SUPPLEMENTAL MATERIAL

Pharmacogenomics of intracellular methotrexate polyglutamates in patient leukemia cells in vivo.

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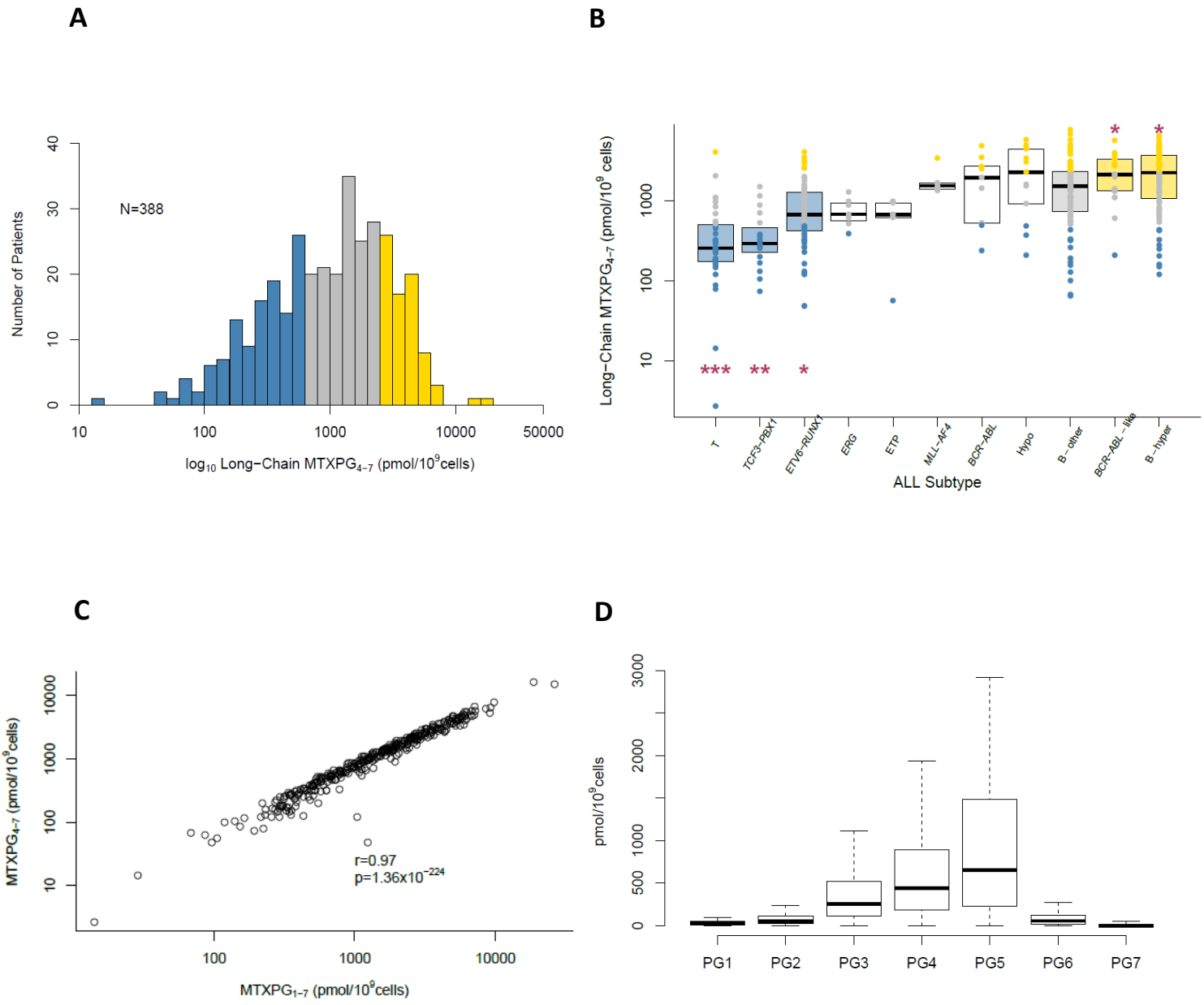
Supplemental Figure 1. Intracellular long-chain methotrexate polyglutamate ($MTXPG_{4-7}$) accumulation in bone marrow acute lymphoblastic leukemia (ALL) cells from 388 newly diagnosed patients with B-lineage ALL, measured at 42 h following in vivo treatment with HDMTX.

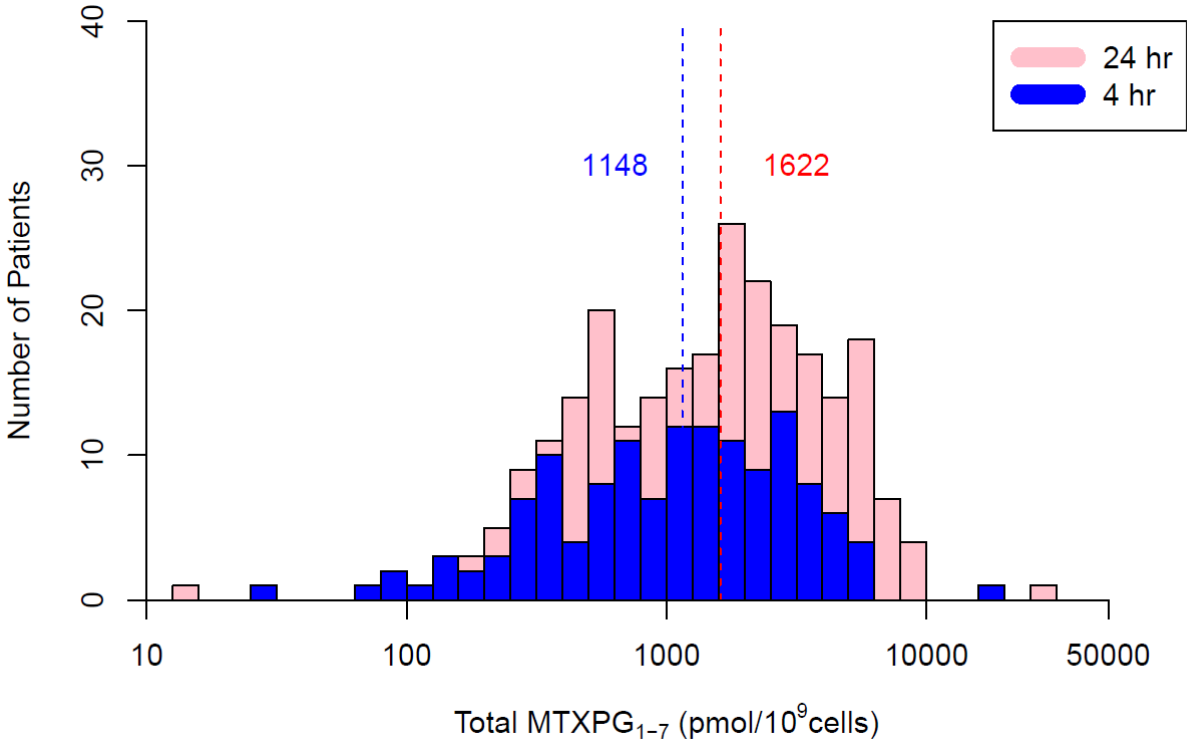
(A) Histogram of long-chain methotrexate polyglutamate ($MTXPG_{4-7}$) accumulation. Samples with low MTXPG accumulation (lower quartile, in blue) ranged from 2.69 to 441.75 pmol of long-chain MTXPG per 10^9cells, while samples with high MTXPG accumulation (upper quartile, in gold) had MTXPG levels of 2,261.46-16,626.7 pmol/10^9cells**. (B)** Long-chain polyglutamates in leukemia cells for 11 major subtypes of ALL. Each box includes data between the 25^{th} and 75^{th} percentiles, with horizontal line indicating the median. Whiskers indicate range of observations. *, **, and *** indicate t-test p-values <0.05, <1x10-5 and <1x10-10, respectively. **(C)** Correlation between Total ($MTXPG_{1-7}$) and Long-chain ($MTXPG_{4-7}$) MTXPG accumulation**.** $MTXPG_{1-7}$ value (x-axis) for each ALL sample is plotted with respect to its corresponding $MTXPG_{4-7}$ value (y-axis) (Pearson´s correlation r=0.974 p= 1.36 x 10^{-224}). **(D)** Distribution of MTXPGs by number of glutamates. Each box depicts MTXPGs with different number of glutamates in primary ALL cells from 340 patients treated with HDMTX. Each box includes data between the 25^{th} and 75^{th} percentiles, with horizontal line indicating the median. Whiskers indicate range of observations.

Supplemental Figure 1



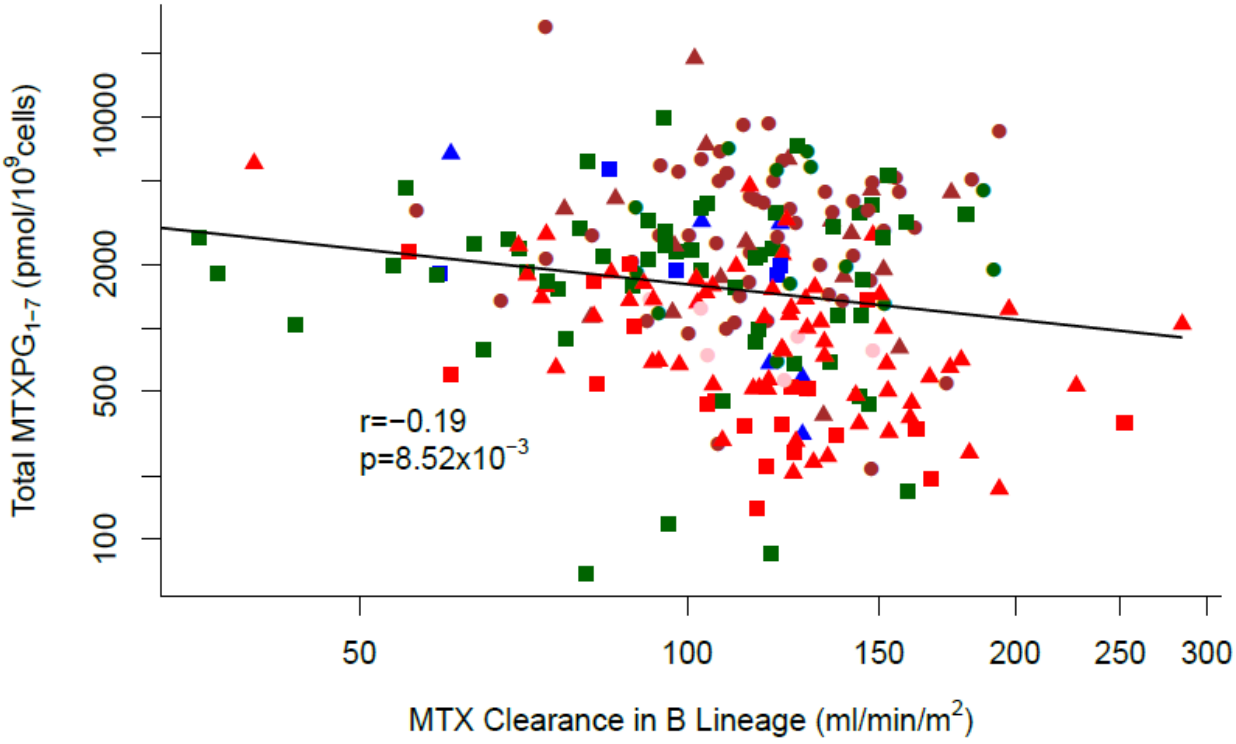
Supplemental Figure 2. Histogram of total methotrexate polyglutamate ($MTXPG_{1-7}$) accumulation in ALL cells from 388 patients after in vivo MTX treatment ($1gm/m^2$) given by either a 4 h (blue) or 24 h (red) intravenous infusion.

The blue dotted line represents the mean MTXPG accumulation in the group of patients who were administered a 4 h infusion of HDMTX. The red dotted line represents the mean MTXPG accumulation in the group of patients who were administered a 24 h infusion.



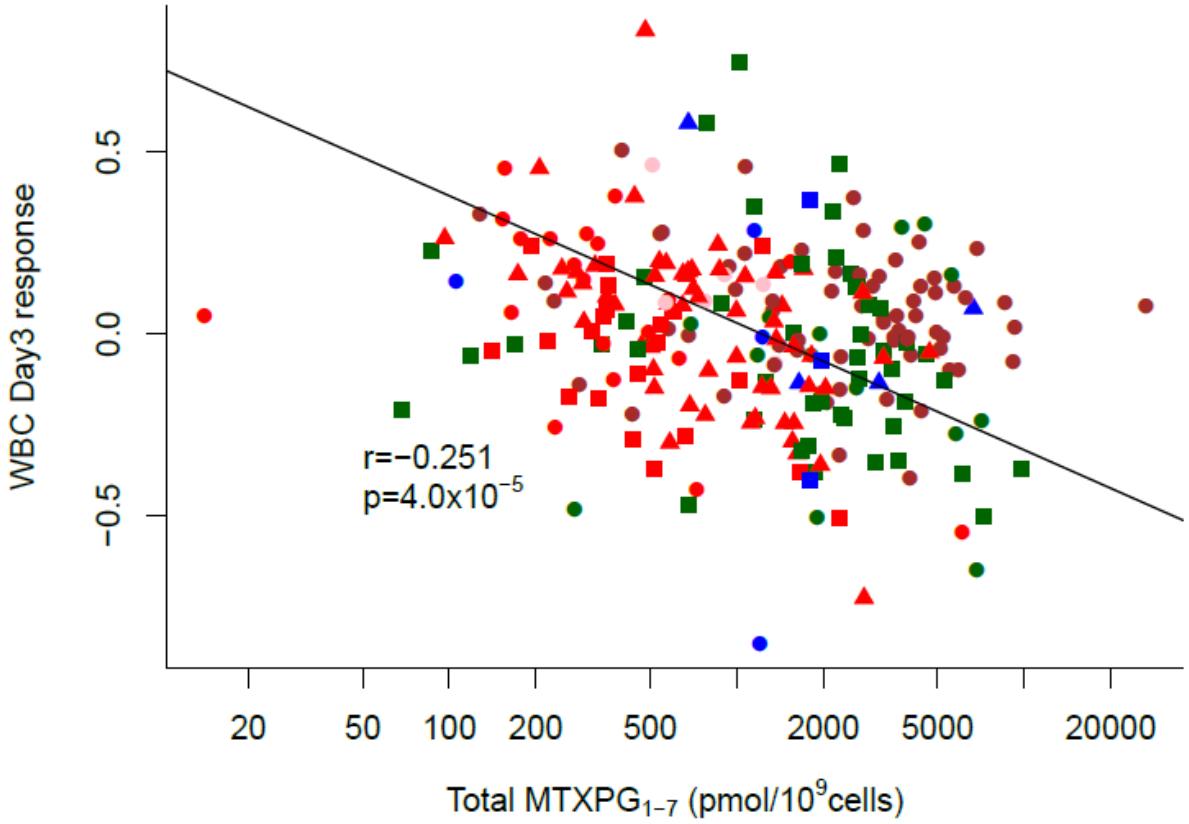
Supplemental Figure 3. Correlation between total MTXPG accumulation in ALL cells in vivo after HD-MTX treatment and MTX systemic clearance.

Total $MTXPG_{1-7}$ value (y-axis) for each ALL sample is plotted with respect to its corresponding MTX clearance observed in the patients following HD-MTX administration (x-axis). The line and p-value are from Pearson´s correlation. The symbols are defined in Figure 3.



Supplemental Figure 4. Correlation between intracellular MXPG and antileukemic effect of single agent treatment with HDMTX.

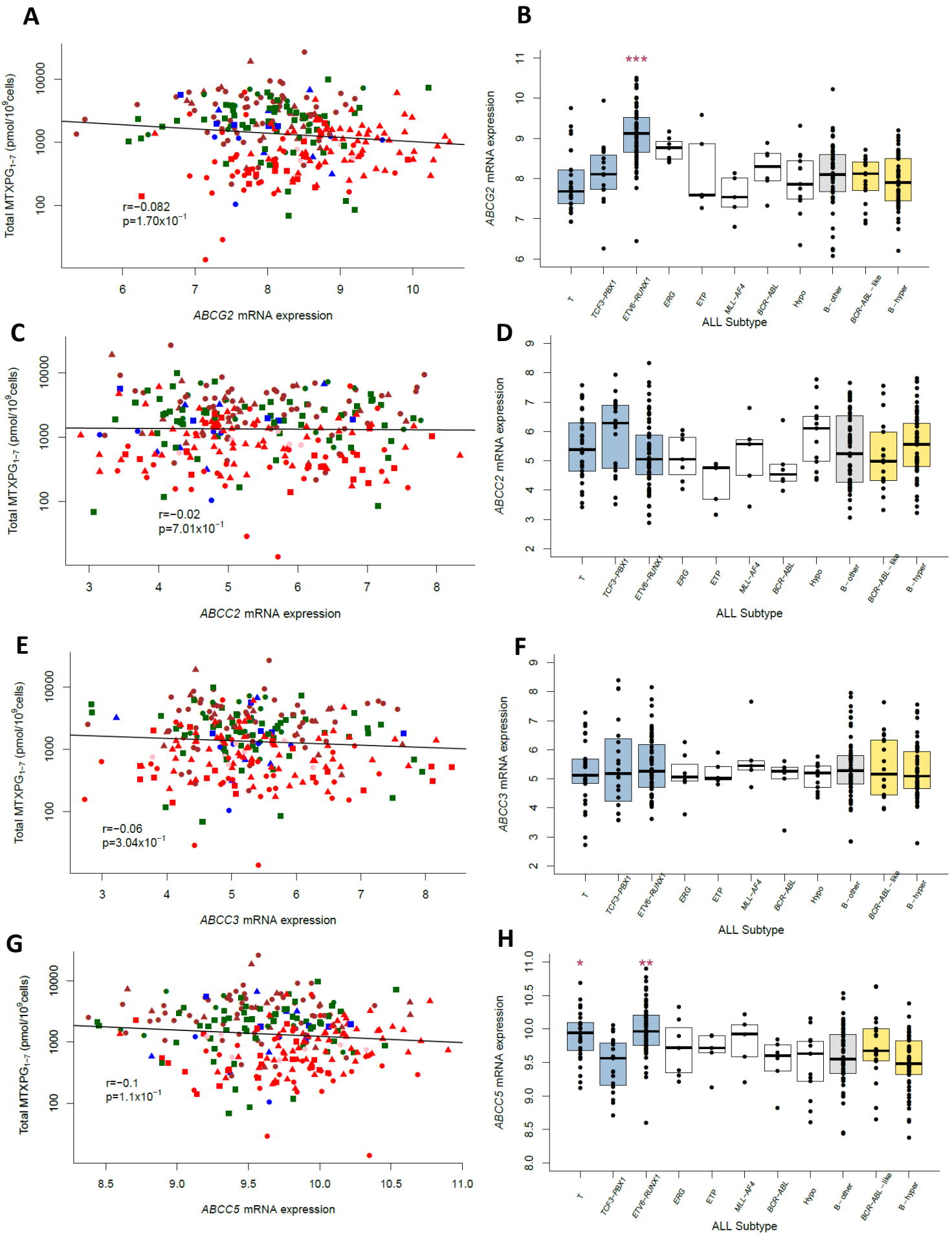
Relation between MTXPG concentrations in ALL cells in vivo (X-axis) and the antileukemic effects of MTX measured as the drop in ALL cells after 3 days of single agent treatment with HDMTX (1 gm/m^2). Each point represents a patient's ALL sample, Line depicts best-fit linear model (r=-0.251), Pearson´s correlation p=4 x10^{-5} Data are the same as shown in Fig 2c, except that ALL each subtype is depicted by a different symbol, as defined in Figure 3.



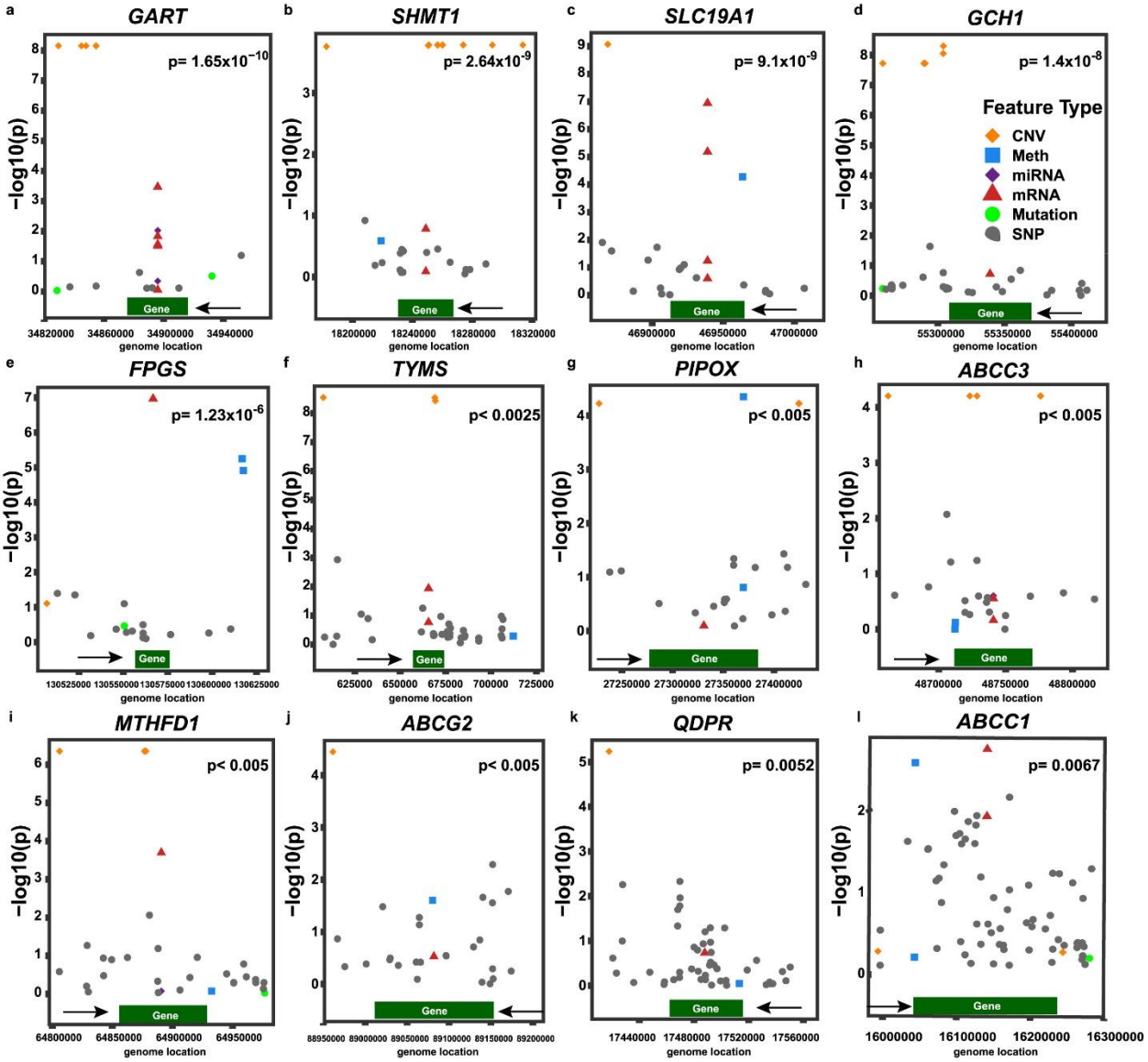
Supplemental Figure 5. Lack of correlation between intracellular MXPG and leukemia cell expression of mRNA encoding various ABC transporters

Panels A, C, E, G depict the relation between intracellular MTXPG and the expression of mRNA encoding ABC transporters. Lines depicts best-fit linear model, Pearson´s correlation p-values. Panels B, D, F, H depict the expression of mRNA encoding each ABC transporter in each of the eleven major ALL subtypes. The symbols are defined in Figure 3.

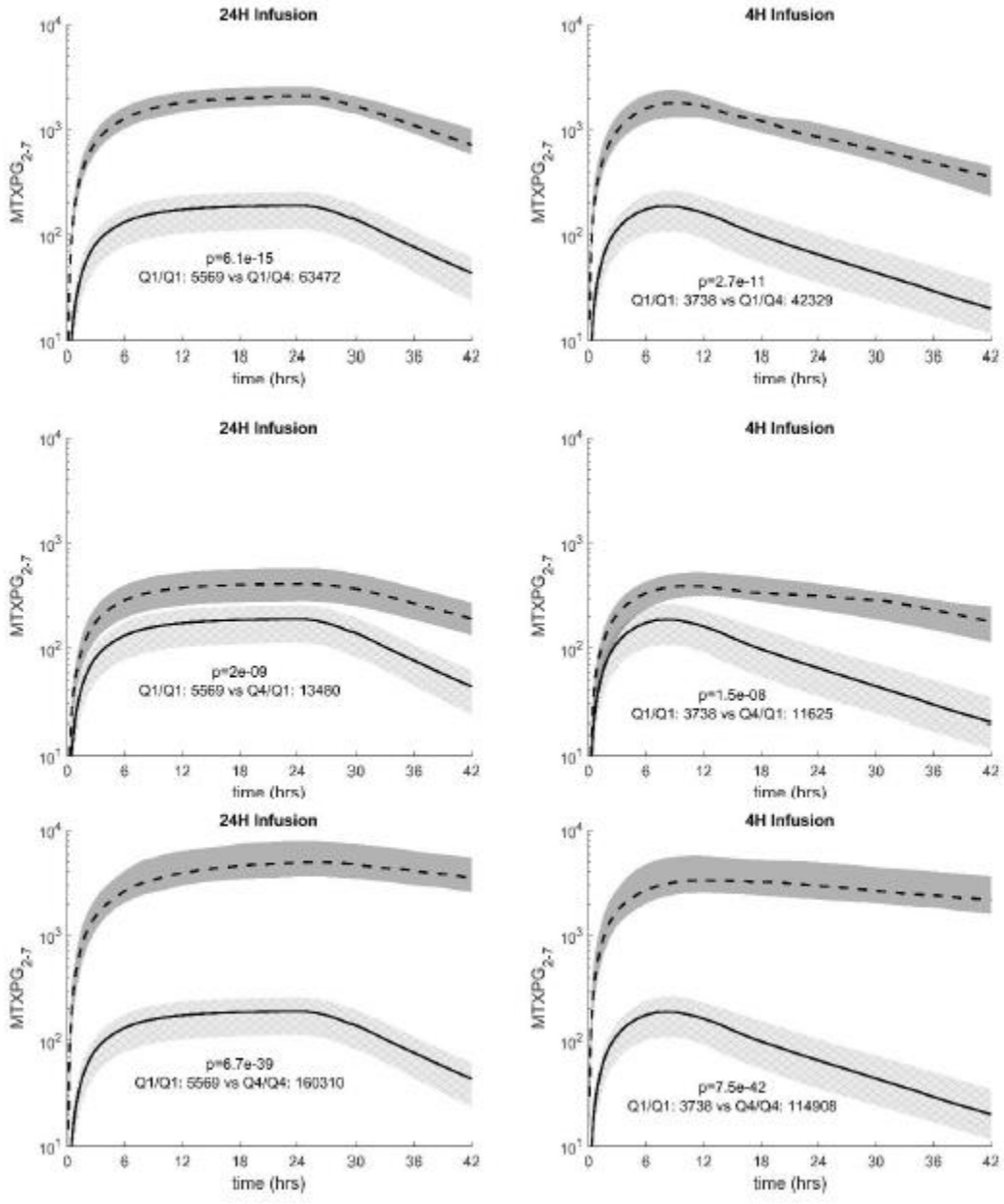
Supplemental Figure 5.



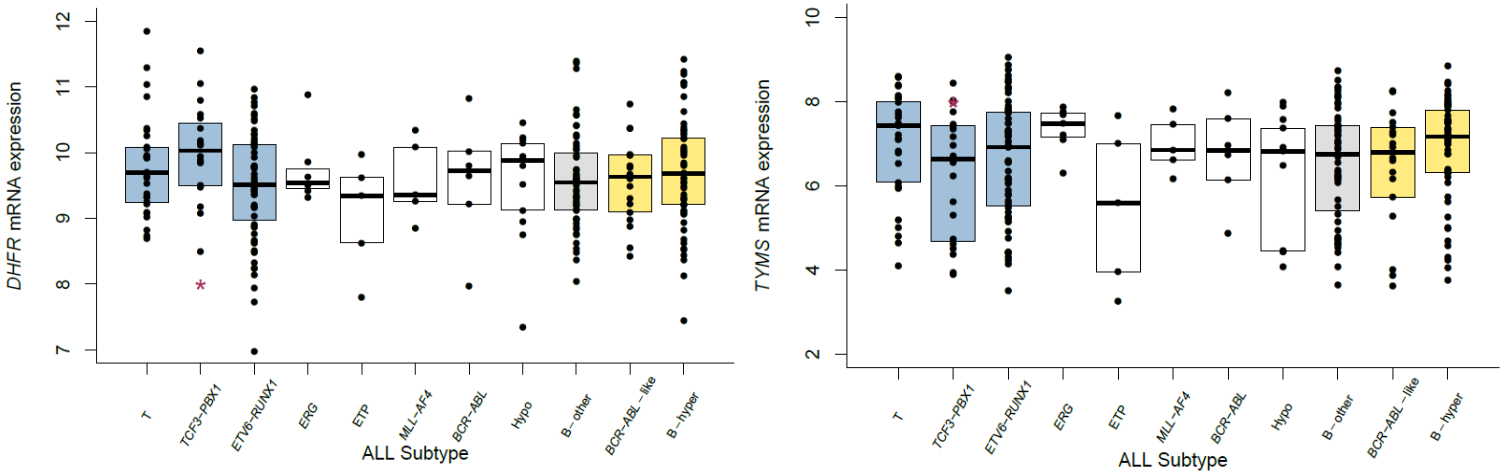
Supplemental Figure 6: Regional plots of genomics variants associated with in vivo accumulation of MTXPG in leukemia cells, aligned with each folate pathway gene achieving statistical significance by the Truncated Aggregation of P-values statistic (TAP). Each panel depicts -log10 p-values for the association of the indicated genomic feature with MTXPG, and the aggregated gene-level p-value (TAP statistic) based on all genomic features is shown for each gene at the top right. The six types of genomic features interrogated for each gene are each depicted by a different symbol, as indicated in the "Feature Type" key. The chromosomal location of each gene is indicated by the green box, and the chromosomal location of each feature type is indicated on the X-axis. The direction of transcription is indicated by the arrow.



Supplemental Figure 7: Model simulated accumulation of MTXPG in ALL cells, in vivo, after a 24H (left column) or a 4H (right column) intravenous infusion of HDMTX (1.0 gm/m2). Results are based on 1000 model simulations for each infusion length. Simulations of intracellular MTXPG are depicted for patients with different combinations of low (Q1=bottom quartile) or high (Q4=top quartile) expression of folate influx/efflux transporter ratio (*SLC19A1*/(*ABCC1*+*ABCC4*) and *FPGS*. P-values compare the model simulated AUC for intracellular MTXPG (indicated in each plot) in each group. Q1/Q4 = Q1 for Transporter ratio/Q4 for *FPGS*, etc.



Supplemental Figure 8: Expression of MTX's intracellular target proteins. mRNA expression of the MTXPG targets dihydrofolate reductase (*DHFR*) and thymidylate synthase (*TYMS*) in different subtypes of ALL. There were no significant differences in *TYMS* expression when comparing each subtype to the B-other subtype. There were also no significant differences in *DHFR*, except for *TCF3-PBX1* which had significantly higher *DHFR* when compared to B-other ALL (t-test p=0.036).



Supplemental Table 1. mRNA expression of folate pathway genes in ALL cells and their association with intracellular MTPG in primary ALL cells, in vivo.

Gene	Probe ID	Association with MTXPG (p-value*)	Estimate
FPGS	202945_at	1.22E-07	0.80
SLC19A1	211576_s_at	5.04E-06	0.55
MTHFD1	202309_at	3.43E-04	0.57
ABCC1	202804_at	7.97E-04	-0.57
ATIC	208758_at	1.7E-03	-0.48
PPAT	201913_s_at	5.0E-03	0.51
GART	212379_at	0.011	0.42
SPR	203458_at	0.017	0.26
MTHFD2L	220346_at	0.025	-0.13
ABCC4	203196_at	0.031	-0.17
TYMS	217684_at	0.037	0.23
FTH1	214211_at	0.042	-0.32
SARDH	207889_at	0.085	-0.25
MTR	203774_at	0.108	0.40
ATPIF1	218671_s_at	0.123	-0.31
ADA	204639_at	0.126	-0.18
ALPPL2	210431_at	0.160	0.22
SHMT1	217304_at	0.196	-0.17
SLCO1B3	206354_at	0.205	-0.10
QDPR	209123_at	0.208	0.18
DHFR	48808_at	0.226	0.16
SLC22A6	210343_s_at	0.239	0.13
ALPI	207140_at	0.239	0.18
GGH	203560_at	0.247	-0.14
GCH1	204224_s_at	0.253	-0.11
ABCG2	209735_at	0.266	-0.12
SLC22A8	221298_s_at	0.307	0.10
MTHFD2	201761_at	0.323	0.10
SHMT2	214095_at	0.327	-0.13
MTHFS	203433_at	0.409	0.12
PTS	209694_at	0.463	-0.10
CBS	212816_s_at	0.483	-0.05
FTCD	220604_x_at	0.508	-0.06
FOLR1	204437_s_at	0.546	0.08
ABCC2	206155_at	0.596	-0.04
SLCO1A2	211481_at	0.715	0.04
ABCC3	208161_s_at	0.787	0.04
FOLR2	204829_s_at	0.801	0.02
PIPOX	221605_s_at	0.815	-0.02
ALDH1L1	205208_at	0.840	0.03

ALPP	210431_at	0.891	0.01
AMT	204294_at	0.939	0.01
ALPL	215783_at	0.940	-0.00
SLCO1B1	210366_at	0.954	-0.00
MTHFR	206800_at	0.978	0.00
MTRR	203200_s_at	0.980	-0.00
ABCB1	209993_at	0.997	0.00

**P-value from linear model of MTXPG versus gene expression, with MTX systemic clearance and MTX infusion time as covariates.*

Supplemental Table 2. Assessment of the multivariable model for intracellular MTXPG.

B-ALL

	Coefficient	95% Confidence Interval	P-value
InfusionTime (4H vs. 24H)	-0.176	(-0.266, -0.086)	0.0013
ALL Subtype (vs. B-other)			$7.2x10^{-11}$
TCF3-PBX1	-0.365	(-0.542, -0.188)	
ETV6-RUNX1	-0.042	(-0.177, 0.093)	
ERG	-0.190	(-0.459, 0.08)	
MLL-AF4	0.055	(-0.258, 0.369)	
BCR-ABL	-0.089	(-0.376, 0.199)	
Hypo	0.286	(0.078, 0.494)	
BCR-ABL-like	0.229	(0.044, 0.414)	
B-hyper	0.294	(0.16, 0.429)	
Clearance	-0.292	(-0.439, -0.145)	0.0022
FPGS expression	0.162	(0.08, 0.243)	0.020
SLC19A1/(ABCC1 + ABCC2)	0.074	(0.032, 0.117)	0.0012

B and T-ALL

	Coefficient	95% Confidence Interval	P-value
Infusion Time (4H vs. 24H)	-0.178	(-0.267, -0.089)	0.0015
ALL Subtype (vs. B-other)			$1.1x10^{-11}$
T	-0.482	(-0.659, -0.305)	
TCF3-PBX1	-0.373	(-0.561, -0.185)	
ETV6-RUNX1	-0.062	(-0.204, 0.08)	
ERG	-0.177	(-0.464, 0.109)	
ETP	-0.188	(-0.524, 0.148)	
MLL-AF4	0.045	(-0.288, 0.379)	
BCR-ABL	-0.097	(-0.403, 0.209)	
Hypo	0.277	(0.056, 0.497)	
BCR-ABL-like	0.227	(0.032, 0.423)	
B-hyper	0.278	(0.135, 0.421)	
Clearance	-0.243	(-0.384, -0.103)	0.044
FPGS expression	0.127	(0.047, 0.206)	0.0021
SLC19A1/(ABCC1 + ABCC2)	0.081	(0.038, 0.123)	0.00036

The p-value shown for each variable is derived from comparing the full model with all covariate to the same model but with the corresponding variable removed. In every case, a significant p-values indicates that the FULL MODEL performs better than the model without the removed covariate.Dependent variable is log10 transformed Total Methetraxate polyglutamate accumulation (pmol/10^9 cells). Clearance, *FPGS* expression and transporter ratio (*SLC19A1*/(*ABCC1* + *ABCC2*)) are log-transformed. P-values are based on F-test comparing full model vs. the model excluding the predictor.

Supplemental Table 3. Assessment of the multivariable model for leukemia response.

B and T-ALL, using **measured** MTXPG accumulation

	Coefficient	95% Confidence Interval	P-value
Subtype (vs. B-other)			0.023
T	0.003	(-0.133, 0.139)	
TCF3-PBX1	-0.136	(-0.272, 0.001)	
ETV6-RUNX1	0.030	(-0.07, 0.129)	
ERG	0.154	(-0.046, 0.355)	
ETP	-0.119	(-0.36, 0.121)	
MLL-AF4	0.046	(-0.226, 0.318)	
BCR-ABL	0.278	(0.005, 0.551)	
Hypo	0.038	(-0.114, 0.189)	
BCR-ABL-like	0.004	(-0.13, 0.138)	
B-hyper	0.167	(0.068, 0.266)	
Observed log10(Total MTXPG)	-0.204	(-0.282, -0.127)	5.60E-07

B and T-ALL, using predicted MTXPG accumulation

	Coefficient	95% Confidence Interval	P-value
Subtype (vs. B-other)			0.03
T	-0.068	(-0.232, 0.096)	
TCF3-PBX1	-0.204	(-0.363, -0.045)	
ETV6-RUNX1	-0.004	(-0.113, 0.104)	
ERG	0.120	(-0.088, 0.329)	
ETP	-0.153	(-0.403, 0.097)	
MLL-AF4	0.075	(-0.205, 0.354)	
BCR-ABL	0.219	(-0.06, 0.497)	
Hypo	0.089	(-0.073, 0.25)	
BCR-ABL-like	0.056	(-0.088, 0.2)	
B-hyper	0.219	(0.109, 0.329)	
Predicted log10(Total MTXPG)	-0.376	(-0.56, -0.193)	8.15E-05

Dependent variable is ALL cell count response as defined in Methods (Sorich et al, PlosMed. 2009). A unit change indicates 10 fold differences in raw ALL count. The predicted MTXPG is based on the multivariable model including infusion time, clearance, ALL subtype, FPGS expression and transporter ratio. The coefficient of -0.204 indicate that 10-fold increase in Total MTXPG correspond to a WBC Day3 reduction of 37% (1 - 10^-0.204).