HIV-1 Envelope Spike MPER: From a Vaccine Target to a New Druggable Pocket for Novel and Effective Fusion Inhibitors

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Dedicated to the memory of Dr. Leopoldo Flores-Romo

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Abstract

Here we highlight a sound and unique work reported by Chen as a vaccine target, emerges as a novel druggable target for the and co-workers entitled "HIV-1 fusion inhibitors targeting the discovery of HIV-1 fusion inhibitors. The compounds (exempli- membrane-proximal external region of Env spikes" (Xiao et al., fied by dequalinium and dequalinium-inspired analogues) *Nat. Chem. Biol.* **2020**, *16*, 529). In this article, the authors prevent the conformational changes of Env from the prefusion identify, by means of a clever antibody-guided strategy, several species to the intermediate states required for membrane small molecules as fusion inhibitors of HIV-1 replication acting fusion. This work not only paves the way to novel, specific and at the membrane proximal external region (MPER) of the HIV-1 useful anti-HIV-1 inhibitors, but also discloses new therapeutic envelope (Env) spike. MPER, which was previously recognized strategies against other infectious diseases.

Keywords: HIV-1 fusion inhibitors; HIV-1 envelope spike; antibody-guided strategy; drug discovery; small molecules

Treatment of Human Immunodeficiency Virus type 1 (HIV-1) infection by a "cocktail" of antiretrovirals (currently more than 25), known as "highly active antiretroviral therapy", has driven the disease to chronic and manageable. These drug cocktails include inhibitors against targets involved in key steps of the viral life cycle: protease inhibitors, reverse transcriptase (nucleo (s/t)ide or non-nucleoside) inhibitors, integrase inhibitors, and/ or entry and fusion inhibitors.[1] However, antiretroviral therapy still has serious limitations, such as drug resistance, unwanted severe side effects and toxicity, which compromise long-term treatment and incite lack of adherence by the patients, making it necessary to identify additional targets for the design and development of new classes of HIV-1 inhibitors.

Virus entry inhibitors, which impede either the attachment to cellular receptors and co-receptors or the conformational changes required for the fusion of viral and cellular membranes (fusion inhibitors),[2,3] inhibit viral infection and may prevent transmission as microbicides.[4] Maraviroc, an entry inhibitor antagonist of CCR5, and enfuvirtide or T20, a gp41-derived peptide, are FDA-approved entry and fusion inhibitors, respec- tively. T20 and other gp41-peptide mimics suffer from

several drawbacks, such as short plasma half-life, poor bioavailability or rapid emergence of viral drug-resistance (by mutations in gp41), which limit their use in favour of co-receptor inhibitors. To bypass the limitations of peptides, the development of bioavailable small molecules as fusion inhibitors is a promising strategy, and a few examples have been described but none of them have been approved yet.[4,5,6]

The envelope (Env) glycoproteins gp120 and gp41 (i. e., the receptor-binding surface and fusion-promoting membrane subunits, respectively) mediate the HIV-1 entry. The Env spike protein is synthesized as a precursor (gp160), rendering by subsequent trimerization and cleavage gp120 and gp41. The entry is initiated by attachment of gp120 to the cell CD4 receptor and chemokine co-receptors (CCR5 or CXCR4), thus inducing conformational changes in gp41 that activate the fusion of viral and cell membranes.[7,8] In this process, the N terminus of the fusion peptide inserts into the cell membrane in an extended conformation (pre-hairpin intermediate),[9] that contains the transmembrane segment and the fusion peptide in the viral and cell membranes. Refolding of gp41 into a hairpin conformation creates a six-helix bundle (post-fusion state) and leads to membrane fusion. Enfuvirtide and also some broadly neutralizing antibodies (bnAbs) target the pre-hairpin intermediate,[10] suggesting that blockage of gp41 refolding may be a good approach to find novel HIV-1 fusion inhibitors. The membrane proximal external region (MPER) of the HIV-1 Env spike has been widely used as vaccine target. MPER is a hydrophobic, highly conserved region in gp41 required for infectivity and is recognized by several bnAbs, such as 2F5, which binds to the pre-hairpin intermediate state of gp41 and block the viral fusion.[10,11] However, the specific role of these bnAbs in the mechanism of viral fusion remains veiled.

All the above provided the backdrop to ignite the spark for Chen and co-workers to investigate whether it would be possible to discover small molecules able to bind to MPER, mimicking the blocking effect of bnAbs and impede the HIV-1 fusion process.[12]

They cleverly used a neutralizing monoclonal antibody (2F5) targeting HIV-1 gp41 as a guided-search methodology and, in conjunction with high-throughput screening (HTS) involving competition with 2F5, identified several small molecules able to bind to the MPER hydrophobic pocket, interfering the CD4- induced conformational changes needed for membrane fusion. This strategy was proposed on the premises that 1) the formation of the antibody-antigen complex involves molecular interactions similar to those formed by drugs binding to their protein targets, 2) the binding affinity of the antibody-antigen complex, though involving a large interface, is mainly due to a specific set of residues (hot spots), suggesting that a small molecule may mimic the interaction of an antibody and compete for antigen binding, and 3) antibodies target inhibitory or neutralizing epitopes on a protein, which may be different from drug-binding sites. This strategy could thus be useful to expand the range of druggable sites not attainable by conven- tional

methods for disease-related proteins.

As a proof-of-concept of this antibody-guided screening approach, the authors used a gp-41-inter construct captured by covalently-linked enfuvirtide (as the pre-hairpin intermediate conformation of gp41).[10] They developed a HTS fluorescence polarization assay that detected the binding of 2F5 and any small-molecule competitor for binding the antibody epitope (gp41-inter). Chen and colleagues screened around 162,100 compounds and identified 146 “hits” in the first round, which were further refined by surface plasmon resonance (SPR) to select compounds that bind to gp41-inter but not to 2F5, and those that inhibited cell-cell fusion mediated by HIV-1 Env but not the simian immunodeficiency virus Env. They identified dequalinium (**1**, Figure 1) as a hit compound (11 μM affinity for gp-41 inter) that showed no binding for 2F5, inhibited cell-cell fusion mediated by HIV-1 Env (IC_{50} = 13.8 μM) but not SIV Env, while lacking cytotoxicity up to 50 μM.

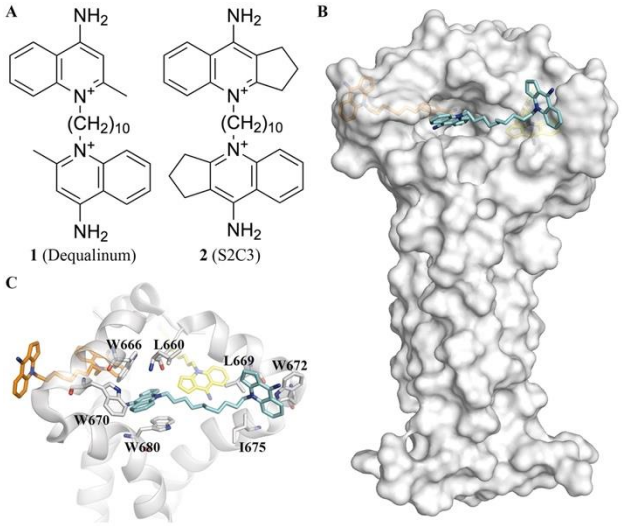

Figure 1. A) Structures of dequalinium (**1**) and S2C3 (**2**). B) Representation of the NMR structure of MPER (shown as white surface; PDB entry 6V4T)[12] bound to S2C3 (shown as sticks with carbon atoms in cyan; the other two S2C3 compounds bound to the trimeric structure are shown as yellow and orange sticks). C) Details of hydrophobic residues proximal to S2C3 in the MPER binding pocket.

To further optimize **1**, structure-activity relationship studies were used to disclose compound S2C3 (**2**, Figure 1), a more potent analogue (2.0 μM affinity for gp-41 inter). Inhibition of viral infectivity and SPR competition studies with 2F5 and 4E10 suggested that S2C3 inhibits the membrane fusion by interfer- ing the CD4-induced conformational changes, and confirmed that MPER is the target in HIV-1 Env protein. Finally, NMR studies with a MPER construct (gp41 fragment containing the MPER and the transmembrane domain (TMD) reconstituted in bicelles)[13] confirmed that **2** binds to a pocket formed by highly conserved hydrophobic residues in MPER (Figure 1). The NMR- based structure of the S2C3-MPER complex was validated by site-directed mutagenesis.[13]

In summary, Chen and co-workers reported the proof-of- concept of a clever antibody-guided strategy to find small molecule HIV-1 fusion inhibitors interacting with a widely used vaccine target (the MPER region) of the HIV-1 Env spike. The results of their work identified dequalinium and a more potent analogue

(compound **2**) that effectively inhibit HIV-1 infection, and demonstrated their binding to a non-conventional site, a hydrophobic pocket formed exclusively by highly conserved residues of the MPER, thus preventing the conformational changes required for membrane fusion. These results are sound and open a new avenue to find new fusion inhibitors of viral replication. Importantly they unveil a new antibody-based druggable target different from those used so far based on enzyme and protein allosteric and catalytic sites.

Acknowledgements

F.J.L. and M.-J.C. thank the Spanish MINECO/FEDER (SAF2015- 64629-C2-1-R, SAF2017-88107, MDM2017-0767, AEI-FEDER UE), MICIU (PID2019-104070RB-C21) and Generalitat de Catalunya (2017SGR1746) for financial support.

Conflict of Interest

The authors declare no conflict of interest

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