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(54) Title: IMMUNOTHERAPY COMPOSITION AND REGIMEN FOR TREATING HEPATITIS C VIRUS INFECTION

(57) Abstract: The present invention relates to the field of immunotherapy and more specifically to a composition comprising non-structural (NS) HCV antigens or expression vector(s) thereof for use for treating ahepatitis C virus (HCV) infection. The present invention as also relates to a therapeutic regimen comprising the step of administrating to a subject such an immunotherapy composition as well as a kit of parts comprising a plurality of containers and instructions for carrying out said therapeutic regimen. The immunotherapy composition, kit and therapeutic regimen can be used in combination with antiviral therapy (e.g. interferon therapy and/or ribavirin).

WO 2013/045668 A2

IMMUNOTHERAPY COMPOSITION AND REGIMEN FOR TREATING HEPATITIS C VIRUS INFECTION

FIELD OF THE INVENTION

The present invention relates to the field of immunotherapy and more specifically to a composition comprising non-structural (NS) HCV antigens or expression vector(s) thereof for use for treating a hepatitis C virus (HCV) infection. The present invention also relates to a therapeutic regimen comprising the step of administrating to a subject such an immunotherapy composition as well as a kit of parts comprising a plurality of containers and instructions for carrying out said therapeutic regimen. The immunotherapy composition, kit and therapeutic regimen can be used in combination with antiviral therapy (e.g. interferon therapy and/or ribavirin).

BACKGROUND OF THE INVENTION

HCV belongs to the *Flaviviridae* family and is an enveloped virus with a positive single-stranded ribonucleic acid (RNA) genome of approximately 9,600 nucleotides which organization is common to all HCV strains and isolates (Virology, 1996, Fields ed., third edition, Lippencott-Raven publishers, pp 945-51 or subsequent editions). The genomic RNA is translated into one single polyprotein of 3010 to 3030 amino acids according to the genotype that is proteolytically cleaved by viral and cellular proteases to yield the functional polypeptides, respectively three structural proteins (core protein, and envelope glycoproteins E1 and E2), a small integral membrane protein p7 which seems to function as an ion channel and six non-structural (NS) proteins NS2, NS3, NS4A, NS4B, NS5A and NS5B, which mediate the intracellular processes of the virus life cycle. More specifically, NS3 (631 amino acids long from positions 1027 to 1657 in the HCV-1 polyprotein precursor) comprises two distinct structural domains, namely an N-terminal domain endowed with an active serine protease activity that is involved in the maturation of the viral polyprotein and a C-terminal domain comprising a helicase activity that plays a role in the replication of the viral genome. NS4 (315 amino acids long from positions 1658 to 1972 in the HCV-1 polyprotein precursor) is cleaved by the NS3-associated protease in NS4A and NS4B polypeptides. They are both hydrophobic and found associated with membranes. The

NS4A polypeptide (positions 1658 to 1711) is a co-factor of NS3 protease whereas the function of NS4B (positions 1712 to 1972) is yet unknown. NS5 (1039 amino acids from positions 1973 to 3011 in the HCV-1 polyprotein precursor) is cleaved by the NS3-associated protease in NS5A and NS5B polypeptides which both have been found within the replication complex associated to the ER membrane. NS5B (from positions 2421 to 3011) acts as a RNA-dependent RNA polymerase, which presumably permits the synthesis of a negative stranded RNA intermediate and positive-stranded progeny copies. NS5A (from positions 1973 to 2420) is a phosphoprotein likely involved in the regulation of the RNA dependent RNA polymerase. Further, it has been implicated in HCV antiviral resistance.

The World Health Organization estimated in 2011 that about 200 million people are chronically infected by HCV worldwide and 3 to 4 million persons are newly infected each year. HCV infection is associated with a high rate of chronicity and it is estimated that 75% of seropositive persons have circulating virus (viremia) and chronic liver disease. Severe complications may develop in 20% of chronically infected patients with a risk of hepatocarcinoma of 1-4% per year which makes hepatitis C the leading cause of liver transplantation in the US and Europe.

The current antiviral therapy (SOC for "standard of care") for chronically infected HCV patients in most countries is a combination of pegylated Interferon alpha (PEG-IFN-α) and ribavirin (Neyts, 2006, Antiviral Res. 71, 363-371). However, this therapy is costly, long (24 weeks or 48 weeks depending on the infecting HCV genotype), associated with significant side effects leading to premature ending of treatment in 10% of cases and inadequate for a significant number of patients (e.g. those with decompensated liver cirrhosis, autoimmune diseases, history of depression and pregnancy). Moreover, only 40 to 50% of genotype 1-infected patients are responsive to SOC achieving sustained virologic response (SVR; undetectability of HCV RNA 6 months after end of SOC) and 70 to 80% for genotypes 2 and 3. During the course of SOC treatment, the kinetic of virus eradication is biphasic with a rapid phase of viral load reduction during the first weeks reflecting virus clearance in blood followed a gradual phase reflecting clearance of infected liver cells. Viral load reduction is predictive of the patient's response to SOC. The treatment is generally terminated in

null responders and non-responders patients that fail to achieve respectively at least one log_{10} and <2 log_{10} IU/mL reduction after 12 weeks of treatment with SOC.

Recent efforts to improve SOC efficacy have now focused on additional compounds targeting the virally encoded proteins. Several NS3/4A, NS5A and NS5B-inhibitors are in Phase II clinical development or even Phase III for the most advanced. Anti NS3/4A telaprevir/Icivio or Invivek (Vertex) and boceprevir/Victrelis (Merck) both received very recently the FDA and European Commission approvals for the treatment of genotype-1 chronic hepatitis C virus, in combination with peginterferon alfa and ribavirin and are now available in the US and in Europe. Although increasing the sustained virological response (SVR) when used with pegylated interferon alpha and ribavirin (e.g. by 25-30% in genotype 1 infected patients), these compounds also have significant side effects and may contribute to the generation of mutations in the targeted HCV proteins and thus of drug-resistant HCV variants.

One may expect that immunotherapy approaches be complementary to antiviral therapy with the aim of providing specific protective immunity in chronic HCV infection. Several candidates have now emerged based on recombinant proteins, synthetic peptides, heat-killed expressing yeasts, DNA and viral vectors (Torresi et al, 2011, J. Hepatol 54, 1273-1285). In this respect, WO2008/113606 describes a therapeutic approach relying on an attenuated non-replicative vaccinia virus Ankara strain (MVA) encoding HCV NS3, NS4 and NS5B antigens (named TG4040) which is suitable for 3 repeated administrations separated by a period of time from 3 to 10 days. In particular, a schedule including 3 injections at one week interval represents an optimized protocol to induce HCV-specific IFN-g producing T cells. A fourth injection after 4 or 6 months was able to enhance T cell responses. In animal models, this vaccine induced vigorous and specific T-cell based responses, which were sustained for as long as 6 months (Fournillier et al., 2007, Vaccine 25, 7339-7353). In addition, TG4040 was capable of conferring partial protection in a HCV-surrogate challenge assay based on a recombinant *Listeria monocytogenes* expressing HCV NS3 protein.

TG4040 has now completed a phase I trial in France. The dose-escalation part of the study involved 15 treatment naïve patients chronically infected with the genotype 1 HCV. The patients received three weekly subcutaneous injections of TG4040

(according to the accelerated protocol described in WO2008/113606) with an escalation of doses ranging from 10^6 pfu to 10^8 pfu and patients treated with the highest dose also received a fourth injection of TG4040 at month 6. Eight out of 15 patients experienced a decrease in viral load ranging from 0.5 to 1.2 $\log_{10}$ IU/mL with the largest viral load reduction occurring in the two patients exhibiting the most significant HCV-specific T-cell response; whereas no immune responses to NS3 and NS4B viral antigens were detected for those patients with no or minimal change in viral load (Honnet et al. Oral presentation at the 60th Annual Meeting of the AASLD November 3, 2009; Habersetzer et al., 2011 Gastroentelology 141, 890-899).

Another approach recently investigated involves a yeast-based immunotherapy composition encoding HCV antigens which is suitable for repeated weekly and monthly administrations in combination with interferon and ribavirin (WO2010/033841). Various therapeutic settings are disclosed, with the HCV NS3 and Core-expressing yeast being administrated before SOC initiation concurrently or after SOC treatment.

One may expect that HCV will continue to be a serious global health threat for many years due to the chronic and persistent nature of the infection, its high prevalence, the continuing transmission of HCV and the significant morbidity of the associated diseases. Thus, there is an important need to develop more effective, less toxic and shorter therapeutic approaches combining immunotherapeutic approaches and antiviral therapy, for improving treatment of HCV infections or HCV-associated diseases or disorders, and especially chronic HCV infections.

The objective problem posed in the present invention is seen as being the provision of an optimized therapeutic regimen for administering an immunotherapy composition, optionally in combination with one or more antiviral compound(s). It is assumed that this optimized therapeutic regimen may permit to prime or stimulate an effective immunity before initiating antiviral therapy and to maintain the primed immunity during continuation of the antiviral treatment.

This technical problem is solved by the provision of the embodiments as defined in the claims.

Other and further aspects, features and advantages of the present invention will be apparent from the following description of the presently preferred embodiments of the invention. These embodiments are given for the purpose of disclosure.

SUMMARY OF THE INVENTION

One embodiment of the present invention concerns an immunotherapy composition for use for treating an HCV infection, especially a chronic HCV infection. The immunotherapy composition is administered to a subject in need thereof pursuant to a repeated administration pattern comprising from 4 to 8 administrations separated from each other by a period of time varying from 3 to 10 days and optionally followed by from 4 to 15 administrations separated from each other by a period of time varying from 3 to 5 weeks. In one aspect of this embodiment, the immunotherapy composition comprises a therapeutically effective amount of an active ingredient selected from the group consisting of (a) a polyprotein NS3/NS4 of a hepatitis C virus with a polypeptide NS5B of a hepatitis virus C; (b) an expression vector comprising a nucleotide sequence encoding a polyprotein NS3/NS4 of a hepatitis C virus and a nucleotide sequence encoding a polypeptide NS5B of a hepatitis virus ; or (c) an expression vector comprising a nucleotide sequence encoding a polyprotein NS3/NS4 of a hepatitis C virus with an expression vector comprising a nucleotide sequence encoding a polypeptide NS5B of a hepatitis virus. In one aspect, the administration pattern comprises 4 to 8 administrations separated from each other by a period of time varying from 3 to 10 days. Alternatively and in a preferred aspect, the administration comprises 4 to 8 administrations separated from each other by a period of time varying from 3 to 10 days followed by from 4 to 15 administrations separated from each other by a period of time varying from 3 to 5 weeks.

In a further aspect of the embodiments described above, the immunotherapy composition can be used in combination with antiviral therapy, and especially with a pegylated interferon alpha2 (PEG-IFNα2) and ribavirin.

Another embodiment of the present invention relates to a therapeutic regimen comprising the steps of administering said immunotherapy composition, optionally in combination with antiviral therapy.

Still a further aspect of the present invention includes a kit of parts for use for treating an HCV infection and preferably a chronic HCV infection, wherein said kit comprises a plurality of containers and instructions for administering to a subject said immunotherapy composition or carrying out said therapeutic regimen.

Still yet a further aspect of the present invention concerns a method to increase by at least 5% the proportion of subjects who achieved complete early virologic response (cEVR) in a population of subjects chronically infected with HCV, as compared to cEVR in a population of subjects chronically infected with HCV and treated only with antiviral therapy.

In still another aspect, the present invention when used in combination with antiviral therapy also permits to act upon safety aspects, e.g. by providing a method of reducing the duration of antiviral therapy and/or reducing the doses or regimen of at least one of the antiviral compounds in the population of subjects chronically infected with HCV treated according to the present invention as compared to a population of subjects chronically infected with HCV and treated only with antiviral therapy, thus resulting in a reduced proportion of antiviral-associated adverse events (e.g. at least 5%) in the population of HCV patients treated according to the invention.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to an immunotherapy composition comprising a therapeutically effective amount of an active ingredient selected from the group consisting of:

- A polyprotein NS3/NS4 of a hepatitis C virus with a polypeptide NS5B of a hepatitis virus C;
- An expression vector comprising a nucleotide sequence encoding a polyprotein NS3/NS4 of a hepatitis C virus and a nucleotide sequence encoding a polypeptide NS5B of a hepatitis virus ;
- An expression vector comprising a nucleotide sequence encoding a polyprotein NS3/NS4 of a hepatitis C virus with an expression vector comprising a nucleotide sequence encoding a polypeptide NS5B of a hepatitis virus;

for use as a medicament intended to treat an HCV infection wherein the administration pattern of the medicament comprises from 4 to 8 administrations of said immunotherapy composition separated from each other by a period of time varying from 3 to 10 days and optionally followed by from 4 to 15 administrations of said immunotherapy composition separated from each other by a period of time varying from 3 to 5 weeks.

Definitions

As used herein throughout the entire application, the terms "a" and "an" are used in the sense that they mean "at least one", "at least a first", "one or more" or "a plurality" of the referenced compounds or steps, unless the context dictates otherwise.

The term "and/or" wherever used herein includes the meaning of "and", "or" and "all or any other combination of the elements connected by said term".

The term "about" or "approximately" as used herein means within 10%, preferably within 8%, and more preferably within 5% of a given value or range.

The terms “amino acids”, “residues” and “amino acid residues” are synonyms and encompass natural amino acids as well as amino acid analogs (e.g. non-natural, synthetic and modified amino acids, including D or L optical isomers).

The terms “polypeptide”, “peptide” and “protein” refer to polymers of amino acid residues which comprise at least nine or more amino acids bonded via peptide bonds. The polymer can be linear, branched or cyclic and may comprise naturally occurring and/or amino acid analogs and it may be interrupted by non-amino acids. As a general indication, if the amino acid polymer is more than 50 amino acid residues, it is preferably referred to as a polypeptide or a protein whereas if it is 50 amino acids long or less, it is referred to as a “peptide”.

The term “polyprotein” as used herein refers to the fusion with one another of two or more polypeptides/peptides in a single polypeptide chain. Preferably, the fusion is performed by genetic means, i.e. by fusing in frame the nucleotide sequences encoding each of said polypeptides/peptides. By "fused in frame", it is meant that the expression of the fused coding sequences results in a single protein without any translational terminator between each of the fused polypeptides/peptides. The fusion can be direct (i.e. without any additional amino acid residues in between) or through a linker

(e.g. 3 to 30 amino acids long peptide composed of repeats of amino acid residues such as glycine, serine, threonine, asparagine, alanine and/or proline).

Within the context of the present invention, the terms “nucleic acid”, “nucleic acid molecule”, “polynucleotide” and “nucleotide sequence” are used interchangeably and define a polymer of any length of either polydeoxyribonucleotides (DNA) (e.g., cDNA, genomic DNA, plasmids, vectors, viral genomes, isolated DNA, probes, primers and any mixture thereof) or polyribonucleotides (RNA) (e.g., mRNA, antisense RNA) or mixed polyribo-polydeoxyribonucleotides. They encompass single or double-stranded, linear or circular, natural or synthetic polynucleotides. Moreover, a polynucleotide may comprise non-naturally occurring nucleotides and may be interrupted by non-nucleotide components.

As used herein, when used to define products, compositions and methods, the term "comprising" (and any form of comprising, such as "comprise" and "comprises"), "having" (and any form of having, such as "have" and "has"), "including" (and any form of including, such as "includes" and "include") or "containing" (and any form of containing, such as "contains" and "contain") are open-ended and do not exclude additional, unrecited elements or method steps. Thus, a polypeptide "comprises" an amino acid sequence when the amino acid sequence might be part of the final amino acid sequence of the polypeptide. Such a polypeptide can have up to several hundred additional amino acids residues. "Consisting essentially of" means excluding other components or steps of any essential significance. Thus, a composition consisting essentially of the recited components would not exclude trace contaminants and pharmaceutically acceptable carriers. A polypeptide "consists essentially of” an amino acid sequence when such an amino acid sequence is present with optionally only a few additional amino acid residues. "Consisting of” means excluding more than trace elements of other components or steps. For example, a polypeptide "consists of” an amino acid sequence when the polypeptide does not contain any amino acids but the recited amino acid sequence.

The term “immunotherapy composition” as used herein refers to a formulation comprising at least one of the therapeutic agents described herein (e.g. expression vector(s) encoding a NS3/NS4 polyprotein and a NS5B polypeptide) and optionally one or more pharmaceutically acceptable vehicle (e.g. carrier, diluent, adjuvant, etc),

capable of inducing or stimulating an immune response upon administration to a subject pursuant to the administration pattern described herein. The induced or stimulated immune response can be innate or specific, humoral or cellular and can be assessed by various measurements including, but not limited to, the production of antibodies, and/or the production of cytokines (e.g. IFN-g) and/or the activation of cytotoxic T cells, B, T-lymphocytes, antigen presenting cells, helper T cells, dendritic cells, NK cells, etc.

As used herein, "HCV" means "hepatitis C virus", a *flaviviridae* member which causes hepatitis C (also known as non-A, non-B hepatitis).

The term "NS polypeptide" refers to a non-structural (NS) polypeptide originating from any HCV genotype, subtype or isolate identified at present time. This term encompasses either a native NS polypeptide encoded by an open reading frame of a cDNA or RNA fragment derived from a HCV virus as well as to a NS derivative and any fragment thereof (e.g. immunogenic peptide).

For purpose of illustration, a "NS5B polypeptide" refers to a native NS5B polypeptide encoded by any HCV genotype, subtype or isolate (e.g. from approximately amino acid 2421 to approximately amino acid 3011 in the HCV-1 polyprotein precursor) as well as any derivative or fragment thereof. A "NS3/NS4 polyprotein" refers to a fusion between a NS3 polypeptide and a NS4 polypeptide (e.g. encompassing both NS4A and NS4B) encoded by or originating from any HCV genotype, subtype or isolate (e.g. from approximately amino acid 1027 to approximately amino acid 1972 in the HCV-1 polyprotein precursor) as well as any derivative or fragment thereof. For sake of clarity, the amino acid stretches referred herein in connection with HCV polypeptides are given with respect to their positions in HCV-1 polyprotein precursor (as described by Choo et al., 1991, Proc. Natl. Acad. Sci. USA 88, 2451-2455 or in GenBank under accession number M62321). However, unless the context clearly dictates otherwise, when it is referred herein to a NS polypeptide or a peptide thereof by reference to HCV-1 polyprotein precursor, this means the NS polypeptide or the peptide thereof of HCV-1, a NS polypeptide or the peptide thereof of any other HCV genotype, subtype or isolate or a modified NS polypeptide or peptide thereof.

As used herein, the term "native" when used in connection with a NS polypeptide or polyprotein or in connection with a NS-encoding nucleotide sequence refers to an amino acid sequence or to a nucleotide sequence that can be found, isolated,

obtained from a source in nature as distinct from one being artificially modified or mutated by man in the laboratory (i.e. mutant or derivative). Such sources in nature include biological samples (e.g. blood, plasma, sera, semen, saliva, tissue sections, biopsy specimen etc.) collected from a subject infected or that has been exposed to HCV, cultured cells, tissue cultures as well as recombinant materials. Recombinant materials include without limitation HCV isolates (e.g. available in depositary institutions), HCV genome, genomic RNA or cDNA libraries, vectors containing HCV genome or fragment(s) thereof).

The term "derivative" or "mutant" as used herein refers to a polypeptide/nucleotide sequence exhibiting one or more modification(s) with respect to the native counterpart. For illustrative purposes, a "NS derivative" refers to a NS polypeptide that has been artificially mutated or altered by man in the laboratory. Any modification(s) can be envisaged, including substitution, insertion and/or deletion of one or more nucleotide/amino acid residue(s), non-natural arrangements (e.g. fusion with non-HCV polypeptides/peptides) as well as any combination of these possibilities. When several mutations are contemplated, they can concern consecutive residues and/or non-consecutive residues. Mutation(s) can be generated by a number of ways known to those skilled in the art, such as site-directed mutagenesis (e.g. using the Sculptor™ in vitro mutagenesis system of Amersham, Les Ullis, France), PCR mutagenesis, DNA shuffling and by chemical synthetic techniques (e.g. resulting in a synthetic nucleic acid molecule).

As used herein, the term "identity" refers to an exact amino acid to amino acid or nucleotide to nucleotide correspondence between two polypeptides or nucleotide sequences. The percentage of identity between two sequences is a function of the number of identical positions shared by the sequences, taking into account the number of gaps which need to be introduced for optimal alignment and the length of each gap. Various computer programs and mathematical algorithms are available in the art to determine the percentage of identity between amino acid sequences, such as for example the Blast program available at NCBI or ALIGN in Atlas of Protein Sequence and Structure (Dayhoffed., 1981, Suppl., 3 482-489). Programs for determining homology between nucleotide sequences are also available in specialized data base (e.g. Genbank, the Wisconsin Sequence Analysis Package, BESTFIT, FASTA and GAP

programs). For illustrative purposes, "at least 80% sequence identity" as used herein means 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100%.

The term "expression vector" as used herein refers to a vehicle, preferably a nucleic acid molecule or a viral particle that contains the elements necessary to allow delivery, propagation and expression of the HCV nucleotide sequences of interest within a host cell or a subject. This term encompasses extrachromosomal vectors (e.g. multicopy plasmids; vaccinia virus vectors) and integration vectors (which are designed to integrate into the host cell genome and produce additional copies of the nucleic acid molecules when the host cell replicates). For the purpose of the invention, the vectors may be of naturally occurring genetic sources, synthetic or artificial, or some combination of natural and artificial genetic elements.

As used herein, the term "isolated" refers to a protein, polypeptide, peptide, polynucleotide, vector, etc., that is removed from its natural environment (i.e. separated from at least one other component(s) with which it is naturally associated or found in nature). For example, a nucleotide sequence is isolated when it is separated of sequences normally associated with it in nature (e.g. dissociated from a genome) but it can be associated with heterologous sequences.

As used herein, the term "host cell" should be understood broadly without any limitation concerning particular organization in tissue, organ, or isolated cells. Such cells may be of a unique type of cells or a group of different types of cells such as cultured cell lines, primary cells and proliferative cells. In the context of the invention, the term "host cells" include prokaryotic cells, lower eukaryotic cells such as yeast, and other eukaryotic cells such as insect cells, plant and mammalian (e.g. human or non-human) cells as well as cells capable of producing the NS polypeptides and the expression vector(s) comprised in the immunotherapy composition for use in the invention. This term also includes cells which can be or has been the recipient of the vectors described herein as well as progeny of such cells.

The term "subject" or "patient" is used interchangeably and generally refers to a vertebrate, particularly a member of the mammalian species and especially domestic animals, farm animals, sport animals, and primates including humans for whom any product and method of the invention is needed or may be beneficial such as subjects

who have been diagnosed as being or at risk of being infected with an HCV and thus are susceptible of having or at risk of having a disease or condition caused by or associated with an HCV infection. In a preferred embodiment, the subject is a human patient chronically infected with an HCV virus or alternatively co-infected with an HCV virus and another virus (e.g. the human immunodeficiency virus HIV).

The term "treating" (and any form of treating such as "treatment", "treat") as used herein refers to prophylaxis (e.g. prevention of a subject at risk of being infected with HCV) and/or therapy (e.g. cure or control of viral replication or disease progression for example in a subject diagnosed as being infected with an HCV). Treatment requires administer externally or internally to a subject a therapeutic agent such as the immunotherapy composition described herein, optionally in association with antiviral therapy, especially the one currently used in the treatment of chronic HCV infection including pegylated IFNα, and/or ribavirin and/or protease inhibitors and/or polymerase inhibitors, etc.

The term "administering" (or any form of administration such as "administered") as used herein refers to the delivery of a therapeutic agent such as the immunotherapy composition described herein to a subject.

The term "administration pattern" as used herein refers to a series of administrations to be carried out in a subject according to the modalities described herein.

HCV Sequences

A number of HCV sequences are suitable for use in the present invention including such sequences that are readily available to investigators in the field, including, but not limited to, HCV sequences described in Genbank and PubMed. Extensive phylogenetic analyses have led to the classification of HCV isolates into 6 major genotypes (1 to 6) with several subtypes (a, b, c, etc.....) within each genotype (Simmons et al., 2005, Hepatology 42, 962-973). HCV sequences originating from any genotype and subtype can be used in the embodiments described herein.

Exemplary HCV of genotype 1a include, without limitation, HCV-1 (Choo et al., 1991, Proc. Natl. Acad. Sci. USA 88, 2451-2455), -J1 (Okamoto et al., 1992, Nucleic Acids Res. 20, 6410-6410), –H (Inchauspé et al., 1991, Proc. Natl. Acad. Sci. 88,

10292-10296) and the isolate described in WO2004/048402. Exemplary HCV of genotype 1b, include without limitation, HCV-JA (Kato et al., 1990, Proc. Natl. Acad., Sci. 87, 9524-9528) and BK (Takamizawa et al., 1991, J. Virol. 65, 1105-1113). Exemplary HCV of genotype 1c include without limitation, HCV-G9 (Okamoto et al., 1994, J. Gen. Virol. 45, 629-635). Exemplary HCV of genotype 2 include without limitation, HCV-J6 (genotype 2a; Okamoto et al., 1991, J. Gen. Virol. 72, 2697-2704), HCV-J8 (genotype 2b; Okamoto et al., 1992, Virology 188, 331-341) and HCV-BEBE1 (genotype 2c; Nako et al., 1996, J. Gen. Virol. 141, 701-704). Exemplary HCV of genotype 3 include, without limitation, HCV-NZL1 (genotype 3a; Sakamoto et al., 1994, J. Gen. Virol. 75, 1761- 1768) and HCV-Tr (genotype 3b; Chayama et al., 1994, J. Gen. Virol. 75, 3623-3628). Exemplary HCV sequences from genotype 4 include, without limitation, HCV-ED43 genotype 4a; Chamberlain et al., 1997, J. Gen. Virol. 78, 1341-1347). Exemplary HCV sequences of genotype 5 HCV-EUH1480 (genotype 5a; Chamberlain et al., 1997, Biochem. Biophys. Res. Commun. 236, 44-49). Exemplary HCV sequences from genotype 6 include, without limitation, HCV-EUHK2 (genotype 6a; Adams et al., 1997, Biochem. Biophys. Res. Commun. 234, 393-396).

It is intended that the present invention is not limited to these exemplary HCV viruses. Indeed the nucleotide and amino acid sequences of any or all of the HCV NS sequences used in accordance with the present invention can vary between different HCV isolates, subtypes and genotypes and it is intended that natural genetic variation is included within the scope of the invention as well as non-natural modification(s), as described hereinafter.

NS polypeptides

The immunotherapy composition for use in the present invention comprises at least non-structural (NS) NS3, NS4 and NS5B HCV polypeptides, with a specific preference for NS3 and NS4 HCV polypeptides in a fused configuration (i.e. NS3/NS4 polyprotein) or expression vector(s) for expression of such NS3, NS4 (e.g. in the form of a NS3/NS4 polyprotein) and NS5B polypeptides. Preferably, the present invention excludes the use of a HCV core polypeptide or an expression vector encoding a HCV core polypeptide.

In the context of the invention, each of the HCV NS3, NS4 and NS5B polypeptides comprised or encoded by the composition for use in the present invention may individually originate from any HCV genotype, subtype, or isolate identified at present time, such as those described herein or any fragment thereof (e.g. immunogenic fragments).

The present invention also encompasses NS derivatives with modification of one or more amino acid residue(s) (consecutive or not) involved directly or indirectly in an enzymatic activity exhibited by a native NS polypeptide, with the aim of disrupting said enzymatic activity. For example, modification(s) within an enzymatic catalytic site may permit to reduce or ablate the associated enzymatic activity. Amino acid residues that are critical for such functional properties may be identified by routine methods and the reduction or ablation of the modified enzymatic activity can be easily determined in appropriate assays using methods known to those of skill in the art. In this respect, the catalytic residues involved in NS3-mediated protease and helicase activities have been identified and protease-defective NS3 derivatives have been described in the art (e.g. Bartenshlager et al., 1993, J. Virol. 67, 3835-3844 and Tomei et al., 1993, J. Virol. 67, 4017-4026) as well as helicase-defective NS3 derivatives (e.g. Kim et al., 1997, J. Virol. 71, 9400 and Lin and Kim, 1999, J. Virol. 73, 8798-8807). Polymerase-defective NS5B derivatives are also available (e.g. Lohmann et al., 1997, J. Virol. 71, 8416-8428).

Other suitable NS derivatives may exhibit a modified cell presentation (e.g. plasma membrane anchorage by addition of appropriate signal and trans-membrane peptides) and/or modified post-translational processing with respect to the native NS counterpart.

Desirably, the resulting NS derivative retains immunogenic properties, in particular a capacity to stimulate a cell-mediated immune response, within the same range as or alternatively higher than the native NS counterpart. It is thus advisable to avoid modification(s) in portions rich in B, CTL and/or T_H epitopes that can be detrimental to the immunogenic property.

In one embodiment, at least two of the NS3, NS4 and NS5B polypeptides comprised in or encoded by the composition for use according to the invention, may originate from HCV of different genotypes, subtypes or isolates. Such a configuration may permit to provide protection against a broader range of HCV genotypes or to adapt the composition or therapeutic regimen to a specific geographic region by using HCV genotype(s) that is/are endemic in this region or to a specific population of patients. In this regard, genotypes 1a, 1b, 2 and 3 are the most prevalent in North America, Europe and Asia; genotype 4 is predominant in North and Central Africa; genotype 5 has so far been mostly identified in South Africa and genotype 6 isolates have been found primarily in Vietnam and Hong Kong. For example, using a NS3/NS4 polyprotein from a genotype 1 HCV and a NS5B polypeptide from genotype 3 or vice versa may permit to design a composition useful for treating Asian, North American and European subjects.

In another embodiment, the NS3, NS4 (e.g. NS3/NS4 polyprotein) and the NS5B polypeptides comprised in or encoded by the composition for use according to the invention originate from the same HCV genotype, subtype or isolate. Suitably, they all originate from a genotype 1 HCV, preferably from a genotype 1b with a specific preference for HCV-JA isolate origin.

Of particular interest is a NS3/NS4 polyprotein comprising an amino acid sequence having at least 80% of identity, advantageously at least 85% of identity, preferably at least 90% of identity, more preferably at least 95% of identity and even more preferably 98% of identity with the amino acid sequence shown in SEQ ID NO: 1. Still more preferred is a NS3/NS4 polyprotein comprising the amino acid sequence shown in SEQ ID NO: 1.

Alternatively or in combination, the NS5B polypeptide comprises an amino acid sequence having at least 80% of identity, advantageously at least 85% of identity, preferably at least 90% of identity, more preferably at least 95% of identity and even more preferably 98% of identity with the amino acid sequence shown in SEQ ID NO: 2. Still more preferred is a NS5B polypeptide comprising the amino acid sequence shown in SEQ ID NO: 2.

The NS polypeptides (e.g. the NS3/NS4 polyprotein and NS5B polypeptide) can be produced by recombinant means, employing the expression vectors (or infectious

viral particles) described herein. Typically, the method comprises (a) introducing an expression vector into a suitable host cell to produce a transfected or infected host cell, (b) culturing *in-vitro* said transfected or infected host cell under conditions suitable for growth of the host cell, (c) recovering the cell culture, and (d) optionally, purifying the NS polypeptide and polyprotein from the recovered cell and/or culture supernatant.

Expression vectors which are appropriate for the recombinant production of the NS polypeptides include, without limitation, bacteriophage, plasmid or cosmid vectors for expression in prokaryotic host cells such as bacteria (e.g. *E. coli, Bacillus subtilis or Listeria*); vectors for expression in yeast (e.g. *Saccharomyces cerevisiae*, *Saccharomyces pombe*, *Pichia pastoris*); baculovirus vectors for expression in insect cell systems (e.g. Sf 9 cells); viral and plasmid vectors for expression in plant cell systems (e.g. Ti plasmid, cauliflower mosaic virus CaMV; tobacco mosaic virus TMV); as well as viral and plasmid vectors for expression in higher eukaryotic cells or organisms. Typically, such vectors are commercially available (e.g. in Invitrogen, Stratagene, Amersham Biosciences, Promega, etc.) or available from depositary institutions such as the American Type Culture Collection (ATCC, Rockville, Md.) and have been the subject of numerous publications describing their sequence, organization and methods of producing, allowing the artisan to apply them. Introduction of an expression vector in the host cell may be carried out by a variety of means, including, but not limited to microinjection, $CaPO_4$- mediated transfection, DEAE-dextran-mediated transfection, lipofection/liposome fusion, gene gun, transduction, electroporation, viral infection, and the like. Host cells can be cultured in conventional fermentation bioreactors, flasks, and petri plates. Culturing can be carried out at a temperature, pH and oxygen content appropriate for a given host cell. No attempts will be made here to describe in detail the various expression systems, host cells and methods known for the recombinant production of polypeptides.

NS nucleotide sequences

The nucleotide sequences encoding the NS3, NS4 and NS5B polypeptides can be generated from any source using sequence data accessible in the art and the sequence information provided herein. A preferred embodiment is directed to the use of a nucleotide sequence encoding a NS3/NS4 polyprotein and a nucleotide sequence

encoding a NS5B polypeptide. Such nucleotide sequences may be isolated independently from HCV-containing cells, cDNA and genomic libraries, viral genomes or any prior art vector known to include it, by conventional molecular biology or PCR techniques. Alternatively, they may also be generated by chemical synthesis in automatized process (e.g. assembled from overlapping synthetic oligonucleotides or synthetic gene). Modification(s) may be generated by a number of ways known to those skilled in the art, such as chemical synthesis, site-directed mutagenesis, PCR mutagenesis, DNA shuffling, etc.

Nucleic acid modifications may also be envisaged for improving level expression in a particular host cell or subject. It has been indeed observed that, when more than one codon is available to code for a given amino acid, the codon usage patterns are highly non-random and the utilisation of codons may be markedly different between different hosts. As the nucleotide sequences encompassed by the invention are mostly of viral origin (HCV), they may have an inappropriate codon usage pattern for efficient expression in host cells such as bacterial, lower or higher eukaryotic cells. Typically, codon optimisation can be performed by replacing one or more "native" (e.g. HCV) codon corresponding to a codon infrequently used in the host cell or subject of interest by one or more codon encoding the same amino acid which is more frequently used in the host cell or subject of interest. It is not necessary to replace all native codons corresponding to infrequently used codons since increased expression can be achieved even with partial replacement. Moreover, some deviations from strict adherence to optimised codon usage may be made to accommodate the introduction of restriction site(s) into the resulting nucleotide sequences.

Further, expression in the host cell or subject can still be improved through additional modifications of the nucleotide sequences aimed to prevent clustering of rare, non-optimal codons and/or to suppress or modify at least partially negative sequence elements which are expected to negatively influence expression levels (e.g. AT-rich or GC-rich sequence stretches; unstable direct or inverted repeat sequences; RNA secondary structures; and/or internal cryptic regulatory elements such as internal TATA-boxes, chi-sites, ribosome entry sites, and/or splicing donor/acceptor sites).

In a preferred embodiment, the nucleotide sequence encoding the NS3/NS4 polyprotein comprises a nucleotide sequence having at least 80% identity, suitably at

least 85% of identity, advantageously at least 90% of identity, preferably at least 95% of identity, more preferably 98% of identity and, even more preferably, 100% of identity with the nucleotide sequence shown in SEQ ID NO: 3. Alternatively or in combination, the nucleotide sequence encoding the NS5B polypeptide comprises a nucleotide sequence having at least 80% identity, suitably at least 85% of identity, advantageously at least 90% of identity, preferably at least 95% of identity, more preferably 98% of identity and, even more preferably, 100% of identity with the nucleotide sequence shown in SEQ ID NO: 4.

Expression Vectors

A preferred aspect of the present invention is directed to an immunotherapy composition comprising one or two expression vector(s) encoding the at least NS3, NS4 and NS5B polypeptides described herein (e.g. the NS3/NS4 polyprotein and the NS5B polypeptide).

In the context of the invention, the term expression vector has to be understood broadly including plasmid and viral vectors.

A "plasmid vector" as used herein refers to a replicable DNA construct for use in various expression systems, “shuttle” vectors functioning in both prokaryotic and/or eukaryotic cells as well as “transfer” vectors for transferring polynucleotide(s) in a viral vector. Usually plasmid vectors contain selectable marker genes that allow host cells carrying the plasmid vector to be selected for or against in the presence of a corresponding selective drug. A variety of positive and negative selectable marker genes are known in the art. By way of illustration, an antibiotic resistance gene can be used as a positive selectable marker gene that allows a host cell to be selected in the presence of the corresponding antibiotic and a suicide gene can be used as a negative selectable marker gene that allows a host cell expressing such suicide gene to be killed upon addition of the corresponding prodrug.

Representative examples of suitable plasmid vectors include, without limitation, pBR type plasmids (e.g. pBR322), pUC (Gibco BRL), pBluescript (Stratagene), pREP4, pCEP4 (Invitrogen), pCI (Promega), pVAX (Invitrogen) and pgWiz (Gene Therapy System Inc).

The present invention also encompasses vectors (e.g. plasmid DNA) complexed to lipids or polymers to form particulate structures such as liposomes, lipoplexes or nanoparticles.

The term "viral vector" as used herein refers to a nucleic acid vector that includes at least one element of a virus genome and may be packaged into a viral particle or to a viral particle. The terms “virus”, “virions”, viral particles” and “viral vector particle” are used interchangeably to refer to viral particles that are formed when the nucleic acid vector is transduced into an appropriate host cell or cell line according to suitable conditions allowing the generation of infectious viral particles. In the context of the present invention, the term “viral vector” has to be understood broadly as including nucleic acid vector (e.g. DNA viral vector) as well as viral particles generated thereof. The term “infectious” refers to the ability of a viral vector to infect and enter into a host cell or subject. Viral vectors can be replication-competent, or can be genetically disabled so as to be replication-defective or replication-impaired. The term "replication-competent" as used herein encompasses replication-selective and conditionally-replicative viral vectors which are engineered to replicate better or selectively in specific host cells (e.g. infected cells).

Suitable viral vectors for use in this invention can be generated from a variety of different viruses (e.g. retrovirus, adenovirus, adenovirus-associated virus (AAV), poxvirus, herpes virus, measle virus, foamy virus, alphavirus, vesicular stomatis virus, etc). As described above, the term "viral vector" encompasses vector DNA, genomic DNA as well as viral particles generated thereof.

In one embodiment, the expression vector comprised in the composition for use in the invention is a poxviral vector. It can be obtained from any member of the *poxviridae* such as Orthopoxviruses, Parapoxviruses, Avipoxviruses (e.g. canarypox ALVAC and fowlpox), Capripoxviruses, Leporipoxviruses, Suipoxviruses, Molluscipoxviruses and Yatapoxviruses. Preferably, the poxvirus is an Orthopoxvirus with a specific preference for a vaccinia virus, especially a vaccinia virus (VV) selected from the group consisting of the VV Western reserve, Copenhagen (Goebel et al., 1990, Virol. 179, 247-266; Johnson et al., 1993, Virol. 196, 381-401), Wyeth and modified Ankara (MVA) (Antoine et al., 1998, Virol. 244, 365-396) strains, the latter being preferred in particular MVA 575 (ECCAC V00120707) and MVA BN (ECCAC

V00083008). The genomic sequences of a vast number of Poxviridae are described in the literature and in specialized data bank (e.g. Gene bank accession numbers NC_006998, M35027, U94848 and NC_005309 NC_001132 corresponding respectively to Western reserve, Copenhagen, MVA and Canarypox sequences) and the general conditions for constructing recombinant poxvirus are well known in the art.

The nucleotide sequences encoding the NS3/NS4 polyprotein and the NS5B polypeptide can be inserted in the same vector or independent vectors and in any location of the poxviral genome, preferably in a non-essential locus. When inserted in the same expression vector, they may be positioned in sense or antisense orientation each other. Thymidine kinase gene is particularly appropriate for insertion in VV Copenhagen and any deletion I-VI for insertion in MVA vector, insertion in deletion II or III being preferred.

Another viral vector suitable for use in the invention is an adenoviral vector. It can be derived from a variety of human or animal adenoviruses (e.g. canine, ovine, simian, etc). Any serotype can be employed. Desirably, the adenoviral vector is replication-defective and originates from a human Ad, and more particularly from a human Ad of a rare serotype, or from a chimpanzee Ad. Representative examples of human adenoviruses include subgenus C Ad2 Ad5 and Ad6, subgenus B Ad11, Ad34 and Ad35 and subgenus D Ad19, Ad24, Ad48 and Ad49. Representative examples of chimp Ad include without limitation AdCh3 (Peruzzi et al., 2009, Vaccine 27, 1293-1300), AdCh63 (Dudareva et al., 2009, Vaccine 27, 3501-3504) and any of those described in the art (see for example WO03/000283; WO03/046124; WO2005/071093; WO2009/073103; WO2009/073104; WO2009/105084; WO2009/136977 and WO2010/086189). Replication-defective adenoviral vectors can be obtained as described in the art, e.g by deletion of at least a region of the adenoviral genome or portion thereof essential to the viral replication, with a specific preference for deletion of E1 region (e.g. extending from approximately positions 459 to 3510 by reference to the sequence of the human adenovirus type 5 disclosed in the GeneBank under the accession number M 73260 and in Chroboczek et al., 1992, Virol. 186, 280-285). The present invention also encompasses vectors having additional deletion(s)/modification(s) within the adenoviral genome, especially in the non-essential

E3 region or in other essential E2, E4 regions as described in WO94/28152; Lusky et al., 1998, J. Virol 72, 2022-2032).

Other suitable viral vectors are morbillivirus which can be obtained from the paramyxoviridae family, with a specific preference for measles virus. Various attenuated strains are available in the art (Brandler et al, 2008, CIMID 31, 271-291; Singh et al., 1999, J. Virol. 73(6), 4823-4828), such as and without limitation, the Edmonston A and B strains (Griffin et al., 2001, Field's in Virology, 1401-1441), the Schwartz strain (Schwarz A, 1962, Am J Dis Child. 103, 216), the S-191 or C-47 strains (Zhang et al., 2009, J Med Virol. 81(8): 1477-1483). Insertion between P and M genes is particularly appropriate.

In accordance with the present invention, the nucleotide sequences comprised in the composition for use in the invention are in a form suitable for expression of the encoded NS polypeptides (e.g. the NS3/NS4 polyprotein and NS5B polypeptide) in a host cell or subject, which means that each of the nucleotide sequence is placed under the control of appropriate regulatory sequences. As used herein, the term "regulatory elements" refers to any element that allows, contributes or modulates the expression of a nucleotide sequence in a given host cell or subject, including replication, duplication, transcription, splicing, translation, stability and/or transport of the nucleotide sequence or its derivative (e.g. mRNA).

It will be appreciated by those skilled in the art that the choice of the regulatory sequences and especially the promoter can depend on such factors as the vector itself, the host cell, the level of expression desired, etc. In the context of the invention, the promoter can be constitutive directing expression of the nucleotide sequence in many types of host cells or specific to certain host cells (e.g. liver-specific regulatory sequences) or regulated in response to specific events or exogenous factors (e.g. by temperature, nutrient additive, hormone, etc) or according to the phase of a viral cycle (e.g. late or early). One may also use promoters that are repressed during the vector production step in response to specific events or exogenous factors, in order to circumvent potential toxicity of the expressed polypeptide(s).

Promoters suitable for constitutive expression in mammalian cells include but are not limited to the cytomegalovirus (CMV) immediate early promoter (Boshart et al.,

1985, Cell 41, 521-530), the RSV promoter, the adenovirus major late promoter, the phosphoglycero kinase (PGK) promoter (Adra et al., 1987, Gene 60, 65-74), the thymidine kinase (TK) promoter of herpes simplex virus (HSV)-1 and the T7 polymerase promoter. Vaccinia virus promoters are particularly adapted for expression in poxviral vectors. Representative example include without limitation the vaccinia 7.5K, H5R, 11K7.5 (Erbs et al., 2008, Cancer Gene Ther. 15, 18-28), TK, p28, p11 and K1L promoter, as well as synthetic promoters such as those described in Chakrabarti et al. (1997, Biotechniques 23, 1094-1097), Hammond et al. (1997, J. Virological Methods 66, 135-138) and Kumar and Boyle (1990, Virology 179, 151-158) as well as early/late chimeric promoters. Promoters suitable for measle-mediated expression include without limitation any promoter directing expression of measle transcription units (Brandler and Tangy, 2008, CIMID 31, 271-291). Liver-specific promoters include without limitation those of HMG-CoA reductase (Luskey, 1987, Mol. Cell. Biol. 7, 1881-1893); sterol regulatory element 1 (SRE-1; Smith et al., 1990, J. Biol. Chem. 265, 2306); albumin (Pinkert et al., 1987, Genes Dev. 1, 268-276); phosphoenol pyruvate carboxy kinase (PEPCK) (Eisenberger et al., 1992, Mol. Cell Biol. 12, 1396-1403); alpha-1 antitrypsin (Ciliberto et al., 1985, Cell 41, 531-540); human transferrin (Mendelzon et al., 1990, Nucleic Acids Res. 18, 5717-5721); and FIX (US 5,814,716) genes.

Those skilled in the art will appreciate that the regulatory elements controlling the expression of the NS (NS3/NS4 and NS5B) nucleotide sequences may further comprise additional elements for proper initiation, regulation and/or termination of transcription (e.g. polyA transcription termination sequences), mRNA transport (e.g. nuclear localization signal sequences), processing (e.g. splicing signals), and stability (e.g. introns and non-coding 5' and 3' sequences), translation (e.g. an initiator Met, tripartite leader sequences, IRES ribosome binding sites, Shine-Dalgarno sequences, etc.) into the host cell or subject and purification steps (e.g. a tag).

In a preferred embodiment, the composition for use in the invention comprises a MVA expression vector into the genome of which are inserted preferably in deletion III and in the same orientation, (a) a nucleotide sequence encoding the NS3/NS4 polyprotein placed under the control of the vaccinia pH5R promoter and (b) a nucleotide sequence encoding the NS5B polypeptide placed under the control of the

vaccinia p7.5K promoter. Preferably, the nucleotide sequence encoding the NS3/NS4 polyprotein comprises the nucleotide sequence shown in SEQ ID NO: 3 and the nucleotide sequence encoding the NS5B polypeptide comprises the nucleotide sequence shown in SEQ ID NO: 4.

If needed, the composition for use in the invention can further comprise one or more transgene(s), e.g. a gene of interest to be expressed together with the NS (NS3/NS4 and NS5B)-encoding nucleotide sequences in a host cell or subject aimed to improve therapeutic or protective activity to an HCV infection or any disease or condition caused by or associated with an HCV infection. Suitable transgenes include, but are not limited to, immunomodulators such as cytokines and any other antigen originating from a potentially co-infecting organism (e.g. HIV, tuberculosis mycobacterium, etc). If a transgene is used, it can be expressed from the expression vector encoding NS3/NS4 polyprotein and/or the NS5B polypeptide or from an independent vector for use in combination which can be the same or different with respect to the NS3/NS4 or NS5B-expressing vector.

According to another preferred embodiment, the expression vector(s) comprised in the composition for use in the invention is in the form of infectious viral particles. Typically, such viral particles are produced by a process comprising the steps of (a) introducing the viral expressing vector of the invention into a suitable host cell, (b) culturing said host cell under suitable conditions so as to allow the production of said infectious viral particle, (c) recovering the produced viral particle from the culture of said cell, and (d) optionally purifying said recovered viral particle.

A vast number of host cells can be used for producing the expression vector(s) in used in the invention. The host cells may be cultured as adherent cells or in suspension, in presence or absence of carriers and in conventional bioreactors, wave bioreactors, microcarriers, roller bottles, flasks or petri plates. Culturing is carried out at a temperature, pH and oxygen content appropriate for a given cell using batch, fed-batch, continuous systems, hollow fiber, and the like.

For example, poxviral vectors may be produced in avian cells such as chicken embryonic Fibroblast (CEF) prepared from chicken embryos obtained from fertilized eggs and duck cell lines (see e.g. WO03/076601, WO2007/077256, WO2009/004016,

WO2010/130756 and US2011-008872). The avian cells are typically cultured at a temperature comprised between 30°C and 37°C in an appropriate culture system to enable cell growth. The cultured cells are then infected with the expression pox vector and cultured under conditions which enable virus amplification (transcription of the viral genome, production of the viral proteins and packaging into infectious viral particles) for a time period varying from 1 to 14 days. In particular, the culture medium and temperature conditions used during the infection steps can be the same as or different from the culture medium and temperature used during the cell growth and/or virus amplification steps.

Replication-defective adenoviral vectors may be produced in a cell line providing *in trans* the defective function(s). Suitable cell lines for complementing E1-deleted adenoviral vectors include the 293 cells (Graham et al., 1997, J. Gen. Virol. 36, 59-72) as well as the HER-96 and PER-C6 cells (e.g. Fallaux et al., 1998, Human Gene Ther. 9, 1909-1917; WO97/00326) or any derivative of these cell lines.

A number of culture media are available in the art such as Dulbecco's Modified Eagle's Medium (DMEM, Invitrogen) or Basal Medium Eagle (BME, Invitrogen) which can be optionally supplemented with e.g. serum (e.g. Fetal Calf Serum (FCS)) and/or amino acid(s) (e.g. L-Glutamine). Cells can also be cultured in a medium free from animal product. Many media free from animal product have been already described and some of them are commercially available such as for instance 293 SFM II; 293-F Cells, SFM Adapted; 293-H Cells, SFM Adapted; 293fectin™ Transfection Reagent; CD 293 AGT™; CD 293 Medium; FreeStyle™ 293 Expression System; FreeStyle™ 293 Medium; FreeStyle™ 293-F Cells, SFM Adapted; VP-SFM; VP-SFM AGT™; Adenovirus Expression Medium (AEM) Growth Medium for PER.C6® Cells; CD 293 AGT™; CD 293 Medium ; SFM Adapted; EPISERF® Medium; OptiPro™ SFM (all available from Invitrogen).

The produced vectors may be recovered from the culture supernatant and/or from the host cells. The cells may be lysed to allow the liberation of the viral particles outside the producing cells. The disruption of the cell membrane may be carried out by various techniques well known in the art, including but are not limited to freeze/thaw, hypotonic lysis, detergent-mediated lysis, sonication and microfluidization, etc.

If needed, the recovered vectors may be purified. Various steps may be implemented, whatever the order of such steps, such as a clarification step allowing under suitable conditions the withdrawal of the cellular debris (e.g. by depth filtration), a concentration step allowing concentration of the vectors in the crude preparation (e.g. by microfiltration or ultrafiltration); a nuclease treatment step allowing the degradation of cellular DNA (e.g. by using benzonase) and/or one or more chromatographic step(s) (e.g. ion exchange, gel filtration, affinity, hydroxyapatite, etc. with a specific preference for anion exchange). For illustrative purposes, purification of poxviral particles can be performed as described in WO2007/147528 and WO2010/130753 whereas adenoviral vectors can be purified as described in WO96/27677, WO98/00524, WO98/22588, WO98/26048, WO00/40702, EP1016700 and WO00/50573. The conditions and technology to be used depend on factors such as net charge, molecular weight, hydrophobicity, hydrophilicity and will be apparent to those having skill in the art. Moreover, the level of purification will depend on the intended use.

Typically, the therapeutic agent comprised in or encoded by the immunotherapy composition for use in the invention is administered in a "therapeutically effective amount" which means an amount sufficient for producing a therapeutic benefit whether administered alone or in combination with one or more antiviral compounds. The therapeutically effective amount may vary as a function of various parameters, in particular the type of therapeutic agent (polypeptide, plasmid or viral vector), the mode of administration; the disease state; the age and weight of the subject; the nature and extent of symptoms; the kind of concurrent treatment (e.g. interferon and/or ribavirin and/or antiprotease therapy); and/or the frequency and dose of treatment. Further refinement of the calculations necessary to determine the appropriate amount is routinely made by a practitioner, in the light of the relevant circumstances.

For general guidance, suitable therapeutically effective amount of a viral vector-comprising composition varies from about 10^5 to about 10^{13} vp (viral particles), iu (infectious unit) or pfu (plaque-forming units) per dose depending on the vector and the quantitative technique used. For example, individual doses for administration of a vaccinia virus-based composition comprise from approximately $5x10^5$ to approximately 10^9 pfu, advantageously from approximately $5x10^6$ pfu to approximately 10^8 pfu,

preferably from approximately $5x10^6$ pfu to approximately $5x10^7$ pfu; and more preferably in an amount of approximately 10^7 pfu of poxvirus or MVA. Preferred doses for adenoviral vector compositions contain from about 10^5 to about 10^{12} vp, with a specific preference for doses of about $5x10^8$, about 10^9, about $5x10^9$, about 10^{10}, about $5x10^{10}$ vp or about 10^{11} vp. Techniques available to evaluate the quantity of vp, iu and pfu present in a dose are conventional in the art. The quantity of vaccinia virus vector present in a sample can be determined by routine titration techniques using permissive cells (e.g. BHK-21 or CEF) and suitable coloration (e.g. using neutral red; Talavera, 1992, Methods Mol. Biol. 8, 235-248) or immunostaining (e.g. using anti-MVA antibodies; Caroll et al., 1997, Virology 238, 198-211). The number of adenovirus particles is usually determined by measuring the A260 absorbance or by HPLC, iu titers by quantitative DBP immunofluorescence and pfu by counting the number of plaques following infection of permissive cells. Preferably the vp/iu ratio is below 50 in accordance with FDA guidelines.

A composition based on vector plasmids may be administered in doses of between 1µg and 25mg, advantageously between 10µg and 20mg, desirably between 100µg and 2mg, with a specific preference for doses comprising between 200µg and 1mg. Techniques available to evaluate the amount of plasmid vector present in a sample are conventional in the art (e.g. by spectrophotometry). A protein composition may be administered in doses of between 10ng and 20 mg, with a special preference for a dosage from about 0.1µg to about 2 mg of the immunogenic polypeptide per kg body weight.

The immunotherapy composition for use in the present invention is suitable for a variety of modes of administration, including parenteral and mucosal routes. Parenteral administration is preferred and especially intradermal, intraepithelial, intranasal, scarification, subcutaneous or intramuscular route, with a specific preference for subcutaneous route.

Moreover, the route of administration, the therapeutic agent and the therapeutically effective amount can vary for each administration of the immunotherapy composition. For illustrative purposes, the 4 to 8 administrations separated by a 3-10 days period of time may be carried out with a MVA vector-based immunotherapy

composition whereas the 4 to 15 administrations separated by a 3-5 weeks period of time may use an adenovirus vector-based or a plasmid vector-based immunotherapy composition. One may also envisage administration pattern combining various administration routes, e.g. subcutaneous and intramuscular administrations.

It is nevertheless preferred that all administrations of the immunotherapy composition are carried out by the same route of administration, preferably selected from the group consisting of intradermal, intramuscular and subcutaneous, the latter being preferred. Subcutaneous administration is especially suited for composition comprising viral expression vectors such as MVA and intramuscular for composition comprising plasmid expression vectors.

Administration may be carried out using conventional needles or devices adapted to the administration route, optionally, using one or more compound(s) or device(s) capable of facilitating or improving delivery of the active agent(s) (polypeptides or expression vector(s)). Suitable compounds include, but are not limited to, polycationic polymers (e.g. chitosan, polymethacrylate, PEI, etc) and cationic lipids (e.g.DC-Chol/DOPE, transfectam lipofectin available from Promega®) and suitable devices include without limitation those permitting electroporation- and pressure-mediated delivery. Administration via electroporation is particularly suited for intramuscular delivery of plasmid vector-based composition.

The immunotherapy composition for use in the invention is preferably administered to the subject according to an administration pattern comprising from 4 to 8 administrations separated from each other by approximately one week (designated herein after "weekly" administrations) preferably followed by from 4 to 15 administrations separated from each other by approximately one month (designated herein after "monthly" administrations). "Approximately one week" refers to 6, 7 or 8 days whereas "approximately one month" refers to a period of time of 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34 or 35 days. In the context of the invention, the period of time between two administrations may vary within the defined range.

A particularly preferred administration pattern comprises 6 weekly administrations followed by 5 to 12 monthly administrations, more preferably, 6 weekly

administrations followed by 6 to 11 monthly administrations and even more preferably 6 weekly administrations followed by 7 monthly administrations.

Immunotherapy composition

In one embodiment, the immunotherapy composition for use in the invention comprises a pharmaceutically acceptable vehicle further to the therapeutically effective amount of the active agent(s).

The term "pharmaceutically acceptable vehicle" is intended to include any and all carriers, solvents, diluents, excipients, adjuvants, dispersion media, coatings, antibacterial and antifungal agents, absorption delaying agents and the like compatible with administration in mammals and in particular human subjects.

Desirably, the composition for use in the invention is buffered at a physiological or slightly basic pH (e.g. from approximately pH 7 to approximately pH 9) in order to be appropriate for human or animal use. Suitable buffers include without limitation phosphate buffer (e.g. PBS), bicarbonate buffer and/or Tris buffer.

The composition for use in the invention can further comprise a diluent appropriate for human or animal use. It is preferably isotonic, hypotonic or weakly hypertonic and has a relatively low ionic strength. Representative examples include, but are not limited to, sterile water, physiological saline (e.g. sodium chloride), Ringer's solution, glucose, trehalose or saccharose solutions, Hank's solution, and other aqueous physiologically balanced salt solutions (see for example the most current edition of Remington : The Science and Practice of Pharmacy, A. Gennaro, Lippincott, Williams&Wilkins).

The pharmaceutically acceptable vehicle(s) may also permit to preserve the stability of the immunotherapy composition under the conditions of manufacture and long-term storage (i.e. for at least 6 months, with a preference for at least two years) at freezing (e.g. -70°C, -20°C), refrigerated (e.g. 4°C) or ambient temperatures whatever the composition form (e.g. frozen, liquid or solid). Solid (e.g. dry powdered or lyophilized) compositions can be obtained by a process involving vacuum drying and freeze-drying. For illustrative purposes, sterile phosphate buffer saline (PBS) and physiological saline are adapted to the preservation of plasmid vectors whereas buffered formulation including NaCl and sugar are appropriate for the preservation of viral

vectors, e.g. 10 mM Tris pH 8.5 with 1M saccharose, 150mM NaCl, 1mM $MgCl_2$ and 54 mg/l Tween 80 for adenoviral vector and Tris 10 mM pH 8 with saccharose 5 % (W/V), Sodium glutamate 10 mM, and NaCl 50 mM for MVA vectors.

Additional pharmaceutically acceptable excipients may be used for providing desirable pharmaceutical or pharmacodynamic properties, including for example osmolarity, viscosity, clarity, colour, sterility, stability, rate of dissolution of the formulation, delay release or absorption into a subject, promoting transport across the blood barrier or penetration in a particular organ (e.g. liver).

In addition, the immunotherapy composition for use in the invention may comprise one or more adjuvant(s). An adjuvant refers to a component capable of amplifying the immunogenic nature of an immunogen (e.g. HCV NS polypeptides) whether specific or non-specific, humoral or cellular. Desirably such adjuvant(s) is/are suitable for enhancing immunity, including a T cell-mediated immunity through the toll-like receptors (TLR), such as TLR-7, TLR-8 and TLR-9. Representative examples of useful adjuvants include without limitation alum, mineral oil emulsion such as Freunds complete and incomplete (IFA), lipopolysaccharide or a derivative thereof (Ribi et al., 1986, Immunology and Immunopharmacology of Bacterial Endotoxins, Plenum Publ. Corp., NY, p407-419), saponins such as QS21 (Sumino et al., 1998, J. Virol. 72, 4931-4939; WO 98/56415), imidazo-quinoline compounds such as Imiquimod (Suader, 2000, J. Am Acad Dermatol. 43: S6), S-27609 (Smorlesi, 2005, Gene Ther. 12: 1324-1332) and related compounds such as those described in WO2007/147529, cytosine phosphate guanosine oligodeoxynucleotides such as CpG (Chu et al., 1997, J. Exp. Med. 186, 1623-1631; Tritel et al., 2003, J. Immunol. 171, 2538-2547) and cationic peptides such as IC-31 (Kritsch et al., 2005, J. Chromatogr Anal. Technol Biomed Life Sci. 822, 263-270).

Combination with other therapeutic modalities

In a preferred embodiment, the immunotherapy composition is for use in combination with other therapeutic modalities intended to treat a subject infected with HCV.

The term "combination" and variation such as "combined use" refers to the action of administering in the same subject two or more entities, one of which being the

immunotherapy composition described herein. The two or more entities of the combination may be administered via different routes, dosage and according to different time schedule and for a different period of time.

The immunotherapy composition for use in the invention is preferably used in combination with one or more antiviral compound(s). An "antiviral compound" may be defined as a chemical compound or a biological molecule which is capable of inhibiting at least one step of the HCV cycle life either directly (e.g. by inhibiting a viral enzyme) or indirectly (e.g. by mimicking a viral substrate such as nucleoside and nucleotide analogs or inhibiting a cell protein required for the virus replication or entry) or capable of blocking virus infection (e.g. blocking antibodies), with the aim of reducing the quantity of HCV virus in an infected subject either in a transient or sustained way.

Representative examples of antiviral compounds are selected from the group consisting of interferon, ribavirin, NS3/4A protease inhibitors, NS5A inhibitors, NS5B polymerase inhibitors, nucleoside analogs, nucleotide analogs, immunomodulators such as interleukins and chemokines and host cell enzyme inhibitors such as cyclophilin inhibitors, or any functional analog thereof (e.g. Tiribavirin™; Valeant). Preferred antiviral compounds for use in combination with the immunotherapy composition described herein are interferon and/or ribavirin and optionally a NS3/4A protease inhibitor.

Typically, the antiviral therapy is provided to the subject for 24 to 48 weeks according to the genotype of the infecting HCV (e.g. 48 weeks for genotype 1 and 24 weeks for genotype 2 and 3), the type of antiviral therapy (bi or tritherapy) and tolerance of the subject. Preferably, the antiviral therapy comprises providing interferon and ribavirin for a period of 48 weeks (biotherapy). When the antiviral therapy further involves a NS3/4A protease inhibitor (tritherapy) such as Merck's boceprevir (Victrelis) or Vertex's telaprevir (Incivek or Incivio), the period of antiviral therapy may be reduced to 24 weeks. Alternatively, the antiviral therapy may be IFN-free providing one or more antiviral compounds in the absence of any interferon (e.g. NS3/4A protease and/or polymerase inhibitors). The antiviral therapy may also comprise providing one or more nucleoside / nucleotide analog(s) in the absence of any interferon or ribavirin.

Although any interferon can be provided in the context of the invention, it is desirably an interferon-alpha (IFNα), preferably a pegylated interferon-alpha and more preferably a pegylated interferon-alpha 2a (e.g. PEGASYS^{R}) or a pegylated interferon-alpha 2b (e.g. PEGINTRON^{R}). Interferon lambda may also be envisaged and, preferably, a pegylated interferon lambda. The dose, route and timing of administration can vary according to the type of interferon and the manufacturers and it is within the reach of the physician to adapt the proper modalities from one subject to another. Pegylated interferon alpha is usually provided by intramuscular or subcutaneous route in an amount of approximately 180µg per week for pegylated interferon-alpha 2a and of approximately 1.5µg per kilogram and per week for pegylated interferon-alpha 2b.

Ribavirin (e.g. COPEGUS^{R}) is a synthetic nucleoside analogue which chemical name is 1-β-D-ribofuranosyl-1-H-1, 2, 4-triazole-3-carboxamide. Ribavirin is commercially available in tablets for oral administration. Ribavirin is preferably provided orally at a dose ranging from approximately 1000 to approximately 1200 mg per day (e.g. 5 or 6 tablets of 200mg) depending on the subject weight (5 tablets of 200mg for subjects <75kg and 6 tablets of 200mg for subjects > 75kg) or other factors such as tolerance.

Viral protease inhibitors include but are not limited to NS3/4A protease inhibitors Telaprevir^{TM} (Vertex), boceprevir^{TM} (Merck), TMC435 (Tibotec), BI 201335 (Boehringer Ingelheim), ABT-450 (Abbott), BMS-650032 (Bristol-Myers Squibb) and RG7227 (danoprevir; Roche). For purpose of illustration, boceprevir^{TM} and telaprevir^{TM} are preferably provided orally at a dose of approximately 2400 and 2250 mg per day, respectively.

Inhibition of the NS5B polymerase disrupts HCV RNA synthesis and thus prevents the synthesis of genomic HCV RNA required for the production of new virions. Polymerase inhibitors can be assigned to two broad categories based on their mechanism of action and chemical structure: nucleoside analogs that bind competitively to the active site of the polymerase and non-nucleosidic analogs that are non-competitive inhibitors and bind to a number of discrete sites on NS5B. NS5B inhibitors in development include, but are not limited to, RG7128 (mericitabine; Roche), GS-9190 (Gilead), PSI-7977 and PSI-938 (Pharmasset), and VX-222 (Vertex).

The viral protein NS5A which is involved in viral replication and viral assembly is also a target of new antivirals in development such as BMS-790052 (Bristol-Myers Squibb).

In addition to the viral targets, host cell proteins involved in virus replication are also targets for drug developers. In particular cyclophilins (peptidyl-prolyl cis trans isomerases) involved in many cellular processes such as protein folding and trafficking are also required for HCV replication. DEB-025 (alispovir; Novartis) and SCY-635 (Scynexis) are host cyclophilin inhibitors undergoing clinical development.

In the context of the invention, the immunotherapy composition for use in the invention may be administered before providing antiviral therapy or concomitantly or after. Completely separated (e.g. antiviral therapy is provided after immunotherapy composition and started after completion of the immunotherapy treatment) or partially overlapping administrations of the immunotherapy composition and the antiviral therapy are encompassed by the present invention. In a preferred embodiment, administrations of the immunotherapy composition for use in the invention are started before providing said antiviral therapy, e.g. at least 4 weeks, desirably, at least 8 weeks, advantageously at least 10 weeks and preferably at least 12 weeks before providing one or more antiviral compound(s). In this consideration, a particularly suitable administration pattern comprises 6 weekly followed by one monthly administrations of the immunotherapy composition before providing antiviral therapy approximately 12 weeks after the first administration of the immunotherapy composition.

Additional administrations of the immunotherapeutic composition may be carried out over the period of antiviral therapy (e.g. 24 or 48 weeks), a portion of this period of time (i.e. less than 24 or 48 weeks) and may be pursued once the course of the anti-viral therapy has ended. More preferably, the immunotherapy composition for use in the invention is administered at monthly periodicity for 18 to 24 weeks, with a specific preference for about 21 weeks, following the initiation of the antiviral therapy, (e.g. interferon and ribavirin with optionally an NS3/4A inhibitor).

In the context of the invention, interferon may be provided to the subject the same day as the immunotherapy composition (e.g. the weekly PEG-IFNα2 may coincide with administration of the composition described herein) or interferon

injections and administrations of the immunotherapy composition can be separated by one or few days (2, 3, 4, 5 or 6 days).

An even more preferred administration pattern comprises 13 administrations of the immunotherapy composition for use in the invention, preferably at week 0, 1, 2, 3, 4, 5, 9, 13, 17, 21, 25, 29 and 33 and initiating interferon and ribavirin at week 12.

Still more preferably, the immunotherapy composition comprises a MVA vector encoding a NS3/NS4 polyprotein and a NS5B polypeptide and is administered to the subject by subcutaneous route at a dose comprising from about $5x10^6$ to about $1x10^8$ pfu, said interferon is a PEG-IFNα2 provided by intramuscular or subcutaneous route and said antiviral compound is ribavirin provided orally. More particularly preferred, the immunotherapy composition comprises approximately 10^7 pfu of a MVA vector in the genome of which is incorporated a nucleotide sequence encoding a NS3/NS4 fusion protein comprising an amino acid sequence having at least 95% identity with the amino acid sequence shown in SEQ ID NO: 1 and a nucleotide sequence encoding a NS5B polypeptide comprising an amino acid sequence having at least 95% identity with the amino acid sequence shown in SEQ ID NO: 2; said interferon is pegylated interferon alpha 2a provided to the subject weekly by subcutaneous route in an amount of approximately 180μg per week and said ribavirin is provided orally in an amount of 1000 to 1200 mg per day.

In another embodiment, the immunotherapy composition may be used in combination with IFN-free antiviral therapy, i.e. in combination with one or more antiviral compounds as described above except any IFN, e.g. in combination with ribavirin and/or viral protease inhibitor and/or polymerase inhibitor and/or nucleoside/nucleotide analogs. In this consideration, the ability of the immunotherapy composition to induce innate and adaptative immunity may complement the mechanism of action of the antiviral compounds and the efficacy of the immunotherapy composition is not expected to be affected if antiviral escape mutants emerge as point mutations will not interfere with the overall immunity provided by the immunotherapy composition. Such a modality may permit to reduce the IFN-associated side effects.

In another embodiment, the immunotherapy composition may be used in combination with ribavirin-free antiviral therapy, i.e. in combination with one or more antiviral compounds as described above except ribavirin or any analog thereof, e.g. in

combination with IFN and/or viral protease inhibitor and/or polymerase inhibitor and/or nucleoside/nucleotide analogs.

Therapeutic regimen

Another aspect of the present invention relates to a therapeutic regimen comprising the steps of administering to a subject the immunotherapy composition for use in the invention pursuant to the administration pattern described herein. Preferably the therapeutic regimen is carried out in combination with providing the subject with antiviral therapy as described herein, and especially the antiviral therapy currently used in the art for treating HCV infection and particularly chronic HCV infection (e.g. PEG-IFNα and ribavirin with optionally a NS3/4A inhibitor).

In the context of the invention, the immunotherapy composition is used or the therapeutic regimen carried out according to the modalities described herein to provide a therapeutic benefit against the HCV infection as compared to not using said immunotherapy composition (e.g. as compared to only providing antiviral therapy).

A therapeutic benefit may be ascertained by the ability of the immunotherapy composition or therapeutic regimen described herein to allow a reduction or alleviation of one or more symptoms usually associated with an HCV infection (e.g. liver inflammation, liver cytolysis, liver fibrosis, cirrhosis and/or hepatocarcinoma), diminishment of extent of disease, stabilized (i.e., not worsening) state of disease, preventing spread of disease, delay or slowing of disease progression or severity, amelioration or palliation of the disease state, reduction of the toxicity linked to the SOC, viral clearance (whether transient or sustained) as well as survival extension as compared to the expected symptoms, disease progression and survival if not receiving the immunotherapy composition or the therapeutic regimen described herein.

More particularly, the immunotherapy composition or therapeutic regimen described herein may contribute to the control of the HCV infection in combination with antiviral therapy, e.g. by increasing the proportion of patients that achieve viral load reduction or eradication at a given time point or by reducing the time period of antiviral therapy needed to achieve this goal, and/or by reducing the amount of antiviral therapy to achieve this goal and/or by reducing the frequency of antiviral therapy, etc.

As a general indication, the therapeutic benefit can be easily assessed by measuring any relevant marker(s) indicative of HCV infection typically considered by physicians or other skilled healthcare staff, for example, by quantifying HCV nucleic acid, and/or viral antigens at various time points in a biological sample collected from a subject. For example, a therapeutic benefit may be assessed upon comparing the level of such marker(s) measured at selected time points in the group of subjects being treated according to the invention with the level of the same marker(s) measured at the same time points and under the same conditions in the subjects not using said immunotherapy composition or therapeutic regimen. A particularly appropriate marker to assess the therapeutic benefit is to carry out repeated measurements of viral load in blood at various time points, translating such measurements in classic efficacy metrics like rapid viral response, early viral response, complete early viral response, end of treatment response and/or sustained viral response indicative of or associated with clearance (i.e. cure).

The "Viral load" is usually determined by quantifying HCV ribonucleic acid (RNA) in a given sample (e.g. blood or serum sample) collected from a subject, using a quantitative RT-PCR assay or by any other art-recognized methodology. Although the lower limit of quantitation (LLOQ) determining HCV-RNA unquantifiability may vary among the various assays, it is desirably less than 29 international units/mL (IU/ml), and preferably ≤15 IU/mL. A particularly suitable RT-PCR assay is the Roche TaqMan R assay (as described in the appended Examples) providing a LLOQ of 15 IU/ml and a lower limit of detection (LLOD) determining HCV RNA undetectably of less than 10 IU/mL.

As a general guidance, it is usual practice to determine the viral load in a subject at baseline (i.e. prior to initiation of the therapeutic regimen or the use of the immunotherapy composition according to the invention) and during the course of the therapeutic regimen or immunotherapy composition use. In the context of the embodiment where the immunotherapy composition is used and the therapeutic regimen carried out in combination with antiviral therapy, viral load may be estimated at various time points (e.g. before providing the antiviral therapy and after 2, 4 or 5, 12, 24, 36 and 48 weeks of antiviral therapy and 12 and 24 weeks after the end of antiviral therapy).

For example, a reduction of HCV-RNA of at least 0.5 $\log_{10}$ IU/mL between 2 time points may be predictive of a therapeutic benefit.

More specifically, a therapeutic benefit is ascertained when there is an increase of at least 5%, and preferably at least 10% of the proportion of the subjects achieving a significant reduction in the level of serum HCV RNA quantified after at a given time point (e.g. clearance of HCV RNA).

Advantageously, the immunotherapy composition is used or the therapeutic regimen carried out with the aim of increasing by at least 5%, and preferably at least 10% the proportion of subjects achieving ultra virological response (UVR) compared to only providing antiviral therapy for 2 weeks.

Desirably, the immunotherapy composition is used or the therapeutic regimen carried out with the aim of increasing by at least 5%, and preferably at least 10% the proportion of subjects achieving rapid virologic response (RVR) or even better of maintaining an extended RVR after 12 weeks of antiviral therapy compared to only providing antiviral therapy for 4 weeks.

Preferably, the immunotherapy composition is used or the therapeutic regimen carried out with the aim of increasing by at least 5%, and preferably at least 10% the proportion of subjects achieving early virologic response (EVR) or even better a complete early virologic response (cEVR) compared to only providing antiviral therapy for 12 weeks.

More preferably, the immunotherapy composition is used or the therapeutic regimen carried out with the aim of increasing by at least 5%, and preferably at least 10% the proportion of subjects achieving a sustained virologic response (SVR) compared to only providing antiviral therapy for 24 or 48 weeks.

"At least 5%" means 5% or any number above 5 comprised between 6 and 100, and at least 10% means 10% or any number between 11 and 100, with a specific preference for at least at least 11%, at least 12%, at least 13%, at least 14%, at least 15%, at least 16%, at least 17%, at least 18%, at least 19%, at least 20%, at least 21%, at least 22%, at least 23%, at least 24%, at least 25%, at least 26, at least 27%, at least 28%, at least 29%, at least 30%, at least 31%, at least 32%, at least 33%, at least 34% or at least 35%.

The expression “Ultra Virological Response” (UVR) as used herein refers to undetectability of HCV RNA after 2 weeks of antiviral therapy.

The expression “rapid virologic response” or “RVR” as used herein refers to undetectability of HCV RNA after 4 weeks of antiviral therapy.

The expression "extended rapid virologic response" or "eRVR" as used herein refers to undetectability of HCV RNA after both 4 and 12 weeks of antiviral therapy.

The expression “early virologic response” or “EVR” as used herein refers to a $\geq$ 2 log 10 IU/mL reduction in HCV RNA after 12 weeks of antiviral therapy.

The expression “complete early virologic response” or “cEVR” is defined herein as undetectability of the HCV RNA 12 weeks after 12 weeks of antiviral therapy.

The expression “end of treatment response” or “ETR” is defined herein as HCV RNA undetectable after completion of antiviral therapy.

The expression “sustained virologic response” or “SVR” as used herein refers to the maintenance of undetectability of HCV RNA 12 or 24 weeks after antiviral therapy completion.

Quantification of the viral load is preferably determined at the different time points using a RT-PCR assay providing a LLOD of less than 10UI/mL or any other methodology that is recognized in the art to have an equivalent sensitivity.

Alternatively or in combination, the therapeutic benefit can also be assessed by determining the level of liver enzyme activity (e.g. alanine aminotransferase (ALT) and/or aspartate aminotransferase (AST)) in the blood or serum of a subject and by assessing the fibrosis level in the liver (e.g. by Fibroscan^{R}). For example, a normalization of such enzymes or a reduction of the elasticity and/or fibrosis stage may be indicative of a better liver status.

In an advantageous aspect, the immunotherapy composition is used and the therapeutic regimen carried out with the goal of reducing the duration of antiviral therapy and/or reducing the doses or regimen of at least one of the antiviral compounds in the population of subjects chronically infected with HCV treated according to the present invention as compared to a population of subjects chronically infected with HCV and treated only with antiviral therapy, thus resulting in a reduced proportion of

antiviral-associated adverse events (e.g. at least 5%) in the population of HCV patients treated according to the invention.

In another aspect, the immunotherapy composition is used and the therapeutic regimen carried out with the goal of inducing or stimulating the immune system in the treated subject over the baseline status or over the expected status with only providing antiviral therapy (e.g. PEG-IFNα and ribavirin).

The induced or stimulated immune response can be non-specific (innate) and/or specific (anti-HCV and optionally anti-vector responses), humoral and/or cellular, with a specific preference for inducing or stimulating innate or specific T cell responses mediated by CD8+ T cells, CD4+ T cells or both CD8+ and CD4+ T cells. The specific anti-HCV immune response is preferably directed against one or more epitope present in any of NS3, NS4 and NS5B polypeptides. It is expected that the multiple and repeated administrations of the immunotherapy composition before providing the antiviral therapy permit to induce an effective innate and/or specific immune response in the treated subject that may be maintained upon the administrations performed during the course of antiviral therapy.

The ability of the above-described immunotherapy composition or therapeutic regimen to induce or stimulate an immune response in a treated subject can be evaluated by a variety of assays which are standard in the art (for a general description of techniques available to evaluate the onset and activation of an immune response, see for example Coligan et al., 1992 and 1994, Current Protocols in Immunology; ed J Wiley & Sons Inc, National Institute of Health). Measurement of cellular immunity can be performed by measurement of cytokine profiles produced by activated effector cells including those derived from CD4+ and CD8+ T-cells (e.g. quantification of IL-10 or IFNg-producing cells by ELIspot on PBMC obtained from the blood samples collected at various endpoints)), by determination of the activation status of immune effector cells (e.g. T cell proliferation assays by a classical [^{3}H] thymidine uptake), by assaying for antigen-specific T lymphocytes in a sensitized subject (e.g. peptide-specific lysis in a cytotoxicity assay). Humoral response may be determined by antibody binding and/or competition assays (e.g. see Harlow, 1989, Antibodies, Cold Spring Harbor Press).

In another aspect, the invention also concerns a method to increase by at least 5%, and preferably at least 10%, the proportion of subjects who achieved complete early virologic response (cEVR) in a population of subjects chronically infected with HCV, as compared to cEVR in a population of subjects chronically infected with HCV and treated only with antiviral therapy (e.g. interferon and ribavirin), the method comprising administering to the population of subjects the immunotherapy composition or carrying out the therapeutic regimen described herein so as to induce or stimulate a T cell-mediated immune response against one or more epitope present in HCV NS3, NS4 and/or NS5B polypeptide.

In another preferred embodiment, the immunotherapy composition is used and the therapeutic regimen carried out according to the invention for treating a chronically infected subject.

The term "Chronic HCV infection" refers to an infection with HCV persisting for more than 6 month in a subject. Although the natural course of chronic HCV infection/chronic hepatitis shows variability from a subject to another, chronic HCV infection often leads to liver-associated diseases (liver inflammation, liver fibrosis and ultimately hepatocarcinoma).

Preferably, the subject is chronically infected with an HCV being from the same genotype or subtype as any HCV from which originate the NS polypeptides comprised in or encoded by the immunotherapy composition for use in the present invention, with a specific preference for an infecting HCV of genotype 1 (1a or 1b). Alternatively, the subject is infected with an HCV from a different genotype of the HCV from which originates the NS therapeutic agent (e.g. an infecting HCV of genotype 3). More preferably, the subject is naïve of any prior interferon-based treatment for HCV before using the immunotherapy composition or carrying out said therapeutic regimen.

In another preferred embodiment, the subject to be treated is a naïve subject at baseline, and especially naïve of prior IFN therapy before using the immunotherapy composition or carrying out the therapeutic regimen described herein.

"Naïve" means that the subject or patient who is to be treated according to the modalities described herein has not been previously treated with any antiviral

compound approved for treating HCV infection. Thus, an IFN-naïve subject has not been previously treated with any IFNα compound approved for treating HCV infection.

Preferably, such "naïve" subject to be treated has a high viral load at baseline, i.e. before using the immunotherapy composition or carrying out the therapeutic regimen described herein.

In the context of the invention, a "high viral load at baseline" refers to a level of HCV RNA higher than 400 000 IU/mL, and preferably higher than 600 000 IU/mL.

Alternatively, the subject to be treated may be a prior non-responder or partial responder to a treatment for HCV (e.g. PEG-IFNα and ribavirin).

In another preferred embodiment, the subject to be treated is non cirrhotic. (i.e. having a fibrosis status below 4 (F0, F1, F2 or F3) as determined by routine exploration techniques such as fibroscan or biopsy).

In another aspect, the invention provides a kit of parts for use for treating an HCV infection and preferably a chronic HCV infection, wherein said kit comprises a plurality of containers and instructions for administering to a subject the immunotherapy composition or for carrying out the therapeutic regimen according to the modalities described herein.

Preferably, the kit comprises a) an immunotherapeutic composition comprising a vaccinia MVA vector as defined above; b) antiviral compounds such as an interferon and ribavirin; and c) directions for administering each of (a) and (b).

The invention has been described in an illustrative manner, and it is to be understood that the terminology which has been used is intended to be in the nature of words of description rather than of limitation. Obviously, many modifications and variations of the present invention are possible in light of the above teachings. It is therefore to be understood that within the scope of the appended claims, the invention may be practiced in a different way from what is specifically described herein.

All of the above cited disclosures of patents, publications and database entries are specifically incorporated herein by reference in their entirety to the same extent as if each such individual patent, publication or entry were specifically and individually indicated to be incorporated by reference.

LEGENDS OF THE FIGURES

Figure 1 illustrates a schematic representation of a randomized, open label phase II study in treatment-naïve patients with chronic genotype 1 hepatitis C. Arm A subjects were treated with the SOC consisting of Pegylated interferon alfa-2a (Pegasys®) and ribavirin (Copegus®). Arm C patients received multiple sub-cutaneous injections of TG4040 according to two different kinetics of administration (weekly and monthly basis) in combination with SOC.

Figure 2 illustrates a schematic representation of the virologic response rate (the percentage of patients with undetectable HCV RNA) measured in control Arm A (SOC treatment) and Arm C (SOC + TG4040) patients.

Figure 3 illustrates a schematic representation of the slope of viral load decline during the 12 weeks following SOC administration in control Arm A (SOC treatment) and Arm C (TG4040 + SOC) patients.

EXAMPLES

EXAMPLE 1: TG4040 Phase I study without SOC

TG4040 is described in WO2004/111082 and WO2008/113606, all of which are hereby expressly incorporated by reference in their entireties. TG4040 is a Modified Vaccinia virus Ankara (MVA) vector that comprises two expression cassettes cloned in the deletion III of the MVA backbone in the same orientation, the first cassette expressing a fusion between NS3 and NS4 proteins under the ph5r promoter and the second expressing NS5B under the p7.5 promoter. The NS3, NS4 and NS5B polypeptides originate from genotype 1b HCV-JA strain (Kato et al, 1990, Proc Natl Acad Sci USA 87, 9524–9528). TG4040 was stored at -80°C in Tris-HCl 10mM with saccharose 5%, sodium glutamate 10mM and NaCl 50mM buffered at pH 8. The TG4040 is supplied in individual clear glass vials. Each vial is intended for single use (i.e. one injection to one patient).

This example describes the results of a dose-escalating phase I study with TG4040 (TG4040.01) in non-cirrhotic, treatment-naïve patients chronically infected with hepatitis C virus (HCV genotype 1a or 1b). Three doses of TG4040 were administered by sub-cutaneous (SC) route, respectively 10^6, 10^7 and 10^8 pfu

corresponding to cohorts 1, 2 and 3. The dose 10^8 pfu was further explored in three extension cohorts (4, 5 and 6). Fourty patients included in this analysis received 3 weekly SC injections of TG4040 and a boost injection done 2, 4 or 6 months after the first injection at 10^8 pfu only. More specifically,

- cohort 1 (C1): 3 patients received 3 injections at 10^6 pfu, at day 1, day 8 and day 15;
- cohort 2 (C2): 3 patients received 3 injections at 10^7 pfu, at day 1, day 8 and day 15;
- cohort 3 (C3): 9 patients received 3 injections at 10^8 pfu, at day 1, day 8 and day 15 and one boost injection at month 6;
- cohort 4 (C4): 7 patients received 3 injections at 10^8 pfu, at day 1, day 8 and day 15 and one boost injection at month 6. Of note, those patients had a more advanced liver disease with high alanine aminotransferase (ALT) levels (between 2 and 5 times of the upper limit of normal [ULN] in the 2 months prior to inclusion) and fibrosis Stage $\geq$ F2;
- cohort 5 (C5): 9 patients received 3 injections at 10^8 pfu, at day 1, day 8 and day 15 and one boost injection at month 2;
- cohort 6 (C6): 9 patients received 3 injections at 10^8 pfu, at day 1, day 8 and day 15 and one boost injection at month 4.

The mean age of the 40 patients was 39.9 years. More male patients (57.5%) were enrolled. Genotype 1a and 1b were equally distributed in each dose group.

Virologic, biologic and immunologic markers were assessed at baseline (before the first TG4040 injection) and at different time points. Viral load (HCV-RNA quantification) was evaluated by quantitative RT-PCR (using the real-time Roche's TaqMan® assay) , MVA and HCV-specific cellular immune responses were assessed by ELISpot IFN-g and anti-MVA humoral responses were assessed both by ELISA (for total antibodies) and by a biological assay based on BHK-21 cells infection (for neutralizing antibodies).

Results

TG4040 was shown to be safe and well-tolerated. There was no SAE related to TG4040. The incidence of drug-related injection site induration was more common in

the highest dose groups (10^8 pfu) with 29 events in 18/34 patients (53%) compared to the low and medium dose groups (10^6 pfu or 10^7 pfu) with two events in 2/6 (33%) patients. The second most frequently reported adverse drug reaction (ADR) was injection site inflammation with 33 events reported by 16/40 patients (40%) followed by injection site erythema with 20 events in 13/40 (33%) patients.

Viral load decrease

Mean viral load at baseline was 5.02 log10 IU/mL in cohort 1, 4.97 log10 IU/mL in cohort 2 and 5.60 log10 IU/mL in cohort 3. When taking into account decreases in viral loads superior to 0.5log10IU/mL, a higher vaccine efficacy was noted with three weekly injections of the low and the medium doses as 100% of the patients (6/6) demonstrated at least once a viral load decrease superior to this cut-off. In contrast, three weekly injections of the highest dose led to a decrease superior to 0.5log10IU/mL in 21% of the patients (7/34). These results may be explained by a higher anti-MVA neutralizing antibody response when the highest dose is administered. Of note, for half of the 13 patients having shown at least one significant viral decrease, the NADIR was observed at Day 37, i.e. 3 weeks after the last TG4040 prime injection. The boost injection performed at the highest dosage in Cohorts 3 to 6 demonstrated no effect in term of viral load decrease.

ELISpot results

ELISpot IFN-γ assay was performed on patients' peripheral blood mononuclear cells (PBMC) after stimulation with 3 peptide pools from TG4040-derived NS3 and NS4b antigens, 2 peptide pools from TG4040-unrelated NS5a and a positive control CEF peptide pool. In cohorts 1 to 3, these results show that:

- Responses to TG4040-derived and –unrelated antigens were detectable at baseline in 3 out of 15 patients.
- After primary injections of TG4040, an increase in responses to TG4040-derived antigens but not to TG4040-unrelated NS5a antigen was observed, peaking at D22 and above the cut-off for 6 patients.

The detailed analysis per patient showed that significant responses were observed in cohorts 1 & 2 and that, for some of these patients, decrease of viral load and TG4040-specific immune responses were co-incident. Of note, cohort 3 patients with no viral load variation in response to TG4040 displayed only very weak and sporadic

TG4040-related immune responses if any. Moreover, the most significant TG4040 induced immune responses (IFN-γ effectors) are concomitant with the highest viral load decrease in these naive chronically infected patients. It can however not be concluded at this level that the highest dose does not lead to HCV-specific immune responses as a further optimization of the method used in Cohorts 4, 5 and 6 allowed the measurement of significant responses respectively in 71% (5/7), 89% (8/9) and 56% (5/9) of the patients. After the boost injection, the HCV-specific cellular responses were maintained in term of intensity in Cohort 5 (2-month boost) and further increased in Cohort 6 (4-month boost) in 78% (7/9) of the patients, whereas the boost was not effective in Cohort 4 (6-month boost).

Altogether, TG4040.01 study showed a favourable safety profile as well as promising antiviral efficacy associated with immunogenicity. Based on the efficacy combined with fewer reactions at injection site, the medium dose of 10^7 pfu was selected for further investigations.

EXAMPLE 2: TG4040 Phase II study in combination with SOC

This example describes a randomized, open label phase II study in treatment-naïve non cirrhotic patients with chronic genotype 1 hepatitis C. The patients were stratified by age (> versus ≤ 50 years) and by baseline viral load (> versus ≤ 400 000 IU/mL). The study included multiple sub-cutaneous injections of TG4040 according to two different kinetics of administration (weekly and monthly injections) in combination with the standard of care consisting of Pegylated interferon alfa-2a (Pegasys®) and ribavirin (Copegus®). The objectives of this study are to compare the efficacy and safety of TG4040 in combination with SOC versus SOC alone. A schematic illustration of this study is given in Figure 1.

The rationale behind the described study is to stimulate anti-HCV immunity by administrating multiple and repeated injections of TG4040 (6 weekly and 1 monthly) prior to initiating SOC and also to prevent the viral load rebound observed in TG4040.01 phase I study after the last vaccine injection (around 3 weeks). Administrations of TG4040 were pursued at a monthly periodicity after the introduction of SOC so as to maintain TG4040-mediated immunity in the treated patients while permitting SOC-mediated viral clearance.

Materials and Methods

TG4040 (described above in Example 1) was injected subcutaneously at 10^7 pfu.

Ribavirin (Copegus®) is a nucleoside analogue with antiviral activity. The chemical name of ribavirin is 1-ß-D-ribofuranosyl-1*H*-1, 2, 4-triazole-3-carboxamide. It is available as a oval-shaped, film-coated tablet for oral administration. Each tablet contains 200 mg of RBV and the following inactive ingredients: pregelatinized starch, microcrystalline cellulose, sodium starch glycolate, cornstarch and magnesium stearate. The daily dose of RBV will be 1000-1200 mg/day (weight-based) per os.

Pegylated interferon alfa-2a (Pegasys®) is a covalent conjugate of recombinant alfa-2a interferon with a single branched bis-monomethoxy polyethylene glycol (PEG) chain. The dose of Peg-IFN alfa 2a will be weekly SC injections of 180 µg supplied as prefilled single use syringes (180µg/0.5mL).

Study design

90 treatment naïve chronic HCV genotype 1 patients stratified according to age and viral load were enrolled. They underwent a 1:2 randomization with 31 patients were enrolled in the control arm A (PegIFN/RBV) and 59 patients enrolled in arm C to receive 10 to 13 injections of TG4040 (10^7 pfu) starting 12 weeks before initiation of PegIFN/RBV therapy.

In arm C, TG4040 was administered to the patients at the dose of 10^7 pfu pursuant to the following schedule, 6 SC injections once-weekly for 6 weeks followed by 4 to 7 SC injections every 4 weeks. Injections were done on thighs or arms, left and right, alternately. SOC was introduced twelve weeks after TG4040 initiation (corresponding to 7 TG4040 injections) and provided for 48 weeks. Altogether, patients in arm C received 33 weeks of TG4040 and 48 weeks of SOC. Twelve weeks after SOC initiation, patients continued TG4040 plus SOC if they achieved cEVR or not. At any time, patients who discontinued SOC due to intolerance were proposed to continue TG4040 alone every 4 weeks for up to 48 weeks. Of note, following to a safety amendment, injections of TG4040 were stopped during the trial; patients received an average of 12 injections.

In control **Arm A**, patients received the SOC consisting of subcutaneous administration of pegylated interferon alfa-2a (Pegasys®) at the dose of 180 µg once-weekly and oral administration of ribavirin (Copegus®) tablets at the dose of 1000-1200 mg/day, weight-based, for 48 weeks. After 12 weeks of SOC, patients who achieved EVR (defined as a viral load (VL) decrease ≥ 2 log10 IU/mL) continued SOC for 36 weeks whereas null patients who experienced no EVR stopped SOC treatment. At any time, patients who discontinued SOC due to intolerance were proposed to start TG4040 alone once-weekly for 6 weeks followed by a single sub-cutaneous injection every 4 weeks for up to 48 weeks (based on Arm C schedule).

All patients were followed up to 24 weeks after end of treatment or after having discontinued all study medication.

Methods

Follow-up included safety, immune parameters evaluation and HCV-RNA monitoring over time and relative to baseline.

Safety was assessed by evaluating the overall incidence of adverse event (AEs) and severe adverse events (SAEs) as well as laboratory assessments for each arm and for the study as a whole.

Virologic evaluation was performed by quantifying the serum Hepatitis C Virus (HCV) ribonucleic acid (RNA) on serum samples using Roche TaqMan® assay. The limit of detection is 10 IU/mL serum. Viral load was estimated at various end points, in particular after 2 weeks of SOC (evaluation of the percentage of patients who achieved ultra virologic response (UVR)), after 4 weeks of SOC (evaluation of the percentage of patients who achieved rapid virologic response (RVR)) after 12 weeks of SOC (evaluation of the percentage of patients who achieved the early virologic response (EVR) and the complete EVR (cEVR)), and after 24 weeks of SOC (the week of potential stopping rule application), at the end of SOC treatment (evaluation of the percentage of patients who achieved end of treatment response (ETR)), 12 and 24 weeks after the end of SOC treatment (evaluation of the percentage of patients who achieve the sustained virologic response 12 and 24 weeks after the end of SOC (SVR12 and SVR24)). Moreover, the slope of the different phases of viral clearance was

assessed (e.g. between baseline and week 1, between baseline and week 4, between week 4 and week 12 of SOC).

Kinetics of induction and duration of immune responses was evaluated at different time points.

in Arm A: at screening, at baseline and at Week 2, Week 12, Week 24 of SOC therapy and 24 weeks after end of SOC (i.e. at Week 72).

in Arm C: at screening, at baseline and at Week 9 (i.e. 4 weeks after the 6 weekly injections of TG4040), at Week 12 (before SOC initiation), at Week 14 (i.e. 2 weeks after SOC initiation), at Week 24 (i.e. 12 weeks of SOC), at Week 36 (i.e. 24 weeks of SOC) and at Week 84 (24 weeks after end of SOC).

HCV-specific cellular immune parameters was addressed by ELISpot IFN-γ after in vitro restimulation of PBMC with vaccine-derived antigens as well as vaccine-unrelated HCV reagents (15-mer peptides overlapping by 11 amino-acids will be used). Additional immune and inflammatory parameters may be analyzed to gain further into details in the pathways involved in TG4040 mechanism of action, e.g. by analysis of soluble mediators in serum including cytokines, of anti-MVA antibody and of MVA-specific cellular immune response. Total anti-MVA antibodies (plasma IgG) and/or neutralizing capacity of these antibodies was evaluated respectively by ELISA and by inhibition of in vitro infection of BHK21 cell line with MVA-GFP. At each time-point, 5 mL of blood was drawn for this purpose. Cellular mediated response to MVA was measured by ELISpot IFN-γ. Soluble mediators in serum were assessed by ELISA/Luminex technology. A total of 45 mL of blood was drawn for immunology evaluation: 5 mL for the humoral immune response and for soluble mediators' measurement and 40 mL for the cellular immune response.

Statistical analysis

The study assessed independently the cEVR rate for evaluable patients. The sample size was determined using a three outcome one stage design based on the following assumptions:

- the null hypothesis H_0 in terms of cEVR response rate (r) is $r < 40\%$;
- the alternative hypothesis H_A of efficacy is $r > 60\%$;
- the probability of a false positive result is equal to $\alpha = 2.5\%$ and the probability of false negative result is equal to $\beta = 10\%$;

- according to final evaluable set of patients (53 in Arm C), the null hypothesis was accepted if cEVR was observed in 25 patients or less and rejected if cEVR was observed in at least 29 patients;

- the 40% threshold was chosen based on the literature.

Results

The enrolled patients were distributed according to Table 1

	Arm A (n = 31)	Arm C (n = 59)
Mean Age in years	41	43.6
Gender, (n females / n males)	15/16	27/32
Caucasian, n (%)	30 (96.8)	59 (100)
Mean Baseline HCV RNA in log 10 IU/mL (SD)	5.96 (0.68)	5.71 (0.81)
HCV genotype, n (%) 1a 1b 1a/b	 6 (19.4) 25 (80.6) 0	 15 (25.4) 44 (74.6) 0
IL-28B n C-C / n non C-C (All data non available)	7/17	16/32
F3 Fibrosis, n (%) (Biopsy or Fibroscan)	1 (3.2)	7 (11.8)
High Baseline ALT (≥ 2 ULN), n (%)	5 (16.1)	16 (27.1)
Patients evaluables	30 (96.8%)	53 (89.8%)

Initial preliminary data have shown that twenty seven patients out of 59 who have received at least one injection of TG4040 in arm C displayed a more than 0.5 log10 IU/mL viral load decrease at one or more time-points, ranging from 0.50 to more than 2 log10 IU/mL consistent with phase I trial results. Interestingly, the antiviral effect was maintained during several weeks in half of the responding patients.

After SOC initiation in Arm C, in marked contrast and unexpectedly, a high proportion of patients reached the HCV RNA undetectability (or cEVR) in serum (less

than 10 IU/mL) after 10 administrations of TG4040 (6 weekly and one monthly before initiation of SOC and 3 monthly during the course of the 12 weeks of SOC). Under SOC, the Arm C patients also demonstrated sharp decrease of their viral load starting extremely early.

Preliminary data including primary end point achievement (see Figures 2 and 3)

The positive effect of TG4040 pre-vaccination on viral suppression was seen as early as one week after SOC initiation: the slope of viral load decline was significantly greater in Arm C, leading to 1.4 log10IU/mL decrease compared to 0.9 log10IU/mL in Arm A. Following respectively 1 and 2 weeks of SOC administration, the proportion of patients of Arm C having reached more than 1 $\log_{10}$ IU/mL decrease is 56 and 67% versus 35 and 58% in Arm A (W2 response).

Following 4 or 5 weeks of SOC administration in Arms A and C respectively, the proportion of patients of Arm C having reached HCV RNA undetectability is 23.6% (13/55 patients) versus 6.5% (2/31) in Arm A (RVR response).

Among the 31 and 59 enrolled patients in Arms A and C respectively, 30 and 53 patients were evaluable for cEVR. Following 12 weeks of SOC administration, the proportion of patients of Arm C having reached cEVR is 64% (34/53 patients) versus 30% in Arm A. The difference between cEVR rates in Arms A and C is statistically different (chi-quare test; $p = 0.003$).

When considering the patients who have started SOC treatment (ITT population), the proportion of patients of Arm C having reached undetectability after 24 weeks of SOC is 76.4% (42/55) compared to 67.7% (21/31) in Arm A. The advantage of Arm C pre-vaccination is also observed at ETR evaluation with 69% (38/55) compared to 64% (20/31) in Arm A.

Altogether, such data confirm the efficacy of the exemplified therapeutic regimen combining multiple and repeated administrations of TG4040 and SOC and providing TG4040 before initiating SOC. The primary objective of the study, the cEVR improvement was reached both in evaluable and ITT populations of Arm C with a good balance in terms of IL-28B polymorphism distribution (about 70% of non C-C per arm)

While TG4040 displayed significant but limited effect on viral load when used as stand alone (Example 1), the phase II study of Example 2 revealed a strong synergistic effect when TG4040 is used with PEG-IFN and ribavirin-based therapy. The administration pattern did not show any sign of liver's enzymes levels exacerbation, with grade 1 and reversible fluctuations of those enzymes were seen during TG4040 pre-vaccination period. Two safety events were observed in two Arm C patients, that may be explained by the exacerbation of IFN effects when TG4040 is added to SOC combination.

These results support the benefit of TG4040 as an active immunotherapy to be used as such or in combination with antiviral compounds, especially IFN-free antiviral cocktails.

CLAIMS

1. An immunotherapy composition comprising a therapeutically effective amount of an active ingredient selected from the group consisting of:
   - A polyprotein NS3/NS4 of a hepatitis C virus with a polypeptide NS5B of a hepatitis virus C;
   - An expression vector comprising a nucleotide sequence encoding a polyprotein NS3/NS4 of a hepatitis C virus and a nucleotide sequence encoding a polypeptide NS5B of a hepatitis virus ;
   - An expression vector comprising a nucleotide sequence encoding a polyprotein NS3/NS4 of a hepatitis C virus with an expression vector comprising a nucleotide sequence encoding a polypeptide NS5B of a hepatitis virus;

   for use as a medicament intended to treat an HCV infection wherein the administration pattern of the medicament comprises from 4 to 8 administrations of said immunotherapy composition separated from each other by a period of time varying from 3 to 10 days and optionally followed by from 4 to 15 administrations of said immunotherapy composition separated from each other by a period of time varying from 3 to 5 weeks.

2. The immunotherapy composition for use according to claim 1, wherein said NS3/NS4 polyprotein and said NS5B polypeptide or said nucleotide sequences encoding said NS3/NS4 polyprotein and said NS5B polypeptide originate from the same HCV genotype, preferably from a genotype 1, and more preferably from a genotype 1b.

3. The immunotherapy composition for use according to claim 2, wherein said NS3/NS4 polyprotein comprises an amino acid sequence having at least 80% of identity with the amino acid sequence shown in SEQ ID NO: 1.

4. The immunotherapy composition for use according to claim 2, wherein said NS5B polypeptide comprises an amino acid sequence having at least 80% of identity with the amino acid sequence shown in SEQ ID NO: 2.

5. The immunotherapy composition for use according to claim 3, wherein the nucleotide sequence encoding the NS3/NS4 polyprotein comprises a nucleotide sequence having at least 80% identity with the nucleotide sequence shown in SEQ ID NO: 3.

6. The immunotherapy composition for use according to claim 4, wherein the nucleotide sequence encoding the NS5B polypeptide comprises a nucleotide sequence having at least 80% identity with the nucleotide sequence shown in SEQ ID NO: 4.

7. The immunotherapy composition for use according to anyone of claims 1 to 6, wherein said expression vector is a plasmid vector or a viral vector.

8. The immunotherapy composition for use according to claim 7, wherein said viral vector is a poxviral vector, preferably a vaccinia virus vector selected from the group consisting of the the Western reserve, Copenhagen, Wyeth and modified Ankara (MVA) strains.

9. The immunotherapy composition for use according to claim 8, wherein said immunotherapy composition comprises a MVA expression vector into the genome of which are inserted, preferably in deletion III and in the same orientation, (a) a nucleotide sequence encoding the NS3/NS4 polyprotein placed under the control of the vaccinia pH5R promoter and (b) a nucleotide sequence encoding the NS5B polypeptide placed under the control of the vaccinia p7.5K promoter.

10. The immunotherapy composition for use according to anyone of claims 1 to 9, wherein said immunotherapy composition further comprises a pharmaceutically acceptable vehicle.

11. The immunotherapy composition for use according to anyone of claims 1 to 10, which is suitable for parenteral administration and especially intradermal, scarification, subcutaneous or intramuscular route.

12. The immunotherapy composition for use according to anyone of claims 8 to 11, wherein individual doses for administration of a vaccinia virus comprising composition comprise from approximately $5x10^6$ pfu to approximately 10^8 pfu, preferably from approximately $5x10^6$ pfu to approximately $5x10^7$ pfu; and more preferably in an amount of approximately 10^7 pfu of poxvirus or MVA vector.

13. The immunotherapy composition for use according to anyone of claims 1 to 12, wherein said administration pattern comprises from 4 to 8 administrations of said immunotherapy composition separated from each other by approximately one week followed by from 4 to 15 administrations separated from each other by a period of time of approximately one month.

14. The immunotherapy composition for use according to claim 13, wherein said administration pattern comprises 6 weekly administrations followed by 5 to 12 monthly administrations, more preferably, 6 weekly administrations followed by 6 to 11 monthly administrations and even more preferably 6 weekly administrations followed by 7 monthly administrations.

15. The immunotherapy composition for use according to anyone of claims 1 to 14, wherein said immunotherapy composition is used in combination with antiviral therapy.

16. The immunotherapy composition for use according to claim 15, wherein said antiviral therapy comprises providing one or more antiviral compounds selected from the group consisting of interferon, ribavirin, NS3/4A protease inhibitors, NS5A inhibitors, NS5B polymerase inhibitors, nucleoside analogs, nucleotide analogs, immunomodulators and host cell enzyme inhibitors or any functional analog thereof.

17. The immunotherapy composition for use according to claim 16, wherein said one or more antiviral compounds are interferon and/or ribavirin and optionally a NS3/4A protease inhibitor.

18. The immunotherapy composition for use according to claim 17, wherein said interferon is an interferon-alpha (IFNα), preferably a pegylated interferon-alpha and more preferably a pegylated interferon-alpha 2a or a pegylated interferon-alpha 2b or an interferon lambda.

19. The immunotherapy composition for use according to claim 18, wherein said pegylated interferon-alpha is provided by intramuscular or subcutaneous route in an amount of approximately 180µg per week for pegylated interferon-alpha 2a and of approximately 1.5µg per kilogram and per week for pegylated interferon-alpha 2b.

20. The immunotherapy composition for use according to claim 18, wherein said ribavirin is provided orally at a dose ranging from approximately 1000 to approximately 1200 mg per day.

21. The immunotherapy composition for use according to claim 16 or 17, wherein said NS3/4A protease inhibitor is provided orally at a dose of approximately 2250 or 2400 mg per day.

22. The immunotherapy composition for use according to anyone of claims 17 to 21, wherein said interferon and said ribavirin are concurrently provided to the subject for a period of between 24 and 48 weeks, and preferably for 48 weeks.

23. The immunotherapy composition for use according to anyone of claims 15 to 17 and 20-22 wherein said antiviral therapy is free of interferon.

24. The immunotherapy composition for use according to anyone of claims 15 to 23, wherein said immunotherapy composition is administered at least 4 weeks, advantageously at least 8 weeks, desirably at least 10 weeks and preferably at least 12 weeks before providing the antiviral therapy.

25. The immunotherapy composition for use according to claim 24, wherein said administration pattern comprises 6 weekly followed by one monthly administrations of the immunotherapy composition before providing the antiviral therapy approximately 12 weeks after the first administration of the immunotherapy composition.

26. The immunotherapy composition for use according to anyone of claims 15 to 25, wherein said immunotherapy composition is administered at monthly periodicity for 18 to 24 weeks and preferably for about 21 weeks following the initiation of the antiviral therapy.

27. The immunotherapy composition for use according to anyone of claims 24 to 26 , wherein said administration pattern comprises 13 administrations of the immunotherapy composition, preferably at week 0, 1, 2, 3, 4, 5, 9, 13, 17, 21, 25, 29 and 33 and providing interferon and ribavirin at week 12.

28. The immunotherapy composition for use according to any one of claims 15 to 27, wherein said immunotherapy composition comprises a MVA vector encoding a NS3/NS4 polyprotein and a NS5B polypeptide and is administered to the subject by subcutaneous route at a dose comprising from about $5x10^6$ to about $1x10^8$ pfu, said interferon is a PEG-IFNα2 provided by intramuscular or subcutaneous route and said antiviral compound is ribavirin provided orally.

29. The immunotherapy composition for use according to claim 28, wherein said immunotherapy composition comprises approximately 10^7 pfu of a MVA vector in the genome of which is incorporated a nucleotide sequence encoding a NS3/NS4 fusion protein comprising an amino acid sequence having at least 95%

identity with the amino acid sequence shown in SEQ ID NO: 1 and a nucleotide sequence encoding a NS5B polypeptide comprising an amino acid sequence having at least 95% identity with the amino acid sequence shown in SEQ ID NO: 2; said interferon is pegylated interferon alpha 2a provided to the subject weekly by subcutaneous route in an amount of approximately 180µg per week and said ribavirin is provided orally in an amount of 1000 to 1200 mg per day.

30. A therapeutic regimen comprising the steps of administering to a subject the immunotherapy composition for use according to anyone of claims 1 to 29.

31. The therapeutic regimen according to claim 30, wherein said therapeutic regimen is carried out in combination with providing the subject with antiviral therapy as defined in any one of claims 15 to 29.

32. The immunotherapy composition for use according to anyone of claims 15 to 29 or the therapeutic regimen according to claim 30 or 31, wherein the proportion of subjects achieving a rapid virologic response (RVR) is increased by at least 5%, and preferably at least 10%, compared to only providing antiviral therapy for 4 weeks.

33. The immunotherapy composition for use according to anyone of claims 15 to 29 or the therapeutic regimen according to claim 30 or 31, wherein the proportion of subjects achieving en early virologic response (EVR) or a complete early virologic response (cEVR) is increased by at least 5%, and preferably at least 10%, compared as only providing antiviral therapy for 12 weeks.

34. The immunotherapy composition for use according to anyone of claims 15 to 29 or the therapeutic regimen according to claim 30 or 31, wherein the proportion of subjects achieving a sustained virologic response (SVR) is increased by at least 5%, and preferably at least 10%, compared to only providing antiviral therapy for 24 or 48 weeks.

35. The immunotherapy composition for use according to anyone of claims 1 to 29 and 32 to 34 or the therapeutic regimen according to anyone of claims 30 to 34, for use for inducing or stimulating the immune system in the subject over the baseline status or over the expected status with only providing antiviral therapy.

36. The immunotherapy composition for use according to anyone of claims 1 to 29 and 32 to 35 or the therapeutic regimen according to anyone of claims 30 to 35, for use for treating a chronically infected subject.

37. The immunotherapy composition for use according to claim 36 or the therapeutic regimen according to claim 36, wherein the subject is chronically infected with of genotype 1 HCV.

38. The immunotherapy composition for use according to claim 36 or 37 or the therapeutic regimen according to claim 36 or 37, for use in a subject naïve of any prior interferonα-compound approved for treating an HCV infection before initiating said administration pattern.

39. The immunotherapy composition for use according to anyone of claims 36 to 38 or the therapeutic regimen according to anyone of claims 36 to 38, wherein the subject has a high viral titer at baseline.

40. A kit of parts for use for treating an HCV infection and preferably a chronic HCV infection, wherein said kit comprises a plurality of containers and instructions for administering to a subject an immunotherapy composition for use according to anyone of claims 1 to 29 and 32 to 39, or in accordance with the therapeutic regimen according to anyone of claims 30 to 39.

41. The kit according to claim 40, comprising: a) an immunotherapeutic composition as defined in anyone of claims 1 to 29 and 32 to 39; b) antiviral compounds such as an interferon and ribavirin; and c) directions for administering each of (a) and (b).

42. A method to increase by at least 5%, and preferably at least 10%, the proportion of subjects who achieved complete early virologic response (cEVR) in a population of subjects chronically infected with hepatitis C virus (HCV), as compared to cEVR in a population of subjects chronically infected with HCV and treated only with antiviral therapy, the method comprising administering to the population of subjects the immunotherapy composition for use according to anyone of claims 1 to 29 and 32 to 39 or a therapeutic regimen according to anyone of claims 30 to 39 so as to induce or stimulate a T cell-mediated immune response against one or more epitope present in HCV NS3, NS4 and/or NS5B polypeptide.

Figure 1

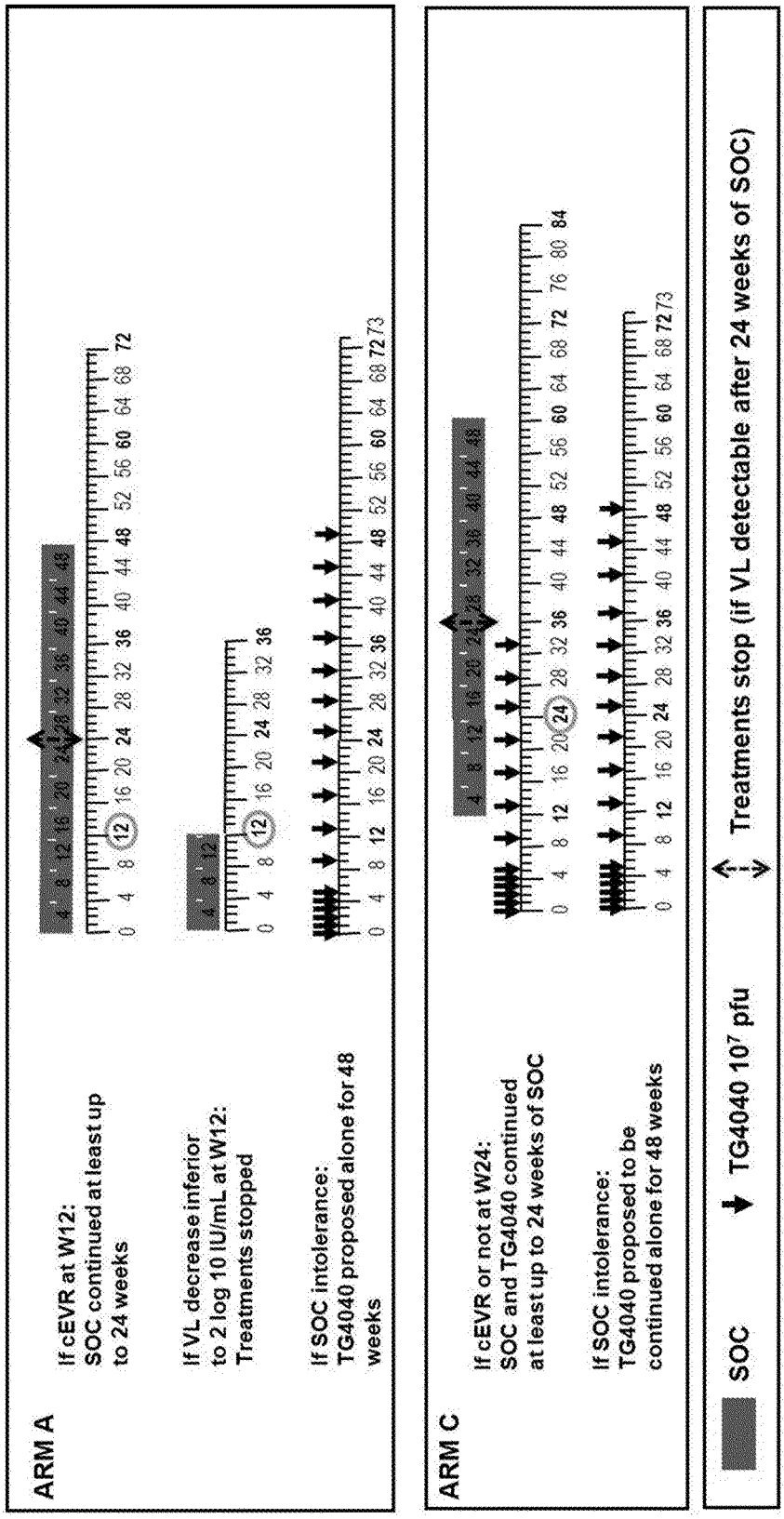

ARM A
If cEVR at W12:
SOC continued at least up to 24 weeks
If VL decrease inferior to 2 log 10 IU/mL at W12:
Treatments stopped
If SOC intolerance:
TG4040 proposed alone for 48 weeks
ARM C
If cEVR or not at W24:
SOC and TG4040 continued at least up to 24 weeks of SOC
If SOC intolerance:
TG4040 proposed to be continued alone for 48 weeks
SOC
TG4040 10^7 pfu
Treatments stop (if VL detectable after 24 weeks of SOC)

Figure 2

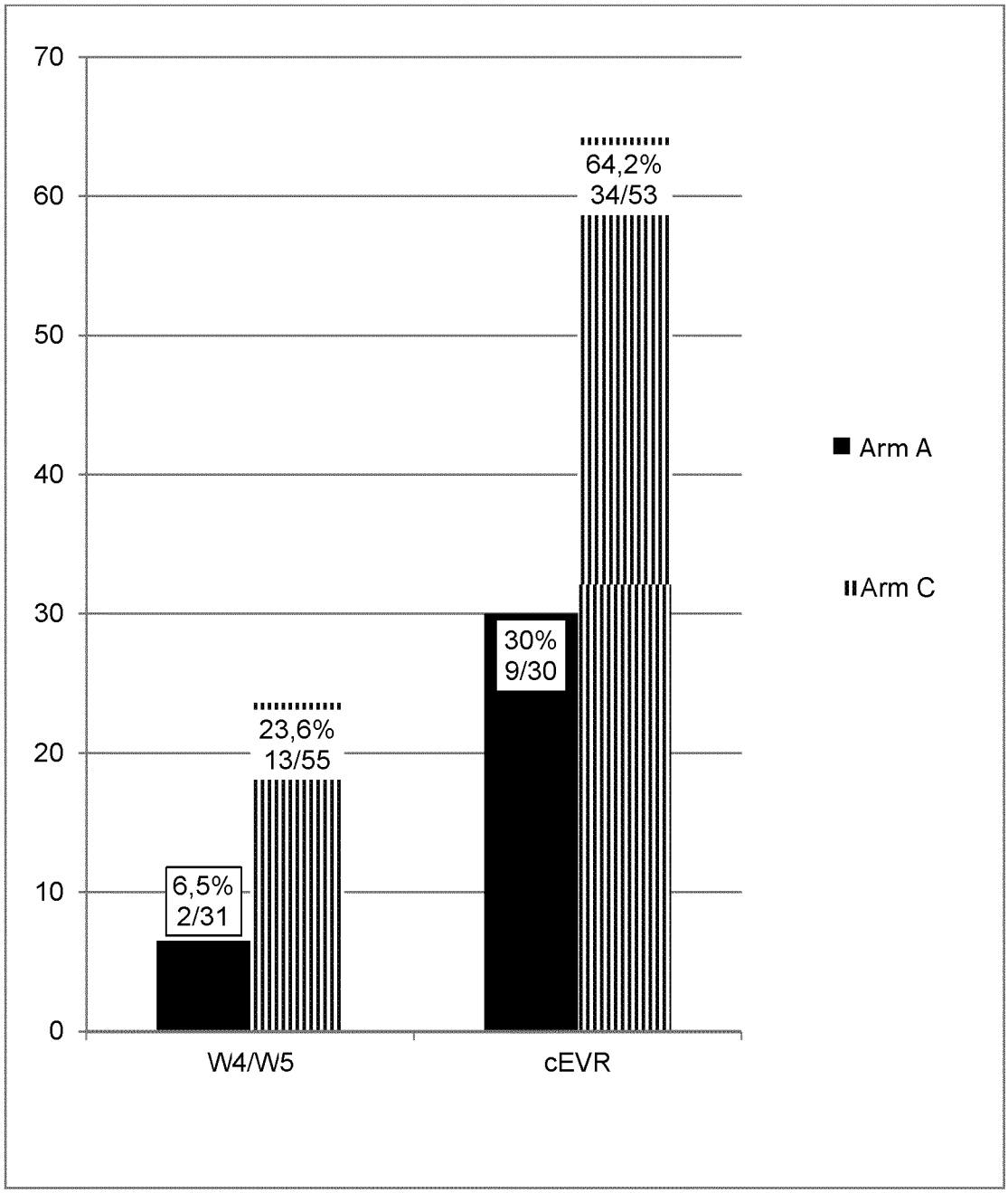
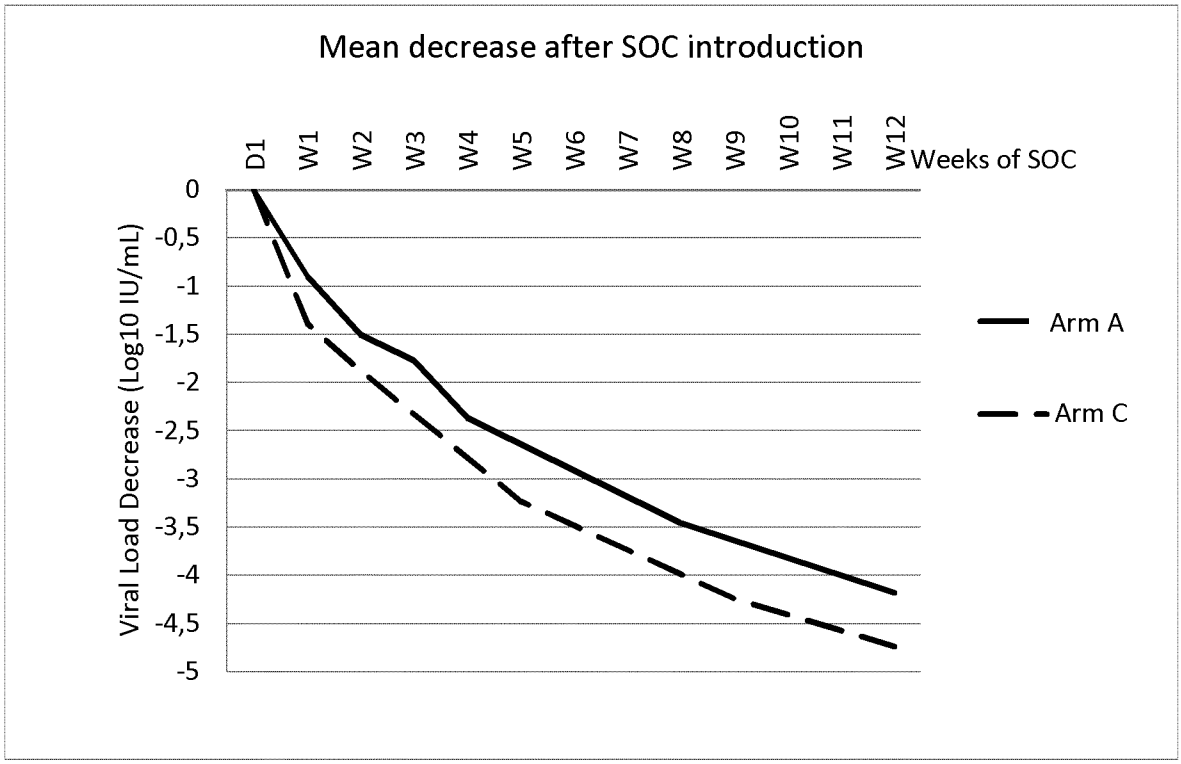

70
60
50
40
30
20
10
0
64,2%
34/53
30%
9/30
23,6%
13/55
6,5%
2/31
W4/W5
cEVR
Arm A
Arm C

Figure 3


Mean decrease after SOC introduction
D1
W1
W2
W3
W4
W5
W6
W7
W8
W9
W10
W11
W12
Weeks of SOC
0
-0,5
-1
-1,5
-2
-2,5
-3
-3,5
-4
-4,5
-5
Viral Load Decrease (Log10 IU/mL)
Arm A
Arm C