Insights into HIV-1 Capsid Inhibitors in early clinical development as antiretroviral agents

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1. Introduction

Almost 30 years after the discovery of the first approved antiretroviral (ARV) agent Zidovidine, more than 25 additional agents in five drug classes have been discovered, revolutionising the management of HIV infection [1]. During this drug development process, researchers have made extraordinary progress understanding the virus's life cycle, interaction with CD4 cells and resistance mechanisms. However, the majority of currently available ARVs suppress viral replication by blocking viral enzymes, and until now early stages of HIV infection, such as capsid disassembly and nuclear import have not been successfully targeted [2]. Targeting novel steps in the HIV lifecycle offers new treatment classes and opportunities to circumvent viral resistance when it occurs.

In recent years, the critical role of HIV-1 capsid protein (CA) in the viral replication cycle has been more clearly understood. CA, as one of the structural proteins, appears to have an important role in multiple processes essential for viral replication from viral uncoating, nuclear import to re-assembly, finely regulating HIV-1 replication, emerging as an attractive target for drug development[3, 4]. A highly potent capsid inhibitor, with long-acting potential, is being developed for human use and has entered pre-clinical and phase I clinical trials. This review aims to summarize recent advances in the discovery of HIV-1 capsid inhibitors as ARVs.

2. Role of capsid protein in the HIV life cycle

HIV-1 is an enveloped RNA virus of the family *Retroviridae*. The HIV-1 viral genome contains *gag, pol* and *env* genes. The *Pol* gene encodes the viral enzymes protease (PR), reverse transcriptase (RT) and integrase (IN). *Env* encodes the proteins forming the viral envelope.

Gag encodes the virion structural proteins, matrix (p17 MA), capsid (p24 CA), nucleocapsid (p7 NC), p6 and spacer peptides Sp1 and Sp2[2]. The mature capsid of HIV is a cone-shaped shell, composed of hexameric and pentameric rings of p24 CA, that carries the viral genome and associated enzymes for delivery into the host. Although exact timing and mechanism of capsid uncoating are not clearly established, after capsid uncoating the viral genome and the replicative enzymes are released [2]. There is experimental evidence suggesting that uncoating occurs right after the entry of the virus and that capsid is not uncoated until it reached to the nuclear core [2-4]. While there is an incomplete understanding of these early steps, reverse transcription and later stages of the viral cycle critically depend on the optimal stability of the capsid[5]. CA appears to facilitate reverse transcription by controlling core stability, preventing premature uncoating, which could subsequently abort reverse transcription. Apart from capsid disassembly, CA plays multiple essential roles in the HIV replication cycle, including nuclear transport of the pre-integration complex, virus production and capsid re-assembly and maturation [2-4]. It may also be important to modulate recognition by the innate and intrinsic immune system[6]. A comprehensive mapping of the *gag* suggests complete functional conservation of *gag* sequences across all subtypes, with the highest level of conservation in CA with minimal genetic variability at the N-terminal domain (NTD) of the CA as a binding pocket [7].

3. Capsid inhibitors

Although traditionally in drug development protein-protein interactions are thought to be complicated and antiviral action was expected to be weak, in the recent years, research in identifying CA targeting inhibitors has gained momentum due to the vast potential of this new target. Over the last 15 years, several approaches and a variety of CA targeting HIV inhibitors

have been described (Table 1). However, the antiviral mechanism of some CA inhibitors has not been conclusively determined. Additionally, some CA inhibitors were shown to have low potency resulting in two major pharmaceutical companies discontinuing antiviral research efforts on CA- inhibitors precluding their further clinical development.

3.1 Pre-clinical development

The first report of a CA-targeting HIV inhibitor emerged in 2003 through a computational search for small molecules that bound to the CA-NTD. CAP-1 appeared to inhibit mature capsid assembly in vitro but did not destabilise the core [8]. A significant discovery transpired demonstrating the crystal structure of the capsid hexamer, outlining the molecular basis of capsid assembly [9]. Soon after, the crystal structure of novel small CA-inhibitor molecules (BI-1 and BI-2) were identified, convincingly binding to the capsid. However, the potency of BI-1 was modest precluding further clinical development [10].

In 2010, PF-3450074 (PF74), binding to a novel pocket in the NTD of the CA demonstrated broad-spectrum inhibition of HIV isolates, with submicromolar potency (EC50 = 8–640 nM)[11]. This compound was shown to primarily interfere with early events by destabilizing the HIV-1 capsid and also late events in the virus lifecycle by disturbing the formation of mature core. PF74's antiviral activity was shown to be influenced by another CA- binding host factor, cyclophilin A. And there is a strong suggestion that a major antiviral mechanism of PF74 is to disturb the binding of host factors to the incoming HIV capsid. This highlights the need for additional studies to further understand the antiviral mechanism of PF74.

In 2017, a new CA-targeting HIV inhibitor (GS-CA1) was described, which has a dual antiviral mechanism with broad-spectrum inhibition across all HIV clades [12]. This CA inhibitor appears to exhibit high antiviral potency in human peripheral blood mononuclear cells (EC50 = 140 pM). In vitro metabolic stability and PK profiles in multiple preclinical species were favourable with low systemic drug clearances and long half-lives (7.2–18.7 hr). These findings, combined with low aqueous solubility, implied a prospect for long-acting potential. In a humanised mouse model, GS-CA1 monotherapy showed high antiviral efficacy, outperforming long-acting rilpivirine. This work also identified five amino acid substitutions (L56I, M66I, Q67H, N74D and A105E) associated with in-vitro resistance to GS-CA1. Further work assessing the prevalence of nucleotide substitutions associated with in vitro resistance to GS-CA1 suggested the absence of these gag mutations in treatment naïve patient samples at baseline and at the time of virological failure in patients receiving a first-line PI-based regimen using ultra-deep sequencing of the CA region [13]. This work highlights that there is no need for pre-treatment genotypic resistance testing before using the CA-inhibitors, particularly GS-CA1.

Most recently, GS-6207, an analogue of GS-CA1, was described. In-vitro pharmacological profile suggested that it has potent and selective antiviral activity in MT-4 cells (EC50 = 0.1 nM, CC50 = 27 μM) and in 23 clinical isolates with a mean EC50 of 0.05 nM (0.02 - 0.16 nM) [14]. These results indicate a potent antiviral activity (picomolar), almost over 10 times more than currently available ARVs. In vitro experiments demonstrated low solubility, low lipophilicity and high metabolic stability in human hepatocytes. In-vivo PK profile was ascertained following a single subcutaneous administration in various animal models suggesting low clearance, moderate volume distribution and long half-life (15 - 38 hr). Animal experiments of extended release formulation suggested that it displays long-acting

pharmacokinetics supporting once every 3 month administration in humans. In-vitro resistance emergence to GS-6207 exhibited a selection of mutations either alone or in combination (L56I, M66I, Q67H, K70N, N74D, N74S and T107N), with Q67H and N74D being the mostly observed mutations [15]. The residues at which the GS-6207-selected mutations occurred in vitro were highly conserved regions of the CA. All GS-6207-selected variants showed reduced susceptibility to GS-6207, but these variants remained fully susceptible to ARVs in other classes including dolutegravir, efavirenz and tenofovir alafenamide. In 6 clinical isolates, GS-6207 selected for the viral breakthrough with N74D in 8% (3/36) of samples at 8-fold 95% effective concentration (EC95), which was lower than emtricitabine (81%) and rilpivirine (33%) and comparable to efavirenz (8%). Capsid deep sequencing showed very low baseline prevalence (<1%) of GS-6207-resistant variants in isolates from treatment-naïve patients and none that confer high-level resistance to GS-6207. This is the first compound that progressed into clinical development.

3.2 Clinical studies

A Phase I randomised control study was conducted to establish safety, tolerability and pharmacokinetics of GS 6207 following a single subcutaneous administration in healthy volunteers [16]. Participants were randomised into four arms compromising different doses of GS 6207 (30, 100, 300, 450mg) or placebo in an 8:2 ratio. Ten participants were enrolled in each arm with comparable demographics. Across all groups, the majority of participants were middle-aged, white men with a median BMI of 26 except in the Cohort 100mg in which 60% of the participants were female. Following a single subcutaneous administration, all doses maintained systemic exposure for over 24 weeks, and doses >=100mg had plasma concentrations above the protein adjusted 95% effective concentrations (paEC95) of

3.87ng/mL at 12 weeks suggesting that 12 weekly administration of this suspension could be an option. The median half-life was 35.5, 30.2, 43.1, 39.9 days in ascending doses. In the blinded safety analysis, no deaths or serious adverse events were reported. All reported adverse events were mild to moderate. The most common adverse event was mild injection site reactions.

Results from an ongoing Phase Ib randomised double-blind placebo-controlled dose-finding study (NCT03739866) was presented at the IAS Conference in July 2019[17]. Twenty-four individuals living with HIV who were treatment-naïve or who had not used ARV therapy for <12 weeks were randomised to receive a single subcutaneous dose of GS 6207 (20, 50, 150, 450, or 750 mg) or placebo in a 3:1 ratio (n=8 per group) through to day10 at which point all participants were started on bictegravir/emtricitabine/tenofovir alafenamide (B/F/TAF). The primary endpoint was the maximum $\log^{10}$ reduction in HIV-1 RNA over 10 days after the dose. Preliminary data for the 50, 150, and 450 mg cohorts including 6 active and 2 placebo participants showed comparable demographics and baseline characteristics across all arms. The median age was 34 years (19-59), and 58% were white, 17 of 24 (71%) were naive to ARVs, and the mean baseline HIV-1 RNA was 4.48 $\log^{10}$ copies/mL. At day 10, mean maximum HIV-1 RNA reduction was 1.8, 1,8 and 2.2 $\log^{10}$ copies/mL in the 50, 150, and 450 mg cohorts, respectively. In the 50 and 450mg cohort, average GS-6207 concentrations on day 10 were 1.1- to 9.9-fold above the paEC95 against wild-type HIV-1. Based on this preliminary data, no serious adverse events or events leading to discontinuation occurred. No Grade 3 or 4 laboratory abnormalities were reported.

4. Expert opinion:

In an era where virological suppression can be achieved in the majority of patients receiving currently available ARVs, novel treatments are expected to deliver enhanced tolerability, safety and convenient formulations. Given the changing emphasis in the management of HIV towards improving the quality of life, potent and long-acting formulations of ARVs with a high barrier to resistance are of interest. Previous studies discussed in this review have demonstrated the importance of capsid protein as a novel pharmacological target. Multiple essential roles of the capsid protein in the viral replication cycle signify how a CA targeting inhibitor can disturb the core stability, thereby interfering with early as well as late events of HIV infection. In addition, higher sequence conservation suggests that CA-inhibitors will require mutations that disturb the fitness of the virus, thus providing a higher barrier to resistance, making CA highly attractive therapeutic target. While many CA inhibiting small molecules and compounds have been discovered, only a few have been confirmed as a potent antiviral agent. This signifies the challenges of protein-protein interactions as well as the novelty of the CA target. However, further work is essential to understand the role of CA in post-nuclear entry steps of HIV-1 infection and delineate the antiviral mechanism of various CA inhibitors including proposed binding mode, structural units, host factors and antiviral affinity.

There is only one compound that entered clinical development. This promising CA inhibitor, GS-6207, with dual antiviral activity destabilises the HIV-1 capsid and disturbs the formation of the mature core. It has shown potent antiviral action in in vitro studies. More recently, a subcutaneous formulation of GS 6207 has entered early clinical trials and demonstrated promising antiviral activity as well as safety through day 10 in individuals living with HIV. This Phase Ib study with encouraging preliminary data in people living with HIV is still ongoing. The preliminary results of the Phase 1b study and PK analysis of GS-6207 suggest prolonged plasma exposure after cessation of dosing with measurable concentrations up to 24

weeks in all administered doses. This long plasma tail may be a concern for increased risk of resistance if the planned injection is not repeated before the plasma concentrations drop below the paEC95. Although GS-6207 will likely progress to the next stage of clinical development, further characterisation of resistance mechanisms of this new ARV target is essential.

Antiviral agents are not without side effects, and there are long-term safety concerns for almost all antiretrovirals although to a lesser extent with the newer ARV options. There is a shift towards limiting drug exposure by providing different formulations and combinations. These include class sparing regimens as well as long-acting formulations. From this perspective, CA inhibitors with potent antiviral activity used as class sparing regimens will have a vital role in the management of HIV to circumvent side effects, but most importantly provide a prospect for individuals for whom few treatment options remain. However, future phase 2 and 3 clinical trials are needed to ascertain the long-term efficacy, safety and tolerability of this CA inhibitor in treatment naïve and treatment-experienced patients.

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This study is the first clinical trial (Phase 1b) studying the GS-6207, long-acting HIV capsid inhibitor, in people living with HIV.

Table 1: Capsid inhibitors in pre-clinical and clinical development

CA-targeting inhibitor	Binding site	Antiviral mechanism
CAP-1	CA-NTD	Interferes with late events by inhibiting the ability of CA to self-assemble
PF74	CA-NTD	interferes with early and late events in the virus lifecycle by destabilizing the HIV-1 capsid
BI compounds **BI-1** **BI-2**	CA-NTD	interferes with early and late events in the virus lifecycle by destabilizing the HIV-1 capsid
Peptide inhibitors (CAI) **NYAD-1**	CA-CTD	inhibits the assembly of immature and mature-like virus particles in vitro
C-A1	point mutation at CA 105	inhibits viral gene expression and it also inhibits HIV integration
CAK026, I-XW-053	the actual CA binding site for these inhibitors has not been determined, and it is possible that they target additional viral proteins	inhibits HIV reverse transcription in target cells
C1	CA-NTD	inhibits HIV-1 replication by acting at a late step to disrupt proper assembly of the mature viral capsid, without altering Gag processing Though the antiviral mechanism is not fully understood

Ebselen		inhibits HIV reverse transcription in target cells; it also results in impaired uncoating based on a cell fractionation assay.
GS-CA1 ***GS-6207 is the only compound that reached clinical development**	NTD-CTD	a dual antiviral mechanism like PF74, but with much greater potency