Intracellular Targets made druggable by TCR-like Antibodies, TCR Fusion Proteins & Cell-Penetrating Biologics 2018:
an industry analysis of technologies, stakeholders, deals & trends

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readouts that will allow for not just binding but signaling to be used to identify the most relevant therapeutic leads.

Single variable domain T-cell receptors (svd-TCRs)

In December 2015, ITS launched single variable domain T-cell receptors (svd-TCRs), a novel therapeutic modality for the purposes of targeting MHC/peptide complexes (Press Release Dec 3, 2015). ITS has previously used its HuTARG™ platform to generate fully human TCR-like monoclonal antibodies (T-MAbs) and fully human TCRs that bind to specific MHC/peptide complexes. Now the company has applied its HuTARG platform to the de novo generation of large repertoires of svd-TCRs for drug discovery and has isolated first-in-class svd-TCRs specific to MHC complexes that contain the NY-ESO peptide. Svd-TCRs are small single domain molecules that overcome the challenges of utilizing natural heterodimeric T-cell receptors; the scaffold can be easily engineered as a fusion protein or engineered as a chimeric antigen receptor (CAR). Not only will svd-TCRs have utility in T-cell therapies for cancer but also for T-cell redirected applications and future applications where specific cell types are targeted to sites of inflammation or healing to modify the disease process.

ITS believes that its mammalian cell-based platform gives it an unique competitive advantage for cell based therapies; the HuTARG technology generates large de novo repertoires from a single chromosomal site with high and uniform expression allowing large numbers of drug candidates to be assessed simultaneously in the context of a mammalian cell. As diversity is generated within the cell, the cloning of and manipulation of large recombinant libraries is no longer required. In addition to making it dramatically easier to generate diverse repertoires the HuTARG platform also has the advantage that scaffolds are expressed in a mammalian system allowing for high expression and manufacturability to be evaluated as part of all screens.

4.4.5 MAR (MHC Antigen Receptor) and TriTE Technology

Chinese company Timmune Biotech is using proprietary **MAR** (MHC Antigen Receptor) technology as an antibody technology for MHC antigen targeting. MAR antibodies represent a class of fully human single domain antibodies that bind to MHC antigen complex with the same binding mechanism as TCR. MAR is also called "TCR-like antibody". By binding to MHC antigen complexes, MAR can be used to target at intra-cellular targets. Timmune has generated a series of MAR antibodies including MARs binding to WT1, gp100, NY-ESO-1, and MAGE-A3.

Initially, the scientists developed a T cell receptor (TCR) -like **murine** IgG2a monoclonal antibody, m8F4, which binds to the PR1 peptide/HLA-A2 complex, and targets both HLA-A2+ leukemia cell lines and primary HLA-A2+ patient blasts of various AML subtypes.

PR1 (VLQELNVTV) is a human leukocyte antigen-A2 (HLA-A2)-restricted leukemia-associated peptide from proteinase 3 (P3) and neutrophil elastase (NE) that is recognized by PR1-specific cytotoxic T lymphocytes that contribute to cytogenetic remission of acute myeloid leukemia (AML). Murine 8F4 binds with high specific binding affinity (dissociation constant K_D = 9.9 nM) to a conformational epitope of PR1/HLA-A2, with contact residues near P1 of the PR1 peptide and the N-terminus of the α2-helical domain of HLA-A2 (Sergeeva, 2011). Flow cytometry and confocal microscopy of 8F4-labeled cells showed significantly higher PR1/HLA-A2 expression on AML blasts compared with normal leukocytes (P = .046). The high affinity of 8F4 for PR1/HLA-A2 was attributed to its generation by direct immunization instead of use of Fab or scFv antibody phage-display libraries (relatively low affinities).

To generate mouse anti-PR1/HLA-A2 monoclonal antibodies, PR1 (VLQELNVTV) had been refolded with recombinant HLA-A2 and β2-microglobulin. Two 6-week-old mice were injected subcutaneously or intraperitoneally with a 300-μL suspension composed of 20 μg of purified PR1/HLA-A2 monomer mixed with either 12 μg of AbISCO-100 adjuvant or complete Freund adjuvant. The mice were immunized at 2-week intervals for a total of 4 times by intraperitoneal injection of antigen plus adjuvant, followed by an intraperitoneal injection of antigen alone 3 days before harvest of splenocytes. Three days before the final boost, serum was tested for polyclonal immune response using ELISA. Clones (n = 950) were isolated by limiting dilution. After screening with parallel ELISA, selected clones were transferred to 24-well plates and grown in DMEM containing 4mM l-glutamine, 20% fetal bovine serum, and 10% hybridoma cloning factor.

Sergeeva et al. (2016b) also explored whether 8F4 was active in vivo against chemotherapy-resistant AML, including secondary AML. In a screening model, coincubation of AML with 8F4 ex vivo prevented engraftment of all tested AML subtypes in immunodeficient NSG (NOD scid IL-2 receptor γ-chain knockout) mice. In a treatment model of established human AML, administration of 8F4 significantly reduced or eliminated AML xenografts and extended survival compared with isotype antibody-treated mice. Moreover, in secondary transfer experiments, mice

(neo)epitopes via the human leukocyte antigen (HLA) complex on the cancer cell surface, without harming healthy tissue.

The Morphosys technologies include bispecific platforms for oncology applications. The anti-CD3 bispecific format utilizes Morphosys‘ proprietary anti-CD3 binding moieties. The anti-4-1BB bispecific format selectively stimulates natural killer (NK) and tumor specific T-cells within the tumor environment by using proprietary 4-1BB binding moieties.

6.3.4 Pure MHC

Austin, Texas, based Pure MHC is a a platform technology company funded and managed by Emergent Technologies with expertise in disease-specific target identification and generation of T-cell receptor mimic antibodies. The Pure MHC target discovery technology was developed by Chief Scientist, William Hildebrand of the University of Oklahoma Health Sciences Center. He founded two additional subsidiary companies, Pure Transplant and Pure Vaccine Solutions in 2003 which, when merged with the technology of Receptor Logic in 2013, gave rise to the creation of Pure MHC, LLC. The “Pure” companies were founded on the principle that a ready source of native HLA proteins can contribute to modulating and understanding autoimmune, transplant, allergy, pathogen, and vaccine-derived immune responses. Pure MHC is closeley related with Pure Protein, founded in 1999 and led by William Hildebrand, PhD. Pure Protein has technology to manufacture soluble HLA proteins that are used in a proprietary method of novel immunogenic epitope discovery and validation. By the merge with with Receptor Logic, Pure MHC also holds IP on a proprietary method for generating T-cell receptor mimics from defined epitopes. Together, these technologies provide the capability to identify novel disease targets, validate “Usual Suspect” targets, and subsequently “drug” those same targets with monoclonal antibodies (T cell receptor (TCRm)) and other immunotherapeutic modalities. Such modalities include antibody drug conjugates and bispecific antibodies, T cell eliciting vaccines, and via T cells expressing a chimeric antigen receptor derived from the TCRm.

Soluble HLA (sHLA) is Pure MHC’s key enabling technology that allows for the expression of a pure source of HLA in a diseased cell of choice. Using sensitive mass spectroscopy techniques referred to as **Deep Ligand Sequencing**, Pure MHC compares thousands of peptides recovered from HLA complexes extracted from cancer, infected, and normal cells and then determines with high confidence which peptides are unique (and therefore represent novel disease targets) to the

tumor antigens of less than US$ 50 mln. Adicet Bio acquired technology and manpower from Applied Immune Technologies (AIT) for less than US$ 50 mln.

Table 25: Acquisition of Technology Companies in the Field

Acquiring Company	Acquired Company	Year	Technology & Assets	Terms
Adicet Bio	Applied Immune Technologies	2016	EpiTarget for IC target discovery; TCRL mAbs	nd
Agenus	PhosImmune	2015	Phosphopeptide tumor antigen library	$ 9.9 mln upfront cash + stock +< $35 mln M
NantCell	Altor BioScience	2017	TCR-Il-2 fusion protein + Superantigen technology	$ 290 mln mainly in stock
Juno Therapeutics	AbVitro	2016	Single cell sequencing platform to discover TCRs	$ 78 mln cash + $ 45 mln stock
Kite Pharma	T Cell Factory	2015	TCR-GENErator	$ 24 mln upfront + M

IC, intracellular; M, milestones

AIT can discover novel intracellular targets with the EpiTarget technology and generate TCR-like antibodies. T-cell companies Juno Therapeutics and Kite Pharma also acquired companies with technology to discover and generate T-cell receptors.

Many of technology companies in the field are early stage and in need of financing indicating a low value for a potential acquisition.

7.3 Collaboration and Licensing Deals between Technology Companies

Typical collaborations of technology companies are between one company which has intracellular targets and a second company with the complementary technology to generate a therapeutic against it, as shown in Table 26. A preferred partner for providing intracellular targets is immatics which has the industry's most productive intracellular target discovery technology XPRESIDENT.

Two different examples of companies with complementary technologies are the collaboration between Feldan and Elasmogen, where one company has the delivery vehicle to pass the cell membrane and the other company the specific binding molecule against challenging targets. This pattern can also be seen in the collaboration between Patrys and the Walter and Eliza Hall Institute of Medical Research.

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