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 leukocyte antigen (HLA) molecules. These peptide-HLA complexes are monitored and recognized by T cell receptors (TCRs) expressed by T cells. The specificity of TCRs can be mimicked by monomeric antibodies that exhibit similar peptide-specific, HLA-restricted recognition and are termed TCR-like antibodies (TCRLs).

Adicet has established a hydridoma-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 tumors. Here we present two highly specific TCRLs targeting two HLA-A*02-restricted peptides: Tyrosinase p83 (Tyr) and MAGE-A4AA-36. 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 targets positive pools.
- TCRLs are specific to peptide-MHC complexes

Multiple Application of TCRLs
- scFv for chimeric antigen receptors
- Bispecific T-cell engaging antibodies
- Antibody-drug conjugates

Tyrosinase is an Attractive Target for Melanoma

- mRNA Expression in Melanoma and Normal Skin
- TYR expression in melanoma specimens (qPCR)
- Normal skin
- Expression in the majority of primary and metastatic melanomas (>75% by mRNA), including stages 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 inc:
  Melanoma patient specimens 68% (75%) and Melanoma cell lines 67% (68%) Tyr mRNAs
- Normal eye (retina, choroid and iris) and skin

MAGE-A4 as a Target for Multiple Solid Tumors

- mRNA Expression in Various Cancers
- Specific Killing of Multiple Tumor HLA-A2+ / MAGE-A4+ Cell Lines by CD3-TCRL Bispecific Compound
- 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.

Summary

- Two HLA-A*02-restricted target peptides: Tyrosinase 369-377 (Tyr) and MAGE-A432-239 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.