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Original Research Article

CellCelector™ as a platform in isolating primary B cells for antibody discovery

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ABSTRACT

The most robust strategy in antibody discovery is the use of immunized animals and the ability to isolate and immortalize immune B-cells to hybridoma for further interrogation. However, capturing the full repertoire of an immunized animal is labor intensive, time consuming and limited in throughput. Therefore, techniques to directly mine the antibody repertoire of primary B-cells are of great importance in antibody discovery. In the current study, we present a method to isolate individual antigen-specific primary B-cells using the CellCellector™ single-cell isolation platform from XenoMouse® (XM) immunized with a recombinant therapeutic protein, EGFR. We screened a subset of CD138+ B-cells and identified 238 potential EGFR-specific B-cells from 1189 antibody-secreting cells (ASCs) and isolated 94 by CellCellector. We identified a diverse set of heavy chain complementarity-determining region sequences and cloned and expressed 20 into a standard human immunoglobulin G1 antibody format. We further characterized and identified 13 recombinant antibodies that engage soluble and native forms of EGFR. By extrapolating the method to all 400 000 CD138+ B-cells extracted from one EGFR immunized XM, a potential 1196 unique EGFR-specific antibodies could be discovered. CellCelector allows for interrogating the B-cell pool directly and isolating B-cells specific to the therapeutic target of interest. Furthermore, antibody sequences recovered from isolated B-cells engage the native and recombinant target, demonstrating the CellCellector can serve as a platform in antibody discovery.

Statement of Significance: Several tools allow for the interrogation of B-cells; however, they are custom made, require specialists to operate and can be expensive to be broadly utilized. We demonstrated the CellCelector™ platform can rapidly screen and isolate EGFR-specific B-cells and validated antibodies for specificity to native and soluble forms of EGFR.

KEYWORDS: antibody discovery; CellCelector™; B-cell; ASC; antibody generation

INTRODUCTION

A main source of monoclonal antibodies for research and therapeutic development relies on hybridoma technology. Primary B-cells, which are short lived and a challenge to culture, are fused with myeloma cells to generate immortal hybridoma cells that continuously secret antibodies [1, 2]. The hybridoma process generally requires extensive cell culture, substantial laboratory space and multiple operators, thus making the process labor intensive, time consuming and costly. Additionally, 1 out of 5000 B-cells survive fusion, become immortalized and secrete antibody using optimized electrofusion protocols [3]. By interrogating B-cells directly, the drawbacks of hybridoma technology can be overcome.

Sources of antibody repertoires include human donors [4], animal hosts [5] and (of significance for therapeutic discovery) engineered animals with human antibody sequences [6]. One of the first fully human transgenic

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antibody-generating animals, XenoMouse®, has demonstrated its impact through the approval of panitumumab (Vectibix®), Evolocumab (Repatha®) and Denosumab (Prolia®/Xgeva®) [7, 8]. XenoMouse®-derived antibody sequences reveal a broad and diverse utilization of different V, D and J genes similar to their utilization in humans [9, 10]. The utilization of the large human repertoire in XenoMouse® is manifested by the production of antigen-specific, high-affinity human antibodies, 10^{-9} to 10^{-11} M, to numerous antigens with broad mechanisms of action [7], including IL13 [11], β-Klotho [12] and TRAILR2 [13].

Several tools are available for direct B-cell interrogation of their antigen specificity using custom microfluidic chambers [14], microencapsulation [4, 15], custom microwell devices [16, 17] and more recently nanofluidic optoelectronic technique built on the Beacon™ platform [18]. These methods allow for the direct isolation and screening of single B-cells, without immortalization or library generation. Antibody sequences are generally recovered using single-cell polymerase chain reaction (PCR) [19] or barcode-based next-generation sequencing followed by recombinant cloning and expression [20]. Disadvantages of these tools are that they are custom made, require specialists to operate and in the case with the Beacon™ platform expensive to be broadly utilized in antibody discovery.

In this report, we describe a high-throughput approach that allows for the combined screening of the phenotype and antigen specificity of antibodies secreted from primary B-cells. In this approach, B-cells are deposited into CellCelector nanowells with sub-nanoliter (800 pL) volume. The B-cells are interrogated for EGFR specificity and immunoglobulin (Ig) secretion. In combination with automated fluorescent microscope, EGFR-positive B-cells are identified and retrieved by automated micromanipulation (picking). Single-cell real time (RT) PCR is performed to amplify Ig variable heavy and light chain (VH:VL) genes, which are then cloned into an expression vector and expressed for validation for EGFR specificity. This methodology provides a fast, efficient and economical platform for isolation and validation of antigen-specific antibodies.

MATERIALS AND METHODS

EGFR immunization, ASC harvest and enrichment

For this study, extracellular portion of human EGFR (amino acid sequence 25-645 conjugated 6xHis) was used as immunogen and protein for BLI characterization. Mice were housed in groups at a facility that has received a Certificate of Good Animal Practice from the Canadian Council on Animal Care (CCAC). Animals were cared for in accordance with the CCAC Guidelines. All research protocols were reviewed and approved by the Amgen Institutional Animal Care and Use Committee. Three XenoMouse® animals were immunized with decreasing concentrations of antigen spaced over 4.5 months; Boost 1 consisted of 50 μg of antigen emulsified in CFA delivered subcutaneously. After 14 days, the mice received a second boost with 25 μg of antigen emulsified in Sigma Adjuvant System (Sigma Aldrich) delivered half intraperitoneal and half sub-cutaneous. A third boost of 12.5 μg of antigen emulsified in Sigma Adjuvant system was delivered 14 days later, half intraperitoneal and half sub-cutaneous. The mice remained dormant for 3 months and then received two boosts containing 5 μg of antigen in Sigma Adjuvant System spaced 1 week apart. A final boost of 25 μgs of antigen was delivered half intraperitoneal and half sub-cutaneous in the absence of adjuvant. Mice were euthanized and spleens and lymph nodes were harvested for B-cell processing.

The spleen and lymph nodes were processed into a single-cell suspension using a GentleMACS cell dissociator (Miltenyi Biotech). The RBCs were lysed using Pharmlyse buffer (BD Bioscience) according to the manufacturer's instructions. Non-B-cells and IgM expressing B-cells were removed by magnetic depletion as described previously [18]. CD138+ B-cells were isolated using an EasySep™ Mouse CD138 Positive Selection kit (Stemcell Technologies). The isolated B-cells were then counted for addition to nano-well plate.

B-cell loading and EGFR-positive identification on CellCelector

A six-well plate with 60 000 nanowells per well was primed with 100% ethanol and centrifuged for 2 min at 700 g to remove residual air micro bubbles formed in the nanowells. Ethanol was removed, leaving enough liquid covering the surface (1–2 mL), and washed with 3 mL of phosphate-buffered saline (PBS) (added and centrifuged for 2 min at 700 g). The nanowells were washed with PBS four more times and with culture media (IMDM (Gibco, #12440-053) supplemented with 15% Super Low immunoglobulin (IgG) fetal bovine serum (FBS) (HyClone, #SH30898-03), HEPES (Gibco, #15630-080), Sodium Pyruvate (Gibco, #11360-070), Pen-step-Glut (Gibco, #10378-016), MEM-NEAA (Gibco, #11140-050), 2-ME (Gibco, #21985-023) and Hybridoma Fusion and Cloning Supplement (Roche, #11363735001)) three times. A suspension of B-cells (60×10^3 cells/mL), Gt anti-Human IgG (H + L) Polystyrene (3.12 um) beads (1.2×10^6 beads/mL) (Spherotech, #HUP-30-5), Goat anti-Human IgG-AlexaFluor 647 (AF647) (Jackson ImmunoResearch, #109-605-098) (5 μg/mL), biotinylated-EGFR (5 μg/mL) and Streptavidin AlexaFluor 488 (AF488) (5 μg/mL) was prepared in culture media. One milliliter of the suspension was dispensed dropwise across one well of the six-well plate. Cells settled for 5 min at RT and centrifuged for 5 min at 300 g. Cells were incubated for 4 h at 37°C, 5% CO_2. Cells were imaged on the CellCelector platform, identified for IgG expression ($AF647^+$) and antigen binding ($AF488^+$), picked and dispensed into a well of a 96-well PCR plate (Axygen, #PCR-96-HS-AC-C) containing 5 μL lysate buffer (TCL buffer (Qiagen, #1031576) and 100-fold dilution of 0.5 M DTT (ThermoFisher, #R0861)) and 10 μL QX Mineral Oil (Qiagen, #929605). Cells were frozen at −80°C for molecular recovery.

Molecular recovery of antibody heavy and light chains

RNA was purified using Agencourt RNA Clean XP kit and eluted directly into a 9 μL RT reaction with Maxima

RNaseH minus RT. A RACE-A dT primer with adaptor (P1, biot-AAGCAGTGGTATCAACGCAGAGTACTTT TTTTTTTTTTTTTTTTTTTTTTTTTTTVN) and a 5′ template switching primer (P2, Biot-GTGGTATCAACGC AGAGTACACGACGCTCTTCCGATCTrGrGrG) were used in the RT reaction. The RT reaction was incubated at 42°C for 90 min, followed by 10 cycles of 50°C for 2 min followed by 42°C for 2 min, then heat-inactivated at 75°C for 15 min and held at 4 °C. The 9 μL RT reaction product was added to a PCR mix using KAPA HiFi HotStart Ready mix with primer P3 (biot-AAGCAGTGGTATCAACGCAGAGT) in a reaction, amplifying the complementary DNA (cDNA) in 30 μL total. The cDNA amplification was performed at 98°C for 3 min, followed by 20 cycles of 98°C for 15 s, 65°C for 30 s, 72°C for 6 min, with a final incubation at 72°C for 10 min, and held at 4°C. PCR clean-up was performed using the Agencourt AMPure PCR kit. Proprietary beads were used to wash and remove PCR contaminants and the cDNA amplification product was eluted in 15 μL of water. A 1 μL portion of the cDNA amplification product was either used to sequence the gamma and light chains or generate amplicons for cloning into the bidirectional vector using primers specific to the antibody constant regions. PCR conditions were 98°C for 3 min, followed by five cycles of 98°C for 20 s, 65°C for 45 s, 72°C for 45 s, 10 cycles of 98°C for 20 s, 60°C for 45 s, 72°C for 45 s, 10 cycles of 98°C for 20 s, 58°C for 45 s, 72°C for 45 s and final extension of 5 min at 72°C, and held at 4°C. Sequencing was done at Genewiz and analyzed using in-house software.

Construction of expression vector: Golden gate assembly

Heavy and lights chain amplicons with Esp3I restriction sites were subjected to Golden Gate assembly. A total of 20 ng of each vector fragment was mixed with 0.5 μL T4 ligase (Invitrogen, #15224017), 0.5 μL Esp3I (Invitrogen, #ER0452) and 1 μL of Fast Digest Buffer 10x (Invitrogen, #ER0452) to which ATP is added at a final concentration of 5 μM. Water is added until a final volume of 10 μL. PCR conditions followed 30 cycles of 37°C for 2 min, 16°C for 3 min, 37°C for 5 min, then heat-inactivated at 80°C for 5 min and held at 4°C. A total of 1.5 μL of the reaction mixture is transformed into 15 μL of TOP10 competent bacteria (Lucigen, #60107-1). The bacteria are placed on ice for 30 min, heat-shocked for 40 s at 42°C and placed on ice for 3 min. A total of 950 μL of SOC-rich media is added to the bacterial culture and incubated at 37°C for 1 h under agitation. A total of 100 μL of the culture is plated on Carbenicillin-X-Gal agar plates (Teknova, #L2906) and incubated overnight.

Antibody quantification

Antibodies in 293 T expression media were quantified using the Octet® HTX instrument. An IgG1 standard was prepared at 100 μg/mL in 293 T expression media and diluted two-fold to 1.5625 μg/mL. The IgG1 standard was run in triplicate following the quantification standard method on the Octet® instrument. Samples with unknown concentrations were run neat. For quantification analysis, the ForteBio Data Analysis v11.0 software was used.

Affinity assay using BLI

The Octet® HTX instrument was used to determine affinities of EGFR antibodies. Prior to the binding measurements, the sensor tips were pre-hydrated for 10 min in Octet running buffer containing 10 mM Tris (Fisher, #BP152-1), 150 mM NaCl (Fisher, #S271-10), 1 mM $CaCl_2$ (Fisher, #BP510-500), 0.1 mg/mL BSA (BioShop, #ALB001.1) and 0.1% Triton-X 100 (Calbiochem, #9410-OP), pH 7.4. Streptavidin (SA) biosensors (ForteBio, #18-5021) were used to load biotinylated anti-human Fc antibody (Invitrogen, #A18827) followed by one baseline step of 60 s in Octet running buffer. Antibodies were captured by the anti-human Fc antibody and submerged in running buffer for 60 s prior to being placed in wells containing EGFR at concentrations ranging from 1.5625 to 100 nM for 10 min and followed by 10 min of dissociation time in Octet running buffer. Antibodies and antigen concentrations were all prepared in Octet running buffer. SA biosensors were used once without regeneration. For data evaluation, the ForteBio Data Analysis v11.0 software was used. The kinetic rate constants, association rate constant (ka, M^{-1} s^{-1}), dissociation rate constant (kd, s^{-1}) and the equilibrium rate constant (KD, M) were determined using a 1:1 Langmuir model.

Binding assay by flow cytometry

Binding was performed on CHO cells transfected with EGFR. CHO cells were suspended in 10 mL of High Glucose DMEM culture media (Gibco, #21068-028) at 1 × 10^6 cells/mL. Cells acclimated to the new media for 3 h at 37°C, 5% CO2, 95% humidity. For 10 mL of CHO cells, a total of 10 μg of vector DNA was prepared with 10 μL of 3 mg/mL of PEI MAX™ (Polysciences Inc, #24765-2) in a total of 0.5 mL of culture media. Cells were incubated for 3 h at 37°C, 5% CO2, 95% humidity before adding sodium butyrate to a final concentration of 5 mM and incubated ON. The following day, CHO cells were centrifuged at 2000 rpm for 2 min, supernatant removed and resuspended to 1 × 10^6 cells/mL in FACS buffer (PBS (HyClone, #SH30256.02), 2% FBS (Sigma, #F2442)). To a V-bottom assay plate (Costar, #3897), 50 μL of CHO suspension (final 50 000 cells/well) and 50 μL of test antibody were added to each well and incubated for 1 h at 4°C. CHO cells were washed twice with 150 μL FACS buffer and 50 μL of secondary solution (5 μg/mL Goat α Human-AlexaFluor 647 (Jackson ImmunoResearch, #109-605-098) and 2.5 μg/mL 7AAD (Sigma-Aldrich, #A9400-5MG) in FACS buffer) was added and were incubated for 1 h at 4°C. CHO cells were washed twice with 150 μL FACS buffer and resuspended in 70 μL of FACS buffer and run using iQue 2. Data were analyzed using FCS Express 7.

RESULTS

Isolation of EGFR-specific B-cells

To demonstrate the utility of the CellCelector to identify therapeutically relevant antibodies, we used EGFR as our model antigen. EGFR is a well-known target in cancer therapy with known anti-EGFR therapeutic antibodies in the clinic, such as cetuximab, panitumumab,

nimotuzumab and necitumumab [21]. We obtained two million antibody-secreting cells (ASCs), CD138 positive, from three mice immunized with the ECD of EGFR that demonstrated titers to EGFR (Supporting Fig. 1). The expression of CD138 (Syndecan-1) has been used to broadly define the ASC population [22]. We loaded 60 000 cells into one well of a six-well plate containing approximately 60 000 nanowells/well, to achieve an average occupancy of one cell per nanowell. A mixture of anti-human IgG Fc specific capture beads, an anti-human IgG conjugated Alexa Fluor 647 (AF647), biotinylated EGFR and Alexa Fluor 488 (AF488)–conjugated streptavidin was incubated with cells. We scanned a total of 52 000 nanowells in brightfield and fluorescence to identify nanowells containing B-cells that secret antibodies ($AF647^+$) and are positive for binding to EGFR ($AF488^+$). We identified 1189 nanowells containing ASCs (IgG-AF647 positive) and 238 nanowells containing EGFR-specific ASCs (AF488 and AF647 double positive). We selected a representative sampling and exported 94 EGFR-positive ASCs for molecular recovery of IgG heavy and light chains (Fig. 1, Supporting Figs 2 and 3).

VH and VL recovery, cloning and recombinant expression

To sequence IgG heavy and light chains, a Rapid Amplification of cDNA Ends (RACE) RT-PCR protocol was performed to obtain sufficient cDNA for heavy and light chain amplification. Separate reactions were performed for heavy, kappa and lambda chains. We recovered 84 heavy chain sequences (89% recovery rate) and 74 wells with light chain sequences (79% recovery rate). Of the 74 wells with light chain sequences 57 were kappa and 53 lambda, indicating the presence of more than one B-cell in some nanowells. We constrained our analysis and focused on nanowells that contained single cells to eliminate the recovery of noncognate VH and VL pairs caused by multiple ASCs within a single nanowell. We identified 35 potential EGFR-specific antibody sequences that utilized diverse germline segments (Supporting Fig. 4). From nucleotide sequence alignment of all 35 sequences, we found two pairs of heavy chains sequences that were identical (Clones B07 + E08 and E07 + F04), one pair of heavy chains that were different by 1 amino acid (Clones C05 + H09) and one triplicate of heavy chains identical in sequence (Clones E09 + E10 + G07). However, light chains were different among identical heavy chain sequence pairs, and of the triplicate HC sequences, E09 and G07 utilized almost identical lambda light chains (different in 1 amino acid and 1 nucleotide base) and E10 utilizing a kappa light chain. Thus, by screening B-cells directly, we obtained unique sequences allowing for the interrogation of a repertoire without fast replicating clones potentially narrowing diversity, as what may occur with hybridoma. We successfully cloned, expressed and purified 20 unique antibody sequences (Fig. 2). These 20 sequences were cloned into an expression vector using a human IgG1 isotype, transiently transfected into human embryonic kidney 293 T cells and harvested after 5 days the antibody containing culture media. The supernatant was quantified using biolayer interferometry

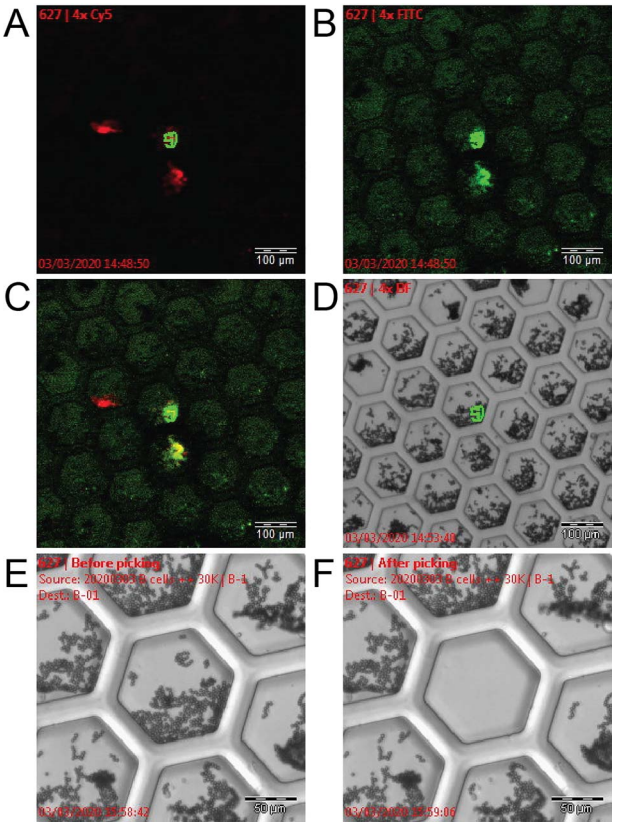


Figure 1. Representative fluorescent and brightfield images of an $EGFR^+$ ASC to be picked from a nanowell and deposited into well-B1 of a 96-well PCR plate containing lysis buffer for molecular recovery. Images are centered and a green outline is auto-assigned to the well and B-cell to be picked. (A) Image of fluorescence specific 647 nm emission (RED); it is observed two neighboring wells contain ASCs. (B) Image of fluorescence specific 488 nm emission (GREEN); it is observed one neighboring well contains an ASC specific to EGFR. (C) Overlay of images from both 647 and 488 nm channels and (D) brightfield image used to pick an $EGFR^+$ ASC. Brightfield images (E) before and (F) after an $EGFR^+$ ASC is picked from nanowell. Brightfield images demonstrate all contents within the nanowell are picked. Images C-F for all 94 $EGFR^+$ ASC clones picked can be found in Supporting Figs 2 and 3.

(BLI) and concentrations were determined to range from 22 to 398 mg/L (Supporting Table 1). The 20 antibodies were used for further characterization without further purification.

Characterization of recombinant antibodies to EGFR

The 20 expressed antibodies were assayed for antigen-specific binding using flow cytometry and for affinity to recombinant monomeric EGFR using BLI. Flow cytometry was performed using binding to EGFR transiently expressed on CHO cells. In this assay, 10 antibodies specifically bound EGFR-CHO cells with greater than 100-fold geomean shift over parental CHO cells, three antibodies bound 3.5 to 15.6-fold more to EGFR-CHO and the remaining seven antibodies bound to EGFR-CHO cells less than two-fold over parental CHO (Fig. 3A). Next, we determine affinities of antibodies to EGFR titrated from ~1.5 to 100 nM. Similarly, to flow cytometry

A

Antibody Name (Well ID)	VH Germline	VK Germline
A04	VH3|3-21/D2|2-15|RF2/JH6	VL3|3r/JL2
B01	VH1|1-08/D5|5-18|RF3/JH3	VK4|B3/JK4
B06	VH5|5-51/D1|1-26|RF3/JH6	VK2|A19/JK2
B08	VH3|3-33/D6|6-6|RF1/JH6	VK1|L19/JK4
B09	VH4|4-30.1/D4|4-17|RF2/JH6	VK1|A20/JK5
B12	VH1|1-08/D4|4-11|RF3/JH6	VK4|B3/JK4
C09	VH3|3-53/D7|7-27|RF1/JH4	VL1|1g/JL2
C10	VH3|3-23/D5|5-24|RF3/JH6	VK4|B3/JK4
C11	VH3|3-23/D5|5-24|RF3/JH6	VL1|1e/JL2
D03	VH4|4-61/D4|4-11|RF2/JH4	VK1|L19/JK1
D08	VH3|3-33/D6|6-6|RF1/JH6	VK2|A19/JK2
E08	VH3|3-33/D4|4-17|RF2/JH6	VL3|3l/JL2
E09	VH3|3-33/D4|4-17|RF2/JH6	VL3|3l/JL2
E11	VH3|3-48/D2|2-8|RF2/JH6	VK1|L19/JK1
F04	VH3|3-21/D4|4-11|RF2/JH4	VL7|7a/JL2
F11	VH3|3-33/D5|5-24|RF3/JH6	VL3|3m/JL2
G04	VH3|3-33/D4|4-17|RF2/JH6	VL3|3l/JL2
G07	VH3|3-33/D4|4-17|RF2/JH6	VL3|3l/JL2
H01	VH4|4-39/D4|4-23|RF3/JH4	VL2|2b2/JL2
H03	VH3|3-11/D5|5-24|RF3/JH4	VL3|3r/JL2

B

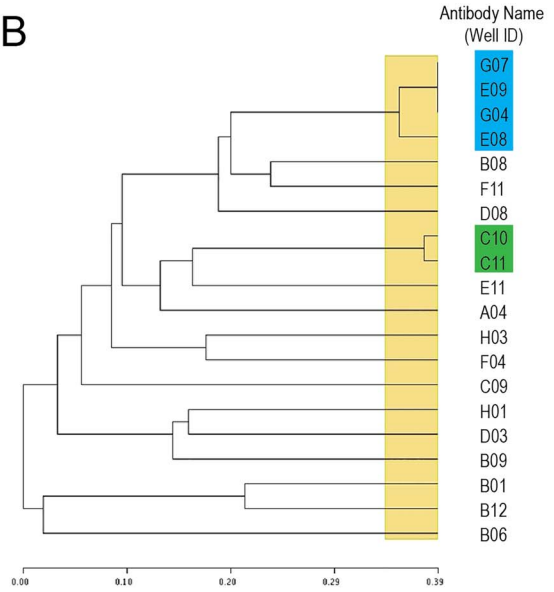


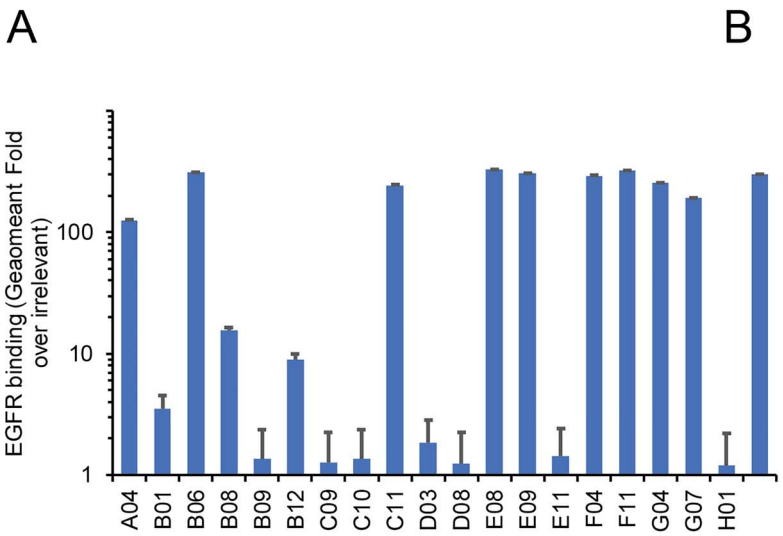
Figure 2. Sequence characterization of single-cell, recovered, EGFR-specific clones chosen for cloning. (A) Table of Variable Heavy (VH) and Light (VL) germline usage for each clone. (B) VH CDR dendrogram illustrating the sequence diversity of the 20 clones. The yellow box indicates a chosen maximum distance between two antibodies to allow grouping and is based on a calculation of similarity equal to the number of mismatches divided by the sequence length. The tree length is on the x-axis and antibodies with a distance less than 0.1 from each other were grouped together resulting in 16 separate antibody clades.

binding assay, the 13 antibodies that bound EGFR-CHO cells greater than 3.5 fold than parental CHO cells demonstrated affinities ranging from 0.656 to 9.46 nM to EGFR (Fig. 3B and C, Supporting Fig. 5). Therefore, we were able to confirm isolated sequences from primary B-cells bind to native EGFR on-cells and soluble EGFR protein.

DISCUSSION

Routinely, the CellCelector platform has been used for the isolation of single stem, primary and tumor cells of human origin [23, 24]. Here, we described the CellCelector platform to screen and isolate ASCs therapeutic antibodies derived from single B-cells originating from immunization of XenoMouse. We believe the CellCelector platform can thus be leveraged to characterize human ASCs harvested from peripheral blood of patients undergoing an active disease or expanded to ASCs from other animal or transgenic species. Furthermore, expanding assays (such as antibody competition, epitope binning, receptor-ligand blocking) and using the seven-color imaging capabilities will be necessary steps to realize the full capabilities of the CellCelector platform. Current assays are in place to add antigen-expressing cells and could replace the soluble-bead system described here. The use of reporter cells allows for more physiologically relevant screening scenarios for therapeutically interesting complex membrane targets, as well as functional, reporter-style, cell-dependent assays.

The CellCelector platform stands in contrast to microfluidic based approaches that have been developed and commercialized for antibody discovery. One example is the 10x Chromium instrument, which encapsulates individual B-cells within individual micro-droplets with a unique barcoded gel bead. B-cells are lysed and RT-PCR performed to generate amplicons for NGS [25]. Bioinformatic analysis deconvolutes heavy and light chains originating from a single micro-droplet. Since no screens are performed to identify target-specific B-cells, associating amino acid sequence to target specificity is of challenge. LIBRA-seq overcomes this limitation by utilizing a pool of antigens labeled with the same fluorophore, and each antigen is conjugated with a unique barcoded DNA oligo [26]. Fluorescence-activated cell sorting (FACS) is performed before encapsulation of single B-cells [26]. In the LIBRA-seq technique, antigen barcodes and heavy-light chain amplicons are tagged with a common barcode associated with individual micro-droplet thus linking antibody sequences to antigen specificity. However, these methods identify memory B-cell populations, neglecting plasma B-cells, which often contain higher affinity antibodies. While LIBRA-seq is limited to soluble proteins, the gel encapsulated microenvironment (GEM) assay allows to screen B-cell populations for soluble and on-cell targets in micro-droplets [27]. The GEM assay encapsulates a single ASC and either beads, cells or other particles immobilized within the matrix of the micro-droplet. Antibody is secreted from the ASC and diffuses locally within the micro-droplet where it binds to its target, and the target-bound antibody is detected with a labeled secondary antibody [27]. Unfortunately, micro-droplets containing ACS of interest are harvested manually under a microscope. Unlike microfluidic-based techniques that place greater emphasis on paring heavy and light chains, with the CellCelector platform, greater emphasis is placed on identifying ACS of interest through initial screens and automating the harvesting for the ASCs of interest.



B

Antibody Name (Well ID)	Affinity kon(1/Ms)	kdis(1/s)	KD (nM)
A04	2.7E+06	1.8E-03	0.7
B01	3.3E+05	6.2E-04	1.9
B06	8.0E+05	3.5E-03	4.4
B08	8.4E+04	1.0E-04	1.2
B09			Not Binding
B12	7.6E+04	5.9E-04	7.8
C09			Not Binding
C10			Not Binding
C11	2.1E+05	2.3E-03	11
D03			Not Binding
D08			Not Binding
E08	2.0E+05	1.0E-03	5.1
E09	1.1E+05	1.0E-03	9.5
E11			Not Binding
F04	1.2E+05	3.4E-04	2.8
F11	1.2E+06	1.3E-03	1.1
G04	2.8E+05	2.6E-04	0.9
G07	2.4E+05	1.1E-03	4.7
H01			Not Binding
H03	1.7E+06	1.3E-03	0.8

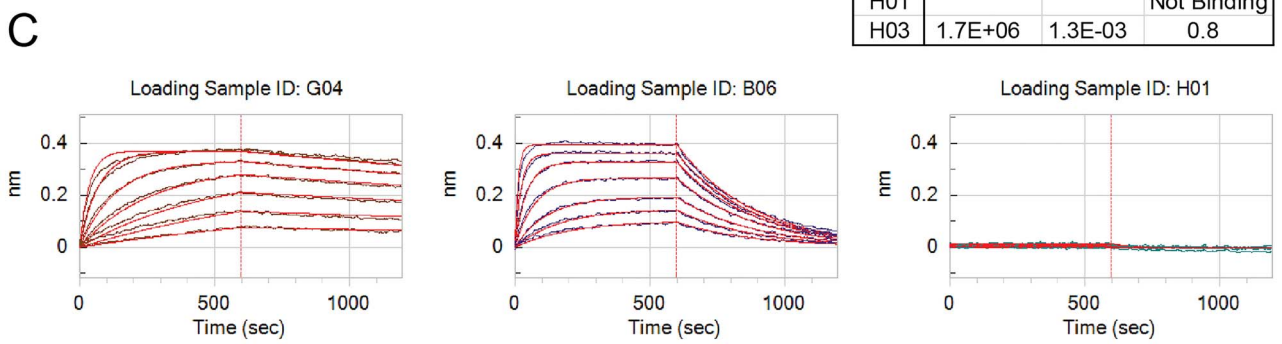


Figure 3. Characterization of 20 expressed clones. (A) Binding of antibodies to EGFR expressing CHO cells by flow cytometry. EGFR binding was determined using the geomean of antibodies to EGFR-CHO over parental CHO cells. Antibodies were tested at 50 nM in triplicate. (B) Affinity of antibodies to soluble EGFR determined using BLI. Antibodies were captured to an anti-human Fc antibody loaded on streptavidin biosensors and binding measured to EGFR titrated from 1.56 to 100 nM. Antibodies binding to cells >3.5-fold also bind to soluble EGFR. (C) Three representative sensorgrams of antibodies demonstrating high affinity (<1 nM), moderate affinity (1–10 nM), and no binding to soluble EGFR. All sensorgrams can be found in **Supporting Fig. 5.**

We analyzed 13% (52 000) of the total CD138$^+$ population (400 000) from one mouse and identified 0.5% (238) potential EGFR$^+$ B-cells. Out of 238 potential EGFR$^+$ B-cells we cloned 20 sequences and validated 13 (65%) sequences were positive for EGFR. If we extrapolate to all CD138$^+$ population, there is a potential 1196 EGFR$^+$ unique sequences that could be identified through screening greater number of plates and using plate architecture with higher nanowell density. This contrasts with traditional hybridoma generation workflows in terms of quantity and diversity of recovered antibody sequences. It was reported in 1989, an optimized hybridoma electrofusion protocol capable of generating one hybridoma from 5000 input splenic lymphocytes [3]. If we assume there has been a 20–100-fold improvement in hybridoma generation efficiency over the past 30 years, this would have resulted in one to five EGFR$^+$ hybridoma clones identified. By sampling the B-cell pool directly, we could avoid diversity loss and selection of identical clones arising from overgrowth of faster growing clones.

Utilizing the CellCelector platform to screen and isolate ASCs deposited into nanowells for antigen positive hits was accomplished within 1 day, followed by integrating with rapid VH-VL recovery and recombinant expression methods that can be accomplished in 5–7 days [28–30]. We believe obtaining antigen selective antibodies can be accomplished in as little as 3–4 weeks. Thus, the CellCelector platform can be used to identify and isolate B-cells specific to a therapeutic target in antibody discovery. We anticipate this technology can be readily adapted to the discovery of antibodies to other therapeutic targets.

SUPPLEMENTARY DATA

Supplementary Data are available at *ABT* Online.

AUTHORS' CONTRIBUTIONS

W.L.M. and K.O. conceived and designed the study with input from S.G. and K.M. All authors, W.L.M., K.O.,

C.N., W.C.C., S.G., K.M. and V.W., conducted and analyzed experiments. W.L.M. generated figures and wrote the manuscript with edits from all co-authors.

DATA AVAILABILITY

The data underlying this article are available in the article and in its online supplementary material.

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CONFLICTS OF INTEREST STATEMENT

W.L.M., K.O., W.C.C., S.G. and K.M. are employees and stockholders of Amgen Inc. V.W. is a former employee of Amgen Inc. C.N. is an employee and stockholder of ALS Automated Lab Solutions GmbH.

ETHICS AND CONSENT STATEMENT

Not applicable.

ANIMAL RESEARCH

Mice were housed in groups at a facility that has received a Certificate of Good Animal Practice from the Canadian Council on Animal Care (CCAC). Animals were cared for in accordance with CCAC Guidelines. All research protocols were reviewed and approved by the Amgen Institutional Animal Care and Use Committee.

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