

## OVERVIEW

# Baseline and time-updated factors in preclinical development of anionic dendrimers as successful anti-HIV-1 vaginal microbicides

Ignacio Rodríguez-Izquierdo<sup>1</sup> | Daniel Sepúlveda-Crespo<sup>2</sup>  |  
 Jose María Lasso<sup>3</sup>  | Salvador Resino<sup>2</sup>  | Ma Ángeles Muñoz-Fernández<sup>1,4,5</sup> 

<sup>1</sup>Instituto de Investigación Sanitaria Gregorio Marañón (IISGM), Madrid, Spain

<sup>2</sup>Unidad de Infección Viral e Inmunidad, Centro Nacional de Microbiología, Instituto de Salud Carlos III, Madrid, Spain

<sup>3</sup>Department of Plastic Surgery, HGUGM, Madrid, Spain

<sup>4</sup>Spanish HIV HGM BioBank, Madrid, Spain

<sup>5</sup>Section of Immunology, Laboratorio InmunoBiología Molecular, Hospital General Universitario Gregorio Marañón (HGUGM), Madrid, Spain

## Correspondence

Ma Ángeles Muñoz-Fernández,  
 Laboratorio InmunoBiología Molecular,  
 Hospital General Universitario Gregorio  
 Marañón. Spanish HIV HGM Biobank.  
 CIBER-BBN. IISGM, C/Dr. Esquerdo  
 46, Madrid 28007, Spain.  
 Email: [mmunoz.hgugm@gmail.com](mailto:mmunoz.hgugm@gmail.com)

## Abstract

Although a wide variety of topical microbicides provide promising in vitro and in vivo efficacy, most of them failed to prevent sexual transmission of human immunodeficiency virus type 1 (HIV-1) in human clinical trials. In vitro, ex vivo, and in vivo models must be optimized, considering the knowledge acquired from unsuccessful and successful clinical trials to improve the current gaps and the preclinical development protocols. To date, dendrimers are the only nanotool that has advanced to human clinical trials as topical microbicides to prevent HIV-1 transmission. This fact demonstrates the importance and the potential of these molecules as microbicides. Polyanionic dendrimers

**Abbreviations:** 7-AAD, 7-aminoactinomycin D; AIDS, acquired immunodeficiency syndrome; API, active pharmaceutical ingredient; ATP, adenosine triphosphate; BLT, marrow-liver-thymus; cART, combined antiretroviral therapy; CC<sub>50</sub>, compound's concentration required for the reduction of cell viability by 50%; COVID-19, coronavirus disease 2019; DC, dendritic cell; EC<sub>50</sub>, half-maximal effective concentration; EC<sub>90</sub>, 90% effective concentration; EMA, European Medicines Agency; FDA, Food and Drug Administration; FITC, fluorescein isothiocyanate; FIV, feline immunodeficiency virus; GFP, green fluorescent protein; HCV, hepatitis C virus; HEC, hydroxyethylcellulose; HIV-1, human immunodeficiency virus type 1; HIV-2, human immunodeficiency virus type 2; h-mice, humanized mice; HPMC 4000, hydroxypropylmethylcellulose 4000; HPV, human papillomavirus; HSC, hematopoietic stem cell; HSV-2, herpes simplex virus type 2; hu-HSC, human RAG; hu-PBL, human peripheral blood lymphocyte; hu-Thy/Liv, human fetal thymus-liver tissue; MERS-CoV, Middle East respiratory syndrome coronavirus; MØ, macrophage; MTS, 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt; MTT, 3-(4,5-dimethylthiazol-2-yl)2,5-diphenyl tetrazolium bromide; NHP, nonhuman primate; NOD, nonobese diabetic; NSG, NOD *scid* gamma; PAA, poly(alkylideneamine); PAMAM, poly(amidoamine); PBMC, peripheral blood mononuclear cell; PEG 6000, polyethylene glycol 6000; PLL, poly(L-lysine); PPH, phosphorus-containing dendrimer; PPI, poly(propylene imine); RSV, respiratory syncytial virus; SARS-CoV-2, respiratory syndrome coronavirus 2; *Scid*, severe combined immunodeficiency; SHIV, simian/human immunodeficiency virus; SIV, simian immunodeficiency virus; STIs, sexually transmitted infections; T/F, transmitted/founder; TI, therapeutic index; TLR, toll-like receptor; UNAIDS, Joint United Nations Programme on HIV/AIDS; WHO, World Health Organization; WST-1, 4-[3-(4-Iodophenyl)-2-(4-nitro-phenyl)-2H-5-tetrazolio]-1,3-benzene sulfonate; XTT, sodium 3'-[1-(phenylaminocarbonyl)-3,4-tetrazolium]-bis(4-methoxy-6-nitro) benzene sulfonic acid hydrate.

Ignacio Rodríguez-Izquierdo and Daniel Sepúlveda-Crespo contributed equally to this manuscript.

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**Funding information**

Acción Estratégica en Salud, Plan Nacional de Investigación Científica, Desarrollo e Innovación Tecnológica (2013-2016) and cofinanced by Instituto de Salud Carlos III (ISCIII) and Fondo Europeo de Desarrollo Regional (FEDER), Grant/Award Number: RD16/0025/0019; Centro de Investigación Biomédica en Red en Enfermedades Infecciosas, Grant/Award Number: CB21/13/00044; COST CA17140 Cancer Nanomedicine-“From the Bench to Bedside”; EPIICAL project; Fondo de Investigación Sanitaria (FIS), Grant/Award Number: PI16/01863; Gordon and Betty Moore Foundation, Grant/Award Number: 5334; Instituto de Salud Carlos III, Grant/Award Numbers: CD20CIII/00001, PI20CIII/00004, RD16CIII/0002/0002; Ministerio de Economía y Competitividad, Grant/Award Number: CGL2013-40564-R; RETIC, Grant/Award Number: PT17/0015/0042

**Edited by:** Emily Friebe, Managing Editor and Gregory M. Lanza, Co-Editor-in-Chief

are highly branched nanocompounds with potent activity against HIV-1 that disturb HIV-1 entry. Herein, the most significant advancements in topical microbicide development, trying to mimic the real-life conditions as closely as possible, are discussed. This review also provides the preclinical assays that anionic dendrimers have passed as microbicides because they can improve current antiviral treatments' efficacy.

This article is categorized under:

Nanotechnology Approaches to Biology > Nanoscale Systems in Biology  
Therapeutic Approaches and Drug Discovery > Nanomedicine for Infectious Disease  
Toxicology and Regulatory Issues in Nanomedicine > Regulatory and Policy Issues in Nanomedicine

**KEYWORDS**

acceptability, dendrimer, HIV-1, preclinical steps, regulation, vaginal microbicide

## 1 | INTRODUCTION

With almost 40 years of combating the human immunodeficiency virus type 1 (HIV-1) and acquired immunodeficiency syndrome (AIDS) epidemic, HIV-1 remains a significant global public health problem and is the most challenging infectious disease to eliminate. The 2020 Joint United Nations Programme on HIV/AIDS (UNAIDS) report estimates that 38 million people are living with HIV-1 worldwide (UNAIDS, 2020b). Sub-Saharan Africa remains the worst-affected region of HIV-1 infections, where women and girls represent 59% of the HIV-1-infected population (UNAIDS, 2020a). Moreover, the coronavirus disease 2019 (COVID-19) pandemic has raised concerns about the increase in new HIV-1 infections and HIV-associated mortality due to potential effects from disruptions in HIV-1 programs (Drain & Garrett, 2020; El-Sadr & Justman, 2020; Jewell, Mudimu, et al., 2020; Jewell, Smith, et al., 2020). Various factors have contributed to disruptions in HIV care and service during the COVID-19 pandemic: (i) strict quarantine measures, (ii) lack of access to health facilities, (iii) blocking of transport in many cities to prevent the spread of COVID-19 (Guo et al., 2020), (iv) stock out of cART due to the closure of pharmaceutical companies (WHO, 2020), and (v) changing the focus of healthcare personnel to care for COVID-19 patients instead of people living with HIV (Kowalska et al., 2020). The World Health Organization (WHO) has reported that, as of July–April 2020, several countries had interruptions in cART services (over 11 million HIV people affected), had a high risk of cART interruption, or presented a low stock of cART (almost 26 million HIV people) (WHO, 2020). Some mathematical models assessed the impact of COVID-19 on HIV epidemiology, indicating that a 6-month interruption in cART could lead to 500,000 further deaths from HIV-related illnesses (Jewell, Mudimu, et al., 2020) or that HIV-related deaths would increase by 10% over the next 5 years (Hogan et al., 2020).

## 2 | THE IMPORTANCE OF VAGINAL MICROBICIDES TO PREVENT HIV-1

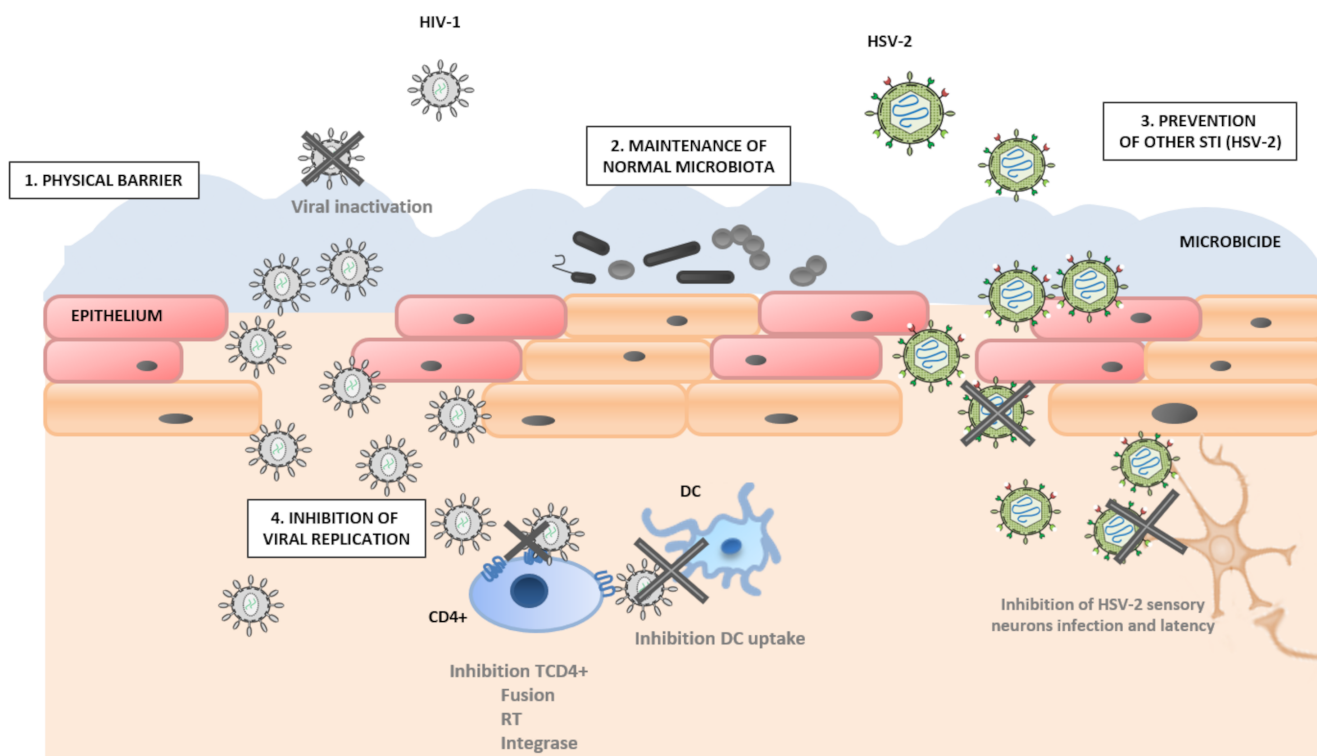
Sexual transmission is the main route of HIV-1 infection (Davari et al., 2020; LeMessurier et al., 2018).

In the absence of an approved vaccine to prevent HIV-1 transmission (Laher et al., 2020; Medlock et al., 2017), and despite the great efforts made by researchers during recent years (Baden et al., 2020; Dieffenbach & Fauci, 2020;

Pantaleo et al., 2019; Pitisuttithum & Marovich, 2020), combined antiretroviral therapy (cART) remains the primary strategy to treat and control HIV-1 infection (Giacomelli et al., 2019; Phanuphak & Gulick, 2020). However, cART is costly and poses a challenge to apply in low-resource countries (Kahn et al., 2011). Moreover, several concerns, including the long-term toxicity, development of resistance mutations, and drug–drug interactions, have shown a challenge for finding new therapeutic approaches that prevent and control the HIV-1/AIDS epidemic (Back & Marzolini, 2020; Battini & Bollini, 2019; Chawla et al., 2018; Gibas et al., 2019). In this sense, the use of long-lasting, female-controlled, and efficacious topical microbicides among women has emerged as a new strategy to overcome the HIV-1 epidemic in the last years.

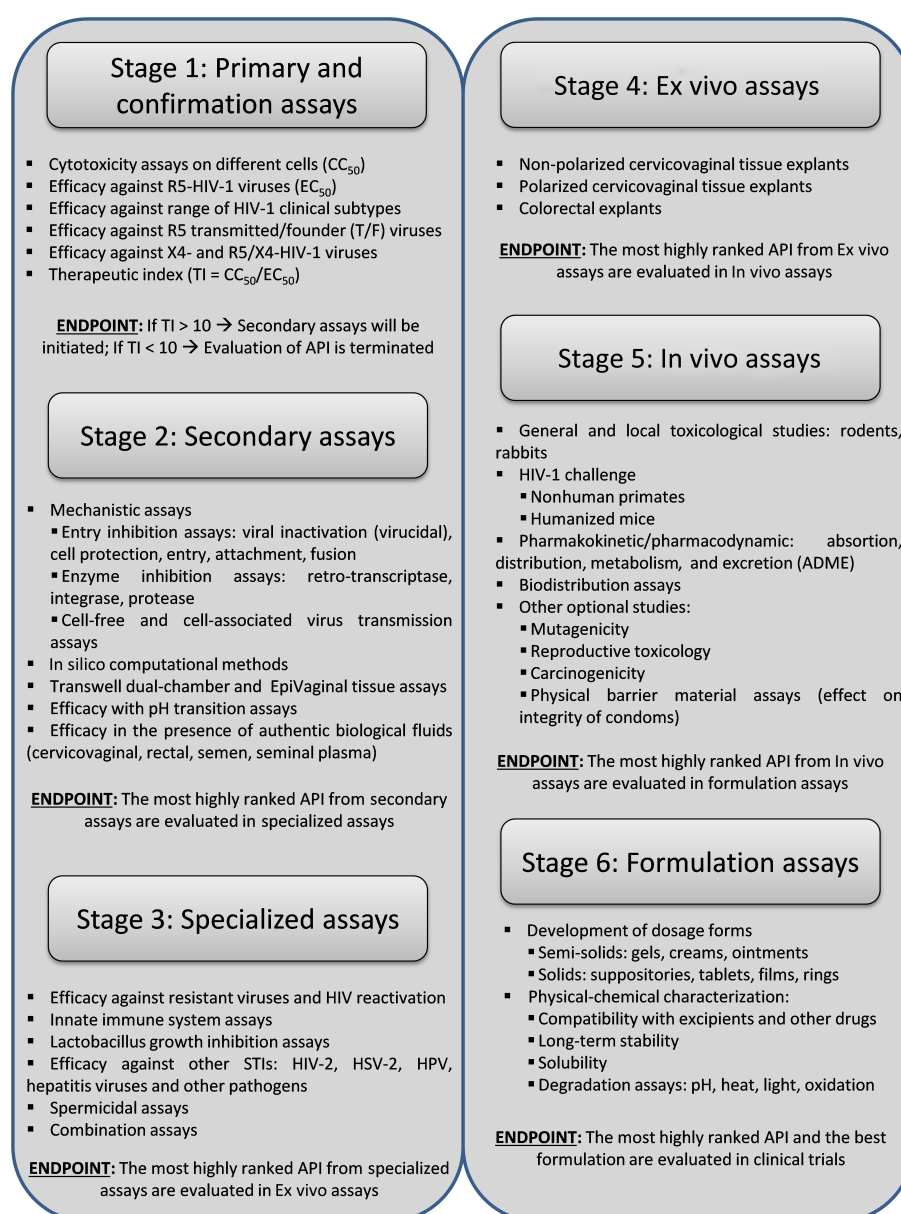
A microbicide is a compound formulated as a gel, ointment, cream, insert (suppository, tablets, ovules), film, or ring applied vaginally/rectally to reduce the risk of HIV-1 and other STIs, such as genital herpes (herpes simplex virus type 2 [HSV-2]), human papillomavirus (HPV), hepatitis C virus (HCV), gonorrhea, chlamydia, or syphilis (Coutinho et al., 2017; Garcia & Wray, 2020; Patel & Rohan, 2017). A microbicide must act as a lubricant coat that maintains vaginal defenses, inactivates HIV-1, and prevents other STIs in the presence of vaginal fluids and semen. Moreover, a microbicide must be readily accessible, easy to use, long shelf-life, and compatible with latex. It must also be (among other features) stable at higher temperatures, odorless, colorless, and tasteless (Antimisiaris & Mourtas, 2015). In the infection process, the virus may cross the epithelial barrier through breaches caused by microabrasions, microtrauma, or genital ulcers, or through the thinning and disruption of the multilayer lining by a pre-existing inflammation (Hladik & Doncel, 2010; Thurman & Doncel, 2011). Therefore, the microbicide must also block HIV-1 capture and transmission by dendritic cells (DCs), HIV-1 entry to the host cell, and HIV-1 replication before the integration process (Figure 1).

Several antiretroviral drugs are currently in advanced stages of development as microbicides, either vaginally or rectally (Al-Khouja et al., 2020; Bunge et al., 2020; A. Y. Liu et al., 2019; MTN, 2020; Pleasants et al., 2020). However, there is no commercially available microbicide due to the lack of effectiveness of these products in preclinical and clinical trials for many reasons (Baeten et al., 2020; Musekiwa et al., 2020; Notario-Perez et al., 2017).



**FIGURE 1** Key factors for microbicides to reduce the risk of HIV-1 transmission. The microbicide must act as a physical barrier against HIV-1 and other sexually transmitted infections (STIs). The microbicide must maintain vaginal defenses and inhibit HIV-1 replication. Once HIV-1 has crossed the epithelial barrier, the microbicide must prevent the HIV-1 uptake by dendritic cells (DCs), HIV-1 binding, fusion, or any process before the integration

To date, regulatory requirements for approval and licensure for microbicides have not been specified because guidance from the Food and Drug Administration (FDA) and the European Medicines Agency (EMA) date back years ago and are out of date (EMA, 2011; EMA, 2013; USFDA, 2014; WHO, 2009). Following these guidelines, we offer a scientific basis for developing a preventive anti-HIV-1 microbicide (Figure 2). In this way, we provide a more up-to-date approach, considering the problems and novel discoveries involved in such studies. One possible reason for clinical failure may be that preclinical steps with microbicides do not reflect the environment encountered during heterosexual transmission of HIV-1 (Roan & Munch, 2015). Here, we discuss ways to improve preclinical assays (including in vitro, ex vivo, and in vivo models) of active pharmaceutical ingredients (API) as topical microbicides to mimic the real-life conditions of HIV-1 transmission in people. A decreased drug solubility and stability, reduced drug cellular uptake and internalization, lack of adherence, mucus, and tissue penetration, and lack of protection from biological factors like metabolizing enzymes are other reasons for clinical failure (das Neves et al., 2016; Patel & Rohan, 2017). Recent progress in developing better and more promising novel strategies based on nanotechnology opens a new perspective to overcome these drawbacks and improve current systems for dealing with HIV-1 infection (Brako et al., 2017; das Neves et al., 2016; Macchione et al., 2020; Notario-Perez et al., 2017; Sanchez-Rodriguez, Vacas-Cordoba, et al., 2015).



**FIGURE 2** Updated summary of the main steps of the preclinical microbicide development



### 3 | NANOTECHNOLOGY AND NANOMEDICINE: THE REVOLUTION

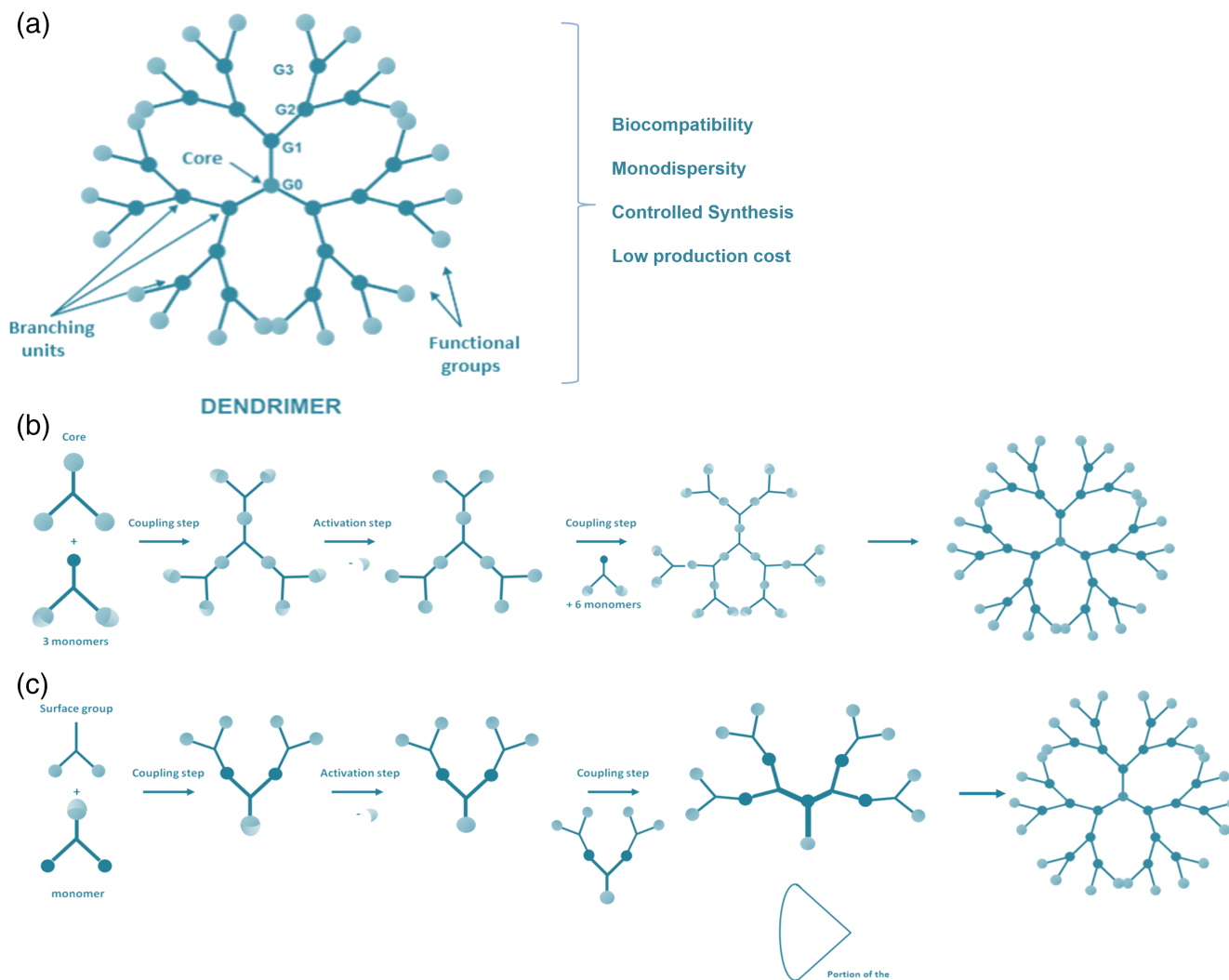
Nanotechnology is a multidisciplinary field defined as the intentional design, synthesis, characterization, and applications of materials and devices by controlling their size and shape with length scales in the 1–100 nm range (Bayda et al., 2019; Patra et al., 2018). Nanotechnology is considered an emerging, exponential, and cutting-edge technology with enormous potential in medical applications. Its application to medicine (nanomedicine) takes advantage of that nanomaterials are similar in size to biological structures to open up a wide field of research for the diagnosis and treatment of diseases (Soares et al., 2018), such as cancer (Bahreyni et al., 2020; Khot et al., 2020), Alzheimer (Binda et al., 2020; Zhang et al., 2020) and other neurological disorders (Sim et al., 2020), HIV infection (Macchione et al., 2020; Roy et al., 2015), pulmonary diseases (Doroudian et al., 2020), and currently against COVID-19 (Heinrich et al., 2020; Witika et al., 2020). Examples of nanomaterials used in medicine include liposomes, polymeric micelles, nanoparticles, quantum dots, nanogels, superparamagnetic iron oxide crystals, dendritic structures, carbon nanotubes, and nanoshells (Marchesan & Prato, 2013). Dendritic structures include mainly hyperbranched polymers, dendrigraft polymers, dendrons, and dendrimers (Carlmark et al., 2009; Ma et al., 2016). In particular, the interest in dendrimers has continuously increased over time as promising candidates for many applications in nanomedicine (Malkoch & García-Gallego, 2020).

### 4 | DENDRIMERS: WHAT AND WHY?

Dendrimers are hyper-branched, well-organized, and nano-sized molecules with a tree-like structure that present a central core, interior layers with repeated units (so-named generations), and functional groups at the periphery (Svenson & Tomalia, 2005; Figure 3a). The size of dendrimers is reached by a controlled synthesis of a stepwise growth of generations, contrary to what happens with classical polymerization processes (Dias et al., 2020). The synthesis of dendrimers can follow either a divergent or convergent approach (Abbasi et al., 2014; Sandoval-Yanez & Castro Rodriguez, 2020). The divergent method comprises the growth of the dendrimer originated from a core outward. This process involves the activation of groups at the periphery and the addition of branching units and is repeated until the desired dendrimer size (or generation) is reached (Mekelburger et al., 1992; Newkome et al., 1985; Newkome et al., 1994; Tomalia et al., 1985) (Figure 3b). The convergent method comprises dendrons' synthesis through reiterative reactions coupling to a multifunctional central core in the last synthesis step (Hawker & Frechet, 1992; Figure 3c). Other less explored strategies for dendrimer synthesis have been reported, such as double exponential growth (Kawaguchi et al., 1995), hypercores and branched monomers growth (Wooley et al., 1991), orthogonal coupling or lego chemistry (Grande et al., 2014; Maraval et al., 2003), click chemistry (Anandhan et al., 2019; Anandkumar & Rajakumar, 2017; Deraedt et al., 2015; Juárez-Chávez et al., 2019; Lowe, 2014), and onion peel approach (Bagul et al., 2017; Sharma et al., 2015).

The diameter of dendrimers increases linearly with the generation, reaching sizes averaging 1–20 nm (Kaminskas et al., 2011; Svenson & Tomalia, 2005). An important characteristic that does not follow a fixed rule among researchers in this field is defining the dendrimer's size. The unquestionable fact is that generations define dendrimers' size, and the most used nomenclature refers to when each generation corresponds to a layer of branching units (Sepulveda-Crespo, Cena-Diez, et al., 2017; Sepulveda-Crespo, Gomez, et al., 2015). Dendrimers constitute a versatile platform with several intrinsic properties, including nanoscale size and shape, monodispersity (well-defined molecular structure), multivalency (high density of functional end-groups provides many simultaneous interactions with biological membranes and drugs), high biocompatibility, high aqueous and nonpolar solubility, high reactivity, and structural flexibility (core compositions can vary). The main advantages/drawbacks from the synthesis process, physicochemical characterization, and other specific properties from dendrimers have been widely reported and described in detail in excellent seminal reviews (Chis et al., 2020; Dias et al., 2020; Mishra, 2011; Nanjwade et al., 2009; Palmerston Mendes et al., 2017; Sepulveda-Crespo, Cena-Diez, et al., 2017; Sepulveda-Crespo, Gomez, et al., 2015).

Dendrimers offer unique opportunities as biological agents, especially as carriers for chemical drugs or peptides (Hu et al., 2020; Sepulveda-Crespo et al., 2016; Shcharbin et al., 2020), as powerful tool for gene silencing (Dong et al., 2020; Ellert-Miklaszewska et al., 2019), as antibacterial agents (Abd-El-Aziz et al., 2020; Alfei & Schito, 2020; Mlynarczyk et al., 2020; Sanz Del Olmo et al., 2020), as well as to have intrinsic antiviral activities, such as against HIV-1 (Cena-Diez et al., 2017; Guerrero-Beltran et al., 2019; Rodriguez-Izquierdo et al., 2019; Sepulveda-Crespo, Serramia, et al., 2015; Vacas-Cordoba et al., 2016), HIV-2 (Briz et al., 2015), HSV-2 (Cena-Diez, Vacas-Cordoba, et al., 2016; Guerrero-Beltran et al., 2020; Rodriguez-Izquierdo et al., 2020), HCV (Javadi et al., 2019; San Anselmo et al., 2020; Sepulveda-Crespo,



**FIGURE 3** Structure and methods for the synthesis of dendrimers. (a) Schematic representation of dendrimer components. Synthesis of dendrimers following the (b) divergent approach or (c) the convergent method

Jimenez, et al., 2017), HPV (Donalisio et al., 2010), influenza virus (Farabi et al., 2020; Hatano et al., 2014), respiratory syncytial virus (RSV) (Gazumyan et al., 2000), or Middle East respiratory syndrome coronavirus (MERS-CoV) (Kandeel et al., 2020).

## 5 | POLYANIONIC DENDRIMERS A POTENTIAL TOOL AS ANTI-HIV MICROBICIDES

For anti-HIV microbicide applications, dendrimers must be nontoxic, nonimmunogenic (unless used in vaccine development), cross biological membranes, stay in tissue or blood circulation during long periods, and reach specific targets (Dias et al., 2020; Nanjwade et al., 2009). The efficacy, membrane permeability, hemolytic and cytotoxic effects of dendrimers depend on their size, core chemistry, and mainly type and charge of the functional groups at the periphery (Duncan & Izzo, 2005). Cationic dendrimers have been shown to interact with the negative charge of biological membranes, causing membrane disintegration, loss of cytoplasmic proteins, cell destabilization, and cell lysis (Duncan & Izzo, 2005; K. Jain et al., 2010; Labieniec-Watala & Watala, 2015; Madaan et al., 2014). Thus, there are many strategies to reduce or mask dendrimers' charges and overcome these drawbacks for delivery purposes (Janaszewska et al., 2019). Other reports have shown that anionic dendrimers are less toxic than cationic dendrimers (Janaszewska et al., 2019; Jevprasesphant et al., 2003). In addition to the overall advantages of dendrimers, anionic structures have more excellent

stability than cationic dendrimers and mimic biological receptors or cofactors, constituting ‘authentic’ molecular decoys. In the context of viral infections, anionic dendrimers act as entry inhibitors, blocking HIV replication. These dendrimers nonspecifically interact with HIV envelope proteins and other STIs, preventing electrostatic recognition of the target cell in the early stages of infection. Moreover, they can act against the cationic residues of cell receptors, modifying their structure and avoiding an effective interaction with viruses. In addition to having an antiviral activity per se, anionic dendrimers can trap drugs by encapsulation or charge interactions and deliver drugs within the cell, improving intracellular traffic (Sepulveda-Crespo, Cena-Diez, et al., 2017; Sepulveda-Crespo, Gomez, et al., 2015). Therefore, these dendrimers are excellent molecules as efficient anti-HIV microbicides (Cena-Diez et al., 2019; Maciel et al., 2019; Nandy et al., 2015; Sepulveda-Crespo, Serramia, et al., 2015).

The main dendrimers designed as anti-HIV microbicides and their characteristics (generations, central core, number, and functional end-groups) have been described previously (Sepulveda-Crespo, Cena-Diez, et al., 2017; Sepulveda-Crespo, Gomez, et al., 2015). Amphiphilic dendrimers (S. Han et al., 2012), Boltorn hyperbranched dendritic polymers (Berzi et al., 2012), carbosilane dendrimers (Relano-Rodriguez & Munoz-Fernandez, 2020), glycodendrimers (Ordanini et al., 2016), metallodendrimers (Garcia-Gallego et al., 2015), peptide dendrimers (Bon et al., 2013), phosphorus-containing (PPH) dendrimers (Perez-Anes et al., 2009), poly(alkylideneamine) (PAA) dendrimers (Maciel et al., 2019), poly(amidoamine) (PAMAM) dendrimers (Witvrouw et al., 2000), poly(propylene imine) (PPI) dendrimers (Rosa Borges et al., 2010), and poly(L-lysine) (PLL) dendrimer (Telwatte et al., 2011) are the structures most commonly used as anti-HIV microbicides. The API concentration that results in 50% inhibition in HIV-1 infection ( $EC_{50}$ ), HIV-1 strains and cell lines used, and potential targeting sites were reported in these works (Sepulveda-Crespo, Cena-Diez, et al., 2017; Sepulveda-Crespo, Gomez, et al., 2015). In this review, we provide all in vitro, ex vivo, and in vivo assays that have been performed with these dendrimers as anti-HIV microbicides. We highlight the preclinical steps needed to reach the efficient clinical translation of promising anionic dendrimers as microbicides to prevent HIV-1 infection. These data are summarized in the text in the form of tables for easy reading and clear understanding (Tables 1–3).

## 6 | IN VITRO PRECLINICAL STUDIES

### 6.1 | Screening: selecting the best model

To optimize the in vitro algorithm with many API with potential as topical microbicides, the cell line(s) and HIV-1 variant(s) must be carefully selected.

Initial screening with a large number of API must be performed using permissive cells to HIV-1 infection: epithelial cell lines from the human vagina (Vx2/E6E7), ectocervix (Ect1/E6E7), endocervix (End1/E6E7), uterus (HEC-1A, SiHa, CaSki) (Gali, Delezay, et al., 2010) or rectum (Caco-2, HT-29, SW1463) (Mukhopadhyaya et al., 2016), peripheral blood mononuclear cells (PBMCs),  $CD4^+$  T lymphocytes and cells from mononuclear phagocyte lineage (monocytes, macrophages [M $\phi$ s], and DCs; Coutinho et al., 2017). As R5-HIV-1 viruses predominate during the early stages of HIV sexual transmission, primary and established human cells that express CCR5 co-receptor should be used. TZM.bl reporter cell-based assay with a readout of HIV-1 infection through luminescence is considered the gold-standard assay (Roan & Munch, 2015). Another single-round infectivity assay with the enhanced green fluorescent protein (GFP) gene is used. The infectivity is measured by quantifying the number of GFP expressing cells by flow cytometry (Relano-Rodriguez et al., 2019). Other single-round infection systems with reporter genes incorporated into the viral particles are  $\beta$ -galactosidase (Bartolo et al., 2018), secreted alkaline phosphatase, or chloramphenicol acetyl transferase (Soezi et al., 2015). MT-2, MT4, or CEM-SS cells are also highly permissive for HIV-1 by developing HIV-induced syncytia that can be evaluated (Busso et al., 1991; Fernandez et al., 2019; Nara & Fischinger, 1988; Szucs et al., 1988).

Subsequent evaluations must be conducted using several laboratory-adapted R5-HIV-1 isolates and the most geographically prevalent HIV-1 clinical subtypes (A, B, and C). It is also essential to use R5 transmitted/founder (T/F) viruses because around 80% of heterosexual transmissions are established from this single HIV-1 variant (Joseph et al., 2015). Currently, a collection of full-length T/F HIV-1 infectious molecular clones are available (Parrish et al., 2013). Once primary infection occurs (R5-HIV-1), X4-HIV-1 viruses emerge late in the infection, and both HIV-1 variants are present in sexual fluids. Therefore, API must be active against laboratory-adapted and clinical viruses with different tropism, CXCR4-, and dual (R5/X4)-tropic viruses.

## 6.2 | Primary assays: cytotoxicity and efficacy

It is crucial to define the working concentration of API that can suppress HIV transmission in cell culture. Several standard assays are available to measure metabolic markers that estimate the number of viable cells in culture: tetrazolium reduction (MTT, MTS, XTT, or WST-1), resazurin reduction, cell-permeable protease detection, ATP detection, or bioluminescent nonlytic real-time detection (Riss et al., 2004). Possible highly active API can be not toxic to mitochondria with these colorimetric cell viability assays but could affect other enzymatic pathways. Therefore, additional Vybrant and Live/Dead or 7-aminoactinomycin D (7-AAD) cytotoxicity assays to eliminate false active API should be performed (C. S. Lackman-Smith et al., 2010; Lecoeur et al., 2001). Vybrant and Live/Dead assay measures plasma membrane integrity and intracellular esterase activity, whereas 7-AAD penetrates the damaged cell membranes, stains the DNA, and can be measured by flow cytometry.

Primary assays include a measurement of viability ( $CC_{50}$ : cytotoxic concentration of API that causes the reduction of the viable cells by 50%) and efficacy ( $EC_{50}$ ) with appropriate viruses and cells to generate the therapeutic index (TI). The TI is the ratio between API amount that causes toxicity and therapeutic effect ( $TI = CC_{50}/EC_{50}$ ). API evaluation is concluded if TI fails to meet the cut-off  $TI > 10$  (Muller & Milton, 2012).  $EC_{90}$  (90% inhibition) can also be calculated to select a rational dose for pharmacokinetic/pharmacodynamic assays.

## 6.3 | Secondary assays

APIs identified as the most effective and not toxic in the initial screening are selected to evaluate their unknown mechanism of anti-HIV-1 action. Mechanistic assays should be included, such as virucidal activity, entry, attachment, fusion, reverse transcription, integration, proteolytic processing, maturation, and/or budding. In silico computational methods, including molecular docking, molecular dynamics, and free energy calculations, should also be performed (Guerrero-Beltran et al., 2018; Nandy et al., 2015). Cell lines enabling multi-round infections like activated PBMCs isolated from healthy individuals are required to evaluate the activity of API targeting late stages of replication. Other cells supporting HIV-1 infection, such as U373-MAGI-CCR5E cells, B-THP-1/DC-SIGN cells, H9 cells, A3R5 cells, and C8166 cells, can also be used to evaluate the activity of API. Focal immunoassay, quantification of viral antigen (p24), or reverse transcriptase activity are used to titrate HIV-1 (McMahon et al., 2009). Cervicovaginal epithelial cells do not express CD4 and CCR5/CXCR4 receptors. However, the prevalent infectious forms of HIV-1 present in semen and cervicovaginal secretions can cross the epithelium and infect the target cells. Since cell-associated HIV-1 transmission is more efficient than the cell-free virus, cell-associated HIV-1 transmission inhibition assays, including CD4-dependent and CD4-independent assays, should be performed (Gupta et al., 2013). For further studies of cell-free and cell-associated HIV-1 transmission, a dual-chamber culture system as a sensitive model of a tight epithelial monolayer mimicking human vagina epithelium is used. The model evaluates API permeability and transport across the epithelial cell monolayer by measuring transepithelial electrical resistance. In this assay, API must cross the monolayer to the lower tissue culture chamber with PBMCs protecting against HIV-1 infection (Gali, Arien, et al., 2010; Mesquita et al., 2009). The organotypic 3D EpiVaginal tissue model (Cena-Diez et al., 2017; Ugaonkar et al., 2015) is a validated in vitro method used to evaluate the toxicity, absorption, and permeability of API into and through the tissue. Vaginal organoids can be probably used soon (just as it happens with other organs [Lancaster & Huch, 2019; Simian & Bissell, 2017; Takebe & Wells, 2019]) to mimic structural, functional and dynamic characteristics of vagina, to test anti-HIV-1 drugs and their interactions with stem cells, and even so to research vaginal microbiome (Ali, Syed, Jamaluddin, et al., 2020; Ali, Syed, & Tanwar, 2020).

The healthy female genital tract's pH is acidic (pH: 4.0–6.0), whereas the pH of normal semen is slightly alkaline (pH: 7.2–8.0). During heterosexual transmission, the acidic vaginal pH is neutralized by semen, providing an environment that facilitates bacterial vaginosis and yeast infections (Sabatte et al., 2011). On the other hand, a study has shown that the presence of semen reduces the sensitivity of HIV by diverse microbicides (Zirafi et al., 2014). Therefore, it is critically important to verify the biological activity of API over a wide range of pH and in the presence of natural simulated semen/seminal plasma (final concentration of 12.5–25% [Cena-Diez, Garcia-Broncano, et al., 2016; Garcia-Broncano et al., 2017]), rectal and cervicovaginal fluids to mimic the effects of the introduction of semen to the vagina. Since the microbicide will be applied before HIV-1 infection and heterogeneity between biological samples, adjusting the viral load or diluting the biological matrix material is needed.

## 6.4 | Specialized assays

A microbicide should also be effective against several resistant HIV-1 viruses, not interfere with normal flora in the vagina or immune system, and prevent other STIs.

Resistant viruses pre-existing in the vaginal/rectal fluids or tissues can cross the microbicidal barrier due to the presence of defined mutations that causes a loss of antiviral susceptibility. In this sense, assays to evaluate API's antiviral activity against resistant and multidrug-resistant viruses should be included: viruses are passaged in the presence of increasing concentrations of API, with a starting concentration that allows virus replication at a low level (Arien et al., 2016; Rodriguez-Izquierdo et al., 2019). On the other hand, latently infected resting CD4<sup>+</sup> T cells are the most source of HIV-1 reactivation, leading to a rebound of the viral load if treatment is interrupted. Moreover, the non-suppressive regimens can provide the emergence of drug-resistance mutations, being the major obstacle to HIV-1 eradication. Therefore, “shock and kill” assays to increase HIV-1 reactivation and that, at the same time, avoid new HIV-1 infections should be performed (Relano-Rodriguez et al., 2019).

The innate immune system constitutes the first defense against viral infection, especially after disrupting the mucosal epithelium during sexual intercourse. The cervicovaginal epithelial cells, DCs, MØs, and lymphocytes are vital in early HIV recognition. Therefore, several assays evaluating cytokines, toll-like receptor (TLR) expression, and differentiation, maturation, and activation of the primary immune cells localized in the vaginal mucosa should be performed (Martin-Moreno et al., 2019). Given the importance of maintaining the normal flora in the vagina and its low pH through lactic acid production, it is essential to determine the impact of API on the microbiome (Donahue Carlson et al., 2017; Klatt et al., 2017; Taneva et al., 2018) and the efficient growth H<sub>2</sub>O<sub>2</sub>-producing *Lactobacilli* sp. (Petrova et al., 2013). It is also essential to evaluate API's ability to inhibit other STIs, such as HIV-2, HSV-2, HPV, hepatitis viruses, and other pathogens associated with vaginosis or candidiasis (chlamydia, gonorrhea, or syphilis; Briz et al., 2015; Cena-Diez, Vacas-Cordoba, et al., 2016; Guerrero-Beltran et al., 2017; Sepulveda-Crespo, Jimenez, et al., 2017; Telwatte et al., 2011).

The API activity may extend as a spermicidal candidate because it would provide a women-controlled method to prevent or delay unwanted pregnancies. The spermicidal potential of API should be evaluated through some parameters, such as the minimum effective spermicidal concentration, sperm motility and viability, sperm membrane permeability and intracellular pH, induction of sperm cell apoptosis, changes at sperm membrane and sperm mitochondrial transmembrane, and topographical imaging of membrane domains over the sperm head (Cena-Diez et al., 2019; Chakraborty et al., 2014; R. K. Jain et al., 2010).

Preclinical microbicide development also includes combination prevention strategies. Drugs must be combined in a correct molar ratio to avoid chemical incompatibilities, drug–drug interactions, or competitiveness on the same target. Combination assays enhance efficacy with lower concentrations, decrease toxicity and underlying side effects, and reduce the probability or delay of the development of resistance. It is essential to evaluate synergistic, additive, or antagonistic properties of the combination assays using any software packages: Calcsyn, Compusyn, MacSynergy II, MixLow R package,... (Fouquier & Guedj, 2015). At this point, API identified as highly active with a defined mechanism of action are advanced for further ex vivo assays.

## 6.5 | Summary of in vitro assays performed with dendrimers

The first conclusion is that cytotoxicity and efficacy assays were the first and most repeated studies (see Table 1). A few dendrimers were not evaluated for toxicity or presented in the manuscript (Clayton et al., 2011; Domenech et al., 2010; Garcia-Vallejo et al., 2013; Morales-Serna et al., 2010). Few cell lines in most cytotoxicity studies were used. A single cellular model was used in most cases, and the best models were not selected according to the guidelines discussed in this manuscript. It may be due to either unavailability in labs or the aim to obtain preliminary results to optimize other more potent dendrimers. Ideally, preliminary studies should select vaginal (or rectal) epithelial cell lines and a primary cell model or an immortalized cell line that resembles this cell type (Chonco et al., 2012; Dezzutti et al., 2004; Sanchez-Rodriguez, Diaz, et al., 2015). Efficacy tests were aimed at inhibition assays against different HIV-1 isolates or binding and kinetic studies in the presence of recombinant proteins. Overall, very few studies showed the values of CC<sub>50</sub> and EC<sub>50</sub>, and consequently TI values. This parameter must be mandatory for any research that seeks to demonstrate the anti-HIV activity of dendrimers (and any other drug candidates).



TABLE 1 Primary in vitro assays performed with dendrimers as vaginal microbicides against HIV-1

In vitro assays Dendrimer	Classification	Efficacy against R5 transmitted/ founder against R5-HIV-1	Efficacy against X4-HIV-1 R5/X4-HIV-1	Mechanistic assays	In silico computational assays	Epithelial tissue assays	pH transition assays	Efficacy in authentic biological fluids	Efficacy against HIV-1 resistant viruses/ HIV-1 reactivation	STIs assays	Other assays	Innate immune system assays	Vaginal normal flora assays	Combination assays	References
BH30sucMan	Boltorn														(Arce et al., 2003; Tabarani et al., 2006)
Dendron12	Boltorn														(Berzi et al., 2012; Sattin et al., 2010)
BH3OPSGal	Boltorn														(Morales-Serna et al., 2010)
G2-S16	Carbosilane														(Armaiz et al., 2014; Cena-Diez, Garcia-Broncano, et al., 2016; Cena-Diez et al., 2017; Cena-Diez et al., 2019; Chonco et al., 2012; Guerrero-Beltran et al., 2020; Guerrero-Beltran et al., 2019; Guerrero-Beltran et al., 2018; Gutiérrez-Ulloa et al., 2020; Jaramillo-Ruiz et al., 2016; Martin-Moreno et al., 2019; Relano-Rodriguez et al., 2019; Relano-Rodriguez & Munoz-Fernandez, 2020; Rodriguez-Izquierdo et al., 2020; Sepulveda-Crespo, Jimenez, et al., 2017; Sepulveda-Crespo et al., 2014;

TABLE 1 (Continued)

In vitro assays	Dendrimer	Classification	Efficacy against R5-HIV-1	Efficacy against R5 transmitted/1 and/or founder HIV-1	Efficacy against X4-HIV-1	Efficacy against R5/X4-HIV-1	Mechanistic assays	In silico computational assays	In vitro chamber/ dual-transwell assays	Efficacy with pH transition biological fluids	Efficacy in the presence of authentic HIV-1 viruses/ reactivation assays	Efficacy against resistant HIV-1	Efficacy against other STIs	Spermicidal assays	Innate immune system assays	Vaginal normal flora assays	Combination assays	References
	G2-STIE16	Carbosilane																Sepulveda-Crespo, Sanchez-Rodriguez, et al., 2015; Sepulveda-Crespo, Serramia, et al., 2015) (Cena-Diez et al., 2017; Galán et al., 2014; Garcia-Broncano et al., 2017; Sanchez-Rodriguez, Diaz, et al., 2015; Sepulveda-Crespo, Jimenez, et al., 2017; Sepulveda-Crespo et al., 2014; Sepulveda-Crespo, Sanchez-Rodriguez, et al., 2015)
	G2-CTE16	Carbosilane																(Galán et al., 2014; Sanchez-Rodriguez, Diaz, et al., 2015; Sepulveda-Crespo, Jimenez, et al., 2017)
	G1-NSI6	Carbosilane																(Armáiz et al., 2014; Briz et al., 2015; Cordoba et al., 2013; Vacas-Cordoba et al., 2014; Vacas-Cordoba et al., 2016; Vacas-Cordoba et al., 2013)

(Continues)

TABLE 1 (Continued)

In vitro assays	Cytotoxicity	Efficacy against R5-HIV-1	Efficacy against R5 transmitted/ founder	Efficacy against X4-HIV-1 and/or R5/X4-HIV-1	Mechanistic assays	In silico computational assays	EpiVaginal tissue assays	Transwell dual-chamber/ EpiVaginal transition assays	Efficacy with pH authentic biological fluids	Efficacy in the presence of resistant HIV-1 reactivation	Efficacy against HIV-1	Efficacy against other STIs	Spermicidal assays	Innate immune system assays	Vaginal normal flora assays	Combination assays	References
Dendrimer	Classification																
G2-Sh16	Carbosilane																(Armáiz et al., 2014; Briz et al., 2015; Cordoba et al., 2013; Garcia-Broncano et al., 2017; Guerrero-Beltran et al., 2018; Relano-Rodriguez et al., 2019; Vacas Cordoba et al., 2013, 2014, 2016)
G0-Sh4	Carbosilane																(Cena-Diez et al., 2019; Guerrero-Beltran et al., 2018; Relano-Rodriguez et al., 2019)
G2-S24P	Carbosilane																(Sepulveda-Crespo et al., 2018; Sepulveda-Crespo, Jimenez, et al., 2017; Sepulveda-Crespo et al., 2014; Sepulveda-Crespo, Sanchez-Rodriguez, et al., 2015)
G2-S8T	Carbosilane																(Gutierrez-Ulloa et al., 2019)
G2-C18PEG	CPEGC																(Kandi et al., 2019)
NP/Ag-G2-C18PEG	CPEGC																(Ardestani et al., 2015)
[G1]-CO2Na	GATG																(Domenech et al., 2010)
Bol13.4	Glycodendrimer																
ROD3.5.6	Glycodendrimer																(Varga et al., 2014)

TABLE 1 (Continued)

In vitro assays	Dendrimer	Classification	Efficacy against R5-HIV-1	Efficacy against R5 transmitted/ founder	Efficacy against X4-HIV-1 and/or R5/X4-HIV-1	Mechanistic assays	In silico computational assays	EpiVaginal tissue assays	Transwell dual-chamber/ EpiVaginal transition assays	Efficacy with pH authentic biological fluids	Efficacy against HIV-1 reactivation viruses/ HIV-1 reactivation STIs	Efficacy against other STIs	Spermicidal assays	Innate immune system assays	Vaginal normal flora assays	Combination assays	References
	ROD3.7.6	Glycodendrimer															(Ordanini et al., 2015; Ordanini et al., 2016)
	G1-C8	PAA															(Ordanini et al., 2015; Ordanini et al., 2016)
	G1-S8	PAA															(Maciel et al., 2019)
	Lewis <sup>x</sup> -PAMAM	PAMAM															(Maciel et al., 2019)
	Sulfo-6	PAMAM															(Garcia-Vallejo et al., 2013)
	SPL2923	PAMAM															(Clayton et al., 2011)
	SPL6195	PAMAM															(Hantson et al., 2005; Witvrouw et al., 2000)
	SPL7304	PAMAM															(Witvrouw et al., 2000)
	SB105-A10	Peptide dendrimer															(McCarthy et al., 2005)
	Trp(5a-5f) and modifications	Peptide dendrimer															(Bon et al., 2013; Donaliso et al., 2010; Luganini et al., 2011)
	SCSLD3	PLL															(Martinez-Gualda et al., 2017, 2019, 2020; Rivero-Buceta et al., 2015)
	PLDG3-PSCel	PLL															(S. Han et al., 2012)
	SPL7013	PLL															(Shuqin Han et al., 2010)
																	(Barnes et al., 2008; Dezzutti

(Continues)

TABLE 1 (Continued)

In vitro assays	Classification	Efficacy against R5-HIV-1	Efficacy against R5 transmitted/1 and/or founder HIV-1	Efficacy against X4-HIV-1	Mechanistic assays	In silico computational assays	EpiVaginal tissue assays	Transwell dual-chamber/ EpiVaginal with pH transition assays	Efficacy in the presence of authentic biological fluids	Efficacy against resistant HIV-1 reactivation	Efficacy against other STIs	Spermicidal assays	Innate immune system assays	Vaginal normal flora assays	Combination assays	References
SPL7115	PLL															et al., 2004; Gong et al., 2005; C. Lackman-Smith et al., 2008; McCarthy et al., 2005; Nandy et al., 2015; O'Loughlin et al., 2010; Price et al., 2011; Rupp et al., 2007; Telwatte et al., 2011; Tyssen et al., 2010; Ziafi et al., 2014)
PPH-3d-G1	PPH															(Tyssen et al., 2010) (Perez-Anes et al., 2010)
PPH-5c-Gc1	PPH															(Perez-Anes et al., 2009)
PSGal64mer	PPI															(Kensinger, Catalone, et al., 2004; Kensinger, Yowler, et al., 2004)
MVC-GBT	PPI															(Rosa Borges et al., 2010)
MVC-3SL	PPI															(Rosa Borges et al., 2010)
SPL7320	PPI															(McCarthy et al., 2005)
Metallo dendrimer G2S	PPI															(Garcia-Gallego et al., 2015)
Metallo dendrimer G2C	PPI															(Garcia-Gallego et al., 2015)
Viol36	Viologen															(Asaftei et al., 2012; Asaftei & De Clercq, 2010)



TABLE 1 (Continued)

In vitro assays	Efficacy against R5	Efficacy against X4-HIV-1	Efficacy against R5/HIV-1	Mechanistic assays	In silico computational assays	EpiVaginal assays	Transwell dual-chamber/	Efficacy with pH transition assays	Efficacy in authentic biological fluids	Efficacy against HIV-1 resistant viruses/ reactivation	Efficacy against HIV-1	STIs	Other STIs	Innate immune system assays	Vaginal normal flora assays	Combination assays	References
Dendrimer	Efficacy against founder R5-HIV-1	Efficacy against R5-HIV-1	Efficacy against R5-HIV-1	Cytotoxicity assays	In silico computational assays	EpiVaginal assays	Transwell dual-chamber/ assays	Efficacy with pH transition assays	Efficacy in authentic biological fluids	Efficacy against HIV-1 resistant viruses/ reactivation	Efficacy against HIV-1	STIs	Other STIs	Innate immune system assays	Vaginal normal flora assays	Combination assays	(Asaftei & De Clercq, 2010; Asaftei et al., 2012)
Viol7																	

Note: The assays carried out are represented with a green shading; not carried out with a gray shading. Abbreviations: CPEGC, citric acid-polyethylene glycol-citric acid; GATG, gallic acid-triethylene glycol; PAA, poly(alkylideneamine); PAMAM, poly(amidoamine); PLL, poly(L-lysine); PPH, phosphorus-containing dendrimers; PPI, poly(propylene imine).

Another aspect to be taken into account are studies to elucidate the mechanism of action of dendrimers. All dendrimers inhibit HIV-1 infection, particularly at the viral entry-level. Some studies have performed additional assays and inhibited HIV-1 entry, retro-transcriptase, and integration processes (McCarthy et al., 2005; Witvrouw et al., 2000) or inhibited dimerization of HIV-1 capsid protein (Domenech et al., 2010). It is possible to elucidate the potential binding sites of dendrimers to virus proteins or cell receptors, thus preventing the virus's binding from hosting cells and subsequent infection with computational models (Guerrero-Beltran et al., 2018; Nandy et al., 2015; Sanchez-Rodriguez, Diaz, et al., 2015).

There are a few dendrimers that continue to advance in *in vitro* assays to reach clinical trials. Those that do not advance are either because the expected results are not obtained or because the authors prefer to optimize the synthesis of other potential dendrimers, as is the case of tryptophan dendrimers (Martinez-Gualda et al., 2017, 2019, 2020; Rivero-Buceta et al., 2015). The importance of conducting further assays has been demonstrated over the years, especially with efficacy studies in the presence of cervicovaginal fluids or semen or innate immunity tasks. The most advanced dendrimers are G2-STE16, G2Sh16, and SPL7013, although none reaches the projection of G2-S16 dendrimer with all the *in vitro* assays of the algorithm presented in this manuscript and very encouraging results. SPL7013 showed excellent results in each of the *in vitro* assays carried out. Still, some crucial studies are lacking, such as testing its efficacy against several resistant viruses, T/F viruses, and demonstrating that SPL7013 does not alter innate immunity.

## 7 | EX VIVO PRECLINICAL STUDIES

Due to the sexual transmission being the primary mode of HIV-1 infection, human tissue explants from cervicovaginal and colorectal samples must be incorporated into preclinical protocols to eliminate potential discrepancies with *in vitro* models (Arien et al., 2012) (see Table 2). Tissue explants models evaluate API's efficacy against different HIV-1 isolates, toxicity (histology), cytokine changes, and viral evolution, thus understanding the basic mechanisms of HIV-1 transmission, pathogenesis, and antimicrobial factors (Anderson et al., 2010; Dezzutti, 2015; Fernandez-Romero et al., 2015). Inflammation processes and mucosal response to API can be evaluated using polychromatic flow cytometry and multiplex cytokine assays (Merbah et al., 2011; Richardson-Harman et al., 2009).

The system of cervicovaginal tissue is considered the gold standard model for microbicide development. Several explants systems have been described with polarized or nonpolarized exposure of virus and API (Dezzutti, 2015). The nonpolarized tissues are submerged in a medium containing HIV-1, creating a worst-case scenario where other STIs could damage epithelium, such as HSV-2, HPV, other viruses, bacteria, or fungi (Rollenhagen et al., 2014; Tugizov et al., 2013). This model determines the effective API's concentration, irritation, and the API's ability to block HIV-1 infection of the immune cells that migrate out from tissue. In the polarized tissues, HIV-1 and API are applied directly to the epithelium, mimicking humans' transmission. Cell viability and tissue permeability need to be continuously monitored due to this model is deteriorating quickly.

There are other explant models where target cells are activated before HIV-1 infection to start symptoms of inflammation. However, these tissues also deteriorate after 48 h of culture (Cummins Jr. et al., 2007). Some studies use 3D human-made organotypic ectocervical-vaginal tissues to evaluate API toxicity (Ayehunie et al., 2006). However, these models lack the entire epithelial thickness and the correct balance of the immune system's cells. Therefore, novel models of reconstructed endocervical and ectocervical tissues have been developed (Ayehunie et al., 2006; Bouschbacher et al., 2008; Stoddard et al., 2009).

Cervicovaginal explants are in constant evolution and provide additional information. However, the effective concentration of API may be challenging to determine due to the heterogeneity of mucosal tissue and different HIV-1 replication rates in the mucosal tissue (Dezzutti et al., 2017). These explants show several limitations; thus, new protocols will be needed to overcome these weaknesses and exploit the strengths (see Table 3; Anderson et al., 2010; Dezzutti, 2015; Grivel & Margolis, 2009).

Due to several heterosexual and homosexual transmissions occurring across the colorectal epithelial lining, colorectal explants are used as a model to study API toxicity and efficacy (Ham et al., 2015; Mukhopadhyaya et al., 2016; Scott et al., 2016). However, the majority of the problems encountered with cervicovaginal explants apply to colorectal explants. Finally, as in the case of *in vitro* studies, semen, cervicovaginal secretions, and normal flora must be included to study their API activity effects (Introini et al., 2017; Nahui Palomino et al., 2017).

## 7.1 | Summary of ex vivo assays performed with dendrimers

Of all the dendrimers presented in this manuscript with in vitro potential anti-HIV-1 activity as microbicides, only two were tested for ex vivo activity in explants against HIV-1 (see Table 4).

Dendron 12 inhibited HIV-1 infection of human cervical tissues (Berzi et al., 2012), whereas SPL7013 prevented HIV-1 infection in human cervical explants and human colorectal explant culture (Abner et al., 2005; Cummins Jr. et al., 2007). Not even the most advanced dendrimer in in vitro studies (G2-S16) was tested ex vivo. This intermediate step could save time and costs before going through in vivo studies. However, ex vivo validation has not been mandatory to transfer any candidate for a microbicide to the clinic. On the other hand, there are many varieties of explant models that require the standardization of protocols. The concerns generated by the explants' limitations and weaknesses, which have not been resolved to date, can be the main reasons for the absence of ex vivo assays with dendrimers.

Moreover, the possible emergence of vaginal organoids and their probable easy maintenance in vitro may rule out ex vivo systems if these drawbacks are not resolved. In this regard, the solution could be to develop an engineered human tissue as a gold standard model to study the inflammatory and inhibitory processes derived from HIV-1 infection. In other words, a single explant model that can recapitulate mucosal cell diversity, immune response, and inter-donor variation (Herrera, 2019).

## 8 | IN VIVO PRECLINICAL STUDIES

Another challenge in preclinical microbicide testing is the employment of a correct animal model. The absence of a validated animal model is the major obstacle for selecting and evaluating API that advances human clinical trials. Before testing in animals or collecting tissue (and cells) from animals, the study must be approved by the Institute Animal Ethics Committee, considering that the protocols for conducting experiments and regulatory requirements vary according to the local governing body. This review discusses the two most commonly used animal models for HIV-1 research: nonhuman primates (NHPs) and humanized mice (h-mice).

### 8.1 | HIV-1 challenge: nonhuman primates

NHPs are the first animal model to study the pathogenesis and transmission of HIV-1. NHPs, particularly Asian macaques, have been widely used for HIV-1 microbicide testing. Their genital tract anatomy, immunology, and physiology show remarkable similarities to human structures (Thippeshappa et al., 2020). NHPs have a high susceptibility to simian immunodeficiency virus (SIV, e.g., SIV<sub>mac239</sub>, SIV<sub>mac251</sub>) and engineered simian/human immunodeficiency virus chimeras (SHIV, e.g., SHIV<sub>89.6P</sub>, SHIV<sub>162P3</sub>, SHIV<sub>KU1</sub>, SHIV<sub>AD8</sub>, SHIV<sub>BAL</sub>, SHIV<sub>1157</sub>) capable of replicating and causing macaques' disease (Hatzioannou et al., 2009; Veazey & Lackner, 2017). The most common and accepted NHPs are the rhesus macaque (*Macaca mulatta*), the pig-tailed macaque (*Macaca nemestrina*), and the cynomolgus macaque, also

**TABLE 2** Strengths and weaknesses of different commercially available models for vaginal research as microbicides

Characteristics	VK2/E6E7	EpiVaginal	Vaginal explant
Model	Cell line	Vaginal	Tissue
Assay	In vitro	In vitro	Ex vivo
Real conditions	No	Closely	Yes
Maintenance	Months	Weeks	Weeks
Reproducibility	High	High	Variable
Homogeneity	High	Lower	Lower
Tissue structure	No	Similar to native human vaginal tissue	Complete
Relevance to humans	Moderate	High	High
Commerciality	Yes	Yes	No

Note: Herein a vaginal cell line (VK2/E6E7), a commercial vaginal model (EpiVaginal), and ex vivo tissue explants are compared.

known as long-tailed or crab-eating macaque (*Macaca fascicularis*) (see Table 5 for analyzing their main characteristic for HIV-1 research).

Rhesus macaques are the most used NHPs for microbicide testing due to their availability. SIV infections result in high viral loads, a progressive loss of CD4<sup>+</sup> T cells, and a disease progression rate similar to that observed in HIV-1-infected humans. Rhesus macaques are used in microbicide studies for topical and systemic distribution and to evaluate API efficacy (Calenda et al., 2017; McBride et al., 2017; Zhao et al., 2017). Pig-tailed macaques have a vaginal flora similar to humans and are more susceptible to the same STIs. Viral loads and CD4<sup>+</sup> T cell depletion rates are slightly more vulnerable to SIV transmission, and disease progress more rapidly than rhesus macaques. Pig-tailed macaques are commonly used to study immunology, pathogenesis, toxicity, and efficacy of API (Cole et al., 2010; Moss et al., 2012). Cynomolgus macaques are smaller and less expensive than rhesus macaques, but researchers employ them less. The viral load and CD4<sup>+</sup> T cell turnover are less pathogenic than those observed in rhesus or pig-tailed macaques. The smaller size of the vagina of cynomolgus macaques makes vaginal biopsies more complex, being only useful for efficacy studies (Bouchemal et al., 2015; Murphy et al., 2014; Murphy et al., 2018).

NHP models have important limitations due to high costs, not being readily available, limited supply for large-scale screening of many API, more complicated for international transport, and the inability to employ HIV-1 and drug-resistant HIV-1 isolates challenge studies (Garcia-Tellez et al., 2016). Therefore, it is unclear whether NHP models precisely predict what would occur in humans, though our knowledge from HIV-1 infection or pathogenesis has, to a large extent, been possible due to macaque investigation models.

## 8.2 | HIV-1 challenge: humanized mice (h-mice)

Small-model animals (rodents, rabbits, and cats) can be used to evaluate potential microbicide candidates rapidly. Feline immunodeficiency virus (FIV) served as a surrogate model for HIV-1 infection in humans due to similarities in

**TABLE 3** Advantages, limitations, and potential solutions to improve HIV-1 transmission research on cultures from human mucosal explants. (Modified from Anderson et al. (2010), Dezzutti (2015), and Grivel and Margolis (2009))

Advantages	Disadvantages	Possible solutions
Real tissue architecture	<ul style="list-style-type: none"> <li>Lack of physiological variables</li> </ul>	<ul style="list-style-type: none"> <li>Add the desired parameters, such as semen or cervical mucus</li> </ul>
Presence of lymphocyte subtypes, dendritic cells, and other immune cells	<ul style="list-style-type: none"> <li>Donor variability</li> <li>Integrity of tissue deteriorates after 3 weeks</li> <li>Physiological conditions deteriorate in culture</li> </ul>	<ul style="list-style-type: none"> <li>Use tissue as fresh as possible</li> <li>Check cellular status and viability</li> </ul>
Measurement of HIV-1 infection and replication without activation	<ul style="list-style-type: none"> <li>Not mimic in vivo systemic conditions</li> <li>Tissue explants collected using surgical methods must differentiate ectocervix versus endocervix (ectocervix contains fewer HIV-1 target cells)</li> <li>Endocervix produces mucus in culture</li> </ul>	<ul style="list-style-type: none"> <li>Ectocervix must be a more transparent color</li> <li>Mucus produced by the endocervix could be used for further studies as a delivery method for HIV-1 physiological infection</li> </ul>
Presence of the immune system	<ul style="list-style-type: none"> <li>Absence of immune cells migration or recruitment</li> <li>Disparate number, localization, or types of HIV-1 target immune cells</li> </ul>	<ul style="list-style-type: none"> <li>Implement immune-histological analysis of tissue to identify types of HIV-1 target cells avoiding data interpretation</li> </ul>
Response to exogenous hormones and spectrum of cytokine release	<ul style="list-style-type: none"> <li>Donor status of hormones and innate immunity</li> <li>Evaluation of cytokine and hormone response in tissue could be difficult</li> </ul>	<ul style="list-style-type: none"> <li>Test the natural state of donor tissue</li> <li>Digest tissue and analyze isolated cells</li> </ul>
Tissue infection by other sexually transmitted infections	<ul style="list-style-type: none"> <li>Absence of microbiome to study bacterial interactions</li> </ul>	<ul style="list-style-type: none"> <li>Include conditions and protocols for microbiome efficacy</li> </ul>
Use of antibiotic and controlled parameters	<ul style="list-style-type: none"> <li>Amphotericin B inhibits HIV-1 infection</li> </ul>	<ul style="list-style-type: none"> <li>Use amphotericin-free antibiotic cocktail</li> </ul>

**TABLE 4** Primary ex vivo and in vivo assays performed with dendrimers as vaginal microbicides against HIV-1

Ex vivo and in vivo assays	Dendrimer	Classification	Cervicovaginal/colorectal explants		HIV-1 challenge in vivo: humanized mice		HIV-1 challenge in vivo: Other STIs challenge in vivo		In vivo toxicological studies		Biodistribution: in vivo studies		Other in vivo studies		References
Dendron12	Boltorn														(Berzi et al., 2012)
G2-S16	Carbosilane														(Briz et al., 2015; Cena-Diez et al., 2017, 2019; Cena-Diez, Vacas-Cordoba, et al., 2016; Chonco et al., 2012; Guerrero-Beltran et al., 2020; Relano-Rodriguez & Munoz-Fernandez, 2020; Rodriguez-Izquierdo et al., 2020; Sepulveda-Crespo, Serramia, et al., 2015)
G2-STE16	Carbosilane														(Galán et al., 2014; Sanchez-Rodriguez, Diaz, et al., 2015)
G2-CTE16	Carbosilane														(Galán et al., 2014; Sanchez-Rodriguez, Diaz, et al., 2015)
G1-NS16	Carbosilane														(Vacas Cordoba et al., 2013)
G2-Sh16	Carbosilane														(Vacas Cordoba et al., 2013)
G0-Sh4	Carbosilane														(Cena-Diez et al., 2019; Cena-Diez, Vacas-Cordoba, et al., 2016)
G1-C8	PAA														(Maciel et al., 2019)
G1-S8	PAA														(Maciel et al., 2019)
SPL7013	PLL														(Abner et al., 2005; Bernstein et al., 2003; Cummins Jr. et al., 2007; Jiang et al., 2005; McCarthy et al., 2005; Patton et al., 2006)

Note: The assays carried out are represented with a green shading; not carried out with a gray shading. Abbreviations: PAA, poly(alkylideneamine); PLL, poly(L-lysine).



the pathogenesis with HIV-1. However, cats are not susceptible to HIV-1. They are not used because FIV lacks specific accessory genes present in HIV-1, uses CD134 rather than CD4 as a primary receptor, and can infect B cells and CD8<sup>+</sup> T-cells (Hatzioannou & Evans, 2012). Mice, rats, and rabbits are relatively inexpensive and easy to handle. However, cells from these animals do not provide essential cofactors to support robust HIV-1 replication. Although these small-animal models offer advantages in terms of high reproductive rates, low maintenance costs, and the ability to conduct studies using inbred, genetically identical animals, they are distantly related to humans. Therefore, h-mice are the best small-animal models for HIV/AIDS research and recently to evaluate HIV-1 latent reservoirs and persistence (Deruaz & Tager, 2017; Flerin et al., 2019; Llewellyn et al., 2019; Marsden, 2020; Schmitt & Akkina, 2018). H-mice are genetically immunocompromised mice transplanted with human hematopoietic stem cells (HSCs), lymphoid tissue, or peripheral blood lymphocytes cells obtaining an identity, metabolic system, and functionality similar to humans (Ibeh et al., 2016; Marsden & Zack, 2017; Masse-Ranson et al., 2018; Victor Garcia, 2016; Weichseldorfer et al., 2020). Several h-mice models include the severe combined immunodeficiency (*scid*) mice with adult human peripheral blood lymphocytes (hu-PBLs) or human fetal thymus-liver tissue (hu-Thy/Liv), the human RAG (hu-HSC), and the bone marrow-liver-thymus (BLT) models. Hu-PBLs-SCID and hu-Thy/Liv-SCID mice models can be infected with HIV-1 by intraperitoneal injection or into the human implants to assess the efficacy of drugs and monoclonal antibodies against HIV-1 infection (Safrit et al., 1993; Stoddart et al., 2014; Yoshida et al., 2003). However, these mice models cannot be used for studying mucosal transmission (Hatzioannou & Evans, 2012).

H-BLT mice and h-RAG (Rag2<sup>-/-</sup>γc<sup>-/-</sup>) mice models have been developed to test microbicides against HIV-1 (Deruaz & Luster, 2013). BLT mice are generated from nonobese diabetic (NOD) *scid* or NOD *scid* gamma (NSG) mice, implanted with fetal thymus-liver cells, and transplanted with human CD34<sup>+</sup> HSCs from the same donor. This model mimics T cell development in humans because human T cells develop within the human thymus. More importantly, BLT mice can be infected with HIV-1 topically and are widely used (Denton et al., 2008, 2011; Destache et al., 2016; Gallay et al., 2017; Hur et al., 2012). Despite the significant advantages of the BLT mice model, several limitations must be addressed (see Table 6; Lavender et al., 2018). H-RAG mice model engrafted with CD34<sup>+</sup> HSCs is a model that increases tolerance to transplanted human HSCs and reduces graft-versus-host-disease (Lavender et al., 2013). This model does not develop tumors like mice with the mutation *scid*, and enhances human immune cells dissemination in the peripheral blood, liver, spleen, and vagina (Veselinovic et al., 2012). However, humoral responses to HIV-1 are not detectable, and secondary lymphoid tissues development is limited (Berges et al., 2006; Nochi et al., 2013).

### 8.3 | Other in vivo studies

Before introducing the microbicide candidate to clinical trials, safety and pharmacokinetic (absorption, distribution, metabolism, and excretion; Fu et al., 2020; Huang et al., 2020; Pandey et al., 2020) assays should be evaluated to estimate the margin of safety and risks.

Effects on vaginal tissue at several dose levels (single and repeated doses) and two different animal species (one rodent and one nonrodent) should be evaluated. Signs of inflammation, damage to the vaginal mucosa, or alteration of epithelial cells should be assessed following the scoring system described previously by Eckstein et al. (1969). There is

**TABLE 5** Comparison of different nonhuman primates used in HIV-1 studies as microbicides

Characteristics	Rhesus macaques	Pigtail macaques	Cynomolgus macaques
Microbicide studies	Most widely used	Widely used	Limited
Vaginal	Yes	Yes	No
Rectal	Yes	Yes	Yes
Disease progression	Similar to human	Rapid progression	Less pathogenic
Viral load	High	Slightly higher	Lower
CD4 <sup>+</sup> T depletion	Progressive	Rapid	Rapid
Vaginal anatomy	Similar to human	Similar to human	Vaginal vault smaller
Breeding time	Seasonal	Year-round	Seasonal
Availability	Wide	Limited	Wide

**TABLE 6** Advantages and limitations of bone marrow-liver-thymus (BLT) humanized mice for HIV-1 studies as microbicides. (Modified from Karpel et al. (2015), Marsden (2020), and Weichseldorfer et al. (2020))

Advantages	Disadvantages
Reconstitution with human T cells that develops within a human thymus	Any interaction with components of the mouse immune system will not reflect lymphoid tissue development in humans
Support robust HIV-1 infection. High levels of sustained viremia	Not valid for studies involving long-term chronic infection or prolonged immunization
Test CD4 <sup>+</sup> T cell depletion and virus-specific humoral and cellular immune responses	Restrictions on the use of fetal tissues in some parts of the world
Test human-specific genes and cytokines	Pharmacokinetic and metabolism in mice and humans are different
Study HIV-1 by the mucosal route	Limited sample volumes and cell numbers
Study latent HIV reservoirs	Not produced high levels of IgG antibodies, cytotoxic T cell responses, macrophage or natural killer cells
Study large amounts of animals in the same group compared to macaques	Mice are individually humanized for a limited lifespan
	Development of graft-versus-host disease after 6 months engraftment (may alter T and B cell activation states)

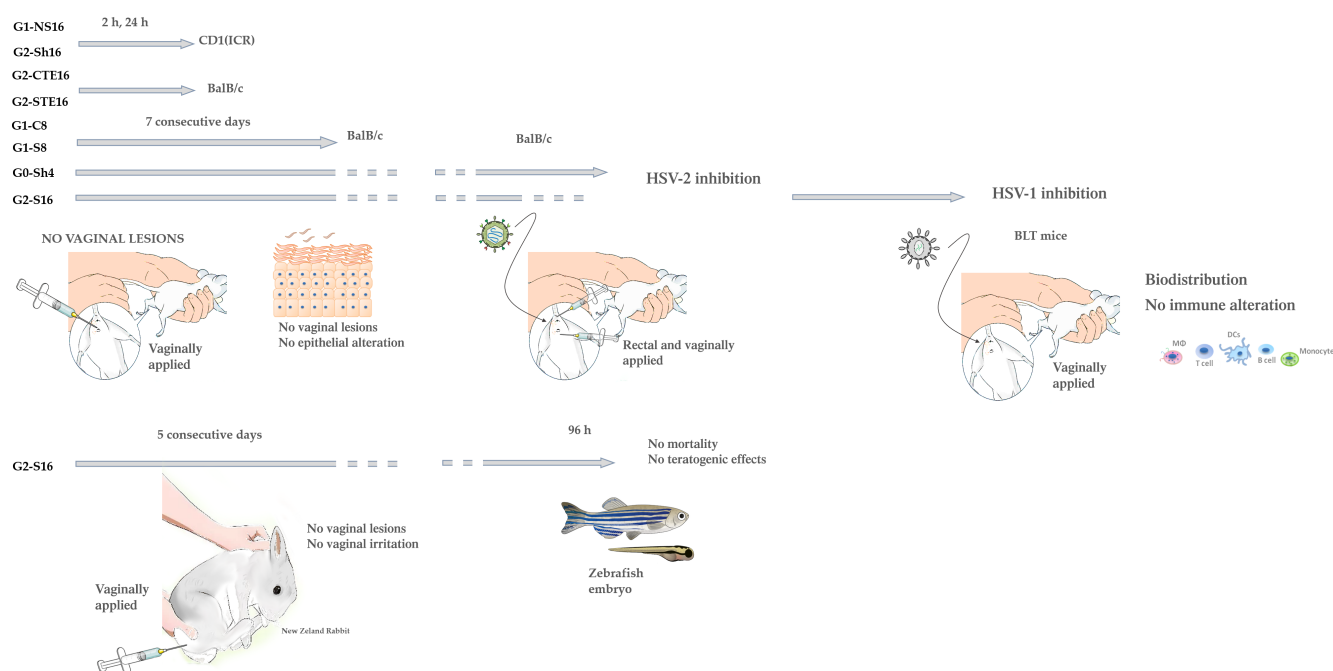
no established standard intravaginal administration. However, it is recommended to assess vaginal toxicity and irritation at two and 24 h after application, 5 or 7 days after daily application, or even after 14 days with two consecutive intravaginal administrations (Amaral et al., 2006; Dhondt et al., 2005; Fields et al., 2014; Nuttall et al., 2008).

API should be labeled with any probes to determine whether API crosses the epithelial barrier. Biodistribution studies must label API with fluorophores (fluorescein isothiocyanate [FITC]) to analyze the compound across the animal organism (das Neves et al., 2014; Henry et al., 2016). If API does not cross the epithelial barrier, only toxicological assays will be carried out. If API crosses the epithelial barrier, a battery of six assays of safety and activity that cannot be studied in humans should be included: mutagenicity, carcinogenicity, one (and two)-generation reproduction toxicity, neurotoxicity, embryotoxicity, and genetic studies (Lard-Whiteford et al., 2004). Mutagenicity and genetic studies evaluate chromosomal and structural DNA changes, such as duplications, insertions, or translocations (da Silva Dantas et al., 2020; Nakamura et al., 2020; Prado-Ochoa et al., 2020), and identify gene mutations (Gao et al., 2020; Marcelino et al., 2020; Park et al., 2020). Carcinogenicity studies evaluate tumors' development after 18 in mice or hamsters or 24 months in rats (Dekant et al., 2020; Prado-Ochoa et al., 2020; Saleh et al., 2020). Reproduction toxicity studies analyze the morphology and motility of male sperm, parturition, the number of live and dead pups, and the sex of the puppies. The same procedure is repeated to obtain the offspring of the second generation (Ganiger et al., 2007; Q. Liu et al., 2018; Montagnini et al., 2018; Wang et al., 2019). Neurotoxicity studies evaluate neuropathological lesions and neurological dysfunctions (loss of memory, sensory defects, and learning and memory dysfunctions) after 28 or 90 days of administration (Ishtiaq et al., 2021; Xu et al., 2021). Embryotoxicity studies evaluate embryofetal effects (hemorrhagic bullae, malformations, deformities, and mortality) between the 8th and 14th day of pregnancy (Carvalho et al., 2020; Maziero et al., 2020). In addition to these studies, compatibility with condoms, hypersensitivity, and photosensitivity studies should also be evaluated.

## 8.4 | Summary of in vivo assays performed with dendrimers

All dendrimers that reach this in vivo step were toxicologically tested to evaluate inflammation and vaginal irritation parameters after a short or prolonged vaginal exposure (see Table 4). G1-C8 and G1-S8 dendrimers showed no damage or alteration into the vaginal epithelium after seven consecutive days of exposure in a BALB/c mouse model (Maciel et al., 2019). G2-STE16 and G2-CTE16 in a female BALB/c mouse model (Galán et al., 2014; Sanchez-Rodriguez, Diaz, et al., 2015), and G2-Sh16 and G1-NS16 in CD1(ICR) mice (Vacas Cordoba et al., 2013), did not show irritation or vaginal lesions after two and 24 h vaginal application. G0-Sh4, like G2-S16 dendrimer, did not generate alteration of epithelial cells, inflammation, or damage to the vaginal mucosa after 7 days with daily application in BALB/c mice.

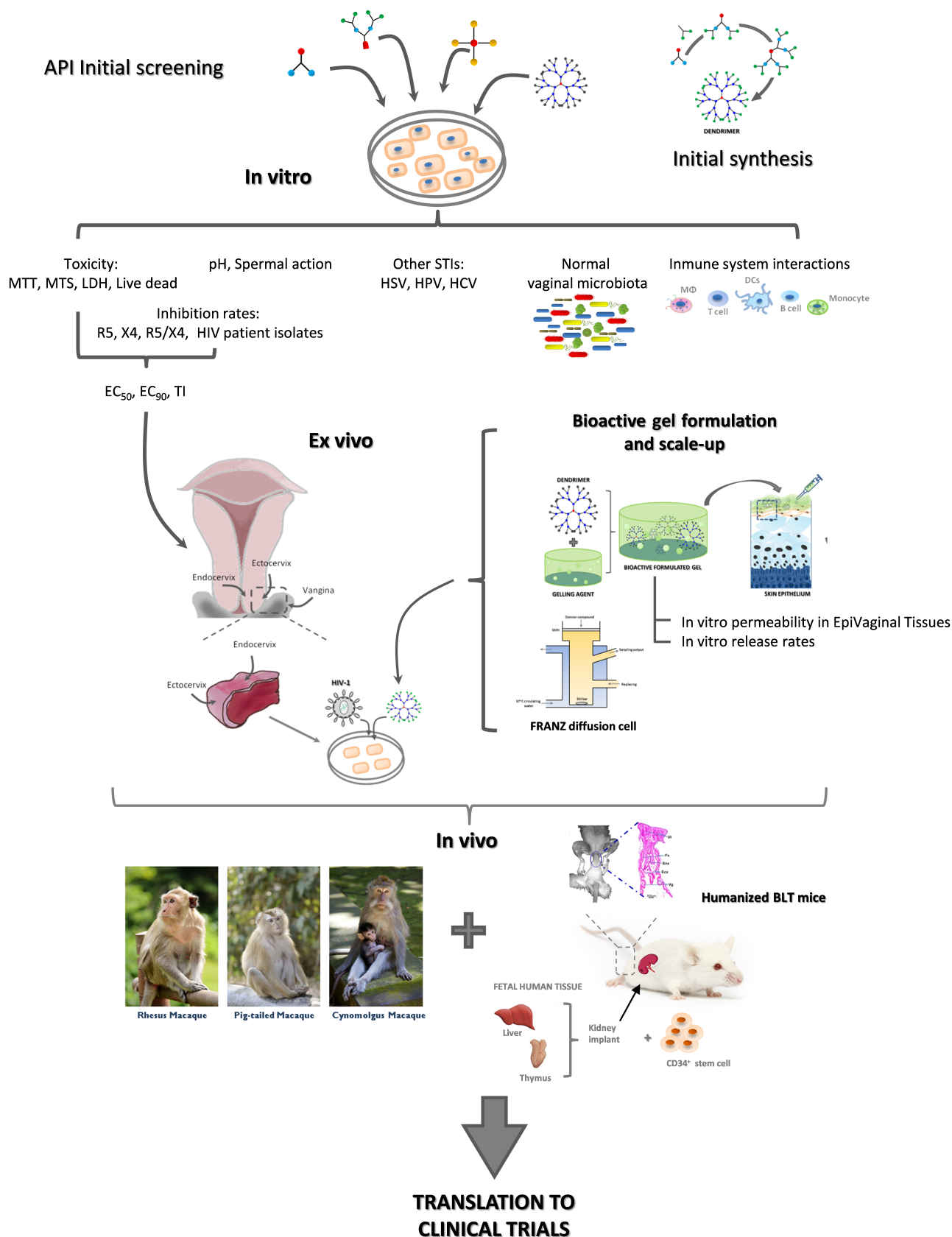
Remarkably, G0-Sh4 halted the same mouse model's HSV-2 infection both vaginally and rectally (Cena-Diez et al., 2019; Cena-Diez, Vacas-Cordoba, et al., 2016). The G2-S16 is the dendrimer with more toxicological studies vaginally. G2-S16 did not show toxicity and vaginal irritation after daily intravaginal administration during five consecutive days in New Zealand White rabbits (Chonco et al., 2012). Moreover, one or two successive doses of G2-S16 after 7 days postapplication did not disrupt epithelial cells nor produce damage in the vaginal mucosa in BALB/c mice (Briz et al., 2015; Sepulveda-Crespo, Serramia, et al., 2015), maintaining intact vaginal microbiome (Guerrero-Beltran et al., 2020). And more importantly, G2-S16 dendrimer prevented HIV-1 vaginal transmission in BLT-mice and HSV-2 in BALB/c female mice (Cena-Diez et al., 2019; Cena-Diez, Vacas-Cordoba, et al., 2016; Rodriguez-Izquierdo et al., 2020; Sepulveda-Crespo, Serramia, et al., 2015). G2-S16 dendrimer is the only dendrimer with vaginal



**FIGURE 4** Summary of main in vivo assays performed with polyanionic carbosilane dendrimers as vaginal microbicides

**TABLE 7** Advantages and limitations of vaginal dosage forms for anti-HIV-1 vaginal microbicides. (Modified from Antimisiaris and Mourtas (2015), Garg et al. (2010), and Rohan et al. (2014))

Formulation	Advantages	Disadvantages
Gel Cream Ointment	<ul style="list-style-type: none"> <li>Self-controlled</li> <li>Lubricant effects</li> <li>Low side-effects</li> <li>Low systemic absorption</li> <li>Low cost</li> <li>Combination system</li> </ul>	<ul style="list-style-type: none"> <li>Applicator required</li> <li>Not uniformity of API distribution</li> <li>Administration with frequency</li> </ul>
Tablet Suppository Film	<ul style="list-style-type: none"> <li>Self-controlled</li> <li>No applicator is required</li> <li>Low side-effects</li> <li>Rapid or sustained release</li> <li>Combination system</li> </ul>	<ul style="list-style-type: none"> <li>Absorption in the vaginal epithelium is dependent on local hydration</li> <li>Not uniformity of API distribution</li> <li>Vaginal irritation due to contact with solids</li> </ul>
Ring	<ul style="list-style-type: none"> <li>Self-controlled</li> <li>No applicator is required</li> <li>Long-term application</li> <li>Rapid release</li> <li>Combination system</li> </ul>	<ul style="list-style-type: none"> <li>High cost</li> <li>Uncomfortable placement</li> <li>Not uniformity of API distribution</li> <li>Difficult sustained release</li> <li>Complex manufacturing</li> </ul>



**FIGURE 5** Key preclinical steps in the development of a vaginal microbicide against HIV-1 infection to lead to clinical trials. API, active pharmaceutical ingredient; DCs, dendritic cells; EC50, half-maximal effective concentration; EC90, 90% effective concentration; HCV, hepatitis C virus; HPV, human papillomavirus; HSV, herpes simplex virus; LDH, lactate dehydrogenase; MΦ, macrophages; MTS, 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; TI, therapeutic index

biodistribution studies in BALB/c mice and teratogenic studies in a zebrafish model. G2-S16 did not cross the vaginal barrier even after 20 h of vaginal application and did not show mortality, sublethal, or teratogenic effects in any dose and at 96 h after intravaginal application in the zebrafish embryos (Cena-Diez et al., 2017; for a detailed summary, see Figure 4).

SPL7013 is another of the most advanced dendrimers in *in vivo* assays. SPL7013 was not toxic vaginally and rectally after a 4-day repeat dose in a female pig-tailed macaque model (Patton et al., 2006) and did not irritate 5-day repeat dose intravaginal administration in rabbits (Bernstein et al., 2003). More importantly, SPL7013 prevented vaginal transmission in pig-tailed macaques after cell-free SHIV<sub>89</sub> infection (Jiang et al., 2005; McCarthy et al., 2005) and HSV-2 infection in female Swiss Webster mice (Bernstein et al., 2003). The lack of more relevant vaginal toxicological studies in different conditions, doses and times, could be the leading cause of failure in clinical trials as vaginal microbicide: poor acceptability, modifications in the microbiome, inflammation, and epithelial damage (Carballo-Dieguez et al., 2012; Cohen et al., 2011; McGowan et al., 2011; Moscicki et al., 2012; Pellett Madan et al., 2015).

## 9 | VAGINAL FORMULATIONS: DEVELOPMENT AND CHARACTERIZATION

Microbicide formulation or delivery system plays a key role because API must be stable in the vehicle, reach the right activity site, distribute throughout the vaginal compartment, and remain there for a sufficient time. Liquid formulations (e.g., suspensions or solutions) are inappropriate due to their short retention and contact time in the vaginal surface to exert their activity. Therefore, appropriate formulations for vaginal delivery of microbicides should include semi-solid (gels, creams, ointments) or solid (tablets, films, suppositories, rings) dosage forms (see Table 7).

The gel is a suspension of inorganic or organic particles interpenetrated by an aqueous or nonaqueous liquid (Antimisiaris & Mourtas, 2015; Garg et al., 2010; Rohan et al., 2014). Its high lubrication capacity and wide acceptance contribute to it being the most widely studied formulation as vaginal microbicide (Al-Khouja et al., 2020; Bunge et al., 2018; Delany-Moretwe et al., 2018; Halwes et al., 2016). The tablets are polymers that gel in the presence of vaginal fluids and overcome stability concerns related to gels (Antimisiaris & Mourtas, 2015; Garg et al., 2010; Rohan et al., 2014). Tablets protect the vagina instantly (fast-dissolving tablets) or in a controlled and sustained manner to promote better adherence and less use (Clark et al., 2014; McConville et al., 2013; McConville et al., 2016). Fast-dissolving films are water-soluble polymers that release the API to dissolve in the vaginal mucosa. Although vaginal films do not require an applicator to use, their possible influence during sexual intercourse is a disadvantage (Antimisiaris & Mourtas, 2015; Garg et al., 2010; Rohan et al., 2014). However, their formulation as a vaginal microbicide has been studied (Notario-Perez et al., 2019; Notario-Perez et al., 2020; Patki et al., 2020). Despite the microbicide formulation's progress, and most vaginal drugs have been in the form of gels, vaginal rings have gained acceptance in the last years due to a controlled release for a long time (Bunge et al., 2020; Katz et al., 2020; Roberts et al., 2020; Thurman et al., 2019; Vincent et al., 2018). Recently, there has been a growing interest in alternative dosage forms such as nanogels (das Neves & Sarmento, 2015; Destache et al., 2016; Kovarova et al., 2015; Lai & He, 2016) or electrospun fibers (Tyo et al., 2017; Tyo et al., 2019).

Physicochemical properties of vehicles should be evaluated: type of polymer used (Carbopol 934, hydroxypropylmethylcellulose 4000 [HPMC 4000], hydroxyethylcellulose [HEC], polyethylene glycol 6000 [PEG 6000]), dose volume, viscosity, yields stress, pH, osmolality, appearance, odor, and impurities. Vehicles must maintain the acidic pH of the vagina once in contact with semen, be bioadhesive to hold the vaginal surface in the long term, and have a relatively thick viscosity (El-Enin et al., 2020; Nelson, 2018). The API solubility and compatibility with formulation excipients and other drugs and long-term stability should also be evaluated in preformulation research. Other studies, such as API capacity across a wide range of pH or degradation assays (acid/base, heat, light, oxidative stress), should also be evaluated. Therefore, assessing the best API dosage form (API monodispersity, dose volume, product dimensions, or impurities) is mandatory.

SPL7013 has been formulated in three different gel prototypes containing a mucoadhesive carbopol gel (VivaGel®). SPL7013 and carbopol were tested for buffering capacity, pH, osmolality, and viscosity. The prototypes differed only in propylene glycol and glycerin (Bernstein et al., 2003; Mumper et al., 2009; Telwatte et al., 2011). The rest of the carbosilane and PAA dendrimers tested in *in vivo* assays were formulated in a universal HEC gel with encouraging results (Briz et al., 2015; Cena-Diez, Vacas-Cordoba, et al., 2016; Maciel et al., 2019; Sanchez-Rodriguez, Diaz, et al., 2015;



## BOX 1 List of drugs based on dendrimers as anti-HIV-1 microbicides

### Boltorn

- *BH30sucMan*: G3 with 2,2-bis(methylol)propionic acid (bis-MPA) as a core and 32 mannoses at the periphery
- *Dendron12*: G3 with 3-azidopropanoic acid as a core and four pseudotrimannoside groups at the periphery
- *BH3OPSGal*: G3 with bis-MPA as a core and functionalized with 32  $\beta$ -Galceramide groups at the periphery

### Carbosilane

- *G2-S16*: G1 with silicon as a core and 16 sulfonate groups at the periphery
- *G2-STE16*: G2 with silicon as a core and 16 sulfonate groups at the periphery
- *G2-CTE16*: G2 with silicon as a core and 16 carboxylate groups at the periphery
- *G1-NS16*: G1 with silicon as a core and 16 naphthylsulfonate groups at the periphery
- *G2-Sh16*: G1 with silicon as a core and 16 sulfate groups at the periphery
- *G0-Sh4*: G0 with silicon as a core and four sulfate groups at the periphery
- *G2-S24P*: G2 with a polyphenolic core and 24 sulfonate groups at the periphery
- *G2-S8T*: G2 with a triazole core and eight sulfonate groups at the periphery

### Citric acid-polyethylene glycol-citric acid (CPEGC)

- *G2-C18PEG*: G2 with polyethylene glycol as core and 18 carboxylate groups
- *NPAg-G2-C18PEG*: Nanoconjugated silver G2 dendrimer with polyethylene glycol as core and 18 carboxylate groups

### Gallic acid-triethylene glycol (GATG)

- [*G1*]-*CO2Na*: G1 with a GATG core and nine benzoate groups at the periphery

### Glycodendrimers

- *Bol13.4*: G1 with pentaerythritol as a core and six pseudodimannoside groups at the periphery
- *ROD3.5.6*: G0 with an aromatic rod-like core and six pseudodisaccharide groups at the periphery
- *ROD3.7.6*: G0 with an aromatic rod-like core and two pseudodisaccharide groups at the periphery

### Poly(alkylideneamine) (PAA)

- G1-C8: G1 with 1,6-diaminohexane/hexamethylenediamine as a core and eight carboxylate groups at the periphery
- G1-S8: G1 with 1,6-diaminohexane/hexamethylenediamine as a core and eight sulfonate groups at the periphery

### Poly(amidoamine) (PAMAM)

- *LewisX*: G5 with ethylenediamine as a core and 14–16 glycan groups at the periphery
- *Sulfo-6*: G2 with ethylenediamine as a core and 16 sialic acid groups or 11 sulfate groups at the periphery
- *SPL2923*: G4 with ammonia as a core and 24 1-(carboxymethoxy) naphthalene-3,6-disulfonate groups at the periphery
- *SPL6195*: G4 with ethylenediamine as a core and 32 benzene dicarboxylate groups at the periphery
- *SPL7304*: G4 with benzhydrylamine amide as a core and 32 1-(carboxymethoxy) naphthalene-3,6-disulfonate groups at the periphery

### Peptide dendrimers

- *SB105-A10*: G1 with benzhydramine amide as a core and four sequence peptide chain (ASLRVRIKK) at the periphery
- *Trp(5a-5f) and modifications*: G1 with carboxylic acid with an aminotriester as a branching unit and 9–18 tryptophans at the periphery

### Poly(L-lysine) (PLL)

- *SPL7013 (VivaGel®)*: G4 with benzhydramine amide as a core and 32 1-(carboxymethoxy) naphthalene-3,6-disulfonate groups at the periphery
- *SCSLD3*: G3 with stearylamine as a core and 32 cellobioses at the periphery. Degree of sulfation: 2.3
- *PLDG3-PSCel*: G3 with benzhydramine amide as a core and 24 cellobioses at the periphery. Degree of sulfation: 1.9
- *SPL7115*: G2 with benzhydramine amide as a core and 8 1-(carboxymethoxy) naphthalene-3,6-disulfonate groups at the periphery

### Phosphorus-containing (PPH) dendrimers

- *PPH-3d-G1*: G1 with cyclotriphosphazene as a core and 12 galactosylceramide, *N*-hexadecylaminolactitol groups at the periphery
- *PPH-5c-Gc'1*: G1 with cyclotriphosphazene as a core and 12 phosphonic acid moiety and lateral alkyl chain at the periphery

### Poly(propylene imine) (PPI)

- *PSGal64mer*: G5 with 1,4-diaminobutane as a core and 44 galactoses and 2 sulfate groups at the periphery
- *MVC-GBT*: G5 with 1,4-diaminobutane as a core and 46 globotrioses at the periphery
- *MVC-3SL*: G5 with 1,4-diaminobutane as a core and 28 3' sialyllactoses at the periphery
- *SPL7320*: G4 with benzhydramine amide as a core and 32 1-(carboxymethoxy) naphthalene-3,6-disulfonate groups at the periphery
- *MD-G2S*: G2 metallodendrimer with ethylenediamine as a core and 16 sulfonate groups at the periphery
- *MD-G2C*: G2 metallodendrimer with ethylenediamine as a core and 16 carboxylate groups at the periphery

### Viologen

- *Viol36*: G1 with a benzyl as a core and 6 ethyl groups at the periphery
- *Viol7*: G1 with a benzyl as a core and 6 thymine groups at the periphery

Sepulveda-Crespo, Serramia, et al., 2015). However, not all gel's physicochemical properties have currently been evaluated.

## 10 | CONCLUSIONS AND FUTURE PERSPECTIVES

A better knowledge based on the sexual needs of women living with HIV will lead to better approaches to prevent HIV-1 transmission. In this sense, topical vaginal microbicides address these issues, considering that funding in this field has increased over the years. Different formulations have been developed to protect against HIV infection but should be used appropriately. The great advantage of topically applied microbicides is to avoid negotiation with the partner. A microbicide should prevent transmission of HIV-1 and other STIs in the vaginal mucosa and systemically in the case of injuries.

An exhaustive preclinical evaluation of API as a microbicide candidate is vital to save costs and time before reaching clinical trials. Preclinical steps start optimizing API's structure–activity relationship using *in vitro*, *ex vivo*, and *in vivo* assays and developing a suitable vaginal delivery system. However, candidates can fail due to an inadequate optimization of their structure or an incomplete preclinical characterization. Considering the correct optimization of API structure, new models, assays, and endpoints that mimic the vagina and the penis' environmental conditions during sexual intercourse have been developed and incorporated into preclinical steps.

This algorithm (see Figure 5) identifies potential compounds as vaginal microbicides. The algorithm designed must also consider the risk of HIV-1 transmission rectally. Microbicide candidates, such as anionic dendrimers that inhibit viral entry, are primary compounds. pH transition assays with acidic, essential, and seminal plasma, different cytotoxicity assays to check if API damages the mitochondria or the integrity of the plasma membrane, assays with T/F viruses are some of the assays that have been updated in this preclinical algorithm. Remarkably, combination assays with different mechanisms of action, such as the HIV-1 entry (anionic dendrimers) and other processes before integration, are highly recommended.

There is no perfect model that fully recapitulates how humans become HIV-infected or how the dendrimer would act in humans. Currently, h-mice are excellent models for evaluating mucosal HIV transmission *in vivo* due to engrafted parts of the human immune system. However, their short lifespan and low sample volumes that can be obtained are their main limitations. A better understanding of the human and mouse immune systems will lead to better h-mice models.

Despite its good results in the different *in vitro*, *ex vivo*, and *in vivo* assays, the anionic SPL7013 dendrimer failed in the first clinical trials due to signs of inflammation and epithelial damage (Carballo-Dieguez et al., 2012; Cohen et al., 2011; McGowan et al., 2011; Moscicki et al., 2012; Pellett Madan et al., 2015). However, it is intended to take advantage of its enormous benefits as a molecule and is currently being used against bacterial vaginosis (Chavoustie et al., 2020; Waldbaum et al., 2020). Remarkably, Starpharma wants to use SPL7013 as a COVID-19 nasal spray after showing significant activity against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the virus that causes COVID-19 (Starpharma, 2021). This news represents more than enough reasons to continue believing in anionic dendrimers as potential candidates as vaginal microbicides against HIV-1 and other viruses. However, they must go through extensive preclinical assays before reaching clinical trials (Box 1).

## ACKNOWLEDGMENT

This work has been (partially) funded by the RD16/0025/0019 projects as part of Acción Estratégica en Salud, Plan Nacional de Investigación Científica, Desarrollo e Innovación Tecnológica (2013–2016) and cofinanced by Instituto de Salud Carlos III (ISCIII) and Fondo Europeo de Desarrollo Regional (FEDER), RETIC PT17/0015/0042, Fondo de Investigación Sanitaria (FIS) (grant no. PI16/01863) and EPIICAL project. COST CA17140 Cancer Nanomedicine–“From the Bench to Bedside.” This work has also been supported by the Ministry of Economy and Competitiveness #CGL2013-40564-R and Gordon and Betty Moore Foundation grant no. 5334. This work was also funded by research grants from ISCIII (grant numbers PI20CIII/00004, and RD16CIII/0002/0002) to Salvador Resino. The study was also funded by the Centro de Investigación Biomédica en Red en Enfermedades Infecciosas (CB21/13/00044). DS-C is a “Sara Borrell” researcher from ISCIII (grant no. CD20CIII/00001).

## CONFLICT OF INTEREST

The authors have declared no conflicts of interest for this article.

## AUTHOR CONTRIBUTIONS

**Ignacio Rodríguez-Izquierdo:** Data curation (equal); investigation (equal); writing – original draft (equal). **Daniel Sepúlveda-Crespo:** Data curation (equal); formal analysis (lead); investigation (equal); methodology (lead); writing – original draft (equal). **Jose María Lasso:** Supervision (equal); validation (equal); writing – review and editing (equal). **Salvador Resino:** Supervision (equal); validation (equal); visualization (equal); writing – review and editing (equal). **Ma Ángeles Muñoz-Fernández:** Conceptualization (lead); funding acquisition (lead); investigation (equal); project administration (lead); resources (equal); supervision (equal); validation (equal); visualization (equal); writing – review and editing (equal).

## DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

**ORCID**

Daniel Sepúlveda-Crespo  <https://orcid.org/0000-0002-8053-6045>

Jose María Lasso  <https://orcid.org/0000-0001-9728-7826>

Salvador Resino  <https://orcid.org/0000-0001-8783-0450>

Ma Ángeles Muñoz-Fernández  <https://orcid.org/0000-0002-0813-4500>

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**FURTHER READING**

Relaño-Rodríguez, I., & Muñoz-Fernández, M. Á. (2020). Emergence of nanotechnology to fight HIV sexual transmission: The trip of G2-S16 Polyanionic Carbosilane dendrimer to possible pre-clinical Trials. *International Journal of Molecular Sciences*, 21(24), 9403. <https://doi.org/10.3390/ijms21249403>

Sepúlveda-Crespo, D., Ceña-Díez, R., Jiménez, J. L., & Muñoz-Fernández, M. Á. (2017). Mechanistic studies of viral entry: An overview of dendrimer-based microbicides as entry inhibitors against both HIV and HSV-2 overlapped infections. *Medicinal Research Reviews*, 37(1), 149–179. <https://doi.org/10.1002/med.21405>

**REFERENCES**

- Abbasi, E., Aval, S. F., Akbarzadeh, A., Milani, M., Nasrabadi, H. T., Joo, S. W., Hanifehpour, Y., Nejati-Koshki, K., & Pashaei-Asl, R. (2014). Dendrimers: Synthesis, applications, and properties. *Nanoscale Research Letters*, 9(1), 247. <https://doi.org/10.1186/1556-276X-9-247>
- Abd-El-Aziz, A. S., El-Ghezlani, E. G., & Abdelghani, A. A. (2020). Design of organoiron dendrimers containing paracetamol for enhanced antibacterial efficacy. *Molecules*, 25(19), 4514. <https://doi.org/10.3390/molecules25194514>
- Abner, S. R., Guenther, P. C., Guarner, J., Hancock, K. A., Cummins, J. E., Jr., Fink, A., Gilmore, G. T., Staley, C., Ward, A., Ali, O., Binderow, S., Cohen, S., Grohskopf, L. A., Paxton, L., Hart, C. E., & Dezzutti, C. S. (2005). A human colorectal explant culture to evaluate topical microbicides for the prevention of HIV infection. *The Journal of Infectious Diseases*, 192(9), 1545–1556. <https://doi.org/10.1086/462424>
- Alfei, S., & Schito, A. M. (2020). From nanobiotechnology, positively charged biomimetic dendrimers as novel antibacterial agents: A review. *Nanomaterials (Basel)*, 10(10), 2022. <https://doi.org/10.3390/nano10102022>
- Ali, A., Syed, S. M., Jamaluddin, M. F. B., Colino-Sanguino, Y., Gallego-Ortega, D., & Tanwar, P. S. (2020). Cell lineage tracing identifies hormone-regulated and Wnt-responsive vaginal epithelial stem cells. *Cell Reports*, 30(5), 1463–1477. <https://doi.org/10.1016/j.celrep.2020.01.003>
- Ali, A., Syed, S. M., & Tanwar, P. S. (2020). Protocol for in vitro establishment and long-term culture of mouse vaginal organoids. *STAR Protocols*, 1(2), 100088. <https://doi.org/10.1016/j.xpro.2020.100088>
- Al-Khouja, A., Shieh, E. C., Fuchs, E. J., Marzinke, M. A., Bakshi, R. P., Hummert, P., Ham, A., Buckheit, K. W., Breakey, J. C., Weld, E. D., Chen, H., Caffo, B. S., Buckheit, R. W., & Hendrix, C. W. (2020). Examining the safety, pharmacokinetics, and pharmacodynamics of a rectally administered IQP-0528 gel for HIV pre-exposure prophylaxis: A first-in-human study. *AIDS Research and Human Retroviruses*, 37, 444–452. <https://doi.org/10.1089/AID.2020.0188>
- Amaral, E., Perdigao, A., Souza, M. H., Mauck, C., Waller, D., Zaneveld, L., & Faundes, A. (2006). Vaginal safety after use of a bioadhesive, acid-buffering, microbicidal contraceptive gel (ACIDFORM) and a 2% nonoxynol-9 product. *Contraception*, 73(5), 542–547. <https://doi.org/10.1016/j.contraception.2005.12.006>
- Anandhan, R., Reddy, M. B., & Sasikumar, M. (2019). Development of novel triazole based dendrimer supported spiroborate chiral catalysts for the reduction of (E)-O-benzyl oxime: An enantioselective synthesis of (S)-dapoxetine. *New Journal of Chemistry*, 43(38), 15052–15056. <https://doi.org/10.1039/C9NJ03217G>
- Anandkumar, D., & Rajakumar, P. (2017). Synthesis and anticancer activity of bile acid dendrimers with triazole as bridging unit through click chemistry. *Steroids*, 125, 37–46. <https://doi.org/10.1016/j.steroids.2017.06.007>
- Anderson, D. J., Pudney, J., & Schust, D. J. (2010). Caveats associated with the use of human cervical tissue for HIV and microbicide research. *AIDS*, 24(1), 1–4. <https://doi.org/10.1097/QAD.0b013e3283333acfb>
- Antimisariaris, S. G., & Mourtas, S. (2015). Recent advances on anti-HIV vaginal delivery systems development. *Advanced Drug Delivery Reviews*, 92, 123–145. <https://doi.org/10.1016/j.addr.2015.03.015>
- Arce, E., Nieto, P. M., Diaz, V., Castro, R. G., Bernad, A., & Rojo, J. (2003). Glycodendritic structures based on Boltorn hyperbranched polymers and their interactions with lens culinaris lectin. *Bioconjugate Chemistry*, 14(4), 817–823. <https://doi.org/10.1021/bc034008k>
- Ardestani, M. S., Fordoei, A. S., Abdoli, A., Ahangari Cohan, R., Bahramali, G., Sadat, S. M., Siadat, S. D., Moloudian, H., Nassiri Koopaei, N., Bolhasani, A., Rahimi, P., Hekmat, S., Davari, M., & Aghasadeghi, M. R. (2015). Nanosilver based anionic linear globular dendrimer with a special significant antiretroviral activity. *Journal of Materials Science. Materials in Medicine*, 26(5), 179. <https://doi.org/10.1007/s10856-015-5510-7>

- Arien, K. K., Kyongo, J. K., & Vanham, G. (2012). Ex vivo models of HIV sexual transmission and microbicide development. *Current HIV Research*, 10(1), 73–78.
- Arien, K. K., Venkatraj, M., Michiels, J., Joossens, J., Vereecken, K., Van der Veken, P., Heeres, J., De Winter, H., Heyndrickx, L., Augustyns, K., & Vanham, G. (2016). Resistance and cross-resistance profile of the diaryltriazine NNRTI and candidate microbicide UAMC01398. *The Journal of Antimicrobial Chemotherapy*, 71(5), 1159–1168. <https://doi.org/10.1093/jac/dkv501>
- Arnáiz, E., Vacas-Córdoba, E., Galán, M., Pion, M., Gómez, R., Muñoz-Fernández, M. A. Á., & de la Mata, F. J. (2014). Synthesis of anionic carbosilane dendrimers via “click chemistry” and their antiviral properties against HIV. *Journal of Polymer Science Part A: Polymer Chemistry*, 52(8), 1099–1112. <https://doi.org/10.1002/pola.27090>
- Asaftei, S., & De Clercq, E. (2010). Corrections to “Viologen” dendrimers as antiviral agents: The effect of charge number and distance. *Journal of Medicinal Chemistry*, 53(15), 5895–5895. <https://doi.org/10.1021/jm100741h>
- Asaftei, S., Huskens, D., & Schols, D. (2012). HIV-1 X4 activities of polycationic “Viologen” based dendrimers by interaction with the chemokine receptor CXCR4: Study of structure–activity relationship. *Journal of Medicinal Chemistry*, 55(23), 10405–10413. <https://doi.org/10.1021/jm301337y>
- Ayehunie, S., Cannon, C., Lamore, S., Kubilus, J., Anderson, D. J., Pudney, J., & Klausner, M. (2006). Organotypic human vaginal-ectocervical tissue model for irritation studies of spermicides, microbicides, and feminine-care products. *Toxicology In Vitro*, 20(5), 689–698. <https://doi.org/10.1016/j.tiv.2005.10.002>
- Back, D., & Marzolini, C. (2020). The challenge of HIV treatment in an era of polypharmacy. *Journal of the International AIDS Society*, 23(2), e25449. <https://doi.org/10.1002/jia2.25449>
- Baden, L. R., Stieh, D. J., Sarnecki, M., Walsh, S. R., Tomaras, G. D., Kublin, J. G., McElrath, M. J., Alter, G., Ferrari, G., Montefiori, D., Mann, P., Nijs, S., Callewaert, K., Goepfert, P., Edupuganti, S., Karita, E., Langedijk, J. P., Wegmann, F., Corey, L., ... Travers, Hvtv H. P. X. Study Team. (2020). Safety and immunogenicity of two heterologous HIV vaccine regimens in healthy, HIV-uninfected adults (TRAVVERSE): A randomised, parallel-group, placebo-controlled, double-blind, phase 1/2a study. *Lancet HIV*, 7(10), e688–e698. [https://doi.org/10.1016/S2352-3018\(20\)30229-0](https://doi.org/10.1016/S2352-3018(20)30229-0)
- Baeten, J. M., Hendrix, C. W., & Hillier, S. L. (2020). Topical microbicides in HIV prevention: State of the promise. *Annual Review of Medicine*, 71, 361–377. <https://doi.org/10.1146/annurev-med-090518-093731>
- Bagul, R. S., Hosseini, M. M., Shiao, T. C., & Roy, R. (2017). “Onion peel” glycodendrimer syntheses using mixed triazine and cyclotriphosphazene scaffolds. *Canadian Journal of Chemistry*, 95(9), 975–983. <https://doi.org/10.1139/cjc-2017-0220>
- Bahreyni, A., Mohamud, Y., & Luo, H. (2020). Emerging nanomedicines for effective breast cancer immunotherapy. *Journal of Nanobiotechnology*, 18(1), 180. <https://doi.org/10.1186/s12951-020-00741-z>
- Barnes, T. J., Ametov, I., & Prestidge, C. A. (2008). Naphthalene sulfonate functionalized dendrimers at the solid-liquid interface: Influence of core type, ionic strength, and competitive ionic adsorbates. *Langmuir*, 24(21), 12398–12404. <https://doi.org/10.1021/la8020996>
- Bartolo, I., Diniz, A. R., Borrego, P., Ferreira, J. P., Bronze, M. R., Barroso, H., Pinto, R., Cardoso, C., Pinto, J. F., Diaz, R. C., Broncano, P. G., Muñoz-Fernandez, M. A., & Taveira, N. (2018). Evaluation of the fusion inhibitor P3 peptide as a potential microbicide to prevent HIV transmission in women. *PLoS One*, 13(4), e0195744. <https://doi.org/10.1371/journal.pone.0195744>
- Battini, L., & Bollini, M. (2019). Challenges and approaches in the discovery of human immunodeficiency virus type-1 non-nucleoside reverse transcriptase inhibitors. *Medicinal Research Reviews*, 39(4), 1235–1273. <https://doi.org/10.1002/med.21544>
- Bayda, S., Adeel, M., Tuccinardi, T., Cordani, M., & Rizzolio, F. (2019). The history of nanoscience and nanotechnology: From chemical-physical applications to nanomedicine. *Molecules*, 25(1), 112. <https://doi.org/10.3390/molecules25010112>
- Berges, B. K., Wheat, W. H., Palmer, B. E., Connick, E., & Akkina, R. (2006). HIV-1 infection and CD4 T cell depletion in the humanized Rag2<sup>-/-</sup>gamma c<sup>-/-</sup> (RAG-hu) mouse model. *Retrovirology*, 3, 76. <https://doi.org/10.1186/1742-4690-3-76>
- Bernstein, D. I., Stanberry, L. R., Sacks, S., Ayisi, N. K., Gong, Y. H., Ireland, J., Mumper, R. J., Holan, G., Matthews, B., McCarthy, T., & Bourne, N. (2003). Evaluations of unformulated and formulated dendrimer-based microbicide candidates in mouse and Guinea pig models of genital herpes. *Antimicrobial Agents and Chemotherapy*, 47(12), 3784–3788. <https://doi.org/10.1128/aac.47.12.3784-3788.2003>
- Berzi, A., Reina, J. J., Ottria, R., Sutkeviciute, I., Antonazzo, P., Sanchez-Navarro, M., Chabrol, E., Biasin, M., Trabattoni, D., Cetin, I., Rojo, J., Fieschi, F., Bernardi, A., & Clerici, M. (2012). A glycomimetic compound inhibits DC-SIGN-mediated HIV infection in cellular and cervical explant models. *AIDS*, 26(2), 127–137. <https://doi.org/10.1097/QAD.0b013e32834e1567>
- Binda, A., Murano, C., & Rivolta, I. (2020). Innovative therapies and nanomedicine applications for the treatment of Alzheimer’s disease: A state-of-the-art (2017–2020). *International Journal of Nanomedicine*, 15, 6113–6135. <https://doi.org/10.2147/IJN.S231480>
- Bon, I., Lembo, D., Rusnati, M., Clo, A., Morini, S., Miserocchi, A., Bugatti, A., Grigolon, S., Musumeci, G., Landolfo, S., Re, M. C., & Gibellini, D. (2013). Peptide-derivatized SB105-A10 dendrimer inhibits the infectivity of R5 and X4 HIV-1 strains in primary PBMCs and cervicovaginal histocultures. *PLoS One*, 8(10), e76482. <https://doi.org/10.1371/journal.pone.0076482>
- Bouchemal, K., Aka-Any-Grah, A., Dereuddre-Bosquet, N., Martin, L., Lievin-Le-Moal, V., Le Grand, R., Nicolas, V., Gibellini, D., Lembo, D., Pous, C., Koffi, A., & Ponchel, G. (2015). Thermosensitive and mucoadhesive pluronic-hydroxypropylmethylcellulose hydrogel containing the mini-CD4 M48U1 is a promising efficient barrier against HIV diffusion through macaque cervicovaginal mucus. *Antimicrobial Agents and Chemotherapy*, 59(4), 2215–2222. <https://doi.org/10.1128/AAC.03503-14>
- Bouschbacher, M., Bomsel, M., Verronese, E., Gofflo, S., Ganor, Y., Dezutter-Dambuyant, C., & Valladeau, J. (2008). Early events in HIV transmission through a human reconstructed vaginal mucosa. *AIDS*, 22(11), 1257–1266. <https://doi.org/10.1097/QAD.0b013e3282f736f4>
- Brako, F., Mahalingam, S., Rami-Abraham, B., Craig, D. Q., & Edirisinghe, M. (2017). Application of nanotechnology for the development of microbicides. *Nanotechnology*, 28(5), 052001. <https://doi.org/10.1088/1361-6528/28/5/052001>



- Briz, V., Sepulveda-Crespo, D., Diniz, A. R., Borrego, P., Rodes, B., de la Mata, F. J., Gomez, R., Taveira, N., & Munoz-Fernandez, M. A. (2015). Development of water-soluble polyanionic carbosilane dendrimers as novel and highly potent topical anti-HIV-2 microbicides. *Nanoscale*, 7(35), 14669–14683. <https://doi.org/10.1039/c5nr03644e>
- Bunge, K. E., Dezzutti, C. S., Hendrix, C. W., Marzinke, M. A., Spiegel, H. M. L., Moncla, B. J., Schwartz, J. L., Meyn, L. A., Richardson-Harman, N., Rohan, L. C., & Hillier, S. L. (2018). FAME-04: A phase 1 trial to assess the safety, acceptability, pharmacokinetics and pharmacodynamics of film and gel formulations of tenofovir. *Journal of the International AIDS Society*, 21(8), e25156. <https://doi.org/10.1002/jia2.25156>
- Bunge, K. E., Levy, L., Szydlo, D. W., Zhang, J., Gaur, A. H., Reirden, D., Mayer, K. H., Futterman, D., Hoesley, C., Hillier, S. L., Marzinke, M. A., Hendrix, C. W., Gorbach, P. M., Wilson, C. M., Soto-Torres, L., Kapogiannis, B., Nel, A., Squires, K. E., & Team, Mtn-Ipm Study. (2020). Brief report: Phase IIa safety study of a vaginal ring containing dapivirine in adolescent young women. *Journal of Acquired Immune Deficiency Syndromes*, 83(2), 135–139. <https://doi.org/10.1097/QAI.0000000000002244>
- Busso, M., Thornthwaite, J., & Resnick, L. (1991). HIV-induced syncytium formation requires the formation of conjugates between virus-infected and uninfected T-cells in vitro. *AIDS*, 5(12), 1425–1432. <https://doi.org/10.1097/00002030-199112000-00003>
- Calenda, G., Villegas, G., Barnable, P., Litterst, C., Levendosky, K., Gettie, A., Cooney, M. L., Blanchard, J., Fernandez-Romero, J. A., Zydowsky, T. M., & Teleshova, N. (2017). MZC gel inhibits SHIV-RT and HSV-2 in macaque vaginal mucosa and SHIV-RT in rectal mucosa. *Journal of Acquired Immune Deficiency Syndromes*, 74(3), e67–e74. <https://doi.org/10.1097/QAI.0000000000001167>
- Carballo-Dieguez, A., Giguere, R., Dolezal, C., Chen, B. A., Kahn, J., Zimet, G., Mabragana, M., Leu, C. S., & McGowan, I. (2012). "tell Juliana": Acceptability of the candidate microbicide VivaGel(R) and two placebo gels among ethnically diverse, sexually active young women participating in a phase 1 microbicide study. *AIDS and Behavior*, 16(7), 1761–1774. <https://doi.org/10.1007/s10461-011-0028-6>
- Carlmark, A., Hawker, C., Hult, A., & Malkoch, M. (2009). New methodologies in the construction of dendritic materials. *Chemical Society Reviews*, 38(2), 352–362. <https://doi.org/10.1039/b711745k>
- Carvalho, M., Caixeta, G. A. B., Lima, A. R. S., Teófilo, M. N. G., Cruvinel, W. M., Gomes, C. M., Fleury, L. F. F., Paula, J. A. M., & Amaral, V. C. S. (2020). Assessing the safety of using the dry extract of *Justicia pectoralis* Jacq. (Acanthaceae) during pregnancy of Wistar rats. *Journal of Ethnopharmacology*, 268, 113618. <https://doi.org/10.1016/j.jep.2020.113618>
- Cena-Diez, R., Garcia-Broncano, P., de la Mata, F. J., Gomez, R., & Munoz-Fernandez, M. A. (2016). Efficacy of HIV antiviral polyanionic carbosilane dendrimer G2-S16 in the presence of semen. *International Journal of Nanomedicine*, 11, 2443–2450. <https://doi.org/10.2147/IJN.S104292>
- Cena-Diez, R., Garcia-Broncano, P., Javier de la Mata, F., Gomez, R., Resino, S., & Munoz-Fernandez, M. (2017). G2-S16 dendrimer as a candidate for a microbicide to prevent HIV-1 infection in women. *Nanoscale*, 9(27), 9732–9742. <https://doi.org/10.1039/c7nr03034g>
- Cena-Diez, R., Martin-Moreno, A., de la Mata, F. J., Gomez-Ramirez, R., Munoz, E., Ardoy, M., & Munoz-Fernandez, M. A. (2019). G1-S4 or G2-S16 carbosilane dendrimer in combination with Platycodin D as a promising vaginal microbicide candidate with contraceptive activity. *International Journal of Nanomedicine*, 14, 2371–2381. <https://doi.org/10.2147/IJN.S188495>
- Cena-Diez, R., Vacas-Cordoba, E., Garcia-Broncano, P., de la Mata, F. J., Gomez, R., Maly, M., & Munoz-Fernandez, M. A. (2016). Prevention of vaginal and rectal herpes simplex virus type 2 transmission in mice: Mechanism of antiviral action. *International Journal of Nanomedicine*, 11, 2147–2162. <https://doi.org/10.2147/IJN.S95301>
- Chakraborty, D., Maity, A., Jha, T., & Mondal, N. B. (2014). Spermicidal and contraceptive potential of desgalactotigonin: A prospective alternative of nonoxynol-9. *PLoS One*, 9(9), e107164. <https://doi.org/10.1371/journal.pone.0107164>
- Chavoustie, S. E., Carter, B. A., Waldbaum, A. S., Donders, G. G. G., Peters, K. H., Schwebke, J. R., Paull, J. R. A., Price, C. F., Castellarnau, A., McCloud, P., & Kinghorn, G. R. (2020). Two phase 3, double-blind, placebo-controlled studies of the efficacy and safety of Astodimer 1% gel for the treatment of bacterial vaginosis. *European Journal of Obstetrics, Gynecology, and Reproductive Biology*, 245, 13–18. <https://doi.org/10.1016/j.ejogrb.2019.11.032>
- Chawla, A., Wang, C., Patton, C., Murray, M., Punekar, Y., de Ruiter, A., & Steinhart, C. (2018). A review of Long-term toxicity of antiretroviral treatment regimens and implications for an aging population. *Infectious Disease and Therapy*, 7(2), 183–195. <https://doi.org/10.1007/s40121-018-0201-6>
- Chis, A. A., Dobrea, C., Morgovan, C., Arseniu, A. M., Rus, L. L., Butuca, A., Juncan, A. M., Totan, M., Vonica-Tincu, A. L., Cormos, G., Muntean, A. C., Muresan, M. L., Gligor, F. G., & Frum, A. (2020). Applications and limitations of dendrimers in biomedicine. *Molecules*, 25(17), 3982. <https://doi.org/10.3390/molecules25173982>
- Chonco, L., Pion, M., Vacas, E., Rasines, B., Maly, M., Serramia, M. J., Lopez-Fernandez, L., De la Mata, J., Alvarez, S., Gomez, R., & Munoz-Fernandez, M. A. (2012). Carbosilane dendrimer nanotechnology outlines of the broad HIV blocker profile. *Journal of Controlled Release*, 161(3), 949–958. <https://doi.org/10.1016/j.jconrel.2012.04.050>
- Clark, M. R., Peet, M. M., Davis, S., Doncel, G. F., & Friend, D. R. (2014). Evaluation of rapidly disintegrating vaginal tablets of Tenofovir, Emtricitabine and their combination for HIV-1 prevention. *Pharmaceutics*, 6(4), 616–631. <https://doi.org/10.3390/pharmaceutics6040616>
- Clayton, R., Hardman, J., LaBranche, C. C., & McReynolds, K. D. (2011). Evaluation of the synthesis of sialic acid-PAMAM glycodendrimers without the use of sugar protecting groups, and the anti-HIV-1 properties of these compounds. *Bioconjugate Chemistry*, 22(10), 2186–2197. <https://doi.org/10.1021/bc200331v>
- Cohen, C. R., Brown, J., Moscicki, A. B., Bukusi, E. A., Paull, J. R., Price, C. F., & Shiboski, S. (2011). A phase I randomized placebo controlled trial of the safety of 3% SPL7013 gel (VivaGel(R)) in healthy young women administered twice daily for 14 days. *PLoS One*, 6(1), e16258. <https://doi.org/10.1371/journal.pone.0016258>

- Cole, A. M., Patton, D. L., Rohan, L. C., Cole, A. L., Cosgrove-Sweeney, Y., Rogers, N. A., Ratner, D., Sassi, A. B., Lackman-Smith, C., Tarwater, P., Ramratnam, B., Ruchala, P., Lehrer, R. I., Waring, A. J., & Gupta, P. (2010). The formulated microbicide RC-101 was safe and antivirally active following intravaginal application in pigtailed macaques. *PLoS One*, 5(11), e15111. <https://doi.org/10.1371/journal.pone.0015111>
- Cordoba, E. V., Arnaiz, E., De La Mata, F. J., Gomez, R., Leal, M., Pion, M., & Munoz-Fernandez, M. A. (2013). Synergistic activity of carbosilane dendrimers in combination with maraviroc against HIV in vitro. *AIDS*, 27(13), 2053–2058. <https://doi.org/10.1097/QAD.0b013e328361fa4a>
- Coutinho, C., Sarmiento, B., & das Neves, J. (2017). Targeted microbicides for preventing sexual HIV transmission. *Journal of Controlled Release*, 266, 119–128. <https://doi.org/10.1016/j.jconrel.2017.09.030>
- Cummins, J. E., Jr., Guarner, J., Flowers, L., Guenther, P. C., Bartlett, J., Morken, T., Grohskopf, L. A., Paxton, L., & Dezzutti, C. S. (2007). Preclinical testing of candidate topical microbicides for anti-human immunodeficiency virus type 1 activity and tissue toxicity in a human cervical explant culture. *Antimicrobial Agents and Chemotherapy*, 51(5), 1770–1779. <https://doi.org/10.1128/AAC.01129-06>
- da Silva Dantas, F. G., Araujo, R. P., de Almeida-Apolonio, A. A., de Castilho, P. F., Oliveira Galvao, F., Negri, M., Oesterreich, S. A., Cardoso, C. A. L., & Oliveira, K. M. P. (2020). Cytotoxicity, mutagenicity and acute oral toxicity of aqueous *Ocotea minarum* leaf extracts. *Natural Product Research*, 8, 1–5. <https://doi.org/10.1080/14786419.2020.1855642>
- das Neves, J., Araujo, F., Andrade, F., Amiji, M., Bahia, M. F., & Sarmiento, B. (2014). Biodistribution and pharmacokinetics of dapivirine-loaded nanoparticles after vaginal delivery in mice. *Pharmaceutical Research*, 31(7), 1834–1845. <https://doi.org/10.1007/s11095-013-1287-x>
- das Neves, J., Nunes, R., Rodrigues, F., & Sarmiento, B. (2016). Nanomedicine in the development of anti-HIV microbicides. *Advanced Drug Delivery Reviews*, 103, 57–75. <https://doi.org/10.1016/j.addr.2016.01.017>
- das Neves, J., & Sarmiento, B. (2015). Precise engineering of dapivirine-loaded nanoparticles for the development of anti-HIV vaginal microbicides. *Acta Biomaterialia*, 18, 77–87. <https://doi.org/10.1016/j.actbio.2015.02.007>
- Davari, M., Giwa, H. B., Nabizade, A., Taheri, F., & Giwa, A. (2020). Antiretroviral therapy and the risk of sexual transmission of HIV: A systematic review and meta-analysis. *HIV Medicine*, 21(6), 349–357. <https://doi.org/10.1111/hiv.12841>
- Dekant, W., Jean, P., & Arts, J. (2020). Evaluation of the carcinogenicity of dichloromethane in rats, mice, hamsters and humans. *Regulatory Toxicology and Pharmacology*, 18, 104858. <https://doi.org/10.1016/j.yrtph.2020.104858>
- Delany-Moretlwe, S., Lombard, C., Baron, D., Bekker, L. G., Nkala, B., Ahmed, K., Sebe, M., Brumskine, W., Nchabeleng, M., Palanee-Philips, T., Ntshangase, J., Sibiyi, S., Smith, E., Panchia, R., Myer, L., Schwartz, J. L., Marzinke, M., Morris, L., Brown, E. R., ... Rees, H. (2018). Tenofvir 1% vaginal gel for prevention of HIV-1 infection in women in South Africa (FACTS-001): A phase 3, randomised, double-blind, placebo-controlled trial. *The Lancet Infectious Diseases*, 18(11), 1241–1250. [https://doi.org/10.1016/S1473-3099\(18\)30428-6](https://doi.org/10.1016/S1473-3099(18)30428-6)
- Denton, P. W., Estes, J. D., Sun, Z., Othieno, F. A., Wei, B. L., Wege, A. K., Powell, D. A., Payne, D., Haase, A. T., & Garcia, J. V. (2008). Antiretroviral pre-exposure prophylaxis prevents vaginal transmission of HIV-1 in humanized BLT mice. *PLoS Medicine*, 5(1), e16. <https://doi.org/10.1371/journal.pmed.0050016>
- Denton, P. W., Othieno, F., Martinez-Torres, F., Zou, W., Krisko, J. F., Fleming, E., Zein, S., Powell, D. A., Wahl, A., Kwak, Y. T., Welch, B. D., Kay, M. S., Payne, D. A., Gallay, P., Appella, E., Estes, J. D., Lu, M., & Garcia, J. V. (2011). One percent tenofvir applied topically to humanized BLT mice and used according to the CAPRISA 004 experimental design demonstrates partial protection from vaginal HIV infection, validating the BLT model for evaluation of new microbicide candidates. *Journal of Virology*, 85(15), 7582–7593. <https://doi.org/10.1128/JVI.00537-11>
- Deraedt, C., d'Halluin, M., Lesturgez, S., Salmon, L., Goglio, G., Ruiz, J., & Astruc, D. (2015). Alkynyl-functionalized imidazolium for “click” dendrimer functionalisation and palladium nanoparticle stabilization. *European Journal of Inorganic Chemistry*, 2015(8), 1345–1350. <https://doi.org/10.1002/ejic.201403045>
- Deruaz, M., & Luster, A. D. (2013). BLT humanized mice as model to study HIV vaginal transmission. *The Journal of Infectious Diseases*, 208 (Suppl 2), S131–S136. <https://doi.org/10.1093/infdis/jit318>
- Deruaz, M., & Tager, A. M. (2017). Humanized mouse models of latent HIV infection. *Current Opinion in Virology*, 25, 97–104. <https://doi.org/10.1016/j.coviro.2017.07.027>
- Destache, C. J., Mandal, S., Yuan, Z., Kang, G., Date, A. A., Lu, W., Shibata, A., Pham, R., Bruck, P., Rezich, M., Zhou, Y., Vivekanandan, R., Fletcher, C. V., & Li, Q. (2016). Topical tenofvir disoproxil fumarate nanoparticles prevent HIV-1 vaginal transmission in a humanized mouse model. *Antimicrobial Agents and Chemotherapy*, 60(6), 3633–3639. <https://doi.org/10.1128/AAC.00450-16>
- Dezzutti, C. S. (2015). Animal and human mucosal tissue models to study HIV biomedical interventions: Can we predict success? *Journal of the International AIDS Society*, 18, 20301. <https://doi.org/10.7448/IAS.18.1.20301>
- Dezzutti, C. S., James, V. N., Ramos, A., Sullivan, S. T., Siddig, A., Bush, T. J., Grohskopf, L. A., Paxton, L., Subbarao, S., & Hart, C. E. (2004). In vitro comparison of topical microbicides for prevention of human immunodeficiency virus type 1 transmission. *Antimicrobial Agents and Chemotherapy*, 48(10), 3834–3844. <https://doi.org/10.1128/AAC.48.10.3834-3844.2004>
- Dezzutti, C. S., Park, S. Y., Marks, K., Lawlor, S., Russo, J., Macio, I., Chappell, C., & Bunge, K. (2017). Heterogeneity of HIV-1 replication in ectocervical and vaginal tissue ex vivo. *AIDS Research and Human Retroviruses*, 34, 185–192. <https://doi.org/10.1089/AID.2017.0107>
- Dhondt, M. M., Adriaens, E., Roey, J. V., & Remon, J. P. (2005). The evaluation of the local tolerance of vaginal formulations containing dapivirine using the Slug Mucosal Irritation test and the rabbit vaginal irritation test. *European Journal of Pharmaceutics and Biopharmaceutics*, 60(3), 419–425. <https://doi.org/10.1016/j.ejpb.2005.01.012>
- Dias, A. P., da Silva Santos, S., da Silva, J. V., Parise-Filho, R., Igne Ferreira, E., Seoud, O. E., & Giarolla, J. (2020). Dendrimers in the context of nanomedicine. *International Journal of Pharmaceutics*, 573, 118814. <https://doi.org/10.1016/j.ijpharm.2019.118814>



- Dieffenbach, C. W., & Fauci, A. S. (2020). The search for an HIV vaccine, the journey continues. *Journal of the International AIDS Society*, 23(5), e25506. <https://doi.org/10.1002/jia2.25506>
- Domenech, R., Abian, O., Bocanegra, R., Correa, J., Sousa-Herves, A., Riguera, R., Mateu, M. G., Fernandez-Megia, E., Velazquez-Campoy, A., & Neira, J. L. (2010). Dendrimers as potential inhibitors of the dimerization of the capsid protein of HIV-1. *Biomacromolecules*, 11(8), 2069–2078. <https://doi.org/10.1021/bm100432x>
- Donahue Carlson, R., Sheth, A. N., Read, T. D., Frisch, M. B., Mehta, C. C., Martin, A., Haaland, R. E., Patel, A. S., Pau, C. P., Kraft, C. S., & Ofotokun, I. (2017). The female genital tract microbiome is associated with vaginal antiretroviral drug concentrations in human immunodeficiency virus-infected women on antiretroviral therapy. *The Journal of Infectious Diseases*, 216(8), 990–999. <https://doi.org/10.1093/infdis/jix420>
- Donalisio, M., Rusnati, M., Civra, A., Bugatti, A., Allemand, D., Pirri, G., Giuliani, A., Landolfo, S., & Lembo, D. (2010). Identification of a dendrimeric heparan sulfate-binding peptide that inhibits infectivity of genital types of human papillomaviruses. *Antimicrobial Agents and Chemotherapy*, 54(10), 4290–4299. <https://doi.org/10.1128/AAC.00471-10>
- Dong, Y., Chen, Y., Zhu, D., Shi, K., Ma, C., Zhang, W., Rocchi, P., Jiang, L., & Liu, X. (2020). Self-assembly of amphiphilic phospholipid peptide dendrimer-based nanovectors for effective delivery of siRNA therapeutics in prostate cancer therapy. *Journal of Controlled Release*, 322, 416–425. <https://doi.org/10.1016/j.jconrel.2020.04.003>
- Doroudian, M., O' Neill, A., Mac Loughlin, R., Prina-Mello, A., Volkov, Y., & Donnelly, S. C. (2020). Nanotechnology in pulmonary medicine. *Current Opinion in Pharmacology*, 56, 85–92. <https://doi.org/10.1016/j.coph.2020.11.002>
- Drain, P. K., & Garrett, N. (2020). SARS-CoV-2 pandemic expanding in sub-Saharan Africa: Considerations for COVID-19 in people living with HIV. *EClinicalMedicine*, 22, 100342. <https://doi.org/10.1016/j.eclinm.2020.100342>
- Duncan, R., & Izzo, L. (2005). Dendrimer biocompatibility and toxicity. *Advanced Drug Delivery Reviews*, 57(15), 2215–2237. <https://doi.org/10.1016/j.addr.2005.09.019>
- Eckstein, P., Jackson, M. C., Millman, N., & Sobrero, A. J. (1969). Comparison of vaginal tolerance tests of spermicidal preparations in rabbits and monkeys. *Journal of Reproduction and Fertility*, 20(1), 85–93. <https://doi.org/10.1530/jrf.0.0200085>
- El-Enin, A., Elbakry, A. M., Hosary, R. E., & Lotfy, M. A. F. (2020). Formulation, development, and in-vitro/ex-vivo evaluation of vaginal bio-adhesive salbutamol sulfate tablets for preterm labor. *Pharmaceutical Development and Technology*, 25(8), 989–998. <https://doi.org/10.1080/10837450.2020.1767129>
- Ellert-Miklaszewska, A., Ochocka, N., Maleszewska, M., Ding, L., Laurini, E., Jiang, Y., Roura, A. J., Giorgio, S., Gielniewski, B., Priel, S., Peng, L., & Kaminska, B. (2019). Efficient and innocuous delivery of small interfering RNA to microglia using an amphiphilic dendrimer nanovector. *Nanomedicine (London, England)*, 14(18), 2441–2458. <https://doi.org/10.2217/nmm-2019-0176>
- El-Sadr, W. M., & Justman, J. (2020). Africa in the path of Covid-19. *The New England Journal of Medicine*, 383(3), e11. <https://doi.org/10.1056/NEJMp2008193>
- EMA. (2011). *ICH guideline S6 (R1)—Preclinical safety evaluation of biotechnology-derived pharmaceuticals*. Available from [https://www.ema.europa.eu/en/documents/scientific-guideline/ich-s6r1-pre-clinical-safety-evaluation-biotechnology-derived-pharmaceuticals-step-5\\_en.pdf](https://www.ema.europa.eu/en/documents/scientific-guideline/ich-s6r1-pre-clinical-safety-evaluation-biotechnology-derived-pharmaceuticals-step-5_en.pdf).
- EMA. (2013). *ICH guideline M3(R2) on non-clinical safety studies for the conduct of human clinical trials and marketing authorisation for pharmaceuticals*. Available from [https://www.ema.europa.eu/en/documents/scientific-guideline/ich-guideline-m3r2-non-clinical-safety-studies-conduct-human-clinical-trials-marketing-authorisation\\_en.pdf](https://www.ema.europa.eu/en/documents/scientific-guideline/ich-guideline-m3r2-non-clinical-safety-studies-conduct-human-clinical-trials-marketing-authorisation_en.pdf).
- Farabi, K., Manabe, Y., Ichikawa, H., Miyake, S., Tsutsui, M., Kabayama, K., Yamaji, T., Tanaka, K., Hung, S. C., & Fukase, K. (2020). Concise and reliable syntheses of glycodendrimers via self-activating click chemistry: A robust strategy for mimicking multivalent glycan-pathogen interactions. *The Journal of Organic Chemistry*, 85(24), 16014–16023. <https://doi.org/10.1021/acs.joc.0c01547>
- Fernandez, M. V., Delviks-Frankenberry, K. A., Scheiblin, D. A., Happel, C., Pathak, V. K., & Freed, E. O. (2019). Authentication analysis of MT-4 cells distributed by the National Institutes of Health AIDS reagent program. *Journal of Virology*, 93(24), e01390-19. <https://doi.org/10.1128/JVI.01390-19>
- Fernandez-Romero, J. A., Teleshova, N., Zydowsky, T. M., & Robbiani, M. (2015). Preclinical assessments of vaginal microbicide candidate safety and efficacy. *Advanced Drug Delivery Reviews*, 92, 27–38. <https://doi.org/10.1016/j.addr.2014.12.005>
- Fields, S., Song, B., Rasoul, B., Fong, J., Works, M. G., Shew, K., Yiu, Y., Mirsalis, J., & D'Andrea, A. (2014). New candidate biomarkers in the female genital tract to evaluate microbicide toxicity. *PLoS One*, 9(10), e110980. <https://doi.org/10.1371/journal.pone.0110980>
- Flerin, N. C., Bardhi, A., Zheng, J. H., Korom, M., Folkvord, J., Kovacs, C., Benko, E., Truong, R., Mota, T., Connick, E., Jones, R. B., Lynch, R. M., & Goldstein, H. (2019). Establishment of a novel humanized mouse model to investigate in vivo activation and depletion of patient-derived HIV latent reservoirs. *Journal of Virology*, 93(6), e02051-18. <https://doi.org/10.1128/JVI.02051-18>
- Fouquier, J., & Guedj, M. (2015). Analysis of drug combinations: Current methodological landscape. *Pharmacology Research & Perspectives*, 3(3), e00149. <https://doi.org/10.1002/prp2.149>
- Fu, H., Di, Q., Wang, J., Jiang, Q., & Xu, Q. (2020). Toxicokinetics and distribution in female rats after chronic nonylphenol exposure. *Toxicology and Industrial Health*, 36(11), 925–935. <https://doi.org/10.1177/0748233720958963>
- Galán, M., Rodríguez, S., Javier, J., Luis, J., Relloso, M., Maly, M., de la Mata, F. J., Muñoz-Fernández, M. A., & Gómez, R. (2014). Synthesis of new anionic carbosilane dendrimers via thiol-ene chemistry and their antiviral behaviour. *Organic & Biomolecular Chemistry*, 12(20), 3222–3237. <https://doi.org/10.1039/C4OB00162A>
- Gali, Y., Arien, K. K., Praet, M., Van den Bergh, R., Temmerman, M., Delezay, O., & Vanham, G. (2010). Development of an in vitro dual-chamber model of the female genital tract as a screening tool for epithelial toxicity. *Journal of Virological Methods*, 165(2), 186–197. <https://doi.org/10.1016/j.jviromet.2010.01.018>

- Gali, Y., Delezay, O., Brouwers, J., Addad, N., Augustijns, P., Bourlet, T., Hamzeh-Cognasse, H., Arien, K. K., Pozzetto, B., & Vanham, G. (2010). In vitro evaluation of viability, integrity, and inflammation in genital epithelia upon exposure to pharmaceutical excipients and candidate microbicides. *Antimicrobial Agents and Chemotherapy*, 54(12), 5105–5114. <https://doi.org/10.1128/AAC.00456-10>
- Gallay, P. A., Chatterji, U., Kirchhoff, A., Gandarilla, A., Gunawardana, M., Pyles, R. B., Marzinke, M. A., Moss, J. A., & Baum, M. M. (2017). Prevention of vaginal and rectal HIV transmission by antiretroviral combinations in humanized mice. *PLoS One*, 12(9), e0184303. <https://doi.org/10.1371/journal.pone.0184303>
- Ganiger, S., Malleshappa, H. N., Krishnappa, H., Rajashekhar, G., Ramakrishna Rao, V., & Sullivan, F. (2007). A two generation reproductive toxicity study with curcumin, turmeric yellow, in Wistar rats. *Food and Chemical Toxicology*, 45(1), 64–69. <https://doi.org/10.1016/j.fct.2006.07.016>
- Gao, Y., Chen, X., Li, C., Wang, H., Tian, J., & Fu, F. (2020). Toxicological evaluation of, red rice yeast extract, Xuezhikang: Acute, 26-week chronic and genotoxicity studies. *Regulatory Toxicology and Pharmacology*, 114, 104654. <https://doi.org/10.1016/j.yrtph.2020.104654>
- Garcia, M. R., & Wray, A. A. (2020). *Sexually transmitted infections*. StatPearls.
- Garcia-Broncano, P., Cena-Diez, R., de la Mata, F. J., Gomez, R., Resino, S., & Munoz-Fernandez, M. A. (2017). Efficacy of carbosilane dendrimers with an antiretroviral combination against HIV-1 in the presence of semen-derived enhancer of viral infection. *European Journal of Pharmacology*, 811, 155–163. <https://doi.org/10.1016/j.ejphar.2017.05.060>
- Garcia-Gallego, S., Diaz, L., Jimenez, J. L., Gomez, R., de la Mata, F. J., & Munoz-Fernandez, M. A. (2015). HIV-1 antiviral behavior of anionic PPI metallo-dendrimers with EDA core. *European Journal of Medicinal Chemistry*, 98, 139–148. <https://doi.org/10.1016/j.ejmech.2015.05.026>
- Garcia-Tellez, T., Huot, N., Ploquin, M. J., Rasclé, P., Jacquelin, B., & Muller-Trutwin, M. (2016). Non-human primates in HIV research: Achievements, limits and alternatives. *Infection, Genetics and Evolution*, 46, 324–332. <https://doi.org/10.1016/j.meegid.2016.07.012>
- Garcia-Vallejo, J. J., Koning, N., Ambrosini, M., Kalay, H., Vuist, I., Sarrami-Forooshani, R., Geijtenbeek, T. B., & van Kooyk, Y. (2013). Glycodendrimers prevent HIV transmission via DC-SIGN on dendritic cells. *International Immunology*, 25(4), 221–233. <https://doi.org/10.1093/intimm/dxs115>
- Garg, S., Goldman, D., Krumme, M., Rohan, L. C., Smoot, S., & Friend, D. R. (2010). Advances in development, scale-up and manufacturing of microbicide gels, films, and tablets. *Antiviral Research*, 88(Suppl 1), S19–S29. <https://doi.org/10.1016/j.antiviral.2010.09.010>
- Gazumyan, A., Mitsner, B., & Ellestad, G. A. (2000). Novel anti-RSV dianionic dendrimer-like compounds: Design, synthesis and biological evaluation. *Current Pharmaceutical Design*, 6(5), 525–546. <https://doi.org/10.2174/1381612003400704>
- Giacomelli, A., de Rose, S., & Rusconi, S. (2019). Clinical pharmacology in HIV cure research - what impact have we seen? *Expert Review of Clinical Pharmacology*, 12(1), 17–29. <https://doi.org/10.1080/17512433.2019.1561272>
- Gibas, K. M., van den Berg, P., Powell, V. E., & Krakower, D. S. (2019). Drug resistance during HIV pre-exposure prophylaxis. *Drugs*, 79(6), 609–619. <https://doi.org/10.1007/s40265-019-01108-x>
- Gong, E., Matthews, B., McCarthy, T., Chu, J., Holan, G., Raff, J., & Sacks, S. (2005). Evaluation of dendrimer SPL7013, a lead microbicide candidate against herpes simplex viruses. *Antiviral Research*, 68(3), 139–146. <https://doi.org/10.1016/j.antiviral.2005.08.004>
- Grande, J. B., Urlich, T., Dickie, T., & Brook, M. A. (2014). Silicone dendrons and dendrimers from orthogonal SiH coupling reactions. *Polymer Chemistry*, 5(23), 6728–6739. <https://doi.org/10.1039/C4PY00680A>
- Grivel, J. C., & Margolis, L. (2009). Use of human tissue explants to study human infectious agents. *Nature Protocols*, 4(2), 256–269. <https://doi.org/10.1038/nprot.2008.245>
- Guerrero-Beltran, C., Cena-Diez, R., Sepulveda-Crespo, D., De la Mata, J., Gomez, R., Leal, M., Munoz-Fernandez, M. A., & Jimenez, J. L. (2017). Carbosilane dendrons with fatty acids at the core as a new potential microbicide against HSV-2/HIV-1 co-infection. *Nanoscale*, 9(44), 17263–17273. <https://doi.org/10.1039/c7nr05859d>
- Guerrero-Beltran, C., Garcia-Heredia, I., Cena-Diez, R., Rodriguez-Izquierdo, I., Serramia, M. J., Martinez-Hernandez, F., Lluesma-Gomez, M., Martinez-Garcia, M., & Munoz-Fernandez, M. A. (2020). Cationic dendrimer G2-S16 inhibits herpes simplex type 2 infection and protects mice vaginal microbiome. *Pharmaceutics*, 12(6), 515. <https://doi.org/10.3390/pharmaceutics12060515>
- Guerrero-Beltran, C., Prieto, A., Leal, M., Jimenez, J. L., & Munoz-Fernandez, M. A. (2019). Combination of G2-S16 dendrimer/dapivirine antiretroviral as a new HIV-1 microbicide. *Future Medicinal Chemistry*, 11(23), 3005–3013. <https://doi.org/10.4155/fmc-2018-0539>
- Guerrero-Beltran, C., Rodriguez-Izquierdo, I., Serramia, M. J., Araya-Duran, I., Marquez-Miranda, V., Gomez, R., de la Mata, F. J., Leal, M., Gonzalez-Nilo, F., & Munoz-Fernandez, M. A. (2018). Anionic carbosilane dendrimers destabilize the GP120-CD4 complex blocking HIV-1 entry and cell to cell fusion. *Bioconjugate Chemistry*, 29(5), 1584–1594. <https://doi.org/10.1021/acs.bioconjchem.8b00106>
- Guo, W., Weng, H. L., Bai, H., Liu, J., Wei, X. N., Zhou, K., & Sande, A. (2020). Quick community survey on the impact of COVID-19 outbreak for the healthcare of people living with HIV. *Zhonghua Liu Xing Bing Xue Za Zhi*, 41(5), 662–666. <https://doi.org/10.3760/cma.j.cn112338-20200314-00345>
- Gupta, P., Lackman-Smith, C., Snyder, B., Ratner, D., Rohan, L. C., Patton, D., Ramratnam, B., & Cole, A. M. (2013). Antiviral activity of retrocyclin RC-101, a candidate microbicide against cell-associated HIV-1. *AIDS Research and Human Retroviruses*, 29(2), 391–396. <https://doi.org/10.1089/AID.2012.0135>
- Gutierrez-Ulloa, C. E., Sepulveda-Crespo, D., Garcia-Broncano, P., Malý, M., Muñoz-Fernández, M. A., de la Mata, F. J., & Gómez, R. (2019). Synthesis of bow-tie carbosilane dendrimers and their HIV antiviral capacity: A comparison of the dendritic topology on the biological process. *European Polymer Journal*, 119, 200–212. <https://doi.org/10.1016/j.eurpolymj.2019.07.034>
- Gutiérrez-Ulloa, C., Peña-González, C. E., Barrios-Gumiel, A., Ceña-Díez, R., Serramía-Lobera, M. J., Muñoz-Fernández, M. A., de la Mata, F. J., Sánchez-Nieves, J., & Gómez, R. (2020). New synthetic procedure for the antiviral sulfonate carbosilane dendrimer G2-S16 and its fluorescein-labelled derivative for biological studies. *RSC Advances*, 10(34), 20083–20088. <https://doi.org/10.1039/D0RA03448G>

- Halwes, M. E., Steinbach-Rankins, J. M., & Frieboes, H. B. (2016). Pharmacokinetic modeling of a gel-delivered dapivirine microbicide in humans. *European Journal of Pharmaceutical Sciences*, 93, 410–418. <https://doi.org/10.1016/j.ejps.2016.08.037>
- Ham, A. S., Nugent, S. T., Peters, J. J., Katz, D. F., Shelter, C. M., Dezzutti, C. S., Boczar, A. D., Buckheit, K. W., & Buckheit, R. W., Jr. (2015). The rational design and development of a dual chamber vaginal/rectal microbicide gel formulation for HIV prevention. *Antiviral Research*, 120, 153–164. <https://doi.org/10.1016/j.antiviral.2015.06.010>
- Han, S., Kanamoto, T., Nakashima, H., & Yoshida, T. (2012). Synthesis of a new amphiphilic glycodendrimer with antiviral functionality. *Carbohydrate Polymers*, 90(2), 1061–1068. <https://doi.org/10.1016/j.carbpol.2012.06.044>
- Han, S., Yoshida, D., Kanamoto, T., Nakashima, H., Uryu, T., & Yoshida, T. (2010). Sulfated oligosaccharide cluster with polylysine core scaffold as a new anti-HIV dendrimer. *Carbohydrate Polymers*, 80(4), 1111–1115. <https://doi.org/10.1016/j.carbpol.2010.01.031>
- Hantson, A., Fikkert, V., Van Remoortel, B., Pannecouque, C., Cherepanov, P., Matthews, B., Holan, G., De Clercq, E., Vandamme, A. M., Debyser, Z., & Witvrouw, M. (2005). Mutations in both env and gag genes are required for HIV-1 resistance to the polysulfonic dendrimer SPL2923, as corroborated by chimeric virus technology. *Antiviral Chemistry & Chemotherapy*, 16(4), 253–266. <https://doi.org/10.1177/095632020501600405>
- Hatano, K., Matsubara, T., Muramatsu, Y., Ezure, M., Koyama, T., Matsuoka, K., Kuriyama, R., Kori, H., & Sato, T. (2014). Synthesis and influenza virus inhibitory activities of carbosilane dendrimers peripherally functionalized with hemagglutinin-binding peptide. *Journal of Medicinal Chemistry*, 57(20), 8332–8339. <https://doi.org/10.1021/jm5007676>
- Hatzioannou, T., Ambrose, Z., Chung, N. P., Piatak, M., Jr., Yuan, F., Trubey, C. M., Coalter, V., Kiser, R., Schneider, D., Smedley, J., Pung, R., Gathuka, M., Estes, J. D., Veazey, R. S., KewalRamani, V. N., Lifson, J. D., & Bieniasz, P. D. (2009). A macaque model of HIV-1 infection. *Proceedings of the National Academy of Sciences of the United States of America*, 106(11), 4425–4429. <https://doi.org/10.1073/pnas.0812587106>
- Hatzioannou, T., & Evans, D. T. (2012). Animal models for HIV/AIDS research. *Nature Reviews. Microbiology*, 10(12), 852–867. <https://doi.org/10.1038/nrmicro2911>
- Hawker, C. J., & Frechet, J. M. J. (1992). Unusual macromolecular architectures: The convergent growth approach to dendritic polyesters and novel block copolymers. *Journal of the American Chemical Society*, 114(22), 8405–8413. <https://doi.org/10.1021/ja00048a009>
- Heinrich, M. A., Martina, B., & Prakash, J. (2020). Nanomedicine strategies to target coronavirus. *Nano Today*, 35, 100961. <https://doi.org/10.1016/j.nantod.2020.100961>
- Henry, C. E., Wang, Y. Y., Yang, Q., Hoang, T., Chattopadhyay, S., Hoen, T., Ensign, L. M., Nunn, K. L., Schroeder, H., McCallen, J., Moench, T., Cone, R., Roffler, S. R., & Lai, S. K. (2016). Anti-PEG antibodies alter the mobility and biodistribution of densely PEGylated nanoparticles in mucus. *Acta Biomaterialia*, 43, 61–70. <https://doi.org/10.1016/j.actbio.2016.07.019>
- Herrera, C. (2019). The pre-clinical toolbox of pharmacokinetics and pharmacodynamics: In vitro and ex vivo models. *Frontiers in Pharmacology*, 10, 578. <https://doi.org/10.3389/fphar.2019.00578>
- Hladik, F., & Doncel, G. F. (2010). Preventing mucosal HIV transmission with topical microbicides: Challenges and opportunities. *Antiviral Research*, 88(Suppl 1), S3–S9. <https://doi.org/10.1016/j.antiviral.2010.09.011>
- Hogan, A. B., Jewell, B. L., Sherrard-Smith, E., Vesga, J. F., Watson, O. J., Whittaker, C., Hamlet, A., Smith, J. A., Winskill, P., Verity, R., Baguelin, M., Lees, J. A., Whittles, L. K., Ainslie, K. E. C., Bhatt, S., Boonyasiri, A., Brazeau, N. F., Cattarino, L., Cooper, L. V., ... Hallett, T. B. (2020). Potential impact of the COVID-19 pandemic on HIV, tuberculosis, and malaria in low-income and middle-income countries: A modelling study. *The Lancet Global Health*, 8(9), e1132–e1141. [https://doi.org/10.1016/S2214-109X\(20\)30288-6](https://doi.org/10.1016/S2214-109X(20)30288-6)
- Hu, Q., Wang, Y., Xu, L., Chen, D., & Cheng, L. (2020). Transferrin conjugated pH- and redox-responsive poly(amidoamine) dendrimer conjugate as an efficient drug delivery carrier for cancer therapy. *International Journal of Nanomedicine*, 15, 2751–2764. <https://doi.org/10.2147/IJN.S238536>
- Huang, M. C., Turner, K. J., Vallant, M., Robinson, V. G., Lu, Y., Price, C. J., Fennell, T. R., Silinski, M. A., Waidyanatha, S., Ryan, K. R., Black, S. R., Fernando, R. A., & McIntyre, B. S. (2020). Tolerability and age-dependent toxicokinetics following perinatal hydroxyurea treatment in Sprague Dawley rats. *Journal of Applied Toxicology*, 41, 1007–1020. <https://doi.org/10.1002/jat.4087>
- Hur, E. M., Patel, S. N., Shimizu, S., Rao, D. S., Gnanapragasam, P. N., An, D. S., Yang, L., & Baltimore, D. (2012). Inhibitory effect of HIV-specific neutralizing IgA on mucosal transmission of HIV in humanized mice. *Blood*, 120(23), 4571–4582. <https://doi.org/10.1182/blood-2012-04-422303>
- Ibeh, B. O., Furuta, Y., Habu, J. B., & Ogbadu, L. (2016). Humanized mouse as an appropriate model for accelerated global HIV research and vaccine development: Current trend. *Immunopharmacology and Immunotoxicology*, 38(6), 395–407. <https://doi.org/10.1080/08923973.2016.1233980>
- Introini, A., Bostrom, S., Bradley, F., Gibbs, A., Glaessgen, A., Tjernlund, A., & Broliden, K. (2017). Seminal plasma induces inflammation and enhances HIV-1 replication in human cervical tissue explants. *PLoS Pathogens*, 13(5), e1006402. <https://doi.org/10.1371/journal.ppat.1006402>
- Ishtiaq, A., Ali, T., Bakhtiar, A., Bibi, R., Bibi, K., Mushtaq, I., Li, S., Khan, W., Khan, U., Anis, R. A., Anees, M., Sultan, A., & Murtaza, I. (2021). Melatonin abated bisphenol A-induced neurotoxicity via p53/PUMA/Drp-1 signaling. *Environmental Science and Pollution Research International*, 28, 17789–17801. <https://doi.org/10.1007/s11356-020-12129-5>
- Jain, K., Kesharwani, P., Gupta, U., & Jain, N. K. (2010). Dendrimer toxicity: Let's meet the challenge. *International Journal of Pharmaceutics*, 394(1–2), 122–142. <https://doi.org/10.1016/j.ijpharm.2010.04.027>
- Jain, R. K., Jain, A., Kumar, R., Verma, V., Maikhuri, J. P., Sharma, V. L., Mitra, K., Batra, S., & Gupta, G. (2010). Functional attenuation of human sperm by novel, non-surfactant spermicides: Precise targeting of membrane physiology without affecting structure. *Human Reproduction*, 25(5), 1165–1176. <https://doi.org/10.1093/humrep/deq036>



- Janaszewska, A., Lazniewska, J., Trzepinski, P., Marcinkowska, M., & Klajnert-Maculewicz, B. (2019). Cytotoxicity of dendrimers. *Biomolecules*, 9(8), 330. <https://doi.org/10.3390/biom9080330>
- Jaramillo-Ruiz, D., De La Mata, F. J., Gomez, R., Correa-Rocha, R., & Munoz-Fernandez, M. A. (2016). Nanotechnology as a new therapeutic approach to prevent the HIV-infection of treg cells. *PLoS One*, 11(1), e0145760. <https://doi.org/10.1371/journal.pone.0145760>
- Javadi, F., Rahimi, P., Modarresi, M. H., Bolhassani, A., Shafiee Ardestani, M., & Sadat, S. M. (2019). G2 dendrimer as a carrier can enhance immune responses against HCV-NS3 protein in BALB/c mice. *Avicenna Journal of Medical Biotechnology*, 11(4), 292–298.
- Jevprasesphant, R., Penny, J., Attwood, D., McKeown, N. B., & D'Emanuele, A. (2003). Engineering of dendrimer surfaces to enhance trans-epithelial transport and reduce cytotoxicity. *Pharmaceutical Research*, 20(10), 1543–1550. <https://doi.org/10.1023/a:1026166729873>
- Jewell, B. L., Mudimu, E., Stover, J., Ten Brink, D., Phillips, A. N., Smith, J. A., Martin-Hughes, R., Teng, Y., Glaubius, R., Mahiane, S. G., Bansi-Matharu, L., Taramusi, I., Chagoma, N., Morrison, M., Doherty, M., Marsh, K., Bershteyn, A., Hallett, T. B., Kelly, S. L., & HIV Modelling Consortium. (2020). Potential effects of disruption to HIV programmes in sub-Saharan Africa caused by COVID-19: Results from multiple mathematical models. *Lancet HIV*, 7(9), e629–e640. [https://doi.org/10.1016/S2352-3018\(20\)30211-3](https://doi.org/10.1016/S2352-3018(20)30211-3)
- Jewell, B. L., Smith, J. A., & Hallett, T. B. (2020). Understanding the impact of interruptions to HIV services during the COVID-19 pandemic: A modelling study. *EClinicalMedicine*, 26, 100483. <https://doi.org/10.1016/j.eclinm.2020.100483>
- Jiang, Y. H., Emau, P., Cairns, J. S., Flanary, L., Morton, W. R., McCarthy, T. D., & Tsai, C. C. (2005). SPL7013 gel as a topical microbicide for prevention of vaginal transmission of SHIV89.6P in macaques. *AIDS Research and Human Retroviruses*, 21(3), 207–213. <https://doi.org/10.1089/aid.2005.21.207>
- Joseph, S. B., Swanstrom, R., Kashuba, A. D., & Cohen, M. S. (2015). Bottlenecks in HIV-1 transmission: Insights from the study of founder viruses. *Nature Reviews. Microbiology*, 13(7), 414–425. <https://doi.org/10.1038/nrmicro3471>
- Juárez-Chávez, L., Pina-Canseco, S., Soto-Castro, D., Santillan, R., Magaña-Vergara, N. E., Salazar-Schettino, P. M., Cabrera-Bravo, M., & Pérez-Campos, E. (2019). In vitro activity of steroidal dendrimers on Trypanosoma cruzi epimastigote form with PAMAM dendrons modified by “click” chemistry. *Bioorganic Chemistry*, 86, 452–458. <https://doi.org/10.1016/j.bioorg.2019.01.056>
- Kahn, J. G., Marseille, E. A., Bennett, R., Williams, B. G., & Granich, R. (2011). Cost-effectiveness of antiretroviral therapy for prevention. *Current HIV Research*, 9(6), 405–415. <https://doi.org/10.2174/157016211798038542>
- Kaminskas, L. M., Boyd, B. J., & Porter, C. J. (2011). Dendrimer pharmacokinetics: The effect of size, structure and surface characteristics on ADME properties. *Nanomedicine (London, England)*, 6(6), 1063–1084. <https://doi.org/10.2217/nnm.11.67>
- Kandeel, M., Al-Taher, A., Park, B. K., Kwon, H. J., & Al-Nazawi, M. (2020). A pilot study of the antiviral activity of anionic and cationic polyamidoamine dendrimers against the Middle East respiratory syndrome coronavirus. *Journal of Medical Virology*, 92(9), 1665–1670. <https://doi.org/10.1002/jmv.25928>
- Kandi, M. R., Mohammadnejad, J., Shafiee Ardestani, M., Zabihollahi, R., Soleymani, S., Aghasadeghi, M. R., & Baesi, K. (2019). Inherent anti-HIV activity of biocompatible anionic citrate-PEG-citrate dendrimer. *Molecular Biology Reports*, 46(1), 143–149. <https://doi.org/10.1007/s11033-018-4455-6>
- Karpel, M. E., Boutwell, C. L., & Allen, T. M. (2015). BLT humanized mice as a small animal model of HIV infection. *Current Opinion in Virology*, 13, 75–80. <https://doi.org/10.1016/j.coviro.2015.05.002>
- Katz, A. W. K., Naidoo, K., Reddy, K., Chitukuta, M., Nabukeera, J., Siva, S., Zimba, C., & Montgomery, E. T. (2020). The power of the shared experience: MTN-020/ASPIRE trial Participants' descriptions of peer influence on acceptability of and adherence to the Dapivirine vaginal ring for HIV prevention. *AIDS and Behavior*, 24(8), 2387–2399. <https://doi.org/10.1007/s10461-020-02799-0>
- Kawaguchi, T., Walker, K. L., Wilkins, C. L., & Moore, J. S. (1995). Double exponential dendrimer growth. *Journal of the American Chemical Society*, 117(8), 2159–2165. <https://doi.org/10.1021/ja00113a005>
- Kensinger, R. D., Catalone, B. J., Krebs, F. C., Wigdahl, B., & Schengrund, C. L. (2004). Novel polysulfated galactose-derivatized dendrimers as binding antagonists of human immunodeficiency virus type 1 infection. *Antimicrobial Agents and Chemotherapy*, 48(5), 1614–1623. <https://doi.org/10.1128/aac.48.5.1614-1623.2004>
- Kensinger, R. D., Yowler, B. C., Benesi, A. J., & Schengrund, C. L. (2004). Synthesis of novel, multivalent glycodendrimers as ligands for HIV-1 gp120. *Bioconjugate Chemistry*, 15(2), 349–358. <https://doi.org/10.1021/bc034156a>
- Khot, V. M., Salunkhe, A. B., Priel, S., Bauer, J., Thorat, N. D., & Townley, H. (2020). Nanomedicine-driven molecular targeting, drug delivery, and therapeutic approaches to cancer chemoresistance. *Drug Discovery Today*, 26, 724–739. <https://doi.org/10.1016/j.drudis.2020.12.016>
- Klatt, N. R., Cheu, R., Birse, K., Zevin, A. S., Perner, M., Noel-Romas, L., Grobler, A., Westmacott, G., Xie, I. Y., Butler, J., Mansoor, L., McKinnon, L. R., Passmore, J. S., Abdool Karim, Q., Abdool Karim, S. S., & Burgener, A. D. (2017). Vaginal bacteria modify HIV tenofovir microbicide efficacy in African women. *Science*, 356(6341), 938–945. <https://doi.org/10.1126/science.aai9383>
- Kovarova, M., Council, O. D., Date, A. A., Long, J. M., Nochi, T., Belshan, M., Shibata, A., Vincent, H., Baker, C. E., Thayer, W. O., Kraus, G., Lachaud-Durand, S., Williams, P., Destache, C. J., & Garcia, J. V. (2015). Nanoformulations of rilpivirine for topical pericoital and systemic coitus-independent administration efficiently prevent HIV transmission. *PLoS Pathogens*, 11(8), e1005075. <https://doi.org/10.1371/journal.ppat.1005075>
- Kowalska, J. D., Skrzat-Klapaczynska, A., Bursa, D., Balayan, T., Begovac, J., Chkhartishvili, N., Gokengin, D., Harxhi, A., Jilich, D., Jevtovic, D., Kase, K., Lakatos, B., Matulionyte, R., Mulabdic, V., Nagit, A., Papadopoulos, A., Stefanovic, M., Vassilenko, A., Vasylyev, M., ... ECEE Network Group. (2020). HIV care in times of the COVID-19 crisis—Where are we now in central and eastern Europe? *International Journal of Infectious Diseases*, 96, 311–314. <https://doi.org/10.1016/j.ijid.2020.05.013>
- Labieniec-Watala, M., & Watala, C. (2015). PAMAM dendrimers: Destined for success or doomed to fail? Plain and modified PAMAM dendrimers in the context of biomedical applications. *Journal of Pharmaceutical Sciences*, 104(1), 2–14. <https://doi.org/10.1002/jps.24222>

- Lackman-Smith, C., Osterling, C., Luckenbaugh, K., Mankowski, M., Snyder, B., Lewis, G., Paull, J., Profy, A., Ptak, R. G., Buckheit, R. W., Jr., Watson, K. M., Cummins, J. E., Jr., & Sanders-Beer, B. E. (2008). Development of a comprehensive human immunodeficiency virus type 1 screening algorithm for discovery and preclinical testing of topical microbicides. *Antimicrobial Agents and Chemotherapy*, 52(5), 1768–1781. <https://doi.org/10.1128/AAC.01328-07>
- Lackman-Smith, C. S., Snyder, B. A., Marotte, K. M., Osterling, M. C., Mankowski, M. K., Jones, M., Nieves-Duran, L., Richardson-Harman, N., Cummins, J. E., Jr., & Sanders-Beer, B. E. (2010). Safety and anti-HIV assessments of natural vaginal cleansing products in an established topical microbicides in vitro testing algorithm. *AIDS Research and Therapy*, 7, 22. <https://doi.org/10.1186/1742-6405-7-22>
- Laher, F., Bekker, L. G., Garrett, N., Lazarus, E. M., & Gray, G. E. (2020). Review of preventative HIV vaccine clinical trials in South Africa. *Archives of Virology*, 165(11), 2439–2452. <https://doi.org/10.1007/s00705-020-04777-2>
- Lai, W. F., & He, Z. D. (2016). Design and fabrication of hydrogel-based nanoparticulate systems for in vivo drug delivery. *Journal of Controlled Release*, 243, 269–282. <https://doi.org/10.1016/j.jconrel.2016.10.013>
- Lancaster, M. A., & Huch, M. (2019). Disease modelling in human organoids. *Disease Models & Mechanisms*, 12(7), dmm039347. <https://doi.org/10.1242/dmm.039347>
- Lard-Whiteford, S. L., Matecka, D., O'Rear, J. J., Yuen, I. S., Litterst, C., Reichelderfer, P., & International Working Group on, Microbicides. (2004). Recommendations for the nonclinical development of topical microbicides for prevention of HIV transmission: An update. *Journal of Acquired Immune Deficiency Syndromes*, 36(1), 541–552. <https://doi.org/10.1097/00126334-200405010-00001>
- Lavender, K. J., Pace, C., Sutter, K., Messer, R. J., Pouncey, D. L., Cummins, N. W., Natesampillai, S., Zheng, J., Goldsmith, J., Widera, M., Van Dis, E. S., Phillips, K., Race, B., Dittmer, U., Kukolj, G., & Hasenkrug, K. J. (2018). An advanced BLT-humanized mouse model for extended HIV-1 cure studies. *AIDS*, 32(1), 1–10. <https://doi.org/10.1097/QAD.0000000000001674>
- Lavender, K. J., Pang, W. W., Messer, R. J., Duley, A. K., Race, B., Phillips, K., Scott, D., Peterson, K. E., Chan, C. K., Dittmer, U., Dudek, T., Allen, T. M., Weissman, I. L., & Hasenkrug, K. J. (2013). BLT-humanized C57BL/6 Rag2<sup>-/-</sup>γ<sup>-/-</sup>CD47<sup>-/-</sup> mice are resistant to GVHD and develop B- and T-cell immunity to HIV infection. *Blood*, 122(25), 4013–4020. <https://doi.org/10.1182/blood-2013-06-506949>
- Lecoeur, H., Fevrier, M., Garcia, S., Riviere, Y., & Gougeon, M. L. (2001). A novel flow cytometric assay for quantitation and multiparametric characterization of cell-mediated cytotoxicity. *Journal of Immunological Methods*, 253(1–2), 177–187. [https://doi.org/10.1016/S0022-1759\(01\)00359-3](https://doi.org/10.1016/S0022-1759(01)00359-3)
- LeMessurier, J., Traversy, G., Varsaneux, O., Weekes, M., Avey, M. T., Niragira, O., Gervais, R., Guyatt, G., & Rodin, R. (2018). Risk of sexual transmission of human immunodeficiency virus with antiretroviral therapy, suppressed viral load and condom use: A systematic review. *CMAJ*, 190(46), E1350–E1360. <https://doi.org/10.1503/cmaj.180311>
- Liu, A. Y., Norwood, A., Gundacker, H., Carballo-Diequez, A., Johnson, S., Patterson, K., Bekker, L. G., Chariyalertsak, S., Chitwarakorn, A., Gonzales, P., Holtz, T. H., Mayer, K. H., Zorrilla, C., Buchbinder, S., Piper, J. M., Lama, J. R., & Cranston, R. D. (2019). Brief report: Routine use of oral PrEP in a phase 2 rectal microbicide study of tenofovir reduced-glycerin 1% gel (MTN-017). *Journal of Acquired Immune Deficiency Syndromes*, 81(5), 516–520. <https://doi.org/10.1097/QAI.0000000000002066>
- Liu, Q., Lei, Z., Wu, Q., Awais, I., Shabbir, M. A. B., Ahmed, S., Fatima, Z., Wang, X., Pan, Y., Xie, S., & Yuan, Z. (2018). The reproductive toxicity of mequindox in a two-generation study in Wistar rats. *Frontiers in Pharmacology*, 9, 870. <https://doi.org/10.3389/fphar.2018.00870>
- Llewellyn, G. N., Seclen, E., Wietgreffe, S., Liu, S., Chateau, M., Pei, H., Perkey, K., Marsden, M. D., Hinkley, S. J., Paschon, D. E., Holmes, M. C., Zack, J. A., Louie, S. G., Haase, A. T., & Cannon, P. M. (2019). Humanized mouse model of HIV-1 latency with enrichment of latent virus in PD-1(+) and TIGIT(+) CD4 T cells. *Journal of Virology*, 93(10), e02086-18. <https://doi.org/10.1128/JVI.02086-18>
- Lowe, A. B. (2014). Thiol-yne 'click'/coupling chemistry and recent applications in polymer and materials synthesis and modification. *Polymer*, 55(22), 5517–5549. <https://doi.org/10.1016/j.polymer.2014.08.015>
- Luganini, A., Nicoletto, S. F., Pizzuto, L., Pirri, G., Giuliani, A., Landolfo, S., & Gribaudo, G. (2011). Inhibition of herpes simplex virus type 1 and type 2 infections by peptide-derivatized dendrimers. *Antimicrobial Agents and Chemotherapy*, 55(7), 3231–3239. <https://doi.org/10.1128/AAC.00149-11>
- Ma, Y., Mou, Q., Wang, D., Zhu, X., & Yan, D. (2016). Dendritic polymers for theranostics. *Theranostics*, 6(7), 930–947. <https://doi.org/10.7150/thno.14855>
- Macchione, M. A., Aristizabal Bedoya, D., Figueroa, F. N., Munoz-Fernandez, M. A., & Strumia, M. C. (2020). Nanosystems applied to HIV infection: Prevention and treatments. *International Journal of Molecular Sciences*, 21(22), 8647. <https://doi.org/10.3390/ijms21228647>
- Maciel, D., Guerrero-Beltran, C., Cena-Diez, R., Tomas, H., Munoz-Fernandez, M. A., & Rodrigues, J. (2019). New anionic poly(alkylideneamine) dendrimers as microbicide agents against HIV-1 infection. *Nanoscale*, 11(19), 9679–9690. <https://doi.org/10.1039/c9nr00303g>
- Madaan, K., Kumar, S., Poonia, N., Lather, V., & Pandita, D. (2014). Dendrimers in drug delivery and targeting: Drug-dendrimer interactions and toxicity issues. *Journal of Pharmacy & Bioallied Sciences*, 6(3), 139–150. <https://doi.org/10.4103/0975-7406.130965>
- Malkoch, M., & García-Gallego, S. (2020). Chapter 1 Introduction to dendrimers and other dendritic polymers. In *Dendrimer chemistry: Synthetic approaches towards complex architectures* (pp. 1–20). The Royal Society of Chemistry.
- Maraval, V., Pyzowski, J., Caminade, A.-M., & Majoral, J.-P. (2003). "Lego" chemistry for the straightforward synthesis of dendrimers. *The Journal of Organic Chemistry*, 68(15), 6043–6046. <https://doi.org/10.1021/jo0344438>
- Marcelino, J. M., Villas Boas, G. R., Cunha, M., Deus Junior, R., Castro, L. H., Araujo, F. H., Traesel, G. K., Dos Santos, A. C., Souza, R. I., Paes, M., Gubert, P., Guterres, Z. D. R., de Lima, F. F., Silva, T., Silva, R. C., Cardoso, C. A. L., Argandona, E. J., Macorini, L. F., & Oesterreich, S. A. (2020). Determination of preclinical safety of oil obtained from *Pachira aquatica* Aublet (Malvaceae) seeds:

- Histopathological, biochemical, hematological, and genetic toxicity studies in rats. *Drug and Chemical Toxicology*, 1-18, 1–18. <https://doi.org/10.1080/01480545.2020.1845713>
- Marchesan, S., & Prato, M. (2013). Nanomaterials for (nano)medicine. *ACS Medicinal Chemistry Letters*, 4(2), 147–149. <https://doi.org/10.1021/ml3003742>
- Marsden, M. D. (2020). Benefits and limitations of humanized mice in HIV persistence studies. *Retrovirology*, 17(1), 7. <https://doi.org/10.1186/s12977-020-00516-2>
- Marsden, M. D., & Zack, J. A. (2017). Humanized mouse models for human immunodeficiency virus infection. *Annual Review of Virology*, 4(1), 393–412. <https://doi.org/10.1146/annurev-virology-101416-041703>
- Martinez-Gualda, B., Sun, L., Marti-Mari, O., Mirabelli, C., Delang, L., Neyts, J., Schols, D., Camarasa, M. J., & San-Felix, A. (2019). Modifications in the branched arms of a class of dual inhibitors of HIV and EV71 replication expand their antiviral spectrum. *Antiviral Research*, 168, 210–214. <https://doi.org/10.1016/j.antiviral.2019.06.006>
- Martinez-Gualda, B., Sun, L., Marti-Mari, O., Noppen, S., Abdelnabi, R., Bator, C. M., Quesada, E., Delang, L., Mirabelli, C., Lee, H., Schols, D., Neyts, J., Hafenstein, S., Camarasa, M. J., Gago, F., & San-Felix, A. (2020). Scaffold simplification strategy leads to a novel generation of dual human immunodeficiency virus and enterovirus-A71 entry inhibitors. *Journal of Medicinal Chemistry*, 63(1), 349–368. <https://doi.org/10.1021/acs.jmedchem.9b01737>
- Martinez-Gualda, B., Sun, L., Rivero-Buceta, E., Flores, A., Quesada, E., Balzarini, J., Noppen, S., Liekens, S., Schols, D., Neyts, J., Leyssen, P., Mirabelli, C., Camarasa, M. J., & San-Felix, A. (2017). Structure-activity relationship studies on a Trp dendrimer with dual activities against HIV and enterovirus A71. Modifications on the amino acid. *Antiviral Research*, 139, 32–40. <https://doi.org/10.1016/j.antiviral.2016.12.010>
- Martin-Moreno, A., Sepulveda-Crespo, D., Serramia-Lobera, M. J., Perise-Barrios, A. J., & Munoz-Fernandez, M. A. (2019). G2-S16 dendrimer microbicide does not interfere with the vaginal immune system. *Journal of Nanobiotechnology*, 17(1), 65. <https://doi.org/10.1186/s12951-019-0496-9>
- Masse-Ranson, G., Mouquet, H., & Di Santo, J. P. (2018). Humanized mouse models to study pathophysiology and treatment of HIV infection. *Current Opinion in HIV and AIDS*, 13(2), 143–151. <https://doi.org/10.1097/COH.0000000000000440>
- Maziero, J. S., Thipe, V. C., Rogero, S. O., Cavalcante, A. K., Damasceno, K. C., Ormenio, M. B., Martini, G. A., Batista, J. G. S., Viveiros, W., Katti, K. K., Raphael Karikachery, A., Dhurvas Mohandoss, D., Dhurvas, R. D., Nappinnai, M., Rogero, J. R., Lugao, A. B., & Katti, K. V. (2020). Species-specific in vitro and in vivo evaluation of toxicity of silver nanoparticles stabilized with gum Arabic protein. *International Journal of Nanomedicine*, 15, 7359–7376. <https://doi.org/10.2147/IJN.S250467>
- McBride, J. W., Dias, N., Cameron, D., Offord, R. E., Hartley, O., Boyd, P., Kett, V. L., & Malcolm, R. K. (2017). Pharmacokinetics of the protein microbicide 5P12-RANTES in sheep following single-dose vaginal gel administration. *Antimicrobial Agents and Chemotherapy*, 61(10), e00965-17. <https://doi.org/10.1128/AAC.00965-17>
- McCarthy, T. D., Karellas, P., Henderson, S. A., Giannis, M., O'Keefe, D. F., Heery, G., Paull, J. R., Matthews, B. R., & Holan, G. (2005). Dendrimers as drugs: Discovery and preclinical and clinical development of dendrimer-based microbicides for HIV and STI prevention. *Molecular Pharmaceutics*, 2(4), 312–318. <https://doi.org/10.1021/mp050023q>
- McConville, C., Friend, D. R., Clark, M. R., & Malcolm, K. (2013). Preformulation and development of a once-daily sustained-release tenofovir vaginal tablet containing a single excipient. *Journal of Pharmaceutical Sciences*, 102(6), 1859–1868. <https://doi.org/10.1002/jps.23528>
- McConville, C., Major, I., Devlin, B., & Brimer, A. (2016). Development of a multi-layered vaginal tablet containing dapivirine, levonorgestrel and acyclovir for use as a multipurpose prevention technology. *European Journal of Pharmaceutics and Biopharmaceutics*, 104, 171–179. <https://doi.org/10.1016/j.ejpb.2016.05.003>
- McGowan, I., Gomez, K., Bruder, K., Febo, I., Chen, B. A., Richardson, B. A., Husnik, M., Livant, E., Price, C., Jacobson, C., & MTN-004 Protocol Team. (2011). Phase 1 randomized trial of the vaginal safety and acceptability of SPL7013 gel (VivaGel) in sexually active young women (MTN-004). *AIDS*, 25(8), 1057–1064. <https://doi.org/10.1097/QAD.0b013e328346bd3e>
- McMahon, M. A., Shen, L., & Siliciano, R. F. (2009). New approaches for quantitating the inhibition of HIV-1 replication by antiviral drugs in vitro and in vivo. *Current Opinion in Infectious Diseases*, 22(6), 574–582. <https://doi.org/10.1097/QCO.0b013e328332c54d>
- Medlock, J., Pandey, A., Parpia, A. S., Tang, A., Skrip, L. A., & Galvani, A. P. (2017). Effectiveness of UNAIDS targets and HIV vaccination across 127 countries. *Proceedings of the National Academy of Sciences of the United States of America*, 114(15), 4017–4022. <https://doi.org/10.1073/pnas.1620788114>
- Mekelburger, H.-B., Vögtle, F., & Jaworek, W. (1992). Dendrimers, arborols, and cascade molecules: Breakthrough into generations of new materials. *Angewandte Chemie International Edition in English*, 31(12), 1571–1576. <https://doi.org/10.1002/anie.199215711>
- Merbah, M., Introini, A., Fitzgerald, W., Grivel, J. C., Lisco, A., Vanpouille, C., & Margolis, L. (2011). Cervico-vaginal tissue ex vivo as a model to study early events in HIV-1 infection. *American Journal of Reproductive Immunology*, 65(3), 268–278. <https://doi.org/10.1111/j.1600-0897.2010.00967.x>
- Mesquita, P. M., Cheshenko, N., Wilson, S. S., Mhatre, M., Guzman, E., Fakioglu, E., Keller, M. J., & Herold, B. C. (2009). Disruption of tight junctions by cellulose sulfate facilitates HIV infection: Model of microbicide safety. *The Journal of Infectious Diseases*, 200(4), 599–608. <https://doi.org/10.1086/600867>
- Mishra, I. (2011). Dendrimer: A novel drug delivery system. *Journal of Drug Delivery & Therapeutics*, 1(2), 70–74. <https://doi.org/10.22270/jddt.v1i2.46>



- Mlynczyk, D. T., Długaszevska, J., Kaluzna-Mlynczyk, A., & Goslinski, T. (2020). Dendrimers against fungi—A state of the art review. *Journal of Controlled Release*, 330, 599–617. <https://doi.org/10.1016/j.jconrel.2020.12.021>
- Montagnini, B. G., Pernoncine, K. V., Borges, L. I., Costa, N. O., Moreira, E. G., Anselmo-Franci, J. A., Kiss, A. C. I., & Gerardin, D. C. C. (2018). Investigation of the potential effects of triclosan as an endocrine disruptor in female rats: Uterotrophic assay and two-generation study. *Toxicology*, 410, 152–165. <https://doi.org/10.1016/j.tox.2018.10.005>
- Morales-Serna, J. A., Boutoureira, O., Serra, A., Matheu, M. I., Díaz, Y., & Castillón, S. (2010). Synthesis of hyperbranched  $\beta$ -galceramide-containing dendritic polymers that bind HIV-1 gp120. *European Journal of Organic Chemistry*, 2010(14), 2657–2660. <https://doi.org/10.1002/ejoc.201000132>
- Moscicki, A. B., Kaul, R., Ma, Y., Scott, M. E., Daud, I. I., Bukusi, E. A., Shiboski, S., Rebbapragada, A., Huibner, S., & Cohen, C. R. (2012). Measurement of mucosal biomarkers in a phase 1 trial of intravaginal 3% StarPharma LTD 7013 gel (VivaGel) to assess expanded safety. *Journal of Acquired Immune Deficiency Syndromes*, 59(2), 134–140. <https://doi.org/10.1097/QAI.0b013e31823f2aeb>
- Moss, J. A., Malone, A. M., Smith, T. J., Butkyavichene, I., Cortez, C., Gilman, J., Kennedy, S., Kopin, E., Nguyen, C., Sinha, P., Hendry, R. M., Guenther, P., Holder, A., Martin, A., McNicholl, J., Mitchell, J., Pau, C. P., Srinivasan, P., Smith, J. M., & Baum, M. M. (2012). Safety and pharmacokinetics of intravaginal rings delivering tenofovir in pig-tailed macaques. *Antimicrobial Agents and Chemotherapy*, 56(11), 5952–5960. <https://doi.org/10.1128/AAC.01198-12>
- MTN. (2020). *Studies*. Available from <https://www.mtnstopshiv.org/news/studies>.
- Mukhopadhyaya, I., Murray, G. I., Berry, S., Thomson, J., Frank, B., Gwozdz, G., Ekeruche-Makinde, J., Shattock, R., Kelly, C., Iannelli, F., Pozzi, G., El-Omar, E. M., Hold, G. L., & Hijazi, K. (2016). Drug transporter gene expression in human colorectal tissue and cell lines: Modulation with antiretrovirals for microbicide optimization. *The Journal of Antimicrobial Chemotherapy*, 71(2), 372–386. <https://doi.org/10.1093/jac/dkv335>
- Muller, P. Y., & Milton, M. N. (2012). The determination and interpretation of the therapeutic index in drug development. *Nature Reviews. Drug Discovery*, 11(10), 751–761. <https://doi.org/10.1038/nrd3801>
- Mumper, R. J., Bell, M. A., Worthen, D. R., Cone, R. A., Lewis, G. R., Paull, J. R., & Moench, T. R. (2009). Formulating a sulfonated antiviral dendrimer in a vaginal microbicide gel having dual mechanisms of action. *Drug Development and Industrial Pharmacy*, 35(5), 515–524. <https://doi.org/10.1080/03639040802488097>
- Murphy, D. J., Desjardins, D., Boyd, P., Dereuddre-Bosquet, N., Stimmer, L., Caldwell, A., Le Grand, R., Kelly, C., van Roey, J., & Malcolm, R. K. (2018). Impact of ring size and drug loading on the pharmacokinetics of a combination dapivirine-darunavir vaginal ring in cynomolgus macaques. *International Journal of Pharmaceutics*, 550(1–2), 300–308. <https://doi.org/10.1016/j.ijpharm.2018.08.051>
- Murphy, D. J., Desjardins, D., Dereuddre-Bosquet, N., Brochard, P., Perrot, L., Pruvost, A., Le Grand, R., Lagatie, O., Vanhooren, L., Feyaerts, M., van Roey, J., & Malcolm, R. K. (2014). Pre-clinical development of a combination microbicide vaginal ring containing dapivirine and darunavir. *The Journal of Antimicrobial Chemotherapy*, 69(9), 2477–2488. <https://doi.org/10.1093/jac/dku160>
- Musekiwa, A., Fernando, N. B., & Abariga, S. A. (2020). Effectiveness of vaginal microbicides in preventing HIV transmission. *Tropical Medicine & International Health*, 25(7), 790–802. <https://doi.org/10.1111/tmi.13401>
- Nahui Palomino, R. A., Zicari, S., Vanpouille, C., Vitali, B., & Margolis, L. (2017). Vaginal lactobacillus inhibits HIV-1 replication in human tissues ex vivo. *Frontiers in Microbiology*, 8, 906. <https://doi.org/10.3389/fmicb.2017.00906>
- Nakamura, K., Ishii, Y., Takasu, S., Nohmi, T., Shibusaki, M., & Ogawa, K. (2020). Lack of in vivo mutagenicity of acetamide in a 13-week comprehensive toxicity study using F344 gpt delta rats. *Toxicological Sciences*, 177(2), 431–440. <https://doi.org/10.1093/toxsci/kfaa126>
- Nandy, B., Saurabh, S., Sahoo, A. K., Dixit, N. M., & Maiti, P. K. (2015). The SPL7013 dendrimer destabilizes the HIV-1 gp120-CD4 complex. *Nanoscale*, 7(44), 18628–18641. <https://doi.org/10.1039/c5nr04632g>
- Nanjwade, B. K., Bechra, H. M., Derkar, G. K., Manvi, F. V., & Nanjwade, V. K. (2009). Dendrimers: Emerging polymers for drug-delivery systems. *European Journal of Pharmaceutical Sciences*, 38(3), 185–196. <https://doi.org/10.1016/j.ejps.2009.07.008>
- Nara, P. L., & Fischinger, P. J. (1988). Quantitative infectivity assay for HIV-1 and -2. *Nature*, 332(6163), 469–470. <https://doi.org/10.1038/332469a0>
- Nelson, A. L. (2018). An overview of properties of amphora (acidform) contraceptive vaginal gel. *Expert Opinion on Drug Safety*, 17(9), 935–943. <https://doi.org/10.1080/14740338.2018.1515197>
- Newkome, G. R., Keith, J. M., Baker, G. R., Escamilla, G. H., & Moorefield, C. N. (1994). Chemistry within a unimolecular micelle precursor: Boron superclusters by site- and depth-specific transformations of dendrimers. *Angewandte Chemie International Edition in English*, 33(6), 666–668. <https://doi.org/10.1002/anie.199406661>
- Newkome, G. R., Yao, Z., Baker, G. R., & Gupta, V. K. (1985). Micelles. Part 1. Cascade molecules: A new approach to micelles. A [27]-arborol. *The Journal of Organic Chemistry*, 50(11), 2003–2004. <https://doi.org/10.1021/jo00211a052>
- Nochi, T., Denton, P. W., Wahl, A., & Garcia, J. V. (2013). Cryptopatches are essential for the development of human GALT. *Cell Reports*, 3(6), 1874–1884. <https://doi.org/10.1016/j.celrep.2013.05.037>
- Notario-Perez, F., Galante, J., Martín-Illana, A., Cazorla-Luna, R., Sarmiento, B., Ruiz-Caro, R., das Neves, J., & Veiga, M. D. (2020). Development of pH-sensitive vaginal films based on methacrylate copolymers for topical HIV-1 pre-exposure prophylaxis. *Acta Biomaterialia*, 121, 316–327. <https://doi.org/10.1016/j.actbio.2020.12.019>
- Notario-Perez, F., Martín-Illana, A., Cazorla-Luna, R., Ruiz-Caro, R., Bedoya, L. M., Pena, J., & Veiga, M. D. (2019). Development of mucoadhesive vaginal films based on HPMC and zein as novel formulations to prevent sexual transmission of HIV. *International Journal of Pharmaceutics*, 570, 118643. <https://doi.org/10.1016/j.ijpharm.2019.118643>



- Notario-Perez, F., Ruiz-Caro, R., & Veiga-Ochoa, M. D. (2017). Historical development of vaginal microbicides to prevent sexual transmission of HIV in women: From past failures to future hopes. *Drug Design, Development and Therapy*, 11, 1767–1787. <https://doi.org/10.2147/DDDT.S133170>
- Nuttall, J. P., Thake, D. C., Lewis, M. G., Ferkany, J. W., Romano, J. W., & Mitchnick, M. A. (2008). Concentrations of dapivirine in the rhesus macaque and rabbit following once daily intravaginal administration of a gel formulation of [14C]dapivirine for 7 days. *Antimicrobial Agents and Chemotherapy*, 52(3), 909–914. <https://doi.org/10.1128/AAC.00330-07>
- O'Loughlin, J., Millwood, I. Y., McDonald, H. M., Price, C. F., Kaldor, J. M., & Paull, J. R. (2010). Safety, tolerability, and pharmacokinetics of SPL7013 gel (VivaGel): A dose ranging, phase I study. *Sexually Transmitted Diseases*, 37(2), 100–104. <https://doi.org/10.1097/OLQ.0b013e3181bc0aac>
- Ordanini, S., Varga, N., Porkolab, V., Thepaut, M., Belvisi, L., Bertaglia, A., Palmioli, A., Berzi, A., Trabattoni, D., Clerici, M., Fieschi, F., & Bernardi, A. (2015). Designing nanomolar antagonists of DC-SIGN-mediated HIV infection: Ligand presentation using molecular rods. *Chemical communications (Cambridge, England)*, 51(18), 3816–3819. <https://doi.org/10.1039/c4cc09709b>
- Ordanini, S., Zanchetta, G., Porkolab, V., Ebel, C., Fieschi, F., Guzzetti, I., Potenza, D., Palmioli, A., Podlipnik, C., Meroni, D., & Bernardi, A. (2016). Solution behavior of amphiphilic glycodendrimers with a rod-like core. *Macromolecular Bioscience*, 16(6), 896–905. <https://doi.org/10.1002/mabi.201500452>
- Palmerston Mendes, L., Pan, J., & Torchilin, V. P. (2017). Dendrimers as nanocarriers for nucleic acid and drug delivery in cancer therapy. *Molecules*, 22(9), 1401. <https://doi.org/10.3390/molecules22091401>
- Pandey, S. K., Nakka, H., Ambhore, S. R., Londhe, S., Goyal, V. K., & Nirogi, R. (2020). Short-term toxicity study of 1-aminobenzotriazole, a CYP inhibitor, in Wistar rats. *Drug and Chemical Toxicology*, 1-9, 1–9. <https://doi.org/10.1080/01480545.2020.1850755>
- Pantaleo, G., Janes, H., Karuna, S., Grant, S., Ouedraogo, G. L., Allen, M., Tomaras, G. D., Frahm, N., Montefiori, D. C., Ferrari, G., Ding, S., Lee, C., Robb, M. L., Esteban, M., Wagner, R., Bart, P. A., Rettby, N., McElrath, M. J., Gilbert, P. B., ... NIAID HIV Vaccine Trials Network. (2019). Safety and immunogenicity of a multivalent HIV vaccine comprising envelope protein with either DNA or NYVAC vectors (HVTN 096): A phase 1b, double-blind, placebo-controlled trial. *Lancet HIV*, 6(11), e737–e749. [https://doi.org/10.1016/S2352-3018\(19\)30262-0](https://doi.org/10.1016/S2352-3018(19)30262-0)
- Park, J. S., Cho, E. Y., Kim, Y. S., Kwon, E., Han, K. M., Ku, S. Y., Jung, C. W., Yun, J. W., Che, J. H., & Kang, B. C. (2020). In vivo and in vitro safety evaluation of fermented citrus sunki peel extract: Acute and 90-day repeated oral toxicity studies with genotoxicity assessment. *BMC Complementary Medicine and Therapies*, 20(1), 297. <https://doi.org/10.1186/s12906-020-03079-z>
- Parrish, N. F., Gao, F., Li, H., Giorgi, E. E., Barbian, H. J., Parrish, E. H., Zajic, L., Iyer, S. S., Decker, J. M., Kumar, A., Hora, B., Berg, A., Cai, F., Hopper, J., Denny, T. N., Ding, H., Ochsenbauer, C., Kappes, J. C., Galimidi, R. P., ... Hahn, B. H. (2013). Phenotypic properties of transmitted founder HIV-1. *Proceedings of the National Academy of Sciences of the United States of America*, 110(17), 6626–6633. <https://doi.org/10.1073/pnas.1304288110>
- Patel, S. K., & Rohan, L. C. (2017). On-demand microbicide products: Design matters. *Drug Delivery and Translational Research*, 7(6), 775–795. <https://doi.org/10.1007/s13346-017-0385-4>
- Patki, M., Vartak, R., Jablonski, J., Mediouni, S., Gandhi, T., Fu, Y., Cetindag, E., Dave, R., Valente, S. T., & Patel, K. (2020). Efavirenz nanomicelles loaded vaginal film (EZ film) for preexposure prophylaxis (PrEP) of HIV. *Colloids and Surfaces. B, Biointerfaces*, 194, 111174. <https://doi.org/10.1016/j.colsurfb.2020.111174>
- Patra, J. K., Das, G., Fraceto, L. F., Campos, E. V. R., Rodriguez-Torres, M. D. P., Acosta-Torres, L. S., Diaz-Torres, L. A., Grillo, R., Swamy, M. K., Sharma, S., Habtemariam, S., & Shin, H. S. (2018). Nano based drug delivery systems: Recent developments and future prospects. *Journal of Nanobiotechnology*, 16(1), 71. <https://doi.org/10.1186/s12951-018-0392-8>
- Patton, D. L., Cosgrove Sweeney, Y. T., McCarthy, T. D., & Hillier, S. L. (2006). Preclinical safety and efficacy assessments of dendrimer-based (SPL7013) microbicide gel formulations in a nonhuman primate model. *Antimicrobial Agents and Chemotherapy*, 50(5), 1696–1700. <https://doi.org/10.1128/AAC.50.5.1696-1700.2006>
- Pellett Madan, R., Dezzutti, C. S., Rabe, L., Hillier, S. L., Marrazzo, J., McGowan, I., Richardson, B. A., Herold, B. C., & Microbicide Trials Network Biomedical Sciences Working Group and the MTN 004 Protocol Team. (2015). Soluble immune mediators and vaginal bacteria impact innate genital mucosal antimicrobial activity in young women. *American Journal of Reproductive Immunology*, 74(4), 323–332. <https://doi.org/10.1111/aji.12412>
- Perez-Anes, A., Spataro, G., Coppel, Y., Moog, C., Blanzat, M., Turrin, C. O., Caminade, A. M., Rico-Lattes, I., & Majoral, J. P. (2009). Phosphonate terminated PPH dendrimers: Influence of pendant alkyl chains on the in vitro anti-HIV-1 properties. *Organic & Biomolecular Chemistry*, 7(17), 3491–3498. <https://doi.org/10.1039/b908352a>
- Perez-Anes, A., Stefaniu, C., Moog, C., Majoral, J. P., Blanzat, M., Turrin, C. O., Caminade, A. M., & Rico-Lattes, I. (2010). Multivalent cat-anionic GalCer analogs derived from first generation dendrimeric phosphonic acids. *Bioorganic & Medicinal Chemistry*, 18(1), 242–248. <https://doi.org/10.1016/j.bmc.2009.10.058>
- Petrova, M. I., van den Broek, M., Balzarini, J., Vanderleyden, J., & Lebeer, S. (2013). Vaginal microbiota and its role in HIV transmission and infection. *FEMS Microbiology Reviews*, 37(5), 762–792. <https://doi.org/10.1111/1574-6976.12029>
- Phanuphak, N., & Gulick, R. M. (2020). HIV treatment and prevention 2019: Current standards of care. *Current Opinion in HIV and AIDS*, 15(1), 4–12. <https://doi.org/10.1097/COH.0000000000000588>
- Pitisuttithum, P., & Marovich, M. A. (2020). Prophylactic HIV vaccine: Vaccine regimens in clinical trials and potential challenges. *Expert Review of Vaccines*, 19(2), 133–142. <https://doi.org/10.1080/14760584.2020.1718497>

- Pleasant, E., Tauya, T., Reddy, K., Mirembe, B. G., Woeber, K., Palanee-Phillips, T., Zimba, C., Atujuna, M., Montgomery, E. T., & MTN-020/ASPIRE Study Team. (2020). Relationship type and use of the vaginal ring for HIV-1 prevention in the MTN 020/ASPIRE trial. *AIDS and Behavior*, 24(3), 866–880. <https://doi.org/10.1007/s10461-019-02521-9>
- Prado-Ochoa, M. G., Strassburger-Madrigal, M., Camacho-Carranza, R., Espinosa-Aguirre, J. J., Velazquez-Sanchez, A. M., Vazquez-Valadez, V. H., Angeles, E., Alba-Hurtado, F., & Munoz-Guzman, M. A. (2020). Structure-activity relationship (SAR) and in vitro predictions of mutagenic and carcinogenic activities of Ixodidical ethyl-carbamates. *BioMed Research International*, 2020, 2981681. <https://doi.org/10.1155/2020/2981681>
- Price, C. F., Tyssen, D., Sonza, S., Davie, A., Evans, S., Lewis, G. R., Xia, S., Spelman, T., Hodsman, P., Moench, T. R., Humberstone, A., Paull, J. R., & Tachedjian, G. (2011). SPL7013 gel (VivaGel(R)) retains potent HIV-1 and HSV-2 inhibitory activity following vaginal administration in humans. *PLoS One*, 6(9), e24095. <https://doi.org/10.1371/journal.pone.0024095>
- Relano-Rodriguez, I., Juarez-Sanchez, R., Pavicic, C., Munoz, E., & Munoz-Fernandez, M. A. (2019). Polyanionic carbosilane dendrimers as a new adjuvant in combination with latency reversal agents for HIV treatment. *Journal of Nanobiotechnology*, 17(1), 69. <https://doi.org/10.1186/s12951-019-0500-4>
- Relano-Rodriguez, I., & Munoz-Fernandez, M. A. (2020). Emergence of nanotechnology to fight HIV sexual transmission: The trip of G2-S16 polyanionic carbosilane dendrimer to possible pre-clinical Trials. *International Journal of Molecular Sciences*, 21(24), 9403. <https://doi.org/10.3390/ijms21249403>
- Richardson-Harman, N., Lackman-Smith, C., Fletcher, P. S., Anton, P. A., Bremer, J. W., Dezzutti, C. S., Elliott, J., Grivel, J. C., Guenther, P., Gupta, P., Jones, M., Lurain, N. S., Margolis, L. B., Mohan, S., Ratner, D., Reichelderfer, P., Roberts, P., Shattock, R. J., & Cummins, J. E., Jr. (2009). Multisite comparison of anti-human immunodeficiency virus microbicide activity in explant assays using a novel endpoint analysis. *Journal of Clinical Microbiology*, 47(11), 3530–3539. <https://doi.org/10.1128/JCM.00673-09>
- Riss, T. L., Moravec, R. A., Niles, A. L., Duellman, S., Benink, H. A., Worzella, T. J., & Minor, L. (2004). *Cell viability assays* doi:NBK144065 [bookaccession]. Eli Lilly & Company and the National Center for Advancing Translational Sciences.
- Rivero-Buceta, E., Doyaguez, E. G., Colomer, I., Quesada, E., Mathys, L., Noppen, S., Liekens, S., Camarasa, M. J., Perez-Perez, M. J., Balzarini, J., & San-Felix, A. (2015). Tryptophan dendrimers that inhibit HIV replication, prevent virus entry and bind to the HIV envelope glycoproteins gp120 and gp41. *European Journal of Medicinal Chemistry*, 106, 34–43. <https://doi.org/10.1016/j.ejmech.2015.10.031>
- Roan, N. R., & Munch, J. (2015). Improving preclinical models of HIV microbicide efficacy. *Trends in Microbiology*, 23(8), 445–447. <https://doi.org/10.1016/j.tim.2015.05.001>
- Roberts, S. T., Nair, G., Baeten, J. M., Palanee-Phillips, T., Schwartz, K., Reddy, K., Kabwigu, S., Matovu Kiweewa, F., Govender, V., Gaffoor, Z., Singh, N., Siva, S., Naidoo, K., Montgomery, E. T., & MTN-020/ASPIRE Team. (2020). Impact of male partner involvement on women's adherence to the dapivirine vaginal ring during a phase III HIV prevention trial. *AIDS and Behavior*, 24(5), 1432–1442. <https://doi.org/10.1007/s10461-019-02707-1>
- Rodriguez-Izquierdo, I., Gasco, S., & Munoz-Fernandez, M. A. (2020). High preventive effect of G2-S16 anionic Carbosilane dendrimer against sexually transmitted HSV-2 infection. *Molecules*, 25(13), 2695. <https://doi.org/10.3390/molecules25132695>
- Rodriguez-Izquierdo, I., Natalia, C., Garcia, F., & de Los Munoz-Fernandez, M. (2019). G2-S16 sulfonate dendrimer as new therapy for treatment failure in HIV-1 entry inhibitors. *Nanomedicine (London, England)*, 14(9), 1095–1107. <https://doi.org/10.2217/nnm-2018-0364>
- Rohan, L. C., Devlin, B., & Yang, H. (2014). Microbicide dosage forms. *Current Topics in Microbiology and Immunology*, 383, 27–54. [https://doi.org/10.1007/82\\_2013\\_357](https://doi.org/10.1007/82_2013_357)
- Rollenhagen, C., Lathrop, M. J., Macura, S. L., Doncel, G. F., & Asin, S. N. (2014). Herpes simplex virus type-2 stimulates HIV-1 replication in cervical tissues: Implications for HIV-1 transmission and efficacy of anti-HIV-1 microbicides. *Mucosal Immunology*, 7(5), 1165–1174. <https://doi.org/10.1038/mi.2014.3>
- Rosa Borges, A., Wieczorek, L., Johnson, B., Benesi, A. J., Brown, B. K., Kensinger, R. D., Krebs, F. C., Wigdahl, B., Blumenthal, R., Puri, A., McCutchan, F. E., Bix, D. L., Polonis, V. R., & Schengrund, C. L. (2010). Multivalent dendrimeric compounds containing carbohydrates expressed on immune cells inhibit infection by primary isolates of HIV-1. *Virology*, 408(1), 80–88. <https://doi.org/10.1016/j.virol.2010.09.004>
- Roy, U., Rodriguez, J., Barber, P., das Neves, J., Sarmiento, B., & Nair, M. (2015). The potential of HIV-1 nanotherapeutics: From in vitro studies to clinical trials. *Nanomedicine (London, England)*, 10(24), 3597–3609. <https://doi.org/10.2217/nnm.15.160>
- Rupp, R., Rosenthal, S. L., & Stanberry, L. R. (2007). VivaGel (SPL7013 gel): A candidate dendrimer-microbicide for the prevention of HIV and HSV infection. *International Journal of Nanomedicine*, 2(4), 561–566.
- Sabatte, J., Remes Lenicov, F., Cabrini, M., Rodriguez Rodrigues, C., Ostrowski, M., Ceballos, A., Amigorena, S., & Geffner, J. (2011). The role of semen in sexual transmission of HIV: Beyond a carrier for virus particles. *Microbes and Infection*, 13(12–13), 977–982. <https://doi.org/10.1016/j.micinf.2011.06.005>
- Safrit, J. T., Fung, M. S., Andrews, C. A., Braun, D. G., Sun, W. N., Chang, T. W., & Koup, R. A. (1993). Hu-PBL-SCID mice can be protected from HIV-1 infection by passive transfer of monoclonal antibody to the principal neutralizing determinant of envelope gp120. *AIDS*, 7(1), 15–21. <https://doi.org/10.1097/00002030-199301000-00002>
- Saleh, D. M., Alexander, W. T., Numano, T., Ahmed, O. H. M., Gunasekaran, S., Alexander, D. B., Abdelgied, M., El-Gazzar, A. M., Takase, H., Xu, J., Naiki-Ito, A., Takahashi, S., Hirose, A., Ohnishi, M., Kanno, J., & Tsuda, H. (2020). Comparative carcinogenicity study of a thick, straight-type and a thin, tangled-type multi-walled carbon nanotube administered by intra-tracheal instillation in the rat. *Particle and Fibre Toxicology*, 17(1), 48. <https://doi.org/10.1186/s12989-020-00382-y>

- San Anselmo, M., Lancelot, A., Egido, J. E., Claveria-Gimeno, R., Casanova, A., Serrano, J. L., Hernandez-Ainsa, S., Abian, O., & Sierra, T. (2020). Janus dendrimers to assess the anti-HCV activity of molecules in cell-assays. *Pharmaceutics*, *12*(11), 1062. <https://doi.org/10.3390/pharmaceutics12111062>
- Sanchez-Rodriguez, J., Diaz, L., Galan, M., Maly, M., Gomez, R., Javier de la Mata, F., Jimenez, J. L., & Munoz-Fernandez, M. A. (2015). Anti-human immunodeficiency virus activity of thiol-Ene Carbosilane dendrimers and their potential development as a topical microbicide. *Journal of Biomedical Nanotechnology*, *11*(10), 1783–1798. <https://doi.org/10.1166/jbn.2015.2109>
- Sanchez-Rodriguez, J., Vacas-Cordoba, E., Gomez, R., De La Mata, F. J., & Munoz-Fernandez, M. A. (2015). Nanotech-derived topical microbicides for HIV prevention: The road to clinical development. *Antiviral Research*, *113*, 33–48. <https://doi.org/10.1016/j.antiviral.2014.10.014>
- Sandoval-Yanez, C., & Castro Rodriguez, C. (2020). Dendrimers: Amazing platforms for bioactive molecule delivery systems. *Materials (Basel)*, *13*(3), 570. <https://doi.org/10.3390/ma13030570>
- Sanz Del Olmo, N., Pena Gonzalez, C. E., Rojas, J. D., Gomez, R., Ortega, P., Escarpa, A., & de la Mata, F. J. (2020). Antioxidant and antibacterial properties of Carbosilane dendrimers functionalized with polyphenolic moieties. *Pharmaceutics*, *12*(8), 698. <https://doi.org/10.3390/pharmaceutics12080698>
- Sattin, S., Daggetti, A., Thepaut, M., Berzi, A., Sanchez-Navarro, M., Tabarani, G., Rojo, J., Fieschi, F., Clerici, M., & Bernardi, A. (2010). Inhibition of DC-SIGN-mediated HIV infection by a linear trimannoside mimic in a tetravalent presentation. *ACS Chemical Biology*, *5*(3), 301–312. <https://doi.org/10.1021/cb900216e>
- Schmitt, K., & Akkina, R. (2018). Ultra-sensitive HIV-1 latency viral outgrowth assays using humanized mice. *Frontiers in Immunology*, *9*, 344. <https://doi.org/10.3389/fimmu.2018.00344>
- Scott, Y. M., Park, S. Y., & Dezzutti, C. S. (2016). Broadly neutralizing anti-HIV antibodies prevent HIV infection of mucosal tissue ex vivo. *Antimicrobial Agents and Chemotherapy*, *60*(2), 904–912. <https://doi.org/10.1128/AAC.02097-15>
- Sepulveda-Crespo, D., Cena-Diez, R., Jimenez, J. L., & Angeles Munoz-Fernandez, M. (2017). Mechanistic studies of viral entry: An overview of dendrimer-based microbicides as entry inhibitors against both HIV and HSV-2 overlapped infections. *Medicinal Research Reviews*, *37*(1), 149–179. <https://doi.org/10.1002/med.21405>
- Sepulveda-Crespo, D., de la Mata, F. J., Gomez, R., & Munoz-Fernandez, M. A. (2018). Sulfonate-ended carbosilane dendrimers with a flexible scaffold cause inactivation of HIV-1 virions and gp120 shedding. *Nanoscale*, *10*(19), 8998–9011. <https://doi.org/10.1039/c8nr01664j>
- Sepulveda-Crespo, D., Gomez, R., De La Mata, F. J., Jimenez, J. L., & Munoz-Fernandez, M. A. (2015). Polyanionic carbosilane dendrimer-conjugated antiviral drugs as efficient microbicides: Recent trends and developments in HIV treatment/therapy. *Nanomedicine*, *11*(6), 1481–1498. <https://doi.org/10.1016/j.nano.2015.03.008>
- Sepulveda-Crespo, D., Jimenez, J. L., Gomez, R., De La Mata, F. J., Majano, P. L., Munoz-Fernandez, M. A., & Gastaminza, P. (2017). Polyanionic carbosilane dendrimers prevent hepatitis C virus infection in cell culture. *Nanomedicine*, *13*(1), 49–58. <https://doi.org/10.1016/j.nano.2016.08.018>
- Sepulveda-Crespo, D., Lorente, R., Leal, M., Gomez, R., De la Mata, F. J., Jimenez, J. L., & Munoz-Fernandez, M. A. (2014). Synergistic activity profile of carbosilane dendrimer G2-STE16 in combination with other dendrimers and antiretrovirals as topical anti-HIV-1 microbicide. *Nanomedicine*, *10*(3), 609–618. <https://doi.org/10.1016/j.nano.2013.10.002>
- Sepulveda-Crespo, D., Sanchez-Rodriguez, J., Serramia, M. J., Gomez, R., De La Mata, F. J., Jimenez, J. L., & Munoz-Fernandez, M. A. (2015). Triple combination of carbosilane dendrimers, tenofovir and maraviroc as potential microbicide to prevent HIV-1 sexual transmission. *Nanomedicine (London, England)*, *10*(6), 899–914. <https://doi.org/10.2217/nnm.14.79>
- Sepulveda-Crespo, D., Serramia, M. J., Tager, A. M., Vrbanc, V., Gomez, R., De La Mata, F. J., Jimenez, J. L., & Munoz-Fernandez, M. A. (2015). Prevention vaginally of HIV-1 transmission in humanized BLT mice and mode of antiviral action of polyanionic carbosilane dendrimer G2-S16. *Nanomedicine*, *11*(6), 1299–1308. <https://doi.org/10.1016/j.nano.2015.04.013>
- Sepulveda-Crespo, D., Vacas-Cordoba, E., Marquez-Miranda, V., Araya-Duran, I., Gomez, R., Mata, F. J., Gonzalez-Nilo, F. D., & Munoz-Fernandez, M. A. (2016). Effect of several HIV antigens simultaneously loaded with G2-NN16 carbosilane dendrimer in the cell uptake and functionality of human dendritic cells. *Bioconjugate Chemistry*, *27*(12), 2844–2849. <https://doi.org/10.1021/acs.bioconjchem.6b00623>
- Sharma, R., Zhang, I., Abbassi, L., Rej, R., Maysinger, D., & Roy, R. (2015). A fast track strategy toward highly functionalized dendrimers with different structural layers: An “onion peel approach”. *Polymer Chemistry*, *6*(9), 1436–1444. <https://doi.org/10.1039/C4PY01761G>
- Shcharbin, D., Bryszewska, M., Mignani, S., Shi, X., & Majoral, J. P. (2020). Phosphorus dendrimers as powerful nanoplatforams for drug delivery, as fluorescent probes and for liposome interaction studies: A concise overview. *European Journal of Medicinal Chemistry*, *208*, 112788. <https://doi.org/10.1016/j.ejmech.2020.112788>
- Sim, T. M., Tarini, D., Dheen, S. T., Bay, B. H., & Srinivasan, D. K. (2020). Nanoparticle-based technology approaches to the Management of Neurological Disorders. *International Journal of Molecular Sciences*, *21*(17), 6070. <https://doi.org/10.3390/ijms21176070>
- Simian, M., & Bissell, M. J. (2017). Organoids: A historical perspective of thinking in three dimensions. *The Journal of Cell Biology*, *216*(1), 31–40. <https://doi.org/10.1083/jcb.201610056>
- Soares, S., Sousa, J., Pais, A., & Vitorino, C. (2018). Nanomedicine: Principles, properties, and regulatory issues. *Frontiers in Chemistry*, *6*, 360. <https://doi.org/10.3389/fchem.2018.00360>
- Soezi, M., Memarnejadian, A., Aminzadeh, S., Zabihollahi, R., Sadat, S. M., Amini, S., Hekmat, S., & Aghasadeghi, M. R. (2015). Toward the development of a single-round infection assay based on EGFP reporting for anti-HIV-1 drug discovery. *Reports of Biochemistry and Molecular Biology*, *4*(1), 1–9.
- Starpharma. (2021). *COVID-19 & Starpharma: VIRALEZE™ COVID-19 nasal spray*. Retrieved from <https://starpharma.com/coronavirus>.

- Stoddard, E., Ni, H., Cannon, G., Zhou, C., Kallenbach, N., Malamud, D., & Weissman, D. (2009). gp340 promotes transcytosis of human immunodeficiency virus type 1 in genital tract-derived cell lines and primary endocervical tissue. *Journal of Virology*, 83(17), 8596–8603. <https://doi.org/10.1128/JVI.00744-09>
- Stoddart, C. A., Galkina, S. A., Joshi, P., Kosikova, G., Long, B. R., Maidji, E., Moreno, M. E., Rivera, J. M., Sanford, U. R., Sloan, B., Cieplak, W., Wrin, T., & Chan-Hui, P. Y. (2014). Efficacy of broadly neutralizing monoclonal antibody PG16 in HIV-infected humanized mice. *Virology*, 462–463, 115–125. <https://doi.org/10.1016/j.virol.2014.05.036>
- Svenson, S., & Tomalia, D. A. (2005). Dendrimers in biomedical applications—reflections on the field. *Advanced Drug Delivery Reviews*, 57(15), 2106–2129. <https://doi.org/10.1016/j.addr.2005.09.018>
- Szucs, G., Melnick, J. L., & Hollinger, F. B. (1988). A simple assay based on HIV infection preventing the reclustering of MT-4 cells. *Bulletin of the World Health Organization*, 66(6), 729–737.
- Tabarani, G., Reina, J. J., Ebel, C., Vives, C., Lortat-Jacob, H., Rojo, J., & Fieschi, F. (2006). Mannose hyperbranched dendritic polymers interact with clustered organization of DC-SIGN and inhibit gp120 binding. *FEBS Letters*, 580(10), 2402–2408. <https://doi.org/10.1016/j.febslet.2006.03.061>
- Takebe, T., & Wells, J. M. (2019). Organoids by design. *Science*, 364(6444), 956–959. <https://doi.org/10.1126/science.aaw7567>
- Taneva, E., Sinclair, S., Mesquita, P. M., Weinrick, B., Cameron, S. A., Cheshenko, N., Reagle, K., Frank, B., Srinivasan, S., Fredricks, D., Keller, M. J., & Herold, B. C. (2018). Vaginal microbiome modulates topical antiretroviral drug pharmacokinetics. *JCI Insight*, 3(13), e99545. <https://doi.org/10.1172/jci.insight.99545>
- Telwatte, S., Moore, K., Johnson, A., Tyssen, D., Sterjovski, J., Aldunate, M., Gorry, P. R., Ramsland, P. A., Lewis, G. R., Paull, J. R., Sonza, S., & Tachedjian, G. (2011). Virucidal activity of the dendrimer microbicide SPL7013 against HIV-1. *Antiviral Research*, 90(3), 195–199. <https://doi.org/10.1016/j.antiviral.2011.03.186>
- Thippeshappa, R., Kimata, J. T., & Kaushal, D. (2020). Toward a macaque model of HIV-1 infection: Roadblocks, progress, and future strategies. *Frontiers in Microbiology*, 11, 882. <https://doi.org/10.3389/fmicb.2020.00882>
- Thurman, A. R., & Doncel, G. F. (2011). Innate immunity and inflammatory response to trichomonas vaginalis and bacterial vaginosis: Relationship to HIV acquisition. *American Journal of Reproductive Immunology*, 65(2), 89–98. <https://doi.org/10.1111/j.1600-0897.2010.00902.x>
- Thurman, A. R., Schwartz, J. L., Ravel, J., Gajer, P., Marzinke, M. A., Yousefieh, N., Anderson, S. M., & Doncel, G. F. (2019). Vaginal microbiota and mucosal pharmacokinetics of tenofovir in healthy women using tenofovir and tenofovir/levonorgestrel vaginal rings. *PLoS One*, 14(5), e0217229. <https://doi.org/10.1371/journal.pone.0217229>
- Tomalia, D. A., Baker, H., Dewald, J., Hall, M., Kallous, G., Martin, S., Roeck, J., Ryder, J., & Smith, P. (1985). A new class of polymers: Starburst-dendritic macromolecules. *Polymer Journal*, 17(1), 117–132. <https://doi.org/10.1295/polymj.17.117>
- Tugizov, S. M., Herrera, R., Chin-Hong, P., Velupillai, P., Greenspan, D., Michael Berry, J., Pilcher, C. D., Shiboski, C. H., Jay, N., Rubin, M., Chein, A., & Palefsky, J. M. (2013). HIV-associated disruption of mucosal epithelium facilitates paracellular penetration by human papillomavirus. *Virology*, 446(1–2), 378–388. <https://doi.org/10.1016/j.virol.2013.08.018>
- Tyo, K. M., Duan, J., Kollipara, P., Dela Cerna, M. V. C., Lee, D., Palmer, K. E., & Steinbach-Rankins, J. M. (2019). pH-responsive delivery of Griffithsin from electrospun fibers. *European Journal of Pharmaceutics and Biopharmaceutics*, 138, 64–74. <https://doi.org/10.1016/j.ejpb.2018.04.013>
- Tyo, K. M., Vuong, H. R., Malik, D. A., Sims, L. B., Alatassi, H., Duan, J., Watson, W. H., & Steinbach-Rankins, J. M. (2017). Multipurpose tenofovir disoproxil fumarate electrospun fibers for the prevention of HIV-1 and HSV-2 infections in vitro. *International Journal of Pharmaceutics*, 531(1), 118–133. <https://doi.org/10.1016/j.ijpharm.2017.08.061>
- Tyssen, D., Henderson, S. A., Johnson, A., Sterjovski, J., Moore, K., La, J., Zanin, M., Sonza, S., Karellas, P., Giannis, M. P., Krippner, G., Wesselingh, S., McCarthy, T., Gorry, P. R., Ramsland, P. A., Cone, R., Paull, J. R., Lewis, G. R., & Tachedjian, G. (2010). Structure activity relationship of dendrimer microbicides with dual action antiviral activity. *PLoS One*, 5(8), e12309. <https://doi.org/10.1371/journal.pone.0012309>
- Ugaonkar, S. R., Clark, J. T., English, L. B., Johnson, T. J., Buckheit, K. W., Bahde, R. J., Appella, D. H., Buckheit, R. W., Jr., & Kiser, P. F. (2015). An intravaginal ring for the simultaneous delivery of an HIV-1 maturation inhibitor and reverse-transcriptase inhibitor for prophylaxis of HIV transmission. *Journal of Pharmaceutical Sciences*, 104(10), 3426–3439. <https://doi.org/10.1002/jps.24551>
- UNAIDS. (2020a). *Global AIDS update—Seizing the moment—Tackling entrenched inequalities to end epidemics*. Available from [https://www.unaids.org/sites/default/files/media\\_asset/2020\\_global-aids-report\\_executive-summary\\_en.pdf](https://www.unaids.org/sites/default/files/media_asset/2020_global-aids-report_executive-summary_en.pdf).
- UNAIDS. (2020b). *UNAIDS data 2020*. Available from [https://www.unaids.org/sites/default/files/media\\_asset/2020\\_aids-data-book\\_en.pdf](https://www.unaids.org/sites/default/files/media_asset/2020_aids-data-book_en.pdf).
- USFDA. (2014). *Guidance for industry. Vaginal microbicides: Development for the prevention of HIV infection*. Available from <https://www.fda.gov/files/drugs/published/Vaginal-Microbicides-Development-for-the-Prevention-of-HIV-Infection-PDF.pdf>.
- Vacas Cordoba, E., Arnaiz, E., Relloso, M., Sanchez-Torres, C., Garcia, F., Perez-Alvarez, L., Gomez, R., de la Mata, F. J., Pion, M., & Munoz-Fernandez, M. A. (2013). Development of sulphated and naphthylsulphonated carbosilane dendrimers as topical microbicides to prevent HIV-1 sexual transmission. *AIDS*, 27(8), 1219–1229. <https://doi.org/10.1097/QAD.0b013e32835f2b7a>
- Vacas-Cordoba, E., Galan, M., de la Mata, F. J., Gomez, R., Pion, M., & Munoz-Fernandez, M. A. (2014). Enhanced activity of carbosilane dendrimers against HIV when combined with reverse transcriptase inhibitor drugs: Searching for more potent microbicides. *International Journal of Nanomedicine*, 9, 3591–3600. <https://doi.org/10.2147/IJN.S62673>
- Vacas-Cordoba, E., Maly, M., De la Mata, F. J., Gomez, R., Pion, M., & Munoz-Fernandez, M. A. (2016). Antiviral mechanism of polyanionic carbosilane dendrimers against HIV-1. *International Journal of Nanomedicine*, 11, 1281–1294. <https://doi.org/10.2147/IJN.S96352>



- Varga, N., Sutkeviciute, I., Ribeiro-Viana, R., Berzi, A., Ramdasi, R., Daggetti, A., Vettoretti, G., Amara, A., Clerici, M., Rojo, J., Fieschi, F., & Bernardi, A. (2014). A multivalent inhibitor of the DC-SIGN dependent uptake of HIV-1 and dengue virus. *Biomaterials*, 35(13), 4175–4184. <https://doi.org/10.1016/j.biomaterials.2014.01.014>
- Veazey, R. S., & Lackner, A. A. (2017). Nonhuman primate models and understanding the pathogenesis of HIV infection and AIDS. *ILAR Journal*, 58(2), 160–171. <https://doi.org/10.1093/ilar/ilx032>
- Veselinovic, M., Neff, C. P., Mulder, L. R., & Akkina, R. (2012). Topical gel formulation of broadly neutralizing anti-HIV-1 monoclonal antibody VRC01 confers protection against HIV-1 vaginal challenge in a humanized mouse model. *Virology*, 432(2), 505–510. <https://doi.org/10.1016/j.virol.2012.06.025>
- Victor Garcia, J. (2016). Humanized mice for HIV and AIDS research. *Current Opinion in Virology*, 19, 56–64. <https://doi.org/10.1016/j.coviro.2016.06.010>
- Vincent, K. L., Moss, J. A., Marzinke, M. A., Hendrix, C. W., Anton, P. A., Gunawardana, M., Dawson, L. N., Olive, T. J., Pyles, R. B., & Baum, M. M. (2018). Phase I trial of pod-intravaginal rings delivering antiretroviral agents for HIV-1 prevention: Rectal drug exposure from vaginal dosing with tenofovir disoproxil fumarate, emtricitabine, and maraviroc. *PLoS One*, 13(8), e0201952. <https://doi.org/10.1371/journal.pone.0201952>
- Waldbaum, A. S., Schwabke, J. R., Paull, J. R. A., Price, C. F., Edmondson, S. R., Castellarnau, A., McCloud, P., & Kinghorn, G. R. (2020). A phase 2, double-blind, multicenter, randomized, placebo-controlled, doseranging study of the efficacy and safety of Astodrimmer gel for the treatment of bacterial vaginosis. *PLoS One*, 15(5), e0232394. <https://doi.org/10.1371/journal.pone.0232394>
- Wang, X., Zhao, J., Wei, S., Wang, C., Zhang, L., Wang, M., Liu, Y., Fei, C., Xue, F., & Zhang, K. (2019). Determination of ethanamizuril, a novel triazine coccidiostat, in rat plasma by ultra-performance liquid chromatography system-tandem mass spectrometry and its application in a toxicological study. *Biomedical Chromatography*, 33(11), e4652. <https://doi.org/10.1002/bmc.4652>
- Weichseldorfer, M., Heredia, A., Reitz, M., Bryant, J. L., & Latinovic, O. S. (2020). Use of humanized mouse models for studying HIV-1 infection, pathogenesis and persistence. *Journal of AIDS and HIV Treatment*, 2(1), 23–29.
- WHO. (2009). *Regulatory issues in microbicide development*. Available from [https://apps.who.int/iris/bitstream/handle/10665/44324/9789241599436\\_eng.pdf;jsessionid=8233F59F6003F6F8F64FF42BCD1E2416?sequence=1](https://apps.who.int/iris/bitstream/handle/10665/44324/9789241599436_eng.pdf;jsessionid=8233F59F6003F6F8F64FF42BCD1E2416?sequence=1).
- WHO. (2020). *Disruption in HIV, hepatitis and STI services due to COVID-19. Global HIV, hepatitis and STI programmes World Health Organization*. Available from [https://www.who.int/docs/default-source/hiv-hq/disruption-hiv-hepatitis-sti-services-due-to-covid19.pdf?sfvrsn=5f78b742\\_6](https://www.who.int/docs/default-source/hiv-hq/disruption-hiv-hepatitis-sti-services-due-to-covid19.pdf?sfvrsn=5f78b742_6).
- Witika, B. A., Makoni, P. A., Mweetwa, L. L., Ntemi, P. V., Chikukwa, M. T. R., Matafwali, S. K., Mwila, C., Mudenda, S., Katandula, J., & Walker, R. B. (2020). Nano-biomimetic drug delivery vehicles: Potential approaches for COVID-19 treatment. *Molecules*, 25(24), 5952. <https://doi.org/10.3390/molecules25245952>
- Witvrouw, M., Fikkert, V., Pluymers, W., Matthews, B., Mardel, K., Schols, D., Raff, J., Debyser, Z., De Clercq, E., Holan, G., & Pannecouque, C. (2000). Polyanionic (i.e., polysulfonate) dendrimers can inhibit the replication of human immunodeficiency virus by interfering with both virus adsorption and later steps (reverse transcriptase/integrase) in the virus replicative cycle. *Molecular Pharmacology*, 58(5), 1100–1108.
- Wooley, K. L., Hawker, C. J., & Frechet, J. M. J. (1991). Hyperbranched macromolecules via a novel double-stage convergent growth approach. *Journal of the American Chemical Society*, 113(11), 4252–4261. <https://doi.org/10.1021/ja00011a031>
- Xu, X., Fan, R., Ruan, Y., Xu, M., He, J., Cao, M., Li, X., Zhou, W., & Liu, Y. (2021). Inhibition of PLCbeta1 signaling pathway regulates methamphetamine self-administration and neurotoxicity in rats. *Food and Chemical Toxicology*, 149, 111970. <https://doi.org/10.1016/j.fct.2021.111970>
- Yoshida, A., Tanaka, R., Murakami, T., Takahashi, Y., Koyanagi, Y., Nakamura, M., Ito, M., Yamamoto, N., & Tanaka, Y. (2003). Induction of protective immune responses against R5 human immunodeficiency virus type 1 (HIV-1) infection in hu-PBL-SCID mice by intrasplenic immunization with HIV-1-pulsed dendritic cells: Possible involvement of a novel factor of human CD4(+) T-cell origin. *Journal of Virology*, 77(16), 8719–8728. <https://doi.org/10.1128/jvi.77.16.8719-8728.2003>
- Zhang, X., Zhou, J., Gu, Z., Zhang, H., Gong, Q., & Luo, K. (2020). Advances in nanomedicines for diagnosis of central nervous system disorders. *Biomaterials*, 269, 120492. <https://doi.org/10.1016/j.biomaterials.2020.120492>
- Zhao, C., Gunawardana, M., Villinger, F., Baum, M. M., Remedios-Chan, M., Moench, T. R., Zeitlin, L., Whaley, K. J., Bohorov, O., Smith, T. J., Anderson, D. J., & Moss, J. A. (2017). Pharmacokinetics and preliminary safety of pod-intravaginal rings delivering the monoclonal antibody VRC01-N for HIV prophylaxis in a macaque model. *Antimicrobial Agents and Chemotherapy*, 61(7), e02465-16. <https://doi.org/10.1128/AAC.02465-16>
- Zirafi, O., Kim, K. A., Roan, N. R., Kluge, S. F., Muller, J. A., Jiang, S., Mayer, B., Greene, W. C., Kirchhoff, F., & Munch, J. (2014). Semen enhances HIV infectivity and impairs the antiviral efficacy of microbicides. *Science Translational Medicine*, 6(262), 262ra157. <https://doi.org/10.1126/scitranslmed.3009634>

**How to cite this article:** Rodríguez-Izquierdo, I., Sepúlveda-Crespo, D., Lasso, J. M., Resino, S., & Muñoz-Fernández, M. Á. (2022). Baseline and time-updated factors in preclinical development of anionic dendrimers as successful anti-HIV-1 vaginal microbicides. *WIREs Nanomedicine and Nanobiotechnology*, 14(3), e1774. <https://doi.org/10.1002/wnan.1774>