Discovery and Characterization of Potent TIGIT-specific Antibodies Derived from Very Diverse Phage Display Libraries

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Ab candidates targeting TIGIT (T cell immunoreceptor with Ig and ITIM domains) were discovered via phage panning selections using LakePharma’s licensed state-of-the-art Fab naïve (XOMA) and in-house antigen-specific immune libraries.

TIGIT is a challenging antibody immuno oncology target.
- discovery of human and cynomolgus TIGIT cross-reactive Abs has been often problematic

We will present TIGIT-CD155 ligand inhibition data and kinetics of selected human and cyno TIGIT binding antibodies derived from phage panning.

TIGIT suppresses immunity in a malignant microenvironment when the interaction with ligands CD155 (PVR) and CD112 is not properly regulated.
In-licensing of XOMA’s 031 human Fab and 040 human scFv naïve phage libraries
- Routinely selected pM affinity antibodies
- Fully human, very large (>10^{11}), multiple ORFs
- Leads in multiple clinical programs

In-house diverse immune custom phage libraries

High throughput reformatting into different isotypes

High throughput 96-well transfections and high-yield purifications from proprietary CHO cell lines

IgG specificity, K_D assessments (BLI, Carterra)
Phage Panning and Screening Workflow

➢ 4 rounds of selections with XOMA Fab library:
  – Rounds of selections performed with decreasing biotin-huTIGIT coupled to Streptavidin-coated magnetic beads
  – Stringency of selections increased with number and duration of washes in successive selection rounds
  – Bacterial periplasmic extracts (PPEs) screened for human and cyno-TIGIT binding by ELISA (HighRes automation deck)

➢ 3 rounds of selections with LakePharma immune scFv library, following human and cyno-TIGIT mouse immunizations:
  – 3 rounds of selections with Biotin-huTIGIT on Streptavidin-coated ELISA plate wells

A. Phage panning selections with XOMA 031 and LakePharma immune libraries

B. ENRICHMENT OF PHAGE using biotinylated human-TIGIT coupled to streptavidin-coated magnetic beads

C. SCREENING PPEs by ELISA

D. IgG4 reformatting

B - Biotin-human-TIGIT
SA - Magnetic beads coated with streptavidin (SA)
Sequences of TIGIT-binding Abs from naïve and immune library selection campaigns

- **XOMA Fab naïve Library: Sequenced 1116 huTIGIT binders from 12 x 96-well plates of clones**
  - 61 sequence-unique binders identified
  - 30 unique human TIGIT binders, including all VH-unique sequences, were selected for human IgG4 reformatting and characterization
  - No clones were able to recognize cynomolgus TIGIT

- **LakePharma scFv Immune Library: Sequenced 1116 huTIGIT binders from 12 x 96-well plates of clones**
  - 67 sequence-unique binders identified
  - 56 sequence-unique human-TIGIT binders, including all VH-unique clones, selected for human IgG4 reformatting
1116 individual clones of bacterial Fab PPEs screened by ELISA against cynomolgus and human TIGIT, using the HighRes deck

No cynomolgus TIGIT binders from XOMA Fab library selections

Sequencing analysis allowed the selection of 30 unique human TIGIT Fab binders which were reformatted into human IgG4 for further kinetic and functional characterizations
ELISA binding of TIGIT-specific scFv clones in bacterial PPEs following panning with in-house immune phage library

- Following co-immunization of mice with human and cynomolgus TIGIT-Fc using LakePharma’s optimized hybridoma protocols, a diverse scFv phage library was generated with cDNA template from murine spleen mRNA.

- 1116 individual clones of bacterial scFv PPEs screened by ELISA against cynomolgus and human TIGIT protein, using LakePharma’s HighRes automation deck.

Sequencing analysis allowed the selection of 56 unique TIGIT scFv binders that were subsequently reformatted into a chimeric IgG4 isotype for further kinetic and functional characterizations.
High Throughput IgG4 Transient Production using LakePharma’s TunaCHO™ cell line

- TunaCHO™ is LakePharma’s proprietary cell line (same parental cell line as CHO-GSN)
- Consistent anti-TIGIT IgG4 production in 10ml scale transient transfections
- More cost-effective than other premium CHO transient cell line production systems

Superb expression of 86 IgG4-reformatted anti-TIGIT Abs from XOMA and immune phage display libraries (average yield ~4.6mg from 10ml transfections)
Kinetic analysis (sensogram example shown) demonstrates CD155 (PVR) blocking of TIGIT-binding antibodies

- Streptavidin Octet biosensor loaded with Biotin-human TIGIT (3 min)
- Human TIGIT-specific IgG4 Abs added at saturating concentration (8 min)
- Human CD155 ligand allowed to bind (1 min)

CD155-blocking antibodies were discovered following XOMA and in-house immune phage display library selections
K_D distribution of IgG4-reformatted TIGIT-binding antibodies by Carterra LSA

- Binding assays performed using array SPR
- Purified human and chimeric antibodies (4.5 μg/mL) were loaded onto Anti-Human IgG Fc coated CMD50M chip
- Cyno and Human TIGIT were injected as monovalent analytes as a 3-fold dilution series (0.4-300nM) over IgGs captured onto discrete spots to create a 192-spotted array via anti-human IgG Fc-coated CMD50M chip
- No cynomolgus TIGIT IgGs from XOMA library

Log[KD] distribution for two libraries against huTIGIT-His  Log[KD] distribution for two libraries against cyTIGIT-His

Spot 135, Imm_B7P5, K_D = 563pM

TIGIT-binding reformatted antibodies derived from LakePharma’s immune scFv libraries exhibited superior K_D values
Carterra LSA: Human TIGIT IgG binding (affinity ranked from high to low)

HUMAN AND CYNO TIGIT (0.4-300nM, 3-fold dilution series) binding to 192-array of HIGGS captured via anti-HIGG Fc coated CMD50M chip
TIGIT binders: Heat Map indicating IgG interactions

Ligand blockade

$K_D \text{ (nM) huTIGIT}$

$K_D \text{ (nM) cyTIGIT}$

Bin
Epitope Binning of TIGIT-specific IgGs: Network Plots

Color by bin
AlphaLISA ligand CD155 blockade assay for Immune and XOMA library reformatted clones

- TIGIT:CD155 Homogeneous Assay Kit in AlphaLISA format (BPS Bioscience) using biotin-huTIGIT and huCD155 ligand
- Anti-TIGIT IgGs (40-160 μg/ml) incubated with human TIGIT before adding CD155
- Acceptor and donor beads added before monitoring Alpha Counts
- Lower Alpha Count values indicate CD155 blockade

AlphaLISA CD155 blocking assay revealed multiple ligand-blocking antibodies from XOMA and LakePharma immune library selections (IC50s of top blockers not shown)
Cell-Based Immunoblockade Assay (Promega) for Immune and XOMA library reformatted clones

- CD226-induced luminescence is inhibited when Jurkat effector T cells expressing human TIGIT with luciferase reporter are co-cultured with CHOK1 cells expressing CD155
- Anti-TIGIT IgGs (33-132 µg/ml) block interaction of TIGIT/CD155, resulting in CD226-activated luminescence (Higher RLUs indicate CD155 blockade)

TIGIT-CD155 cell-based assay revealed multiple human TIGIT-specific functional antibodies, the vast majority originating from LakaPharma’s immune phage display library (IC50s of top blockers not shown)
Polyspecificity ELISA experimental design[1]:

- Coating with Baculovirus particle (BVP)
- Loading anti-TIGIT Abs (150, 50, 16.7 µg/ml)
- Detection with HRP-conjugated anti-human Fc (2nd Ab)
- Positive control (PC) and negative control (NC) Abs
- RG6058 benchmark anti-TIGIT IgG4
- Background BVP wells coated only with 2nd Ab
- Cut-off: 5x coated background signal

BVP Score Calculation[1]:

- BVP score is determined by normalizing absorbance on control wells with 2nd Ab only
- BVP score = OD$_{450}$ average of antibody (150 µg/mL) / OD$_{450}$ average of 2nd Ab only

Polyspecificity ELISA assessment reveals multiple clones with low BVP score derived from the LakePharma immune library

Multiple TIGIT-blocking antibodies (12) from our immune library phage display selections were able to meet most ligand blocking, affinity, productivity and polyspecificity criteria.
CONCLUSIONS

- We were able to discover multiple TIGIT antibodies following selections with the XOMA naïve Fab and LakePharma scFv immune libraries.
- There was high sequence diversity of TIGIT binders from the XOMA and LakePharma libraries.
- A high number of cynomolgus and human TIGIT cross-reactive binders was discovered following panning with the LakePharma immune libraries.
- Co-immunization of mice with human and cynomolgus TIGIT resulted in higher affinity CD155 blocking antibodies, as shown by both AlphaLISA and cell-based immuno oncology assays.
- Most TIGIT Abs originating from the LakePharma immune library were unable to recognize non-specifically baculovirus particles (low BVP score), possibly predicting fewer developability issues.
- Presumably, affinity maturation of lower affinity TIGIT Abs derived from the XOMA library selections could lead towards the discovery of a diverse repertoire of potent human TIGIT-blocking antibodies.
Numerous Carterra LSA platform advantages

- Great customer and technical support
- High throughput screening
  - Affinities, dissociation and association constants, epitope binning
- Epitope binning can be performed with multiple (384 x 384) antibodies (ligands vs analytes)
- Sensitive kinetic characterization could allow preliminary selection prior to IgG reformatting
  - Pros: Full kinetics using low concentrations of antibody fragments present in crude PPEs
- Analysis software is very detailed and user-friendly
  - (e.g. heat maps, sensograms, iso-affinity plots, binning networks)
- Already multiple requests for Carterra LSA work from LakePharma clients
  - as part of ongoing long-term antibody discovery projects
  - as stand alone requests for $K_D$ or epitope binning analysis projects
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