# $CD127^{+}$ T-Cells In Chronic Hepatitis C Virus Infected Patients

## *Thesis*

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## List of abbreviations

| | |
|---|---|
| **ALT** | alanine aminotranferase. |
| **Anti-HCV** | antibodies to hepatitis C virus |
| **ASR** | analyte specific reagent |
| **AST** | Aspartate aminotranferase.. |
| **Bcl-2** | B-cell leukemia /lymphoma. |
| **bDNA** | brached DNA. |
| **CARD** | Caspase activation and recruitment domain. |
| **cART** | combination antiretroviral therapy. |
| **cDC** | conventional dentritic cell. |
| **CDC** | center for disease control and prevention. |
| **Cdc42** | Cell division control protein 42 homolog. |
| **CFSE** | carboxy-fluroscein-succinimidyl ester. |
| **CLIA** | chemoilluminescence immune assay. |
| **CLDN** | Claudin |
| **CMF-PBS** | calcium- and magnesium-free phosphate-buffered saline. |
| **CMV** | cytomegalo virus |
| **CRH1** | cytokine receptor homology class 1 |
| **CTL** | cytotoxic T lymphocyte. |
| **CTLA4** | cytotoxic T lymphocyte association antigen 4. |
| **EBV** | Epstein-Barr Virus. |
| **ECD** | extra-celluar domain. |
| **EFn** | Ephrin. |
| **eLF** | eukaryotic initiation factor. |
| **ELISA** | enzyme liked immune-sorbent assay |
| **Eomes** | Eomesodermin. |
| **ER** | endoplasmic reticulum. |
| **Erk-2** | extracellular-signal-regulated kinases |
| **ETS** | E-transformation specific squance |
| **FACS** | fluorescence-activated cell sorter. |
| **FDA** | food and drug administration |
| **FDC** | follicular dendritic cells. |
| **Flu** | influenza. |
| **FNIII** | fibronectin type III |

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| **FOXO1** | forkhead box O1 |
| **FoxP3** | forkhead box P3 |
| **FRCs** | fibroblastic reticular cells. |
| **Fsc** | Forward sscatter |
| **GABPα** | GA-binding protein |
| **GC** | glycocorticiod. |
| **Gfi-1** | growth factor independent-1: |
| **GITR** | glycocoticoid-induced TNF receptor. |
| **GTP** | guanine triphosphate. |
| **HBV** | Hepatitis B Virus |
| **HCC** | Hapato-cellular carcinoma |
| **HCV** | Hepatitis C Virus |
| **HCV Ag** | Hepatitis C Virus core antigen. |
| **HCVpp** | HCV pseudoparticles |
| **HCWs** | high risk healthcare workers |
| **HDAC1** | Histone deacetetylase |
| **HDL** | High density lipoprotein. |
| **HIV** | Human Immunodeficiency virus |
| **HLA** | Human leukocyte antigen. |
| **HVR** | Hyper variable region. |
| **ICD** | intracelluar domain. |
| **ICOS** | inducible costimulatory molecule. |
| **IFNα** | interferon alpha. |
| **IFNɣ** | interferon gamma. |
| **IFN$_{\beta}$** | interferon beta. |
| **IgG** | immunoglobulin G. |
| **IL** | interlukine. |
| **IPS** | interferon- beta promoter stimulator |
| **IPS-1** | interferon- beta promoter stimulator protien-1 |
| **IQR** | interquartile range |
| **IRES** | internal ribosome entry site. |
| **IRF3** | INF regulatory factor 3. |
| **ISGS** | interferon stimulating genes. |
| **JaK1 and JaK3** | Janus kinas 1 and 3 |
| **KIR/HLA** | killer cell IG like receptor/.human leukocyte antigen. |

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| **KLRG1** | Killer cell lectin-like receptor subfamily G member 1. |
| **LCMV** | lymphocytic chorromeningitis virus. |
| **LIP** | lymphopenia induced proliferation. |
| **LOD** | low limit of detection. |
| **MAPK** | mitogen-activated protein kinase |
| **MAVS** | Mitochondrial Antiviral signaling protein. |
| **mDC** | myliod dendritic cells. |
| **MHC** | major histocompatability complex. |
| **mIL7Rα** | Membrane bound interlukin-7 receptor |
| **miRNA** | MicroRNAs |
| **MPEC** | memory precursor effector cells. |
| **MQ** | macrophage. |
| **mRNA** | massenger RNA |
| **m-tuberculosis** | Mycobacterium tuberculosis |
| **NAT** | nucleic acid test |
| **NK** | natural killer cell. |
| **NS** | non-structural. |
| **NTR** | non-translated region.. |
| **P56LCK** | p56- lymphocyte-specific protein tyrosine kinase |
| **PAT** | parental anti-chistosomal therapy |
| **PBMCs** | prephira bloodl mononuclear cells. |
| **PBS** | phosphate buffer saline. |
| **PD-1** | programmed death 1 |
| **pDC** | plasmcytoid dentritic cells. |
| **PI3K** | phosphotidylinositide-3 kinase |
| **PKR** | protein kinase receptors.. |
| **PP2A** | protein phosphatase 2 a. |
| **qRT-PCR** | quantative RT-PCR |
| **RA** | rheumatoid arthritis. |
| **RAG** | recombination activating gene |
| **RASA1** | Ras GTPase-activating protein 1 |
| **Ras GAP** | Ras GTPase-activating protein |
| **RBV** | Ribavirine. |
| **RC** | replication complex |
| **RdRp** | RNA-dependent RNA polymerase. |
| **RIBA** | Recombinant immunoblot assay. |

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| **RIG-1** | retinoic acid-inducible gene 1 |
| **RNA** | Ribonucleic acid. |
| **RNAi** | RNA interference. |
| **RSV** | respiratory syncytial virus. |
| **RTE** | recent thymic emigrant. |
| **RT-PCR** | reverse transcriptase polymerase chain reaction. |
| **RUO** | research use only. |
| **rNTP** | ribonucleoside triphosphates |
| **sCD** | Soluble CD |
| **SCID** | severe combined immunodeficiency. |
| **SH2** | src homology. |
| **SIL7R** | soluble interlukin-7 receptor. |
| **SLEC** | short lived effector cell. |
| **SOCS3** | suppressor of cytokine signaling 3 |
| **SR-BI** | scavenger receptor class B type I. |
| **STAST 1** | signaling transducer and activators of transcription. |
| **Ssc** | Side scatter |
| **SVR** | sustained virological rate. |
| **S/co ratio** | significance of signal-to-cut off ratio. |
| **T1D** | type 1 diabetes |
| **TCR** | T cell receptors |
| **TFh** | T-follicular helper. |
| **Th1 and Th2** | type 1 and type 2 helper T cells. |
| **TIM-3** | T-cell immunoglobulin and mucine domain-contianing molecule 3. |
| **TLR** | toll-like receptor. |
| **TMA** | transcription-mediated amplification. |
| **TMD** | transmembrane domain. |
| **TRIF** | Toll/IL-1 domain containing adaptor inducing IFN - $_{\beta}$ |
| **T-reg** | T-regulatory cells. |
| **TSLP** | thymicstromal lymphoid protein. |
| **TSLPR** | thymicstromal lymphoid protein receptors. |
| **VAMP-associated proteins** | vesicle association membrane protein |
| **VISA** | virus induced signaling adapter. |
| **WHO** | World Health Organization. |
| **γc** | Gamma chain |

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# Introduction

Hepatitis C virus (HCV) is associated with viral persistence in the majority of patients and spontaneous resolution in only a minority; although precise mechanisms governing outcome remain incompletely defined, abundant data demonstrated that the vigor and breadth of the cellular immune response in the earliest stages of infection is critical **(*Smyk-Pearson et al., 2006).***

Acute HCV infection is frequently associated with a strong virus-specific CTL response that declines exponentially over time, particularly in those who develop chronic infection ***(Cox et al., 2005).***

Patients with acute HCV infection demonstrated that the loss of CD127 expression on total naive and memory/effector populations and on viral-specific CTLs is an introduction to viral persistence. Patients with acute-resolving HCV infection express levels of CD127 on CD4+ and CD8+ T cells intermediate to normal and patients with acute-to-chronic infection **(*Golden masson et al., 2006).***

HCV-infected individuals showed a lower level of CD127 expression in $T_{CM}$ (T Central Memory) and effector CD4+ T cells . CD127 expression was also decreased in naıve and $T_{EM}$ (T Effector Memory) CD4+ T cells. Chronic HCV infection per se was closely associated with down-regulation of CD127 expression on CD4+ T cells regardless of naıve/memory phenotype. Variations in different fractions of CD4+ T cells, including the phenotypic profile and expression level of CD25 and CD127, may be associated with low efficiency of immune response in chronic HCV infection ***(Shen et al., 2010).***

Attenuation and dysfunction of T cell immune response is considered the main cause of HCV chronicity. In this aspect, it should be useful to monitor the speed and fluctuation of the ‘‘flow strain’’ from naıve T cells to activated effector cells in clinical management of HCV-infected individuals, which will provide more direct information than viral load for evaluating the association between HCV persistence and inefficient immune responses **(*Shen et al., 2010).***

Chronic HCV is characterized by CTLs that are functionally impaired (decreased antiviral cytokine production, cytotoxicity, and proliferative capacity) (Wedemeyer et al., 2002) or anergic, and may exhibit phenotypic features of early stages of differentiation **(*Lucas et al., 2004).***

The expression of the IL-7R α-chain (CD127) is a marker of activated effector CD8 T cells that are more likely to survive and differentiate into protective memory T cells ***(Prlic et al., 2002).*** In humans, data demonstrate that CD127 is a marker of early viral-specific CTLs destined to become memory CTLs ***(van Leeuwen et al., 2005).***

Since the expression of CD25 and CD127 controls naive and memory T cell homeostasis, proliferation, and differentiation, IL-7/IL-7 receptor (CD127) signaling plays a potential role in determining the outcome and severity of HCV infection ***(Shen et al ., 2010).***

# Aim of the work

The aim of the present study is to determine the association between $CD127^{+}$ T-Cells and HCV chronicity .

# Chapter I: Chronic hepatitis C

Hepatitis C virus was discovered in 1989, it infects about 3% of the world population *(**Lavanchy, 2011***). Hepatitis C virus (HCV) is a RNA virus known to infect humans and chimpanzees, causing similar disease in these two species. HCV is most often transmitted parenterally but is also transmitted vertically and sexually **(*Centers for Disease Control and Prevention. MMWR Recomm Rep. 1998).*** HCV is up to 4 times more infectious than Human Immunodeficiency Virus (HIV) **(*Te and Jensen, 2010*).**HCV is a leading cause of chronic hepatitis, liver cirrhosis and hepatocellular carcinoma worldwide (***Ruggieri et al., 2014****).* Globally, approximately 170 million people are chronically infected with the HCV ***(Nguyen and Nguyen, 2013*)**. It is estimated three million new infections occur annually *(**Escobar-Gutiérrez et al., 2013***).

## Disease distribution and epidemiology:

The virus exhibits a very high degree of genetic diversity that is classified six genotypes and sub-classified more than 80 subtypes by phylogenetic analysis. The various genotypes and subtypes of HCV have been associated with different epidemiological and geographical spread patterns. Genotypes 1 and 2 are globally distributed; genotype 3 is predominant in Asia, North America and parts of Europe; similar regional patterns of endemic diversity have been found for genotype 4 in Europe, Middle East and Central Africa, for genotype 5 in parts of Africa and Europe, and for genotype 6 in Southeast Asia and North America **(*Zhao et al., 2012*).**

The estimated prevalence of HCV in Africa is 5.3% **(*Pybus et al., 2003*).** Egypt has the highest worldwide prevalence (17.5%). Egypt unusually high prevalence is attributable to the history of unsterile

injection equipment used for mass treatment of the general population with parenteral antischistosomal therapy (PAT) from the 1920s to the 1980s **(*Frank et al., 2000 ; Global Burden of Hepatitis C Working Group).*** In the United States 2.7-3.9 million peoples are living with HCV infection (***Smith et al., 2012***). In Asia the majority of these individuals come from the western Pacific and Southeast Asia regions, the prevalence is 94.6 million persons combined ***(Nguyen and Nguyen, 2013*)**. The WHO(World Health Organization.) reports that 75% of HCV-infected individuals developing chronic liver disease, 1.6% of them progress to Hepatocellular carcinoma (HCC), a condition with a mortality rate >80% ( ***Maheshwari and Thuluvath, 2010***).

The lack of available information indicates that hepatitis C is still a neglected disease in many countries. However, from the scanty data presented, there is no doubt that HCV is a major health problem that requires greater attention in Africa. With availability of effective therapies against HCV, physicians, researchers and health care decision makers need to improve efforts in diagnosis, management and prevention of HCV in Africa. The relatively high cost of treatment enforces the need for a systematic approach for this condition so that resources are used most effectively ***(Karoney and Siika, 2013)***.

## HCV structure :

HCV is the sole member of the Hepacivirus genus of the Flaviviridae family of small, enveloped, single strand positive sense RNA viruses **(*Lindenbach et al., 2007*).** The 9.6 kb viral genomic RNA contains a single open reading frame flanked by highly structured non-translated regions (NTRs) (Figure 1) (***Briana et al.,2012***).