CROI meeting 2021

Oral abstract presentation:

TRAPPING THE HIV-1 V3 LOOP IN A HELICAL CONFORMATION ENABLES BROAD NEUTRALIZATION

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Background: HIV-1 entry depends on the interaction of the envelope (Env) protein's variable loop 3 (V3) with a co-receptor. While this indispensable function renders the V3 a key target for inhibition, the vigorous antibody response elicited in natural infection is largely non-neutralizing owing to conformational masking of the V3 crown on the prefusion-closed Env trimer. Only a fraction of individuals develops broadly neutralizing antibodies (bnAbs) targeting the V3 base on a closed Env. In the CD4-bound conformation, the V3 becomes fully accessible and conformationally dynamic. Functional relevance of these intermediate V3 conformations and their potential for broad neutralization still remain to be resolved.

Methods: Here we applied the Designed Ankyrin Repeat Protein (DARPIn) technology to select DARPins targeting HIV-1 Env by Ribosome Display. Hits were screened for V3 binders with broad neutralizing capacity. Neutralization breadth was assessed on a 42-multiclade Tier-2 virus panel in the TZM-bl assay. DARPin epitopes were characterized by binding to Env derivatives, deep mutational Env scanning, X-ray crystallography, cryo-EM and molecular dynamics.

Results: We identified 8 distinct V3 specific DARPins with exceptional neutralization breadth of up to 93%. Unlike V3-glycan bnAbs, these broadly neutralizing DARPins (bnDs) bound V3 solely on open but not closed Env. X-ray and cryo-EM structure analyses of bnD.8 and bnD.9 revealed binding to a 3-turn amphipathic alpha-helix in the C-Strand of V3 spanning residues 314 to 324. We termed this novel conformation αV3C. Remarkably, the αV3C helix was trapped by two unrelated bnDs and observed both in complex with V3 peptide and open, CD4-triggered Env trimer. Molecular dynamics simulations indicated that the αV3C helix remains stable in the absence of the bnDs, emphasizing a functional relevance. Comprehensive Env mutation scanning underlined functional importance. Escape mutations accumulated on the contact face of the helix, but no enrichment of putative helix disturbing mutations occurred.

Conclusion: The discovery of post-CD4 engagement acting V3 inhibitors with extraordinary breadth is remarkable. The helical V3 conformation they define sheds light on V3 conformational dynamics after CD4 engagement and reveals a new site of vulnerability on HIV-1 Env. Our findings emphasize the importance of V3 and the open Env conformation as a target for inhibitors and mark the newly defined αV3C helix as a blueprint for epitope-based vaccine design.

CROI meeting 2020

Poster:

CANDIDATE IMMUNOGENS DIFFERENTIALLY ENGAGE HIV BROADLY NEUTRALIZING PLASMA ANTIBODIES

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Background: Deciphering factors that drive broadly neutralizing antibodies (bnAbs) induction remains critical to guide HIV-1 vaccine development. Including 4,484 patients with detailed demographic data alongside plasma samples, the Swiss 4.5K Screen had unique means to distinguish positive, independent drivers of breadth (viral load, infection length, viral diversity, black ethnicity) (Rusert et al. 2016). Here we report on the XbnAb cohort, a sub-cohort of the Swiss 4.5K Screen that includes bnAb inducers and matched non-neutralizing (nnAb) controls. Using the controlled setting of the XbnAb cohort we compared the capacity of candidate immunogens in binding naturally occurring Abs and assess their efficacy in predicting bnAb activity.

Methods: We defined within the Swiss 4.5K Screen the XbnAb cohort, which comprises all identified bnAb inducers (N=304) and matched nnAb controls (N=304; matched for HIV-1 subtype, length of infection, host demographics). Patient plasmas were assessed for binding antibodies (IgG1,2,3) against 47 HIV-1 envelope (Env) antigens (including 29 stabilized soluble Env trimer variants and candidate immunogens provided by lead investigators in the field) and 2 Gag proteins using an in-house Luminex bead assay. EC50 plasma Ab binding activity was established for each antigen and the prediction potential of antigens to distinguish bnAb activity assessed by univariate conditional logistic regressions.

Results: Confirming work from the Swiss 4.5K Screen (Kadelka et al. 2018) we found that IgG1 Env trimer reactivity is generally higher among bnAb inducers. However, levels of significance varied considerably (p=10-3to p=10-16), highlighting substantial differences among candidate immunogens in engaging natural occurring bnAbs. Comparison of wild type and CD4bs-knockout Envs allowed exploring the impact of CD4bs bnAb activity. Of note, IgG1 reactivity of trimeric CD4bs immunogens were good predictors of neutralization breadth, while the monomeric CD4bs tailored immunogens EOD-GT8 and RSC3 did not differentiate bnAb activity.

Conclusion: Focusing on closely matched bnAb and nnAb inducers, the XbnAb cohort captures the essence of the Swiss 4.5K Screen, providing means to derive population relevant information without the need to screen thousands of individuals. Highlighting the unique capacity of the XbnAb cohort we demonstrate a differential capacity of candidate antigens in engaging natural occurring Abs that needs to be considered when selecting immunogens for further development.

Keystone HIV-1 Vaccines Meeting 2019, Whistler, Canada

Poster:

TARGETING DISTINCT CONFORMATIONS OF THE HIV-1 V3 LOOP CROWN CONFERS EXCEPTIONAL NEUTRALIZATION BREADTH

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The HIV-1 V3 loop combines two features rendering it in principle an ideal target for neutralization. V3 is essential for entry and the immunodominant V3 crown gives rise to a cross-reactive antibody (Ab) response in essentially all infected individuals. Yet, with few exceptions, V3-crown Abs lack neutralizing activity as they cannot bypass the effective masking of V3 on the native envelope trimer. Potent neutralization via V3 has only been reported for rare broadly neutralizing Abs (bnAbs) targeting the V3 base. Using the Designed Ankyrin Repeat Protein (DARPin) technology we provide proof that also the masked V3 crown is a target for broad neutralization. Three V3-crown specific, broadly neutralizing DARPins (bnDs) with up to 90% breadth on a multi-clade Tier-2 virus panel (N= 40) were identified. The bnDs access V3 post-CD4 engagement and circumvent V3 shielding. Crystal structures of V3 peptide:bnD complexes revealed two distinct V3 crown conformations targeted by the bnDs. To explore if differential ability to recognize V3 conformations is linked with neutralization capacity of V3 crown Abs, we investigated plasma Ab reactivity with structural V3 crown mimetic peptides in a cohort of chronically HIV-1 infected individuals (N=4281) with established neutralization breadth. Ab binding to individual V3 mimetics varied amongst patients highlighting a differential ability of V3-crown reactive Abs to recognize alternative V3 conformations. V3-crown reactivity was overall positively associated with neutralization breadth with one V3 mimetic showing a stronger association than the others raising the possibility that conformation specific V3-crown bnAbs may exist and need to be actively searched for.

Poster:

RECOGNITION OF OPEN HIV-1 ENVELOPE STATES CREATES OPPORTUNITY FOR BROAD NEUTRALIZATION

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Our knowledge on relevant neutralization sensitive regions and states of the HIV-1 envelope (Env) trimer are largely defined by broadly neutralizing antibodies (bnAbs). bnAbs identified to date essentially cover the entire surface of the closed Env trimer. The preference of many bnAbs in binding to the closed trimer and the high correlation between closed Env binding and neutralization activity have marked the closed Env state as the only relevant target for broad neutralization.

Here we report on two classes of V3 specific inhibitors we developed using the Designed Ankyrin Repeat Protein (DARPin) technology that challenge this view. The broadly neutralizing DARPins (bnDs) of both classes, termed V3-crown and V3-CD4i, show exceptional breadth (up to 100%) on a tier-2 multi-clade virus panel (N=42). Env mutational scanning revealed that the V3-crown bnDs are affected by mutations within the V3 crown, whereas the V3-CD4i bnD binding site includes the V3 stem. This differential specificity results in distinct functional properties in particular in the dependence on Env opening. Both classes require CD4-binding but differ in the extent of Env conformational changes, with V3-CD4i bnDs requiring more extensive transitions and full displacement of the V1V2 loops. This is underlined by a stronger increase in sensitivity of open Env mutants to the V3-CD4i bnDs compared to V3-crown bnDs. Despite these differences both classes of bnDs achieve high breadth and similar potency. Interestingly, mutations facilitating escape to the V3-CD4i but not V3-crown bnDs also cluster in distal HR1 and C1 regions, suggesting escape strategies that either lead to a closing of the trimer or influence conformational shifts.

The novel broadly neutralizing inhibitors we describe here open a new perspective on the in vivo targetable Env conformation. Broad inhibition paired with the dependence on open Env structures underlines that targeting post CD4 attachment is possible and can be highly effective.

Poster:

COMPARATIVE ANTIGENICITY EVALUATION REVEALS DIFFERENTIAL PROPERTIES OF CD4BS SCAFFOLDS IN PRESENTING NEUTRALIZATION RELEVANT DOMAINS

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Despite enormous advances in HIV-1 vaccine research, the ultimate goal, the development of immunogens that elicit broadly neutralizing antibodies (bnAbs), has not been achieved.
The interaction between the viral envelope (Env) protein and the CD4 receptor is a prime target for inhibition for both receptor and Env directed inhibitors and neutralizing antibodies. bnAbs targeting the CD4 binding site (CD4bs) are amongst the broadest bnAbs identified. Elicitation of CD4bs directed bnAb by vaccines is thus intensely researched and a range of immunogens tailored to evoke CD4bs responses are in development. Here we explored the structural and antigenic characteristics of two recently developed CD4bs scaffolds (HKM4 and eOD-GT8) utilizing the designed ankyrin repeat protein (DARPin) technology. DARPins are small (10-18kDa), highly stable proteins designed to form a binding groove that allows interaction with their cognate target in a highly conformation-specific manner. To compare the capacity of the two candidate CD4bs scaffold immunogens in presenting the CD4bs in a relevant conformation, we selected HKM4 and eOD-GT8 specific DARPins from synthetic DARPin libraries (diversity up to 10^{12}) by ribosome display. The selections revealed a qualitative difference between the two immunogens, with HKM4 yielding higher numbers of Env binders than eOD-GT8 (182 and 15 of specific binders of in total 760 clones screened, respectively). The selected DARPins bound SOSIP.664 Envs preferentially in the CD4-triggered open state. Importantly, several of the selected CD4bs reactive DARPins show high neutralization capacity with the top neutralizer from the HKM4 selection reaching 100% in a 15 multi-clade virus panel. Collectively, we revealed differential antigenic capacities of the two candidate CD4bs scaffolds in presenting neutralization relevant domains highlighting the utility of the DARPin technology in studying properties of immunogens to guide vaccine design.

Keystone HIV-1 Vaccines Meeting 2018, Banff, Canada

Poster:

A BROAD PANEL OF HIV-1 ENVELOPE TARGETING DARPINS AS A TOOL BOX FOR VACCINE RESEARCH

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Broadly neutralizing antibodies (bnAbs) are considered as blueprints for vaccine and therapeutics design. As auxiliary characterization tools we recently generated broad and potent designed ankyrin repeat proteins (DARPins) capable of targeting the membrane external proximal region (MPER) within gp41 and the variable loop 3 (V3) within gp120. The ~10-18kD sized DARPins binding to antigens of interest can be selected from high diversity (~10^{12}) libraries by ribosome display. Differential recognition of the Env epitopes compared to the related bnAbs and a highly structure dependent binding mode make the Env DARPins interesting tools for immunogen characterization. In the current study we aimed towards expanding the DARPin tool box by targeting additional Env domains. By selecting against a range of stabilized, soluble Env trimers and Env derivatives including scaffold and chimeric envelope constructs presenting diverse V1V2 loops, we sought to yield DARPins targeting V1V2, quaternary and/or novel epitopes. In total six different trimer-directed and eight V1V2 focused DARPin selections were conducted. A total of 2660 clones were analyzed for specific Env binding and neutralization potential using a 5-virus panel. This extensive screening allowed for selection of a diverse panel of 24 Env DARPins. Epitope mapping revealed that those novel inhibitors target the V3, V2 and at least two other yet to be delineated epitopes in other domains. Neutralization analysis on a 42-virus panel showed extraordinary breadth of up to 100%, indicating highly conserved epitopes or unique modes of action. Together with previously selected MPER and V3 DARPins, this panel is a valuable tool box for the characterization of neutralization sensitive epitopes and may also aid in unraveling novel sites of vulnerability.

University of Zurich[UZH]

EHVA EUROPEAN ALLIANCE HIV VACCINE

HIV R4P Madrid, 2018

Poster:

V3 REACTIVE, BROADLY NEUTRALIZING DARPINS REVEAL EFFICIENTLY TARGETABLE V3 EXPOSURE DURING ENTRY AND PROVIDE NOVEL INSIGHT ON NATIVE HIV-1 ENVELOPE STABILITY AND PLIABILITY

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Stable, soluble Envelope (Env) trimers are considered lead candidates to induce broadly neutralizing antibody (bnAb) responses and immense efforts are put towards identifying promising Env immunogens with high structural definition. Here we describe broadly neutralizing V3-CD4i specific inhibitors that were generated using the Designed Ankyrin Repeat Protein (DARPin) technology. These broadly neutralizing DARPins (BNDs) recognize a distinct V3 conformation that renders them both interesting broad inhibitors that complete the increasing armament of broadly neutralizing agents and novel tools to select Env immunogens with specific conformational traits.

Fourteen HIV Env specific DARPin sub-libraries were generated by Ribosome Display using diverse Env mutants and derivates. 2660 individual clones were screened for binding to Env antigens in ELISA and by neutralization against 5 viruses in the TZM-bl assay. Candidate BNDs were assessed for breadth and potency on a 42-multi-clade virus panel and epitope specificity delineated by ELISA and escape mapping using mutant Env virus libraries (Dingens et al., 2017).

We identified three genetically distinct BNDs with high neutralization breadth of up to 88%. Unlike bnAbs, these BNDs target preferentially the open, CD4 induced (CD4i) state of Env as documented by elevated binding to open and CD4 bound Envs and increased sensitivity of neutralization of Env carrying trimer opening mutations. Mapping of escape mutations revealed that all three BNDs bind the V3 loop. Intriguingly, the BNDs proved to be sensitive to mutations at the trimer interface, in particular in HR1 and C1, indicating that alteration of Env stability and subunit association affect their activity.

The identified BNDs are unique, broadly neutralizing agents as they like non- and weakly neutralizing V3 loop Abs bind to V3 in the CD4i stage. Yet, they excel the best V3-glycan bnAbs in breadth highlighting that targeting of the CD4i stage of V3 can be highly efficient. The identified V3-CD4i BNDs have implication for immunogen design and can be directly employed to select and characterize appropriate Env conformation. The escape pattern of the V3-CD4i BNDs can be instructive in revealing key positions for Env pliability and native (closed) conformation to identify novel trimer stabilizing mutations.

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EHVA EUROPEAN ALLIANCE HIV VACCINE

9th IAS Conference on HIV Science, Paris, 2017

Poster:

NOVEL SELECTION APPROACHES TO IDENTIFY BROADLY NEUTRALIZING DARPINS TARGETING THE HIV-1 ENVELOPE PROTEIN

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Broadly neutralizing antibodies (bnAbs) only evolve in a fraction of HIV-1 infected individuals. They have a unique potential to suppress viremia and inhibiting HIV-1 infection and are hence considered as blueprints for vaccine and therapeutics design. bnAbs recognize the HIV-1 Envelope (Env) proteins gp120 or gp41 within the context of the HIV-1 Env trimer rendering the trimer as major interest for HIV-1 vaccine research as target immunogen. Using the Designed Ankyrin Repeat Protein (DARPin) technology we recently generated broad and potent DARPins capable of targeting the membrane external proximal region (MPER) within gp41 and the variable loop 3 (V3) within gp120. The ~10-18kD sized DARPins binding to antigens of interest can be selected from high diversity (~10^{12}) libraries by ribosome display. The HIV-1 Env specific DARPins we isolated have exceptional breadth inhibiting up to 100% of tested HIV-1 strains and exceeding the breadth of bnAbs targeting the same epitopes. Differential recognition of the Env epitopes compared to the related bnAbs and an in part structure dependent binding mode make the Env DARPins highly interesting tools for immunogen characterization besides a use as antivirals.

To expand the DARPin toolbox we aimed in the current study to select DARPins targeting additional Env domains such as the variable regions 1 and 2 (V1V2). Using a range of stabilized, soluble Env trimers and Env derivatives including scaffold and chimeric envelope constructs presenting diverse V1V2 loops, we sought to yield DARPins targeting quaternary epitopes and/or the V1V2 loop.

In total six different trimer-directed and eight V1V2 focused DARPin selections were conducted. From each obtained sub-library 200 clones were analyzed for specific Env binding and neutralization potential using a 5-virus panel. The screened libraries contained more than 90% Env binders and numerous novel broad and potently neutralizing DARPins that are currently characterized for epitope specificity.

Besides their potential as therapeutic agents by increasing the panel of broadly neutralizing DARPins we have gathered a valuable tool box for the characterization of neutralization sensitive epitopes that may aid in unraveling novel sites of vulnerability.