Alloy Therapeutics

TECHNICAL OVERVIEW 2024

Keyway™ TCR-based Therapeutic Discovery

Increasing target specificity and tackling intracellular targets with Keyway's TCRm Discovery offering

THE TEAM

Keyway Combines TCR Therapeutic Pioneers with Experts in Antibody Discovery

Leadership team experienced in discovery and advancing biologics including first TCRm to clinic for hematological caners

Dongxing Zha

CEO of Keyway TCR Discovery

Led the ORBIT platform at MD Anderson Cancer Center, a Moon Shots platform focused on biologic and cell-based therapies, where he oversaw the team that advanced the first TCRm to the clinic for the treatment of various hematological malignancies.

MERCK · GlycoFi · The University of Texas MD Anderson Cancer Center · Making Cancer History® · Max-Planck-Gesellschaft

Jaafar Haidar

Senior Director, TCR Discovery

Protein engineer with broad experience creating innovative biotherapeutics including cytokine fusions, mAbs, TCRs and bi-specifics (mAb and/or TCR based). Extended expertise in immuno-oncology and effector cell redirection.

Lilly · Dragonfly · LOXO Oncology at Lilly · ImClone Systems

Errik Anderson

Founder, CEO, Chairman of Alloy

Bioengineer, entrepreneur, and investor who has founded or co-founded several venture-backed biotech companies, including Adimab, Alloy Therapeutics, Compass Therapeutics, Alector, Arsanis, and Avitide.

Alloy Therapeutics · ADIMAB · alector · AVITIDE · COMPASS Therapeutics · arsanis

Piotr Bobrowicz

President, CSO of Alloy

Led scientific efforts across Compass Therapeutics (CSO, VP Translational Research); Adimab (Director of Platform Development, Director of Open Innovation); Merck (Group Leader); GlycoFi; University of Wroclaw.

MERCK · ADIMAB · Texas A&M University · GlycoFi · Uniwersytet Wrocławski · COMPASS Therapeutics

We help our partners unlock the future of TCRm medicines.

Keyway unites the world's most creative antibody discovery team with the pioneering minds behind TCR therapeutics.

Copyright © 2024

KEYWAY FEATURES

Keyway is a Fully Integrated Platform to Drug pMHCs in a High Specific Manner

Platform seeks to create TCR-based therapies with ideal drug properties. Full-service, one-stop-shop for TCRm discovery technology and capabilities.

Proprietary technology and workflows

pHLA complex and control TCRm generation, immunization of proprietary humanized ATX-Gx mice, multiple binder recovery methods, biophysical characterization, bispecific or CAR engineering, *in vitro* and *in vivo* preclinical testing

TCRm-specific specificity studies

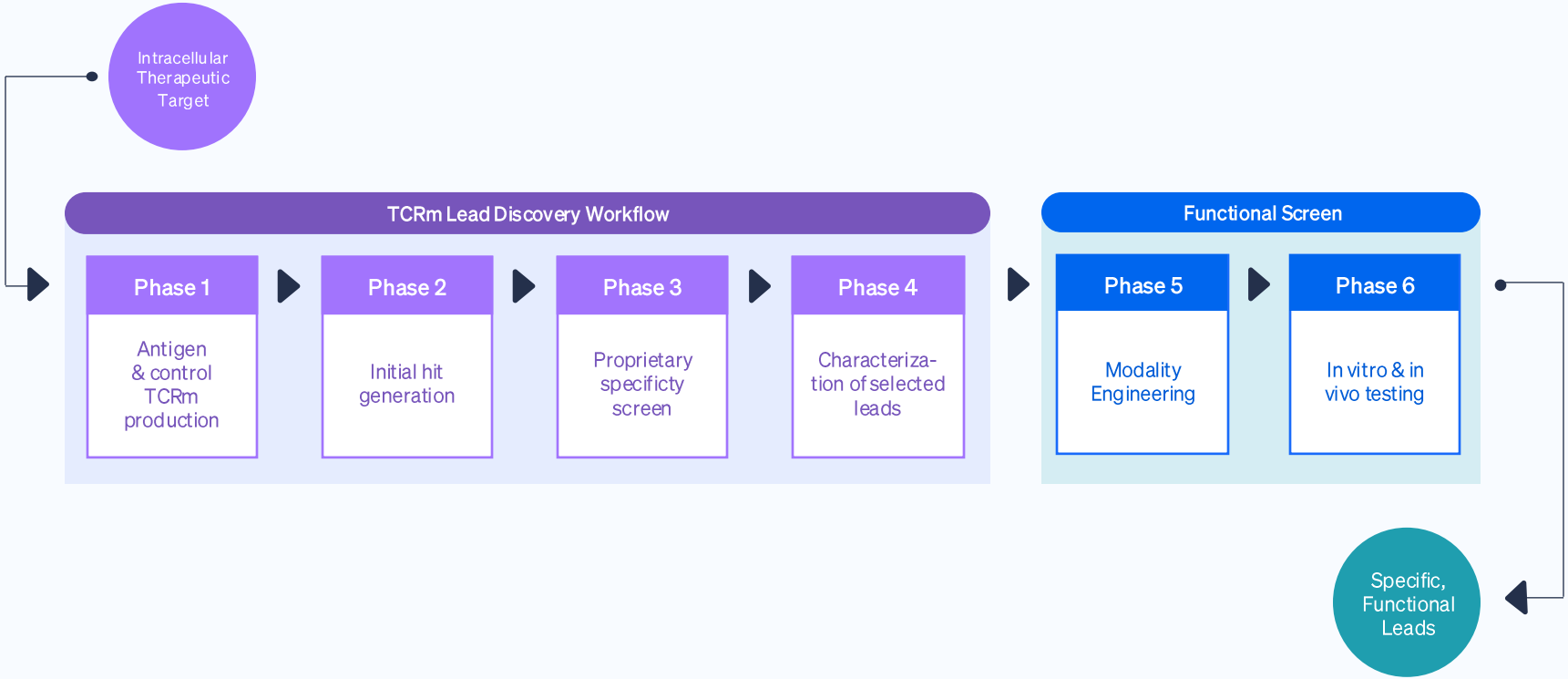
T2 cell pulse assay, EC50 binding assay, display-based alanine scan and X-scan, tumor cell lines binding assay, bioinformatics analysis

Copyright © 2024

KEYWAY DISCOVERY OVERVIWW

Keyway Features an End-to-end TCR-based Therapeutics Discovery Workflow

Tailored Keyway TCRm discovery workflow for the search of functionally-relevant leads via strigent specificity screens

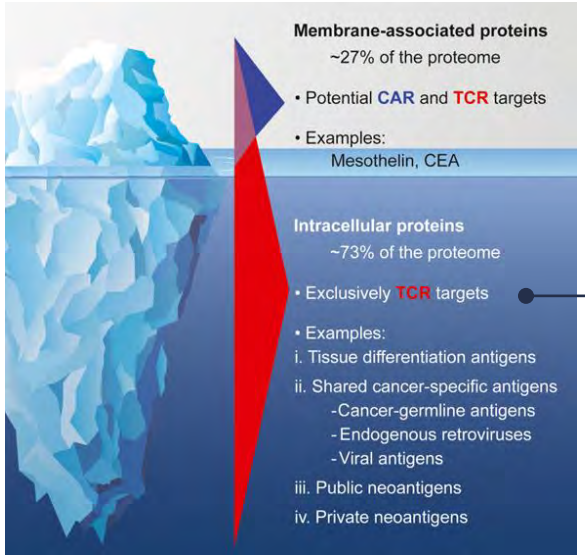


Copyright © 2024

Peptide MHC Complexes Provide Vast Opportunity to Target Beyond Cell Surface

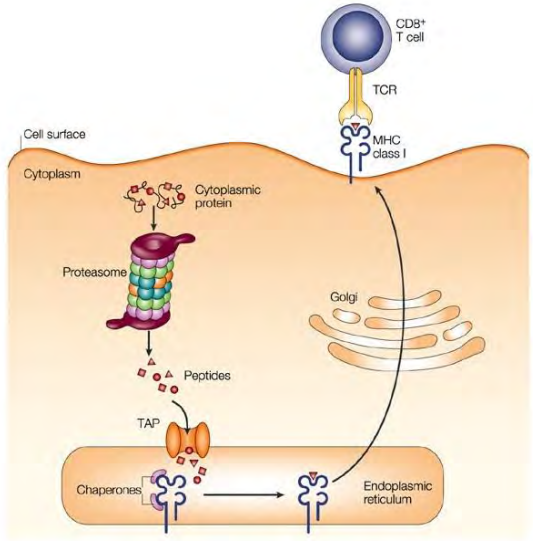
Majority of proteome comprised of intracellular proteins that are inaccessible via conventional biotherapeutic approaches

Membrane-associated proteins
~27% of the proteome



Source: Immunological Reviews, Volume: 290, Issue: 1, Pages: 127-147, First published 29 July 2019, DOI: (10.1111/imr.12772)

Intracellular proteins are loaded into MHC complex and presented on cell surface



Source: Nat Rev Immunol 3, 952–961 (2003). https://doi.org/10.1038/nri1250

Copyright © 2024

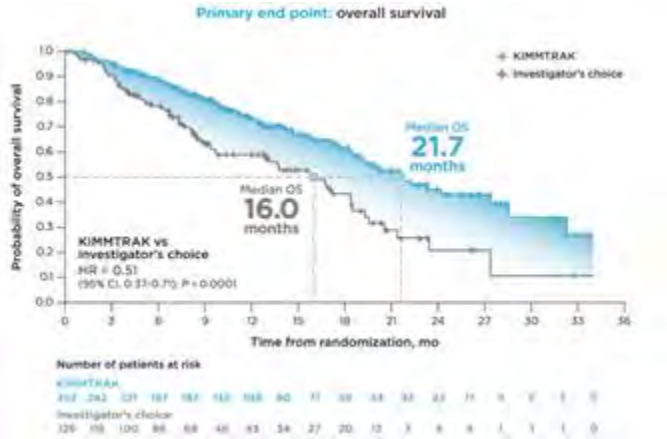
TCR-BASED MODALITIES ARE EMERGING THERAPEUTICS

TCR-based Therapies Unlock Broad New Therapeutic Potential

We envision a future where TCR modalities become as important a treatment paradigm as antibodies in the next 20 years

Opens variety of intracellular targets for precision medicine in liquid & solid tumor

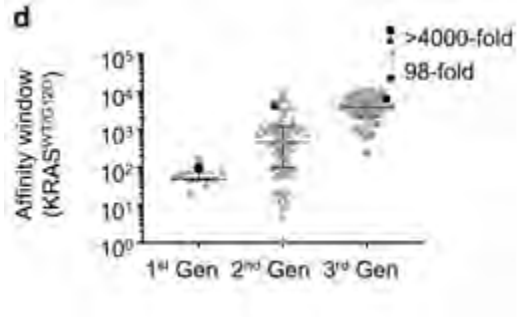
First and only TCRm solid tumor approval: KIMMTRAK bispecific T cell engager redirects immune system to target and kill gp100-expressing uveal melanoma tumor cells expressed via HLA-A2.



Source: Nathan P, Hassel JC, Rutkowski P, et al; IMCgp100-202 Investigators. Overall survival benefit with tebentafusp in metastatic uveal melanoma.

Offers highly specific approach to "undruggable" targets

TCRs are under preclinical and clinical investigation for conventionally "undruggable" targets such as KRAS, p53, viral proteins, and more.



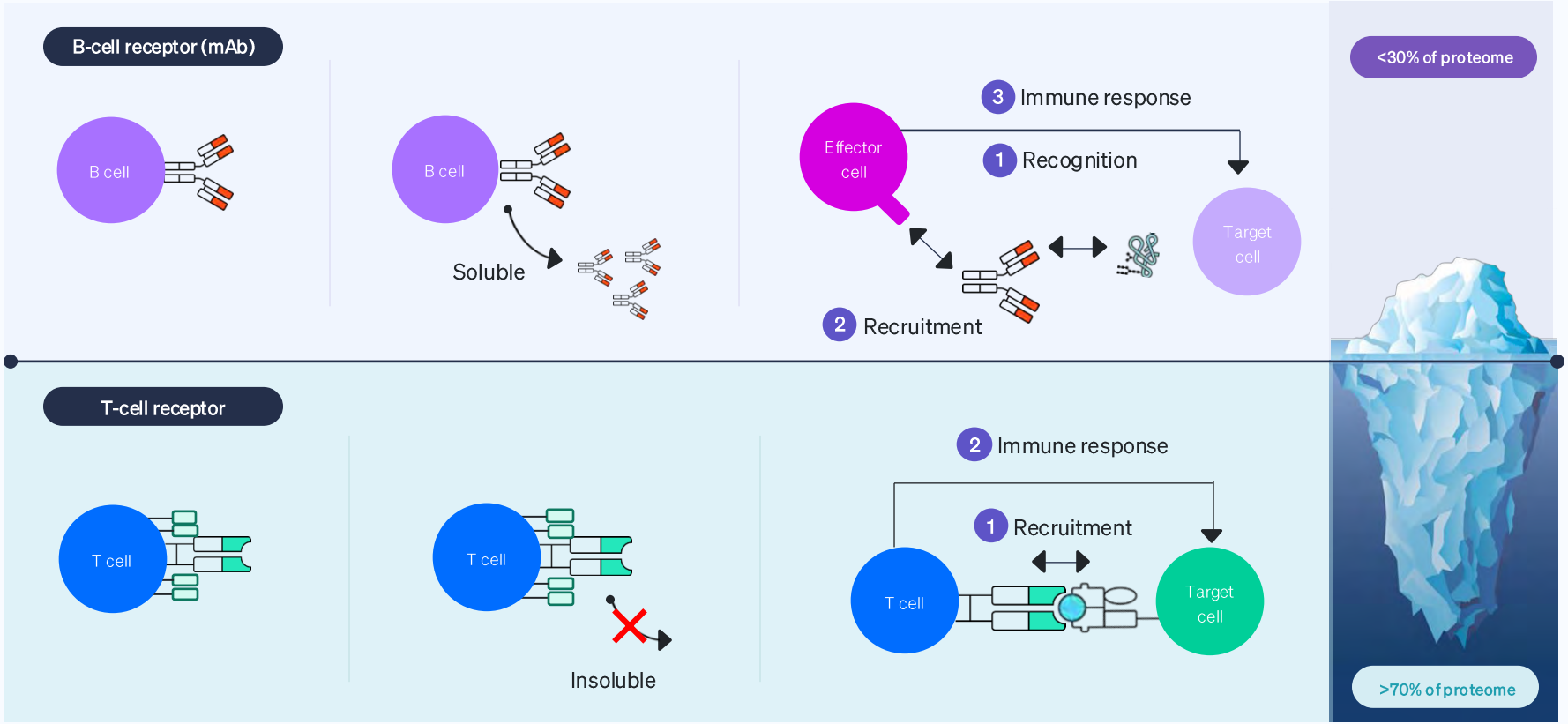
Difference in binding affinity of TCR mutants towards $KRAS^{WT}$ and $KRAS^{G12D}$ plotted as affinity window (K_D $KRAS^{WT}$/ K_D $KRAS^{G12D}$ pHLA) in preclinical model.

Source: Poole, A., Karuppiah, V., Hartt, A. et al. Therapeutic high affinity T cell receptor targeting a KRASG12D cancer neoantigen. Nat Commun 13, 5333 (2022). https://doi.org/10.1038/s41467-022-32811-1

Copyright © 2024

T Cells Evolved to Use TCR to Trigger Response to Intracellular Antigen

Natural mechanisms are the blueprint for therapeutic platforms

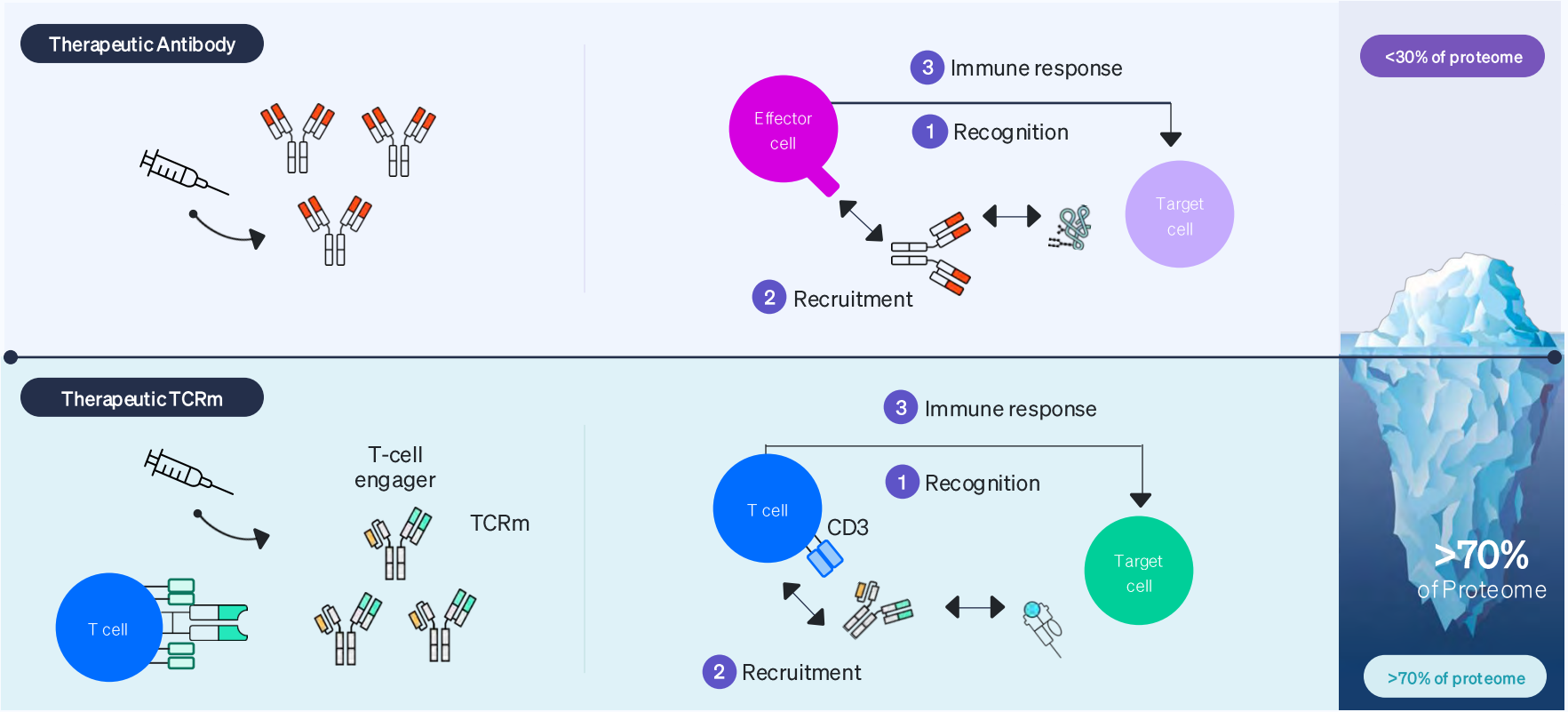


Copyright © 2024

T-CELL RECEPTOR BIOLOGY

TCR Mimics Target pMHCs With the Favorable Drug-like Properties of an Antibody

Natural mechanisms are the blueprint for therapeutic platforms



Copyright © 2024

KEYWAY TCR DISCOVERY

Platform is Designed to Drug pMHC Complexes in a High Specific Manner

Solving the most challenging problems in TCRm discovery with an end-to-end integrated platform and service offering

Solution one

Generating high-quality pHMC complexes

The keyway TCRm Discovery Platform includes the proprietary protocols, workflows, know-how, technology, and materials for the consistent generation of high-quality pHLA complexes. High-quality antigens are crucial for the generation of high-quality therapeutic leads so performing this step correctly and consistently is particularly important.

Solution two

Discovering a diverse array of highly specific, fully-human antibodies

The Keyway TCRm Discovery Platform integrates Alloy's full suite of *in vivo*, *in vitro*, and *in silico* anitbody discovery capabilities in a proprietary workflow tailored to identifying and optimizing a diverse set of antibodies that bind to a specific pMHC complex. The Keyway discovery process counter selects for non-specific binders to related pMHC complezes while also using Alloy's AI/ML workflows to reduce genome-wide polyreacitivty.

Solution three

Performing appropriate specificty testing to select for the best therapeutic leads

The keyway TCRm Discovery Platform includes pMHC display libraries for specificity testing to address off-target non-specificity effects, a major challenge of therapeutic TCRm discovery. Ensuring that therapeutic leads do not bind to off-target epitopes is crucial to the success of such leads in clinical trials.

Copyright © 2024

FEATURES OF KEYWAY DISCOVERY

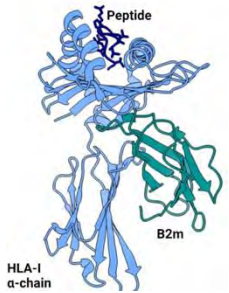
Keyway Generates and Characterizes High-Quality pMHCs

Internal capabilities to produce and characterize pMHC complexes to ensure quality for high specificty TCRm discovery

Solution one

Solution two

Solution three



1 B2m and α-chain Production

HLA-I α-chain and B2M cloning and inclusion body isolation and denaturation

2 In Vitro Refolding

α-chain, B2M, and synthetic peptide refolding

3 pHLA-I Purification

Purification of refolded pMHC(s) complex from the refolding mix

4 Characterization

Binding characterization, purity determination, and mass spec analysis

Copyright © 2024

FEATURES OF KEYWAY DISCOVERY

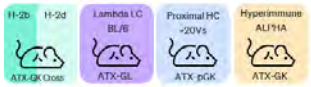
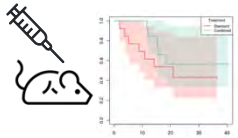
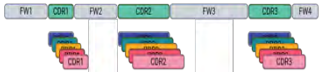
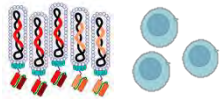
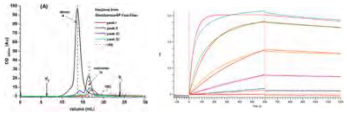
Keyway Discovers Diversity of Specific, Fully-Human mAbs

Keyway TCRm Discovery leverages the entire suite of Alloy antibody discovery tools

Solution one

Solution two

Solution three

	Generate Disversity	Retrieve Diversity	Assess Diversity
In Vivo	 ATX Gx Transgenic Mice	 High-throughput B Cell Screening	 In Vivo Efficacy
In Vitro	 Naïve and Synthetic Display Libraries	 Phage & Mammalian Display Library Screening	 Biophysical Characterization
In Silico	 AI-Driven Library Design	 Immune Repertoire Data Mining	 Modeling and Predicting Structure & Properties

Copyright © 2024

FEATURES OF KEYWAY DISCOVERY

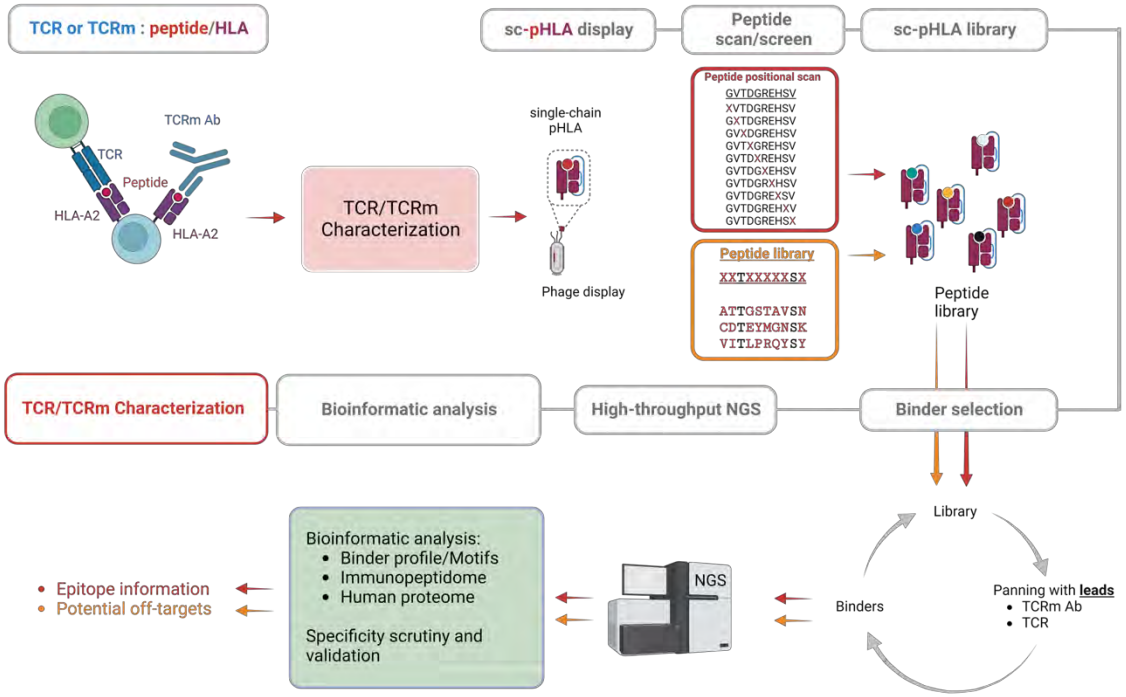
Keyway Performs Specificity Testing to Select Best Leads

pMHC (pHLA) phage display libraries for specificity testing address off-target non-specificity effects

Solution one

Solution two

Solution three

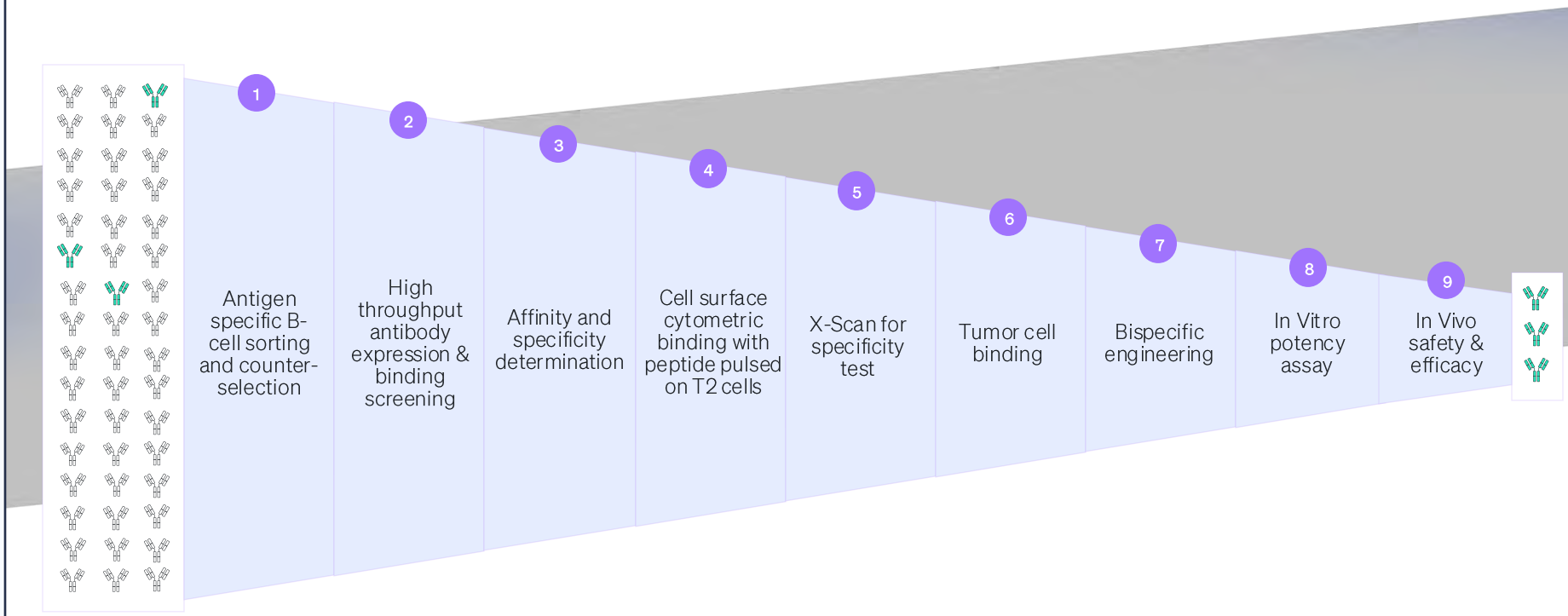


Copyright © 2024

COMPLETE TCR-MIMIC DISCOVERY WORKFLOW

Multiple Layers of Screening Process to Enhance Highly Potent and Specific TCRm

Keyway TCRm antibody characterization funnel



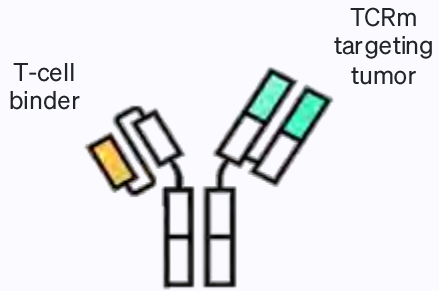
Copyright © 2024

Keyway Can Format TCRm Leads into Bispecific Cell Engager or CAR-T

TCRm modality engineering: Bispecific cell engager or CAR-T

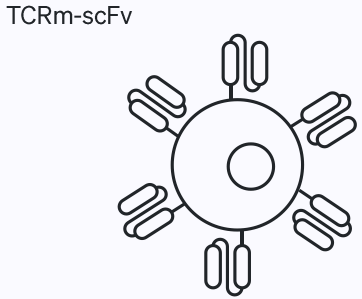
Bispecific Capabilities

- Multiple readily-available high-quality T-cell engager arms (including CD3 for convenient bispecific construction
- High-throughput bispecific generation and screening



CAR-T Capabilities

- mRNA-and lentiviral-based vector system producing CAR-Ts and characterization



Copyright © 2024

SUMMARY

Keyway TCR discovery leverages proprietary technology platforms

Core & value-add services and platforms ready to go — with additional capabilities on the way.

Core services

Keyway TCRm

Proprietary TCRm discovery platform covering pHLA complex generation and the discovery, characterization, engineering, optimization, and selection of the best TCRm leads.

Keyway TCR

TCR display platforms for the discovery of engineered TCRs.

Keyway pHLA

pHLA display platform for the discovery of engineered TCRs.

Keyway TCR Mice

Transgenic murine *in vivo* discovery platform optimized for human TCR-based therapeutics. **Coming soon**

Value-add services

VALUE-ADD

Translational medicine

Accelerates partner programs by providing early insights into functional activity of candidates generated from Keyway TCR discovery projects.

Copyright © 2024

Alloy Therapeutics®

CASE STUDY

Keyway TCRm Discovery

Discovery of Highly Specific TCRm Ab against WT1/HLA-A2

CASE STUDY

Discovery of Highly Specific TCRm Ab Against WT1/HLA-A1

Addressing the challenges of off-target specificity of Keyway TCRm discovery workflow

Case study background & challenge

- WT1 gene is over-expressed in hematological malignancies; National Cancer Institute ranks. WT1 as number one target for cancer IO.
- Discovery of specific binders to WT1/HLA-A2 is challenging due to its high homology to other peptides presented in the context of HLA
- M13L and PIGQ have been identified as top off-target peptides for targeting WT1*

Peptide Name	Sequences
WT1	RMFPNAPYL
M13L (Off-Target Peptide)	RMFPTPPSL
PIGQ (Off-Target Peptide)	RMFPGEVAL

*Ataie et. al. J Mol Biol. 2016 Jan 16; 428(1): 194–205.

Scope & highlight of case study

- Keyway workflow and specificty screens enable the discovery of fully human TCRm Abs with superior specificty versus 3 benchmark anti-WT1/HLA-A2 Abs
  - Roche mAb1 and mAb2 (developed by Roche
  - ESK1 (developed by Eureka)
- Focus on eliminating off-target binding to M13L & PIGQ
- Selected Keyway Ab leads were reformatted into CAR-T where they demonstrated cell-based functions, setting the stage for the further evaluation of TCRm-based CART-T as a therapeutic modality

pHLA monomer in this case study was provided by Immunitrack

Copyright © 2024

CASE STUDY

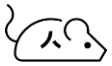
Discovery of Highly Specific TCRm Ab Against WT1/HLA-A2

Tailored Keyway TCRm discovery workflow for the search of specific, functionally-relevant leads

TCRm Lead Discovery Workflow

Phase 1

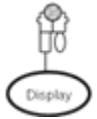
Initial hit generation



Parallel *in vivo* and *in vitro* discovery workflow; ELISA for initial binder screening

Phase 2

Proprietary specificity screen



Selected binders were screened for cell-based specificty against off-target peptides

Phase 3

Evaluation of epitopic coverage

Selected binders were binned against benchmark mAbs in a cross-competition assay

Phase 4

Characterization of selected leads

Highly WT1/HLA-A02-specific leads were selected for binding affinity & EC 50 determination

TCRm-based CAR-T functional screen

Phase 5

CAR-T construction

Leads were selected for formatting into CAR-T

Phase 6

In vitro cell functional assays

CAR-T cell function

pHLA monomer in this case study was provided by Immunitrack

Copyright © 2024

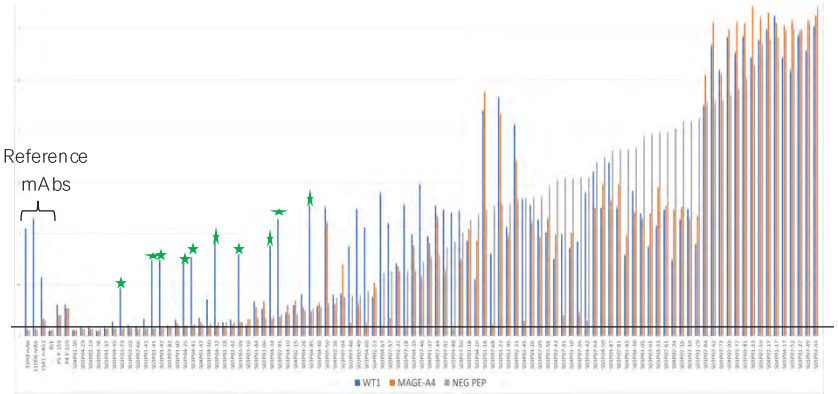
CASE STUDY

Generation of Fully Humans Ab Hits for Binders Toward WT1/HLA-A2

ELISA screening of clones based on a combined *in vitro* and *in vivo* Alloy antibody discovery workflow

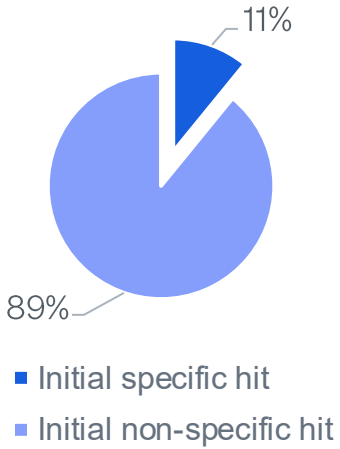
1 Initial ELISA screen for hit identification

Example of ELISA screen to identify hits with initial binding to WT1/HLA-A2 from the vast number of clones generated in Alloy discovery workflow. In this example, 11 mAbs specific for WT1/HLA-A2 representing 3 clonotypes were identified.



2 Preliminary specific hits were selected for further screening

Only a small percentage (11%) of total clones screend by ELISA exhibit satisfactory initial specificty for WT1/HLA-A2 and were selected for more strigent specificity screens.

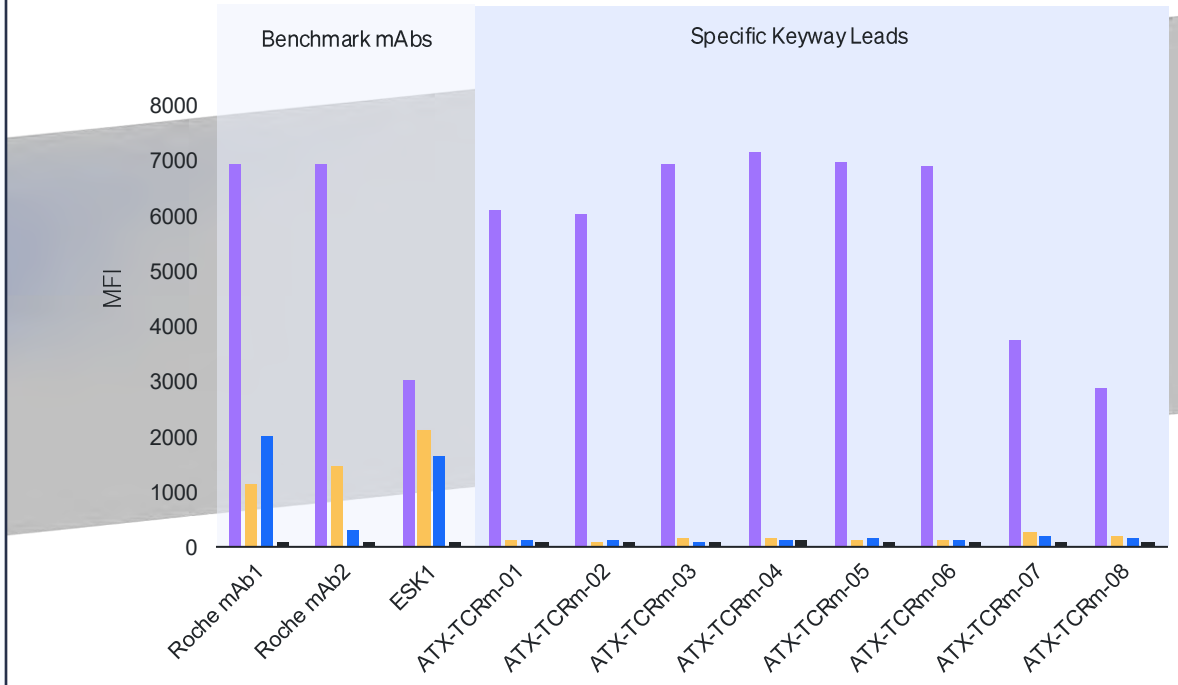


Copyright © 2024

CASE STUDY

TCRm Leads With Superior Specificity to Benchmark Antibodies Were Identified

T2 cell surface peptide pulse assay for specificity screen



- WT1 peptide 50uM
- M13L peptide 50uM
- PIGQ peptide 50uM
- No peptide

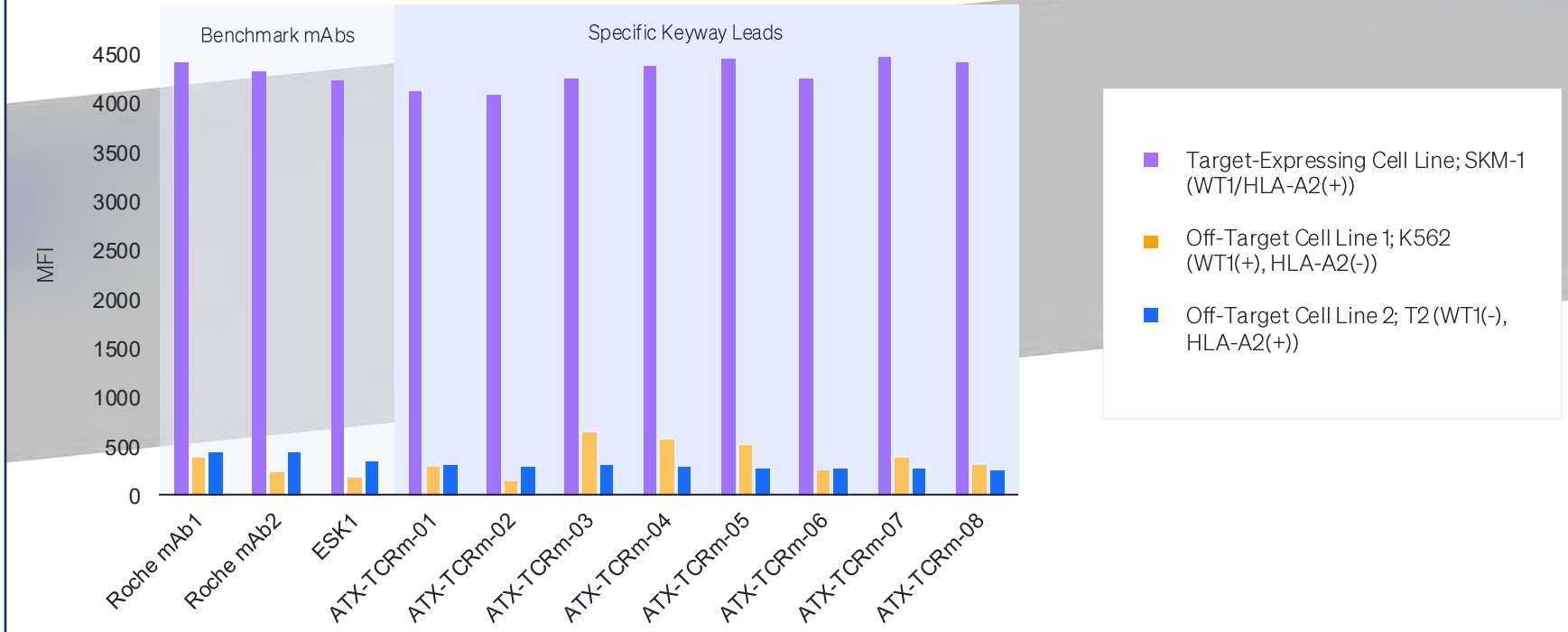
Peptide Name	Sequences
WT1	RMFPNAPYL
M13L	RMFPTPPSL
PIGQ	RMFPGEVAL

Copyright © 2024

CASE STUDY

Tumor Cell Binding Assay Results Corroborate the Superior Peptide Specificity of TCRm Leads

Keyway leads exhibit comparable specific binding to a WT/HLA-A2-expressing cell line compared to benchmark antibodies

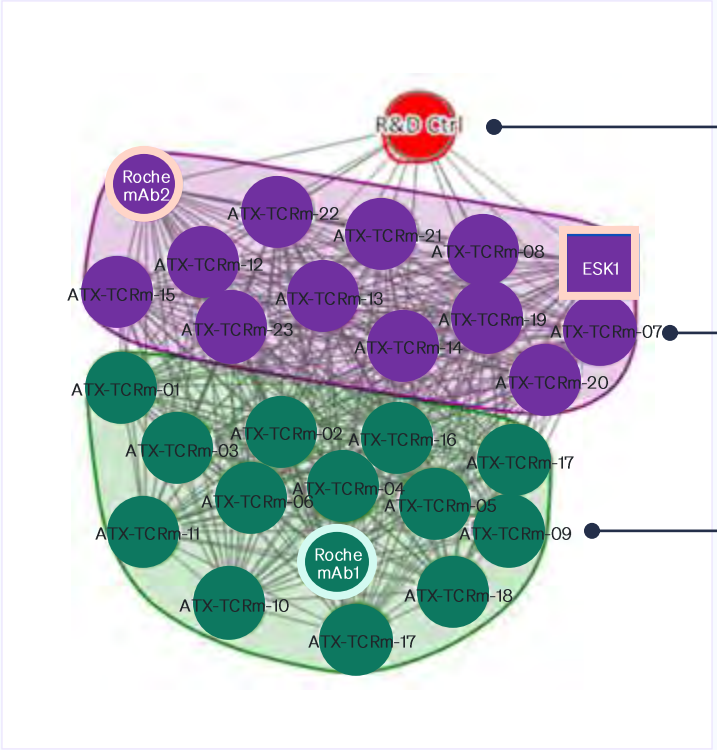


Copyright © 2024

CASE STUDY

Specific TCRm Leads Were Demonstrated to Cover Epitopes of All Benchmark Abs

Binning experiment against benchmark Abs and R&D control was performed by Carterra LSA to indetify preferred leads



R&D control antibdoy recognizes conformational HLA-A2

ESK1 & Roche mAb2

More specific leads are observed in the bin of Roche mAb1 Epitope bin chosen for Keyway preferred leads

Copyright © 2024

CASE STUDY

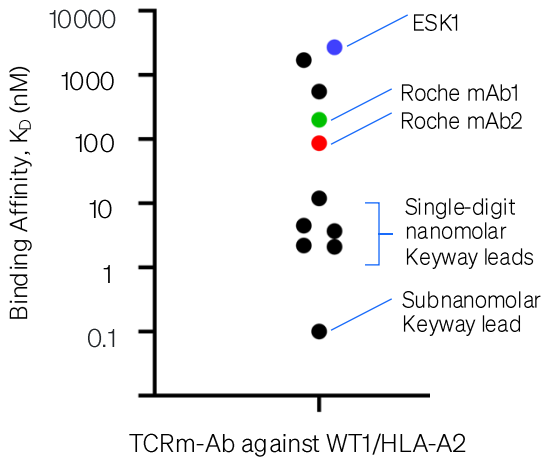
Selected TCRm Leads Exhibit Superior Affinity to WT1/HLA-A2 vs Benchmark Abs

Keyway leads exhibit comparable specific binding to a WT/HLA-A2-expressing cell line compared to benchmark antibodies

Binding kinetics of Keyway TCRm leads and benchmark Abs HLA-WT1

Clone ID	ka (1/Ms)	kd (1/s)	K_D (nM)
ATX-TCRm-01	1.5E+05	6.9E-04	4.5
ATX-TCRm-02	3.2E+05	6.8E-04	2.1
ATX-TCRm-03	1.8E+05	4.1E-04	2.2
ATX-TCRm-04	3.0E+05	3.7E-03	12
ATX-TCRm-05	2.6E+05	9.6E-04	3.7
ATX-TCRm-06	3.0E+05	3.0E-05	0.1
ATX-TCRm-07	5.2E+04	2.8E-02	550
ATX-TCRm-08	3.0E+04	5.1E-02	1700
Roche mAb1	2.4E+05	4.9E-02	200
Roche mAb2	1.9E+05	1.7E-02	87
ESK1	7.7E+04	2.1E-01	2700

Affinity rank of Keyway TCRm leads vs benchmark Abs



Copyright © 2024

CASE STUDY

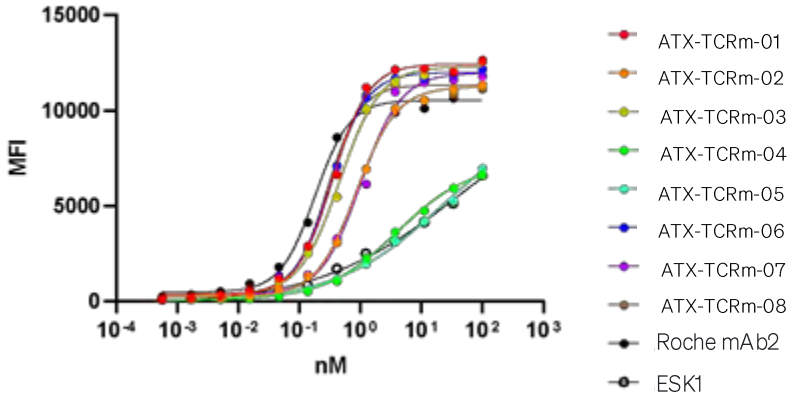
Several Specific TCRm Leads Exhibit Superior Potency vs Benchmark Abs

EC50 determination with WT1 peptide pulsing on T2 cells

EC50 values of selected Keyway TCRm leads

Clone ID	EC50 (nM) @50uM WT1
ATX-TCRm-01	0.355
ATX-TCRm-02	0.867
ATX-TCRm-03	0.461
ATX-TCRm-04	0.327
ATX-TCRm-05	1.020
ATX-TCRm-06	0.288
ATX-TCRm-07	Not Reaching Plateau
ATX-TCRm-08	Not Reaching Plateau
Roche mAb2	0.181
ESK1	Not Reaching Plateau

EC50 comparison of selected Keyway TCRm leads vs benchmark Abs



Copyright © 2024

CASE STUDY

TCRm-based CAR-T Demonstrate Expression, T-Cell Activation Assays, & Cell Killing

Alloy end-to-end capabilities to provide a streamlined workflow for CAR-T cell function evaluation

CAR-T Generation

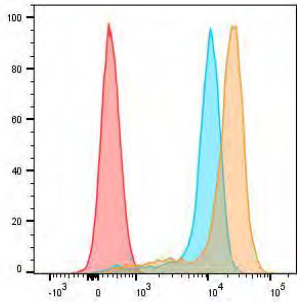
Lead selection

CAR-T modular design

mRNA generation

TCRm-based CAR-T

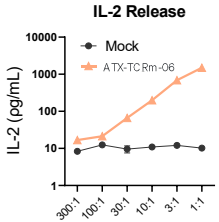
Flow cytometry – CAR expression



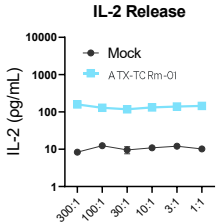
PE-labeled HLA-A2-RMFPNAPYL

Mock
ATX-TCRm-01 electroporated
ATX-TCRm-06 electroporated

ELISA – IL-2 Release

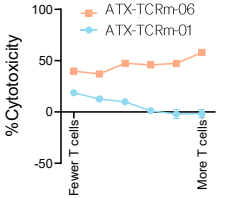


E:T (CAR-J:SKM-1)



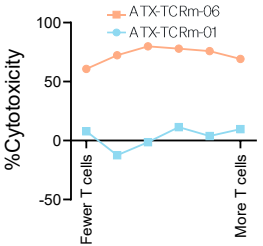
E:T (CAR-J:SKM-1)

Flow cytomertry- cytotoxicity



E:T (CAR-J:SKM-1)

BV-173



E:T (CAR-J:BV-173)

Copyright © 2024

CASE STUDY

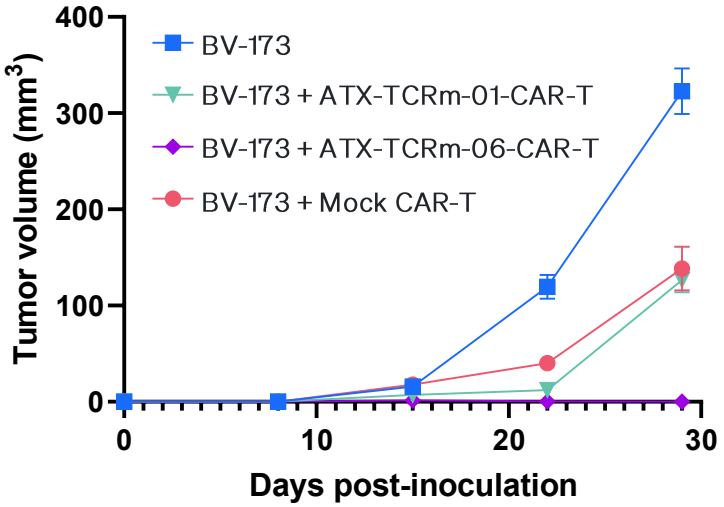
TCRm-Based CAR-T Demonstrate Anti-Tumor Activity in Animal Model

ATX-TCRm06-CAR-T showed significant efficacy against the BV-173 cell line in NSG mice

In vivo functional testing of Keyway CAR-Ts

- BV-173 human B cell leukemia cells were injected into the flanks of NOD *scid* gamma (NSG) immunocompromised mice
- Treatment groups received a 1:1 co-injection of BV-173 cells and indicated CAR-T cells
- Tumor size was monitored using caliper measurements
- **ATX-TCRm-06-CAR-T cells completely ablated tumor growth in NSG mice**
- While both mock- and ATX-TCRm-01-CAR-T cells initially restricted tumor growth compared to non-treated control, the tumors were ultimately capable of significant growth as compared to ATX-TCRm-04-CAR-T treated mice

Keyway CAR-T exhibited tumor suppression efficacies



Copyright © 2024

SUMMARY

Keyway is a One-Stop-Shop to Discover Highly Specific TCRm Therapies

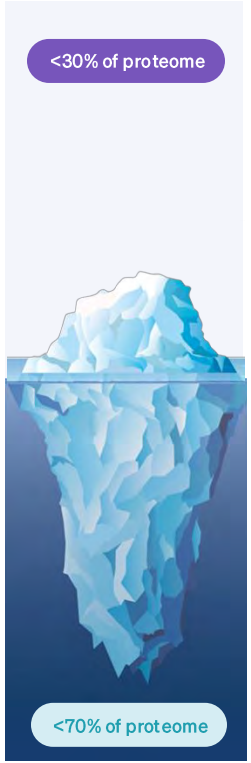
TCRm modality engineering: Bispecific cell engager or CAR-T

1. **TCR therapies unlock broad new therapeutic potential** beyond the cell surface by targeting pMHC complexes

2. **TCR mimics target pMHCs** with the favorable drug-like properties of an antibody

3. **Keyway unites the world's most creative antibody discovery team** with the pioneering minds behind TCR therapeutics to deliver high quality TCRm medicines to our partners

4. **The Keyway TCRm platform comprises:**
   1. Generation of high-quality pMHC complexes
   2. Discovering a diverse array of highly specific, fully-human antibodies
   3. Performing appropriate specificity testing to select for the best therapeutic leads
   4. Modality engineering capabilities to generate TCRm-based bispecific antibodies using proprietary CD3 engagers, as well as TCR-based CAR-Ts
   5. Cell functional assay capabilities for therapeutic lead screening



Copyright © 2024

Alloy Therapeutics

TECHNICAL OVERVIEW 2024

Keyway™ TCR-based Therapeutic Discovery

Increasing target specificity and tackling intracellular targets with Keyway's TCRm Discovery offering