T-cell receptor-like antibodies directed against intracellular tumor targets for immunotherapy of solid tumors

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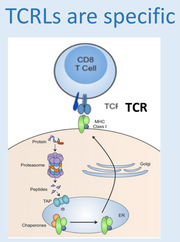
Introduction

Critical to the success of immunotherapy of cancer is the ability to selectively target malignant cells. Peptides from intracellular proteins can be presented on the surface of cells, via human leucocyte antigen (HLA) molecules. These peptide-HLA complexes are monitored and recognized by T cell receptors (TCRs) expressed by T cells. The fine specificity of TCRs can be mimicked by monoclonal antibodies that exhibit similar peptide-specific, HLA-restricted recognition and are termed TCR-like antibodies (TCRLs).

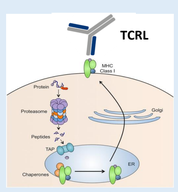
Adicet has established a hybridoma-based platform to derive such TCR-like antibodies and a robust TCRL validation process to isolate target-specific TCRLs. This process includes assessment of antibody affinity and specificity by screening a large panel of irrelevant and similar peptides to ensure the selectivity of TCRLs and eliminate the potential for off-target cross reactivity. In addition, we have established a mass-spectrometry (MS) - based approach, the "EpiTarget platform", to identify novel, disease-specific HLA / peptide complexes in patient tumor specimens. Here we present two highly specific TCRLs targeting two HLA-A*02-restricted peptides: Tyrosinase $_{369\text{-}377}$ (Tyr) and MAGE-A4$_{230\text{-}239}$. These two target peptides were identified and validated by MS and are present in the majority of melanoma specimens (Tyr) and various solid tumors (MAGE-A4). Two highly specific TCRLs against HLA-A*02 / Tyr and HLA-A*02 / MAGE-A4 peptides were identified and converted into CD3-TCRL bispecific T-cell engager format. Both exhibit robust potency *in vitro* against a panel of target positive cell lines and *in vivo* in various xenograft models of melanoma and bladder cancer.

TCRL Platform Technology: Accessing the Intracellular Proteome

- Challenge: Paucity of disease-specific cell surface targets in solid tumors (80% of proteins are intracellular)
- Solution: Targeting disease-specific intracellular proteins highly expands target pool
- TCRLs are specific to peptide-MHC complexes



TCR recognition of MHC-peptide



TCRL recognition of MHC-peptide

Multiple Application of TCRLs

- scFv for chimeric antigen receptors
- Bispecific T-cell engaging antibodies
- Antibody-drug conjugates

EpiTarget- Target Discovery

- Cancer and normal tissues peptide analysis by Mass Spectrometry to mine differential expression of cancer MHC peptides
- Target validation by cross correlating with normal tissue mRNA databases
- Target validation by targeted search of specific peptide by Mass Spectrometry and use of proprietary supportive analytical tools
- Peptide target selection for TCRL

Cancer & Normal tissues → Selection of cancer-specific candidate peptides → Validation of candidate peptide targets

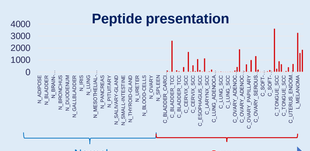
Target Selection and Prioritization

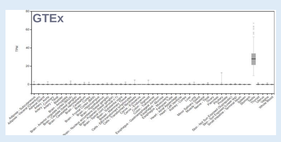
Differential presentation analysis

- Protein libraries
- Mutation libraries
- Peptide libraries
- Spectral libraries
- RNA-seq repositories
- Denovo sequencing

Supportive tools

- mRNA expression
- Databases
- Synthetic peptides
- Similarity analysis
- Literature





Verified Target

TCRL Generation

- MHC-peptide complex generation for selected peptide
- TCRL clones generation by standard hybridoma technology
- Lead clone selection by analysis of binding assays, Alanine Scan, SPR
- TCRL *In Vitro/In Vivo* validation in tumor models using CAR & Bi-Specific modalities
- TCRL - Target Moiety for immune cell product / other modalities

TCRL Selection - High Affinity and High Specificity

Peptide/MHC epitope mapping demonstrates high degree of peptide-dependent interactions
Ala scanning analysis

No binding to multiple peptides with sequence similarity identified by whole proteome mining

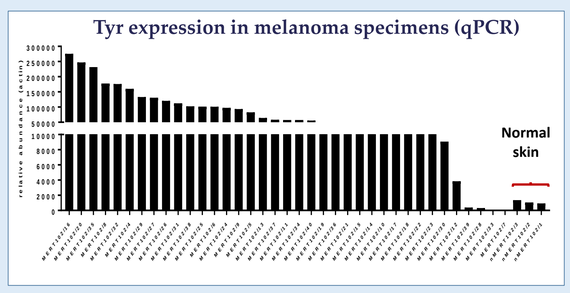
No binding to multiple HLA-A2+ antigen negative cells
A panel of different tumor cell lines and primary normal cells from 12 different essential tissue types

Potent specific *in vitro* & *in vivo* killing of HLA-A2+ Ag+ cells by TCRL-CD3 bi-specific MAb
Demonstrated on cell lines with low presentation levels of peptide-MHC complexes

High Affinity (nM range), High Specificity
Superior TCRLs ✓

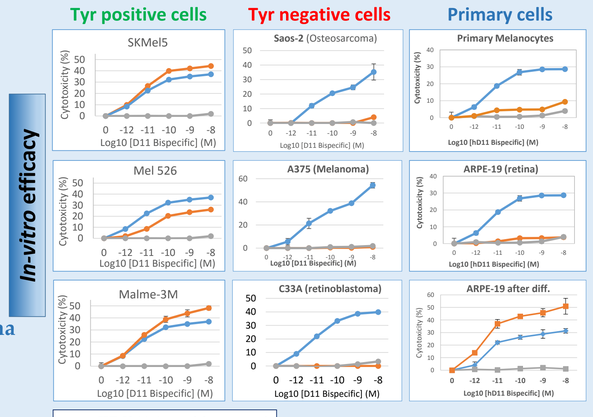
Tyrosinase is an Attractive Target for Melanoma

mRNA Expression in Melanoma and Normal Skin

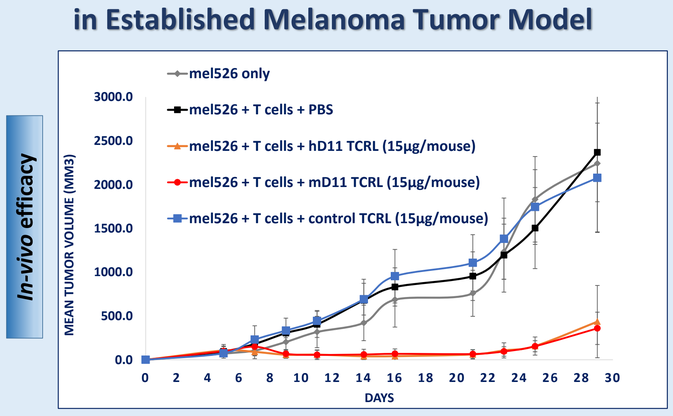


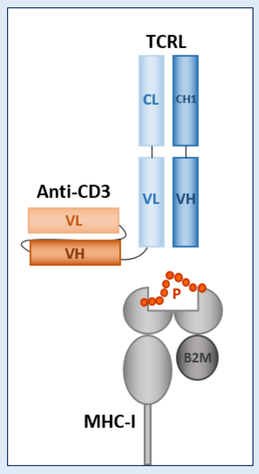
- Expressed in the majority of primary and metastatic melanoma (>70% by mRNA), including stage 3 and 4 disease
- Expression in normal tissues is limited to melanocytes, retina/choroid and inner ear
- Tyr peptide 369-377 Identified by Mass Spec in:
  - Melanoma patient specimens 6/8 (75%) and Melanoma cell lines 6/7 (86%) Tyr mRNA+
  - Normal eye (retina, choroid and iris) and skin

Specific Killing of Melanoma HLA-A2+/Tyr+ Cell Lines but not Tyr- Cell Lines



Murine and Humanized D11 Tyr TCRL-CD3 Bispecific is Efficacious in Established Melanoma Tumor Model

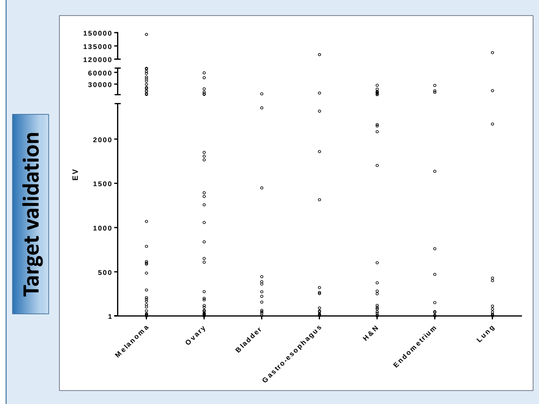




- Mel526 melanoma cells (5x106) inoculated into NOD/SCID mice with ex-vivo expanded T cells (E/T 3:1)
- Upon tumor establishment (70-100mm3) murine, humanized D11 or control Bispecific TCRLs administrated at days 5-14 at the indicated doses (15µg/dose x 10)
- Parental murine and humanized D11 Bispecific exhibit similar anti-tumor activity

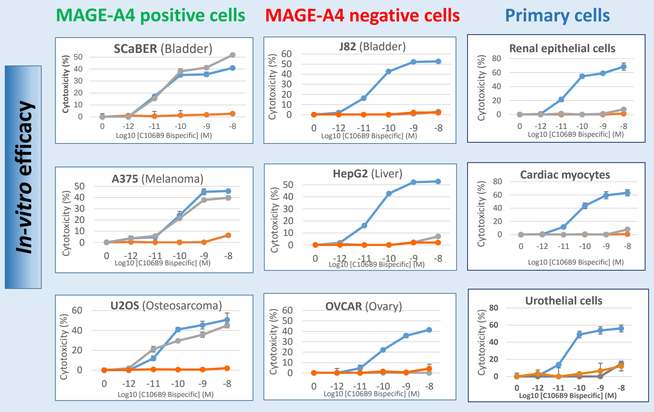
MAGE-A4 as a Target for Multiple Solid Tumors

mRNA Expression in Various Cancers

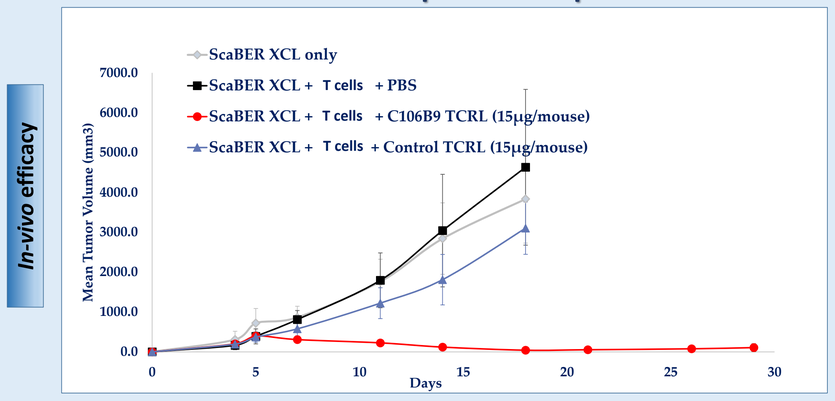


- Identified by Mass Spec in H&N SCC, Bladder TCC, Ovarian, Lung and Esophageal HLA-A2+ patient specimen
- In normal tissues, MAGE-A4 is expressed in placenta and testis.

Specific Killing of Multiple Tumor HLA-A2+/MAGE-A4(+) Cell Lines by C106B9 TCRL-CD3 Bispecific



Regression of Established Bladder Cancer Xenograft by MAGE-A4 TCRL-CD3 BiSpecific Compound



- SCaBER XCL bladder cancer cells (10x106) inoculated into NOD/SCID mice with ex-vivo expanded T cells (E/T 3:1)
- Upon tumor establishment (~200mm3) C106B9-CD3 Bispecific or control TCRLs administered at days 4-9 at the indicated doses (15µg/dose x 6)

Summary

- Two HLA-A*02-restricted target peptides: Tyrosinase 369-377 (Tyr) and MAGE-A4230-239 were identified and validated by Mass Spectrometry to be presented in the majority of melanoma specimens (Tyr) and various solid tumors (MAGE-A4)
- Two highly specific TCRLs against HLA-A*02 / Tyr and HLA-A*02 / MAGE-A4 peptides were identified and converted into CD3-TCRL bispecific T-cell engager format and exhibit robust potency *in vitro* against a panel of target positive cell lines and *in vivo* in xenograft models of melanoma and bladder cancer, respectively
- Identification and validation of additional novel intracellular targets by Epitarget analysis is expected to provide a rich pipeline for TCRL-based treatment modalities for cancer, such as bispecific T-cell engaging antibodies, ADC, and chimeric antigen receptor modified T cells directed against solid tumors
- The TCRL platform complements Adicet's efforts to develop gamma delta T cell-based next generation immunotherapies