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Human anti-MAGEA Antibodies Derived from Tertiary Lymphoid Structures of Cancer Patients

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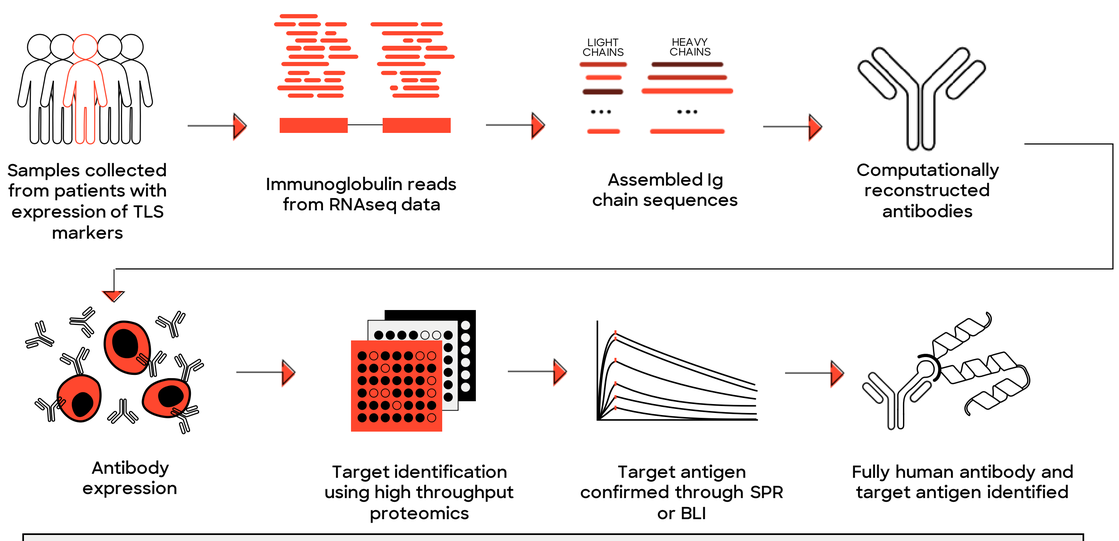
The identification of novel, disease-relevant targets is a tedious and lengthy process. This notion has steered therapeutic development of biologics against a limited set of targets. To date, over 160 antibodies have been approved but most are restricted to around 50 targets. The development of antibodies with low immunogenicity, is by itself, challenging, but paired with the significant burden of target validation has led to a field saturated with antibodies to the same targets. This highlights the current clinical need to discover novel targets by a more thorough screening the human antibody repertoire. We have developed a reverse immunology platform that relies on machine learning-guided reconstruction of antibodies from diseased tissue sources, such as tertiary lymphoid structures (TLS) derived from patient samples with exceptional outcomes in cancer, auto-immune, and infectious diseases. Using immune transcriptomics and artificial intelligence, our reverse immunology pipeline can reconstruct fully human antibodies directly from patient samples by combining heavy and light chains of antibodies isolated from TLS within affected tissue. Reconstructed TLS-derived antibodies are then screened against an array of human proteins to identify their respective targets. Following reconstruction, potential antibodies are de-orphaned by high-throughput screening against thousands of antigens simultaneously. This approach has the dual benefits of identifying promising new targets paired with a corresponding fully human antibody with significant therapeutic potential. Here, we demonstrate the power of our reverse immunology platform by identifying and validating fully human antibodies against melanoma-associated antigens (MAGE), a subset of the cancer-testis antigen (CTA) family that are aberrantly expressed in tumors. Using this method, we were able to identify a MAGEA3/MAGEA4/MAGEA6 specific antibody with high affinity. MAGEA expression has been demonstrated in a range of tumors and is associated with poor prognosis in melanoma as well as non-small cell lung cancer. Anti-MAGEA therapeutics in clinical trials have shown promising results, however; further development is needed to reduce cross-reactivity against non-malignant proteins. Our modified asset displayed a favorable cross-reactivity profile, binding to soluble MAGEA3/MAGEA4/MAGEA6 with low nanomolar/picomolar affinity and a linear epitope.

ABSCI REVERSE IMMUNOLOGY PIPELINE

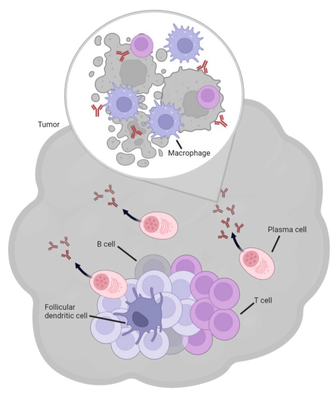
Our workflow identifies antigens targeted by antibodies produced in TLS.



Fully human antibodies can be further developed as next-generation biologics using our AI and synthetic biology Integrated Drug Creation™ platform.

TLS: THE NEXT SOURCE FOR DRUG DISCOVERY INSPIRATION

- **Tertiary Lymphoid Structures (TLS)** are centers of immune activity (B-cell proliferation and antibody production) that develop in chronically inflamed tissues such as tumors[1,2]
- Rapidly growing evidence illustrates correlation between **TLS** antibodies in the tumor microenvironment and positive clinical outcomes[1,2]
- The presence of **TLS** is associated with longer progression-free survival and better response to immune checkpoint inhibitors[1]
- Antibodies from **TLS** have been shown to trigger apoptosis of cancer cells in patients[1]



Absci is the first company utilizing TLS to discover therapeutically relevant antibodies and potential new disease modifying targets.

Absci has generated **the largest library of TLS-derived antibodies and antigen targets.**

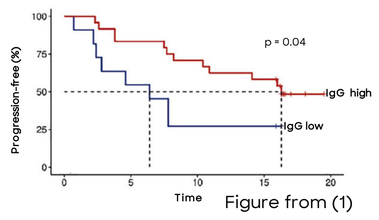
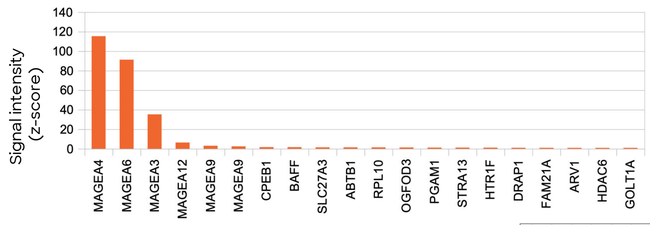


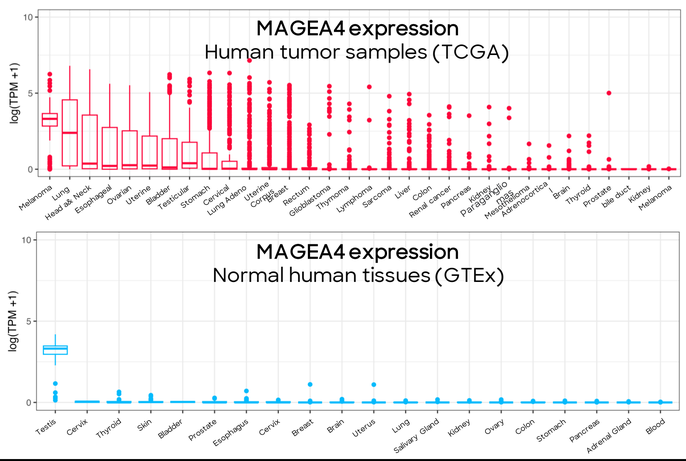
Figure from (1)

INITIAL DE-ORPHANING PROFILE AND MAGE EXPRESSION IN CANCER



Out of hundreds of proteins tested, MAGEA family members predominate the signal during the de-orphaning assay.

MAGEA family members are selectively expressed in many different tumor tissue types and have shown promising results in Anti-MAGEA targeted clinical trials[3,4].



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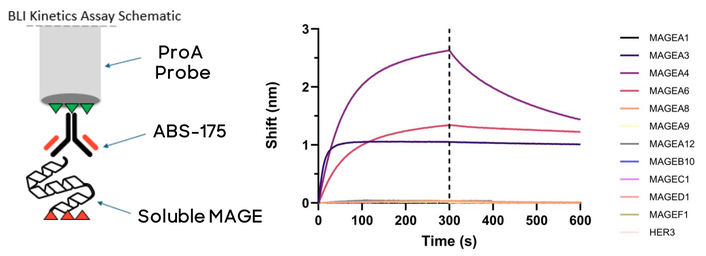
2. Zhang & Wu. “Tertiary lymphoid structures are critical for cancer prognosis and therapeutic response.” *Front. Immunol 13* (2023). DOI: 10.3389/fimmu.2022.1063711
3. Alsalloum A, Shevchenko JA, Sennikov S. “The Melanoma-Associated Antigen Family A (MAGE-A): A Promising Target for Cancer Immunotherapy?” *Cancers* 15.1779 (2023). DOI: 10.3390/cancers15061779
4. Sanderson, Joseph P, et al. “Preclinical evaluation of an affinity-enhanced MAGE-A4-specific T-cell receptor for adoptive T-cell therapy” *OncoImmunology* 9.1 (2020). DOI: 10.1080/2162402X.2019.1682381

TARGET VALIDATION INDICATES HIGH AFFINITY TO SEVERAL MAGEA FAMILY MEMBERS

TLS-derived antibody is cross-reactive against MAGEA3, MAGEA4, & MAGEA6

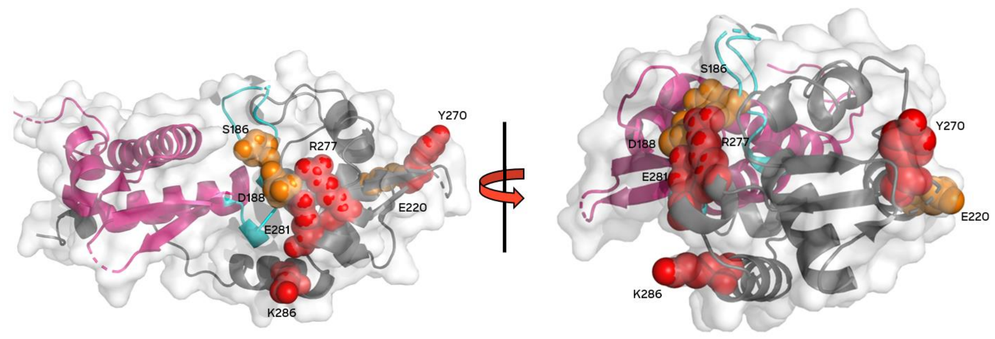


Ligand	Analyte	KD (nM)	R^2	X^2
ABS-175	MAGEA3	0.419	0.999	1.09
ABS-175	MAGEA4	10.2	0.998	9.69
ABS-175	MAGEA6	11.7	0.989	8.22

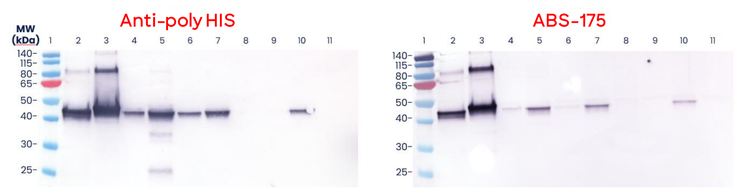
ABS-175 exhibited low nanomolar affinity to MAGEA4/MAGEA6 and picomolar affinity to MAGEA3 by BLI. Asset did not show cross-reactivity against other MAGE family proteins or negative controls tested by BLI (300 nM antigen concentration). Weak affinity was noted to MAGEA8 and MAGEA12 by reduced western blot (~1uM, data not shown).

EPITOPE LIES WITHIN RESIDUES 270-290 OF THE MAGE HOMOLOGY DOMAIN

Alanine scanning of surface-exposed residues revealed critical residues across the MAGEA4 molecule. Putative epitope residues are mostly clustered within the 270-290 amino acid range.



Dual western blots verified surface exposed epitope residues indicated by alanine scanning BLI

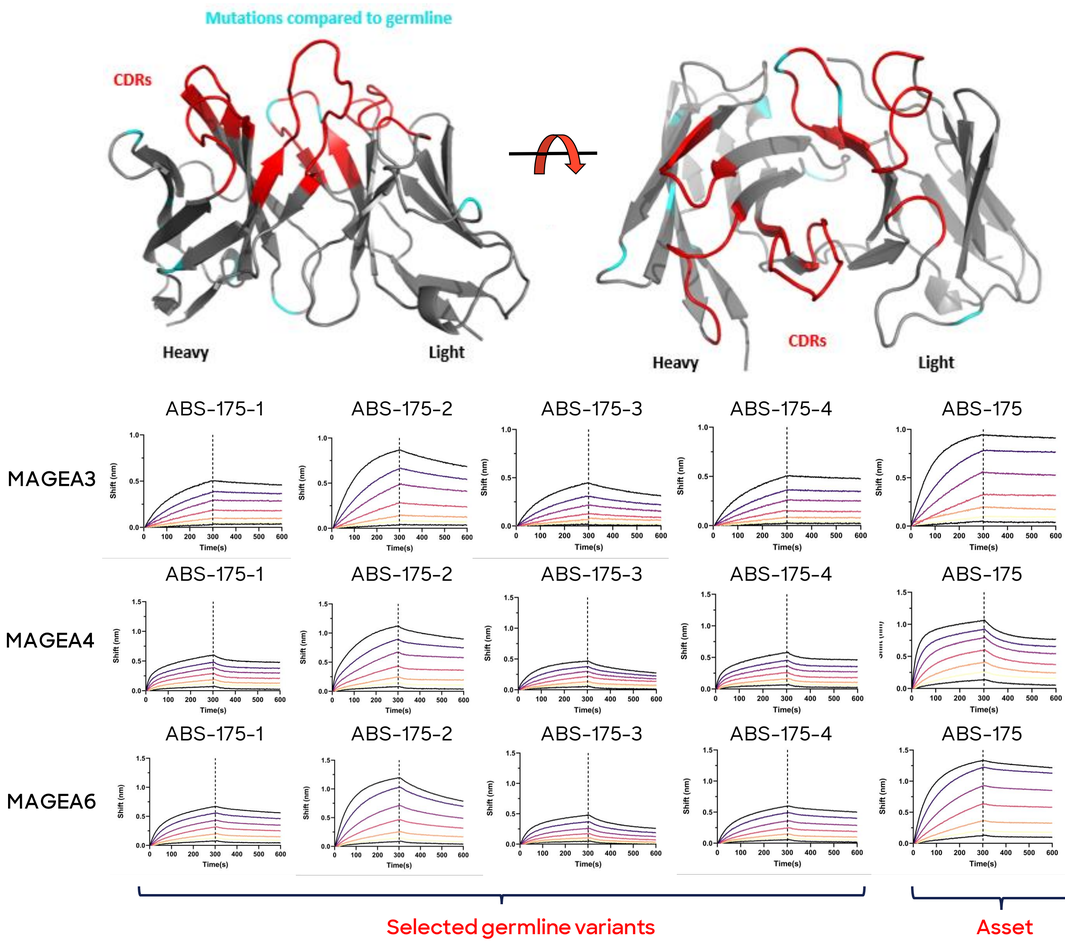


Lane	Sample	Anti-HIS Bind?	ABS-175 Bind?
1	MW Lad	n/a	n/a
2	MAGEA4 - in house	YES	YES
3	MAGEA4 - commercial	YES	YES
4	MAGEA - Y270A	YES	YES/WEAK
5	MAGEA - R277A	YES	YES
6	MAGEA - E281A	YES	YES/WEAK
7	MAGEA - K286A	YES	YES
8	MAGEA - R293A	NO	NO
9	MAGEA - R299A	NO	NO
10	MAGEA - S304A	YES	YES/WEAK
11	MAGEA - E312A	NO	NO

Selected alanine scanning mutants were electrophoresed under reducing conditions then probed with both an anti-poly histidine - HRP conjugated antibody (left) and ABS-175 followed by an anti-human IgG - HRP conjugated antibody (right). Presence of an ~40 kDa indicates presence of MAGEA4 or alanine scanning mutant.

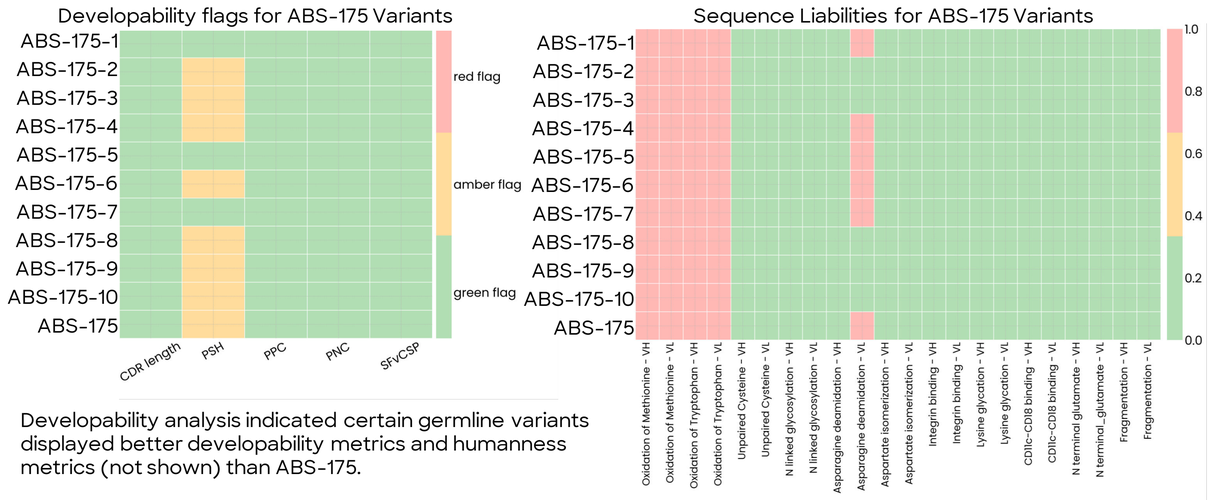
REVERSION OF RNA-SEQ ERRORS TO GERMLINE RESIDUES PRESERVES BINDING

LC and HC mutations reside in hotspots that can alter stability and binding



Potential mutations likely caused by RNA-seq errors rather than somatic hypermutation were noted in the HC FWR and LC FWR/CDR regions of ABS-175. Ten variants of ABS-175 were generated by reverting residues back to germline and retained similar binding profiles by BLI, with marginal impacts on KD against MAGEA4/MAGEA6 and greater impact on KD against MAGEA3.

GERMLINE ENGINEERING RESULTS IN BETTER DEVELOPABILITY AND HUMANNESS METRICS



Developability analysis indicated certain germline variants displayed better developability metrics and humanness metrics (not shown) than ABS-175.