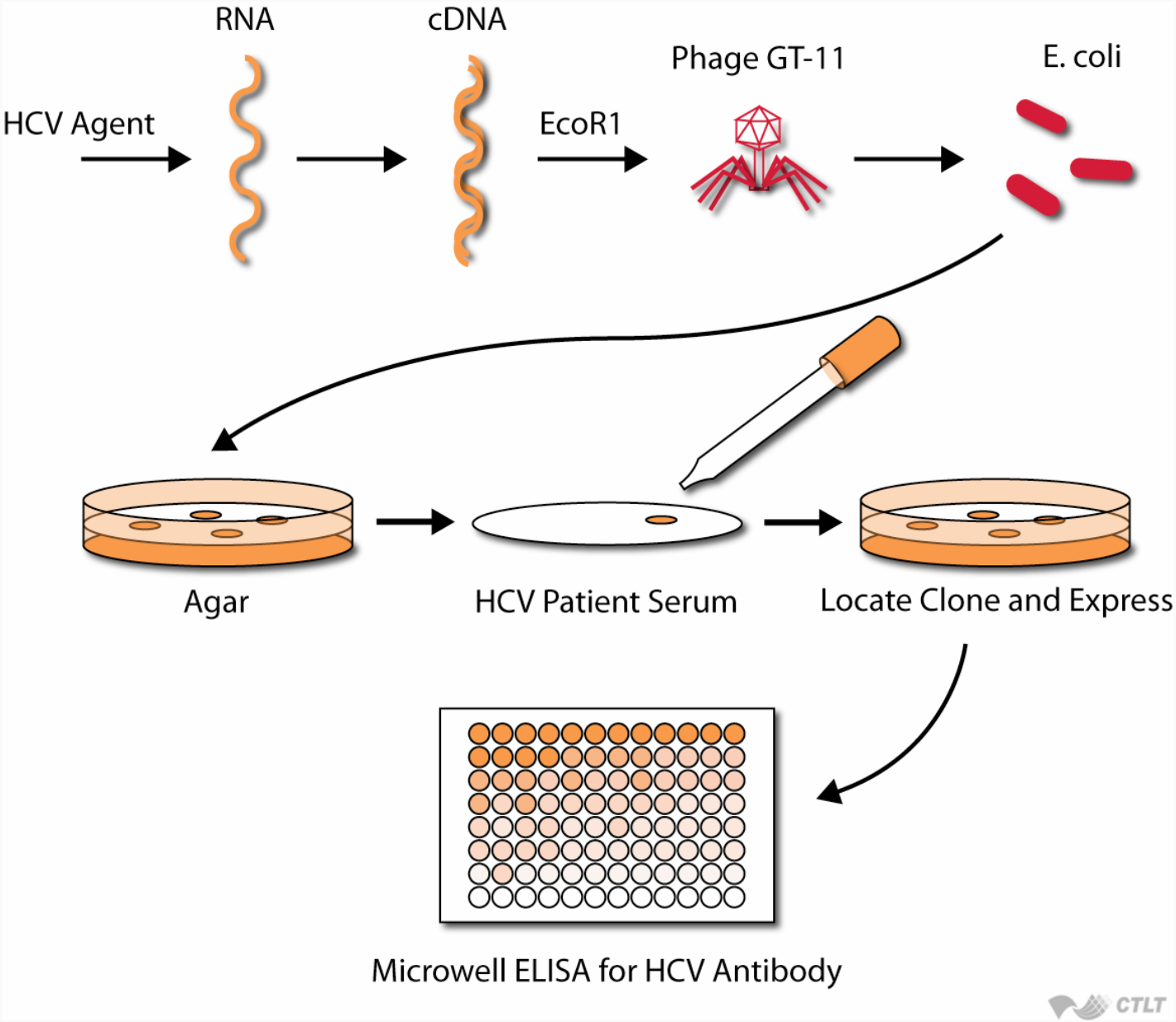
→ 3,200 ml Pool →



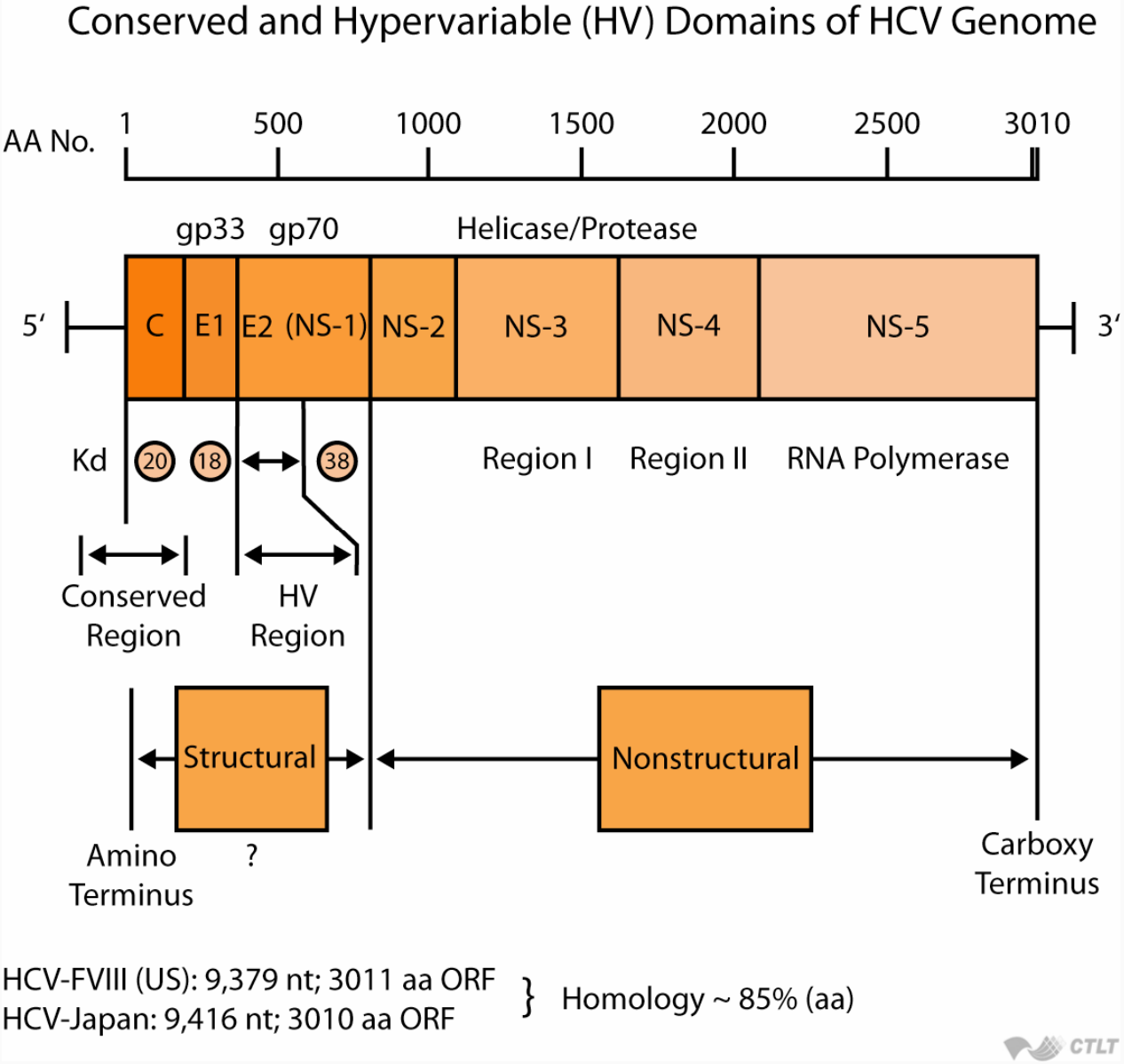
Pool → $\geq 10^6$ CID/ml → Dilute aliquots 4-6x in TENB, pH 8.0 buffer and pellet virus (assumes HCV has S20, w ≥ 150S and is most stable at alkaline pH)

Pelleted Virus → Resuspend → Extract TOTAL RNA (assumes virus has no 3′-polyA tail); Prepare cDNA, insert into λgt_{11}: screen

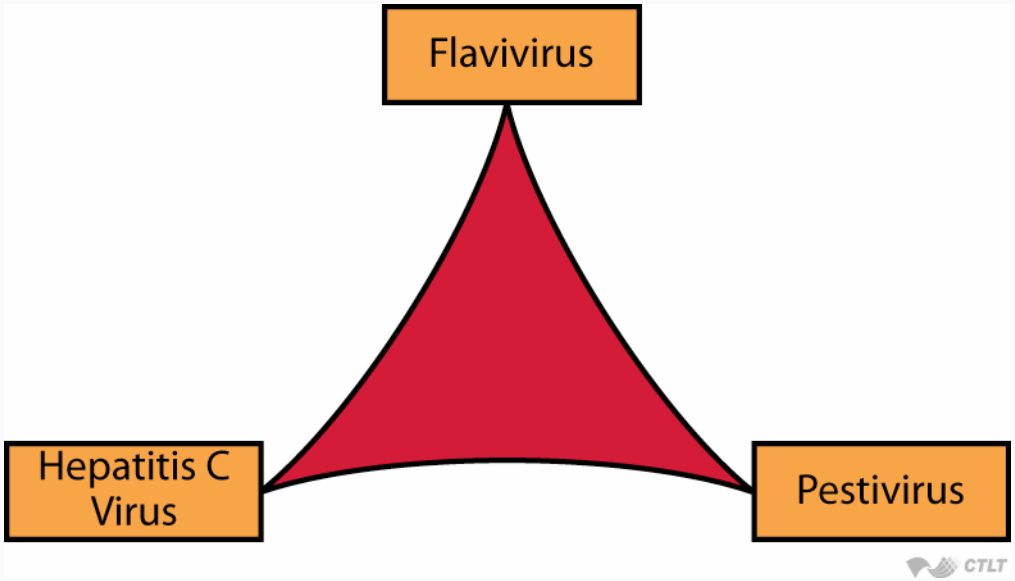
Molecular Cloning and Characterization



Conserved and HV Domains of HCV Genome



The Flaviviridae *Family*



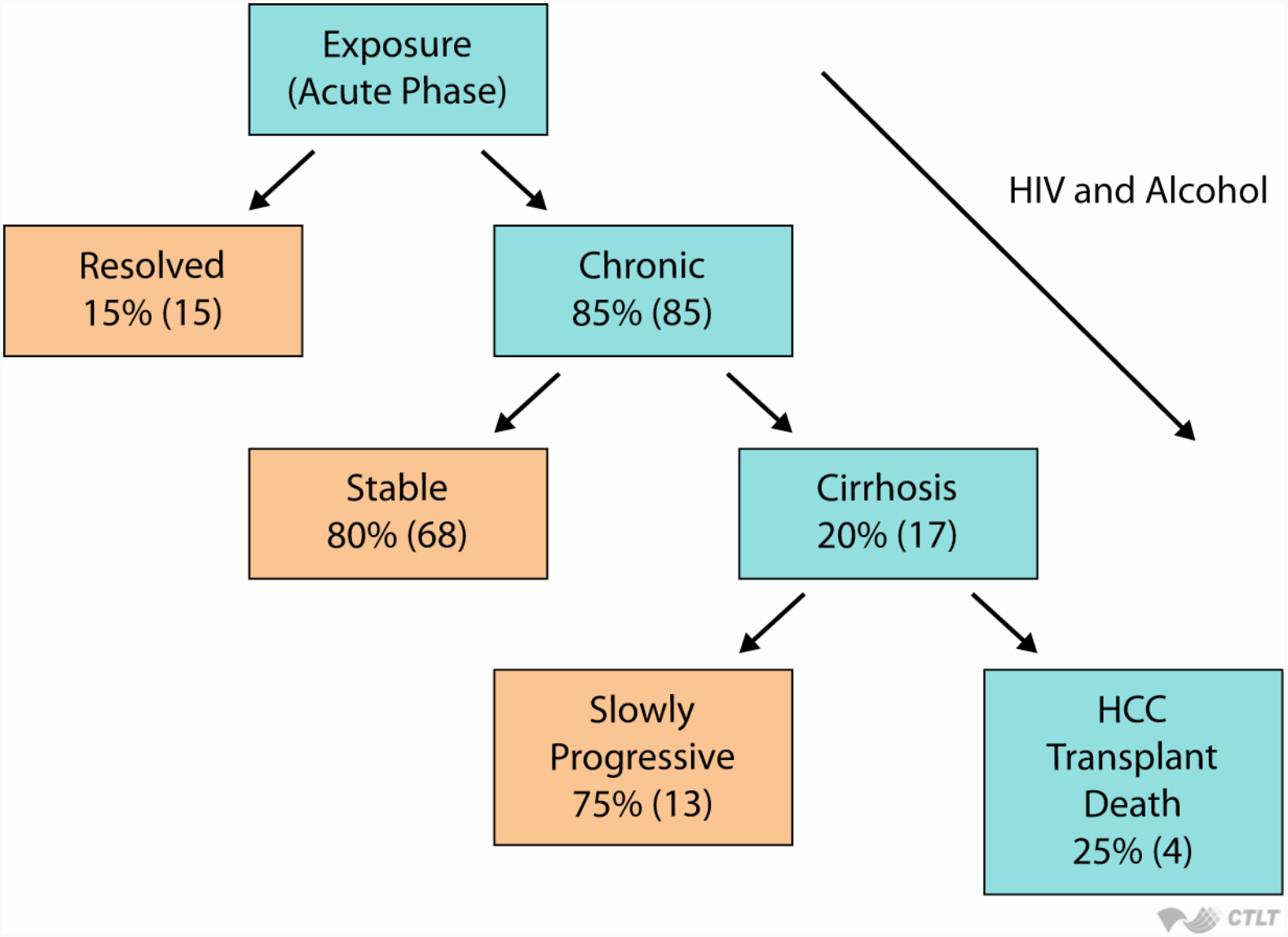
Flaviviruses and Pestiviruses

- Flaviviruses
  - Yellow fever virus
  - Dengue viruses
  - St. Louis encephalitis virus
  - Japanese B encephalitis virus
- Pestiviruses
  - Bovine viral diarrhea
  - Hog cholera virus
  - Border disease virus of sheep

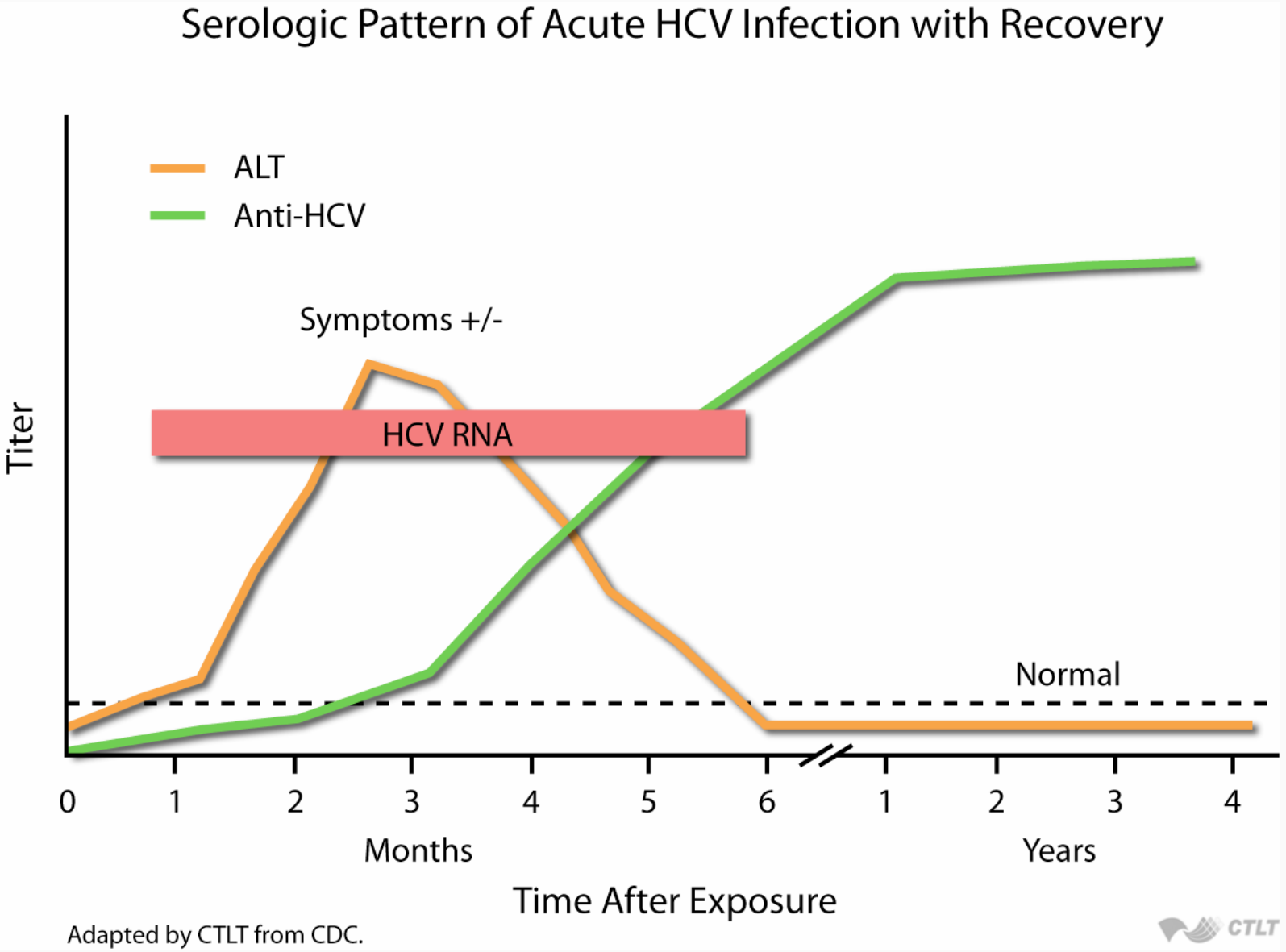
Features of Hepatitis C Virus Infection

Incubation period	Average: 6–7 weeks Range: 2–26 weeks
Acute illness (jaundice)	Mild (20% or less)
Case fatality rate	Low
Chronic infection	75–85%
Chronic hepatitis	70% (most asymptomatic)
Cirrhosis	10–20%
Mortality from CLD	1–5%

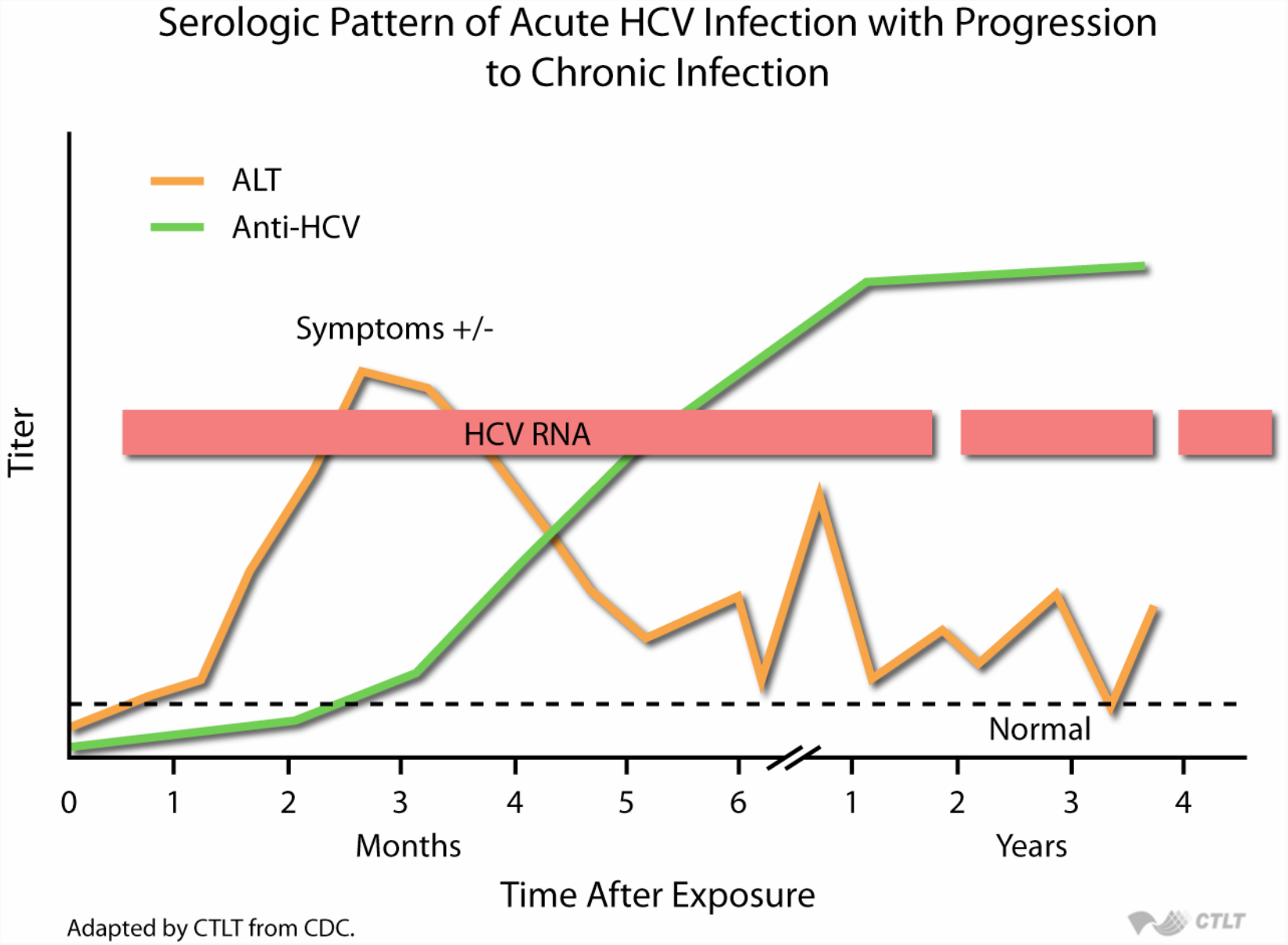
Natural History of HCV Infection



Serologic Pattern of Acute HCV Infection with Recovery



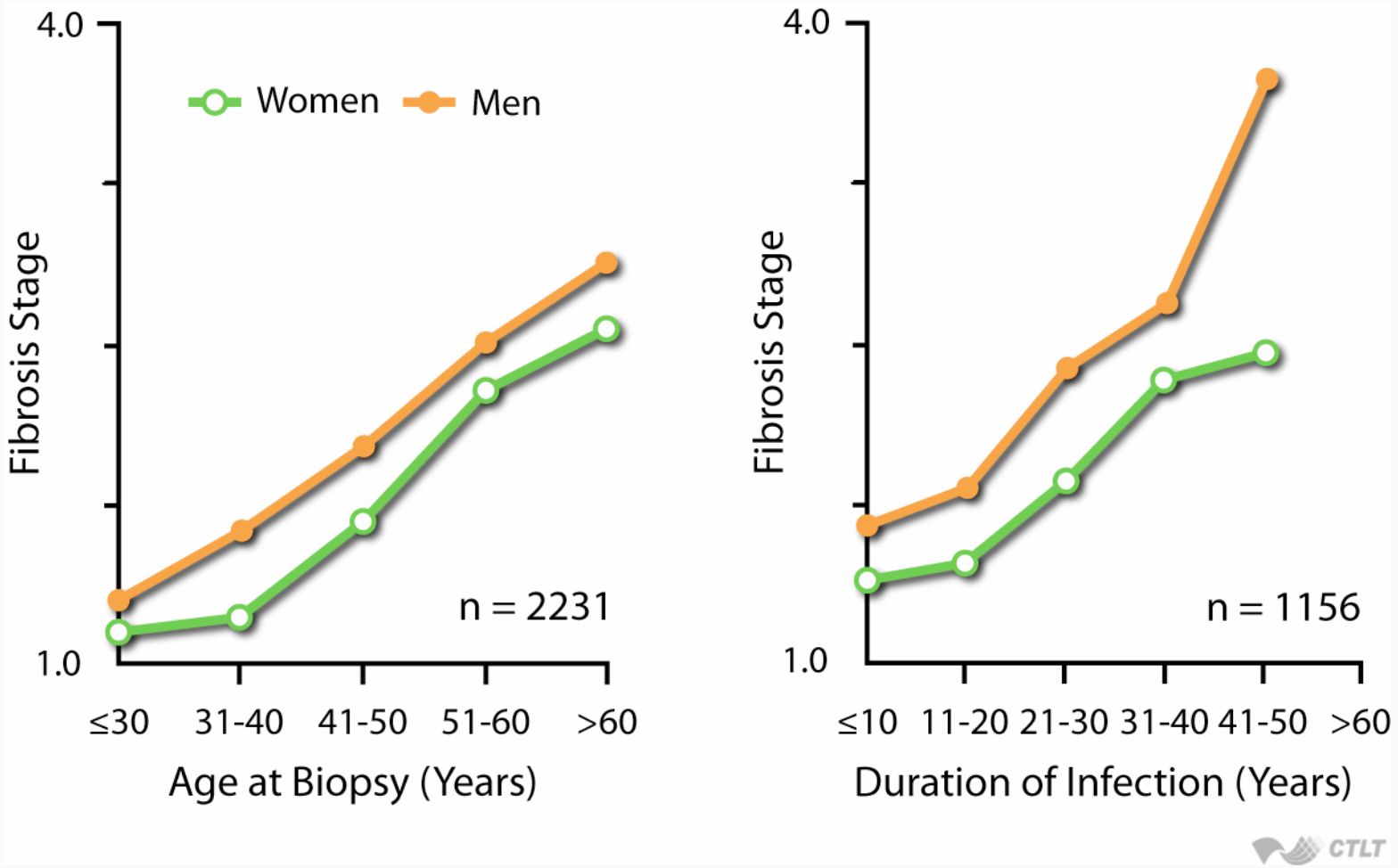
Serologic Pattern: Progression to Chronic Infection



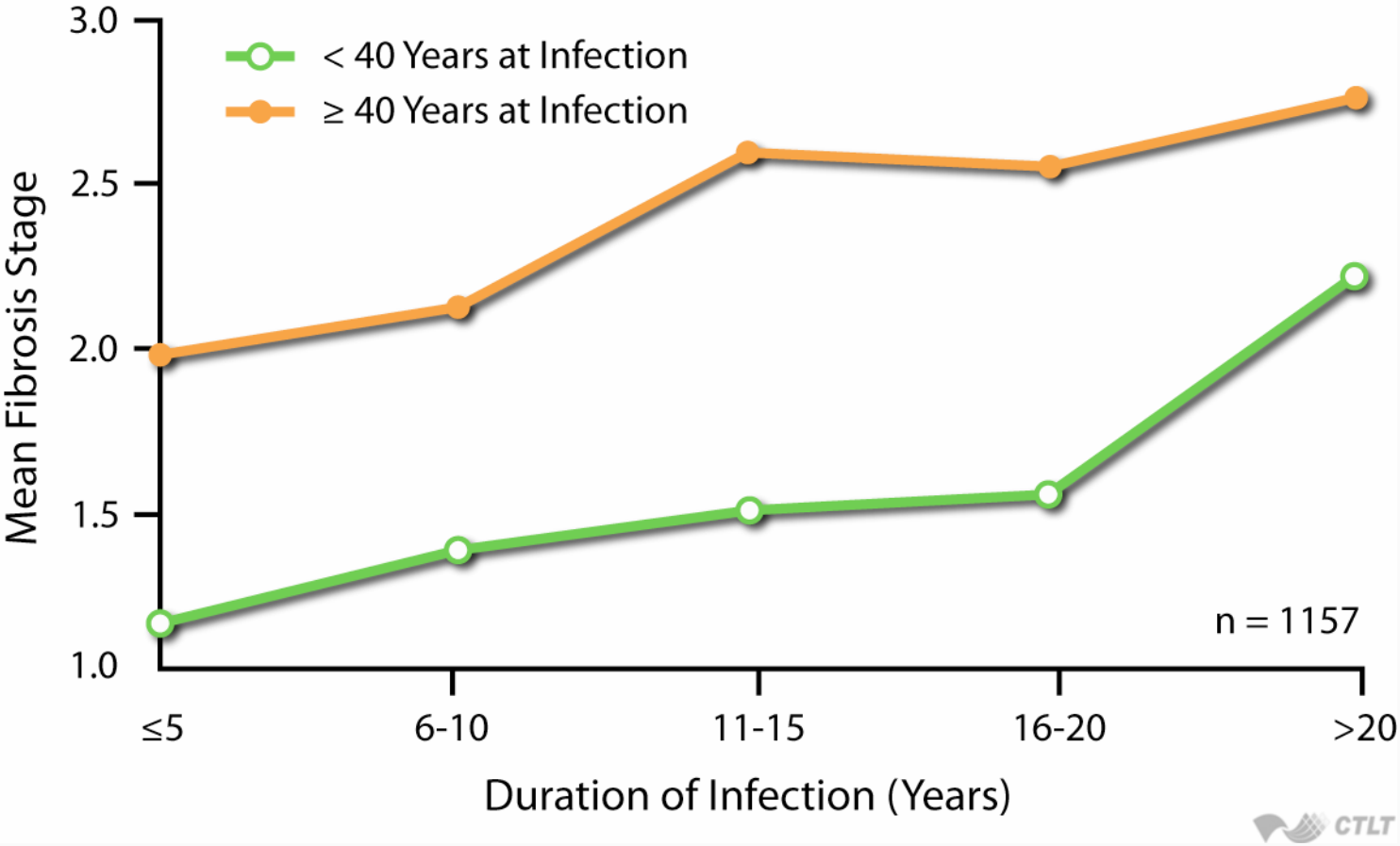
Chronic Hepatitis C

- Factors promoting progression or severity
  - Increased alcohol intake
  - Age >40 years at time of infection
  - HIV co-infection
  - Possible other
    - Male gender
    - Other co-infections (e.g., HBV)

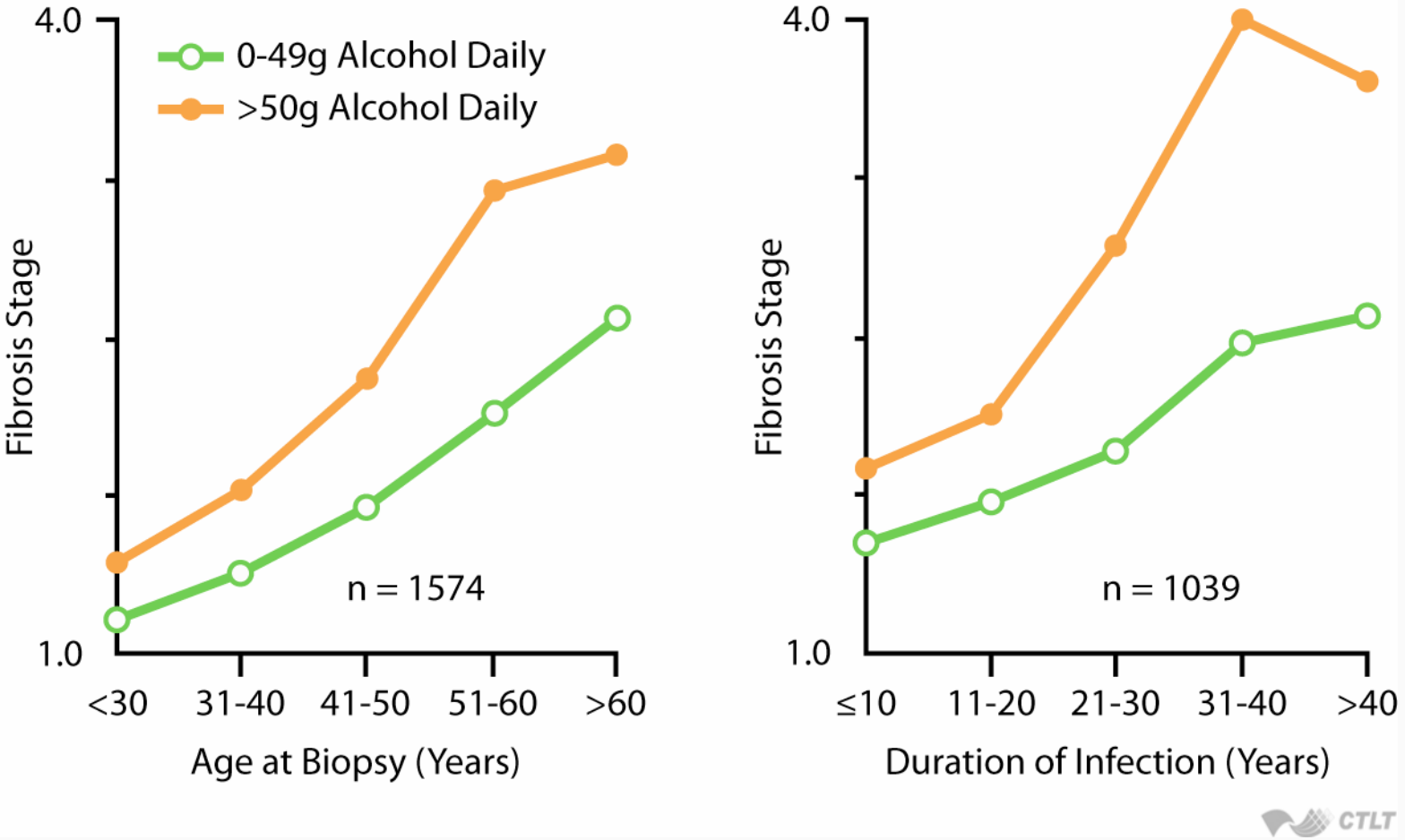
Fibrosis: Gender, Age at Biopsy, Duration of Infection



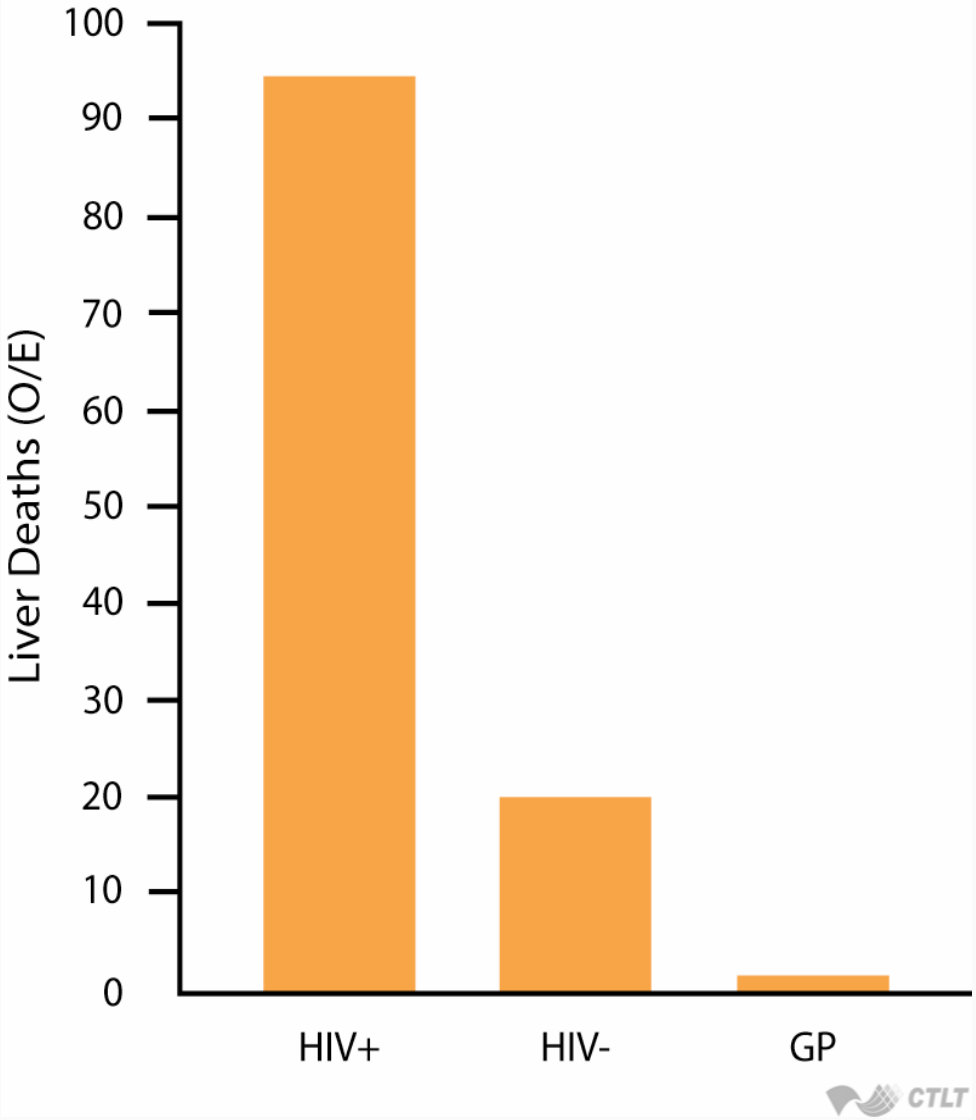
Fibrosis: Age at Infection, Duration of Infection



Fibrosis: Alcohol, Age at Biopsy, Duration of Infection



HCV and HIV: Liver-Related Mortality



- U.K. hemophilia population, 1985–1998
- Liver deaths
  - HIV- up 16.7-fold
  - HIV+ up 94.4-fold
- Risk up after 10 years

Source: Darby, S. C. (1997). *Lancet.*

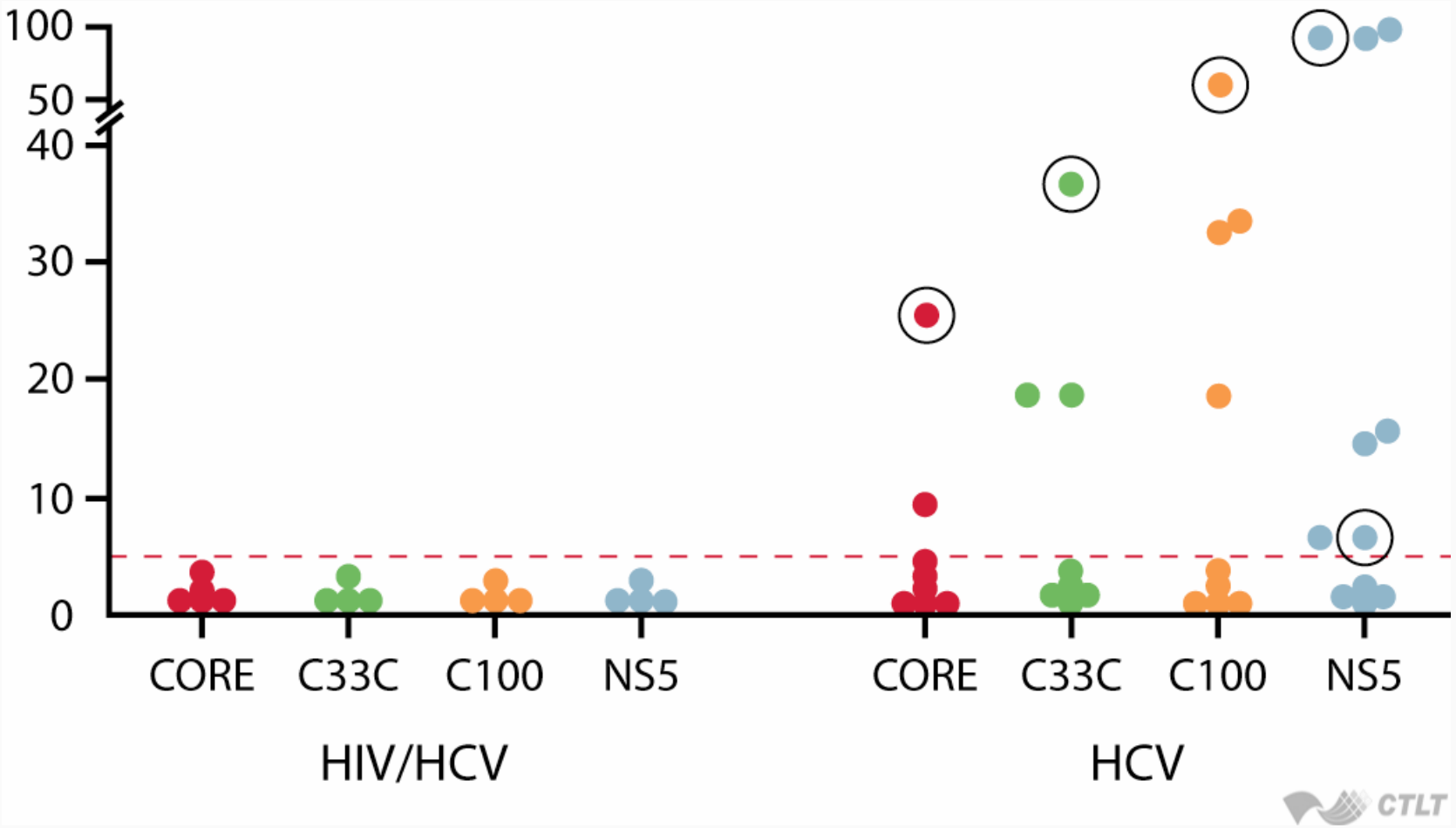
Interaction of HIV and Hepatitis C Virus Infection

1. Both are spread parenterally, so injection drug users and other risk populations (hemophiliacs) are often co-infected
2. HIV infection increases HCV viral load (0.5–1.0 log)
3. Effect of HCV on HIV viral load inconsistent
4. HIV accelerates development of liver fibrosis from HCV
5. Effect of HCV on HIV progression unclear (Swiss cohort = accelerated HIV)

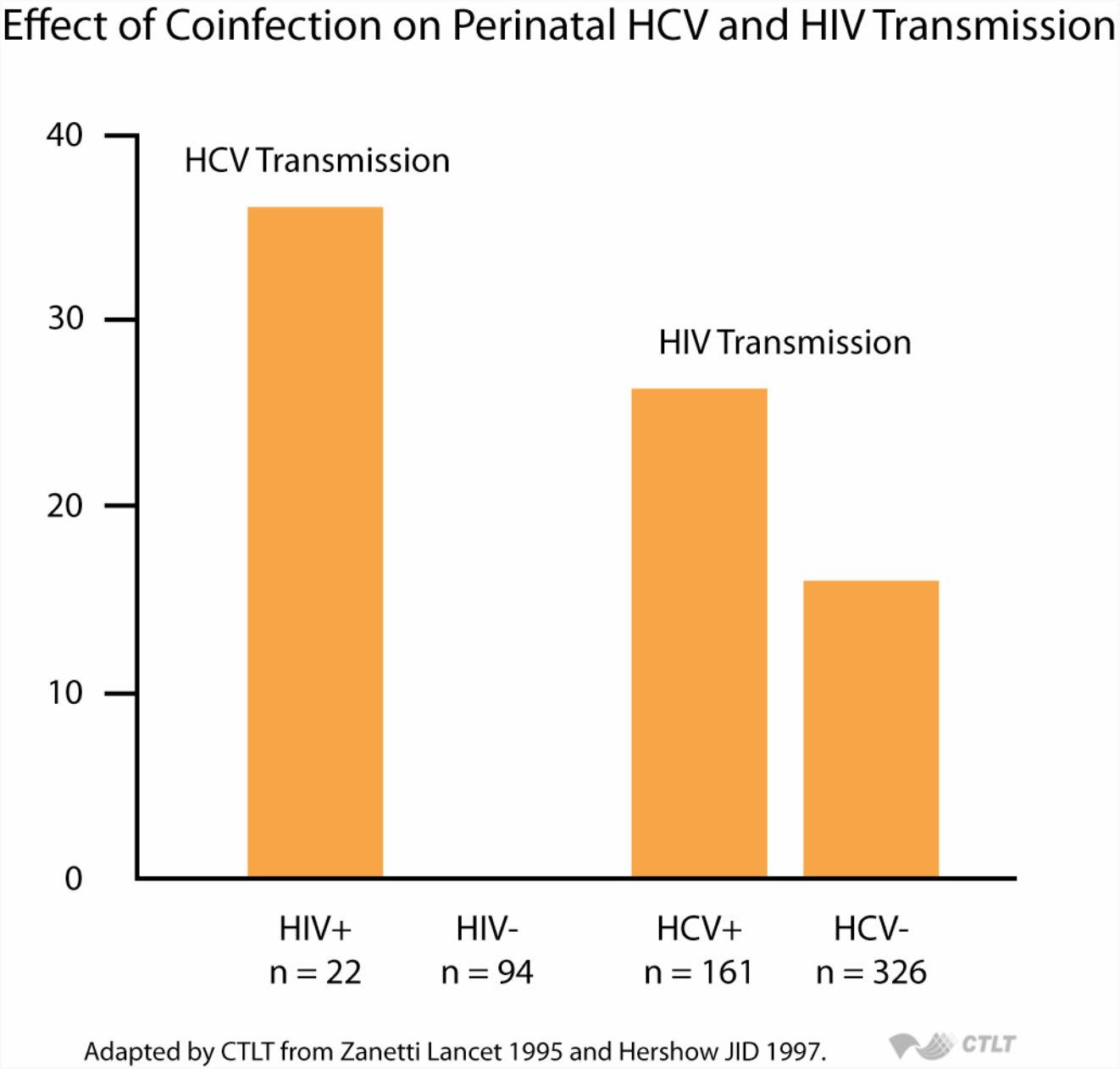
Interaction of HIV and Hepatitis C Virus Infection

6. Response to interferon/ribaviran therapy poorer in HIV-infected subjects
7. Drug interactions: ribaviran and AZT or d4T, ribaviran and DDI
8. HIV infection increases sexual and perinatal transmission of HCV
9. T cell immune responses to HCV decreased in HIV co-infected subjects (both CD8+ CTL and CD4+ proliferative responses)
10. T cell responses to HIV may be increased in HCV co-infected subjects
    - Lauer et al. (2002). *J Virol, 76,* 2817–2826.

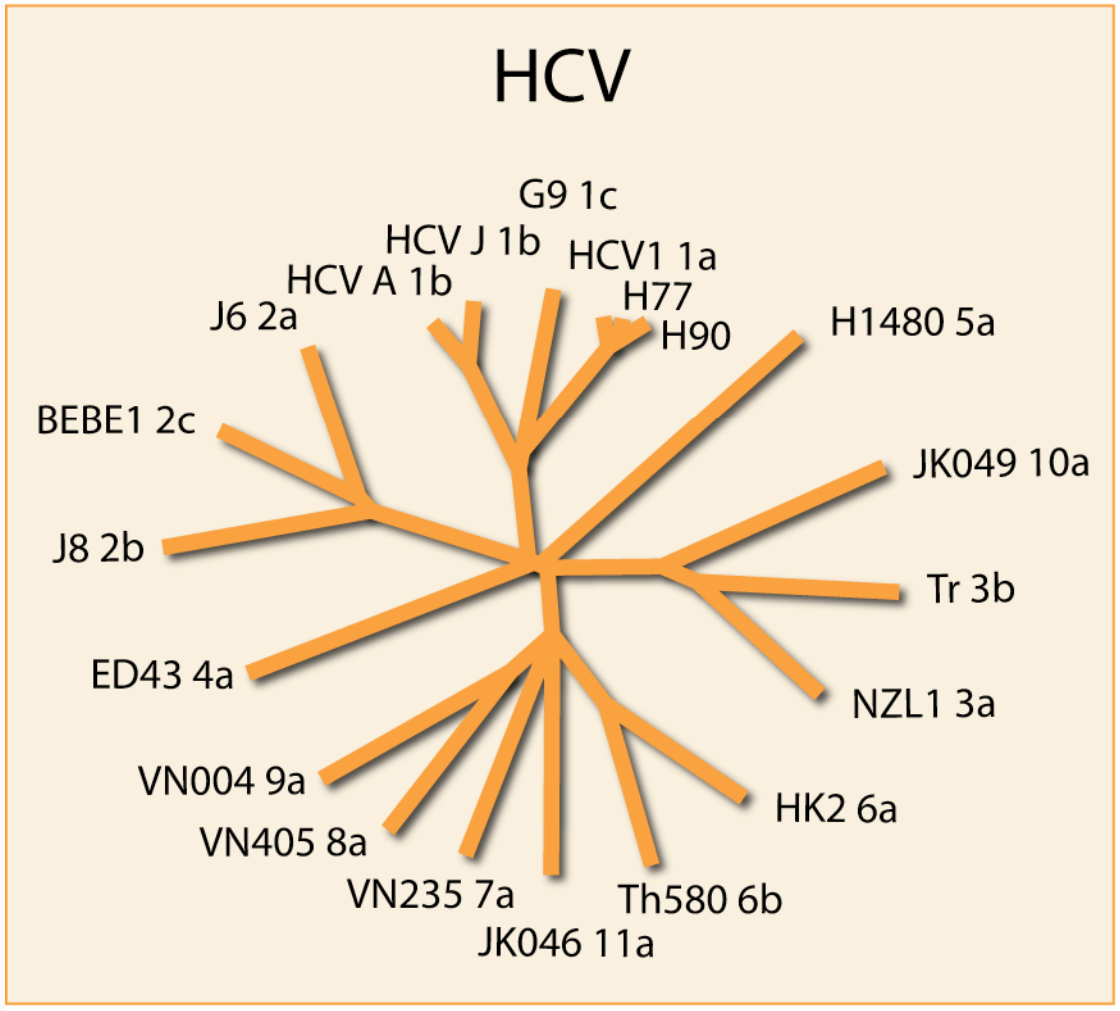
Studies of Cellular Immune Responses

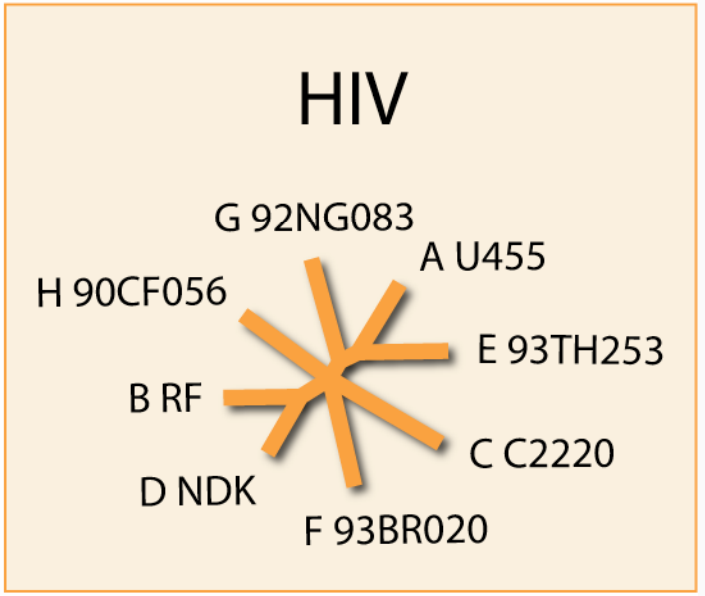


Co-Infection and Perinatal HCV and HIV Transmission



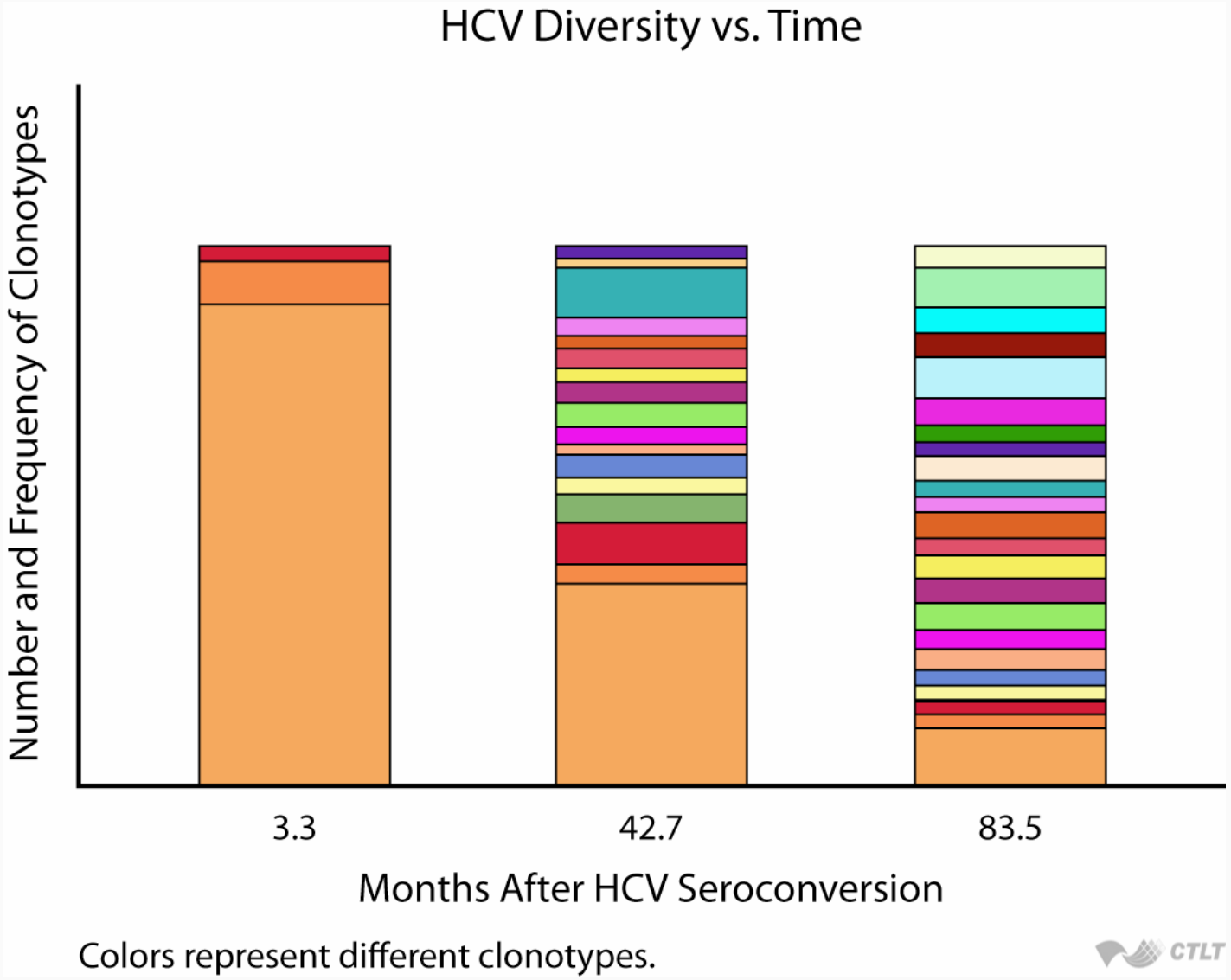
Genetic Diversity of Full Length HCV and HIV Isolates





Distance = 0.1

HCV Diversity vs. Time



Hepatitis C Virus Infection, United States

New infections (cases) per year 1985–1989 1998	 242,000 (42,000) 40,000 (6,500)
Deaths from acute liver failure	Rare
Persons ever infected (1.8%)	3.9 million (3.1–4.8)*
Persons with chronic infection	2.7 million (2.4–3.0)*
Of chronic liver disease—HCV-related	40–60%
Deaths from chronic disease per year	8,000–10,000

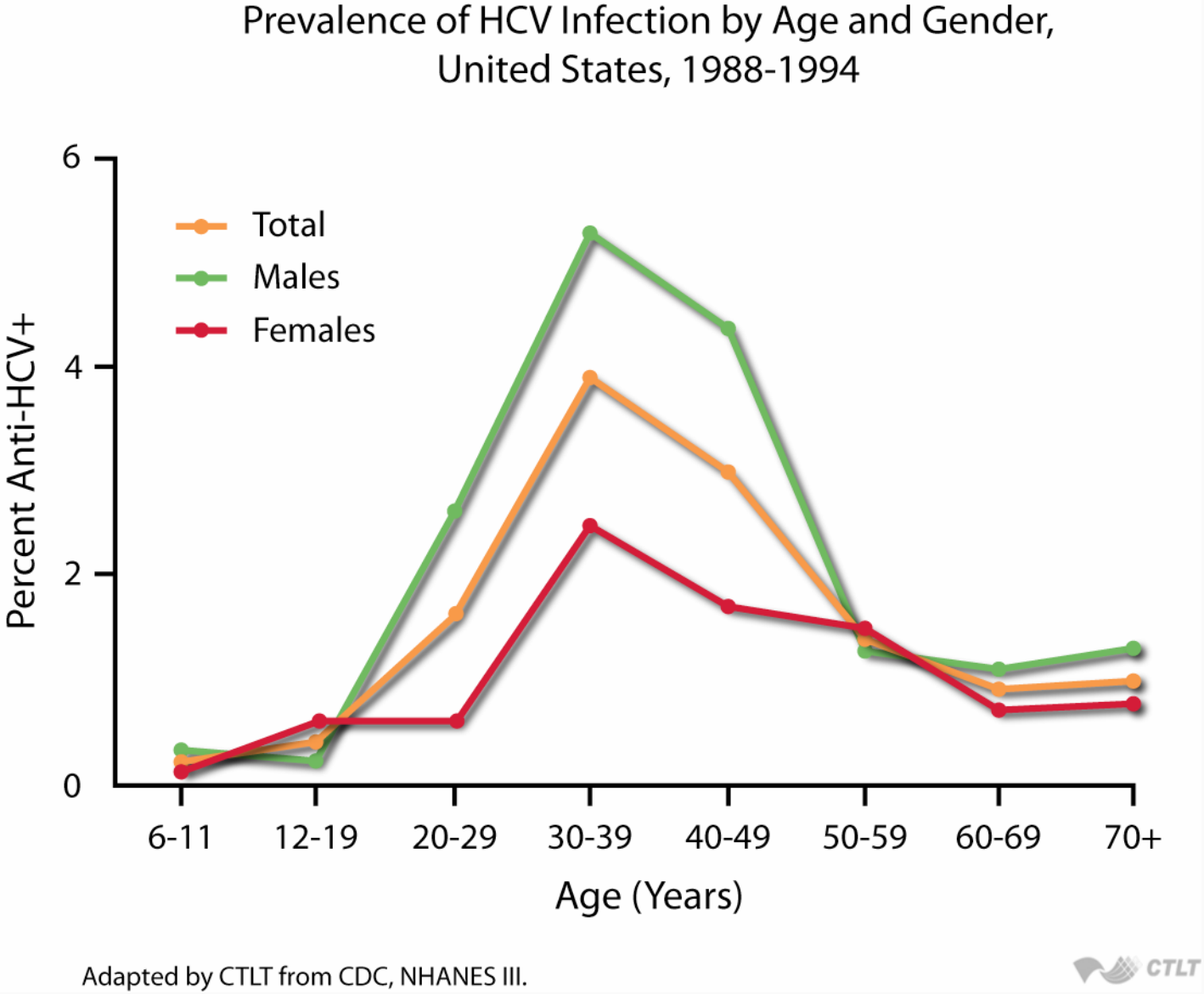
*95% confidence interval

Prevalence of HCV Infection, United States, 1988–1994

Group	Anti-HCV positive	Est. infections millions (95% CI)	Percent of infections
Total	1.8%	3.9 (3.1–4.8)	100%
Race/ethnicity			
White	1.5%	2.4 (1.8–3.1)	61%
Black	3.2%	0.8 (0.6–1.0)	20%
Mexican American	2.1%	0.3 (0.2–0.3)	7%
Other	2.9%	0.5 (0.3–1.0)	13%

Source: (1999). *N Engl J Med, 341*, 556–562.

Prevalence of HCV Infection by Age and Gender, U.S.



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Section B

Transmission of HCV

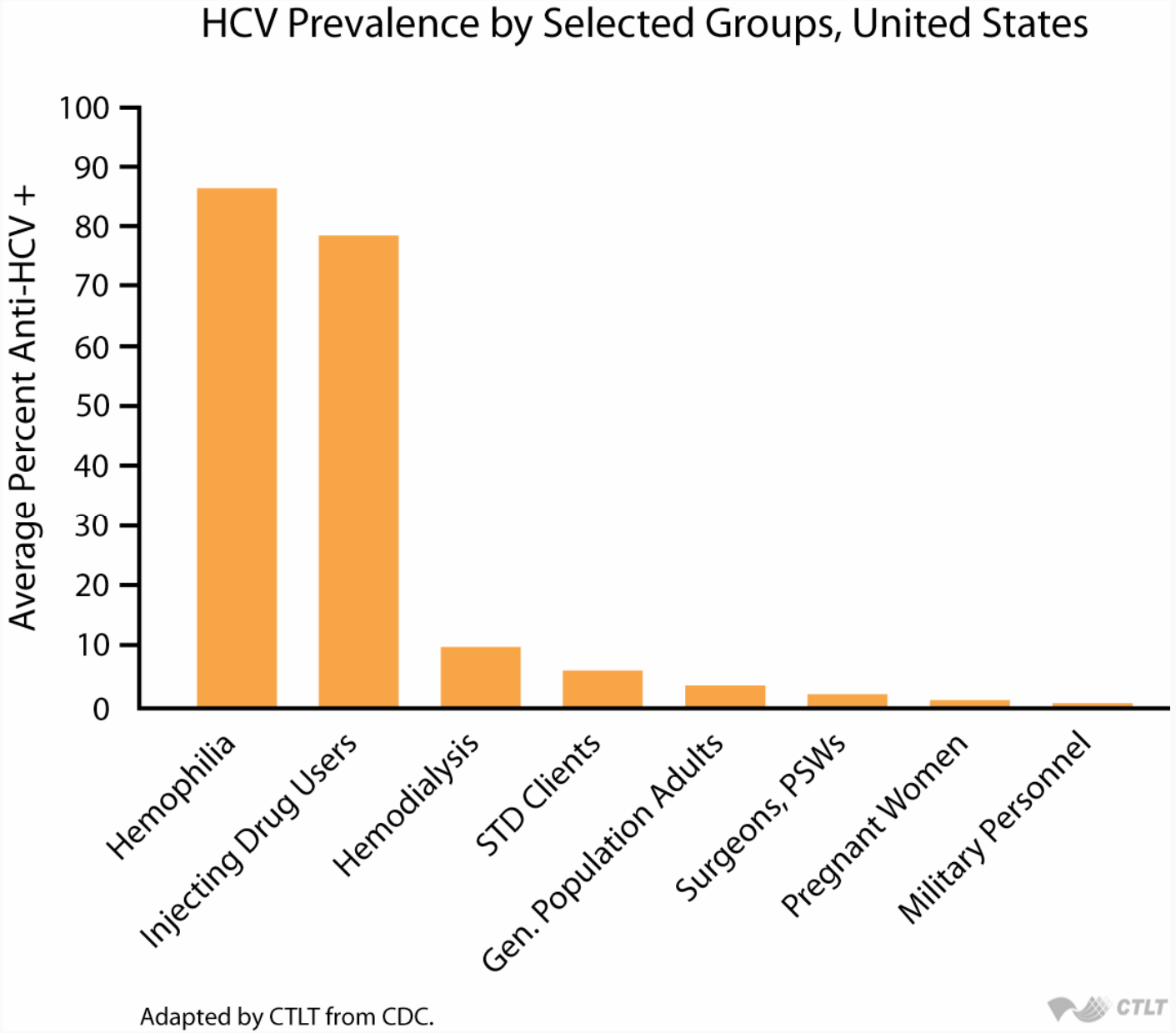
Transmission of HCV

- Percutaneous
  - Injecting drug use
  - Clotting factors before viral inactivation
  - Transfusion, transplant from infected donor
  - Therapeutic (contaminated equipment, unsafe injection practices)
  - Occupational (needlestick)
- Permucosal
  - Perinatal
  - Sexual

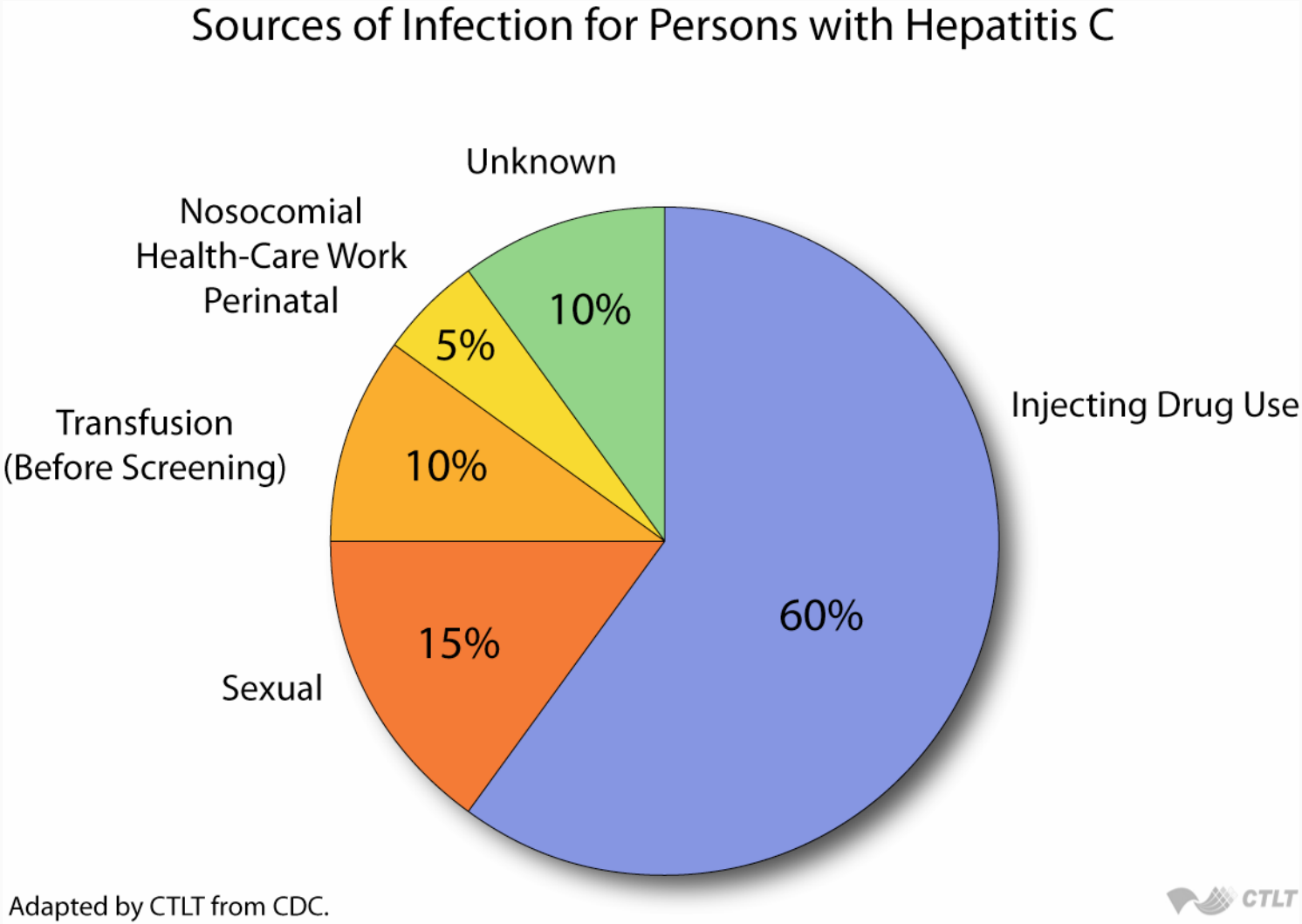
Injecting Drug Use and HCV Infection

- Highly efficient mode of transmission
- Rapidly acquired after initiation
- Four times more common than HIV
- Prevalence of 50–90% after five years
- Predominant risk factor in low-prevalence countries

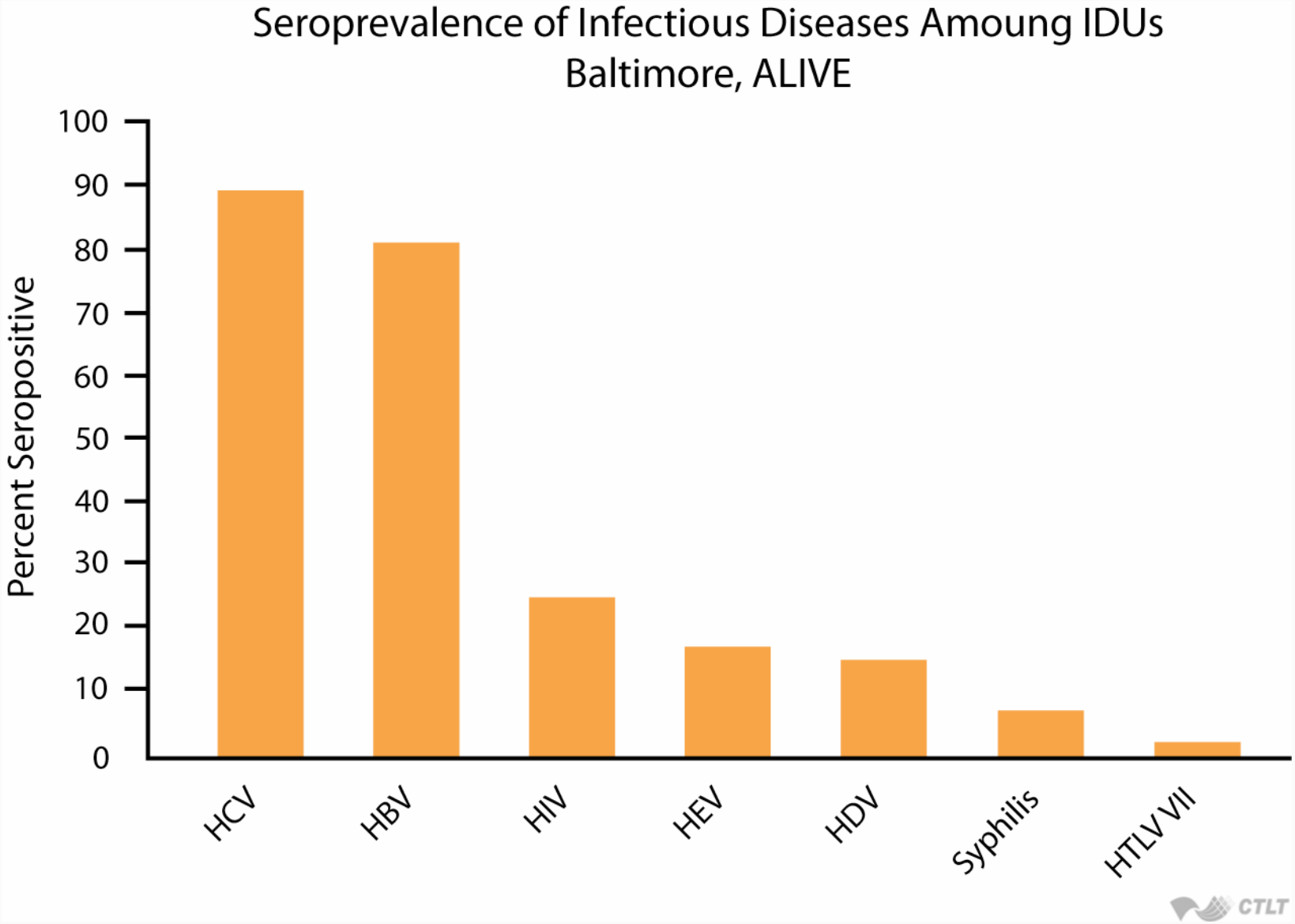
HCV Prevalence by Selected Groups, United States



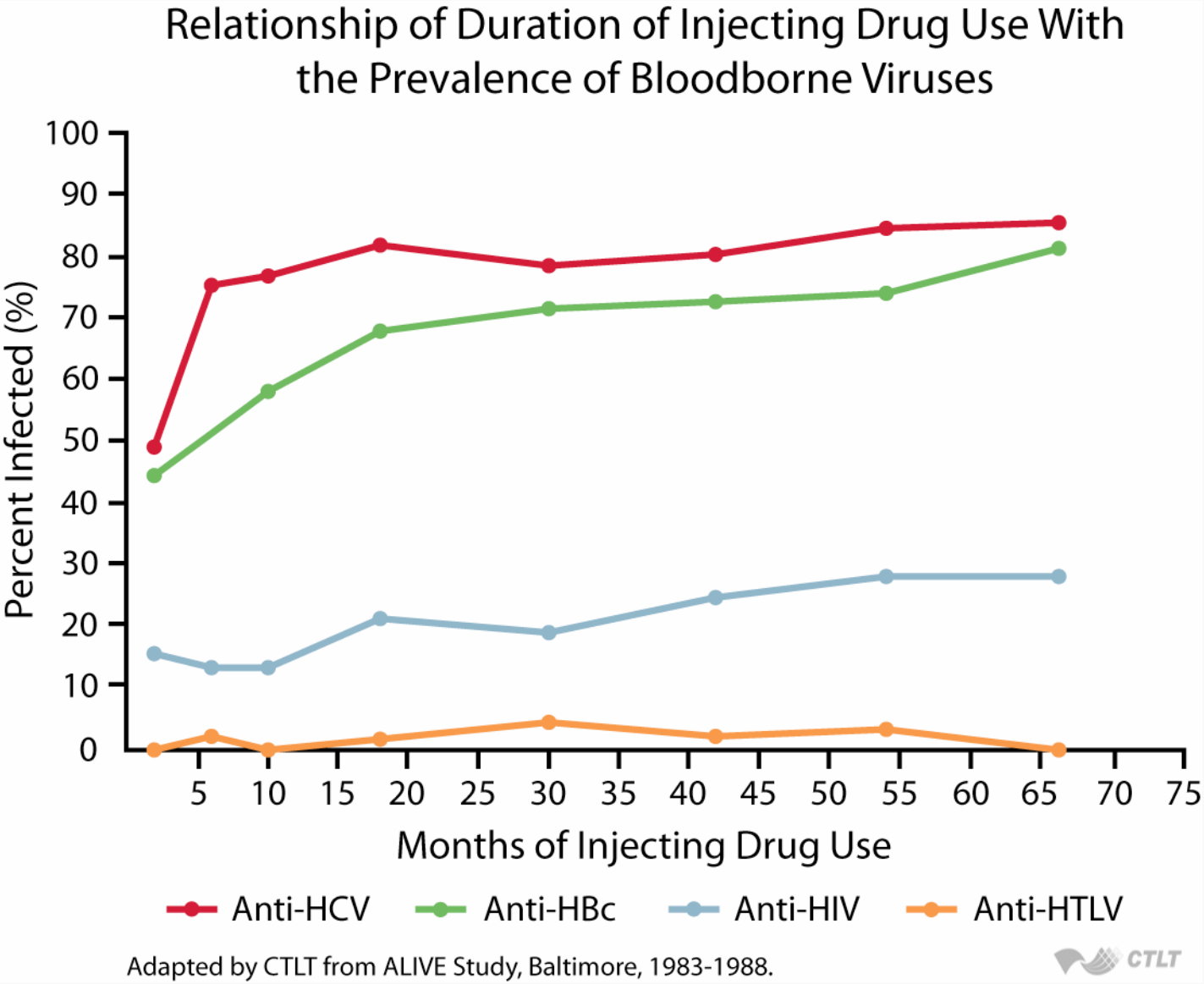
Sources of of Infection for Persons with Hepatitis C



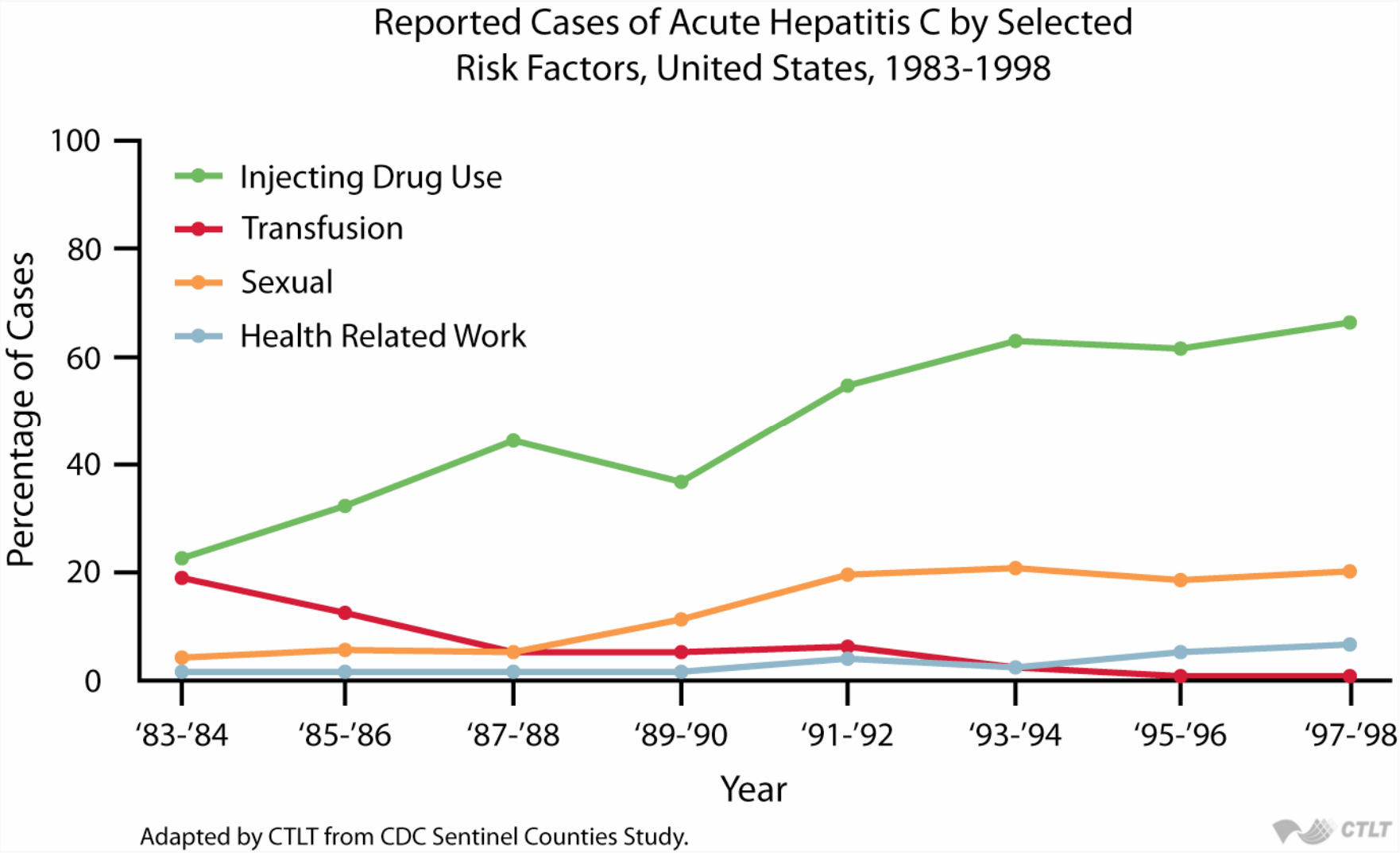
Seroprevalence of Infectious Diseases among IDUs



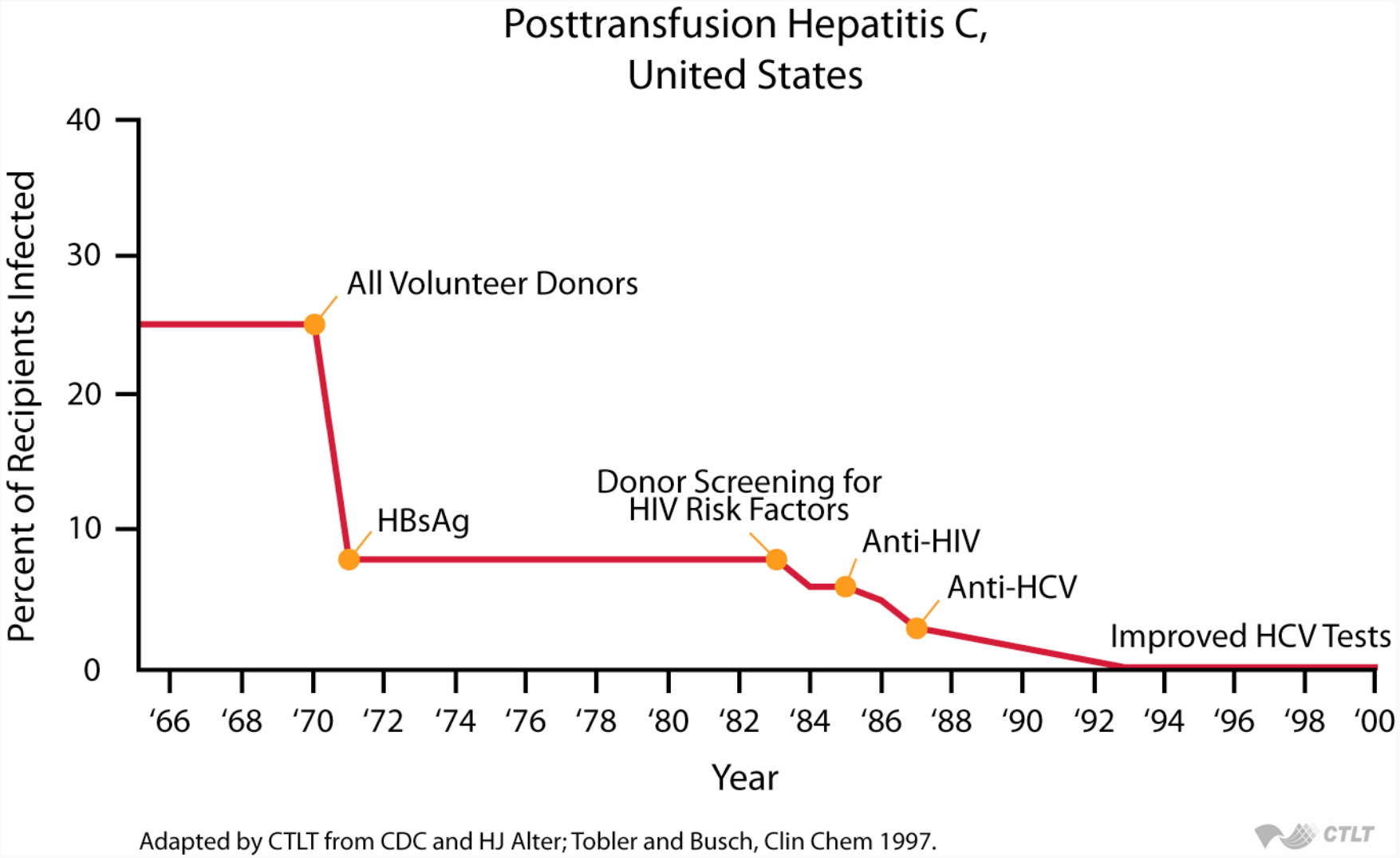
Duration of Injecting Drug Use and Seroprevalence



Reported Acute Cases by Selected Risk Factors

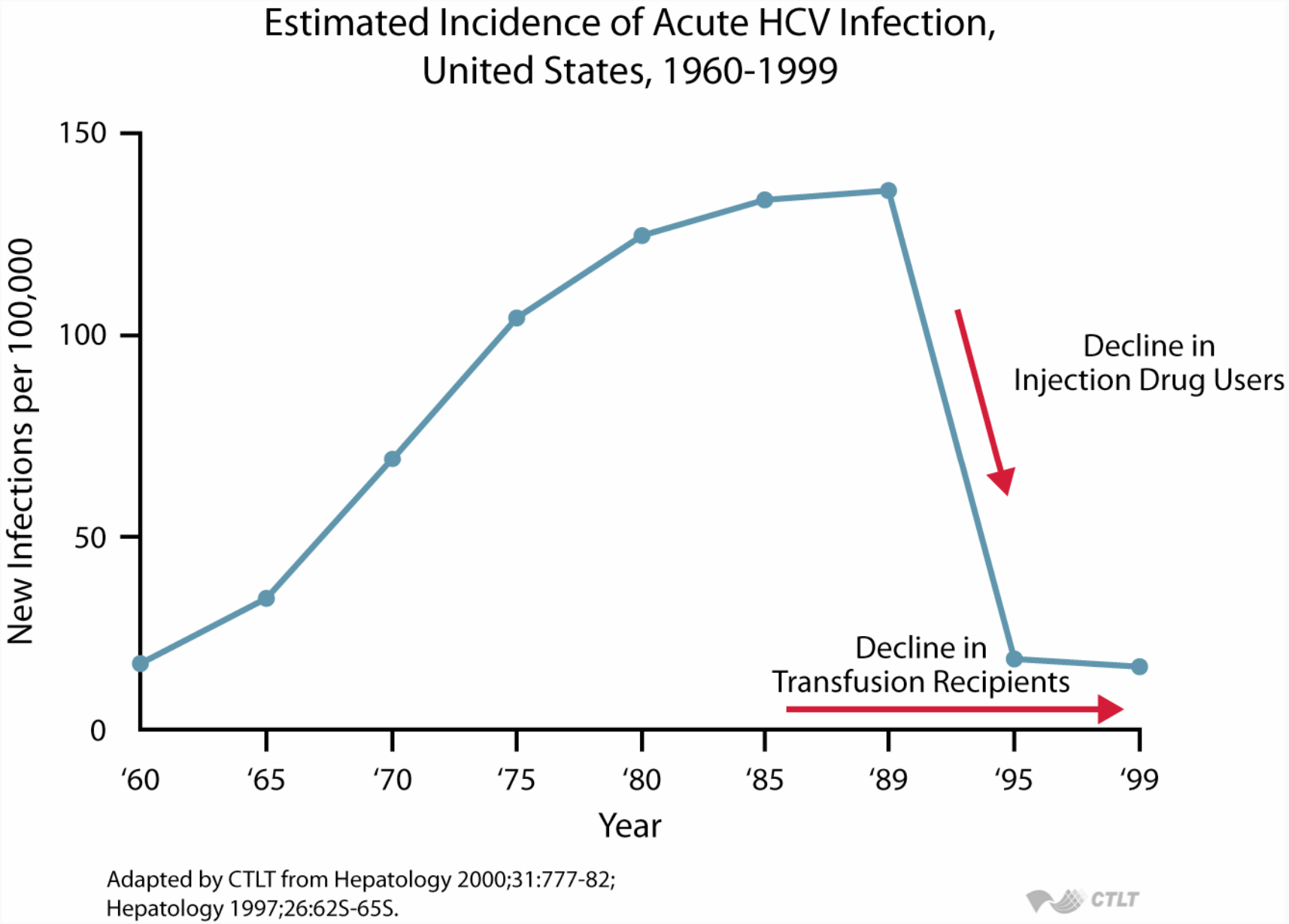


Posttransfusion Hepatitis C, United States



Adapted by CTLT from CDC and HJ Alter; Tobler and Busch, Clin Chem 1997.

Estimated Incidence of Acute HCV Infection, U.S.



Adapted by CTLT from Hepatology 2000;31:777-82;
Hepatology 1997;26:62S-65S.

CTLT

Nosocomial Transmission of HCV

- Recognized primarily in context of outbreaks
- Contaminated equipment
  - Hemodialysis*
  - Endoscopy
- Unsafe injection practices
  - Plasmapheresis,* phlebotomy
  - Multiple-dose medication vials
  - Therapeutic injections

*Reported in the U.S.

Occupational Transmission of HCV

- Inefficiently transmitted by occupational exposures
- Average incidence 1.8% following needle stick from HCV-positive source
  - Associated with hollow-bore needles
- Case reports of transmission from blood splash to eye
  - No reports of transmission from skin exposures to blood
- Prevalence 1–2% among health care workers
  - Lower than adults in the general population
  - 10 times lower than for HBV infection
- Presence of recognized risk factor does not necessarily equate with “increased risk”

HCW-to-Patient Transmission of HCV

- Rare
  - In the U.S., none related to performing invasive procedures
- Most appear related to HCW substance abuse
  - Reuse of needles or sharing narcotics used for self-injection
  - Reported mechanism for transmission of other bloodborne pathogens from some HCWs
- No restrictions routinely recommended for HCV-infected HCWs

Perinatal Transmission of HCV

- Transmission only from women HCV-RNA positive at delivery
  - Average rate of infection is 6%
  - Higher (17%) if woman co-infected with HIV
  - Role of viral titer unclear
- No association with
  - Delivery method
  - Breastfeeding
- Infected infants do well
  - Severe hepatitis is rare

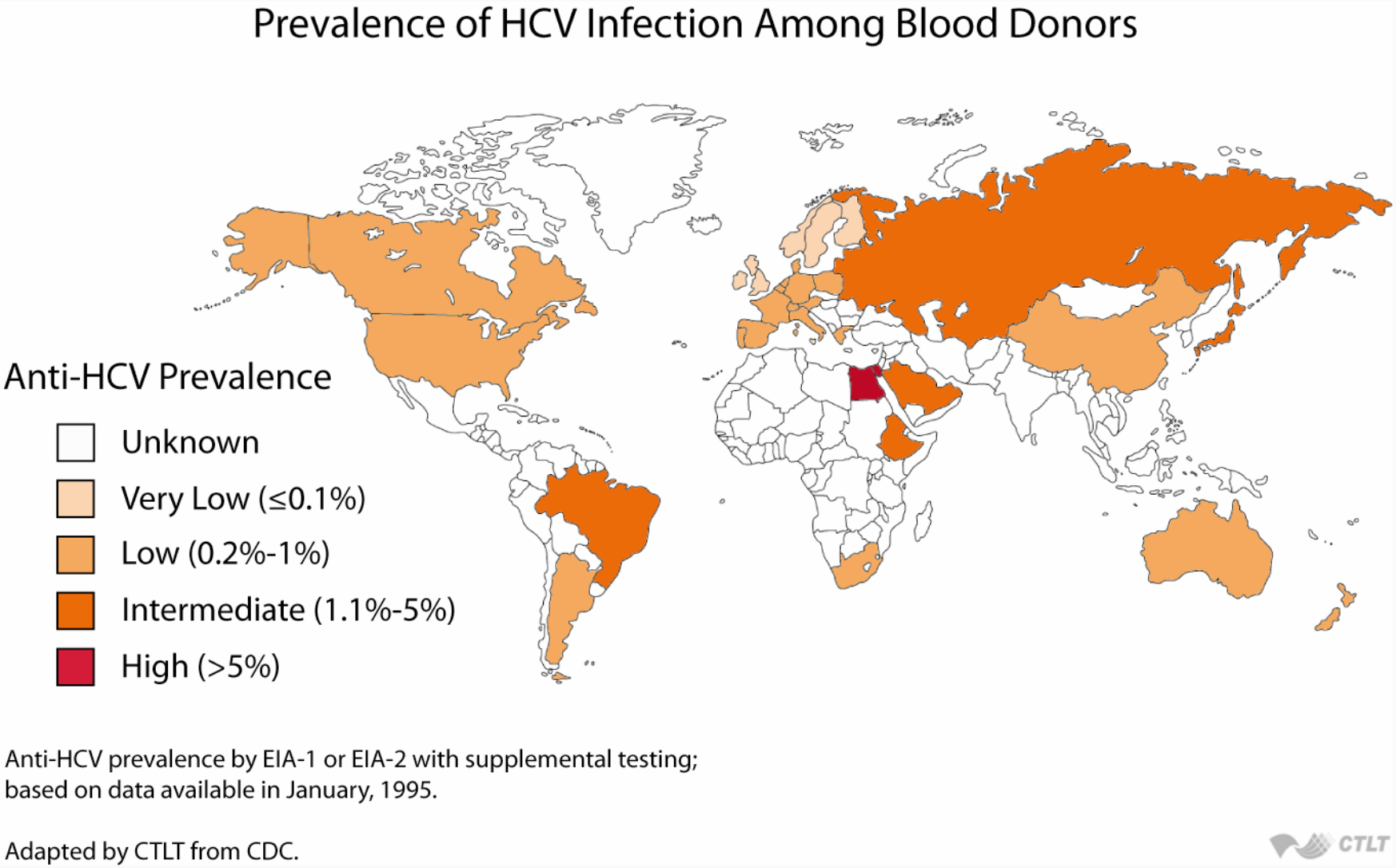
Sexual Transmission of HCV

- Occurs, but efficiency is low
  - Rare between long-term steady partners
  - Factors that facilitate transmission between partners unknown
- Accounts for 15–20% of acute and chronic infections in the United States
  - Sex is a common behavior
  - Large chronic reservoir provides multiple opportunities for exposure to potentially infectious partners

Geographic and Temporal Differences

- Geographic and Temporal differences in the epidemiology of HCV infection
  - HCV infection is endemic in most parts of the world
  - Substantial differences in endemicity of HCV infection
    - Related to frequency and extent to which various risk factors contributed to transmission

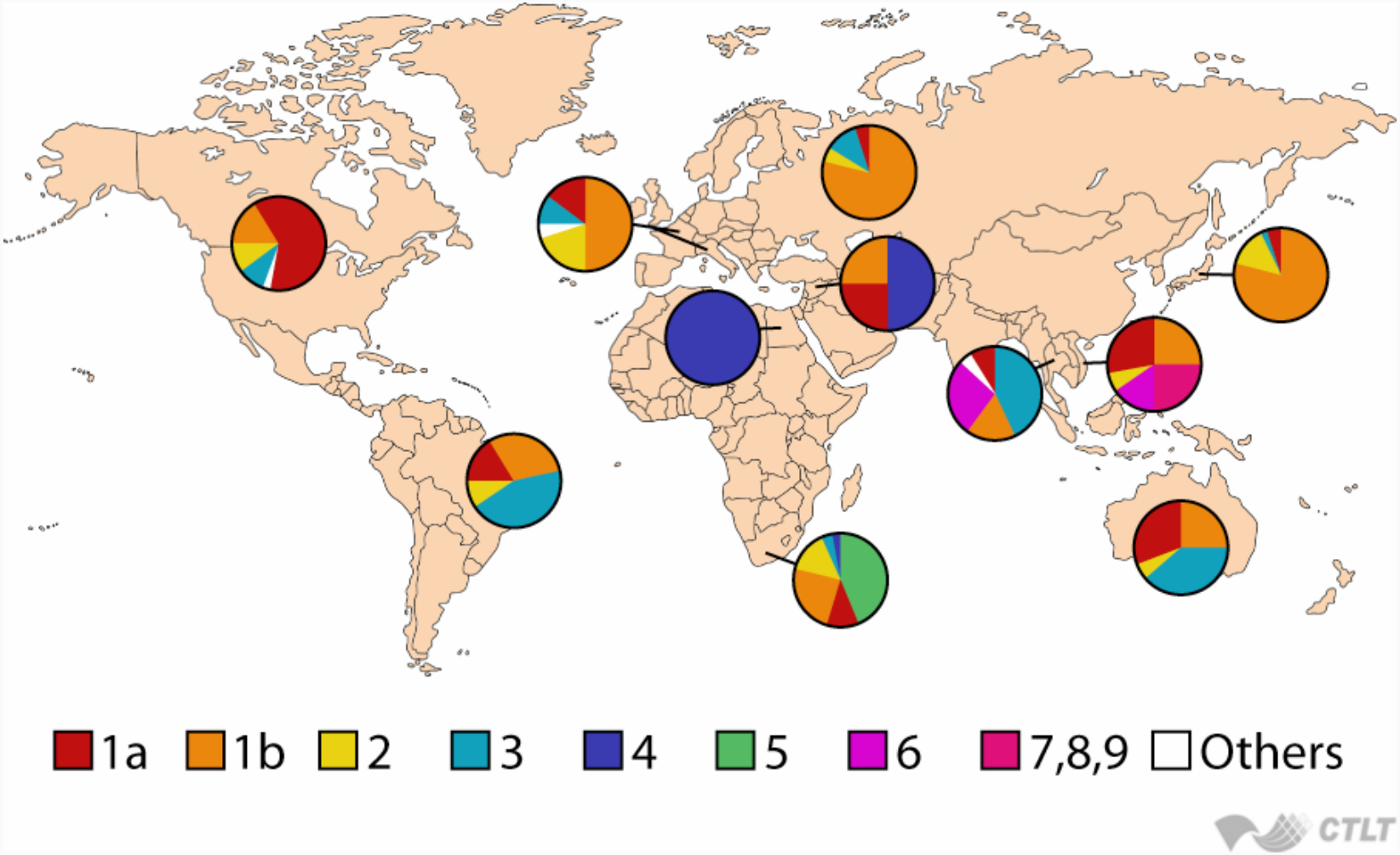
Prevalence of HCV Infection among Blood Donors



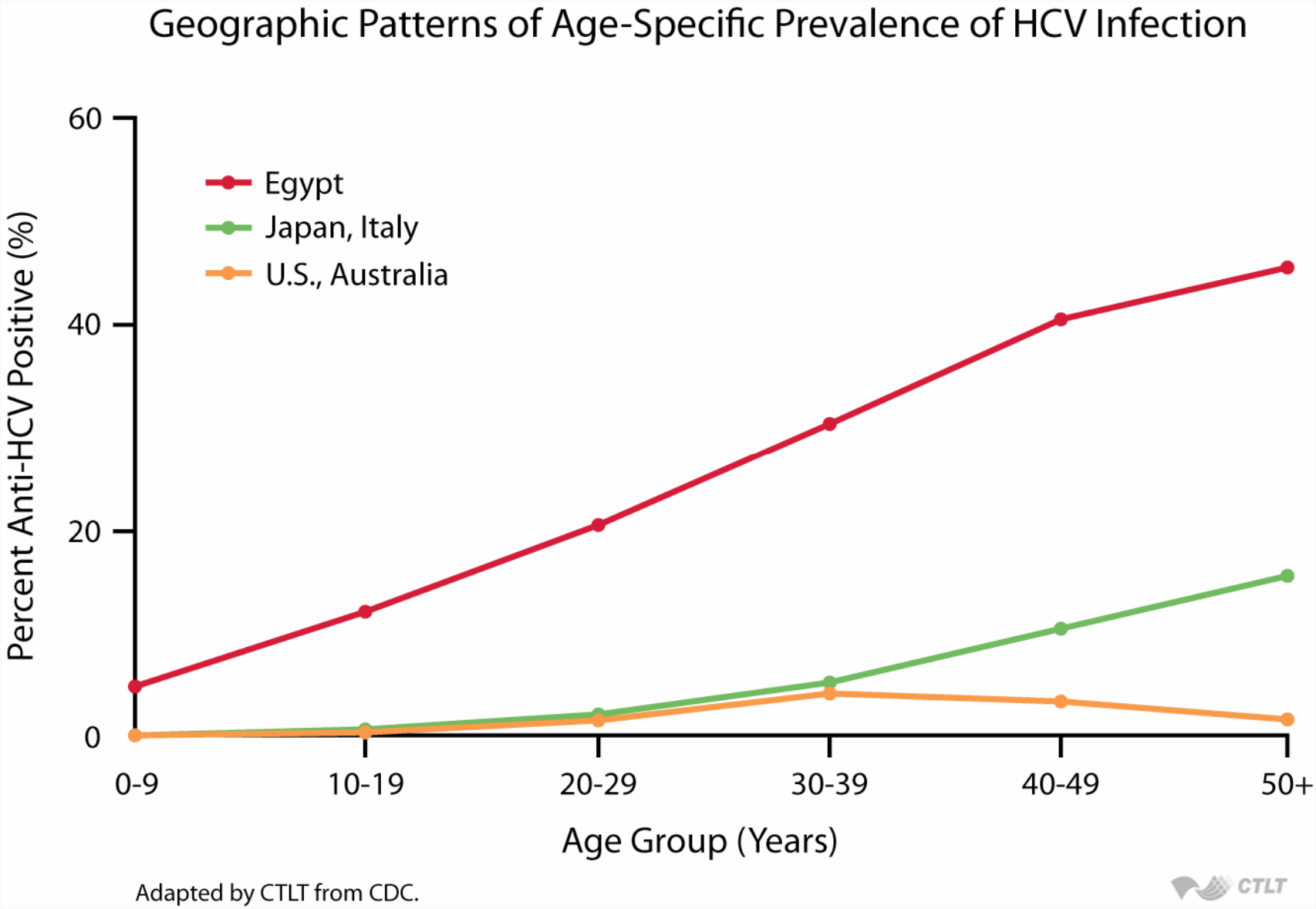
Anti-HCV prevalence by EIA-1 or EIA-2 with supplemental testing; based on data available in January, 1995.

Adapted by CTLT from CDC.

Distributions of Hepatitis C Genotypes



Geographic Patterns of Age-Specific Prevalence of HCV



HCV Infection Related to Therapeutic Injections, Egypt

- HCV infection related to therapeutic injections for schistosomiasis, Egypt

Blood donors	HCV-positive	HCV-negative	OR (95% CI)
Injection therapy	36%	7%	8.9 (2.4–33.5)
Village population	Inject	No inject	PR (95% CI)
HCV-positive	63%	23%	2.8 (2.5–3.2)

Source: Darwish et al. (1993). *Am J Trop Med Hyg;* Abdel-Aziz et al. (2000). Hepatology.

Health Care Related HCV Transmission

- Blood transfusion from unscreened donors
  - Including plasma-derived products not inactivated
- Unsafe injection practices
  - Inadequate sterilization of reusable needles and syringes
  - Sharing of disposable needles and syringes
- Contaminated equipment
  - Inadequate cleaning and disinfection
    - In health care settings
    - Alternative medicine practices, rituals

Unsafe Injections and HCV Infection

- Moderate endemic countries

Percent history reused needles/syringes

Country	HCV-positive	HCV-negative	OR (95% CI)
Italy	63% 89% 76%	31% 53% 72%	3.8 (2.7, 5.3) 7.0 (4.4, 11.2) 1.2 (0.6, 2.5)
Taiwan	26%	8%	4.2 (1.2, 14.5)
Pakistan (≥5 per year)	36%	6%	8.2 (1.9, 41.4)

HCV Infections Attributable to Unsafe Injections

- Proportion of HCV infections attributable to unsafe injections, case-control studies

Country (author)	Year	Age	Pop. attrib. fraction
Taiwan (Ho) (Chen) (Sun)	 1993 1990–1994 1990	 Children Adults Adults	 84%* 20%* 57%
Pakistan (Luby)	1994–1995	All	51%*
Egypt (El Sakka)	1996–1997	All	88%*

*Calculated from data provided by authors

Source: Hutin, Yvan. WHO.

Alternative Medicine and HCV Infection

Acupuncture

Country	HCV-positive	HCV-negative
Cross-sectional Japan	62%* 20%	26% 17%
Case-control Taiwan U.S.A.	5% 0%	1% 1%

*P < .05, performed by unlicensed therapists

Health Care Procedures and HCV Infection

- Low/moderate endemic countries

	Surgery		Dental	
Country	HCV-positive	HCV-negative	HCV-positive	HCV-negative
Case-control				
U.S.A.	10%	12%	24%	24%
Italy	17%*	2%	22%	11%
Cross-sectional				
Italy	56%*	36%	91%*	80%
	77%	57%	90%	90%
Taiwan	13%	3%	24%	28%
Pakistan	No data	No data	33%	39%
Japan	32%*	10%	No data	No data

*P < .05, independent of other risk factors

Cosmetic Procedures and HCV Infection

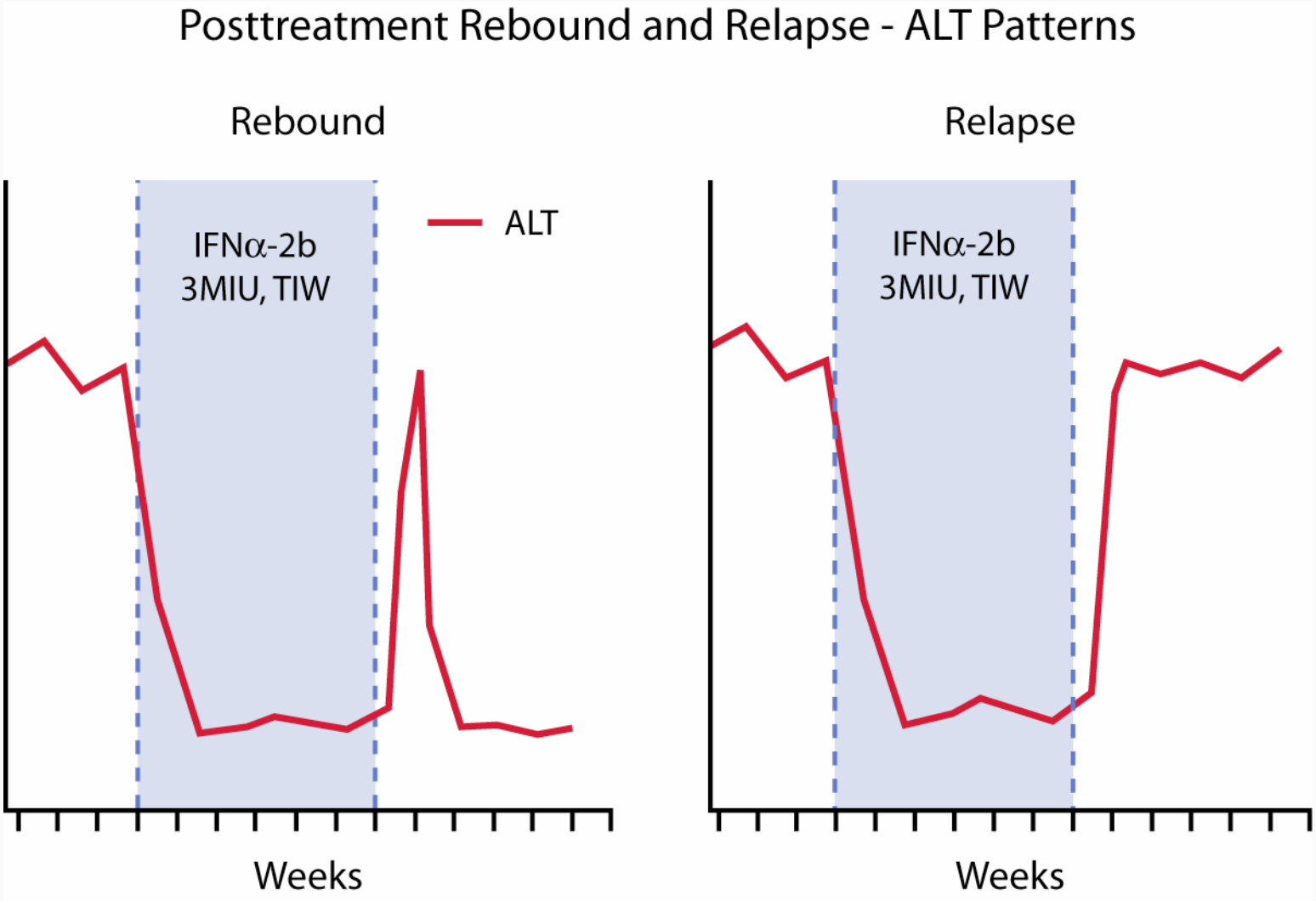
	Tattooing		Body piercing	
Country	HCV-positive	HCV-negative	HCV-positive	HCV-negative
Cross-sectional				
Japan	1%	0%	–	–
	3%	0%	–	–
Pakistan	7%	0%	7%	0%
Case-control				
Taiwan	0%	0%	0%	1%
U.S.A.	1%	1%	3%	3%

Geographic Differences in HCV Transmission Patterns

Importance of exposure by HCV endemicity

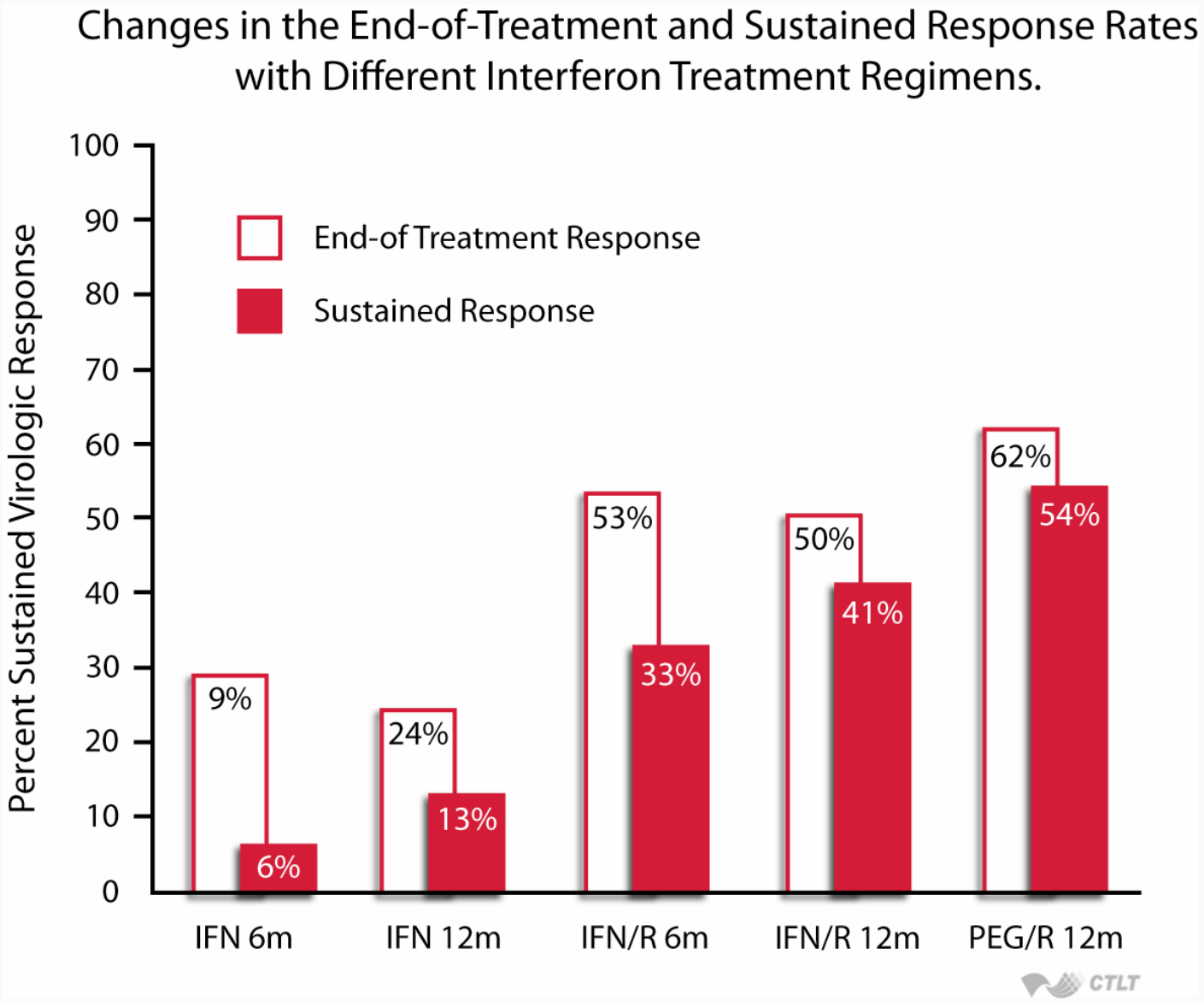
Exposures among prevalent infections	Low	Moderate	High
Injecting drug use	++++	++	+
Transfusions	+++	+++	+++
Health care related	+/–	++++	++++
Unsafe injections	+/–	++++	++++
Folk medicine	–	++	No data

Posttreatment Rebound and Relapse

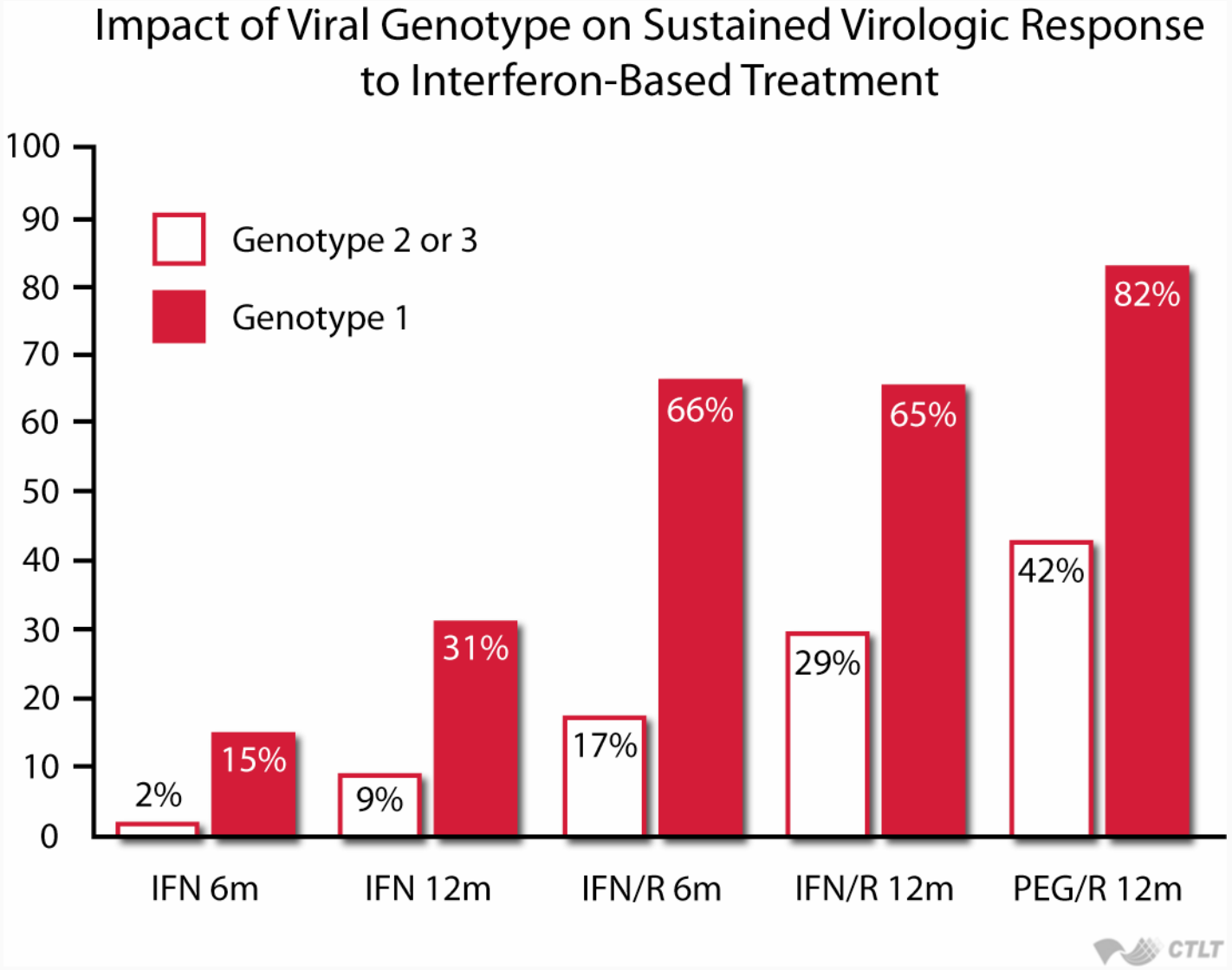


Adapted by CTLT from Davis G, et al. *N Engl J Med.* 1989;321:1501-1506.
Davis G. NHDTP Consensus Conference, Santa Monica, CA, September, 1990.

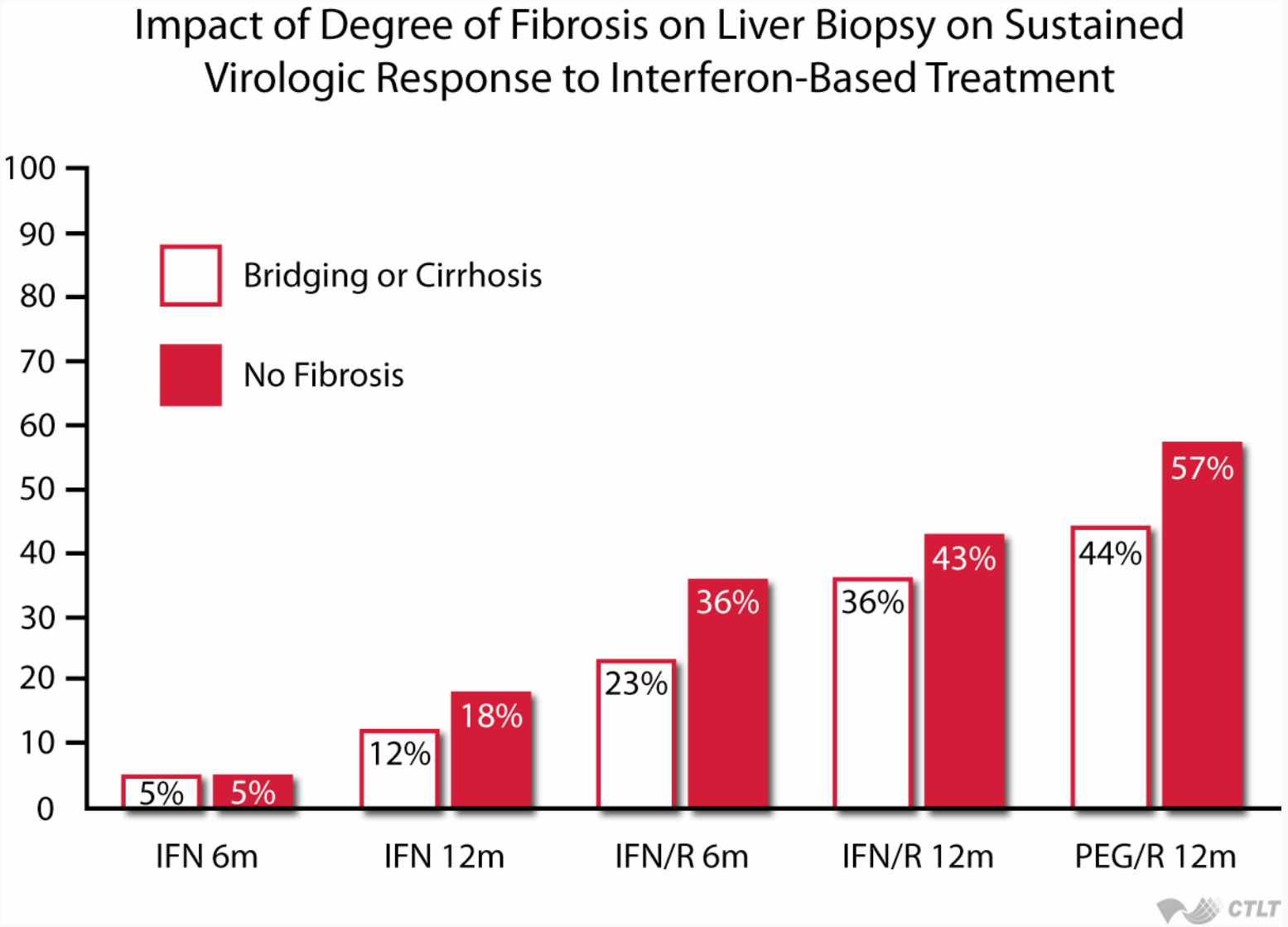
IFN Regimens: End-of-Treatment, Sustained Response Rates



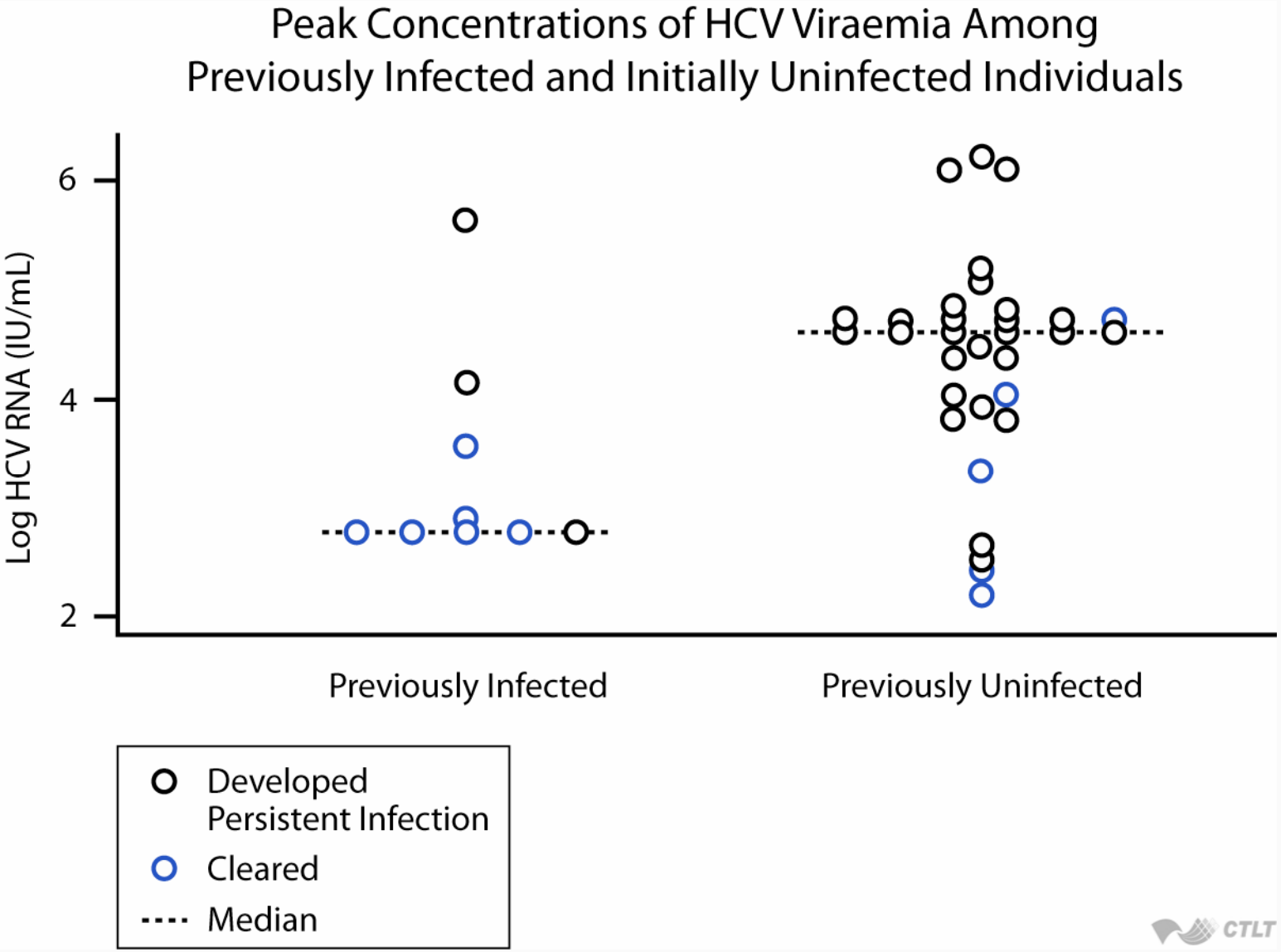
Genotype and Response to Interferon-Based Treatment



Impact of Degree of Fibrosis



Peak Concentrations of HCV Viraemia



Is a Vaccine Possible? Pro (Yes)

- Will it be possible to develop a preventive vaccine for HCV? pro (yes)
  - 30% of persons clear the virus spontaneously
  - The genome of HCV is not integrated into the host genome
  - After HCV infection, CD-8 CTL responses and antibodies appear, but the “protective immune response” or critical epitopes are not known
  - Persons who clear HCV and become re-infected have low viral loads and more likely to clear HCV

Is a Vaccine Possible? Con (No)

- Will it be possible to develop a preventive vaccine for HCV? con (no)
  - After clearance, persons are not immune to reinfection (chimps can be reinfected with the same virus)
  - Great genetic diversity of HCV makes decision on prototype vaccine virus very difficult
  - Immune response drives HCV diversity

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Section C

Hepatitis E

Hepatitis E Virus: Historical Overview

- 1900: Infectious hepatitis reported with high mortality rates in women
- 1955: A common-source outbreak of infectious hepatitis observed in India
  - Longer incubation period than previously observed (40 days)
  - Mean age was older (27 years)
  - High mortality in pregnant women (20% CFR)
- 1980: Reports of large outbreaks in India and Kashmir
  - Seronegative for hepatitis A and B
  - Increased secondary attack rate in household contacts

Virus Characteristics

- HEV is a spherical, non-enveloped, single-stranded RNA virus
- Approximately 27–34 nm in diameter
- Presently unclassified
- May be unstable in external environment/labile

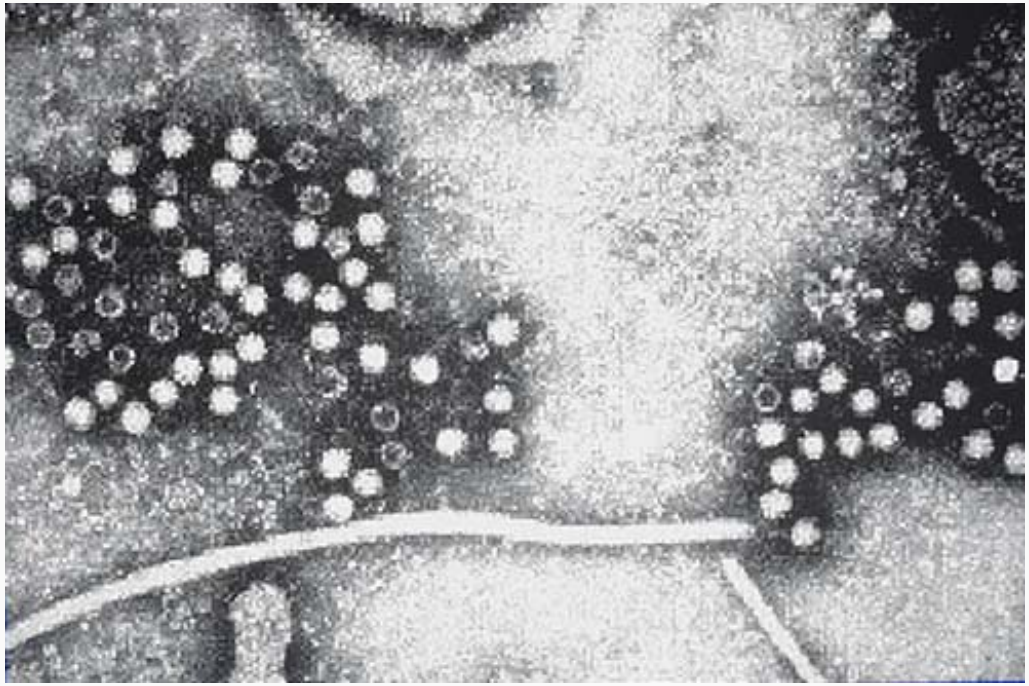


Photo source: Department of Epidemiology, Johns Hopkins School of Hygiene and Public Health.

Background

- Hepatitis E virus (HEV) is the principal cause of enterically transmitted non-A, non-B hepatitis
- Causes epidemic and sporadic disease in many developing countries
- Identified as a distinct virus in 1980
  - Khuroo et al. and Wong et al.
- Cloned and partially sequenced in 1990
  - Reyes et al.
- First complete nucleotide sequence in 1991
  - Tam et al.

Background

- Clinical hepatitis E disease normally seen in adults 15–40 years old (unlike HAV)
- Low seroprevalence in pediatric populations
- Infection rate is 2–4 times higher than disease rate
- Minimal secondary person-to-person transmission observed (illness and/or serology) 2%
- No treatment or vaccine available
- Median cost of infection = $37 (wages/productivity), 35 days lost
  - Clarke et al. (1999).

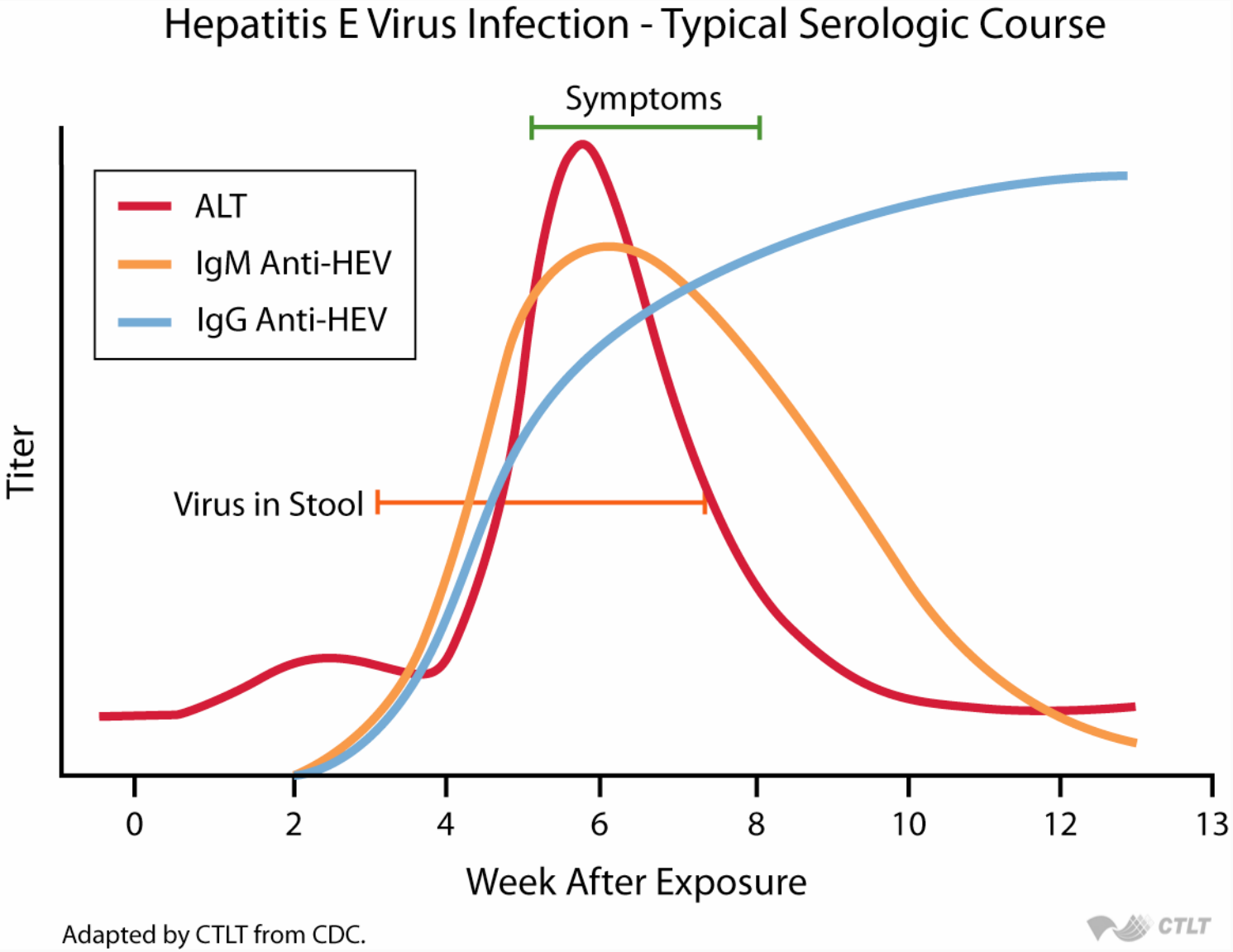
Hepatitis E: Epidemiologic Features

- Most outbreaks associated with fecally contaminated drinking water
- Minimal person-to-person transmission
- U.S. cases usually have history of travel to HEV-endemic areas

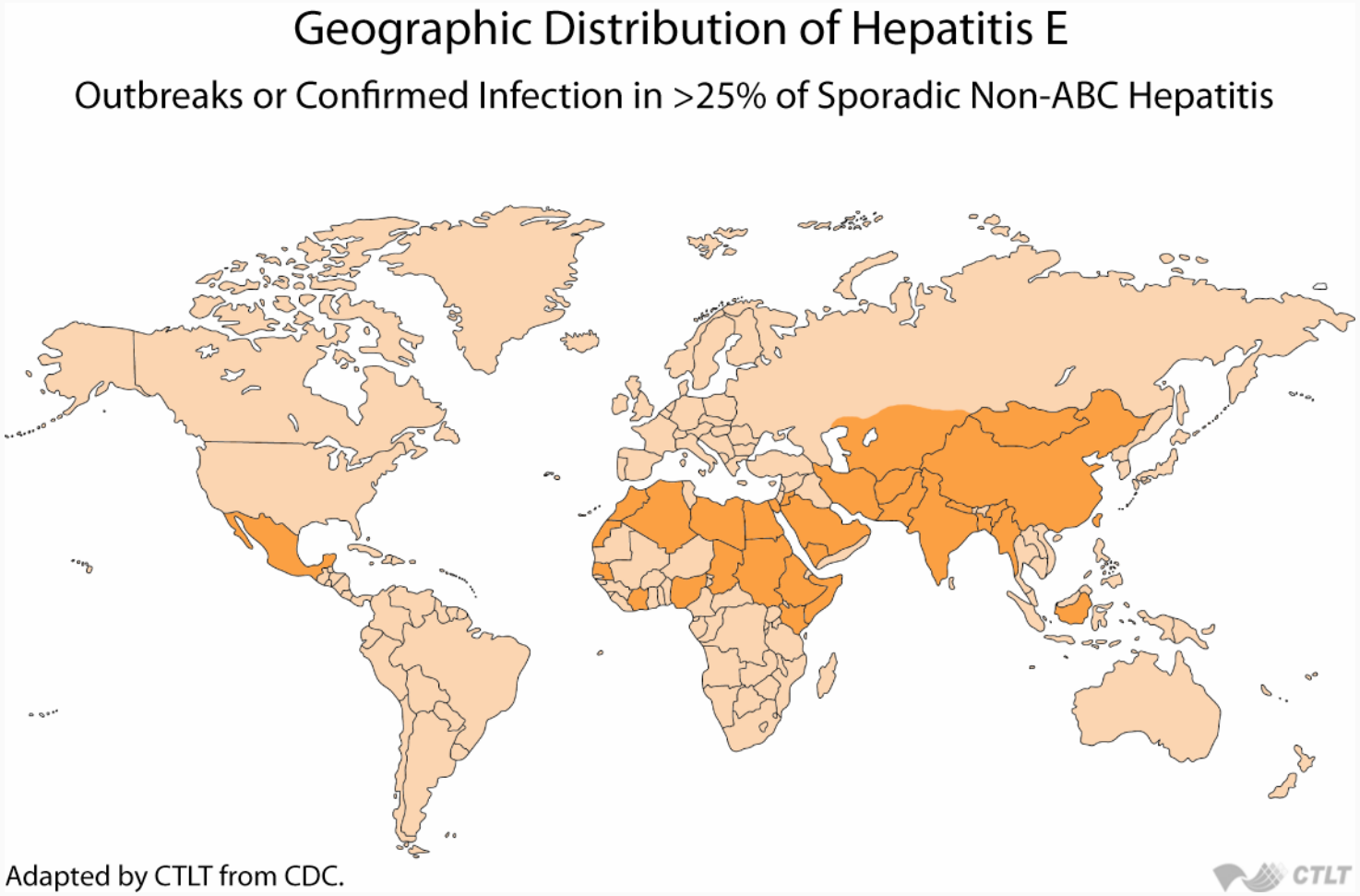
Hepatitis E: Clinical Features

Incubation period	Average: 40 days Range: 15–60 days
Case-fatality rate	Overall: 1–3% Pregnant women: 15–25%
Illness severity	Increased with age
Chronic sequelae	None identified

HEV Infection: Typical Serologic Course



Geographic Distribution of Hepatitis E



Jaundice at Ayurved Hospital (Nepal) and Rainfall