REPORT

Retroviral integration: A critical step in viral life cycle suitable for drug intervention

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ABSTRACT

Retroviral integration is an essential stage in the life cycle of retroviruses. Viruses need to insert their DNA into the chromosomal DNA of the host cells. Retroviral integration is a complex process which involves viral and host proteins and requires the movement of the retroviral DNA from the cytoplasm to the nucleus. The viral integrase is a key enzyme in this process that catalyzes two reactions: 3´ processing of viral DNA that take place in the cytoplasm following reverse transcription and the strand transfer that inserts viral DNA in a host cell chromosome. Development of a successful treatment for the human immunodeficiency virus (HIV) infection using the strand transfer inhibitor, Raltegravir, has demonstrated that retroviral integration is a suitable step for drug intervention. Different types of inhibitors targeting HIV integrase or any of the other components of the retroviral integration process are currently being designed or developed. This work contains a summary of the retroviral integration process as well as, many of the latest advances in this topic which were exposed at the recently held IVth International Meeting on Retroviral Integration.

Keywords: Retroviral integration, HIV-1 integrase, host proteins, LEDGF/p75

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RESUMEN

Integración retroviral: Una etapa crítica del ciclo replicativo viral apropiada para intervención terapéutica. La integración retroviral es una etapa esencial en el ciclo de vida de los retrovirus. Ellos necesitan insertar su material genético en el ADN cromosomal de las células hospederas. La integración retroviral es un proceso complejo donde intervienen proteínas virales y del hospedero y requiere el movimiento del material genético viral desde el citoplasma hasta el núcleo. La integrasa viral es una enzima clave en este proceso que cataliza dos reacciones: El procesamiento 3´ del ADN viral que tiene lugar en el citoplasma después de la transcripción reversa y la transferencia de cadena que inserta el ADN viral en un cromosoma de las células del hospedero. El desarrollo de un tratamiento exitoso contra la infección del VIH con el inhibidor de la transferencia de cadena, Raltegravir, ha demostrado que la integración retroviral es una etapa apropiada para la intervención terapéutica. Actualmente se diseñan diferentes tipos de inhibidores que tienen como blanco la integrasa del VIH o alguno de los otros componentes del proceso de integración retroviral. Este trabajo recoge una reseña del proceso de integración retroviral así como, muchos de los últimos avances en la temática que se expusieron en la recién celebrada IV Conferencia Internacional sobre Integración Retroviral.

Palabras clave: Integración retroviral, integrasa del VIH-1, proteínas del hospedero, LEDGF/p75

*I*ntroduction

HIV/AIDS epidemic rises in developed and developing countries. The treatment of this disease is based on antiretrovirals combination known as Highly Active Antiretroviral Therapy (HAART). This therapy has increased substantially the quality of life and life expectancy of people living with HIV in developed countries. In United Kingdom for example, an analysis of patients aged 20 and over whom started treatment with at least three HIV drugs between 1996 and 2008 showed that life expectancy for an average 20-year-old infected with HIV increased from 30 years to almost 46 between the periods 1996 to 1999 and 2006 to 2008. During the period 1996 to 2008, life expectancy was 40 years for male patients and 50 years for female patients, compared with 58 years for men and nearly 62 years for women in the general UK population [1]. Although the combined therapy allows the reduction of morbidity and mortality, it also has major limitations. It is expensive and requires an infrastructure with a functional health care system allowing the medical monitoring of the success of antiretroviral therapy to prevent or at least delay the emergence of drug-resistant HIV-1 strains. Thus, while antiretroviral combined therapy constitutes an effective approach for the treatment of AIDS and also prevents HIV-1 transmission by reducing the viral loads, it still has little impact on the global spread of the virus and the global number of fatalities caused by AIDS [2]. Even under optimal conditions, HAART has significant drawbacks, e.g. it is frequently associated with significant side effects (such as metabolic and cardiovascular disorders), with immune reconstitution disease, and with the development of resistant HIV-1 strains. Furthermore, HAART requires life-long daily treatment because it does not allow to eliminate resting long-lived cells containing integrated proviruses hence fails to eradicate the virus entirely [2]. Drug resistance is the most important problem of HAART. It limits therapeutic options, carries out rising of viral load, reduction of CD4+ cells, anticipation of symp-

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toms and the mortality risk is increased. According to this, it is important to scientists to have continuity in their researchers to find new drugs with different modes of action.

The current standard of treatment consists of a combination of antiretroviral drugs, which includes nucleoside and non-nucleosides reverse transcriptase inhibitors, protease inhibitors, and/or entry inhibitors. These antiretroviral agents target various stages in the viral life cycle [3]. Recently, a new class of therapeutic agents targeted HIV-1 integration.

On this field, the IVth International Meeting on Retroviral Integration was celebrated last October in the beautiful city of Siena, Italy. The first meeting was celebrated in Bethesda in 1995, then in Paris, 2001; and then in Woods Hole, 2008; where the participants decided to organize the meeting with an interval of 3 years.

*R*etroviral integration

Retroviruses like HIV need to insert their genomes into hosts' genomes to establish productive infections. This process is operated by the intasome, a nucleoprotein complex composed of the viral integrase (IN) tetramer assembled on the viral DNA ends. The intasome engages chromosomal DNA within a target capture complex to carry out strand transfer, irreversibly joining the viral and cellular DNA molecules [4].

Integration of viral DNA into the host cell chromosome involves several coordinated steps (i.e., processing of the viral DNA ends, joining of those ends to target DNA, and repairing the gaps)[5]. The first two reactions are catalyzed by the viral IN protein, whereas the last is mediated by cellular factors. The viral genomic RNA is reverse transcribed to form a molecule of linear double-stranded DNA, the precursor of the integrated provirus. The provirus is co-linear with unintegrated linear viral DNA but differs from the reverse transcription product in missing two or three bases from each end. Flanking the integrated provirus there are repetitions of cellular DNA which are usually 4-6 base pairs in length, depending on the virus. This duplication of cellular sequences that flank the viral DNA is generated as a result of the integration mechanism. Linear viral DNA is contained in a nucleoprotein complex with viral and host proteins in the cytoplasm of infected cells, called pre-integration complex (PIC). These complexes have shown to mediate the integration of viral DNA into target DNA *in vitro*. The provirus is the result of two reactions catalyzed by the viral IN: terminal cleavage and strand transfer [5-7].

IN is sufficient for both 3' end cleavage and joining of the viral DNA to the cellular chromosome or naked target DNA. Most of the IN proteins catalyze the removal of two bases in the 3' end of each strand of viral DNA. This terminal cleavage is necessary for a proper integration allowing the virus to create a standard terminal in the viral DNA that can be heterogeneous due to terminal transferase activity of reverse transcriptase. In addition, the terminal rupture step is coupled to the formation of a stable complex of DNA-IN. After terminal cutting, the hydroxyl is exposed and immediately follows a CA dinucleotide. This CA is conserved between retroviruses which are related to the transposons. Some evidences suggest that more internal Long Terminal Repeat sites are also important for integration. After the processing of the terminal, IN catalyzes the union covalent of hydroxyl groups of DNA to extreme 5' ends of the host cell DNA [5, 8, 9].

IN carries out the terminal rupture and the phase of strand transfer that initiate the integration of viral DNA. Integration of both ends of the viral DNA, followed by the fusion of the segments of DNA to points of joining, yields singlestranded gaps in each union of host-virus DNA and an overhang of two bases derived from viral DNA. For many parasitic reactions of DNA replication, the parasite makes stages only to a point where the guest cannot revert easily, forcing host to complete the work. For retroviral integration, it is reasonable to infer that host DNA repair enzymes complete provirus formation [5, 10, 11].

*H*ost proteins and integration

Several host cell proteins have been suggested to be important for retroviral DNA integration. The HIV-1 IN interactor 1 (Ini1) is a cellular protein identified as able to bind to HIV-1 IN. Ini1 is a member of the SWI/SNF chromatin remodeling complex [12] but it is still uncertain the role that Ini1 plays in normal HIV replication [5]. Other cellular factors have been associated with the PIC, such as the barrier-to-autointegration factor (BAF)[13], high mobility group A1 (HMGA1) [14], the human ortholog of the mouse embryonic ectoderm development gene product (EED)[15], p300 [16] and the cellular transcriptional coactivator Lens epithelium-derived growth factor (LEDGF)/p75 [17]. This one has been reported as an essential HIV integration cofactor [18].

LEDGF/p75 protein

LEDGF/p75 was named for being identified in a human lens epithelial cell library seeking for proteins involved in growth of lens epithelial cells [19]. This protein was found in many other tissues and it is identical to the transcriptional co-activator p75 [20], reason enough to include it into its name. LEDGF/p75 is able to bind strongly to HIV-1 IN [21], and specific for lentiviral IN proteins [22].

The LEDGF/p75-HIV-1 IN interaction is mediated by the IN-binding domain. LEDGF/p75 also contains a PWWP motif (Pro-Trp-Trp-Pro) implicated in protein–protein interactions; a nuclear localization signal (NLS) and an AT-hook motif. These last two involved in DNA-binding and chromosome association [23].

A number of observations implicated LEDGF/p75 as the dominant cellular interactor of lentiviral INs and indicated that the cellular protein was essential for HIV-1 IN-chromatin association, likely acting as a receptor or molecular tether [24].

IVth International Meeting on Retroviral Integration

The IVth International Meeting on Retroviral Integration was organized by Zeger Debyser from the Catholic University of Leuven, Belgium in collaboration with Mauricio Botta from the University of Siena in Italy. The conference was structured in seven sessions with the following topics: Structural biology; Biochemistry of integration; Cellular cofactors, LEDGF/p75;

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Trafficking and nuclear import; Drug discovery; Clinical virology and Other retroviruses and (retro)transposons. In addition was organized a Mini-symposium on gene therapy.

A session of welcome included a Keynote lecture given by Michael Miller from Merck focused on Raltegravir (RAL) and novels HIV-1 IN inhibitors. Session 1 was focused on structural biology and led by Peter Cherepanov from Imperial College in London, UK, whom delivery the Plenary lecture entitled *The mechanism of HIV integration and its inhibition by strand transfer inhibitors: lessons from X-ray crystallography studies using a convenient model system*. This group was able to assemble and crystallize the wild type prototype foamy virus (PFV) intasome by using oligonucleotides mimicking viral DNA ends and PFV IN. The structure evidenced the organization of the retroviral integration apparatus comprising an IN tetramer assembled on a pair of viral DNA ends. Soaking PFV intasome crystals in the presence of clinical HIV-1 IN inhibitors like RAL, Elvitegravir (EVG) and Dolutegravir (DTG), revealed how these small molecules bind to the IN active site and inactivate the viral nucleoprotein complex.

Session 2 was dedicated to biochemistry of integration and was led by Alan Engelman from Dana-Farber Cancer Institute in Boston, USA, whom delivered a remarkable plenary lecture about the HIV-1 IN biochemistry and HIV-1 replication. He posed that detailed characterization of HIV-1 IN mutant viruses is central to understand the mechanistic roles of IN binding factors in HIV-1 replication. They have studied HIV-1 IN mutant proteins that were introduced into HIV-1 molecular clones or bacterial HIV-1 IN expression vectors. Mutant viral infectivities were gauged against wild-type HIV-1. They concluded that most class II HIV-1 IN mutant proteins (mutations referred to as class II do not necessarily kill *in vitro* activities yet nevertheless block virus replication) are defective for concerted DNA integration activity *in vitro*, highlighting the usefulness of this assay for determining replication-defective mutant viral HIV-1 IN activities. Some of the analyzed mutants have been shown to influence preintegration complex nuclear import or host factor LEDGF/p75 binding. Therefore, caution should be exercised when interpreting roles of potential IN binding defects on HIV-1 replication.

Robert Craigie from the NIDDK, National Institutes of Health, Bethesda, USA, delivered an outstanding lecture on studies of nucleoproteins intermediates in HIV-1 DNA integration. They used atomic force microscopy to study stable complexes formed between HIV-1 IN and viral DNA and their interaction with host DNA. A tetramer of HIV-1 IN stably bridges a pair of viral DNA ends. They also observed tetramers of HIV-1 IN associated with single viral DNA ends; time-course experiments suggest that these may be intermediates in intasome assembly. HIV-1 IN tetramers were only observed in tight associations with viral DNA ends. The results suggested that the HIV-1 IN tetramer within the intasome is different from the HIV-1 IN tetramer formed at high concentration in solution in the absence of viral DNA [25].

Another lecture was dictated by Mamuka Kvaratskhelia from Ohio State University, Columbus, USA, titled *Modulation of HIV-1 IN structure and function by LEDGF/p75*. They employed Forster Resonance Energy transfer (FRET) to monitor assembly of individual HIV-1 IN subunits into tetramers in the presence of LEDGF/p75 and viral DNA. The HIV-1 IN-viral DNA and IN-LEDGF/p75 complex yielded significantly different FRET values suggesting two distinct HIV-1 IN conformations in these complexes. The conformation of ternary HIV-1 IN-LEDGF/p75-viral DNA complexes varied depending on the order of the complex assembly. The authors raised that results indicated differential multimerization of HIV-1 IN in the presence of various ligands which could be exploited as a plausible therapeutic target for development of allosteric inhibitors. On the other topic, the authors studied LEDGF/p75 interactions with chromatin. They demonstrated the strong association of LEDGF/p75 with nucleosomes [26].

Another excellent lecture was dictated by Zeger Debyser during Section 3 dedicated to cellular cofactors. The author presented the generation of a human somatic LEDFG/p75 knockout cell line that allows studying spreading HIV-1 infection in the complete absence of its cellular cofactor. Spreading HIV-1 infection in KO cells was abolished and only observed with laboratory strains. Characterization of the residual replication demonstrated a role for HRP-2 as a substitute for LEDGF/p75. However, LEDGF/p75 inhibitors (LEDGFINs) remained fully active even in the absence of LEDGF/75, highlighting their potential as allosteric HIV-1 IN inhibitors. The authors pointed the cellular Transportin-SR2 protein interaction domain of HIV-1 IN in its C-terminal domain, and the HIV-1 IN interaction domain of Transportin-SR2 in a region between amino acids 281-531. The information about the interaction hot spots will fuel the development of novel inhibitors that specifically block the nuclear import of HIV.

The group of Eric Poeschla, from Mayo Clinic College of Medicine in Rochester, USA, examined the mechanism of LEDGF/p75 dominant interference. This group used over-expression of the LEDGF/p75 HIV-1 IN binding domain (IBD); RNAi-mediated depletion of the endogenous protein; selection of viral resistances mutants and other technologies to examine the mechanism of LEDGF/p75 dominant interference effect.

A very attractive lecture, because of its images from infected cells, was presented by Anna Cereseto from The University of Trento, Italy, at Session 4 dedicated to Trafficking and nuclear import. Her group developed a fluorescent microscopy experimental system to detect single viral particles up to the nuclear compartment. They measure individual viral particle movements in live cells. They discovered that PICs move in the nucleus by active transport and that nuclear actin is a possible molecular motor mediating PICs nuclear trafficking [27].

Subsequently, a notable lecture was delivered by Thomas Hope, from the Northwestern University in Chicago, USA. He and his colleagues studied the relationship between HIV reverse transcription, trafficking, uncoating and nuclear import. They used a fluorescent microscopy based uncoating assay which uses antibody staining to detect the association of p24

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capsid protein with HIV-1 viral complexes in cells at various times post-infection. They also used the cyclosporine A washout assay, an owl monkey kidney cell assay based on the restriction of HIV-1 replication by a fusion protein of tripartite motif (TRIM) protein and cyclophilin A [28]. They concluded that reverse transcription, trafficking, nuclear import and uncoating of HIV are kinetically connected and highly interdependent.

Gloria Arriagada, from the Columbia University in New York, USA, talked about the relationship between the SUMO (Small Ubiquitin-Like Modifier) conjugation pathway and early events of the N-tropic Murine Leukemia Virus (N-MLV) life cycle. She reported that the presence of two SUMO interacting motifs (SIMs) in TRIM5α is required for the N-MLV restriction. Results indicated that the presence of intact SIMs in human and rhesus TRIM5α is important for their restriction activities. It was proposed that at least a portion of the antiviral activity of TRIM5α is mediated through the binding of its SIMs to SUMO-conjugated capsid [29].

Session 5 was focused on Drug discovery. The plenary lecture was delivered by Yves Pommier from the National Cancer Institute in Bethesda, USA. He talked about overcoming RAL resistance. He shows that EVG remains active against recombinant HIV-1 IN and viruses bearing the Y143R mutation that confers high resistance to RAL. They presented molecular mechanisms explaining the lack of cross-resistance of EVG and DTG against RAL mutants providing a molecular rationale for the activity of EVG and DTG in patients failing RAL treatment [30].

Chris Pickford from Pfizer in Sandwich, UK discussed about pre-clinical evaluation of HIV replication inhibitors that target the HIV-1 IN-LEDGF/p75 interaction [31]. A panel of viruses containing mutations that confer resistance to HIV-1 IN strand transfer inhibitors did not have a reduced susceptibility to the HIV-1 IN-LEDGF/p75 interaction inhibitors. Combining HIV-1 IN-LEDGF/p75 interaction inhibitors with strand transfer inhibitors (INSTI) in antiviral assays demonstrated that there is an additive effect of these compound classes. The cross-resistance data together with the additive effects support the potential for combined use of HIV-1 IN-LEDGF/p75 interaction inhibitors with strand transfer inhibitors in HIV-infected patients.

Another remarkable lecture was delivered by Louie Lamorte from Boehringer Ingelheim in Canada, regarding the discovery of a novel HIV-1 non-catalytic site in inhibitor (NCINI). This type of compounds comprise a novel class of HIV-1 antiretroviral agents that bind to a conserved allosteric pocket on HIV-1 IN and specifically inhibit the HIV-1 IN 3' processing activity. NCINI have a non-overlapping resistance profile with INSTI. They perform a high-throughput screening to identify inhibitors of the HIV-1 IN 3' processing activity was executed. The potency and absorption distribution metabolism excretion properties of selective hits were optimized through a combination of medicinal chemistry, parallel synthesis and structure guided drug design. The developed candidate BI 224436 exhibited medium inhibitory concentration (IC_{50}) of 15 nM in the Long Terminal Repeat 3' processing assay, an antiviral medium effective concentration (EC_{50}) of 4 to 14 nM against different laboratory strains of HIV-1, and a medium cytotoxic concentration (CC_{50}) greater than 120 μM. The authors concluded that BI 224436 is a potent inhibitor of HIV-1 IN with a novel mechanism of action and resistance profile. It retains antiviral activity against viruses resistant to clinical drugs targeting HIV-1 IN. BI 224436 have been advanced into Phase I clinical trials.

Maurizio Botta, from University of Siena, Dept. of Pharmaceutical and Applied Chemistry, Italy, presented results from his group on a recently disclosed rational design, synthesis and biological evaluation of novel series of HIV-1 IN inhibitors. He detailed that the first part of the study was aimed at identifying a novel class of HIV-1 IN inhibitors acting at the level of the HIV-1 IN-DNA complex formation. In the second part, efforts were directed toward the identification of small molecule HIV-1 IN dimerization inhibitors. They applied molecular modelling approaches including pharmacophores modelling, docking studies and molecular dynamics in order to facilitate hit identification. Botta expressed that the results of the first part of the study lay the foundation for the development of a new generation of HIV-1 IN inhibitors while, to the best of their knowledge, the second part representing the first successful virtual screening and evaluation of small molecule HIV-1 IN dimerization inhibitors.

Another speaker of Session 5, Nouri Neamati from the University of California, USA, spoke about design of cell permeable nanoneedles as HIV-1 IN inhibitors. He explained that his group had previously demonstrated HIV-1 IN inhibition *in vitro* with peptides derived from naturally occurring α-helical regions of the protein. At this event, Neamati showed that hydrocarbon-stapling of these peptides to stabilize their helical structure enables enhanced enzyme inhibitory potency and cell permeability while demonstrating selective inhibition of the LEDGF/p75 interaction. The corresponding unstapled peptides do not show inhibition of replication *in vivo*, although each pair of peptide has similar activity against HIV-1 IN in their *in vitro* assay. The speaker commented that considering peptides enhance potency and cell permeability they may serve as prototypical biochemical probes for development into 'nanoneedles' for the elucidation of HIV-1 IN and host co-factor interactions within their native cellular environment.

Session 6 focused on Clinical Virology. *DTG selects for a R263K mutation in HIV-1 subtype B and AG but not in subtype C viruses* was the plenary lecture in charge of Mark A. Wainberg, from McGill AIDS Centre in Montreal, Canada. Wainberg explained that after week 20 of drug selection with DTG virus remained susceptible to RAL and did not have any known-high resistance mutation. DTG does not appear to select for highly drug resistant variants of HIV-1 and demonstrates a high genetic barrier to the development of resistance. The R263K mutation selected in these studies showed cross-resistance with EVG but this is manifested differently in different subtypes. The R263K mutation alone in subtype B virus causes a modest decrease in the viral susceptibility to DTG. However Wainberg expressed that the lack of selection of R263K in subtype C virus by DTG in

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any of the selections as well as the inactivity for their purified subtype C R263K enzyme warrants further investigation.

Francesca Ceccherini-Silberstein from the University of Tor Vergata Rome, Italy, focused on the new knowledge of resistance to the HIV-1 IN inhibitors. Particular attention was dedicated also to the new technologies and methodologies to enhance detection and interpretation of HIV-1 IN inhibitors resistance. Ceccherini-Silberstein affirmed that resistance tests able to sequence and detect mutations in HIV-1 IN will become crucial for INI proper use in clinical practice.

Session 7 was based on other retroviruses and retro(transposons). The plenary lecture was carried out by Suzanne Sandmeyer from the University of California, Irvine in USA. In order to understand the basis of TFIIIB targeting, Sandmeyer and colleagues were able to map domains responsible for conferring interaction between HIV-1 IN and Brf 1 molecules. These studies showed that a carboxyl-terminal segment from HIV-1 IN aa 415 to 424 was sufficient to confer interaction between GST-tagged HIV-1 IN fragments and his (6)-tagged Brf 1. This subdomain of HIV-1 IN did not interact with Brf 1 lacking amino acids 457 to 469. This result is consistent with targeting of Ty3 HIV-1 IN to Brf 1 bound near the transcription start site via a mechanism similar to that used by Pol III itself. Ty3 exemplifies the close relationship between retroelements and Pol III transcripts. Results indicated that Ty3 is highly specific for loci associated with TFIIIB and that Ty3 provides a very sensitive probe for Pol III promoter elements in the yeast genome.

Zoltan Ivics from Max Debrück Center for Molecular Medicine, in Berlin, Germany, was invited to speak about genetic engineering with sleeping beauty (SB) transposons. SB transposons systems yield efficient stable gene transfer following non-viral gene delivery into primary cell types, including stem cells which are relevant targets for regenerative medicine and gene- and cell-based therapies of complex genetic diseases. His group validated a SB system for its use as a gene vector system to induce pluripotency in both mouse and human cells, and currently explore the utility for the system for personalized cell- and gene- based therapy of monogenic diseases. They applied the SB system for efficient generation of transgenic pigs. The transgenic animals showed normal development and persistent reporter gene expression for more than 12 months. Ivics and his group demonstrated germline transmission, segregation of individual transposons, and continued, copy number-dependent transgene expression in F1 offspring.

The Mini-symposium was based on gene therapy. *Defining the lentiviral integrome in human hematopoietic stem and progenitor cells* was in charge of Fulvio Mavilio from University of Modena, Italy. Mavilio and his group mapped more than 60 000 MLV and HIV integration sites in the genome of human CD34+ hematopoietic stem/progenitor cells and more than 16 000 sites in pheripheral blood T-lymphocytes, and defined genome-wide integration maps in both cells. MLV integrations cluster around regulatory elements of genes involved in hematopoietic functions. On the contrary, HIV integrations are clustered in regions marked by histone modifications associated to the body of transcribed genes. By a rigorous statistical analysis they defined a set of less than 300 genes that are targeted by HIV at significantly higher frequency than matched random controls after normalization for gene length, and a smaller set of genes that are targeted at significantly lower frequency. Functional clustering analysis identified a set of 'high risk' genes in hematopoietic cells, the function of which is more likely to be influenced by lentiviral vector integration in clinical gene therapy. Many of these genes are over-represented in collections of lentiviral vector integrations from patients treated by gene therapy, indicating that lentiviral 'common integration sites' are determined by the HIV target site selection rather than clonal dominance *in vivo*.

Another lecture was dictated by Christof von Kalle from the National Center for Tumor Diseases of Heidelberg, in Germany, on a strategy to overcome remaining safety concerns in somatic gene transfer through sequence specific genome modification using synthetic endonucleases. Endonucleases with exchangeable binding specificities such as homing endonucleases or transcription activator-like effector nucleases, and especially zinc-finger nucleases (ZFN), enable the introduction of specific DNA double strand breaks at almost every desired position in the genome, and, therefore, represent a promising tool for targeted gene transfer applications. A genome-wide analysis of DSB induction by capturing HIV-1 IN-defective lentiviral vectors into genomic DSB during nonhomologous end-joining repair revealed that ZFN cleave their intended target site with very high specificity. Importantly a few other genomic positions showing off-target activity could be identified. The detection of ZFN off-target binding sites enabled identifying the exact nucleotide positions within the ZFN target sequence that tolerate unspecific sequence recognition, thereby contributing to off-target activity.

Mauro Giacca, from the International Center for Genetic Engineering and Biotechnology from Trieste, Italy made an outstanding presentation about *HIV-1 IN stability and nuclear topography regulate viral DNA integration in primary CD4+ T cells*. Giacca explained that three-dimensional immuno fluorescent in situ hybridization (3D Immuno DNA FISH) has indicated that the HIV-1 provirus almost exclusively resides at the periphery of the nucleus in both productive and latent infection. He emphasized that specific interactions are formed between the integrated HIV-1 DNA and the nuclear pore compartment. These interactions are also involved in the transcriptional regulation of the latent provirus. He also commented about previous work from his laboratory which has indicated that in resting T cells, HIV-1 cDNA integration is restricted by the lack of c-Jun N-terminal kinase (JNK), which becomes activated only upon T cell stimulation [32]. JNK phosphorylates the HIV-1 IN serine 57 residue, thus preventing protein ubiquitination and degradation by the proteasome, and is unrelated to other post-translational modifications of HIV-1 IN such as acetylation.

Concluding remarks

Many interesting lectures were delivered during the meeting and more that 30 papers were presented in

32. Manganaro L, Lusic M, Gutierrez MI, Cereseto A, Del Sal G, Giacca M. Concerted action of cellular JNK and Pin1 restricts HIV-1 genome integration to activated CD4+ T lymphocytes. Nat Med. 2010;16(3):329-33.

posters. The conference was a great opportunity to expand knowledge of the retroviral integration process as well as to exchange views and criteria with specialists in the field from different locations of the world.

As mention above, retroviral integration is an essential stage in the life-cycle viral; therefore that to have a deep knowledge of the molecular mechanisms involved in this process is crucial. Therapeutic intervention of the HIV infection in this phase of the viral cycle has been successful using the INSTI RAL so other INSTIs have been designed and are advanced in the development as drug. Significantly, novel inhibitors targeting retroviral integration through other mechanisms are currently under development and rise expectation to improve the limitations of the existing ones.