Relationship between CD33 expression, splicing polymorphism, and *in vitro* cytotoxicity of gemtuzumab ozogamicin and the CD33/CD3 BiTE® AMG 330

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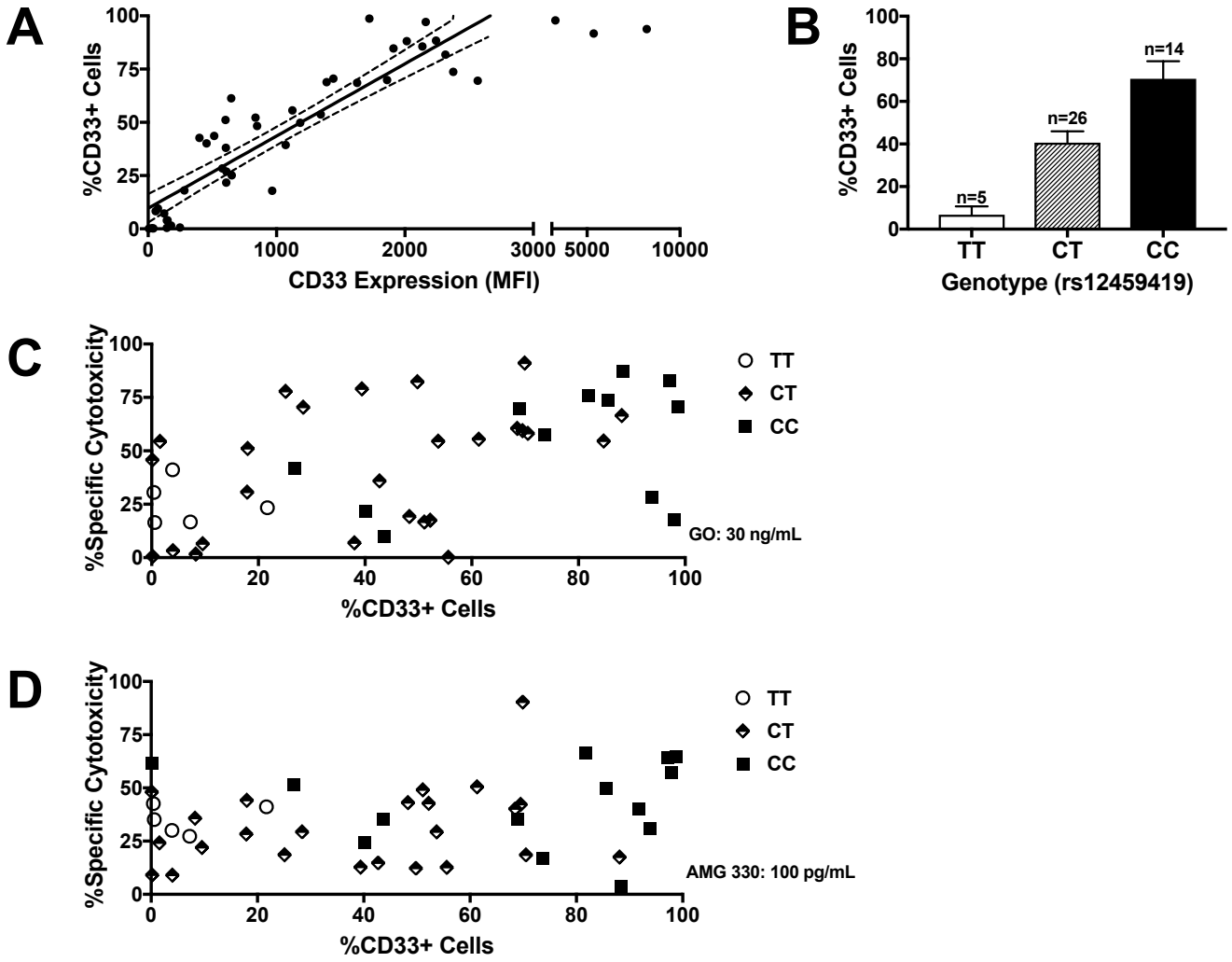
SUPPLEMENTARY TABLE 1. CD33 expression, stratified by rs12459419 genotype, cytogenetic risk, and disease stage

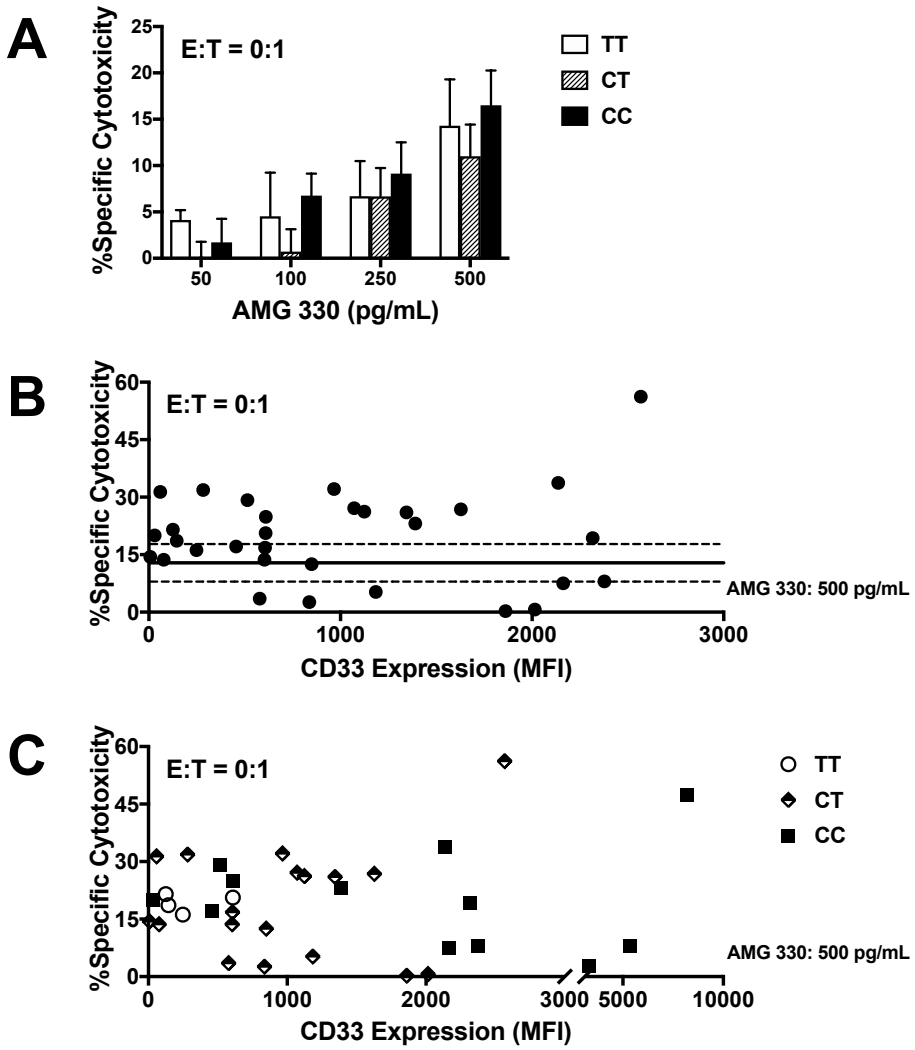
	All Median CD33 MFI (range), n	TT Median CD33 MFI (range), n	CT Median CD33 MFI (range), n	CC Median CD33 MFI (range), n
Newly-diagnosed AML	**651 (30-8206), n=25**	**197 (145-248), n=2**	**649 (41-2014), n=12**	**1723 (30-8206), n=11**
Favorable risk	551 (145-2161), n=4	145, n=1	647, n=1	1308 (455-2162), n=2
Intermediate risk	843 (59-5356), n=14	248, n=1	744 (59-1861), n=8	1723 (610-5356), n=5
Adverse risk	514 (30-8206), n=7	---	400 (41-2014), n=3	1904 (30-8206), n=4
Relapsed/refractory AML	**1018 (7-2567), n=20**	**146 (125-609), n=3**	**1018 (7-2567), n=14**	**2316 (2136-2377), n=3**
Favorable risk	---	---	---	---
Intermediate risk	1075 (77-2567), n=16	146 (125-609), n=3	1075 (77-2567), n=10	2316 (2136-2377), n=3
Adverse risk	611 (7-1124), n=4	---	611 (7-1124), n=4	---

SUPPLEMENTARY FIGURE 1



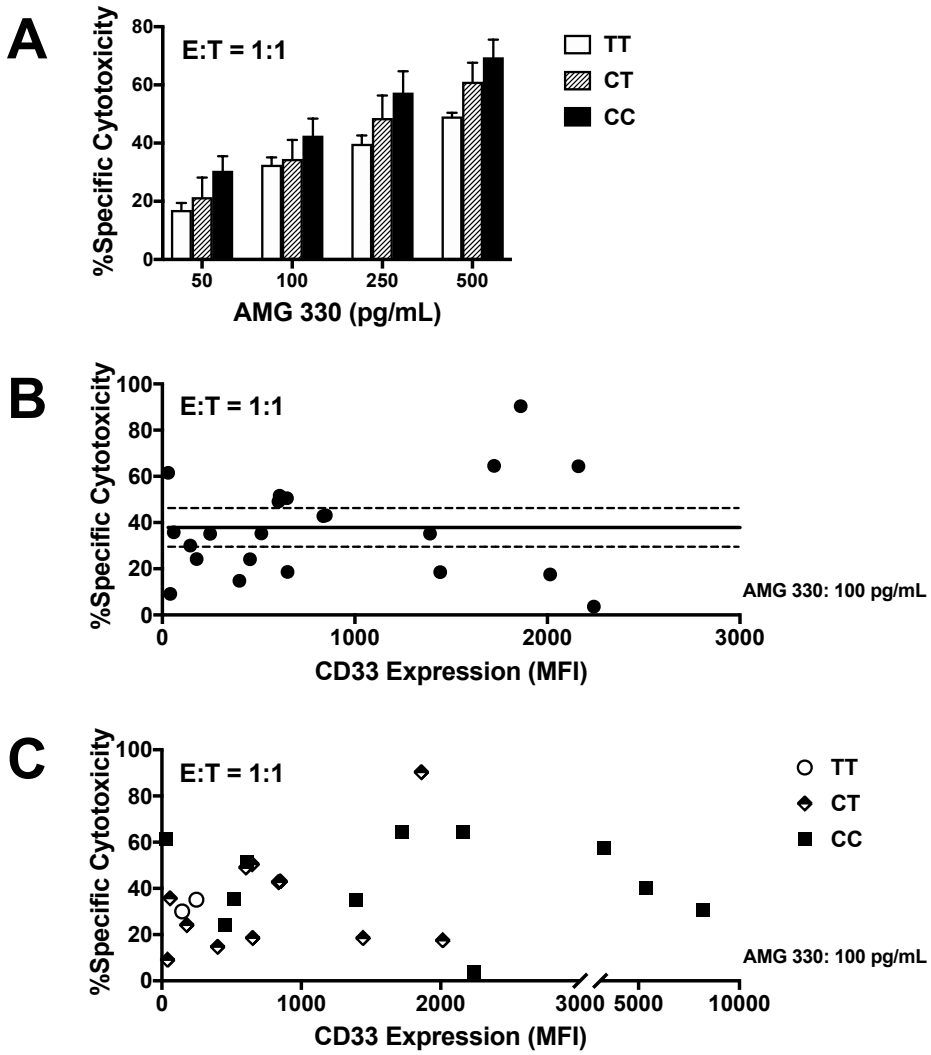
Relationship between %CD33+ blasts, cell surface expression of CD33, CD33 genotype (rs12459419), and *in vitro* GO and AMG 330-induced cytotoxicity in primary AML samples. The percentage of CD33+ blasts was determined essentially as described by Khan et al. (*Leukemia* 2017;31:1059-1068). **(a)** Linear regression between %CD33+ blasts and CD33 expression. **(b)** Percentage of CD33+ blasts in 45 primary AML specimens, stratified by rs12459419 genotype. **(c)** Relationship between cytotoxicity induced by 30 ng/mL of GO/calicheamicin and %CD33+ blasts, stratified by rs12459419 genotype. **(d)** Relationship between cytotoxicity induced by 100 pg/mL of AMG 330 and %CD33+ blasts, stratified by rs12459419 genotype.

SUPPLEMENTARY FIGURE 2



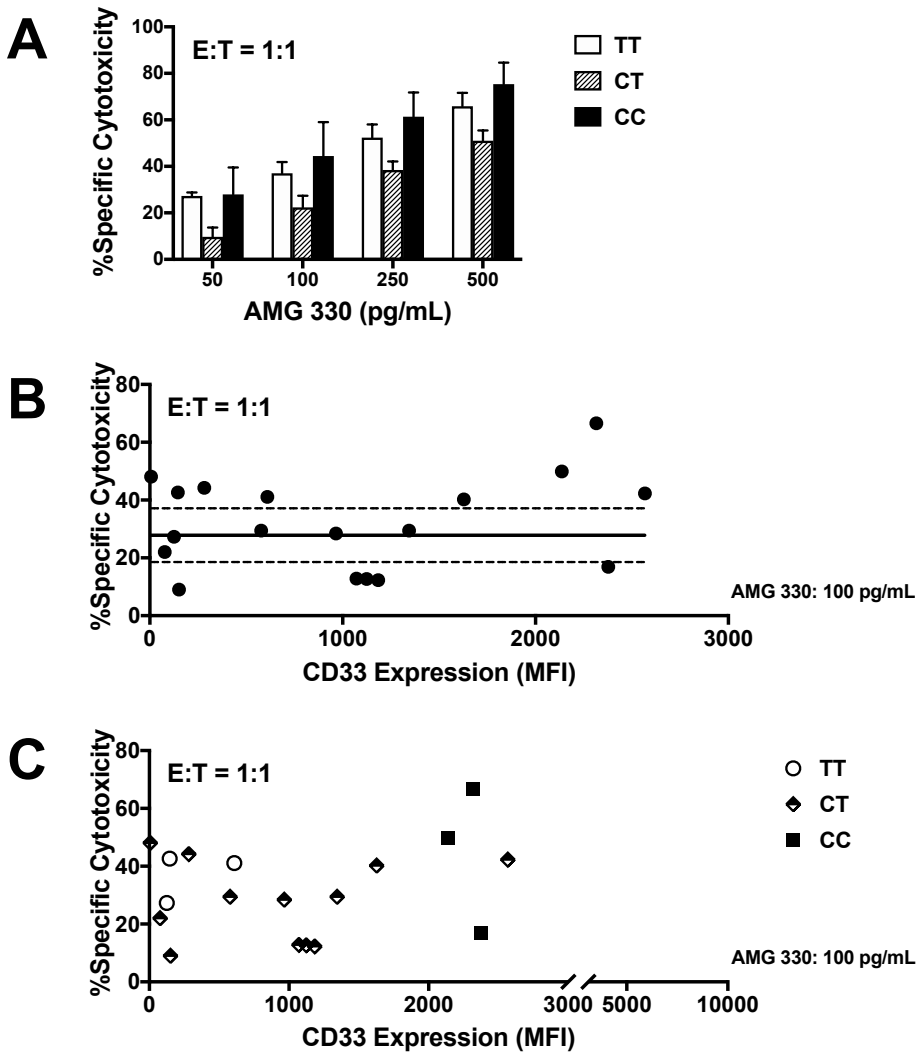
Relationship between cell surface expression of CD33, CD33 genotype (rs12459419), and *in vitro* AMG 330-induced cytotoxicity in primary AML samples in the absence of exogenous (healthy donor) T-cells. (a) Aliquots of primary AML cells were incubated with various doses of AMG 330 as indicated without addition of healthy-donor T-cells. After 2 days, cell numbers and drug-induced cytotoxicity were determined, using DAPI to detect non-viable cells. AML cells were identified by forward/side scatter properties and, negativity for CellVue Burgundy dye. **(b)** Linear regression between CD33 expression and drug-induced cytotoxicity (AMG 330 dose: 500 pg/mL). Dashed line: 95% confidence interval. **(c)** Relationship between cytotoxicity induced by 500 pg/mL of AMG 330 and CD33 expression, stratified by rs12459419 genotype.

SUPPLEMENTARY FIGURE 3



Relationship between cell surface expression of CD33, CD33 genotype (rs12459419), and *in vitro* AMG 330-induced cytotoxicity in primary AML samples from adults with newly-diagnosed AML. (a) Aliquots of pre-treatment primary AML cells obtained from adults with newly-diagnosed AML were incubated with various doses of AMG 330 as indicated together with healthy-donor T-cells at an E:T cell ratio of 1:1. After 2 days, cell numbers and drug-induced cytotoxicity were determined, using DAPI to detect non-viable cells. AML cells were identified by forward/side scatter properties and, negativity for CellVue Burgundy dye. **(b)** Linear regression between CD33 expression and drug-induced cytotoxicity (AMG 330 dose: 100 pg/mL). Dashed line: 95% confidence interval. **(c)** Relationship between cytotoxicity induced by 100 pg/mL of AMG 330 and CD33 expression, stratified by rs12459419 genotype.

SUPPLEMENTARY FIGURE 4



Relationship between cell surface expression of CD33, CD33 genotype (rs12459419), and *in vitro* AMG 330-induced cytotoxicity in primary AML samples from adults with relapsed/refractory AML. (a) Aliquots of pre-treatment primary AML cells obtained from adults with relapsed or refractory AML were incubated with various doses of AMG 330 as indicated together with healthy-donor T-cells at an E:T cell ratio of 1:1. After 2 days, cell numbers and drug-induced cytotoxicity were determined, using DAPI to detect non-viable cells. AML cells were identified by forward/side scatter properties and, negativity for CellVue Burgundy dye. **(b)** Linear regression between CD33 expression and drug-induced cytotoxicity (AMG 330 dose: 100 pg/mL). Dashed line: 95% confidence interval. **(c)** Relationship between cytotoxicity induced by 100 pg/mL of AMG 330 and CD33 expression, stratified by rs12459419 genotype.