Small molecule modulation of CD33

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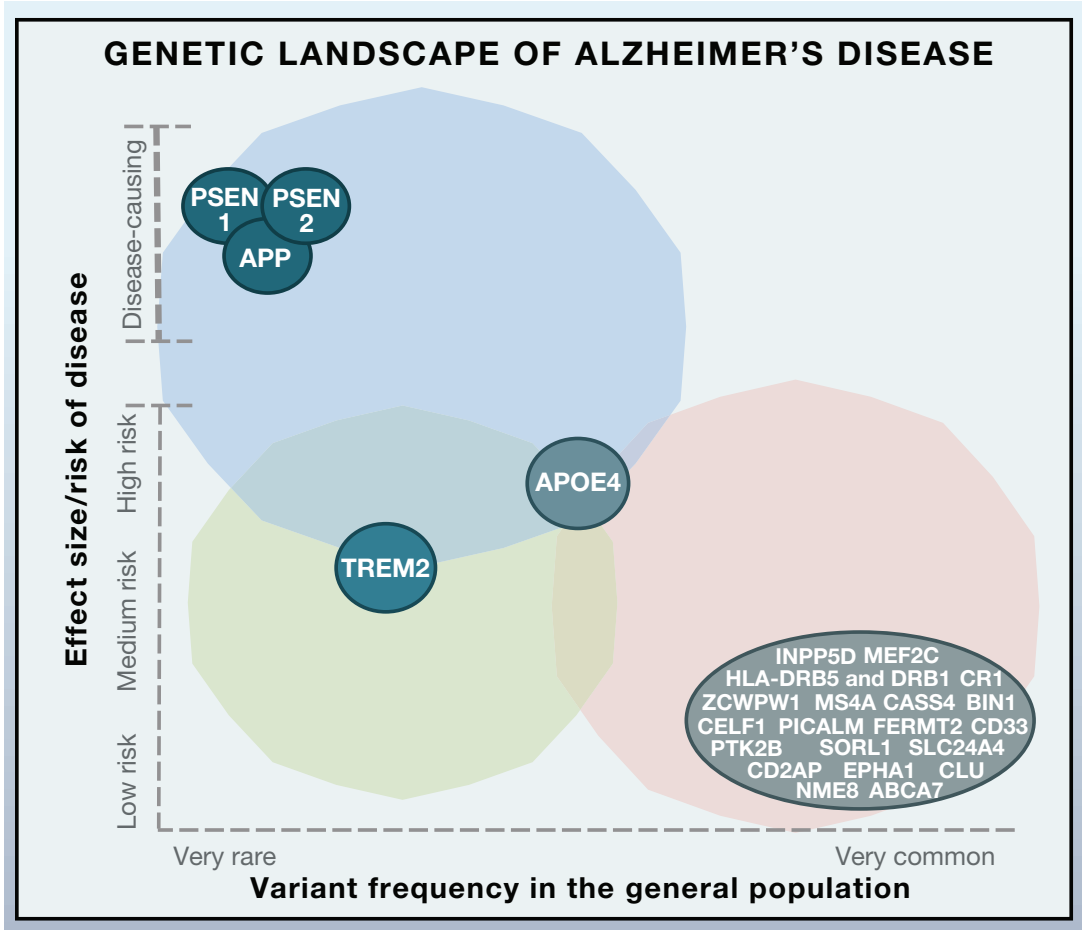
Genetics of LOAD

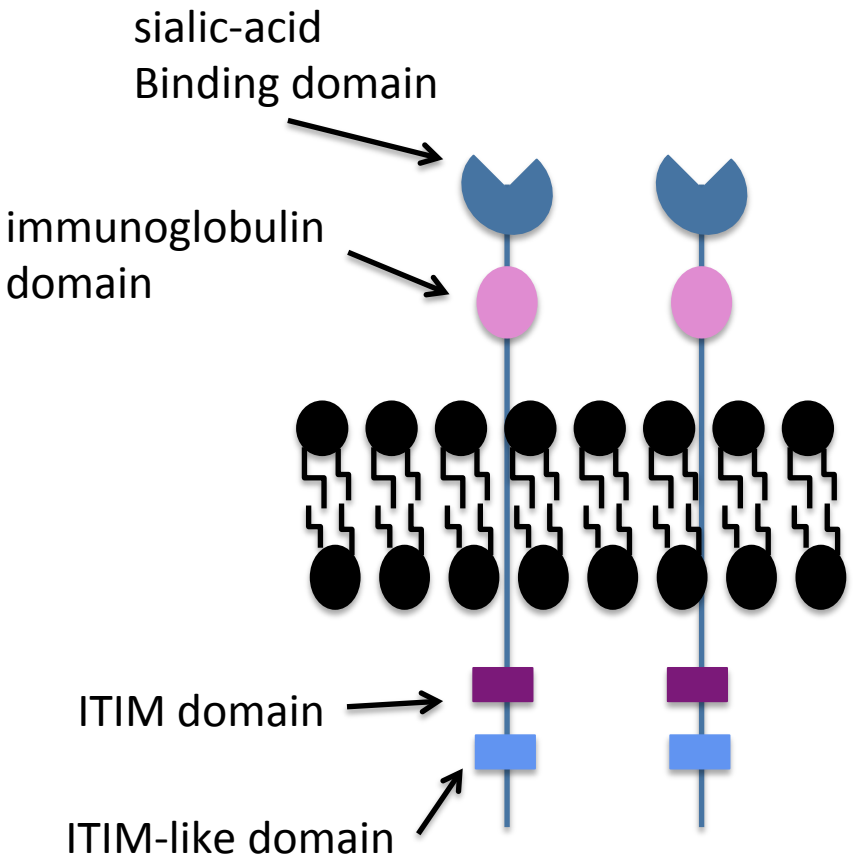
- Late-onset Alzheimer's disease (LOAD) is a highly heterogeneous disorder-
  Complicated genetics - no causative mutations, only risk variants
- Genome-wide association studies (GWAS) have identified genetic variants associated with LOAD



Guerreiro et al. Cell (2013).

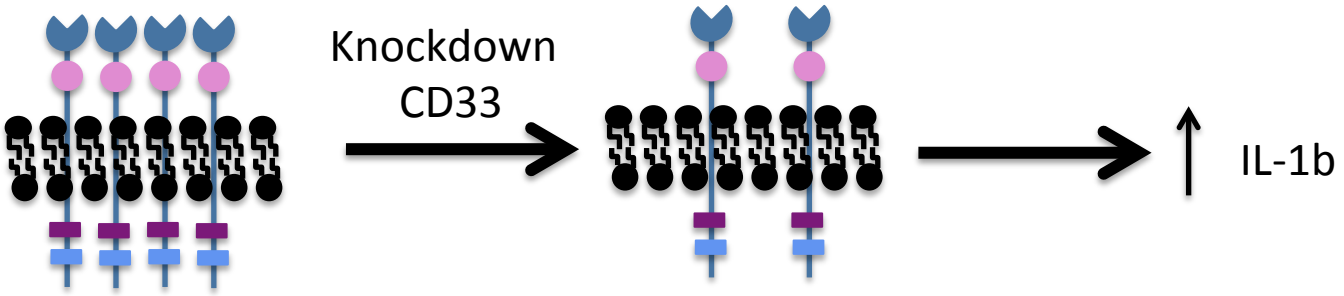
CD33

- **CD33:** inhibitory transmembrane receptor expressed by myeloid cells
  - Often used as a monocyte marker
  - Contains an Immunoreceptor tyrosine based inhibitory motif (ITIM) and a ITIM-like domain
- Binds sialic acids (exact ligand unknown)



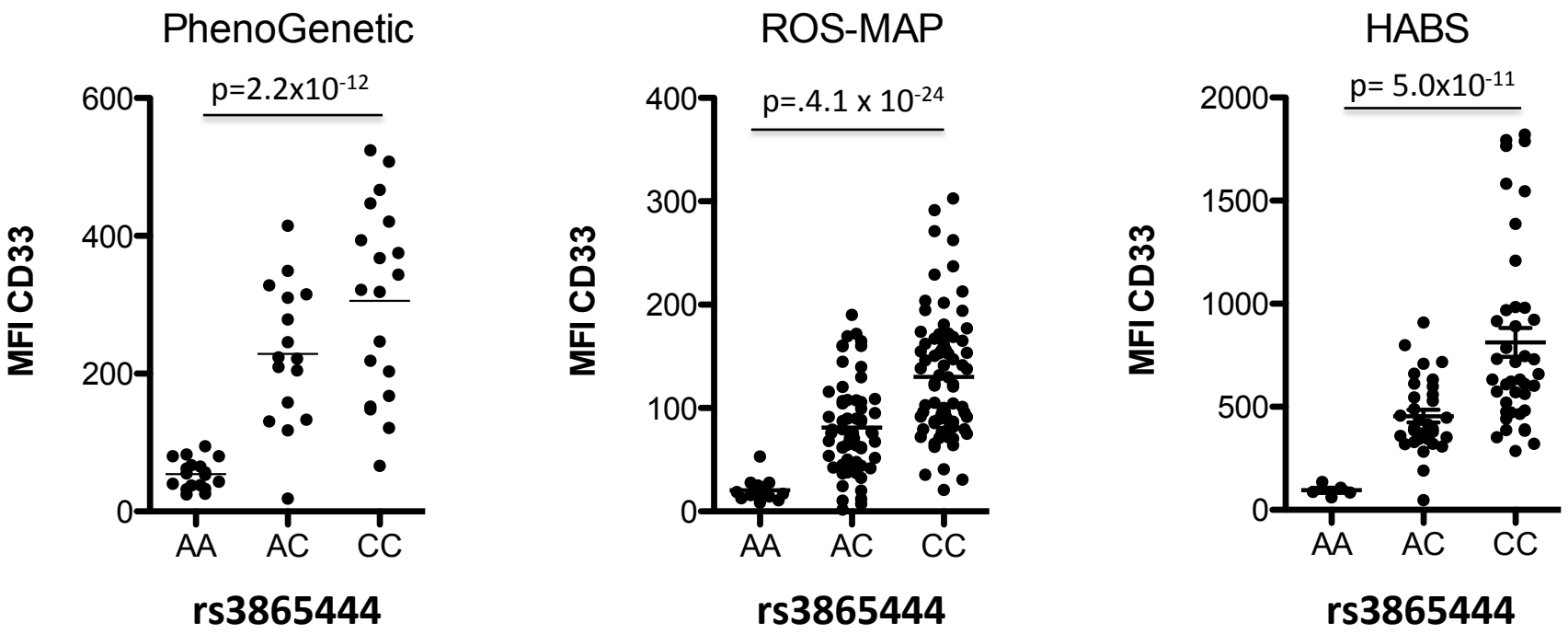
CD33: an innate immune system inhibitory molecule

- Reducing the surface expression of CD33 using siRNA leads to an increase in spontaneous pro-inflammatory cytokine production by monocytes (Lajaunias, 2005)



IS CD33 PROTEIN EXPRESSION INFLUENCED BY THE RS3865444 SNP?

Monocyte CD33 protein expression

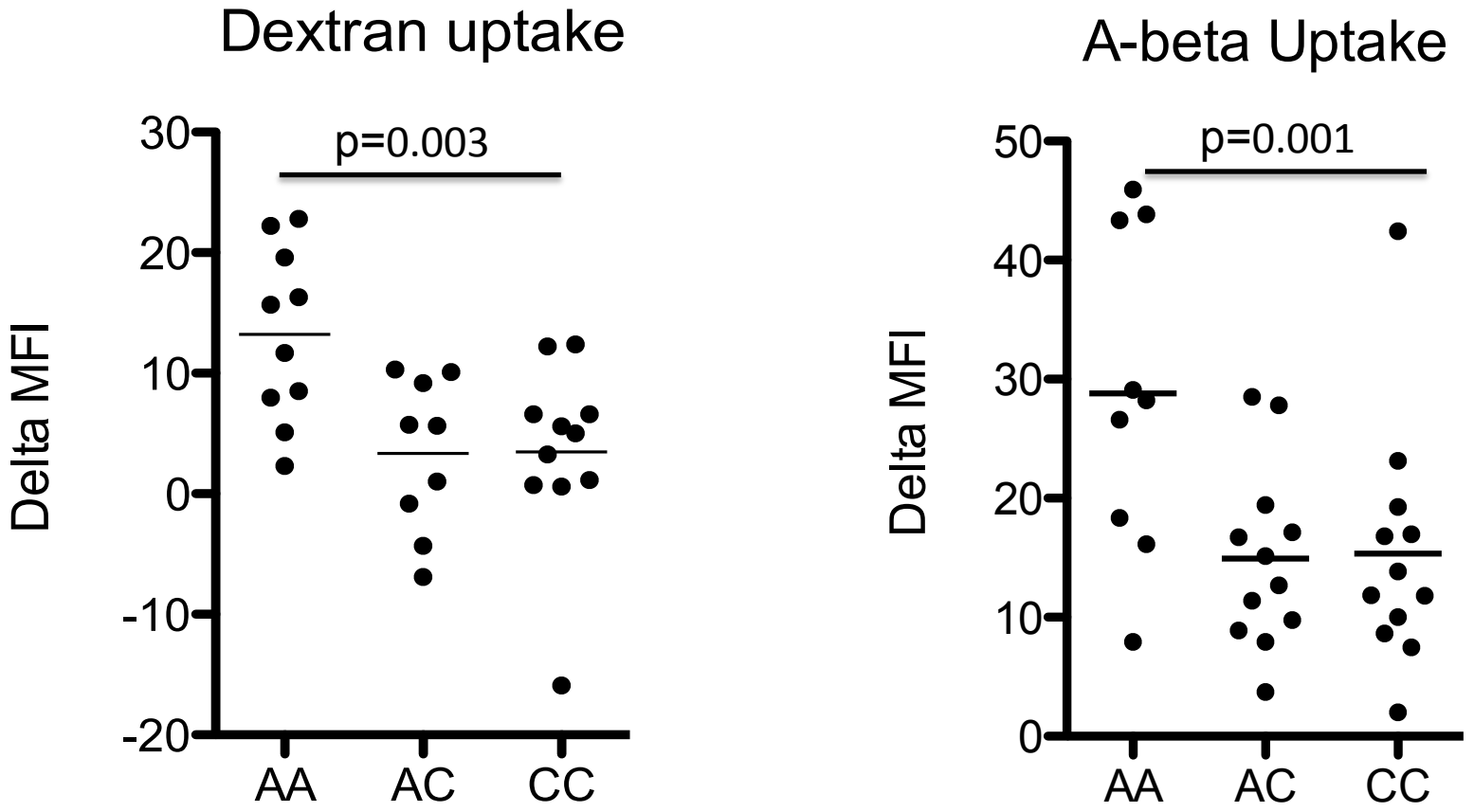


RISK Allele= MORE CD33 PROTEIN

Bradshaw *et al.* (2013) *Nat. Neurosci*.

WHAT DOES THIS MEAN FOR MONOCYTE FUNCTION?

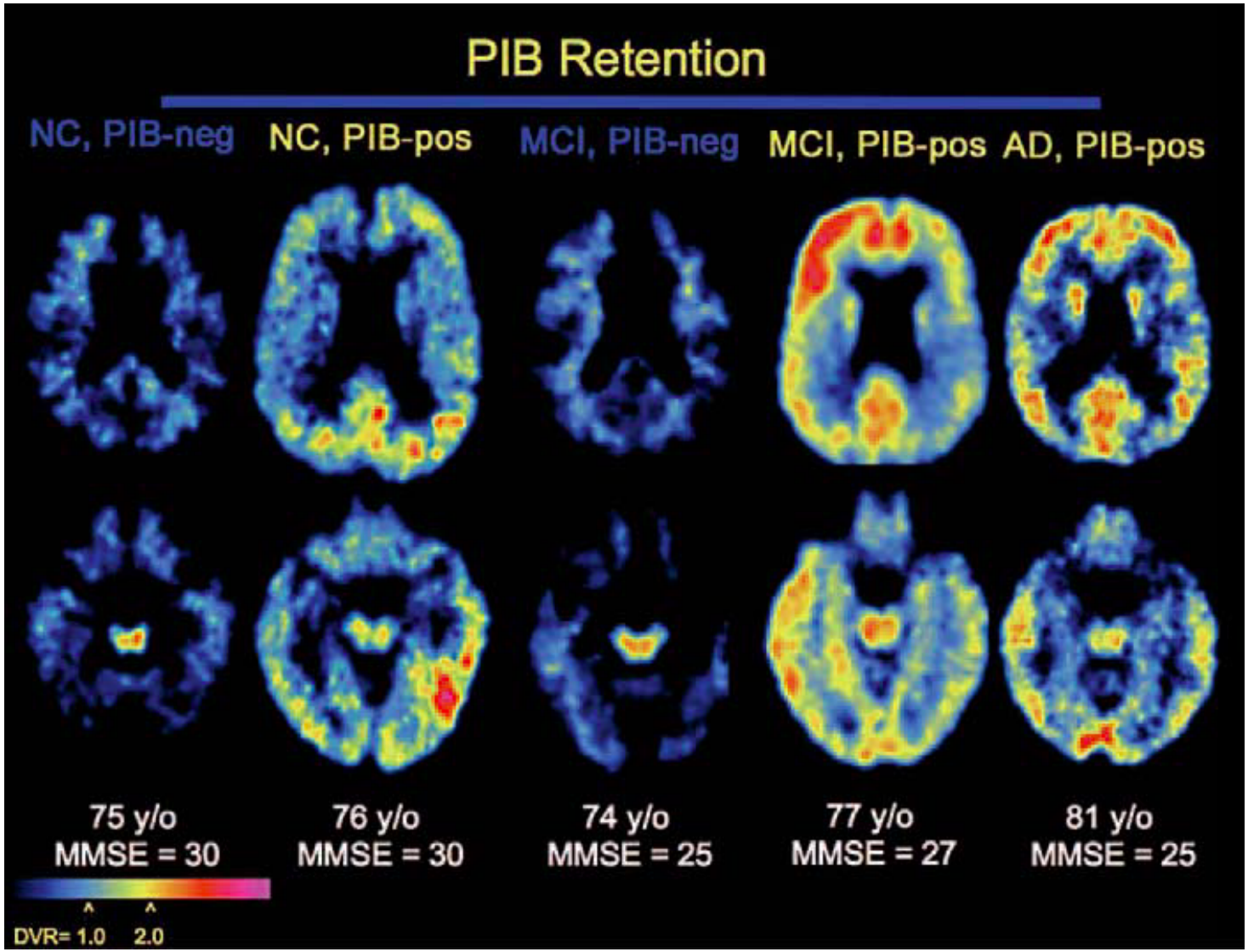
Monocyte uptake ability



Bradshaw *et al. (*2013) *Nat. Neurosci*.

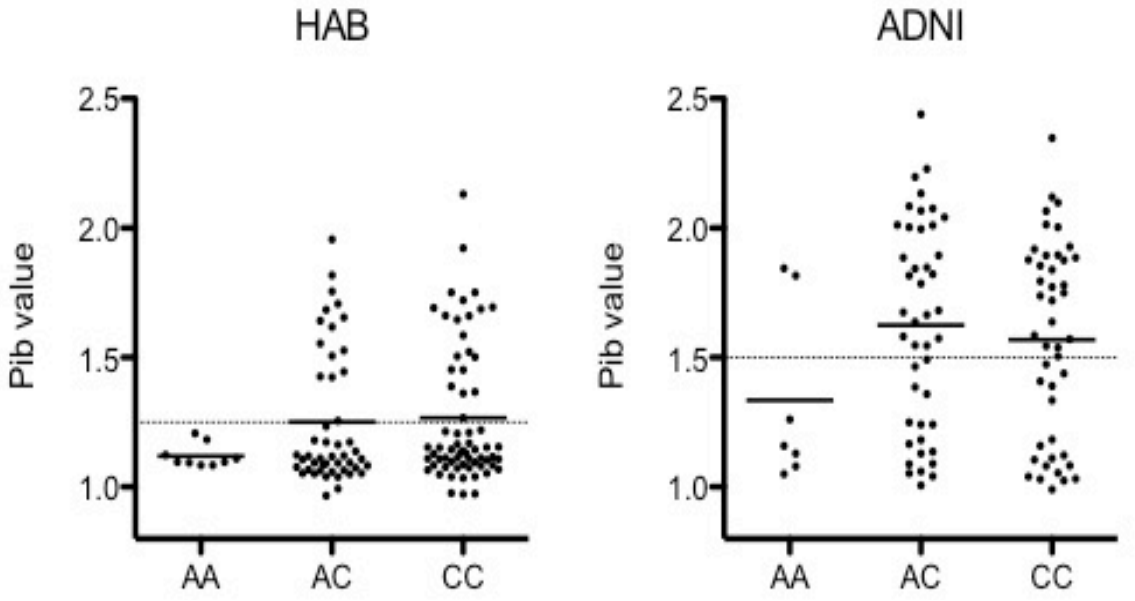
CAN WE CONNECT RS3865444 TO AD PATHOLOGY?

PiB imaging measures fibrillar amyloid in the living



Marshall, GA. Dement Geriatr Cogn Disord. 2011 August; 31(6): 443–450

People homozygous for the protective allele are less likely to be PiB positive



Alzheimer's Disease Neuroimaging Initiative (ADNI): elderly controls, subjects with mild cognitive impairment, and with AD.

Analysis for the rs3865444[C] risk allele	P-value
PiB + versus Pib-	p=0.10
PiB + versus Pib- with "C" dominant	p=0.02
quantitative trait	p=0.22
quantitative trait; dominant model	p=0.02

Bradshaw *et al.* (2013) *Nat. Neurosci*.

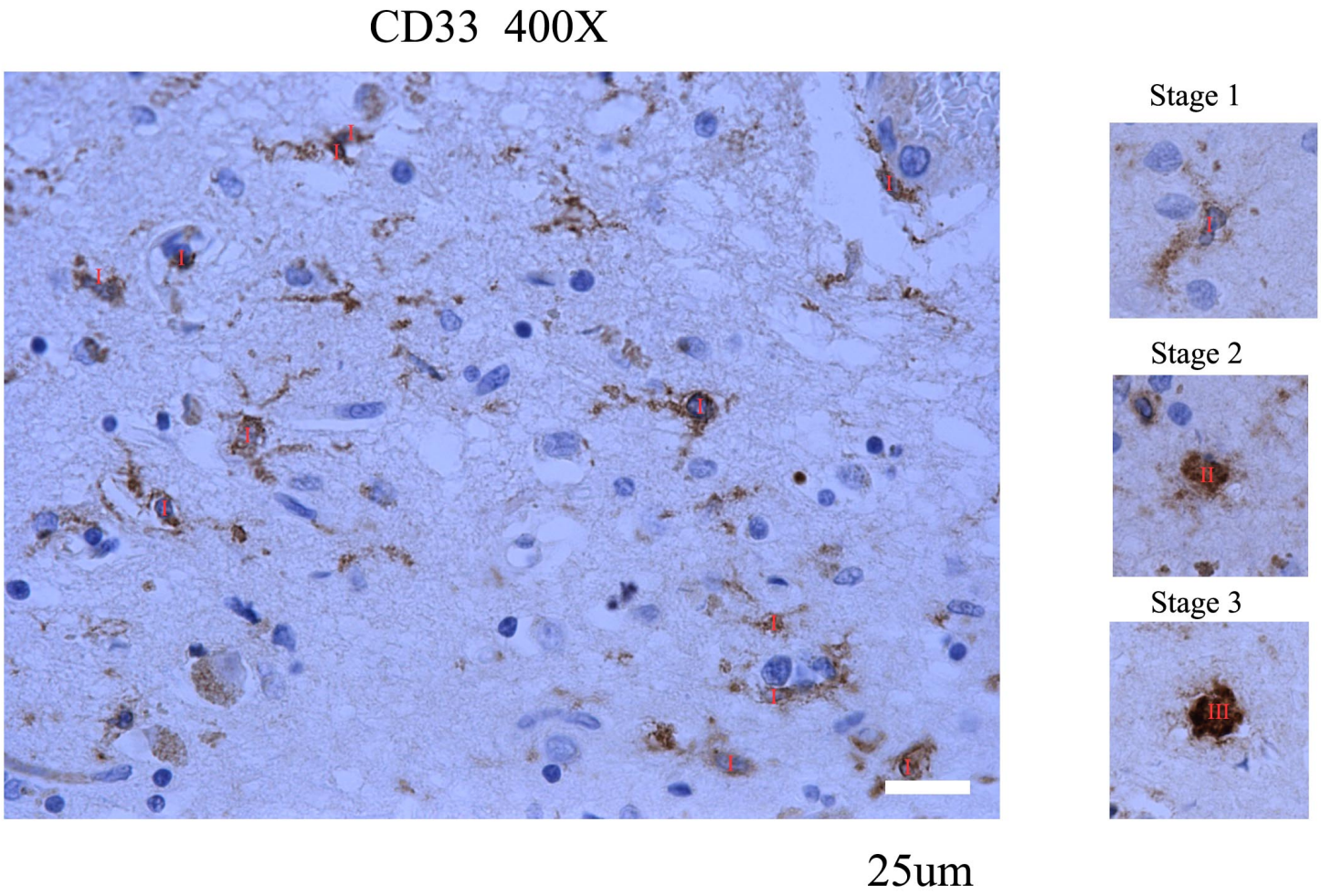
Correlation to autopsy data from ROS-MAP

Neurophathological phenotype*	Association with rs3865444[C]	P-value
Neuritic amyloid plaques	Yes	p=0.01
pathologic diagnosis of AD	Yes	p=0.01
neurofibrillary tangles	No	p=0.16

*n=149

Bradshaw *et al.* (2013) *Nat. Neurosci*.

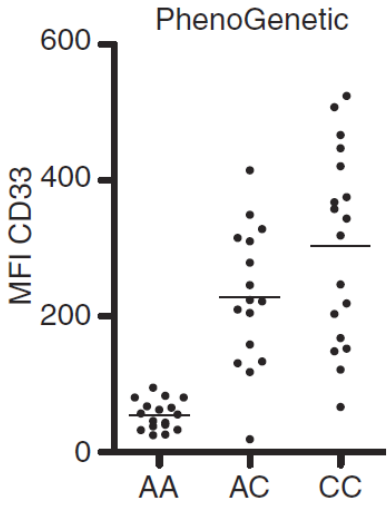
CD33 is expressed by CNS microglia/macrophages

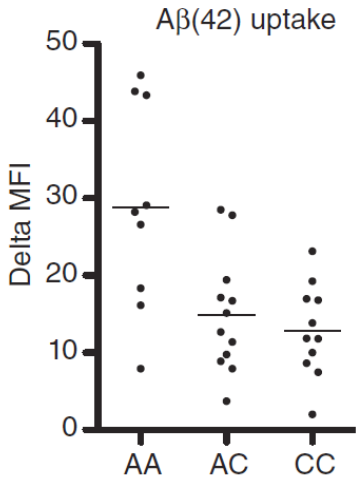


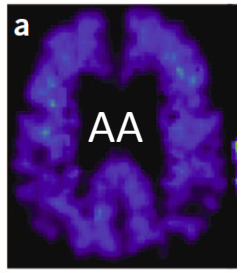
Bradshaw *et al.* (2013) *Nat. Neurosci*.

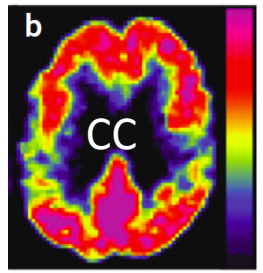
CD33 Summary

rs3865444^{C} risk allele → Greater expression of CD33 → Less Aβ uptake → More Aβ burden in brain





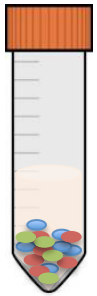


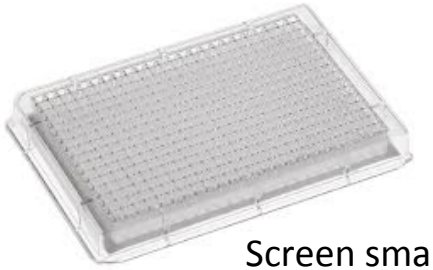


IDENTIFICATION OF SMALL MOLECULES THAT MODULATE CD33

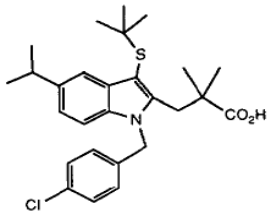
Screen Study Design

PBMCs from a subject with CD33 risk allele

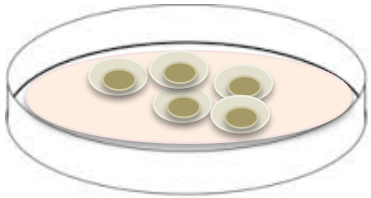




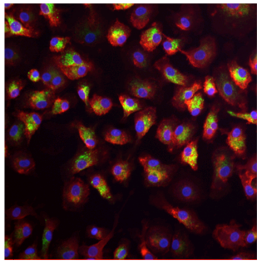
Screen small molecules in triplicate for reduction of CD33



Identify potential leads



Validate in MDM from subjects with the risk allele



Evaluate for modulation of uptake

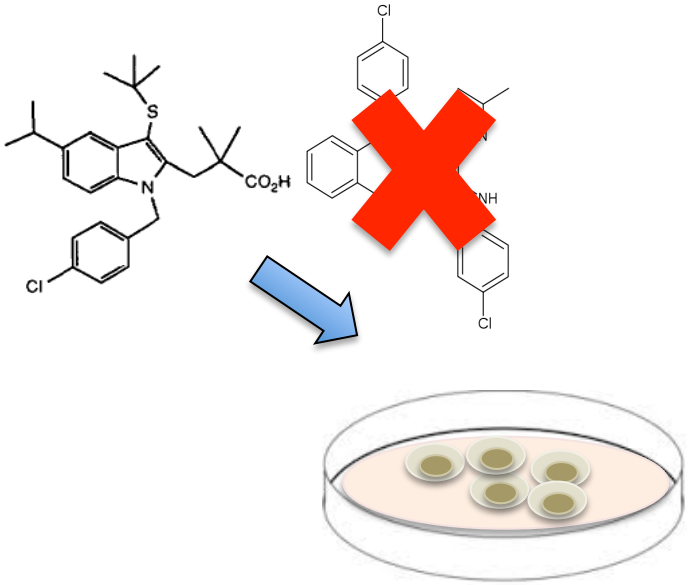
Pilot Screen

- Performed an initial screen of 1200 FDA-approved molecules in human PBMCs
- Identified 5 molecules which reduce monocyte CD33 expression levels in rs3865444^{CC} subject

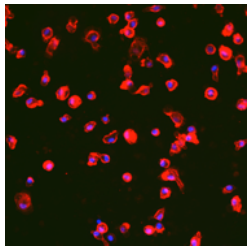
	Drug treated CC samples																						AA control	CC control
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
a	879.40	850.39	830.66	909.46	774.08	755.09	704.14	730.11	720.29	755.70	753.41	746.76	675.71	698.52	676.07	713.49	866.95	821.38	809.76	827.68	892.32	814.84	196.39	697.61
b	832.81	806.62	721.18	766.16	798.28	488.69	692.11	691.37	683.97	713.50	723.72	709.71	757.61	717.70	732.50	692.97	642.29	709.27	756.01	792.15	822.19	807.39	198.97	716.02
c	860.28	777.44	755.71	768.56	774.37	787.98	721.17	711.76	688.68	730.50	666.57	639.62	670.48	687.53	710.74	733.90	758.78	781.75	764.06	762.21	748.27	824.23	207.24	746.18
d	749.91	789.94	773.50	672.56	559.65	547.28	660.33	716.56	615.35	695.80	793.35	675.85	627.25	562.38	670.46	721.59	670.16	701.47	754.57	743.95	612.82	817.08	184.07	814.92
e	786.08	808.49	708.84	790.72	680.27	695.80	636.04	667.20	793.42	686.18	712.57	719.74	700.24	604.42	716.28	645.22	679.86	607.35	837.78	668.34	762.92	793.80	173.14	716.75
f	819.04	838.50	657.46	718.74	703.36	693.87	652.78	660.51	560.98	713.46	764.07	714.00	633.42	584.25	700.61	729.81	657.68	677.33	729.33	902.84	718.37	687.52	244.11	622.95
g	806.84	749.69	693.78	799.43	705.06	728.89	653.52	577.37	680.40	673.12	750.15	723.25	657.24	588.55	647.04	579.22	616.52	681.56	658.17	646.31	778.02	729.39	161.91	808.69
h	793.07	801.22	692.19	741.90	714.20	627.14	661.64	663.25	667.91	709.70	784.54	438.80	687.87	558.14	703.23	662.07	742.27	717.13	674.19	721.26	755.98	734.38	155.81	674.75
i	777.36	831.60	743.72	713.48	670.06	605.09	607.96	758.22	740.65	641.84	750.09	613.81	538.27	688.03	677.51	712.72	658.74	725.25	675.97	597.72	781.41	817.17	188.42	647.04
j	705.17	736.85	818.34	740.32	755.99	635.36	702.13	637.23	722.89	681.50	697.38	743.86	676.39	580.06	674.99	760.47	702.55	656.16	753.95	671.46	783.07	749.58	150.99	737.79
k	711.75	699.29	712.43	757.75	739.44	642.80	703.58	744.56	668.76	554.64	733.78	602.41	641.06	676.78	627.14	730.64	720.36	771.84	701.16	689.64	525.59	757.18	144.57	723.25
l	326.69	717.83	675.31	764.31	795.38	650.99	695.80	690.93	638.78	687.82	757.01	720.40	702.21	726.42	760.57	762.89	731.78	642.15	734.29	637.32	840.63	777.95	226.96	807.14
m	719.60	694.97	671.43	811.83	674.65	529.15	784.59	718.36	687.93	730.10	691.86	596.01	610.18	675.32	698.08	743.63	646.09	631.38	760.01	859.94	789.08	841.39	162.71	835.27
n	694.56	709.50	752.51	737.71	593.83	712.90	591.86	278.82	730.61	755.92	679.04	548.07	631.53	695.27	681.89	772.99	738.41	806.06	834.02	782.60	833.41	828.48	136.98	841.11
o	692.72	623.64	673.92	712.20	716.60	653.92	716.56	783.30	749.27	612.92	679.26	648.47	663.44	689.78	659.49	827.18	787.68	757.36	823.62	800.51	809.57	786.99	139.16	685.02
p	500.79	745.10	624.41	687.14	758.45	673.44	666.34	662.12	699.83	687.74	683.16	655.34	682.30	703.01	752.28	783.61	721.50	756.92	758.90	755.93	803.76	825.55	162.00	735.49

2 compounds were analyzed for CD33 expression in MDM from multiple individuals

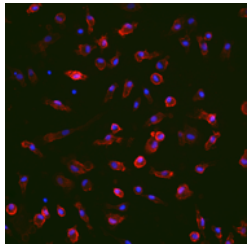
LDNX49 or LDNX55



CD33

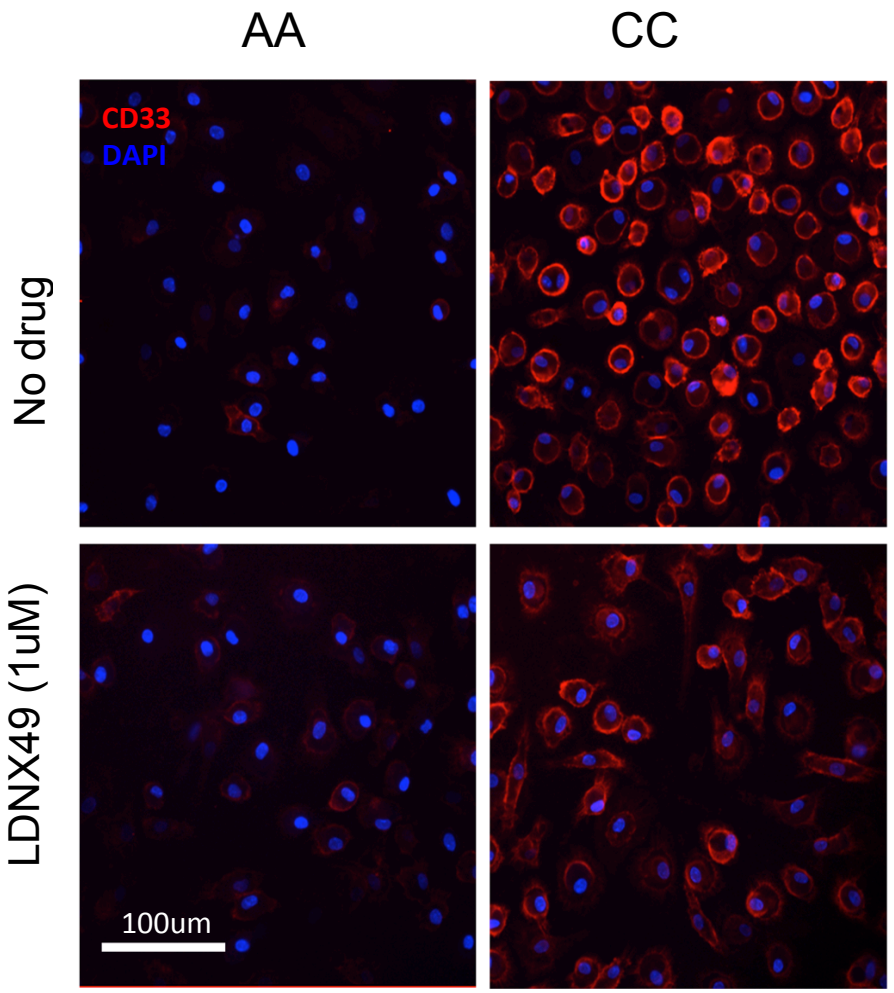


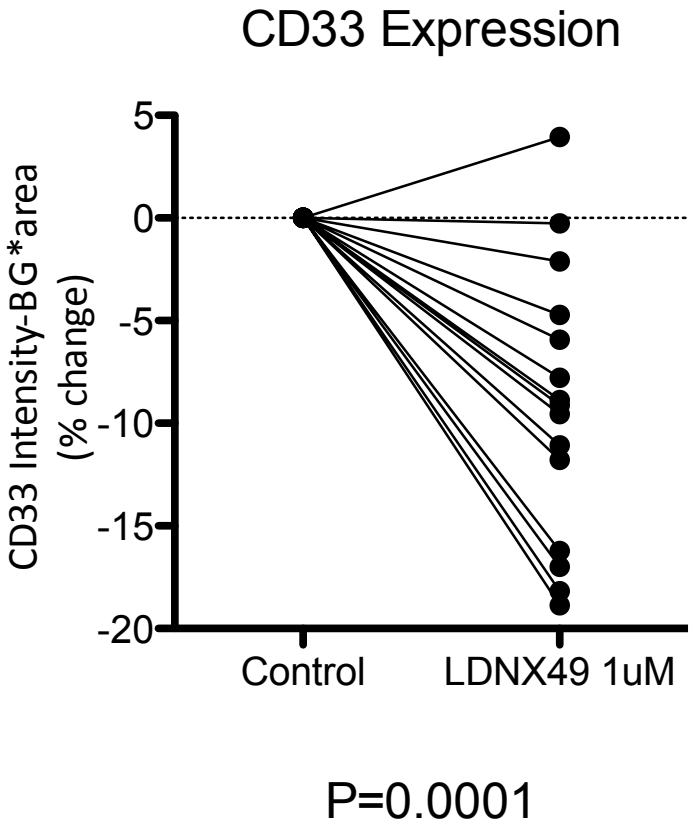
DMSO control



Treatment

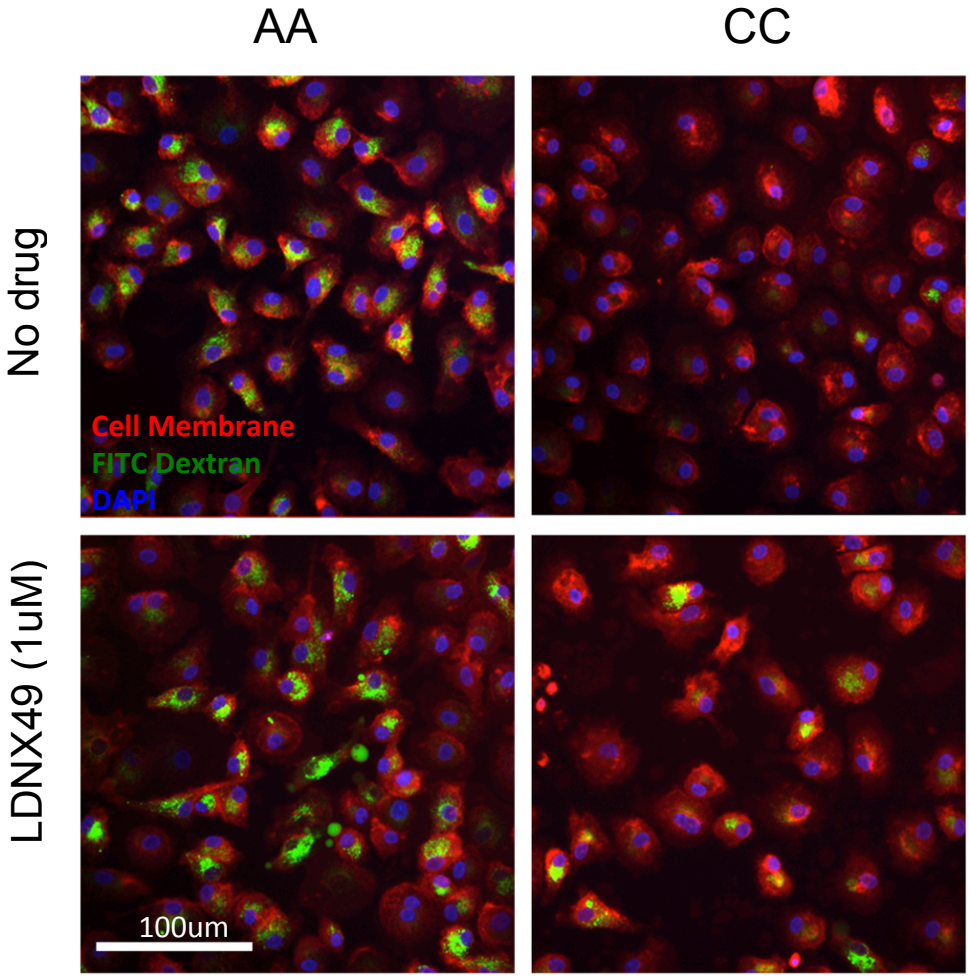
LDNX49 reduces CD33 surface expression in macrophages of subjects carrying the risk allele (CC)

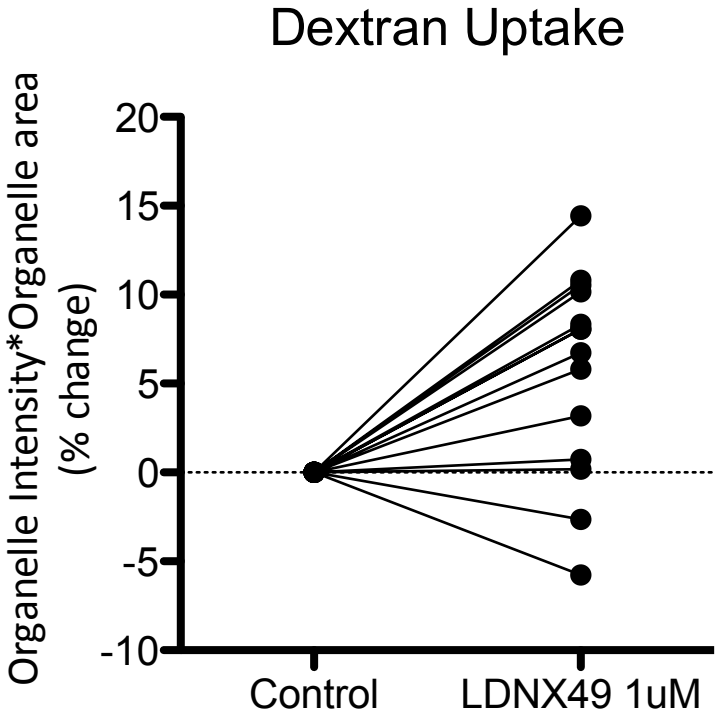




P=0.0001

LDNX49 increases uptake of dextran in macrophages of subjects carrying the risk allele (CC)





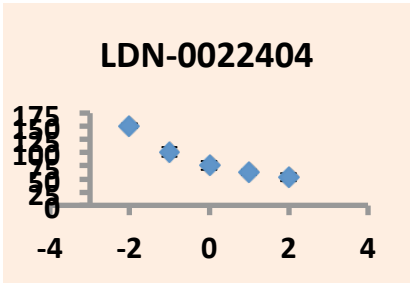
P=0.0028

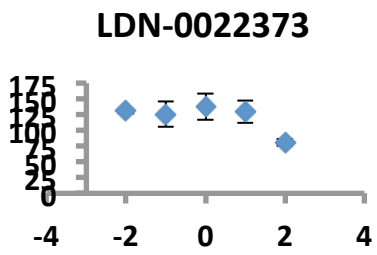
Expanded Screen

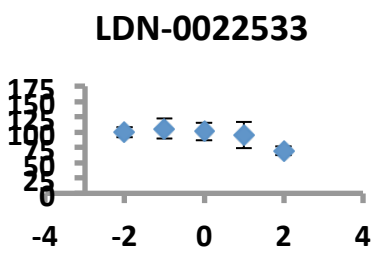
- Performed a screen of 9000 molecules selected for targeting the CNS
- Identified 12 molecules which reduced monocyte CD33 expression levels in rs3865444^{CC} subjects

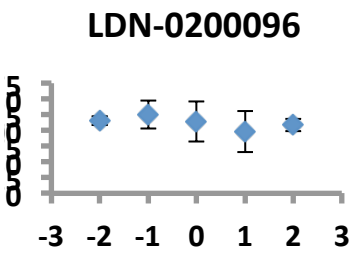
	Drug treated CC samples																						AA control	CC control
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
a	879.40	850.39	830.66	909.46	774.08	755.09	704.14	730.11	720.29	755.70	753.41	746.76	675.71	698.52	676.07	713.49	866.95	821.38	809.76	827.68	892.32	814.84	196.39	697.61
b	832.81	806.62	721.18	766.16	798.28	488.69	692.11	691.37	683.97	713.50	723.72	709.71	757.61	717.70	732.50	692.97	642.29	709.27	756.01	792.15	822.19	807.39	198.97	716.02
c	860.28	777.44	755.71	768.56	774.37	787.98	721.17	711.76	688.68	730.50	666.57	639.62	670.48	687.53	710.74	733.90	758.78	781.75	764.06	762.21	748.27	824.23	207.24	746.18
d	749.91	789.94	773.50	672.56	559.65	547.28	660.33	716.56	615.35	695.80	793.35	675.85	627.25	562.38	670.46	721.59	670.16	701.47	754.57	743.95	612.82	817.08	184.07	814.92
e	786.08	808.49	708.84	790.72	680.27	695.80	636.04	667.20	793.42	686.18	712.57	719.74	700.24	604.42	716.28	645.22	679.86	607.35	837.78	668.34	762.92	793.80	173.14	716.75
f	819.04	838.50	657.46	718.74	703.36	693.87	652.78	660.51	560.98	713.46	764.07	714.00	633.42	584.25	700.61	729.81	657.68	677.33	729.33	902.84	718.37	687.52	244.11	622.95
g	806.84	749.69	693.78	799.43	705.06	728.89	653.52	577.37	680.40	673.12	750.15	723.25	657.24	588.55	647.04	579.22	616.52	681.56	658.17	646.31	778.02	729.39	161.91	808.69
h	793.07	801.22	692.19	741.90	714.20	627.14	661.64	663.25	667.91	709.70	784.54	438.80	687.87	558.14	703.23	662.07	742.27	717.13	674.19	721.26	755.98	734.38	155.81	674.75
i	777.36	831.60	743.72	713.48	670.06	605.09	607.96	758.22	740.65	641.84	750.09	613.81	538.27	688.03	677.51	712.72	658.74	725.25	675.97	597.72	781.41	817.17	188.42	647.04
j	705.17	736.85	818.34	740.32	755.99	635.36	702.13	637.23	722.89	681.50	697.38	743.86	676.39	580.06	674.99	760.47	702.55	656.16	753.95	671.46	783.07	749.58	150.99	737.79
k	711.75	699.29	712.43	757.75	739.44	642.80	703.58	744.56	668.76	554.64	733.78	602.41	641.06	676.78	627.14	730.64	720.36	771.84	701.16	689.64	525.59	757.18	144.57	723.25
l	326.69	717.83	675.31	764.31	795.38	650.99	695.80	690.93	638.78	687.82	757.01	720.40	702.21	726.42	760.57	762.89	731.78	642.15	734.29	637.32	840.63	777.95	226.96	807.14
m	719.60	694.97	671.43	811.83	674.65	529.15	784.59	718.36	687.93	730.10	691.86	596.01	610.18	675.32	698.08	743.63	646.09	631.38	760.01	859.94	789.08	841.39	162.71	835.27
n	694.56	709.50	752.51	737.71	593.83	712.90	591.86	278.82	730.61	755.92	679.04	548.07	631.53	695.27	681.89	772.99	738.41	806.06	834.02	782.60	833.41	828.48	136.98	841.11
o	692.72	623.64	673.92	712.20	716.60	653.92	716.56	783.30	749.27	612.92	679.26	648.47	663.44	689.78	659.49	827.18	787.68	757.36	823.62	800.51	809.57	786.99	139.16	685.02
p	500.79	745.10	624.41	687.14	758.45	673.44	666.34	662.12	699.83	687.74	683.16	655.34	682.30	703.01	752.28	783.61	721.50	756.92	758.90	755.93	803.76	825.55	162.00	735.49

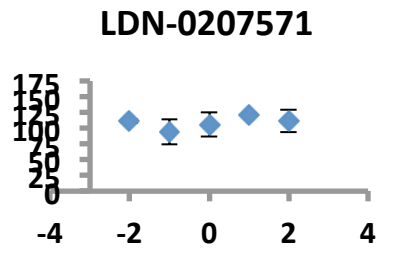
3 compounds prioritized after dose response

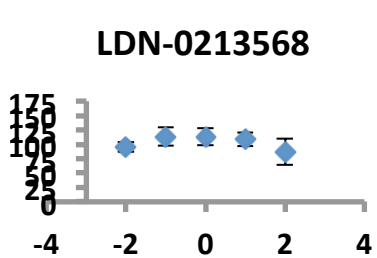


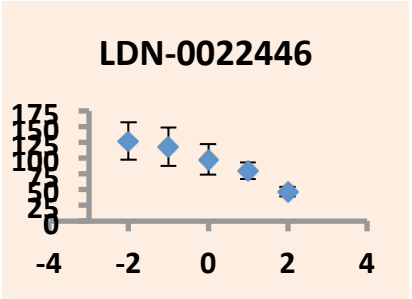


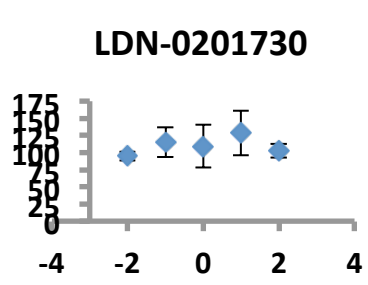


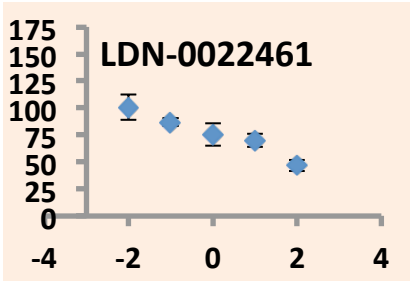


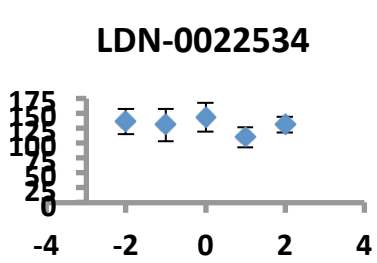


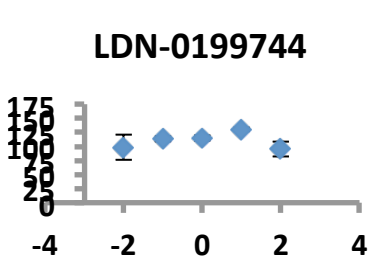


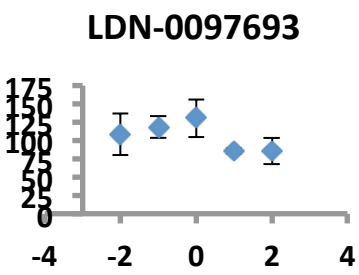












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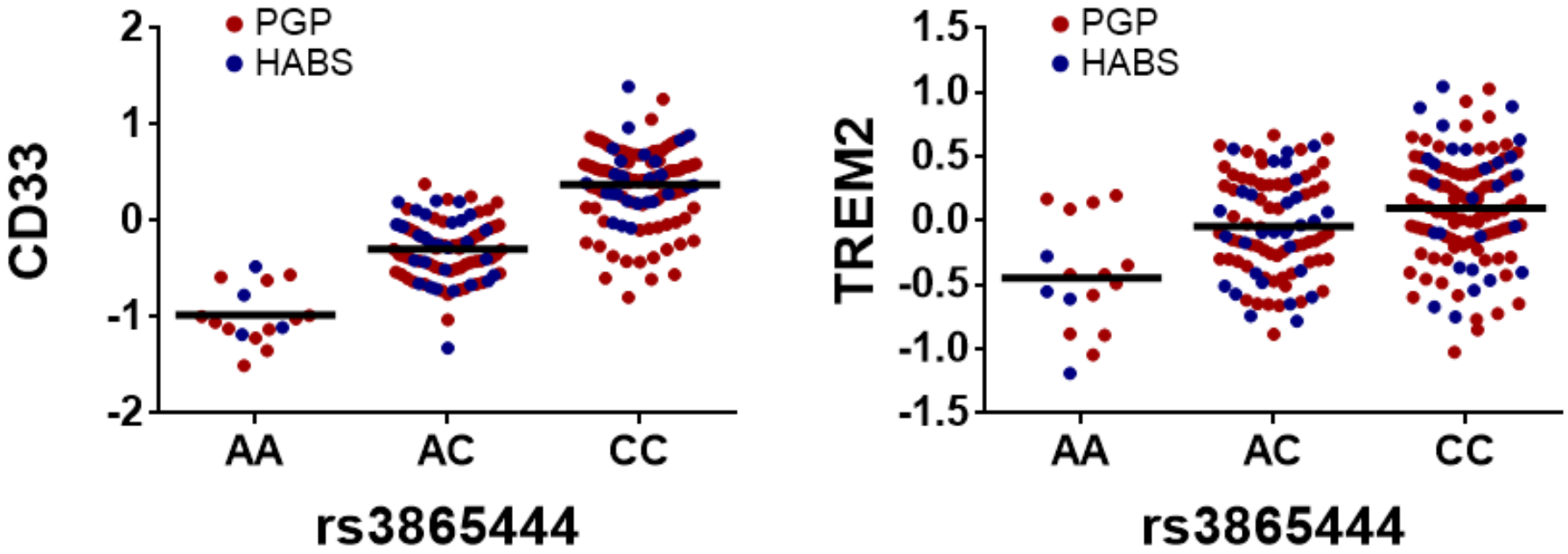
Screen Summary

- Out of 1200 FDA-approved compounds screened, 5 molecules reduced monocyte CD33 expression levels in a rs3865444^{CC} subject to levels that were intermediate between rs3865444^{CC} and rs3865444^{AA} subjects.
- This effect was confirmed *in vitro* in additional subjects for LDNX49.
- Functional studies suggest that LDNX49 also reverses the reduced uptake capacity of macrophages from rs3865444^{CC} subjects.
- Out of 9000 additional compounds 3 molecules were identified that reduce monocyte CD33 levels.

Future Studies

- Validate the newly identified small molecules
- Examine uptake in the presence of the molecules
- Examine additional macrophage functions
- Examine other genetic loci proteins for modulation by identified small molecules

AD risk variant in *CD33* locus, rs3865444[C], associated with increased CD33 and TREM2



	Discovery p-value	Replication p-value	Meta p-value
CD33	6.5×10^{-33}	6.4×10^{-11}	3.1×10^{-42}
TREM2	1.6×10^{-3}	0.01	6.7×10^{-5}

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