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TITLE: Discovery of KCa3.1-Inhibiting Antibodies for the Treatment of Asthma

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14. ABSTRACT

Asthma is a chronic disease associated with inflammation and spasms of the airways in the lungs. It is estimated that 26 million people suffer from asthma in the United States and recent decades have shown a concerning increase in the prevalence of asthma in both children and adults. The costs related to asthma have also been steeply increasing, from ~$12 billion in 1994 to ~$56 billion in 2011. It has also been shown that the frequency of development of asthma is considerably higher in U.S. Service Personnel compared to the civilian population.
For the soldiers that have been deployed to Iraq and Afghanistan it is thought that exposure to fine sand particles, irritants and toxins related to burning trash in "burn pits" may be the cause. Currently asthma cannot be cured and treatment guidelines emphasize the use of preventive anti-inflammatory drugs (e.g., inhaled corticosteroids) to control the disease symptoms. Treatment with corticosteroids helps to manage the disease for a majority of patients

15. SUBJECT TERMS
KCa3.1, Ion channel, Monoclonal antibody, mAbs, Tetrahymena, TetraExpres™, Purification, Formulation, Immunogen, Screening, Phage library, Functional Inhibition, Electrophysiology, Asthma

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1. Introduction

The overarching goal of this grant is the discovery of anti-KCa3.1 antibodies that functionally block the calcium activated potassium channel. Recent *in vitro* and *in vivo* studies have implicated KCa3.1 as a prime target for the treatment of asthma, particularly for patients that do not respond to standard treatments. Therefore, the discovery and development of anti-KCa3.1 monoclonal antibodies represents a potentially novel and effective therapeutic intervention for a number of respiratory diseases including asthma.

2. Key words

KCa3.1
Ion channel
Monoclonal antibody
mAbs
Tetrahymena
TetraExpres™
Purification
Formulation
Immunogen
Screening
Phage library
Functional Inhibition
Electrophysiology
Asthma

3. Overall Project Summary

We are pleased to report that the overarching goal of the research program was successfully met and that TetraGenetics, Inc was able to identify monoclonal antibodies that block the function of the calcium activated ion channel KCa3.1. As detailed below, we were able to meet each of the Specific Aims including the successful expression of KCa3.1 in the TetraExpress™ platform, development of purification and formulation strategies and finally application of those reagents to a phage-display antibody discovery platform. Importantly, of the functionally inhibiting antibodies discovered, one clone demonstrates potent activity as determined by electrophysiology measurements and is thus is a candidate for therapeutic development. The lead antibody is currently undergoing assessments for biological efficacy and biophysical developability.

4. Key Research Accomplishments

The following describes the major goals of the grant and a summary of the results achieved.

Specific Aim 1: Production of human KCa3.1 channel in sufficient quantity and purity to support an antibody discovery campaign-typically 5-10 mg of >90% pure ion channel.
Optimize cell growth conditions for overexpression of human KCa3.1 in *Tetrahymena* and validate resulting protein products via SDS PAGE and Western blotting using either protein specific antibodies or antibodies against the tags. Proprietary growth conditions have enabled overexpression of human voltage-gated ion channels in *Tetrahymena* to levels >100 fold greater than has here-to-fore been possible with conventional host cell systems including insect (Sf9) and mammalian tissue culture cells (CHO and HEK). Recovery of purified KCa3.1 is expected to range from 0.2-0.5 mg per L of culture, and typically 5-10 mg of purified channel is required to support an antibody drug discovery program.

Results Achieved: Recombinant human KCa3.1 was expressed in the TetraExpress™ platform. Purification of the recombinant channel was achieved following detergent extraction of the protein and affinity chromatography (NiNTA). Our initial intent was to formulate the purified channel into an adjuvanted liposome to use as an immunogen in a host animal (chickens). However, we developed an opportunity to directly screen proprietary

phage libraries of either naïve or patient human antibodies developed by ModiQuest. This approach negates the need for follow on humanization of therapeutic antibody candidates. We therefore formulated the purified KCa3.1 onto solid support (magnetic beads) using His-tag chemistry engineered into the protein C-terminus (Figure 1). Once bound to the bead, the channel was reconstituted into a phospholipid bilayer to both stabilize the channel and to preferentially expose the surface loops where antibodies would be expected to exert their functional effects. Additionally, we developed biotinylated liposomes containing KCa3.1 as an alternative screening approach. In addition, a saturating amount of counter-selection reagent was required to support the phage panning strategy and therefore we generated multiple milligrams of a non-related ion channel (Kv1.3) formulated into a soluble liposome (Figure 1).

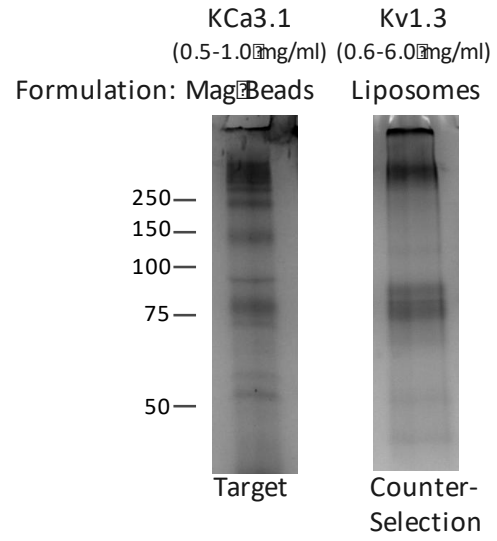

Figure 1. KCa3.1 antibody discovery reagents. Shown are SDS-PAGE blue-stain analysis of magnetic beads containing KCa3.1 (Left panel) and Kv1.3 counter-selection liposomes (Right panel)

Specific Aim II: Generation of anti-KCa3.1 antibodies. A similar strategy will be employed to that used to identify functionally inhibiting anti-Kv1.3 antibodies. The antibodies will be raised in out-bred wild-type chickens with the aim of identifying functionally inhibiting and/or high-affinity human KCa3.1 binding antibodies.

Results Achieved: As noted above our initial campaign was carried out using phage libraries consisting of human antibodies screened against KCa3.1 oriented on magnetic beads. Twelve screening strategies encompassing magnetic and biotinylated liposome formulations on naïve and patient human antibody libraries were carried out.

Strategies incorporating magnetic bead formulations (as opposed to biotinylated liposomes) were found to be superior in recovery of interacting antibody clones and were therefore used for further interrogation. From these screens approximately 400 KCa3.1 specific antibodies were identified of which 34 were sequence unique clones as determined by sequencing of heavy and light chain genes. Given the high output of specific antibodies from these screens and the fact that the antibodies were human (as opposed to chicken as originally intended) we determined it was of higher potential value to forgo the chicken immunization strategy and continue characterization of the human antibodies. The results of successful polyclonal and monoclonal panning strategies are shown in Figure 2.

Specific Aim III: **Identification of anti-KCa3.1 antibodies that inhibit ion channel activity as determined by electrophysiology.** Antibody-containing culture supernatants will be tested for inhibition of human KCa3.1 channel activity. Functional assessment of KCa3.1 binders will be carried out in collaboration with an academic partner that has successfully identified functionally inhibiting Kv1.3 antibodies.

Results Achieved: Functional screening of the 34 sequence unique antibodies derived from the phage libraries was carried out according to plan. In brief, antibodies were evaluated at a single concentration (400nM) by whole-cell patch clamp electrophysiology on mammalian cells expressing human KCa3.1. Two of the 34 antibodies achieved a significant block in KCa3.1 current at the tested concentration (Table 1, note functional antibodies are shown in red text). To confirm the link between genotype and phenotype antibodies were generated by an independent CRO (LakePharma) using light and heavy chain sequences identified in the original screen. Subsequent testing of the LakePharma antibodies confirmed retention of blocking activity. Of the two antibodies, clone 252 was produced at much higher yields in CHO cells than clone 268 in multiple experiments indicating that the latter may suffer developability issues. Therefore Clone 252 was chosen for further investigation. Dose-response analysis of Clone 252 demonstrated an IC50 of approximately 1.8nM (Figure 3).

Training and Professional Development: Nothing to Report

Dissemination of results: TetraGenetics intends to publish the data generated during the course of this award in an appropriate peer-reviewed journal

Polyclonal

Naïve library: Strategy 1

		Strategy 1 – Human naive library – Magnetic liposomes (no competition R1)									
Dilution		R2-in	R3-in	R4-in	R5-in	R6-in	R2-in	R3-in	R4-in	R5-in	R6-in
1:20	A	0.044	0.060	1.195	3.130	2.917	0.063	0.038	0.055	0.054	0.048
1:60	B	0.037	0.045	0.489	2.579	2.121	0.036	0.032	0.036	0.036	0.036
1:180	C	0.046	0.039	0.184	1.663	1.133	0.031	0.033	0.033	0.037	0.031
1:540	D	0.040	0.038	0.074	0.655	0.432	0.032	0.037	0.032	0.035	0.037
1:1620	E	0.033	0.039	0.047	0.251	0.192	0.032	0.039	0.036	0.030	0.031
1:4860	F	0.050	0.040	0.042	0.115	0.092	0.030	0.030	0.031	0.031	0.030
1:14580	G	0.036	0.041	0.040	0.062	0.056	0.031	0.030	0.039	0.040	0.031
blank	H	0.395	0.407	0.135	0.128	0.135	0.136	0.141	0.039	0.034	0.028
		KCa3.1					Kv1.3				

Patient library: Strategy 1

		Strategy 5 – Human patient library – Magnetic liposomes (no competition R1)									
Dilution		R2-in	R3-in	R4-in	R5-in	R6-in	R2-in	R3-in	R4-in	R5-in	R6-in
1:20	A	0.047	0.047	0.228	1.751	1.600	0.040	0.034	0.042	0.043	0.041
1:60	B	0.035	0.041	0.108	0.978	0.831	0.039	0.031	0.032	0.034	0.038
1:180	C	0.041	0.036	0.043	0.331	0.322	0.031	0.039	0.030	0.030	0.042
1:540	D	0.042	0.039	0.045	0.123	0.128	0.029	0.030	0.038	0.029	0.030
1:1620	E	0.040	0.039	0.039	0.066	0.062	0.031	0.033	0.029	0.030	0.030
1:4860	F	0.038	0.037	0.041	0.039	0.043	0.030	0.035	0.030	0.034	0.029
1:14580	G	0.039	0.037	0.030	0.035	0.036	0.031	0.036	0.032	0.030	0.030
Blank	H	0.395	0.407	0.135	0.128	0.030	0.395	0.407	0.039	0.034	0.028
		KCa3.1					Kv1.3				

Monoclonal

Strategy 1 - Output Round 3				Strategy 1 - Output Round 3				Strategy 1 - Output Round 3			
plate ID	well ID	KCa3.1	Kv1.3	plate ID	well ID	KCa3.1	Kv1.3	plate ID	well ID	KCa3.1	Kv1.3
1	B3	1.885	0.047	1	B4	0.530	0.060	2	B2	0.313	0.038
1	H1	1.676	0.071	2	F1	0.527	0.094	1	H11	0.311	0.060
1	C12	1.597	0.064	1	H5	0.521	0.061	1	G6	0.309	0.051
1	D12	1.523	0.078	1	H9	0.520	0.057	1	C5	0.308	0.051
1	C2	1.393	0.045	1	G12	0.517	0.107	1	F2	0.299	0.049
1	G9	1.283	0.046	1	G1	0.510	0.078	2	D1	0.284	0.092
1	B9	1.272	0.059	1	H7	0.505	0.056	1	F3	0.283	0.055
2	H1	1.259	0.241	2	E1	0.504	0.078	1	E9	0.275	0.044
1	G4	1.249	0.050	1	C4	0.504	0.060	1	F4	0.274	0.053
1	C11	1.228	0.050	2	B1	0.503	0.109	2	G1	0.268	0.120
2	C1	1.196	0.097	1	C7	0.499	0.055	2	D3	0.267	0.037
1	F7	1.182	0.042	1	F8	0.497	0.055	1	D10	0.263	0.048
1	H3	1.158	0.053	2	E2	0.493	0.040	2	G6	0.255	0.045
1	E5	1.128	0.042	2	A4	0.491	0.064	2	D2	0.247	0.038
1	E11	1.106	0.046	2	F5	0.490	0.040	2	H5	0.245	0.099
1	B6	1.102	0.042	1	E3	0.472	0.048	1	C10	0.245	0.050
1	A12	1.097	0.086	2	A1	0.472	0.303	1	E8	0.240	0.041
1	F11	1.077	0.042	1	C9	0.470	0.045	1	F10	0.236	0.050
1	C8	1.042	0.048	1	A2	0.461	0.066	1	D1	0.230	0.072
2	A5	1.030	0.083	2	B3	0.441	0.035	1	G8	0.225	0.045
1	E2	1.012	0.054	1	E10	0.440	0.041	1	F5	0.225	0.043
2	D5	0.965	0.036	1	A11	0.431	0.069	2	B5	0.223	0.035
2	B6	0.935	0.034	1	G10	0.429	0.041	1	D7	0.209	0.040
1	C1	0.926	0.055	1	D2	0.423	0.045	2	F2	0.204	0.044
1	E12	0.912	0.059	1	A9	0.421	0.059	1	D8	0.203	0.042
1	F9	0.876	0.047	2	A2	0.420	0.096	1	B10	0.203	0.040
1	G2	0.751	0.071	2	G3	0.416	0.058	1	B1	0.188	0.054
2	A6	0.726	0.100	2	E3	0.411	0.037	2	B4	0.172	0.040
1	C6	0.719	0.043	1	D3	0.405	0.040	2	H3	0.158	0.082
1	A6	0.694	0.068	1	G7	0.400	0.046	2	E6	0.157	0.039
1	A8	0.694	0.082	1	G11	0.398	0.042	2	F4	0.151	0.041
1	B12	0.691	0.074	1	A5	0.392	0.120	2	E4	0.140	0.038
1	A4	0.659	0.080	2	G5	0.389	0.043	1	E1	0.134	0.076
2	C2	0.653	0.039	1	A7	0.384	0.136	1	D5	0.111	0.038
2	A3	0.648	0.081	2	D4	0.376	0.036	2	F6	0.109	0.036
1	G3	0.641	0.061	2	E5	0.376	0.039	1	D11	0.091	0.058
1	A10	0.638	0.098	1	E4	0.374	0.043	1	B11	0.078	0.044
1	B2	0.637	0.042	2	C3	0.364	0.038	2	G2	0.065	0.048
1	D9	0.595	0.039	2	G4	0.359	0.043	1	E7	0.064	0.046
1	B8	0.594	0.063	1	B7	0.353	0.051	1	F6	0.061	0.041
1	A1	0.582	0.072	1	D4	0.351	0.049	1	C3	0.058	0.055
1	F1	0.556	0.083	2	C6	0.350	0.037	1	D6	0.057	0.042
1	F12	0.547	0.069	1	B5	0.324	0.044	2	C4	0.056	0.036
1	A3	0.539	0.073	2	F3	0.321	0.044	1	G5	0.055	0.041
1	E6	0.533	0.045	2	D6	0.319	0.036	2	C5	0.050	0.038

Figure 2. Human naïve and patient phage library panning results. The left panels show ELISA data demonstrating recovery of polyclonal phage from naïve and patient libraries that specifically recognize KCa3.1 (Left side of tables) compared to a non-related ion channel, Kv1.3 (right side of table). The right panel shows ELISA data from subsequently isolated monoclonal phage that specifically recognize KCa3.1 over Kv1.3. These antibodies were sequenced and unique clones were moved forward to functional analysis.

Table 1. Anti-KCa3.1 mAb functional screen

Date	IK mAb clone #	stock vol	stock [uM]	[desired]	vol. test	mAb vol.	Ringer vol.	% blocked		
		~uL		nM	uL	uL	uL	cell 1	cell2	average
11.28.18	237	1400	11.3	300	500	13.274336	486.725664	0		0
11.28.18	238	400	2.07	300	500	72.463768	427.536232	11.45		11.45
12.01.18	239	400	10.2	300	500	14.705882	485.294118	0		0
12.01.18	240	400	8.67	300	500	17.301038	482.698962	0		0
12.01.18	241	400	11.73	300	500	12.787724	487.212276	0		0
12.02.18	242	1100	11.27	300	500	13.309672	486.690328	6.48		6.48
12.02.18	243	400	7.6	300	500	19.736842	480.263158	11.22	0	5.61
12.02.18	244	1400	14.6	300	500	10.273973	489.726027	31.78		31.78
12.02.18	245	300	5.73	300	500	26.17801	473.82199	8.07		8.07
12.02.18	246	400	2.07	300	500	72.463768	427.536232	0		0
12.02.18	247	900	14	300	500	10.714286	489.285714	42.5	0	21.25
01.04.19	248	1400	13.6	300	500	11.029412	488.970588	0		0
01.04.19	249	150	4.33	300	500	34.642032	465.357968	0		0
02.06.19	250	400	3.53	300	500	42.492918	457.507082	9.04		9.04
02.06.19	251	400	3.93	300	500	38.167939	461.832061	22.78		22.78
02.07.19	252	400	6.6	300	500	22.727273	477.272727	74.6		74.6
02.08.19	253	900	13.33	300	500	11.252813	488.747187	12.6	3.59	8.095
02.08.19	254	400	2.67	300	500	56.179775	443.820225	0		0
02.11.19	255	400	2.33	300	500	64.377682	435.622318	14.6		14.6
02.11.19	256	400	2.6	300	500	57.692308	442.307692	30.1		30.1
02.11.19	257	300	3	300	500	50	450	15.8		15.8
02.11.19	258	500	1	300	500	150	350	38.9		38.9
02.12.19	259	400	2.47	300	500	60.728745	439.271255	17.83		17.83
02.12.19	260	1100	9.07	300	500	16.538037	483.461963	20.2		20.2
02.12.19	261	400	3.4	300	500	44.117647	455.882353	0		0
02.13.19	262	200	1	300	500	150	350	0		0
02.13.19	263	400	1.8	300	500	83.333333	416.666667	16.3		16.3
02.13.19	264	400	4.07	300	500	36.855037	463.144963	35.5		35.5
02.13.19	265	400	0.733	300	500	204.63847	295.361528	26.8		26.8
02.13.19	266	400	0.8	300	500	187.5	312.5	12		12
02.13.19	267	400	1.73	300	500	86.705202	413.294798	21		21
02.13.19	268	200	1.33	300	500	112.78195	387.218045	70.5		70.5
02.14.19	269	200	2.6	300	500	57.692308	442.307692	0		0
02.14.19	270	400	0.267	267	400	400	0	0		0

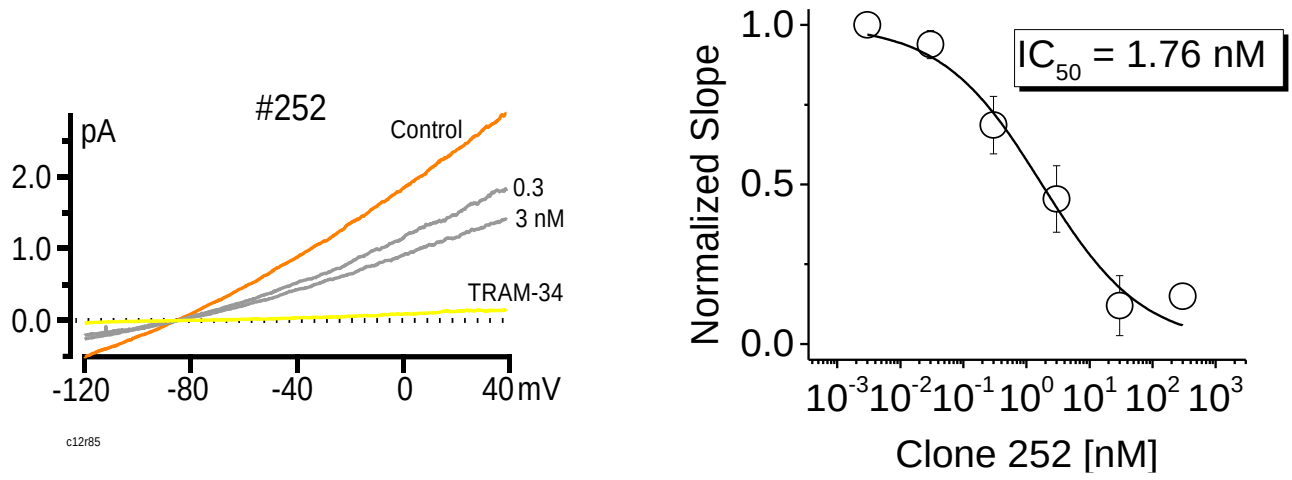

Figure 3. Dose-response analysis of anti-KCa3.1 mAb clone 252. The left panel shows KCa3.1 current traces in the absence (control, orange line) or presence of different concentrations of mAb clone 252 (grey lines). A positive control trace following addition of the KCa3.1 specific small molecule inhibitor TRAM 34 is shown in yellow. The right panel shows calculation of an IC_{50} of around 1.8nM.

Next Reporting Period: Given the success of the current award in achieving its primary goal we requested a 12 month no-cost extension to i) complete the planned characterization of functional anti-KCa3.1 antibodies and ii). further characterize functional antibodies to determine their therapeutic potential as well the next step in their development (e.g. antibody maturation etc.)

We proposed to use the remaining award funds to carry out the following:

1. Confirm functionality of antibody clone 268 by electrophysiology
2. Determine selectivity of functional antibodies against closely related ion channels by electrophysiology.
3. Determine the cross-reactivity of functional antibodies to mouse and rat KCa3.1 (to enable follow on *in vivo* assays)
4. Evaluate cardiac risk of functional antibodies in a human primary cardiomyocyte-based assay
5. Evaluate the biological efficacy of anti-KCa3.1 antibodies in established dose-response human T cell proliferation assays
6. Carry out a Biophysical profile assessment of the functional anti-KCa3.1 antibodies (e.g. thermostability, aggregation analysis, response to various stress conditions)

Work done during the no-cost extension period:
Due to consistent manufacturing liabilities clone 268 was not pursued further as a developable candidate. Instead, all work focused on clone 252

We have been able to show that mAb 252 is not active on KCa related family members nor cardiac channels Nav1.5 or hERG

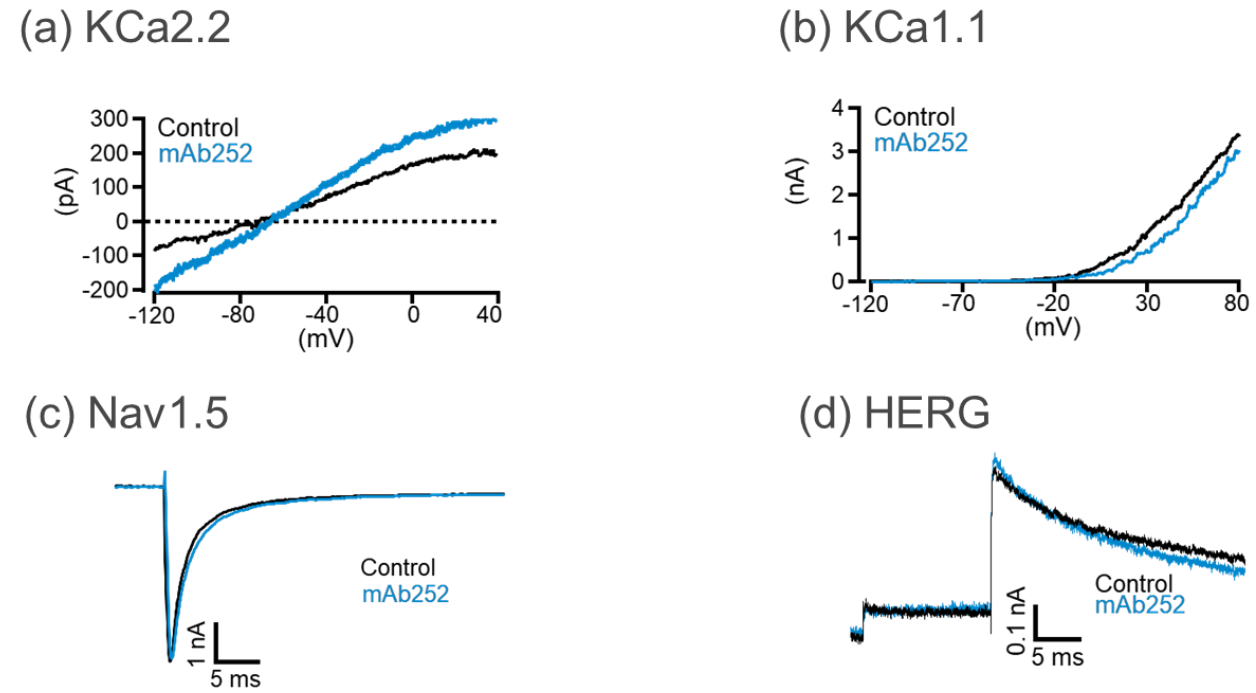

Figure 4. Selectivity analysis of anti-KCa3.1 mAb clone 252 against the related ion channels. (a) KCa2.2 (b) KCa1.1 (c) Nav1.5 and (d) hERG

mAb 252 is not cross-reactive on rodent KCa3.1 channels.

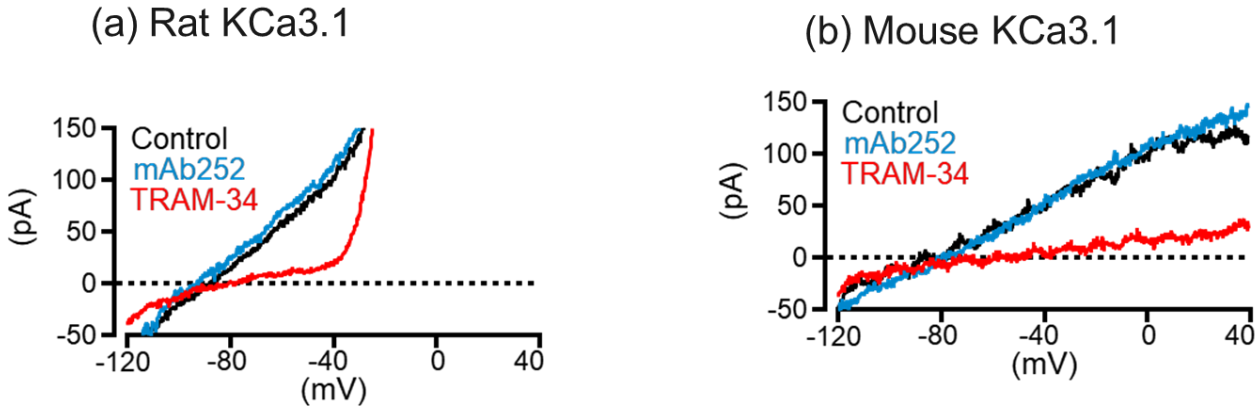

Figure 5. Selectivity analysis of anti-KCa3.1 mAb clone 252 against rodent KCa3.1 channels. (a) Rat (b) Mouse

Clone 252 underwent a developability panel of analytical assays including:

- Melting temperature
- Polyspecificity (BVP Assay)
- Stress analysis (Freeze/thaw and Heat)
- Viscosity measurements (low and high concentration)
- Note: The only developability liability noted was potentially polyspecificity based on BVP score
- SE-UPLC, rCE-SDS

The antibody 252 underwent polyspecificity and thermostability analysis by DSF/DLS. The sample was then split into three sets for different stress conditions. The first set underwent no stress, the second set underwent freeze/thaw stress, and the third set underwent heat stress. After the stress conditions, the samples were analyzed via SE-UPLC, CE-SDS, PTM analysis, and intact mass under reduced & deglycosylated (DGR) condition.

Analytical Results on the Antibody		**TTG-18252**
Original Sample	DSF (Melting Temperature)	71.0°C
	BVP Score	8.3
No Stress	SE-UPLC (Monomer %)	98.87%
	rCE-SDS* (LC + HC purity %)	98.12%
	Viscosity at 6.36 mg/mL	0.98 mPa-s
	Viscosity at 80.22 mg/mL	2.07 mPa-s
After Freeze/Thaw	SE-UPLC (Monomer %)	98.25%
	rCE-SDS* (LC + HC purity %)	98.11%
After Heat Stress	SE-UPLC (Monomer %)	93.12%
	rCE-SDS* (LC + HC purity %)	94.09%

Table 2. Developability Panel for clone 252. Summary of the analytical analysis performed on original sample, no stress, after freeze/thaw stress, and after heat stress. No stress condition is storage at 4°C for 2 weeks. Freeze/thaw stress indicates that the sample went through 5 cycles of freeze and thaw. Heat stress is storage at 40°C for 2 weeks. Reduced CE-SDS (rCE-SDS) is used to represent % purity by adding light chain (LC) and heavy chain (HC) monomer peak %.

TTG-252 antibody showed some degradation after stress conditions. Both aggregation and fragmentation were observed in the SE-UPLC and CE-SDS. The purity percentage remained above 93% and an 8% decrease in the main peak within non-reduced CE-SDS. The antibody TTG-252 has a BVP score of 8.3 which is polyspecific since it is over 5.0 (5x the background signal). A melting temperature of 71°C was observed with a low polydispersity index (PDI) of 0.05 symbolizing the sample was monodispersed. The measured intact mass under reduced and deglycosylated condition matched the calculated MW from the protein sequence for all stress conditions (please see supplemental data section for intact mass results). From the PTM analysis, minor increase in the stoichiometric percentage of oxidation and deamidation from both the stress conditions was observed when comparing to the original sample (please see supplemental data section for PTM results).
A split peak within the CE-SDS profile of the light chain was observed (please see supplemental data section). The split peak is not common, and this is most likely due to PTM that will not affect the developability. No obvious sequence liability was observed except for one deamination site.

5. Impact

Impact on the development of principal disciplines: The successful identification of a functional antibody targeting KCa3.1 confirms the validity of TetraGenetics, Inc.'s approach aimed at producing therapeutic candidate antibodies that modulate ion channel function in general, and potassium channels in particular.

Impact on the development on other disciplines: Nothing to report.

Impact on technology transfer: Nothing to report

Impact on society beyond science and technology: The results described herein have led to the identification of a candidate monoclonal antibody that has the potential to be developed as a novel therapeutic for the treatment of respiratory disease.

6. Changes/Problems

Changes in approach and reasons for change. As noted above our original plan was to discover antibodies using immunized chickens as this was a previously successful strategy in generating functional antibodies targeting another potassium ion channel, Kv1.3. However, we were presented with an opportunity to screen naïve and patient human phage libraries that had potential advantages including speed of antibody recovery and the fact that humanization would not be a down-stream requirement for therapeutic development. Given early success in identifying functionally modulating antibodies that demonstrated significant potency (IC_{50}<5nM) we reasoned that further characterization of the functional antibodies was warranted to determine their potential therapeutic developability.

Actual or anticipated problems or delays and actions or plans to resolve them: Nothing to report

Changes that had a significant impact on expenditures: Nothing to report

Significant changes in use or care of:

human subjects: Not Applicable
vertebrate animals: Not Applicable
biohazards: Nothing to report
and/or select agents: Nothing to report

7. Products

Journal publications: TetraGenetics, Inc. intends to publish the results described above in a peer-reviewed journal.

Books or other non-periodical, one-time publications: Nothing to report

Other publications, conference papers and presentations: Data showing identification of functional KCa3.1 monoclonal antibodies was presented at the Discovery on Target conference in Boston, MA in September, 2019.

Website(s) or other Internet site(s): Nothing new to report

Technologies or techniques: Nothing new to report
Inventions, patent applications, and/or license: TetraGenetics, Inc. intends to file intellectual property covering the composition of matter associated with the discovered anti-KCa3.1 monoclonal antibodies and methods for their use.

Other Products: Nothing to report

8. Participants & other collaborating organizations

Individuals who worked on the project.

Name:	Ashot Papoyan
Project Role	PI
Researcher Identifier (ORCID ID)	0000-0003-0157-376X
Nearest person month worked	4
Contribution to Project	Project Management, molecular biology
Name:	Paul Colussi
Project Role	Scientist
Researcher Identifier (ORCID ID)	0000-0003-1757-1128
Nearest person month worked	1.5
Contribution to Project	Antibody analysis and characterization
Name:	Janna Bednenko
Project Role	Scientist
Researcher Identifier (ORCID ID)	0000-0002-3528-1602
Nearest person month worked	1
Contribution to Project	KCa3.1 expression strain generation and purification
Name:	Ellen Gulezian
Project Role	Scientist
Researcher Identifier (ORCID ID)	N/A
Nearest person month worked	7
Contribution to Project	Molecular biology, KCa3.1 expression strain generation and maintenance
Name:	Joanna Cardarelli
Project Role	Scientist
Researcher Identifier (ORCID ID)	N/A
Nearest person month worked	1
Contribution to Project	KCa3.1 expression strain culture
Name:	Yihui Zhang
Project Role	Scientist
Researcher Identifier (ORCID ID)	N/A
Nearest person month worked	2
Contribution to Project	KCa3.1 antibody analysis

Change in active other support: Nothing to report

Other partner organizations:

Organization Name: University of California, Davis

Location: California, Davis, USA

Partner's contribution to the project: Collaboration. Specifically functional evaluation of anti-KCa3.1 antibodies via electrophysiology measurements.

9. Special Reporting Requirements: Nothing to report

10. Appendices: N/A

Transition Plan Questionnaire

Directions: Please answer all questions that apply for each product under development. Please fill out one document per product.
This is not an application for funding; however, answers will help us understand the outcomes and products from your award.

1. After the award closes, would you be willing to periodically provide voluntary information (via email) regarding the project status (i.e. where the research is headed)? **Yes** ☑ or **No** ☐
These responses will help CDMRP demonstrate the return on its investments and will help demonstrate that the CDMRP is a responsible and successful steward of federal research funding.

2. What **conclusion(s)** does your final data support?

We are pleased to report that the overarching goal of the research program was successfully met and that TetraGenetics, Inc was able to identify monoclonal antibodies that block the function of the calcium activated ion channel KCa3.1. As detailed below, we were able to meet each of the Specific Aims including the successful expression of KCa3.1 in the TetraExpress™ platform, development of purification and formulation strategies and finally application of those reagents to a phage-display antibody discovery platform. Importantly, of the functionally inhibiting antibodies discovered, one clone demonstrates potent activity as determined by electrophysiology measurements and is thus is a candidate for therapeutic development. The lead antibody is currently undergoing assessments for biological efficacy and biophysical developability.

3. Will you/have you applied for/obtained follow-on-funding for this project? **If yes**, please list (a) funding organization, (b) total budget requested/obtained, and (c) title of the funded proposal. *This information will be recorded as an outcome to this award*.

No

4. What will be **the next step(s)** for this project?

Develop the discovered antibody into the pre-IND

5. How would you classify your **lead candidate product**? a
(a) Therapeutic (Small Molecule, Biologic, Cell/Gene Therapy): Biologic
(b) Diagnostic
(c) Device
(d) Research Tool to Address a Research Bottleneck
(e) Knowledge Product (Non-material product such as a compound library, database, something that improves clinical practice, education, etc.)
(f) Other - Please Specify:

6. How does your candidate product aid the Warfighter, Veteran, Beneficiary, and/or General Population?

Treatment of asthma.

7. Therapy / Product Development, Transition Strategies, and Intellectual Property

Describe the steps and relevant strategies required to move the candidate product (knowledge or tangible) to the next phase of development and/or commercialization. Please address any issues with intellectual property.

PIs are encouraged to explore the technical requirements and the current regulatory strategies involved in product development as well as to work with their organization's Technology Transfer Office (or equivalent regulatory/legal office), federal/international regulatory experts, to develop the transition plan and to explore developing relationships with industry, DoD advanced developers (e.g. USAMMDA), and/or other funding agencies to facilitate moving the product into the next phase.